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Biosynthesis of acurin A and B in Aspergillus aculeatus

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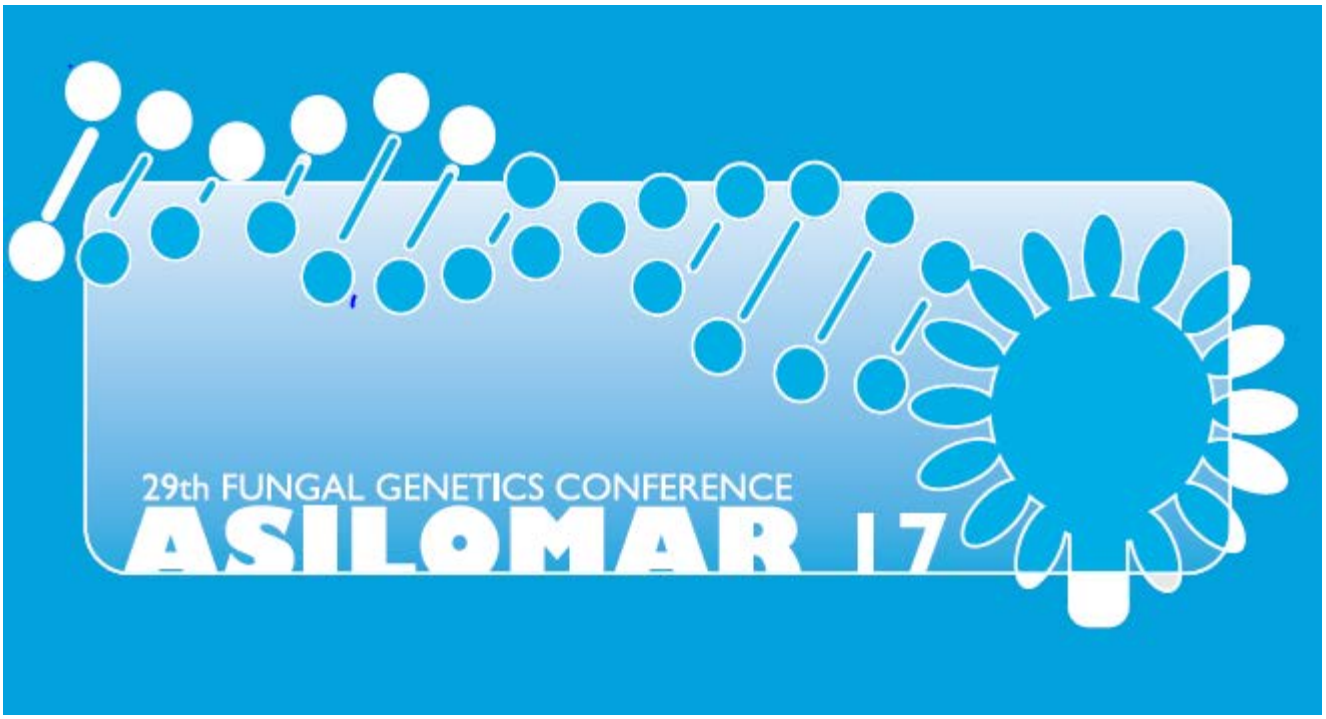

March 14-19, 2017
Pacific Grove, CA

Abstract Book

Genetics Society of America

Wednesday, March 15 9:00 AM – 12:00 NOON
Merrill Hall and Chapel
Plenary Session I: Model Fungi: Biological Insights Beyond the Kingdom

***Neurospora* as a model for circadian clocks in higher organisms.** *J.J. Loros*[1], X. Zhou[1], A. Crowell[1], J.M. Hurley[2], J.M. Emerson[1], B.R. Crane[3], D. Bell-Pedersen[5], M. Freitag[4], L.F. Larrondo[6], J.C. Dunlap[1] 1) Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Rensselaer Polytechnic Institute, Troy, NY; 3) Cornell University, Ithaca, NY; 4) Oregon State University, Corvallis, OR; 5) Texas A&M University, TX; 6) Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile.

Circadian rhythmicity, the cell-intrinsic ability of organisms to track internal time and thereby coordinate activities with the environment, has arisen at least three times in evolution. Neurospora crassa has been an durable system for understanding the molecular bases of these oscillators within the fungal and animal Kingdoms; Neurospora is perhaps the most intensively studied of all circadian cell types. At the core of the circadian system is the heterodimer of White Collar-1 and -2 forming the White Collar Complex (WCC) that drives the expression of the frequency (frq) gene. FRQ complexes with other proteins, including Frequency Interacting RNA Helicase (FRH) and casein kinase 1 (CK1), and in a time-delayed, and highly regulated manner dictated by progressive phosphorylation the complex reduces the activity of the WCC, thus resulting in a negative auto-regulatory feedback loop.

WC-1 and WC-2 interact via PAS domains as do similar positively acting clock-relevant TFs in animal clocks; conserved within the negative element FRQ-FRH complex is the interaction with CK1 and the extensive phosphorylation of a negative element that generates the time delay. FRQ, an intrinsically disordered protein (IDP), is stabilized by FRH which acts non-enzymatically in the clock; separately FRH has a helicase function required for cell viability. The negative elements in fungal and animal clocks are poorly conserved at the protein level, even just among animals, possibly reflecting the fact that many appear to be IDPs for which there is no inherent structural drive for conservation of sequence.

Circadian clocks entrain to daily environmental cycles of light and temperature. Molecular mechanisms of light responsiveness are well understood in Neurospora and the way in which light resets rhythms in general was first worked out in this system. Of central importance for any organism with a clock is the daily control of cellular activities -growth, metabolism, behavior, development and

beyond. Work on Neurospora set the paradigms for understanding clock output, revealing that most of it is through clock-controlled gene expression. As much as 40% of the Neurospora genome is clock controlled at the level of transcript abundance by greater than 50 TFs. Recently a consortium of laboratories have collaborated in using RNA-Seq, ChIP-Seq and bioinformatics to understand the transcriptional networks through which output from the circadian clock in Neurospora is controlled.

Molecular control of fungal senescence: macroautophagy as a "double edged" sword in a complex cellular quality control network. *Heinz D. Osiewacz*, Laura Knuppertz, Alexander Jung, Piet Kramer, Andrea Hamann Molecular Biosciences, J W Goethe University, Frankfurt, DE.

Podospora anserina is a well established model organism to unravel the complex molecular basis of organismal aging. Research with this system uncovered a strong mitochondrial etiology and provided novel perspectives for the elucidation of molecular mechanisms of aging relevant from yeast to humans.

In the last decade, we used *P. anserina* as an *in vivo* system to study the influence on organismal aging of pathways involved in the control of mitochondrial quality. We observed that pathways active in balancing reactive oxygen species, the degradation of proteins by mitochondrial proteases, mitochondrial fission and fusion, programmed cell death, and recently macroautophagy (hereafter termed autophagy) all influence the aging process. Sometimes we obtained results that were according to our expectations. In other cases, they were counterintuitive and called for further investigations and more complex explanations. Recently, a knockout mutant in which the mitochondrial superoxide dismutase isoform was ablated did not show any effect on lifespan compared to the wild type, although the cellular superoxide anion load was strongly increased and mitochondrial respiration was moderately impaired. We found that in this mutant selective autophagy of mitochondria, termed mitophagy, is increased and acts as a compensating "pro-survival" pathway. We also found that the beneficial effect of mild oxidative stress, which is described in different organisms as mitohormesis, in *P. anserina* depends on a functional autophagy machinery. In contrast, increased oxidative stress or stress caused by excessive overexpression of the gene coding for cyclophilin D turns autophagy to a "pro-death" pathway that leads to "autophagic cell death", also termed "programmed cell death type II". In another mutant, general or "bulk" autophagy was found to be induced while in a third mitochondrial quality control mutant both general autophagy and mitophagy were unaffected. Overall, these observations demonstrate a context-dependent flexible function of autophagy in the control of cellular homeostasis that allows an organism to adapt to changing conditions. The elucidation of the underlying regulatory mechanisms and of crosstalks between different pathways is one of the key challenges in this field of research.

***Phytophthora* effectors promote infection by suppressing small RNA silencing in the host.** Y. Zhai[1], D. Choi[1], J. He[2], Y. Hou[1], W. Ye[3], J. Ma[2], *W. Ma*[1], Y. Zhai, D. Choi and J. He contribute equally to this work. 1) University of California, Riverside, CA; 2) Fudan University, Shanghai, China; 3) Nanjing Agricultural University, Nanjing, China.

A broad range of parasites rely on the functions of effector proteins to subvert host immunity and facilitate disease development. The genus *Phytophthora* contains some of the most notorious plant pathogens. Each *Phytophthora* genome is predicted to encode several hundreds to over one thousand effectors, and the majority of them contained the consensus RxLR motif, which allows effector uptake into the host cells. This remarkably large effector repertoire reflects the high level of complexity in *Phytophthora*–plant arms race and demands mechanistic analysis of effector functions in order to gain understanding of *Phytophthora* pathogenesis.

Previously, we discovered RxLR effectors with RNA silencing suppression activity from the soybean pathogen *Phytophthora sojae*. Interestingly, *Phytophthora* Suppressor of RNA silencing 2 (PSR2) has seven tandem repeats, each containing the so-called L-W-Y motifs. Crystal structure of PSR2 revealed that each tandem repeat forms a conserved five-alpha-helix bundle. Genome-wide analysis of RxLR effectors in five *Phytophthora* species identified 20-30 PSR2-like effectors (per species) with various numbers of the repeat units but similar

structural fold, suggesting that PSR2 belongs to a highly conserved and diversified effector family. Other members of the PSR2 effector family also possess RNA silencing suppression activity.

PSR2 promotes infection by specifically affecting the accumulation of secondary small interfering RNAs called phasiRNAs in plants. We characterized PSR2-associating protein(s) in plants in order to understand its virulence mechanism. Our results show that PSR2 interacts with Double-stranded RNA-Binding protein 4 (DRB4), which has a known function in phasiRNA biogenesis by partnering with the endonuclease Dicer-like protein 4 (DCL4). We further determined that genes involved in the phasiRNA pathway positively regulate plant defense against *Phytophthora*. These results suggest that PSR2 promotes infection by targeting phasiRNA biogenesis through its interaction with DRB4.

The impact of modulating translation initiation, elongation and termination on gene expression. *M.S. Sachs* Department of Biology, Texas A&M University, College Station, TX.

The central dogma of molecular biology, generally stated, is that DNA makes RNA (transcription), and RNA makes protein (translation). Studies of model fungi have been instrumental in establishing the strategies and mechanisms that eukaryotes use to control transcription and translation. While the importance of transcriptional control is universally acknowledged, there is now a deepening and widening appreciation of the diverse roles of translation in controlling gene expression. For example, it is now apparent that upstream open reading frames (uORFs), once thought of as rarities, are present in more than 25% of eukaryotic mRNAs, and that the translation of these uORFs can be critical for controlling gene expression levels. It is now apparent that initiation of translation of these uORFs - as well as initiation of translation of the major predicted gene products of mRNAs – can occur at codons other than AUG codons, and these non-AUG initiation events have functional significance. The rate of translation elongation can also be modulated at the level of specific mRNAs by codon-choice or by encoded nascent peptides that stall ribosomes by affecting the function of the ribosome's peptidyltransferase center. Initiation and elongation can both be modulated by controlling the activity of the translation machinery. Events associated with translation termination can have regulatory functions critical for controlling gene expression and also can have a major impact on mRNA stability through the mRNA quality control pathway known as nonsense-mediated mRNA decay (NMD). Here we discuss these processes of translational control and studies in the model fungi that have contributed to our general understanding of how they impact gene expression.

Autocatalytic backbone N-methylation hallmarks a distinct family of ribosomally encoded peptides. N. van der Velden, N. Kalin, M. Helf, J. Piel, M. Freeman, *M. Kunzler* Institute of Microbiology, ETH Zurich, Zurich, CH.

N-Methylated backbone amides alter the physicochemical properties of peptides and are critical for the activity and stability of blockbuster drugs like the immunosuppressant cyclosporin A. In nature, backbone N-methylations have not been observed in proteins, but only in peptides not synthesized by the ribosome. Here, we show that omphalotins, cyclic backbone N-methylated peptides from the mushroom *Omphalotus olearius*, are ribosomally synthesized and post-translationally modified peptides (RiPPs). Expression of a single gene, *ophA*, in *Escherichia coli* revealed auto-α-N-methylation activity of the encoded protein. Remarkably, the sequence destined to be the nematotoxic natural products is encoded in the C-terminus of OphA, and we demonstrate that OphA catalyzes iterative α-N-methylation of this sequence. Thus, OphA is the first observed case of backbone N-methylations as post-translational modifications (PTMs) and the first RiPP precursor found to be capable of catalysis or self-modification. The omphalotins represent, therefore, a new RiPP family, one of the few so far identified in fungi. We also report that OphA can methylate engineered peptide sequences exchanged for the core omphalotin sequence, providing a straightforward and convenient biotechnological strategy to produce custom peptides with backbone N-methylation.

Wednesday, March 15 3:00 PM – 6:00 PM
Merrill Hall
Plant-Fungus Interactions

The two-speed genome of *Verticillium dahliae* mediates emergence of potent virulence factors. *B.P.H.J. Thomma*, L. Faino, J. Li, X. Shi-Kunne, J. Depotter, M. Kramer, G.C. van den Berg, D.E. Cook, H. Rovenich, M.F. Seidl Laboratory of Phytopathology, Wageningen University, Wageningen.

Genomic plasticity enables adaptation to changing environments, which is especially relevant for pathogens that engage in “arms races” with their hosts. In many pathogens, virulence genes reside in highly variable, transposon-rich, physically distinct genomic compartments. However, understanding of the evolution of such compartments, and the role of transposons therein, remains limited. We show that transposons are the major driving force for adaptive genome evolution in the fungal plant pathogen *Verticillium dahliae*, and that highly variable lineage-specific (LS) regions evolved by genomic rearrangements that are mediated by erroneous double-strand repair, often utilizing transposons. Remarkably, LS regions are enriched in active transposons, which may contribute to local genome plasticity. Thus, we provide evidence for genome shaping by transposons, both in an active and passive manner, which impacts the evolution of *V. dahliae* virulence. Based on this knowledge, we are now able to identify crucial virulence factors of *V. dahliae*, which also allows investigating causal relationships between particular effectors and pathotypes.

Effect of cytokinin level manipulation on virulence in the biotrophic fungus *Claviceps purpurea*. *S. Kind*[1], J. Hinsch[1], P. Galuszka[2], P. Tudzynski[1] 1) University of Muenster, Muenster, DE; 2) Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ.

The ergot fungus *Claviceps purpurea* is a biotrophic phytopathogen with a broad host range including economically important crops causing harvest intoxication upon infection. Its infection process is restricted to unfertilized ovaries without causing macroscopic defense symptoms. Thus, sophisticated host manipulation strategies are implicated. The plant hormones cytokinins (CKs) are known to regulate diverse plant cell processes and several plant pathogens alter CK levels during infection. For the first time, we could show that *C. purpurea* synthesizes CKs via two mechanisms and that fungus-derived CKs influence the host-pathogen interaction (Hinsch *et al.*, 2015 and 2016). For a better understanding of the CK biosynthesis and the CK contribution to the plant-fungus interaction we applied different approaches to generate strains with altered CK type composition. The first approach is based on the deletion of the two “LOG” encoding genes which are supposed to be essential for the release of active CKs. We generated both, single and double deletion strains and they were found to be able to produce all types of CKs. Apparently, LOG gene products are dispensable for the formation of CKs. The CK biosynthesis pathway remains unaffected in the second approach as it is based on heterologous overexpression of CK degrading enzymes from maize (Ckx1) and *Rhodococcus fascians* (FasE). Preliminary results indicate that strains overexpressing *Zmckx1* have strongly increased Ckx-activity and drastically reduced CK levels. Evidently, overexpression of Zmckx1 resulted in the formation of functional protein. To our knowledge this is the first time a plant gene was successfully expressed in *C. purpurea*. Additionally, the strains showed reduced virulence dependent on the degree of the overexpression. Although characterization of the strains is not completed yet, so far this approach confirms previous findings that fungal-derived CKs are essential for a compatible plant-fungus interaction.
Hinsch *et al.* (2015) Environ Microbiol: 2935-2951
Hinsch *et al.* (2016) New Phytol: 980-992

Field pathogenomics of wheat yellow rust. *Diane Saunders*[1,2] 1) Crop Genetics, John Innes Centre, Norwich, Norfolk, GB; 2) Earlham Institute, Norwich, Norfolk, GB.

Emerging and re-emerging pathogens pose a continuous threat to food security and human health. Recent advances in next-generation sequencing technologies have provided new opportunities to integrate genomics into the tracking of emerging filamentous plant pathogens. Accordingly, we are leading the genome sequencing of hundreds of isolates of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici* (PST), aimed at improving our understanding of the molecular mechanisms that drive PST evolution. Furthermore, we have developed a robust and rapid “field pathogenomics” strategy to improve filamentous pathogen surveillance. Using gene sequencing of PST-infected wheat leaves taken directly from the field, this technique enabled us to gain insight into the population structure of PST over successive seasons. Our analysis uncovered a dramatic shift in the PST population in the UK and supports the hypothesis that a recent introduction of a diverse set of exotic PST lineages may have displaced the previous populations. Furthermore, we uncovered potential seasonal and varietal specificity for specific genotypes of PST. This knowledge regarding which wheat varieties are susceptible to specific PST isolates, and when those isolates are prevalent throughout the year, represents a powerful tool for disease management.

Historic transposon-driven evolution of a novel non-ribosomal peptide. *D. Berry*[1], H.B. Bode[2], K. Grage[1], W. Mace[3], C.A. Young[4], C.L. Schardl[5], P.P. Dijkwel[1], B. Scott[1] 1) Massey University, Palmerston North, New Zealand; 2) Goethe University, Frankfurt, Germany; 3) AgResearch, Palmerston North, New Zealand; 4) The Samuel Roberts Noble Foundation, Ardmore, OK, USA; 5) University of Kentucky, Lexington, KY, USA.

Epichloë fungi are endophytic symbionts of grasses that produce a variety of bio-protective secondary metabolites. One such metabolite, peramine, is an insect-deterring cyclic dipeptide synthesised by the two-module non-ribosomal peptide synthetase (NRPS) PerA. A historic small transposable element insertion into the 3’ end of the *perA* gene resulted in deletion of sequence encoding the C-terminal reductase domain of PerA, which is required for product release during peramine synthesis. This truncated allele, named *perA-2*, is the only known widespread peramine-negative allelic variant of *perA*; balancing selection and recombination has maintained both *perA* and *perA-2* alleles across multiple speciation events in an example of trans-species polymorphism. We have conducted extensive experiments utilising *Penicillium paxilli* as a heterologous expression system for *perA*, *perA-2*, and a number of synthetic *perA/perA-2* chimeric alleles. We report results demonstrating that the transposon-mediated deletion in *perA-2* is associated with substrate specificity alterations and methylation domain inactivation in PerA-2 proteins. In contrast to previous reports characterising *perA-2* alleles as non-functional, we demonstrate that these evolutionary changes culminate in production of a novel cyclic dipeptide. The structure of this new secondary metabolite will be

described, as will the spontaneous release mechanism that is proposed to take the place of a dedicated product release domain in PerA-2 proteins.

Dissecting the role of the Tox3 effector in the *Parastagonospora nodorum*-wheat interaction. Susan Breen[1], Megan Outram[2], Bostjan Kobe[2], Simon Williams[1], *Peter Solomon*[1] 1) Research School of Biology, The Australian National University, Canberra, ACT, AU; 2) School of Chemistry and Molecular Biosciences, The University of Queensland, QLD, AU.

It had long been thought that necrotrophic pathogenic fungi use a barrage of lytic enzymes to break down plant cells releasing nutrients for growth. However, in recent years it has emerged that some necrotrophic fungi facilitate disease through a strict gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen *Parastagonospora nodorum*, the basis of this host specific interaction is small cysteine-rich effector proteins secreted during infection (ToxA, Tox1 and Tox3). These effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, whilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood.

To characterise these necrotrophic effectors, a search for potential host protein binding partners for the Tox3 effector was conducted. From this work, the wheat TaPR1-1 protein was validated through two independent approaches to interact with Tox3. Subsequent analysis confirmed that Tox3 was able to interact with most acidic and basic TaPR1 proteins in wheat, but not those harbouring a C-terminal extension. Mutagenesis and protein modelling using interacting and non-interacting TaPR1 proteins has predicted several amino acids involved in this interaction and we have now made significant progress in characterising these proteins at the structural level using a crystallography approach.

These findings have raised the question as to the role of the TaPR-1 proteins during infection and the consequence of its interaction with the Tox3 effector. A recent study by Chen *et al*, 2014 showed that the CAPE1 peptide derived from the C-terminus of the tomato PR-1b regulates immune signalling. This peptide is conserved in the TaPR1 proteins but is not required for the interaction with Tox3. The exogenous application of the peptide on wheat leaves significantly enhanced *P. nodorum* disease in a Tox3-dependent manner tentative linking the interaction of Tox3 and TaPR1 with CAPE1 defence signalling. We have now also shown that the CAPE1 peptide directly impacts on the outcome of other diseases, both necrotrophic and biotrophic. These data raise important questions as to the function of the enigmatic PR1 proteins in plant-microbe interactions and highlight the intriguing prospect that they potentially function as carrier proteins to localise the CAPE1 defence signalling peptides to the host apoplast.

Roles of polyamine metabolism in appressorium function. *Raquel Rocha*, Richard Wilson Plant Pathology, University of Nebraska - Lincoln, Lincoln, NE.

Polyamines are a group of ubiquitous and essential metabolites found in all living organisms. In fungi, the most abundant molecules - putrescine, spermidine, and spermine – have roles in conidiation and spore germination in *Aspergillus*, *Mucor,* and *Phycomyces* species. Little is known about how polyamine metabolism affects the development of fungal infection structures. Here, we report that a spermine synthase - encoded by *SMT1* –is required by the rice blast fungus *Magnaporthe oryzae* to generate spermine from spermidine and is essential for developing functional infection cells (appressoria) on rice leaf surfaces. *M. oryzae*, the most important pathogen of cultivated rice, elaborates appressoria at the tips of germ tubes emerging from spores on the leaf surface. The melanin-rich wall of the appressorium allows solutes such as glycerol to accumulate and generate enormous internal turgor pressure. This pressure is translated into mechanical force acting on the penetration peg emerging from a pore at the base of the appressorium, forcing it through the plant cuticle. In contrast, incipient cytorrhysis experiments determined that the appressoria of Δ*smt1* mutant strains generated turgor pressures that were 1.8 higher than wild type (WT), but no penetration pegs were observed breaching rice leaf cuticles by confocal microscopy. These developmental defects were not due to misregulated lipid mobilization or cell cycle progression, which occurred like WT in the D*smt1* mutant strains. Rather, by calculating the ratio of appressoria plasmolysis to cytorrhysis following PEG treatment, we determined that appressorial cell wall porosity was increased 3.5 in Δ*smt1* mutant strains compared to WT. We conclude that polyamine metabolism in *M. oryzae* appressoria, like in *Arabidopsis*, might control cell wall porosity. Perturbing porosity in Δ*smt1* appressoria could affect turgor and/or the rigidity of the cell wall. In turn, these structural defects might affect the polarized growth of the penetration peg from the appressorial pore. Ongoing studies of D*smt1* will, compared to WT, examine the structure of the appressorial cell wall, examine the pore at the base of the appressorium, and determine the status of penetration peg formation. Taken together, our results reveal novel roles for polyamine metabolism in appressorium function that enrich our understanding of the metabolic pathways intrinsic to rice cell colonization by *M. oryzae*.

Characterization of effector candidates from the soybean rust fungus that suppress plant immunity. *M. Qi*[1], T Link[2], R Voegele[2], T Baum[1], S Whitham[1] 1) Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA; 2) Institut für Phytomedizin, Universität Hohenheim, Otto-Sander-Straße 5 70599 Stuttgart, Germany.

Soybean rust (SBR) is an important disease caused by *Phakopsora pachyrhizi* (*Pp*). Like other rust fungi, *Pp* forms specialized cells called haustoria, which are key hubs of molecular exchange with host cells. Haustoria express effector proteins that are transferred to plant cells where they promote fungal growth. We have recently sequenced the *Pp* haustorial transcriptome and predicted candidate effector proteins. To gain insights into functions of *Pp* effector candidates (*Pp*ECs), we have cloned 82 *Pp*EC coding sequences and delivered the proteins into host and non-host plants using a bacterial type III secretion system-based delivery vector. Several *Pp*ECs suppressed immune responses associated with effector- and pattern-triggered immunity, and we are in the process of characterizing these further. One interesting example is *Pp*EC23, a small, cysteine-rich effector candidate that attenuated the hypersensitive response caused by *Pseudomonas syringae* pv *tomato* DC3000 on soybean and tobacco, and it also suppressed basal defense on tobacco and Arabidopsis. *Pp*EC23 interacts with a transcription factor that our data suggests negatively regulates soybean defenses. We are now using host-induced gene silencing and transcriptomics to determine the effects of *Pp*EC23 and the interacting soybean transcription factor in immunity or virulence. The results are expected to provide mechanistic insights into the molecular basis of SBR and may be relevant to other rust diseases.

A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. Ziming Zhong[1], Thierry Marcel[2], Clémence Plissonneau[1], Bruce McDonald[1], Daniel Croll[1], *Javier Palma-Guerrero*[1] 1) Plant Pathology Group, ETH Zürich, Switzerland; 2) UMR BIOGER, INRA, , France.

Zymoseptoria tritici is the causal agent of Septoria tritici blotch (STB), a major pathogen of wheat globally and the most damaging pathogen of wheat in Europe. The *Z. tritici* infection cycle includes an extended asymptomatic phase characterized by slow apoplastic growth followed by a switch to necrotrophy that coincides with the onset of plant cell death. Gene-for-gene (GFG) interactions between this apoplastic pathogen and resistant wheat cultivars have been postulated for many years, but until now none of the genes involved in this interaction were identified. We identified *AvrStb6* by combining QTL mapping based on a cross between two Swiss strains with a genome-wide association study (GWAS) using a natural population of ~100 strains coming from France. We functionally validated *AvrStb6* using ectopic transformations. *AvrStb6* encodes a small, cysteine-rich, secreted protein, with features of fungal effector proteins, which produces an avirulence phenotype on wheat cultivars carrying the *Stb6* resistance gene. *AvrStb6* shows peak of expression during the transition to the necrotrophic phase suggesting that the protein may act as a necrotrophic effector in susceptible cultivars. We found 16 non-synonymous SNPs among the tested strains, indicating that *AvrStb6* is evolving very rapidly. By analyzing the finished genomes of both parental strains used to establish the cross, we found that *AvrStb6* is located in a highly polymorphic subtelomeric region and is surrounded by transposable elements. This genomic environment may facilitate the rapid evolution of *AvrStb6* and enable *Z. tritici* to rapidly adapt to overcome *Stb6* resistance. *AvrStb6* is the first avirulence gene to be functionally validated in *Z. tritici*, contributing to our understanding of avirulence in apoplastic pathogens and the mechanisms of GFG interactions between *Z. tritici* and wheat.

Wednesday, March 15 3:00 PM – 6:00 PM
Chapel
Mating and Clonality in Fungi

Functional link between NADPH oxidase complex and pheromone perception machinery in chemotropic sensing of host plant signals by *Fusarium oxysporum*. Daniela Nordzieke, Stefania Vitale, Mennat El Ghalid, Maria Crespo Ruiz-Cabello, David Turrà, *Antonio Di Pietro* Departamento de Genetica, Universidad de Cordoba, 14071 Cordoba, Spain.

Chemotropism, the ability to re-orient hyphal growth in response to chemical cues, is critical for many aspects of the fungal lifestyle including sexual development and host infection. We previously showed that the root-infecting pathogen *Fusarium oxysporum* senses heme peroxidases secreted by plant roots as chemoattractant signals to direct hyphal growth towards the host. This chemotropic response requires the two pheromone receptors Ste2 and Ste3, as well as elements of the conserved fungal cell wall integrity mitogen-activated protein kinase (CWI MAPK) cascade. Because peroxidases catalyze the reductive cleavage of reactive oxygen species (ROS), we examined the role in chemotropism of the NADPH oxidase complex (NOX), which generates superoxide by transferring electrons across the membrane. Using deletion mutants in different components of the *F. oxysporum* NOX complex, we found that NoxB, NoxR and the tetraspanin Pls1, but not NoxA, are specifically required for peroxidase-induced chemotropism. NOX mutants were impaired in the peroxidase-triggered phosphorylation of the CWI MAPK Mpk1 and the high osmolarity MAPK Hog1. Importantly, directional growth towards peroxidase was restored by exogenous addition of hydrogen peroxide in the *noxB*Δ and *noxR*Δ mutants, but not in *pls1*Δ and *ste2*Δ. Addition of DETC, a specific inhibitor of superoxide dismutase (SOD), an enzyme that converts NOX-derived superoxide to hydrogen peroxide, also abolished peroxidase-induced chemotropism. Our findings reveal a new role for NOX- and SOD in peroxidase-triggered chemotropism and suggest a possible functional link between ROS and pheromone sensing machinery in hyphal growth of a plant pathogen towards the host plant.

Resurrecting a sexual cycle in the human fungal pathogen *Candida glabrata*. *Jane Usher*[1], Alison Day[3], Judith Berman[2], Janet Quinn[3], Ken Haynes[1] 1) Biosciences, University of Exeter, Exeter, Devon, UK; 2) Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Israel; 3) Institute for Cell and Molecular Biosciences, Faculty of Medicine, Newcastle University, UK.

To date no sexual cycle has been descirbed in the pathogenic yeast *Candida glabrata.* This is curious as the population structure contains evidence of recombination, the genome contains mating-type loci that can switch at sites of infection and orthologues of the majority of genes involved in fungal sexual reproduction and meiosis, including many of those missing in other *Candida* specides. The lack of a complete sexual cycle in some hemiascomycete yeasts is surprising as they contain many of the required genes. We naively hypothesized that the functions of the encoded proteins may have changed, as has been previously described for regulators of ribosome biosynthesis in *C. albicans*, resulting in an inability to complete a sexual cycle. To test this hypothesis, we cloned each of the *C. glabrata* genes, functionally annotated to the GO terms mating or meiosis, and attempted to systematically complement the mating/meiosis defects of the orthologous *S. cerevisiae* mutants, as has previously been done for *ste11, ste12* and *ste20* mutants in *C. glabrata.*

Here we show that a complete sexual cycle can be engineered in *C. glabrata.* IME1, encoding the principle regulator of meiosis and IME2, encoding a meiosis specific kinase from *C. glabrata* are unable to complement the mating defects of the orthologous *S. cerevisiae* null mutants. The replacement of the native *C. glabrata* IME1 and IME2 with their orthologs from *S. cerevisiae* results in *C. glabrata* cells that can mate, form stable diploids and undergo sporulation, to give tetrad-like structures containing viable haploid progeny. These progeny, when sequenced show evidence of having undergone recombination. Our results demonstrate that in addition to the loss of orthologous mating and/or meiosis proteins seen in some fungi, those retained may have had their function reassigned, thereby resulting in a reliance on asexual reproduction.

This is the first report of an apparently asexual ascomycetous yeast that has been engineered to successfully complete a sexual cycle. This will not only allow for forward gentic analysis in *C. glabrata*, but offers an approach that may be successful in revealing hidden mating pathways in other species such as *C. albicans* in which stable haploids have recently been described. This data also highlights key evolutionary changes between pathogenic and non-pathogenic fungi.

Genome evolution in asexual laboratory populations of *Saccharomyces cerevisiae*. *Gregory Lang* Biological Sciences, Lehigh University, Bethlehem, PA.

Natural selection optimizes an organism's genotype within the context of its environment. Adaptations to one environment can decrease fitness in another, revealing evolutionary trade-offs. We show that the cost of gene expression underlies a trade-off between growth rate and mating efficiency in the yeast, Saccharomyces cerevisiae. During asexual growth, mutations that eliminate the ability to mate provide a fitness advantage. We have developed a method to track the fraction of sterile cells in a population by assaying for the presence of a mating-specific fluorescent reporter. Using this system, we tracked the emergence and fate of spontaneous sterile mutations in ~600 populations through 1,000 generations. We sequenced forty of these populations at 80-generations resolution and identified hundreds of successful mutations (including sterile mutations). We are currently reconstructing all possible combinations of observed mutations to quantify the fitness effects of each mutation individually and in combination.

***MAT* genes in a lineage of the Basidiomycota unusually enriched in homothallic species.** *M. David Palma*, M. A. Coelho, J. P. Sampaio, P. Gonçalves UCIBIO-REQUIMTE, Departamento de Ciências de Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.

Most fungal species are heterothallic, requiring a combination of two mating types for sexual reproduction. However, a minority of species is homothallic, which means that a single individual is capable of completing the sexual cycle without the need for a compatible partner. The mechanistic basis of homothallism may vary, including mating type switching as observed in *Saccharomyces cerevisiae* or unisexual mating as recently described in *Cryptococcus neoformans,* along with primary or "true" homothallism in which an haploid genome expresses all the

molecular components required for sexual reproduction. Basidiomycetes evolved a complex mating system, usually comprising two genetically independent *MAT* loci encoding two distinct mating compatibility check points, whose molecular underpinnings are known in detail for numerous heterothallic species. However, studies showing how basidiomycete *MAT* genes participate in primary homothallic sexual cycles were lacking. This prompted us to unravel the molecular basis of sexual reproduction in *Phaffia rhodozyma*, a genetically accessible, homothallic yeast species in the Cystofilobasidiales (Agaricomycotina, Basidiomycota). Six *MAT* genes encoding pheromone receptors, pheromone precursors and HD transcription factors were uncovered in the draft genome of *P. rhodozyma*. All were shown by genetic dissection to be functional and involved in sexual reproduction. We subsequently used comparative genomics to uncover *MAT* genes in other species belonging to the Cystofilobasidiales, a lineage comprising an unusually high proportion of homothallic species. Our results indicate that transitions to homothallism probably occurred several times independently in this lineage and reveal a particularly dynamic pattern of *MAT* gene evolution, with high turnover of pheromone receptor alleles and exceptionally large numbers of mature pheromones encoded in the genomes of some of the species.

Whole genome DNA-methylation (methylome) profiling during heterokaryosis in the Agaricomycetes. *R.A. Powers*, T.Y. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

DNA methylation of the 5' position of cytosines, a well-known form of 'epigenetic' modification, has been shown to be important in such diverse processes as the formation of human cancers, development in multicellular eukaryotes, and the silencing of transposons and repetitive elements in plants, animals, and fungi. Despite the importance and apparent conservation of DNA methylation across diverse clades of eukaryotes, we still lack a basic understanding of its roles in the mushroom-forming fungi of the Agaricomycetes. While it has been shown that DNA methylation in CpG (5' Cà G 3') contexts is important for the silencing of repetitive DNA elements in fungi, we lack a comprehensive understanding of DNA methylation at the whole genome level in the Basidiomycota. In particular, we know little of how whole genome DNA methylation ("methylome") patterning differs both during different life-stages within a species, as well as how methylomes differ between taxa. Using whole-genome bisulfite sequencing (WGBS), which involves the treatment of genomic DNA with sodium-bisulfite followed by whole genome next generation sequencing, we have generated genome-wide methylation profiles of two haploid homokaryotic isolates from five taxa across diverse clades of the Agaricomycetes: *Coprinopsis cinerea, Heterobasidion irregulare, Wolfiporia cocos, Coprinellus disseminatus* and *Cyathus stercoreus*. Here, we report that the amount of genomic methylation of cytosines in CpG contexts is species specific and ranges from of a low of ~2% in *Heterobasidion irregulare* to ~12.5% in *Cyathus stercoreus*. Further, we show that there is a high degree of variation in CpG methylation percentages between haploid strains of the same species for most of the species tested. Lastly, we examined the role of changes in methylome patterning during mating and heterokaryosis and show that DNA methylation patterns change during the transition for haploid to heterokaryon, and that these changes are also species specific.

Unpacking the molecular mechanism behind unisexual reproduction in *Huntiella moniliformis*. *A. Wilson*[1], M. van der Nest[1], P.M. Wilken[1], M.J. Wingfield[2], B.D. Wingfield[1] 1) Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa ; 2) Department of Microbiology & Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Unisexual reproduction is a reproductive strategy where an individual fungus is able to produce sexual offspring despite possessing genes that represent a single *MAT* idiomorph. This form of reproduction has been described in a few species of fungi, but was most recently observed in MAT2 individuals of the filamentous ascomycete; *Huntiella moniliformis* (Wilson *et al*. 2015. Fun. Genet. Biol. 80:1-9). This fungus is a member of the family Ceratocystidaceae, a group including well-known pathogenic fungi that infect a wide variety of economically important plants. The underlying molecular mechanisms responsible for unisexuality in filamentous fungi are poorly understood and this study aimed to elucidate these mechanisms using a comparative transcriptomics approach. By sequencing the mRNA from vegetative and sexually-competent cultures of *H. omanensis*, a strictly heterothallic relative of *H. moniliformis*, we were able to identify some of the genes that are important for sexual reproduction in these fungi. By comparing these data to similar data obtained from cultures of *H. moniliformis*, we were able to detect significant differences in gene expression between the heterosexual and unisexual pathways. Most notable was the mating type-independent expression of both the α- and a-factor pheromone genes in the unisexual *H. moniliformis*. This was in contrast to *H. omanensis*, were MAT1 individuals produce the α-factor pheromone and MAT2 individuals the a-factor pheromone in a manner similar to other heterothallic species such as *Neurospora crassa*. *H. moniliformis* cultures also expressed both pheromone receptors at a constitutive level during vegetative growth and sexual reproduction compared to the potentially mating type-specific expression observed in *H. omanensis*. The results suggest that mating type-independent expression of these pheromones plays an important role in the unisexual capabilities of *H. moniliformis*. This can be compared to a unisexual pathway in *C. albicans* where endogenous pheromone production allows for self-activation.

Genome-wide survey of sexual stage-dependent non-coding RNAs in *Fusarium graminearum*. *W. Kim*, F. Trail Department of Plant Biology, Michigan State University, East Lansing, MI.

Although the global functional significance of non-coding RNAs (ncRNAs) remains controversial, there has been growing evidence that ncRNAs play critical roles in morphological transition and meiosis in yeasts. In filamentous fungi, however, little is known about the role of ncRNAs during the sexual development. Here we identified sexual stage-dependent ncRNAs in *F. graminearum*, a model for studying sexual development. To discover novel ncRNAs expressed during the sexual development of *F. graminearum*, transcripts were assembled from time-series transcriptome data across the sexual development, and compared to the reference annotation. Among the novel transcripts not annotated in the reference genome, transcripts harboring little coding potential and differentially expressed (DE) in at least one-time point during the developmental stages were classified as sexual stage-dependent ncRNAs (DE-ncRNAs). A total of 578 DE-ncRNAs were identified and the expression patterns of the DE-ncRNAs were largely developmental stage-dependent, while only 20% of the DE-ncRNAs (113 out of 578) were expressed during asexual spore germination. Interestingly, many of the DE-ncRNA expressions (243 out of 578) peaked at the stage of ascus formation during which meiosis occurs. Many of the DE-ncRNAs were antisense to protein-coding genes, forming 357 sense–antisense pairs. Functional enrichment analyses showed that the sense genes were significantly enriched for the Gene Ontology term 'DNA metabolism', raising a hypothesis that DE-ncRNAs play regulatory roles in DNA synthesis and degradation. In addition, DE-ncRNAs were searched against the RNAcentral database v5 (http://rnacentral.org) to find ncRNAs conserved in other

organisms. Functional roles of selected DE-ncRNAs are currently under investigation. This study will provide a foundation for functional characterization of ncRNAs during the sexual development.

Functions of *PRM1* and *KAR5* in cell-cell fusion and karyogamy drive distinct bisexual and unisexual cycles in the *Cryptococcus* pathogenic species complex. *Ci Fu*, Joseph Heitman Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans and *Cryptococcus deneoformans* are two sibling species belong to the opportunistic human fungal pathogen *Cryptococcus* species complex. *C. neoformans* mainly undergoes bisexual reproduction, whereas *C. deneoformans* undergoes both unisexual and bisexual reproduction. During both sexual cycles, a dramatic yeast-to-hyphal morphological transition takes place and generates either monokaryotic or dikaryotic hyphae, and this process is regulated by a common set of genetic circuits. The unisexual cycle can also generate genotypic and phenotypic diversity *de novo*. Despite the similarities between unisexual and bisexual cycles, there are still genetic and morphological differences, such as an absence of an opposite mating partner and monokaryotic instead of dikaryotic hyphae. The natural populations for both species are mainly α mating type; however, the unisexual cycle is well established in *C. deneoformans* but not in *C. neoformans*, suggesting differences in mating mechanisms may contribute to this. To understand what distinguishes the different modes of sexual cycles, we focused on two cellular processes involved in sexual reproduction: cell-cell fusion and nuclear fusion. We identified orthologs of the plasma membrane fusion protein Prm1 and the nuclear membrane fusion protein Kar5 in both *Cryptococcus* species, and showed that they play conserved roles in cell fusion and karyogamy during sexual reproduction. The involvement of Prm1 and Kar5 indicates that there are fundamental differences between unisexual and bisexual reproduction, and between bisexual reproduction of the two sibling species. Cell fusion and karyogamy are largely dispensable for unisexual reproduction, and the unisexual cycle achieves diploidization early during hyphal development, likely through endoreplication. During the bisexual cycles, in *C. deneoformans*, karyogamy occurs early during the hyphal development whereas it occurred inside the basidium in *C. neoformans*.

Wednesday, March 15 3:00 PM – 6:00 PM
Fred Farr Forum
Circadian Rhythms and Photobiology

Phytochrome-dependent light signaling in *Aspergillus nidulans*. *R. Fischer*, A. Ali, Ch. Streng, Z. Yu Dept of Microbiology, Karlsruhe Institute of Technology (KIT), Karlsruhe, BW, DE.

Bacteria often use two component systems (TCS) as phosphorylation relays to transmit environmental signals from the cell surface to the inner cell. In comparison, microbial eukaryotes use MAP kinase phosphorylation cascades, although TCS are commonly found in the fungal kingdom[1]. Interestingly, in the case of stress-sensing, fungi use a composite signaling cascade comprised of a TCS plus a downstream MAP kinase cascade to trigger gene expression. Besides osmolarity or oxidative stress, fungi sense many other environmental factors, one of which is light[2,3]. Light controls morphogenetic pathways but also the production of secondary metabolites such as penicillin. Here we show that phytochrome-dependent light signaling in *Aspergillus nidulans* uses the stress-sensing signaling cascade to transmit the signal from the cytoplasm into nuclei[4]. In a screening for recessively *blind* mutants, the MAP kinase HogA/SakA was identified by whole-genome sequencing. The phytochrome FphA physically interacted with the histidine-containing phosphotransfer protein YpdA and caused light-dependent phosphorylation of the MAP kinase HogA/SakA and its shuttling into nuclei. In the absence of FphA, HogA/SakA still responded to osmotic stress but not to light. The HogA pathway thus integrates several stress factors and can be considered as a hub for environmental signals. We present evidence that conformational changes of FphA lead to the initiation of the signaling process starting with the phosphotransfer from YpdA to the FphA response regulator. In a screening for dominant mutants, in which light-dependent genes are expressed constitutively, a PHD-finger protein was identified, probably acting as a repressor.

1 Bahn, Y., Xue, C., Idnurm, A., Rutherford, J. & Cardenas, M. E. Sensing the environment: lessons from fungi. *Nat. Rev. Microbiol.* **36**, 57-69 (2007).
2 Rodriguez-Romero, J., Hedtke, M., Kastner, C., Müller, S. & Fischer, R. Fungi, hidden in soil or up in the air: light makes a difference. *Annu. Rev. Microbiol.* **64**, 585-510 (2010).
3 Dasgupta, A., Fuller, K. K., Dunlap, J. C. & Loros, J. J. Seeing the world differently: variability in the photosensory mechanisms of two model fungi. *Environ. Microbiol.* 18:5-20 (2015).
4 Yu, Z., Armant, O. & Fischer, R. Fungi use the SakA/HogA pathway for phytochrome-dependent light signaling. *Nature Microbiol.* 1:16019 (2016).

Light conservatively regulates the ergosterol biosynthetic pathway in the mold pathogens *Aspergillus* and *Fusarium*. *Kevin Fuller*[1], Mike Zegans[2], Robert Cramer[3], Jennifer Loros[4], Jay Dunlap[1] 1) Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Ophthalmology, Dartmouth-Hitchcock Medical Center, Lebanon, NH ; 3) Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH; 4) Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

We have shown that light serves as an important environmental cue that regulates growth, development, and stress resistance in the mold pathogen *Aspergillus fumigatus* (Fuller et al., 2013 & 2016). Whole-genome microarray analysis revealed that several genes in the ergosterol biosynthetic pathway are down-regulated by light in this fungus, including those encoding the sterol 14-demethylase (Erg11/Cyp51b), the hydroxymethylglutaryl CoA synthase (Erg13), and the C4-methyl sterol oxidase (Erg25). qRT-PCR analysis confirmed the light-repression of these genes in the two commonly studied *A. fumigatus* strains, Af293 and CEA10. Furthermore, the influence of light on their expression was lost upon deletion of the *lreA* (blue light receptor) and *fphA* (red light receptor), indicating that these discrete photosystems cooperatively regulate sterol metabolism. Because Erg11 is the target of the azole class of antifungals, we next hypothesized that the light environment could augment the sensitivity of *A. fumigatus* to these drugs. Indeed, E-test and microbroth dilution assays demonstrated that the fungus was more sensitive to voriconazole when cultured in the light.
We reasoned that the influence of light on fungal sensitivity to sterol-targeting drugs may be particularly relevant in the context of fungal eye infections (keratitis). Accordingly, we sought to determine whether the light-repression of ergosterol genes observed in *A. fumigatus* was conserved in species commonly associated with fungal keratitis (namely *Fusarium* species). We identified orthologs of *erg11*, *erg13*, and *erg25* for both *F. oxysporum* and *F. solani* and found that, in both species, all three genes were down-regulated upon light exposure when assessd by qRT-PCR. Taken together, these data suggest that light may play a conserved role in regulating sterol metabolism in fungi and that this could be exploited to a therapeutic end in certain clinical contexts.

Circadian clock control of translation in *Neurospora crassa*. *K.D. Castillo*, S.Z. Caster, D Bell-Pedersen Biology, Texas A&M University, College Station, TX.

The circadian clock controls daily rhythms in a wide variety of physiological and behavioral processes in diverse organisms, allowing anticipation of daily environmental cycles and maintenance of internal temporal order. Rhythms in mRNA accumulation are observed for up to 40% of the eukaryotic genome. Interestingly, about 50% of the oscillating proteins in the mouse liver are produced from mRNAs that are not clock-controlled, suggesting a prominent role for clock regulation of post-transcriptional processes. While the basic mechanisms of circadian transcriptional control are known, the extent of clock control of mRNA translation is understudied. The filamentous fungus *Neurospora crassa* is a simple model organism for determining how the clock controls gene expression. In *N. crassa*, we discovered that the phosphorylation of the highly conserved translation elongation factor 2 (eEF-2), by the serine/threonine kinase RCK-2, is rhythmic in wild-type cells, but not in Δ*rck-2* or clock mutant Δ*frq* cells. These data suggested that clock control of eEF-2 activity plays a key role in rhythmic translation of all, or specific, mRNAs. RNA-seq and ribosome profiling experiments from cultures harvested at different times of the day revealed that clock regulation of eEF-2 activity influences temporal translation of specific mRNAs, including glutathione-S-transferase 3 (*gst-3*). Experiments are currently underway to determine the mechanism of this specificity.

Light-induced gene expression in *Botrytis cinerea* involves GATA-transcription factors and the stress-activated MAP kinase module. *Julia Schumacher*[1], Kim Cohrs[1], Adeline Simon[2], Jaafar Kilani[2], Muriel Viaud[2], Sabine Fillinger[2] 1) IBBP, WWU, Muenster, Germany; 2) BIOGER, INRA, Grignon, France.

Botrytis cinerea is a plant pathogen that exhibits prominent light responses including the formation of the reproduction structures (photomorphogenesis), secondary metabolites/ pigments, and antioxidant enzymes. A complex regulatory network of photoreceptors, transcription factors (TFs) and chromatin modifiers is supposed to initiate, transmit, and fine-tune the responses to different wavelengths of light on the transcriptional level that finally leads to the observable phenotypes. As the formation of the reproduction structures is strictly regulated by light in this fungus - conidia are formed in the light, sclerotia in the dark - the output can be easily monitored. The GATA-type TFs BcWCL1 (as part of the White Collar complex (WCC)) and BcLTF1 are important regulators as their deletions result in light-independent conidiation ("always conidia") due to the deregulation of BcLTF2 (Schumacher et al. 2014; Canessa et al. 2013; Cohrs et al. 2016). Study of light-induced gene (LIG) expression in both deletion mutants highlighted the role of the TFs in activating EARLY and in repressing LATE LIG expression, including *bcltf2* encoding the master regulator of conidiation and further LTFs that may function downstream or in parallel with BcLTF2 in regulating the conidiation process. As the group of LIGs also contained genes that are induced by various stresses in a BcSAK1-dependent manner, the phosphorylation status of the stress-activated MAPK BcSAK1 was studied and shown to increase after exposure to light. Deletion of *bcsak1* impairs LIG expression suggesting that BcSAK1 functions as a co-activator of the WCC in inducing EARLY genes, and as a component releasing the LATE promoters (e.g. *bcltf2)* from repression by BcLTF1 and the WCC resulting in the expression of the conidiation genes. This preliminary model is in agreement with the observations that the deletion of BcSAK1 results in a "never conidia" phenotype (Segmüller et al. 2007), and that the exposure of the wild type to heat and osmotic stress bypasses the requirement of light for conidiation.

Circadian proteomic analysis demonstrates a role for post-translations mechanisms in circadian regulation. *Jennifer M. Hurley*[1], Alexander Crowell[2], Sam Fordyce[1], Sam Purvine[3], Errol Robinson[3], Anil Shukla[3], Erika Zink[3], Scott Baker[3], Jennifer Loros[4], Jay Dunlap[2] 1) Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY; 2) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA; 3) Environmental Molecular Sciences Laboratory, Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA ; 4) Department of Biochemistry and Cellular Biology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA.

The fundamental model of the eukaryotic circadian clock is comprised of a transcriptional/translational negative-feedback loop that controls rhythmic transcription of a substantial fraction of the transcriptome. Based on this model, the prevailing hypothesis in circadian biology has been that by tracking transcriptional rhythms over circadian time, a fundamental understanding of how circadian output mediates behavior, physiology, and metabolism can be obtained. However, data shows that concordance between mRNA and protein levels, including the relationship between circadianly-oscillating mRNAs and proteins, is far from a one-to-one correlation. Therefore, in order to better understand the relationship between circadian output at the level of expression and the level of the proteome, we used Tandem-Mass-Tag Mass Spectrometry to identify global protein levels over a period of 48 hours with a resolution of 2 hours. When we compared this data to our previously-published transcriptomic data, we found significant discordance between rhythmic mRNAs and proteins, demonstrating there are additional factors that determine rhythmicity at the protein level outside of transcription. However, despite this discordance, many trends that are present at the transcriptional level are mirrored at the proteomic level, including that daytime metabolic potential favors catabolism, energy production, and precursor assembly, whereas night activities favor biosynthesis of cellular components and growth. When we analyzed these rhythmic proteins using Gene Ontology (GO), we demonstrated that circadianly-regulated proteins were enriched in many ontological classifications with a great deal of that enrichment in metabolic categories. Due to the strong link between the clock and metabolism, we analyzed proteomic oscillations in a strain that had the gene *csp-1* knocked out, as *csp-1* has been shown to be a potential link between the circadian clock and metabolism. We found that when *csp-1* is knocked out, many metabolic genes are no longer rhythmic, demonstrating that *csp-1* has significant control over the cellular output that is regulated by the circadian clock.

Light controls dimorphic transitions in the human fungal pathogen *Histoplasma capsulatum*. *S. Beyhan*[1,2], M. Voorhies[2], A. Sil[2] 1) J. Craig Venter Institute, La Jolla, CA; 2) University of California, San Francisco, San Francisco, CA.

Histoplasma capsulatum (*Hc*), a dimorphic fungal pathogen, is the most common cause of fungal respiratory infections in immunocompetent host. In the soil, *Hc* grows in a filamentous form. Once inhaled, the filaments and the associated spores convert to a yeast form that expresses virulence genes and causes disease. Host temperature is the key signal that triggers a developmental switch in *Hc*. In the laboratory, cells grow in the filamentous form at room temperature (RT), whereas growth at 37ºC is sufficient to trigger growth in the yeast form. Through previous studies, we identified four key regulators, Ryp1,2,3,4, that are required for yeast-phase growth at 37ºC. Ryp1 and Ryp4 are transcriptional regulators that are conserved in the fungal kingdom. Ryp2 and Ryp3 are both members of the Velvet protein family, which regulates development and secondary metabolism in many fungi. Interestingly, in *A. nidulans*, Velvet family proteins can form a complex with various photosensors to regulate sexual and asexual development in response to light. In *Hc*, the role of light in regulating cell morphology and virulence has not been fully explored. Furthermore, it is unknown whether Velvet family proteins are involved in light response in *Hc.* In this study, our goal is to investigate the role of light in the regulation of phenotypic traits in *Hc*. In our preliminary studies, we found that conidiation was dramatically affected by light. Additionally, we found that the yeast-to-filament transition of *Hc* is light-regulated. Wild-type yeast cells (grown at 37°C) were shifted to RT under light and dark conditions. Morphological changes were monitored by microscopy and transcriptional changes were monitored by RNAseq. As a result, we found that cells that were grown in the dark persisted in the yeast form for a longer period of time before they ultimately switched to filaments. These morphological changes were accompanied by changes in transcript levels of *RYP* genes. We found that *RYP2* and *RYP4* transcript levels were lower in light compared to dark conditions at RT, suggesting that accumulation of these transcripts is light-responsive. Overexpression of *RYP2* or *RYP4* in light conditions was sufficient to prevent the yeast-to-filament transition at RT. Taken together, our results suggest that dark promotes (or light inhibits) yeast-phase growth via regulation of Ryp transcript accumulation, and that light is an important environmental signal for the physiology of *Hc.*

Facultative heterochromatin at the clock gene *frequency*. *William Belden* Dept of Animal Science, Rutgers, The State University of New Jersey, New Brunswick, NJ.

The circadian clock allows organisms to anticipate daily changes in environmental conditions and controls developmental and physiological processes. In *Neurospora*, the core mechanism of the circadian clock is a transcriptional negative feedback loop where White Collar-1 (WC-1) and WC-2 drive rhythmic expression of the *frequency* (*frq*) gene. Part of circadian clock-regulated gene expression involves facultative heterochromatin at the central clock gene; A process that is conserved in species ranging from *Neurospora* to mammals. Facultative heterochromatin formation at *frg* is distinct from heterochromatin at repeated regions throughout the genome and requires the *frq* natural antisense transcript (NAT) *qrf*, components of the COMPASS complex, including the histone H3 lysine 4 methyltransferase SET1, SWD1, and SWD3. Despite these differences, there still a requirement for components of the DCDC complex including the H3 lysine 9 methyltransferase DIM-5. The requirement for both H3K4me and H3K9me in proper regulation of *frq* has led us to examine the genome-wide importance of these modificaitons in transcriptional regulation. Efforts are currently underway to further define the molecular mechanisms of facultative heterochromatin at *frq* and attempts are being made to understand the enigmatic role of COMPASS.

Investigating the complex regulatory network that controls photoinduction of carotenogenesis in *Fusarium*. *Javier Avalos*[1], Obdulia Parra-Rivero[1], Javier Pardo-Medina[1], Macarena Ruger-Herreros[1], Steffen Nordzieke[1], Francisco J. Romero-Campero[2], M. Carmen Limón[1] 1) Genetics, University of Seville, Seville, ES; 2) Computing Sciences and Artificial Intelligence, University of Seville, Seville, ES.

Carotenoids are terpenoid pigments produced by many fungi. As *Neurospora crassa*, species of the genus *Fusarium* accumulate neurosporaxanthin and other carotenoids under light. This production is due to the induced expression of the structural genes *carRA*, *carB*, and *carT*, needed to synthesize neurosporaxanthin. The response is accompanied by the induction of the genes *carX* and *carO*, needed to produce retinal and CarO rhodopsin and organized in a cluster with *carRA* and *carB*. This communication deals with our current knowledge of the molecular mechanisms of this photo-regulation in *F. fujikuroi* and *F. oxysporum*. In contrast to *N. crassa*, carotenogenesis is still able to respond to light in the mutants of the White collar 1 ortholog WcoA, although this protein is mostly responsible for the transcriptional photoinduction. Kinetics experiments on the response to light revealed a biphasic response in *F. fujikuroi*, with an early stage mostly dependent of WcoA and a later stage in which participates the DASH cryptochrome CryD, both stages modulated by the Vvd ortholog VvdA. In addition, the pathway is down-regulated by the RING-finger protein CarS. RNAseq analyses showed that either light or the loss of *carS* function exert mainly an activating effect on the transcriptome of *Fusarium*, with a greater functional diversity in the case of the genes induced by light. The number of the latter decreases drastically in the *carS* mutant (1.1% *vs*. 4.8% in the wild-type), indicating that the deregulation produced by the *carS* mutation affects the light response of many genes. Approximately 27% of the genes activated at least 2-fold by light or by the *carS* mutation are coincident, raising to 40% for an activation threshold of 8-fold, confirming regulatory connections between the regulations by light and by the CarS protein. Upstream to *carS,* we found a previously unidentified transcript, presumably a long non-coding RNA, whose deletion abolishes the photoinduction of the structural genes. Moreover, the deletion of another linked gene coding for a protein of unknown function results in a lower carotenoid accumulation, which was not due to a decreased mRNA photoinduction of the structural genes. Therefore, the available data point to a complex regulation exerted by different interacting elements, either at transcriptional and post-transcriptional levels.

Wednesday, March 15 3:00 PM – 6:00 PM
Kiln
Primary Metabolism and Metabolic Engineering

Expansions and reductions in fungal primary metabolism studied across 100 fungal species. Julian Brandl, Jane Nybo, Tammi C. Vesth, *Mikael Rørdam Andersen* Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs Lyngby, DK.
The primary metabolism of fungi is the power house that drives nearly all cellular functions. Primary metabolism is involved in converting the surroundings of the fungus to a food source as well as delivering metabolite precursors for everything from cellular growth and maintenance over biological responses to external stimuli to producing secreted secondary metabolites and protein effectors. Furthermore, it is known that fungal metabolism is highly versatile. Saphrophytic fungi can grow on a very wide range of carbon and nitrogen sources and utilize this for production of even more diverse range of secondary metabolites and secreted proteins.

Within this project, we have been interested in two main things: 1) What is the "roadmap" of fungal metabolism within a single species? What is the total sum of genes involved in primary metabolism? For studying this, we have been focusing on the industrial workhorse and model fungus Aspergillus niger. 2) What is the diversity of metabolism across fungi? Which gene functions are expanded, reduced, added and lost across species? For this we have used the metabolic "roadmap" of *Aspergillus niger*, and used it to query the genomes of approximately 100 different species, primarily of the genus Aspergillus.

For the initial roadmap of fungal metabolism, we have reconstructed the metabolism of *A. niger* ATCC 1015 at genome-scale. The metabolic reconstruction covers 1801 metabolic conversions, 997 genes, and 1411 metabolites across six compartments. Phenotype arrays have been applied to evaluate the ability to germinate on 180 carbon sources and 92 nitrogen sources. Examining the metabolism shows a high degree of isoenzymes across both central and outer metabolism.

Employing this metabolic network to our database of fungal genomes allows us to examine the diversity of metabolic strategies through the identification of orthologs across species and map this to the species. Our analysis shows that some specialized fungi have a more reduced genome and survives with 1-2 isoenzymes for most of central metabolism, while others apply a diversification strategy and often have 2-5 isoenzymes, even for the highly conserved functions in central metabolism.

Engineering the smut fungus *Ustilago maydis* for the degradation of pectin. P. Stoffels[1,3], E. Geiser[2,3], L.M. Blank[2,3], M. Feldbrügge[1,3], N. Wierckx[2,3], *K. Schipper*[1,3] 1) Institute for Microbiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany ; 2) iAMB – Institute of Applied Microbiology, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany ; 3) Bioeconomy Science Center (BioSC), c/o Forschungszentrum Jülich, 52425 Jülich, Germany.
The microbial conversion of plant biomass components to valuable products in a consolidated bioprocess would greatly increase the ecologic and economic impact of a biorefinery. Plant-pathogenic fungi are promising candidates for biomass valorization, because they contain a vast repertoire of hydrolytic enzymes to sustain their lifestyle. However, expression of the corresponding genes is usually tightly regulated and mostly restricted to the pathogenic phase. We use the biotrophic smut fungus *Ustilago maydis* for the degradation of plant cell wall components by activating its intrinsic enzyme potential during axenic growth. This fungal model organism is equipped with a potent set of hydrolytic enzymes, and moreover, it naturally produces value-added substances such as organic acids and biosurfactants. To achieve the deregulated expression of hydrolytic enzymes during the industrially relevant yeast-like growth in axenic culture, the native promoters of the respective genes were replaced by constitutively active synthetic promoters. This led to an enhanced conversion of biomass components like xylan, cellobiose, and polygalacturonic acid to fermentable sugars. Currently, the intrinsic fungal repertoire is activated and in parallel supplemented with potent heterologous enzymes for the degradation of pectin. Importantly, this can be achieved using both conventional secretion for enzymes of eukaryotic and unconventional secretion for enzymes of bacterial origin. In summary, our results demonstrate the potential applicability of activating the expression of native hydrolytic enzymes from phytopathogens in a biocatalytic process.

A trehalose-regulatory subunit moonlights to regulate cell wall homeostasis through modulation of chitin synthase activity in *Aspergillus fumigatus*. *Arsa Thammahong*, Alayna Caffrey, Sourabh Dhingra, Josh Obar, Robert Cramer Microbiology and Immunology, Dartmouth College, Hanover, NH.
Purpose: Trehalose biosynthesis is a metabolic pathway found in fungi but not humans. Proteins involved in trehalose biosynthesis are essential for human and plant fungal pathogen virulence. Loss of canonical trehalose biosynthesis genes in the human pathogen *Aspergillus fumigatus* alters virulence and cell wall integrity through undefined mechanisms. Here we characterize additional genes, herein called *tslA* and *tslB,* which encode proteins that contain similar protein domains as OrlA (a trehalose-6-phosphate phosphatase), but lack critical catalytic residues for phosphatase activity.
Methods: We utilized a genetics approach to generate null mutants of *tslA* and *tslB*. To observe the phenotypes of these mutants, we used trehalose assays and cell wall perturbing agents. Furthermore, LC-MS/MS and co-immunoprecipitation were performed to define protein-protein interactions of TslA . To further characterize the phenotype of *tslA* null mutant, chitin synthase activity assay and spinning-disk confocal microscope were used to study the chitin content and the localization of CsmA. A chemotherapeutic murine model was utilized to study the host-pathogen interaction.
Results: Loss of *tslA* reduced trehalose content in both conidia and mycelia, impaired cell wall integrity, and significantly altered cell wall structure. Unexpectedly, immunoprecipitation assays coupled with LC-MS/MS revealed a protein interaction between TslA and CsmA, a type V chitin synthase enzyme. TslA regulates not only chitin synthase activity but also CsmA localization. Loss of TslA directly affected the host immune response to *A. fumigatus* characterized by an increase in murine mortality likely due to enhanced immune cell recruitment.
Conclusion: Our data provide a mechanistic model whereby proteins in the trehalose pathway play critical roles in fungal cell wall

homeostasis that alters fungal-host interactions. Future studies are underway to elucidate the mechanism(s) through which OrlA, TslA, TslB, and their interacting partners control fungal cell wall homeostasis and virulence.

Identifying carbohydrate sensing pathways in *Neurospora crassa*. *L.B. Huberman*, S.T. Coradetti, N.L. Glass Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

Identifying and utilizing the nutrients available in the most efficient manner is a challenge common to all organisms. The model filamentous fungus, *Neurospora crassa*, is capable of utilizing a variety of carbohydrates: from simple sugars to the complex sugar chains found in plant cell walls. Expression of the genes necessary for degrading these complex carbohydrates is energetically expensive, so tight regulation of these genes is important. Several transcription factors necessary for cellulose utilization in *N. crassa* have been identified, but the method by which these transcription factors are activated is not clear. We identified a novel repressor of the cellulolytic response, which in combination with other, more general nutrient sensing pathways appears to play an important role in regulating cellulase expression in *N. crassa*.

Global transcriptional regulation and chromatin dynamics of primary metabolism in *Aspergillus nidulans*. *Koon Ho Wong*[1], Ang Li[1], Yingying Chen[1], Liguo Dong[1], Md Ashiqul Alam[3], Fang Wang[1], Kaeling Tan[1], Michael Hynes[4], Joan Kelly[3], Richard Todd[2] 1) Faculty of Health Sciences, University of Macau, Macau; 2) Department of Plant Pathology, Kansas State University, KS; 3) Department of Genetics and Evolution, School of Biological Science, The University of Adelaide, Australia; 4) School of BioSciences, University of Melbourne, Australia.

Fungi adapt their metabolism to nutrient availability primarily via transcriptional control of nutrient uptake and metabolic genes. In *Aspergillus nidulans,* genes involved in carbon and nitrogen metabolism are tightly controlled by carbon catabolite repression (CCR) and nitrogen metabolite repression (NMR), respectively. Two key global regulators are involved in CCR and NMR; the repressor CreA controls expression of carbon metabolic genes, while the activator AreA modulates nitrogen metabolic gene expression. An extensive set of genes is expected to be under the control of the two regulators, which often act in concert with different pathway-specific transcription factors to turn on different subsets of metabolic genes according to nutritional conditions. In an attempt to identify the genome-wide targets of the global and pathway-specific regulators and to learn how they collaborate with each other to bring about the regulation, we have mapped whole-genome binding sites of the two global regulators and some pathway specific factors under different nutrient conditions and integrated the information with genome-wide transcription profiles of wildtype, *creA*? and *areA*? strains. In addition, we have also mapped global chromatin dynamics and chromatin modifications under different nutrient conditions in order to understand the mechanism of regulation at the chromatin level. A summary of CreA and AreA global regulation and chromatin dynamics will be presented.

***Aspergillus fumigatus* carbon catabolite repression is essential for virulence in established infection microenvironments.** *S. Beattie*[1], K. Mark[2], L. Reis[3], S. Dhingra[1], C. Black[6], C. Cheng[2,4,5], G. Goldman[3], R. Cramer[1] 1) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH USA; 2) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH USA; 3) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil; 4) Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Lebanon, NH USA; 5) Institute for Quantitative Biomedical Sciences, Geisel School of Medicine at Dartmouth, Lebanon, NH USA; 6) Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH USA.

Purpose: The filamentous fungus *Aspergillus fumigatus* is responsible for a disproportionate number of cases of invasive mycoses, relative to other filamentous fungi found in the environment in similar or even higher quantities. Therefore, unique aspects of *A. fumigatus* biology must account for the ability to grow within the host and cause disease. Here, we hypothesize that carbon availability during an infection is dynamic, and the ability to optimize carbon utilization within the host is critical for optimal virulence throughout the fungus-host interaction.

Methods: To test this hypothesis, we characterized the carbon catabolite repression (CCR) system of *A. fumigatus* through generation of a *creA* genetic null mutant. Murine models of invasive aspergillosis were used to assess the contribution of CCR to pathogenesis. Transcriptomics, metabolomics, and *in vitro* growth and stress susceptibility assays were further utilized to determine the role of CCR in *A. fumigatus* fitness.

Results: In contrast to what has been observed in yeasts, we identified a novel role for CreA and CCR in the virulence of *A. fumigatus*. While CCR was not required for the establishment of infection, loss of CreA dramatically reduced virulence after infection establishment. Therefore, we hypothesized the virulence defect to be a result of altered *in vivo* host carbon metabolism and observed a critical role for CreA in controlling redox homeostasis in the face of a dynamic nutrient environment.

Conclusions: Collectively, our results support the conclusion that transcriptional regulation of fungal bioenergetics to optimize *in vivo* utilization of carbon and nitrogen sources is critical for human fungal virulence.

Carbon regulation of environmental pH by secreted small molecules that modulate pathogenicity in phytopathogenic fungi. F. Bi[2], S. Barad[1,3], A Dubay[1], D. Kumar[1], J.D. Mínguez[4], E.A. Espeso[5], R. Fluhr[6], *D. Prusky*[1] 1) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Rishon LeZion, IL; 2) Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, and Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Guangzhou 510640, China; 3) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 4) Department of Microbiology and Genetics, CIALE, Universidad de Salamanca, Salamanca, Spain; 5) Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas (C.I.B.), Madrid, Spain; 6) Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot.

Fruit pathogens can either acidify or alkalinize the pH of the colonized host environment. This capability has been used to divide fungal pathogens into acidifying and alkalizing classes. In the present study, we show that diverse classes of fungal pathogens, including; *Colletotrichum gloesporioides* , *Penicillium expansum*, *Aspergillus nidulans, Sclerotinia sclerotiorum*, and *Fusarium oxysporum*—secrete small pH-effector molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonization patterns and select for the activity of PACC-dependent genes. We show that the secretion of pH-effector molecules that contribute to fruit pathogenicity is carbon-dependent. In multiple cross-class organisms, acidification is induced under excess, e.g. 175 mM sucrose. This carbon source is

oxidized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification. In contrast, alkalization occurs under conditions of carbon deprivation, e.g., less than 15 mM sucrose, where catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2)* results in the secretion of ammonia. Functional *?gdh2* mutants show reduced alkalization and pathogenicity during growth under carbon deprivation, but not in high-carbon media or on fruit rich in sugar, whereas analysis of *?gox2* mutants show reduced acidification in carbon-rich media. The induction pattern of *gdh2* was negatively correlated with expression of the zinc finger global carbon regulator repressor *creA*. The present results indicate that differential pH modulation by fungal pathogens is a universal host-dependent mechanism that modulates environmental pH as a tool to enhance host plant colonization.

Role of the urea cycle in the synthesis of nitric oxide. *D. Canovas*[1,2], A.T. Marcos[1], J.F. Marcos[3], T. Schinko[2], J. Strauss[2] 1) Department of Genetics, University of Sevilla, Sevilla, Spain; 2) Division of Microbial Genetics and Pathogen Interactions, Department of Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Sciences Vienna, Austria; 3) Department of Food Science, Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain.

Nitric oxide (NO), and its role in signalling, has been extensively studied in mammals and to some extent in plants. However, little is known about the role of NO in fungi and how it is synthesized in these organisms. Recently, we reported that NO production in *A. nidulans* is coupled to conidiation and requires a functional nitrate reductase (NR) gene (*niaD*) that is upregulated under these conditions even in the presence of the repressing nitrogen source ammonium. NO levels influence the balance between conidiation and sexual reproduction. Here we report that NO levels are also modulated by light, a general environmental cue and a regulator of fungal development. The light-dependent modulation of nitric oxide levels involves NO catabolism by the mitochondrial flavohemoglobin *fhbB*, and *agaA*, an arginase that controls the intracellular concentration of the NO precursor arginine. Addition of arginine to the cultures provokes a transient increase of the production of NO. However, analogues of arginine did not affect the production of NO. Mutants in the urea cycle genes show differences in NO levels compared to the wild type strain. Taken together our findings indicate that light-dependent developmental processes in *A. nidulans* interfere with nitric oxide metabolism which – in addition to nitrate reduction - is modulated by enzymes of the urea cycle.

Reference:

Marcos AT, Ramos MS, Marcos JF, Carmona L, Strauss J, Cánovas D. Nitric oxide synthesis by nitrate reductase is regulated during development in *Aspergillus*. Mol Microbiol (2016) 99:15-33.

Wednesday, March 15 3:00 PM – 6:00 PM
Heather

Magnitude of Fungi Biodiversity

Continental-level population differentiation and environmental adaptation in the mushroom *Suillus brevipes*. *S. Branco*[1], K. Bi[2], H.-L. Liao[3], P. Gladieux[4], H. Badouin[1], C. Ellison[5], N. Nguyen[6], R. Vilgalys[7], K. Peay[8], J. Taylor[9], T. Bruns[9] 1) Departement Genetique et Ecologie Evolutives, Universite de Paris Sud, Orsay, FR; 2) Computational Genomics Resource Laboratory, University of California, Berkeley, CA; 3) North Florida Research and Education Center, University of Florida, Quincy FL ; 4) INRA, UMR BGPI, Montpellier, FR; 5) Department of Genetics, Rutgers University, Piscataway, NJ; 6) Department of Tropical Plant and Soil Sciences, University of Hawai?i at Manoa, Honolulu, HI; 7) Department of Biology, Duke University, Durham, NC; 8) Department of Biology, Stanford University, Stanford, CA; 9) Department of Plant and Microbial Biology, University of California, Berkeley, CA.

Recent advancements in sequencing technology allowed researchers to better address the patterns and mechanisms involved in microbial environmental adaptation at large spatial scales. Here we investigated the genomic basis of adaptation to climate at the continental scale in *Suillus brevipes*, an ectomycorrhizal fungus symbiotically associated with the roots of pine trees. We used genomic data from 55 individuals in seven locations across North America to perform genome scans to detect signatures of positive selection and assess whether temperature and precipitation were associated with genetic differentiation. We found that *S. brevipes* exhibited overall strong population differentiation, with potential admixture in Canadian populations. This species also displayed genomic signatures of positive selection as well as genomic sites significantly associated with distinct climatic regimes and abiotic environmental parameters. These genomic regions included genes involved in transmembrane transport of substances and helicase activity potentially involved in cold stress response. Our study sheds light on large-scale environmental adaptation in fungi by identifying putative adaptive genes and providing a framework to further investigate the genetic basis of fungal adaptation.

Genetic diversity in fungal plant pathogens: interspecific gene exchange, genome plasticity and mobile elements. *Eva Stukenbrock* Environmental Genomics, Max Planck Inst Evolutionary Biology, Plön, Germany.

Population genomic data provide information about past and ongoing evolutionary dynamics of plant pathogens. In the recent years a large number of publications have shed light on evolutionary processes in fungal pathogens. A general conclusion from these studies is that these pathogens have the capacity to evolve very rapidly. Interspecific gene exchange, genome plasticity and mobile elements appear to be important drivers of rapid evolution and adaptation to an extent so far not observed in other Eukaryotes. We study genome evolution in a set of closely related grass pathogens of the genus *Zymoseptoria* to infer mechanisms leading to genetic diversity within and between species. The genus comprises species with distinct population structure and notably different effective population sizes. We have used population genomics analyses and coalescence models to reconstruct the population history of four *Zymoseptoria* species. An extreme case is *Z. pseudotritici*, a young hybrid species. We previously reported a striking pattern of genomic variation in 5 isolates with 1-100 kb regions with no variation at all alternating with similar size regions with 1%-5% variable sites, always arranged into two divergent haplotypes. This is consistent with *Z. pseudotritici* having arisen by hybridization of two divergent haploid spores less than 500 sexual generations ago. Our recent analyses include full genome sequences from additionally 22 isolates. We show that the loss of variation in the hybrid is highly non-random with respect to chromosomes and genes and that selection seems to maintain certain gene combinations. We show that this data set allows us to use the variable regions to study mutation accumulation since speciation, and the size distribution of regions with and without variation illustrates both the process of recombination and mutation in this species. We conclude that *Z. pseudotritici* will be an important model species for studying the consequences of hybrid speciation and that such processes may be common in fungi.

Comparative genomics and transcriptomics of *Russulaceae*. Brian Looney[1,2], Dan Jacobson[1], Kerrie Barry[3], Alan Kuo[3], Igor Grigoriev[3], Francis Martin[4], *Jessy Labbé*[1,2] 1) Bioscience Division Oak Ridge National Laboratory, Oak Ridge TN 37830; 2) University of Tennessee, Knoxville, TN 37996; 3) US DOE Joint Genome Institute, Walnut Creek, CA 94598; 4) INRA UMR1136, 54280 Champenoux, France.

Russulaceae is a diverse fungal family including the genera *Russula*, *Lactarius*, *Lactifluus*, and *Multifurca*, and is one of the most widespread and species rich ECM lineages. In a recent collaborative effort, the Joint Genome Institute has sequenced genomes and transcriptomes of representative groups across *Russulaceae*, including a saprotrophic outgroup. We present here an overview of the first insight into the dense genome sampling within the family to capture specific genomic features and investigate i) to what extent genes involved in plant biopolymer degradation have been maintained within a single, diverse, ECM lineage and ii) to examine functional diversity within this ecologically important clade, specifically focused on their ability to scavenge nitrogen sources from recalcitrant organic matter. Indeed, preliminary evidence suggests that members of this family, though being mutualists, have retained a restricted set of genes coding for lignin peroxidases and copper oxidoreductases which may be responsible for the degradation of lignin derivatives accumulating in soil organic matter.

Patterns of genome evolution across the fungal kingdom. Michiel Konings, *Robin Ohm* Microbiology, Utrecht University, Utrecht, NL.

A comparative genomics analysis of over 200 fungal genomes has revealed large differences in genome evolution across the fungal kingdom. Driving forces behind these differences are variable rates of interchromosomal rearrangements and variable inversion lengths in intrachromosomal rearrangements. Each class of fungi follows its own pattern of genome evolution.

For example, it was previously shown in the class Dothideomycetes (phylum Ascomycota) that a low rate of interchromosomal rearrangements and high rate of intrachromosomal rearrangements leads to mesosyntheny, where gene content but not gene order is conserved. In the class Saccharomycetes (phyum Ascomycota), in contrast, interchromosomal rearrangements occur frequently, quickly degrading conserved syntheny. A similar pattern of genome evolution occurs in the class Agaricomycetes (phylum Basidiomycota) as in Dothideomycetes, with the important difference that the intrachromosomal inversion length is much smaller. This results in a distinct pattern

only found in this class. Moreover, in Agaricomycetes several conserved ancestral chromosomes could be identified with varying patterns of genome evolution, possibly due to their difference in sequence composition. On gene level, conserved gene clusters were identified despite the observed intrachromosomal inversions. Notably, certain transcription factors were over-represented in these conserved gene clusters.

Elucidating fungal endosymbiont interaction mechanisms between *Mortierella elongata* and *Mycoavidus cysteinexigens*. *Jessie Uehling*[1], Fred Dietrich[1], Pawel Misztal[2], Gregory Bonito[3], Tim Tschaplinski[4], Allen Goldstein[2], Chris Schadt[4], Jessy Labbe[4], Rytas Vilgalys[1] 1) Genetics & Genomics, Duke University, Durham NC 27708, USA; 2) Universtiy of California at Berkeley, Berkeley, CA 94270 USA; 3) Michigan State University, East Lansing, MI 48823 USA; 4) Biosciences Division, Oak Ridge National Laboratory, PO Box 2008 MS 6445, Oak Ridge, TN 37831-6445, USA.

Recently efforts to catalog beneficial microbes associated with *Populus* have yielded cultures of diverse fungi including several *Mortierella elongata* (*Mortierellomycotina*) isolates. Some of these fungi including *M. elongata* strain AG77 harbor bacterial endosymbionts belonging to the Burkholderiales. We have sequenced the genome of *M. elongata* AG77 and its bacterial endosymbiont Mycoavidus cysteinexigens (AG77), and used comparative phylogenomics to infer this symbiosis is ancient and grounded in shared metabolites. We have used and developed methods to assemble single genomes from meta-genomic data and inferred several gene losses that make culturing endosymbiont *M. cysteinexigens* (AG77) challenging. To circumvent this research obstacle, we used an antibiotic treatment to create fungal strains with and without their long-term endosymbionts for comparative multi-omic studies. The ability to contrast cleared *M. elongata* strains and their wild type, endosymbiont-housing relatives in the same genetic background allows us to ask the following questions: *1. How does the presence or absence of long-term, co-evolved endosymbionts impact health and physiology of host fungi? 2. Which currently available methods can be used and modified to study interaction dynamics for systems involving multiple intractable microbes?* Our research goals through this work were to develop -omics and imaging pipelines that allow the study of bacterial fungal interaction mechanisms. To this end we have used genome sequencing, transcriptomics, metabolomics, and volatomics for studying fungal endosymbiont interaction dynamics. The results of these experiments will be further discussed.

Horizontal gene transfer: resolving functional diversity in fungal communities. *J.C. Slot* Plant Pathology, The Ohio State University, Columbus, OH.

Horizontal gene transfer (HGT) may have multiple impacts on the functional diversity within fungal communities. HGT may increase the functional diversity among related species through recombination with foreign genetic material. However, HGT may decrease diversity at the molecular and community levels by allowing natural selection to favor the most adaptive genetic mechanisms among species in a community. Gene clusters (co-localized genes contributing to a common function) introduce additional complexity to HGT-enabled functional diversification; they increase the HGT dispersal of individual genes by providing a more complete selectable function than isolated genes, and they may enable complex functional diversity in populations when they exist as 'supergene' polymorphisms. Recent analyses suggest HGT is widespread among fungi with overlapping ecology, implying that many fungal genes and gene clusters have evolutionary ecologies independent of their host lineages. Here, we present novel genetic mechanisms discovered through targeted and systematic searches for likely HGT events involving gene clusters. Specifically, we describe a large diversity of plant secondary metabolite degradation clusters, and a biosynthetic gene cluster responsible for the production of psilocybin, a bioactive component of hallucinogenic mushrooms. We provide evidence that the ecological distributions of gene clusters and their histories of HGT events can indicate the ecological functions of plant and fungal secondary metabolites.

The exciting content in your cup of tea. *I. Skaar*[1], Y Zhang[2], M Sulyok[4], X Liu[5], M Rao[6], J Taylor[3] 1) Mycology, Norwegian Veterinary Institute, Oslo, NO; 2) School of Life Sciences, Shanxi University, Taiyuan, Shanxi, China; 3) Department of Plant and Microbial Biology, University of California, Berkeley, California, USA; 4) Center for Analytical Chemistry, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Tulln, Austria; 5) State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China and; 6) Bureau of Culture and Sports, Puer, Yunnan, China.

Tea is one of the most popular and widely consumed beverages in the world. Tea has important physiological effects on consumers due to the presence of compounds such as polyphenols, amino acids, vitamins, carbohydrates, caffeine, and purine alkaloids, all of which can have health benefits On the other hand, tea may also offer a complex microbiological diversity which can both contribute to the terroir of the tea, but also lead to presence of adverse secondary metabolites. Some teas are produced by microbiological fermentation. An example of such teas is the Pu-erh tea, produced in Yunnan, China, by fermentation of fresh *Camellia sinensis* leaves. The production and quality of Pu-erh tea is consequently closely related to microbial activity, making it important to understand the Pu-erh microbiome.
The fungal diversity in teas and the corresponding fungal metabolites as revealed both by culture dependent and culture-independent approaches will be discussed.

We will present a study of fungal and bacterial communities leaves and two types of Pu-erh teas by high-throughput, rDNA-amplicon sequencing and by quantitative liquid chromatography-tandem mass spectrometry of their metabolites.

We identified 390 fungal and 629 bacterial OTUs from leaves and both Pu-erhs. Major findings were: 1) fungal diversity drops and bacterial diversity rises due to raw or ripened fermentation, 2) fungal and bacterial community composition changes significantly between fresh leaves and both raw and ripened Pu-erh, 3) aging causes significant changes in the microbial community of raw, but not ripened, Pu-erh, and, 4) ripened and well-aged raw, but not young, Pu-erh have similar microbial communities. Twenty-five toxic metabolites, mainly of fungal origin, were detected, with patulin and asperglaucide dominating. We show that principles of community ecology can be applied to microbially fermented foods, that the acceleration of Pu-erh fermentation by the ripened process approximates the slower, traditional process, and that discarding the first preparation of Pu-erh tea and saving the wetted tea to brew a second cup for consumption has a sound mycotoxicological basis.

Environment and host specificity shape a global diversity of marine fungi. *Anthony Amend*, Kaho Tisthammer, Benjamin Wainwright, Geoffrey Zahn University of Hawaii at Manoa, Honolulu, HI.

Fungal diversity in the marine realm has received considerably less attention than terrestrial environments, and has figured rarely into global estimates of fungal diversity. A critical mass of high-throughput sequencing datasets, however, has helped fill in some of the gaps in our knowledge of this often overlooked realm. Over the past five years, our group has examined fungal communities present in the water column, sediments, on corals, sponges and marine algae, while others have conducted systematic global scale surveys of the world's oceans. Using these datasets we address some of the most fundamental questions about marine fungal diversity and the factors that shape it. We show that environmental factors, not geographic distance, describe the majority of variance in community dissimilarly, and that habitats such as sediments, water columns and invertebrate hosts harbor dissimilar communities and unique taxa. We show evidence for host specificity of fungi associated with coral and macro-algal systems, suggesting that species-rich substrates such as coral reefs likely harbor extensive and novel fungal biodiversity. Last, we showcase the prevalence of putatively amphibious fungi, abundant in water and on land. The view that emerges from a synthetic analysis of marine fungal diversity and distributions is that while there is substantial taxonomic overlap between marine and terrestrial systems, biogeographic patterns differ between these realms.

Wednesday, March 15 3:00 PM – 6:00 PM
Nautilus
Fungi in the Microbiome

Crosstalk between gut microbiota and host immunity during fungal dysbiosis. *Iliyan Iliev* Jill Roberts Institute for Reserach in IBD, Department of Medicine, Weill Cornell Medicine, New York, NY.

Commensal microbiota has an enormous effect on wide range of physiological processes. Recent studies demonstrate that together with bacteria and viruses, fungi are important, although smaller, component of the intestinal microbial community. It is currently unknown whether disruption of fungal communities occupying the gastrointestinal tract would affect host immunity. We have previously shown that a polymorphism in the human gene encoding the anti-fungal receptor Dectin-1 is strongly associated with severity of Ulcerative Colitis (UC) and that in a mouse model of colitis fungi can contribute to intestinal inflammation, suggesting that anti-fungal immunity may be an important factor in intestinal inflammation.
Here we show that fungal community dysbiosis takes place during intestinal inflammation. Using in vivo modeling, targeting of gut fungi with drugs and sequencing of gut fungal communities, we identified a define signature associated with targeted or with inflammation-induced gut mycobiota dysbiosis. We further show that targeted disturbance of healthy gut fungal populations resulted in increased intestinal inflammation. We identified a pathway responsible for the regulation in these upstream immunological events. Finally, fungal dysbiosis affected gut commensal bacterial populations suggesting that these communities are interdependent. Taken together our results indicate that disruption of commensal fungal populations in the gut can influence host immunity, affect commensal bacterial communities and contribute to intestinal disease.

The association of fungal communities with chronic non-healing wounds. *L. Kalan*[1], M. Loesche[1], B. Hodkinson[1], G. Ruthel[3], S. Gardner[2], E. Grice[1] 1) Department of Dermatology, University of Pennsylvania, Philadelphia, PA; 2) University of Iowa, College of Nursing, Iowa City, IA; 3) School of Veterinary Medicine, PennVet Imaging Core, University of Pennsylvania, Philadelphia, PA.

Wounds are an under-appreciated but serious complication for a diverse spectrum of diseases. Wound healing requires the execution of highly coordinated and sequential events. Increased microbial bioburden can stall this process in the inflammatory stage resulting in chronic delayed healing, even in the absence of overt infection. As a result, chronic wounds such as diabetic foot uclers (DFUs) have been hearalded a silent epidemic causing significant morbidity and mortality. Advances have been made characterizing the bacterial component of DFUs but few studies have evaluated the fungal component. We longitudinally profiled 100, non-healing DFUs with high-throughput sequencing of the pan-fungal internal transcribed spacer 1 (ITS1) locus. DFUs were sampled every 2 weeks until wound closure, amputation, or the end of the study (26 weeks). We estimate that up to 80% of wounds contain fungi, whereas cultures performed in parallel captured only 5% of colonized wounds. The mycobiome exhibited high levels of temporal instability and heterogeneity between subjects. Fungal diversity increased with antibiotic administration and onset of a clinical complication. Proportions of the phlyum Ascomycota were significantly greater (p=0.015) at the study onset in wounds that took >8 weeks to heal. Wound necrosis was distinctly associated with pathogenic fungal communities (p=0.0001), while a second major group of taxa identified as allergenic filamentous fungi, were associated with low levels of systemic inflammation. Microbe-microbe interactions are hypothesized to promote pathogenesis that impairs wound healing thus we employed directed culturing of wound specimens stably colonized by pathogens. This approach revealed novel inter-kingdom interactions faciltiating biofilm formation of *Candida albicans* with *Citrobacter freundii* and *Trichosporon asahii* with *Staphylococcus simulans*. This study reports that a large proportion of DFU's are colonized by fungal species comprising ubiquitous environmental moulds, skin associated species, and opportunistic pathogens. Little is known about their contribution to stalled healing or interactions with the bacterial microbiome, and may be recognized as important targets for intervention.

Fungi in the built environment. *Rachel Adams*, Despoina Lymperopoulou, Iman Sylvain, John Taylor, Tom Bruns Plant & Microbial Biology, University of California Berkeley, Berkeley, CA.

Built environments harbor an array of fungi to which occupants are exposed. While most fungi we encounter indoors likely do not affect our health, we would like to understand better the situations in which fungi and the built environment interact to affect occupant wellbeing. I will present recent work we are doing to characterize fungi (and bacteria) in residences. In water-damaged buildings, we are working to identify fungi that have originated indoors, for this is key to understanding what is harmful about water-damaging housing. Moreover, the biological state of the fungi (and other microorganisms) detected in these indoor environments through amplicon-based microbiome surveys is often unknown. We have undertaken sampling of different environments within typical residences (non-water damaged) and applied various molecular approaches to try to better understand if the microbes encountered are alive, dormant, or dead. The fungi in wet settings, such as kitchens and bathrooms, as well as dry areas, such as bedrooms and living rooms, were collected and processed using techniques that detect cells with intact membranes (PMA) or that are metabolically active (RNA). These results will help inform to what extent built environments are true ecological habitats or more often an ecological sink of outdoor-sourced fungi. Our work seeks to inform the causes and consequences of fungi in the built environment.

The structure and function of fungal decomposer communities. *J.M. Talbot*[1], C.A. Zeiner[1], R Bandy[1], S. Rainsford[1], D. Segre[1], D. Cullen[2] 1) Biology, Boston University, Boston, MA; 2) USDA Forest Products Laboratory, Madison, WI.

Niche differentiation among species is a key mechanism by which microbial biodiversity may be linked to ecosystem function. We tested a set of widely invoked hypotheses about the extent of niche differentiation in one of the most diverse communities on Earth – decomposer microorganisms – by measuring their response to changes in three abundant litter resources; lignin, cellulose, and nitrogen (N). To do this, we used the model system *Arabidopsis thaliana* to manipulate lignin, cellulose, and N availability and then used high-throughput sequencing to measure the response of microbial communities during decay in the field. In addition, we grew select species in the lab and measured their resource use profiles, as well as competitive ability on *Arabidopsis* litter.

Low concentrations of lignin, cellulose, and N in the litter each caused unique shifts in decomposer community composition after one year

of decay. Fungal communities were dominated by taxa with unique and complementary substrate use profiles. Competitiveness of species in the field mirrored competitive outcome in the laboratory, with species showing unique enzymatic profiles on litter in microcosms. While growth in culture was related to genes coding for metallopeptidases, competitive dominance was achieved by species with high numbers of genes coding for glycosyl hydrolases. Competitive dominance also correlated with increased secretion of fungal hydrocarbons onto litter, suggesting that fungal metabolites and resource exploitation influenced decomposer community composition during decay. Our results contrast the assumption in ecosystem ecology that major C and N degradation mechanisms are uniform across whole decomposer communities and instead suggest that ecosystem functions arise from complementary among groups of dominant and metabolically diverse taxa.

Intestinal mycobiome variation across geography and phylogeny in the snail genus *Conus*. *A. Quandt*, A. Glasco, T. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

This project seeks to use the extensive collections of a hyper-diverse, predatory genus of marine snails, *Conus*, at the University of Michigan Museum of Zoology to fill a gap in our understanding of the fungi that associate with mollusks and how the mycobiome (fungal community) varies across geographic space, phylogenetic diversity, and ecological niches of the hosts. Several species of *Conus* (including *C. ebraeus*), have near global distributions and are particularly well represented in the collections, yet we have no knowledge of whether the mycobiome of such animals will be driven by their geographic location or their phylogenetic relationships. To determine mycobiome compositions, we dissected the intestines and digestive gland tissues from more than 30 *Conus* spp. from over 20 different countries, extracted DNA, and used fungal-specific amplicon-based high throughput sequencing of the ribosomal DNA region. First, we identify which fungi are commonly and uniquely associated with the digestive tracts of *Conus* spp., all of which are dominated by *Malassezia* spp. We also explore whether *Conus* phylogeny or geography causes greater variation in fungal community structure, and discuss techniques for targeted sequencing of preserved museum specimens for studies.

Microbial communities associated with external fur surfaces of bats and the discovery of novel isolates with antifungal activity against *Pseudogymnoascus destructans*. *Paris Hamm*[1], Shiloh Lueschow[2], Ara Winter[3], Nicole Caimi[3], Diana Northup[3], Ernest Valdez[4], Debbie Buecher[5], Christopher Dunlap[2], David Labeda[6], Andrea Porras-Alfaro[1] 1) Department of Biological Sciences, Western Illinois University, Macomb, IL; 2) Crop Bioprotection Research Unit, US Department of Agriculture, Peoria, IL; 3) Department of Biology, University of New Mexico, Albuquerque, NM; 4) United States Geological Survey, Fort Collins Science Center, Albuquerque, NM; 5) Buecher Biological Consulting, Tucson, AZ; 6) Mycotoxin Prevention and Applied Microbiology Research Unit, US Department of Agriculture, Peoria, IL.

White-nose Syndrome (WNS), a bat fungal disease caused by the psychrophilic fungus *Pseudogymnoascus destructans*, has been estimated to have caused the death of more than six million bats in the eastern U.S. and Canada. Fungal and bacterial surveys were conducted to explore bats' natural microbial communities as a possible defense against this pathogen. Our WNS research efforts have mainly focused on the study of microbial communities associated with bats using next generation sequencing, metagenomic analysis, and the evaluation of cultured Actinobacteria to determine antifungal activity against *P. destructans.* Metagenomic analysis showed that fungi on bat fur surfaces belong to Chytridiomycota, Ascomycota, Basidiomycota, Glomeromycota, and Neocallimastigomycota. Distinct fungal and bacteria communities were detected on WNS (+) bats from Illinois and Indiana and WNS (-) bats from New Mexico and Arizona using 454 pyrosequencing. Further, we evaluated the antifungal potential of naturally occurring Actinobacteria isolated from WNS (-) bats. Bacteria colonizing bat fur and membranes were isolated from 12 healthy bat species providing approximately 2,700 isolates. We have screened over 1,300 bacterial isolates using a bi-layer method, of which 36 isolates show antifungal activity against *P. destructans*. Of the antifungal producing Actinobacteria, 32 (89%) were from the genus *Streptomyces*, known for their antibiotic production. Fifteen of the isolates with antifungal activity against *P. destructans* were identified as novel *Streptomyces* species after morphological and multi-gene phylogenetic analysis. This study includes one of the largest surveys of microbial communities associated with healthy bats. Our results show that bats in western North America possess novel bacterial microbiota with the potential to inhibit *P. destructans*.

Functional ecology of mycobiome shifts associated with plant senescence - linking environmental and experimental re-synthesis metatranscriptomics. *Ko-Hsuan Chen*[1], Hui-Ling Liao[2], Hailee B. Korotkin[3], P. Brandon Matheny[3], Francois Lutzoni[1] 1) Department of Biology, Duke University, Durham, NC, USA; 2) Soil and Water Sciences Department, University of Florida, Gainesville, FL, USA; 3) Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA.

The same fungi are often found in healthy and dead tissues of the same plant species. Because plant tissues at different stages of senescence are usually separated by time and space, tracking functional transitions of their mycobiomes is often confounded with extraneous factors. The perennial moss *Dicranum scoparium* has a growth form with a continuous senescence gradient, making it an ideal system to study fungal trophic transitions. Our goal is to understand the function of fungi associated with healthy photosynthetic tissues to advanced states of decomposition. A metatranscriptomic approach was first applied to obtain the metabolic (rRNA) and functional (mRNA) activities of fungi associated with top (photosynthetic), middle (senescent), and bottom (decomposing) layers for the naturally occurring *D. scoparium*. Our study of nutrient-related gene expression in each layer, revealed that the highly expressed genes in the top layer are mostly related to carbon exchange, whereas in the middle and bottom layers we also found highly expressed genes that are involved in nitrogen and phosphate transport. In parallel to our metatranscriptomic study of environmental samples of *D. scoparium*, we isolated fungal strains from the three layers of this moss and grew *D. scoparium in vitro* from spores. By comparing and integrating the fungal rRNA sequences generated by metatranscriptome and culture-based approaches, we assigned fungi into three types of association categories: 1) High activity in photosynthetic tissues, 2) High activity in decomposing tissues and 3) Low activity throughout the gametophyte but abundant when using culture-based detection. Based on these categories, seven fungal strains representing distinct fungal lineages (four Ascomycota, two Basidiomycota, one Mortierellomycotina) were selected to establish fungus-plant re-synthesis pairs in the laboratory with both living and dead gametophytes. For every fungus-plant pairs, growth rates were monitored for two months after fungal colonies reached the moss. Metatranscriptomes of these fungus-plant pairs were obtained at the end of this period.

Fungal-bacterial interactions drive community assembly in cheese rind microbiomes. *Benjamin Wolfe*, Yuanchen Zhang, Casey Cosetta, Erik Kastman Biology, Tufts University, Medford, MA.

Despite a growing appreciation of the widespread co-occurrence of bacteria and fungi in many microbiomes, mechanisms that drive the ecology of bacterial species are largely studied while excluding neighboring microbial eukaryotes. My lab is using tractable microbiomes based on fermented foods to identify the processes and molecular mechanisms by which diverse fungi can shape the ecology of bacterial species and communities. We first use in situ metagenomics and culture-based approaches to identify putative bacterial-fungal interactions in naturally forming communities. We then combine in vitro community reconstructions, comparative genomics, transcriptomics, and metabolomics to identify the molecular mechanisms driving fungal-mediated bacterial competition and cooperation. Results from a variety of cheese rind biofilm models illustrate the diverse ways that fungi drive bacterial community development. In biofilms dominated by closely-related *Staphylococcus* species, the fungus *Scopulariopsis* allows *Staphylococcus* species that are weak competitors and colonizers to outcompete neighbors by altering iron and amino acid availability. In biofilms composed of both non-motile Firmicutes and Actinobacteria as well as motile Proteobacteria, the biophysical networks formed by *Mucor* can facilitate the dispersal of motile community members, leading to communities dominated by Proteobacteria. Most recently, we have found that volatile organic compounds produced by fungi can impact the growth of individual bacterial species and alter the development of multispecies bacterial communities. Collectively, our results demonstrate how fungi can mediate bacterial competition and community development in multispecies microbiomes through a variety of contact-dependent and contact-independent mechanisms. Ongoing work is exploring how manipulation of the bacterial-fungal interactions we have identified can be used to better manage the quality and safety of food microbiomes.

Wednesday, March 15 3:00 PM – 6:00 PM
Scripps
Population Genomics

A framework for comparing genomic rearrangement rates. *C.T. Hann-Soden*[1], L.A. Montoya[1], I Liachko[2], S.T. Sullivan[3], J.W. Taylor[1] 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) University of Washington, Seattle, WA; 3) Phase Genomics, Inc., Seattle, WA.

While sophisticated models exist for inferring the pace and pattern of substitution mutations, methods for inferring genomic rearrangements are being actively developed. We present a distance based framework for estimating the rate and mean size of genomic rearrangements along a phylogeny from draft whole genome sequences. Our framework is analogous to the distance based frameworks developed for estimating substitution rates along a phylogeny. We apply this framework to studying rearrangement rates within the genus *Neurospora*, where numerous transitions from a highly recombining lifestyle to a largely clonal lifestyle have occurred. We find evidence that there are more and larger rearrangements in the clonal lineages relative to the recombining lineages, reflective of weaker selection against rearrangements in clonal lineages. We compare the application of this distance-based framework to the application of more sophisticated models that infer individual rearrangement events within a phylogeny.

Adaptation by mitotic recombination in evolving yeast populations. *T.Y. James* Dept Ecology Evolutionary Bio, Univ Michigan, Ann Arbor, MI.

The role of both mutation and sex in adaptation during microevolution is well known and supported by numerous experimental studies of evolution. However, how often mitotic recombination events are adaptive are poorly studied. Mitotic recombination is here defined to include many forms of loss of heterozygosity (LOH), such as gene conversion, whole chromosomal loss, and crossing over. Why LOH should be adaptive is somewhat counterintuitive, because it leads to a loss of allelic diversity. However, LOH may expose beneficial recessive mutations as well as change epistatic interactions between loci. Moreover, it is observed in essentially all of the species of fungi which have diploid vegetative states, many of which are important pathogens, such as *Candida albicans* and *Batrachochytrium dendrobatidis*. Here, we discuss the development of a model system for studying the role of mitotic recombination during the evolution of diploid fungal pathogens using a yeast-insect system. We show that the system is able to identify genes essential for pathogenicity, and show evidence for LOH during adaptation to the host. These results are compared to adaptation during a 500 generation experiment to diverse in vitro conditions. The data are used to address what form of LOH predominates during the adaptive process, an important question for understanding how precisely genotypes can be fine-tuned by LOH.

Diversity among species of *Coccidioides:* population genomics analysis and local population structure. *B.M. Barker*[1], M.deM. Teixeira[1], D.M. Engelthaler[2], P. Keim[1,2] 1) PMI, Northern Arizona University, Flagstaff, AZ; 2) TGen North, Flagstaff, AZ.

Coccidioides posadasii and *C. immitis* are both filamentous fungi and the causative agents of coccidioidomycosis, commonly known as valley fever. These fungi are primary pathogens that can be responsible for morbidity and mortality in otherwise healthy patients. Over the past several years, valley fever cases have increased dramatically, and the endemic range has expanded. Disease can vary from asymptomatic to lethal. The underlying reasons for disease variation are due in part to intrinsic genetic factors in both the pathogen and host, and represent a major gap in our knowledge. Our overall knowledge of variance in clinically relevant phenotypes, especially in relation to the biology of *Coccidioides*, is limited. We have begun to assess genetic variation within and among species and populations of *Coccidioides.* Our approach is focused on identifying SNPs associated with clinically relevant phenotypes by screening variation found within and among species. *The* strategy should elucidate novel candidate genes in *Coccidioides* stress tolerance using an unbiased approach. Additionally, we have observed local population structure at a smaller scale than previously appreciated. Within Arizona, we detected restricted gene flow among Tucson human clinical, Phoenix human clinical and environmental/veterinary clinical isolates, suggesting that human clinical isolates may not represent total diversity.

Genomic analysis reveals multiple endemic and pandemic lineages of rice blast and gene flow into recombining populations from southeast continental Asia. *Pierre GLADIEUX*[1], Maud THIERRY[2], Adrien RIEUX[3], Sebastien RAVEL[2], Sandrine CROS-ARTEIL[1], Henri ADREIT[2], Joëlle MILAZZO[2], Elisabeth FOURNIER[1], Ryohei TERAUCHI[4], Didier THARREAU[2] 1) BGPI Research Unit, INRA, Montpellier, FR; 2) BGPI Research Unit, CIRAD, Montpellier, FR; 3) PVBMT Research Unit, CIRAD, La Réunion, FR; 4) Iwate Biotechnology Research Center, Kitakami, Iwate, JP.

The rice blast fungus *Magnaporthe oryzae* is the most damaging rice pathogen, and a textbook example of widely distributed, rapidly adapting pathogen, despite limited genetic diversity. The aim of our study was to elucidate the factors and evolutionary changes underlying the emergence, diversification and spread of *M. oryzae* in rice agrosystems. Analyses of population structure based on Infinium-genotyping of 5300 SNPs for 970 isolates collected on five continents identified three main pandemic lineages on *indica* and *japonica* rice, coexisting with multiple lineages with more restricted distributions in Asia and sub-saharan Africa. Analyses of recombination based on resequencing data for a subset of 89 isolates revealed that most lineages are highly clonal, although we found evidence for recombination in a widely distributed lineage infecting japonica and hybrid rice in Yunnan, Laos and Thailand. Because the sequenced isolates were collected between 1973 and 2009 and recombination is limited, we could use a phylogenetic approach to date the emergence and global dispersal of *M. oryzae* using dated tips to calibrate tree nodes. Our analysis provided an accurate estimate of the nucleotide substitution rate. Rice- and Setaria-infecting major lineages diverged ~10000 years before present, matching the oldest archaeological evidence for human exploitation of rice, and rice-infecting lineages radiated ~1200 years ago. Probabilistic and non-parametric 'chromosome painting' revealed gene flow into multiple non-pandemic lineages -including the recombining lineages from southeast continental Asia- from multiple source populations. Gene sets matching putatively migrant mutations were functionally enriched in genes involved in non-self recognition and programmed cell death, suggesting a role of fungal immunity in increasing the genomic diversity of rice blast populations. Despite the general lack of polymorphism and the accumulation of deleterious mutations in rice-infecting lineages, we could identify regions with signatures of balancing selection (increased functional variation) that were functionally enriched in small secreted proteins and proteins involved in

oxidation-reduction processes. Our work provides a population-level genomic framework for defining molecular markers to assist in the control of rice blast and for investigating the molecular underpinnings of phenotypic and fitness differences between divergent lineages.

Evolutionary pathways leading to panzootics of amphibian fungal disease. *Matthew Fisher*[1], Simon O'Hanlon[1], Rhys Farrer[1], Adrien Rieux [2] 1) Dept Infectious Disease Epidemiology, Imperial Col London, London, GB; 2) CIRAD, La Reunion.

The Kingdom Fungi is a biodiverse and essential component of our habitable Planet. However, the last 100 years have witnessed an increasing number of virulent emerging pathogenic fungi across ecosystems, with these infections causing the greatest disease-driven losses of biodiversity ever documented. Fungal life-history characteristics, namely high virulence, environmental persistence, broad host-ranges and flexible genomic architecture, predispose this kingdom to emergence as terminal pathogens across susceptible populations of hosts. Anthropogenic activity is a key factor that perturbs natural cycles of infection by increasing long-distance dispersal of inocula and through environmental forcing of infection dynamics. I demonstrate these concepts by analysing patterns and processes across the backdrop of globally-emerging amphibian-parasitising chytrid fungi. We use comparative genomics to characterise the major evolutionary innovations that have led to the acquisition of pathogenicity across the genus *Batrachochytrium*. Population genomics defines ancient hotspots of amphibian/chytrid biodiversity and pinpoints the origins of panzootic lineages. Tip-dating approaches to calibrating molecular clocks then, for the first time, accurately date the timing of these expansions. These studies answer the questions ‘why’, ‘where from’ and ‘when’ that have long plagued ecologists investigating global amphibian declines. Our findings also argue that ongoing 'fungal pollution' will increasingly cause the attrition of biodiversity unless further steps are taken to tighten global biosecurity for this rapidly emerging class of pathogens.

Can meiotic drive drive speciation? *A.A. Vogan*, Jesper Svedberg, Sandra L. Ament-Velásquez, Hanna Johannesson Systematic Biology, Uppsala University, Uppsala, SE.

Selfish elements can bias their own transmission from one generation to the next in a process referred to as meiotic drive. The driving element will be passed to the majority of offspring, thereby increasing in frequency over generations. The drive can occur regardless of its effect on fitness and may be a detriment to the organism as a whole. This intragenomic conflict could lead to strong selection against individuals carrying the element and promote reproductive isolation between so called killer and sensitive strains.

In ascomycetous fungi, meiotic drive manifests itself as a spore killing phenomenon. Briefly, ascospores carrying a killer element will induce the death of ascospores in the same ascus that do not carry the element. The killing is assumed to have a large cost to fitness, as a cross between killer and sensitive strains would result in the death of roughly half of all sexual progeny. We have examined 4 different spore killers in both *Neurospora* and *Podospora*. All of these examples of drive show patterns that suggest they may promote reproductive isolation between killer and sensitive strains.

In *N. sitophila*, we have indications that two separate defense mechanisms have evolved to protect against drive. Both Tahitian and European populations are polymorphic for spore killing. In Tahiti, a resistance locus has evolved that allows a number of sensitive spores to escape killing. In Europe, no such resistance has been observed. However, sensitive strains appear to be a distinct lineage from killer strains. This suggests that reproductive isolation may be a viable alternative to resistance as a way to protect against spore killing.

In *N. intermedi*a, there are also indications of reproductive isolation between killer and sensitive strains. Crosses between certain populations produce little to no viable progeny when one parent carries a killer element, but produce moderate levels of viable progeny when both parents are sensitive. This pattern is also observed when crosses are conducted between *N. intermedia* and its sister species, *N. metzenbergii.*

The final example is from a population of *P. anserina* in the Netherlands, where the investigated spore killer element appears to be nearly fixed. A small number of individuals can be found that do not have this killer element and again, these isolates appear to be a distinct lineage from the other Dutch strains.

We are currently performing experiments to confirm the link between meiotic drive and speciation.

Comparative population genomics of *Fusarium graminearum* reveals adaptive divergence among cereal head blight pathogens. *A.C. Kelly*, Todd J. Ward ARS, USDA, Peoria.

In this study we sequenced the genomes of 60 *Fusarium graminearum,* the major fungal pathogen responsible for Fusarium head blight (FHB) in cereal crops world-wide. To investigate adaptive evolution of FHB pathogens, we performed population-level analyses to characterize genomic structure, signatures of natural selection and differences in gene content among isolates. Genome-wide patterns of SNP diversity revealed that most isolates with the novel NX-2 (3α-acetoxy, 7α,15-81 dihydroxy-12,13-epoxytrichothec-9-ene) toxin type represent a genetic population (termed NA3) that is distinct from the native (NA1, largely 15-acetyl-deoxynivalenol toxin type) and invasive (NA2, largely 3-acetyl-deoxynivalenol toxin type) populations inhabiting North America, although genetic exchange among populations was documented. The three populations differed in gene content, with 134 genes showing population-specific patterns of conservation. In addition, each population had unique genetic signatures of adaptive selection that were largely focused in hypervariable regions of chromosomes. Sixteen candidate loci, varying in size from 10-40 kb, showed genetic signals of adaptive divergence, in that alleles were highly differentiated among populations but showed reduced diversity within populations. The strongest signals of selective sweeps were observed at the trichothecene biosynthetic gene cluster. However, functional annotation of population-differentiating genomic regions revealed numerous genes involved in host invasion, toxin production and secondary metabolism, and implicated plant hosts, microbial competitors, and temperature and light as major drivers of adaptive divergence. Collectively, our results show that North American populations of *F. graminearum* harbor unique sets of adaptations that contribute to differences in how these pathogens exploit the agricultural landscape.

***Cryptococcus neoformans* genomics influence human survival and immunologic responses.** *Aleeza Gerstein*[1], David Boulware[1], David Meya[2,3], Kirsten Nielsen[1] 1) Department of Microbiology & Immunology, University of Minnesota, Minneapolis, MN; 2) Infectious Diseases Institute, Makerere University, Kampala, Uganda; 3) Makerere University College of Health Sciences, School of Medicine, Department of Medicine, Kampala, Uganda.

Patient outcomes are due to a complex interplay between the quality of medical care, the host immunity factors, and the infecting pathogen's characteristics. Pathogen burden is clearly associated with mortality risk, but other pathogen characteristics, such as genotype and phenotype, have not been fully explored. A number of studies have recently suggested that pathogen genotype may directly influence host survival, even with tremendous variability of other host factors. To further probe this connection, we examined *Cryptococcus neoformans* genotypes from Ugandan clinical isolates collected as part of the Cryptococcal Optimal ART Timing (COAT) trial to examine whether there was a genomic signature in the pathogen associated with patient survival and/or immune response. We focused on ST93, the most frequently observed sequence type (ST) among the isolates, and performed whole-genome sequencing on 41 ST93 isolates, acquired from 22 survivors and 19 from patients who died. Sixteen other isolates from a diversity of ST groups were also sequenced for comparison. We identified 131754 SNPs and 16924 small indels among all isolates, with ~40% in at least one ST93 genome. We used the variants to construct a phylogenetic tree, and surprisingly found that ST93 isolates grouped into two separate clades (clade A and clade B). We focused our analyses on the 219 SNPs and 58 indels that were variable among the ST93 genomes, were unassociated with a centromere, and were predicted to have a fitness effect. Approximately half of these variants deliniated ST93 clade A isolates (123 SNPs and 23 indels). Importantly, a number of these variants were significantly associated with decreased 26-week survival (clade A: 29% vs clade B: 63%, 17 SNPs), decreased CD4 T cell counts nadir (a two base pair deletion), and altered CSF cytokines: a six base pair insertion associated with increased levels of pro-inflammatory interleukin (IL)-6, and a ten base pair deletion associated with increased protective Th1 response of IL-12 and increased protective Th2 response of IL-13. Additional non-clade specific SNPs (n=26) were also each significantly associated with at least two immunologic trait responses. This work demonstrates that fungal genotype may significantly influence human immune responses and survival and provides important candidate genes for future studies on virulence-associated traits in *Cryptococcus*.

Thursday, March 16 9:00 AM – 12:00 NOON
Merrill Hall and Chapel
Plenary Session II: Applied Mycology: Superpowers of Fungal Heroes and Villains

Plant biomass conversion by fungi: a highly diverse and complex process. *Ronald de Vries*[1,2,3] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; 2) Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands; 3) Department of Food and Environmental Sciences, University of Helsinki, Helsinki, Finland.

The conversion of plant biomass by fungi has been a topic of interest for many decades, mainly due to the application of fungal plant biomass degrading enzymes in many biotechnology processes, such as the production of food & feed, paper, textiles, beverages, wine and more recently biofuels and biochemicals. The availability of an increasing number of fungal genome sequences and post-genomic datasets has provided an unprecedented understanding of this process. It revealed that the range of enzymes employed by fungi is significantly higher than was assumed before the availability of genome sequences, but also that the strategies employed by different fungi to degrade similar biomass substrates is highly diverse.

In our group we are particularly interested in understanding the molecular mechanisms underlying this diversity and how the different approaches match the prevailing biomass components. A special emphasis in our studies is in the regulation of the production of the enzymes, as this relates directly to the 'choices' a given species has made with respect to conversion approach. In recent years, several regulators have been identified that are involved in plant biomass conversion and while some are conserved among filamentous ascomycetes, their specific role and set of target genes are far less conserved. The conservation of enzyme systems in basidiomycetes and ascomycetes, while most ascomycete regulators are not present in basidiomycetes, raises questions about the regulatory mechanisms in the latter group and the evolution of the enzyme families versus the regulators that control their production.
In this talk I will present some of our recent data aimed at understanding the diversity of plant biomass conversion by fungi and the highly complex regulatory system that controls this process. This novel insight in how fungi have evolved their plant biomass conversion approaches will provide direct leads on how to improve applications of fungal plant biomass degrading enzymes.

Resistance to antifungal drugs in fungal pathogens. *Dominique Sanglard* Univ of Lausanne and Hosp Ctr, CH-1011 Lausanne.

Diseases caused by fungal pathogens necessitate the use of antifungal agents. The treatment of these diseases by antifungal agents remains a major challenge in hospitals given the restricted arsenal of therapeutic options and the continuing occurrence of drug resistance. We identified in the past several resistance mechanisms in fungal species relevant for human diseases using isolates from treated patients. Our current research has focused on 1) the impact of drug resistance on fungal-host interactions and 2) the understanding of fungal multidrug resistance (MDR) development in the host.
With regards to 1), we showed that the human pathogen *Candida glabrata* can develop rapidly resistance to azoles, which are compounds still widely used in hospitals. Resistance is mediated by several mutations in the transcription factor *PDR1*, which results in the overexpression of targets genes (ABC transporters). The same mutations alter fungal-host interactions by increasing the virulence of *C. glabrata* in several infection models. *PDR1* mutations can augment the expression of at least an important adhesin (*EPA1*) which contributes to establish a more efficient disease. Whole genome sequence analysis of *C. glabrata* strains suggest that this yeast species has accumulated more adhesins than previously anticipated, which may also impact on host-fungal interactions.
2) MDR is a current concern among fungal pathogens. A recent example of MDR by *Candida lusitaniae* came across our investigations. *C. lusitaniae* isolates were obtained during a 3-months therapy with amphotericin B (targeting ergosterol), azoles (targeting sterols) and candins (targeting cell wall). Four resistance profiles from 5 distinct isolates (P1-P5) were identified against 2 to 3 drug classes. To investigate MDR, analysis of specific drug resistance genes or comparisons of genomes by PacBio sequencing were used. The PacBio assembled genomes were compared with each other and with P1, a susceptible isolate. Ten non-synonymous SNPs were detected in these comparisons and revealed i) changes in *ClMRR1* that encodes a putative transcriptional activator in azole-resistant isolates P3 and P4 which correlated with upregulation of a MDR transporter (*MFS7*); ii) Three different mutations in *FKS1* (glucan synthase) in P2, P3 and P5 known to participate to resistance; iii) stop codons in *ERG4* and *ERG3* each of which were related to amphotericin B resistance in P2 and P5. Thus, isolates P2 to P5 accumulated six different drug resistance mutations as compared to P1 that emerged during the short time lapse therapy. This case study illustrates the capacity of a specific species to adapt to drug pressure within a host. MDR emergence is now also becoming a concern in the therapy of *C. glabrata* infections worldwide.

***Malassezia* – what´s the turning point from commensal to pathogen?** *A. Scheynius*, the Malassezia Research Consortium Karolinska Institutet, Stockholm, SE.

Malassezia is a commensal yeast that colonizes the human skin right after birth and predominates the human fungal skin microflora. Fourteen species have so far been identified. One of the species most frequently isolated from human skin is *Malassezia sympodialis*, which is associated with several common skin disorders such as atopic dermatitis (AD) (Saunders CW *et al* 2012). We have found that *M. sympodialis* secretes nanosized exosome-like vesicles, designated MalaEx, that carry allergens and can induce inflammatory cytokine responses (Gehrmann U *et al* 2011). To elucidate *M. sympodialis* host-microbe interactions we assessed whether small RNAs are present in MalaEx and, if so, whether the levels of these RNAs differ in MalaEx isolated from *M. sympodialis* cultured at normal skin pH compared to the higher pH on the skin of AD patients. From small RNA sequence data, we identified a set of reads with well-defined start and stop positions, in a length range of 16 to 22 nucleotides (Rayner S *et al* in press). Bioinformatics analysis indicated that these RNA features appear to have an RNAi-independent route for biogenesis. No significant differences were observed between the MalaEx and their cargo of small RNAs isolated from *M. sympodialis* cultured at the two different pH levels indicating that they are not influenced by the elevated pH level observed on the AD skin.
Complete and accurate genome assembly and annotation is a crucial foundation for comparative and functional genomics. Through long-read DNA sequencing of *M. sympodialis* (ATCC 42132), we have obtained a gap-free genome assembly comprising eight nuclear and one mitochondrial chromosome (Zhu Y *et al* submitted). Proteomics data could be readily integrated with transcriptomics data in standard annotation tools. This increased the number of annotated protein-coding genes by 14% (from 3612 to 4113), compared to using

transcriptomics evidence alone. Manual curation further increased the number of protein-coding genes by 9%. All of these genes have RNA-seq evidence and 87% were confirmed by proteomics. The *M. sympodialis* genome assembly and annotation is at a quality yet achieved only for a few eukaryotic organisms, and constitutes an important reference for future host-microbe interaction studies.

***Fusarium fujikuroi* - a treasure box for new secondary metabolites and non-canonical regulatory mechanisms.** *Bettina Tudzynski*[1], Eva-Maria Niehaus[1], Slavica Janevska[1], Andreas Pfannmüller[1], Lena Studt[1,2], Sarah Rösler[1] 1) Institute of Biology and Biotechnology of Plants, University Münster, Münster, DE; 2) Institut für Angewandte Genetik und Zellbiologie, BOKU Tulln, Austria.

The fungus *F. fujikuroi* causes ''bakanae'' disease of rice due to its ability to produce gibberellins (GAs), a class of phytohormones. However, recent genome sequencing revealed the genetic capacity of this pathogen for the biosynthesis of a whole arsenal of secondary metabolites (SMs). Many of the gene clusters are silent under laboratory conditions, and their products and their roles in the fungus-rice interaction were unknown. To activate these SM gene clusters, we studied the multiple regulatory layers which coordinately control the biosynthesis of each single gene cluster. Key players in their regulation are cluster-specific transcription factors (TFs), mostly belonging to the Zn2-*Cys6* family, global regulators such AreA, PacC and Sge1, components of the Velvet complex, such as Vel1 and Lae1, and histone modifying enzymes affecting the chromatin status in response to environmental conditions. We found that all these factors play differing roles in regulating each individual cluster, and that all clusters are regulated by a cluster-specific network of regulators. For instance, both deletion of the histone deacetylase *HDA1* and replacement of the repressive histone mark H3K27me3 led to the specific activation of the silent beauvericin cluster due to increased acetylation at H3K27. In addition, the expression of beauvericin cluster genes is regulated by the cluster-specific Zn2-*Cys6*-type TF and, surprisingly, by the cluster-specific ABC transporter, both acting as repressors. Furthermore, while the majority of SM biosynthetic genes respond to nitrogen availability, only some of these nitrogen-regulated SM genes depend on the major nitrogen regulators AreA and/or AreB. In contrast to our expectations based on the yeast model, both GATA TFs served as activators for most of these clusters. In addition, genetic manipulation of global regulators (e.g. Vel1, Lae1, AreA and Sge1) affected not only the expression levels of SM genes, but led to altered regulatory mechanisms. For example, nitrogen repression of GA biosynthesis was overcome by over-expressing Lae1 and Sge1. In addition, AreA and AreB affected the accessibility of target gene promoters for pathway-specific TFs, e.g. for the fumonisin-specific TF Fum21. These results suggest that the expression of each single SM gene cluster in *F. fujikuroi* is under control of manifold regulatory mechanisms, all different, and sometimes unexpected.

Symbiosis between a bacterial endophyte and the ancient African crop finger millet results in dramatic trapping and killing of the fungal pathogen *Fusarium graminearum*. Walaa Mousa[1], Charles Shearer[1], Victor Limay-Rios[2], Cassie Ettinger[3], Jonathan Eisen[3], *Manish Raizada*[1] 1) Plant Agriculture, University of Guelph, Guelph, ON, CA; 2) Plant Agriculture, University of Guelph, Ridgetown Campus, Guelph, ON, CA; 3) University of California, Davis Genome Center, Davis, CA.

Unlike its genetic relatives such as corn and wheat, the ancient African crop, finger millet, shows resistance to the fungal pathogen, *Fusarium graminearum*. We recently reported (*Nature Microbiology* 2016; 1:16167) the discovery of a novel plant defence mechanism in which a root-inhabiting bacterial endophyte, strain M6 (*Enterobacter* sp.), detects invading *Fusarium*, and in response stimulates the dramatic growth of nearby root hairs. The root hairs then bend parallel to the root axis, forming a multi-layer, lasagna-like formation, in which the “noodles” are the root hairs and the “filling” consists of microcolonies of the endophyte, a structure we have termed RHESt (root-hair endophyte stacking). The RHESt traps and kills *Fusarium*. Tn5-mutagenesis shows that M6-endophyte killing requires diverse fungicides including phenazine, and resistance to a *Fusarium*-derived antibiotic (fusaric acid), suggestive of long-term, host–endophyte–pathogen co-evolution. This fascinating symbiosis results in reduced deoxynivalenol mycotoxin, and may be protecting millions of subsistence farmers and livestock.

Thursday, March 16 3:00 PM – 6:00 PM
Merrill Hall
Human Pathogenic Fungi

Marker recycling through CRIME. *A.P. Mitchell*, M. Huang Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

We describe here a new approach to marker recycling, a controlled sequence of manipulations in which a genetic marker is selected, then lost, then selected again. Our work was carried out with the fungal pathogen *Candida albicans*, which is typically a diploid and has no complete sexual cycle. The approach makes use of the RNA-guided DNA cleavage activity of CRISPR-Cas9, which was recently engineered for use in *C. albicans* by Valmik Vyas and colleagues (PMID: 25977940). We used the high-speed adaptation for gene deletion described by Kayden Min in our lab (PMID: 27340698) for the specific studies that will be presented. Because the CRISPR-Cas9 system has been implemented in diverse fungi, we believe that CRIME may be applicable to diverse fungi.

To use CRIME, we first created marker cassettes flanked by direct repeats. We used one repeat-flanked marker to create a homozygous mutation in our favorite gene, *OFG1*, by CRISPR-Cas9 transformation. Then, in the *ofg1*/*ofg1* strain, we made a homozygous mutation in a second gene, *OFG2*, by use of a different repeat-flanked marker. In that second transformation, we also included a guide RNA gene to direct cleavage of the marker that lay in the *ofg1* mutant alleles. The idea was that either single-strand annealing or homology-directed repair would then excise the marker we cleaved and leave behind only a copy of the flanking repeats. The idea proved correct: we recovered *ofg1*/*ofg1 ofg2*/*ofg2* double mutants from the transformation that had only the marker in the *ofg2* alleles; they had lost both copies of the marker that had been in the *ofg1* alleles. In fact, we have been able to create successive homozygous mutations in three genes by use of two markers, and still ended up with one marker available for further selection in the triple homozygote. That sequence of steps and analyses required 3 weeks total. Our findings illustrate that CRIME pays, at least in this one context.

We are now modifying our approach to enable wild-type allele reconstruction through a process that we call TIME. We feel that this capability will be critical for geneticists of the future, because CRISPR-Cas9 systems can have off-target effects. Our hope is that geneticists of the future will always remember that, if they do the CRIME, they must do the TIME.

***Candida albicans* Hyr1 binding to c-Met induces oral epithelial cell Invasion while inhibiting neutrophil killing.** *Scott Filler*[1,2], Quynh Phan[1], Norma Solis[1], Marc Swidergall[1], Ashraf Ibrahim[1], Aaron Mitchell[3] 1) Dept of Medicine, Los Angeles Biomedical Res Inst, Torrance, CA; 2) David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

Background. During oropharyngeal candidiasis (OPC) *C. albicans* invades oral epithelial cells. Previously, we found that *C. albicans* Als3 and Ssa1 mediate invasion of oral epithelial cells by binding to E-cadherin and EGFR/HER2. Recently, we discovered that the c-Met receptor tyrosine kinase also mediates epithelial cell endocytosis of *C. albicans,* but functions in a separate pathway from EGFR/HER2. We set out to find the *C. albicans* ligand for c-Met.

Methods. Tyrosine phosphorylation of c-Met and EGFR/HER2 was assessed by immunoblotting with phosphospecific antibodies. To identify the *C. albicans* ligand for c-Met, we used far Western blotting with recombinant c-Met followed by protein sequencing. Epithelial cell endocytosis of *C. albicans* and latex beads coated with recombinant Hyr1 was quantified by a differential fluorescence assay. The extent of *C. albicans* killing by human neutrophils was determined by quantitative culture.

Results. Both the c-Met inhibitor SGX523 and the EGFR inhibitor gefitinib reduced epithelial cell endocytosis of *C. albicans* by ~50%, whereas the combination of SGX523 and gefitinib inhibited endocytosis by ~70%, indicating that c-Met and EGFR function in separate pathways. *C. albicans* hyphae induced tyrosine phosphorylation of c-Met by a process that was independent of EGFR/HER2, Als3 and Ssa1, suggesting that c-Met has a novel ligand. By far Western blotting, we found that the *C. albicans* ligand for c-Met is Hyr1. To verify this result, we determined that an *hyr1*Δ/Δ mutant had reduced capacity to induce phosphorylation of epithelial cell c-Met and invade epithelial cells. Also, latex beads coated with recombinant c-Met were endocytosed by epithelial cells more avidly than control beads coated with BSA. As reported by others, the *hyr1*Δ/Δ mutant had increased susceptibility to killing by human neutrophils. This increased susceptibility was due to failure to activate neutrophil c-Met because inhibition of c-Met with SGX52 enhanced neutrophil killing of WT *C. albicans*, phenocopying the effects of the *hyr1* mutation. Furthermore, activation of c-Met with its natural ligand, hepatocyte growth factor, reduced neutrophil killing of the *hyr1*Δ/Δ mutant to WT levels.

Conclusions. *C. albicans* Hyr1 is the fungal ligand for c-Met and contributes to virulence by two mechanisms. In epithelial cells, activation of c-Met by Hyr1 enhances the endocytosis of *C. albicans*. In neutrophils, activation of c-Met by Hyr1 impairs killing of *C. albicans*.

Taking a host genetics approach to understand how the intracellular fungal pathogen *Histoplasma capsulatum* survives within and lyses host macrophages. *A.L. Cohen*[1], N.V. Prooyen[1], M.C. Bassik[2], A. Sil[1] 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA.

Histoplasma capsulatum (*Hc*) is a pathogenic soil fungus that is inhaled by mammalian hosts, including humans. Subsequently, *Hc* is phagocytosed by macrophages, where it utilizes largely unknown strategies to survive and replicate within the macrophage phagosome. Robust proliferation of *Hc* within the phagosome is followed by host-cell death and the release of live fungal cells. Our goal is to understand how *Hc* manipulates host cell processes, including phagosome maturation, to facilitate intracellular replication within macrophages and successful infection. Our lab and others have shown that, unlike most microbes ingested by macrophages, *Hc* resides within a neutral phagosome, and *Hc*-containing phagosomes do not acquire late lysosomal markers such as cathepsins. However, little is known about the molecular players underlying host manipulation by this fungus. We are taking a two-pronged approach to elucidate host pathways that are targeted by *Hc* during intracellular growth and lysis. First, we are conducting a pooled CRISPR-Cas9 based gene disruption screen in human macrophage-like cells to identify host genes required for death following *Hc* infection. We will then study how the corresponding gene products and pathways are directly or indirectly manipulated by *Hc* to promote intracellular infection. Second, we have discovered that a host lysosomal trafficking regulator, LYST, is required for *Hc* to lyse host macrophages during infection. We are investigating the role

of LYST in allowing *Hc* to promote host cell lysis. These studies will hopefully uncover molecular mechanisms of intracellular fungal pathogenesis as well as novel insights into host cell biology.

***Cryptococcus neoformans* suppresses immune cell infiltration into the brain via a secreted immunomodulatory polysaccharide.** *S.T. Denham*, S. Verma, J.M. Daugherty, R. Reynolds, T.E. Lane, J.C.S. Brown Pathology, University of Utah, Salt Lake City, UT.

The basidiomycetes yeast *Cryptococcus neoformans* is the most prevalent cause of fungal meningitis, responsible for ~1 million diagnosed cases and ~600,000 deaths annually. *C. neoformans* infection begins in the lungs after the inhalation of infectious particles. In immunocompromised individuals, where symptomatic disease is most prevalent, *C. neoformans* systemically disseminates from the lungs. Disseminating *C. neoformans* exhibits a particular tropism for the brain, leading to cryptococcal meningitis (CM). CM patients often suffer high titers of infecting fungal cells within the brain, but exhibit limited neuroinflammation and relatively mild initial symptoms. Consequently, many patients do not seek care until fungal burden is damagingly high and therefore difficult to treat. *C. neoformans* conditionally produces a protective polysaccharide capsule, consisting primarily of the polysaccharide glucuronoxylomannan (GXM). GXM is both attached to the cell surface and secreted into the extracellular space as exo-GXM. GXM has numerous immunosuppressive properties, and its levels in the cerebrospinal fluid (CSF) of human patients negatively correlates with CSF white blood cell count. We thus hypothesized that exo-GXM suppresses inflammation and immune infiltration into the brain during infection. Moreover, this pauci-inflammation of CM is likely not only due to the immunocompromised state of patients, as we and others have found that *C. neoformans* suppresses the immune response of immunocompetent mice.

After infecting C57BL/6J mice intranasally with wild-type *C. neoformans*, fungal cells eventually disseminate to the brain. Upon analysis of infected brains, we discovered very little immune infiltration (neutrophils, macrophages, CD4+, and CD8+ cells), despite high fungal burden and histopathology. We also microscopically observed significant levels of exo-GXM in the brain. When mice were infected intracranially with an acapsular strain (*cap60Δ*), numbers of brain infiltrating immune cells significantly increased over wild-type. Likewise, administration of purified GXM to *cap60Δ* infected mice suppressed immune infiltration and increased fungal burden. We have also identified mutants deficient in exo-GXM production and the regulation of the switch between cell surface retention and exo-GXM secretion. Our results support a model in which *C. neoformans* actively secretes exo-GXM to modulate the host's immune response and promote fungal survival in the brain.

Construction and efficacy of Δ*cps1*, a live attenuated vaccine for coccidioidomycosis. *M. A. Mandel*[1,4], L. F. Shubitz[1], L. Lewis[1], H. T. Trinh[1], A. S. Buntzman[2], J. A. Frelinger[3], J. N. Galgiani[2], M. J. Orbach[1,4] 1) The Valley Fever Center for Excellence, Tucson, AZ; 2) Department of Medicine, University of Arizona, Tucson, AZ; 3) Department of Immunobiology, University of Arizona, Tucson, AZ; 4) The School of Plant Sciences, University of Arizona, Tucson, AZ.

Coccidioidomycosis (Valley Fever, VF) is a fungal disease of mammals endemic to the SW US and parts of Mexico, Central and South America, caused by *Coccidioides immitis* and *C. posadasii*. While most people experience mild flu-like symptoms, this pathogen can cause meningitis and is responsible for 160 deaths and $170 million in hospital costs in CA and AZ annually. Infection results in lifelong immunity, suggesting a vaccine against VF is attainable. We have developed a live attenuated vaccine by deletion of *CPS1*, the ortholog of a *Cochliobolus heterostrophus* virulence factor. Our *Δcps1* strain is non-pathogenic in susceptible mice, and provides dramatic protection against WT infection. Current analyses indicate that Cps1 is a member of the AMP-dependent synthetase/ligase family of proteins related to Dip2 in animals. The role of Cps1 in the parasitic phase is unknown, but evidence shows that the *Δcps1* strain initiates spherule formation but fails to propagate beyond a first generation in the host. We present data showing that vaccination results in long-term survival in mice given lethal doses of *Coccidioides spp.*, and that protection lasts at least 6 months following a single dose of live spores. The *Δcps1* strain forms defective thin-walled spherules that fail to endosporulate and that lyse prematurely. *In silico* analysis suggests Cps1 is a transmembrane protein and transcriptional analysis supports a role as a transcriptional regulator. The original *Δcps1* strain has the complete ORF replaced by the hygromycin phosphotransferase marker. The presence of this marker is a safety and regulatory concern for a live vaccine. To create a new *Δcps1* strain lacking an antibiotic resistance marker, we used Biolog nutritional and genomic data to determine that *Coccidioides spp*. are unable to use sucrose as a carbon source. We demonstrate that the *A. niger sucA* gene can be introduced into *Coccidioides,* allowing selection on sucrose and are using this gain-of-function strategy for the new vaccine strain design.

Discovery of a novel azole-resistance mutation in *Aspergillus fumigatus* and the possible role of sexual reproduction in its evolution. *J. Zhang*[1], Bas Zwaan[1], E Snelders[1], S Schoustra[1], K Dijk[2], J Meis[3,4], F Hagen[3,4], E Kuijper[5], G Kampinga[6], J Zoll[4,7], W Melchers[4,7], P Verweij[4,7], A Debets[1] 1) Laboratory of Genetics, Wageningen University, Wageningen, NL; 2) Department of Medical Microbiology, Vu University Medical Centre, Amsterdam, The Netherlands; 3) Department of Medical Microbiology and Infectious Diseases, CWZ Hospital, Nijmegen, The Netherlands; 4) Expert Centre in Mycology Radboudumc/CWZ (EMRC); 5) Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands; 6) Department of Medical Microbiology, University Medical Centre Groningen, Groningen, The Netherlands; 7) Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands.

We investigated two compost-heaps, one containing azoles and one without azoles, for the presence of azole-resistant and azole-sensitive *A. fumigatus* isolates. The azole-free compost yielded 98% (49/50) sensitive and 2% (1/50) azole resistant isolates, whereas the azole-containing compost yielded 8% (4/45) sensitive and 92% (41/45) resistant isolates. From the latter, 84% (37/45) were of the highly resistant TR_{46} Y121F/ T289A genotype and 8% (4/45) had a novel pan–triazole-resistance harbouring a triple tandem repeat: TR_{46}^3/Y121F/ M172I/T289A/G448S. The stark contrast between the two composts indicates that azole–containing compost is a hot spot for the development and maintenance of azole resistance. Subsequent screening of the clinical *A. fumigatus* collection from the Dutch national surveillance programme indicated that this resistance mechanism was already present in 2012, and is now found in all participating medical centres. We were able to recover this novel TR_{46}^3 mutation among the sexual progeny in the lab, from a cross between two TR_{46} isolates of opposite mating type that were from the same compost. This strongly indicates a role of sex in the emergence of this novel azole–resistance mechanism in *A. fumigatus*. Furthermore, we provide further evidence indicating the potential occurrence of sexual reproduction in compost, but this still needs further confirmation. Our findings alarm the fast development of the azole resistance issue in *A. fumigatus*

and further indicate the role of sexual reproduction of *A. fumigatus* in the development of azole resistance.
Key words: *Aspergillus fumigatus*; novel new mutation; compost; azole resistance; hot spot; sexual reproduction; ascospores; conidiospores

Comparative genomics of CUG-Ser clade species. *Geraldine Butler* Conway Institute, Univ Col Dublin, Dublin, IE.

It has been known for some time that *Candida albicans* and close relatives translate the codon CUG as serine rather than leucine, associated with changes in the $tRNA_{CAG.}$ Recent proteomic and bionformatic analysis has shown that the switch to CUG serine occurred at the base of the Metschnikowiaceae/ Debaryomycetaceae. The discovery of a second codon change (CUG Alanine) in two species outside the clade means that the Metschnikowiaceae should now be referred to as the "CUG-Ser" clade, rather than the "CUG clade" or "CTG clade" as used previously. Metschnikowiaceae include many fully sexual species such as the *Hyphopichia* group and the *Metschnikowia* group. *C. albicans* and closely related asexual species belong to the Candida/Lodderomyces clade, which also includes other major human fungal pathogens, such as *Candida tropicalis* and *Candida parapsilosis*. We used comparative genomic analysis to expand our study of the evolution of mating type in sexual and asexual species within the CUG-Ser clade. We are also exploring within-species diversity using genome sequencing.

Regulation of epigenetic switching and mating in *Candida* species. Matthew Anderson[1], Christine Scaduto[1], Allison Porman[1], Eugenio Mancera[2], Christina Cuomo[3], *Richard Bennett*[1] 1) Brown University, Providence, RI; 2) UCSF, San Francisco, CA; 3) Broad Institute, Cambridge, MA.

We are interested in defining the mechanisms that contribute to both genetic and epigenetic variation in pathogenic *Candida* species. Several *Candida* pathogens, including *C. albicans* and *C. tropicalis*, exhibit "phenotypic switching", in which cells undergo epigenetic and reversible changes in cell state. Such changes impact key phenotypic properties such as sexual competency and interactions with host immune cells. Here, we addressed the molecular mechanisms underlying tristable switching in *C. tropicalis*, in which cells undergo reversible transitions between three different cell states. Our experiments suggest that tristable switching is achieved via a symmetrical self-activating toggle switch (SATS) involving two antagonistic transcription factors, Wor1 and Efg1. In support of this model, ectopic expression of Wor1 or Efg1 is shown to drive transitions between each of the three cell states. Interestingly, stable inheritance of induced cell states is dependent on synergistic cues between expression of transcription factors and chromatin remodeling. This result thereby establishes parallels with the regulation of cell differentiation events in multicellular organisms. We also address how phenotypic switching regulates entry into the program of sexual reproduction in *Candida*. We reveal that differences in pheromone MAPK signaling are responsible for the distinct mating efficiencies of *Candida* white and opaque cell types. Together, these studies establish close links between cell differentiation events in fungi and higher eukaryotes, and define the molecular mechanism by which different cell types display distinct sexual behavior.

Thursday, March 16 3:00 PM – 6:00 PM
Chapel
Big Data (omics)

Big data in MycoCosm. *Igor Grigoriev*, Frank Korzeniewski, Igor Shabalov US Department of Energy Joint Genome Institute, Walnut Creek, CA.

There are 700+ fungal genomes currently available from the US Department of Energy Joint Genome Institute's MycoCosm portal, from projects like the 1000 Fungal Genomes, 300 Aspergillus genomes, and others, which target different scales of fungal diversity. For the majority of these genomes, their annotation employs transcriptomes, often sampled over a range of conditions with most deep sampling for Aspergillus niger and Neurospora crassa (JGI Fungal ENCODE project). For these sequenced genomes, a partnership between two DOE User Facilities, JGI and EMSL, produces proteomics and metabolomics data. In addition, the radidly evolving next generation sequencing platforms enable efficient production of epigenomic data such as DNA methylation. These new types of OMICS data add dimensions to analysis and enable solutions for biological questions of different scales. At the same time, this scale of OMICS data production offers new challenges in data integration, visualization, and analysis.

Exploring fungal dark matter using single-cell genomics. *S. Ahrendt*[1,2], C. A. Quandt[3], D. Ciobanu[2], A. Clum[2], A. Salamov[2], J-F. Cheng[2], T. Woyke[2], T. James[3], I. Grigoriev[1,2] 1) Department of Plant and Microbial Biology, University of California, Berkeley, CA; 2) DOE Joint Genome Insititute, Walnut Creek, CA; 3) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Current estimates suggest that only approximately 100,000 of the estimated 5 million fungal species worldwide have been described, and the overwhelming majority of those fall within the Dikarya. Thus, the diversity of the non-Dikarya lineages has been poorly explored. Our lacking insight into the metabolic potential of these fungi is a reflection of their absence from sequence databases, limiting our ability for meaningful comparative analyses and lifestyle predictions. Exacerbating this issue is the fact that a substantial fraction of these organisms is largely uncultivated, challenging genomic exploration, despite being detected in environmental PCR surveys as a significant component of a community. The difficulties inherent in exploring the genetic make-up of this "fungal dark matter" can be overcome using single-cell sequencing to reconstruct genomes of uncultivated organisms directly isolated from the environment. Environmental fungi, particularly among the zoosporic and other early-diverging fungal lineages, make exceptional targets for single-cell genomic techniques; however, as much of the current single-cell genomic work focuses on mammalian, bacterial, and archaeal systems, there is a pressing need to adopt these protocols for fungi. Here we developed and tested these single-cell methods to sequence the genomes of seven species which cannot be grown in pure culture, including the first representatives of the previously unsequenced Zoopagomycotina subphylum. We show that although there is a large variation in gene space recovery from each single cell (ranging from 6 - 88%), combining multiple cell libraries can increase this recovery to around 90%. Phylogenomic analyses allowed us to place previously unsampled lineages within the fungal tree of life, even when considering partially complete genomes derived from single individual cells. Additionally we explored gene family expansions to identify patterns consistent with lifestyle aspects of these biotrophic organisms.

Integrating ecological and -omics approaches to understand fungal responses to global change. *Serita Frey* Natural Resources and the Environment, University of New Hampshire, Durham, NH.

Soil fungi play key roles in ecosystem C and N cycles through decomposition of organic matter and nutrient exchange with plants. Soil fungi are the primary decomposers of cellulose and lignin, two of the most abundant compounds in plant material. Together with hemicellulose, these compounds form the complex structure referred to as lignocellulose, the decay of which involves a suite of extracellular enzymes. Fungi are the primary producers of these enzymes, and the presence of enzyme-coding genes determines whether a particular fungal species has the genetic machinery to decompose plant tissues. Any environmental disturbance that selectively impacts one group of fungi with a specific genetic capacity will likely influence organic matter decay, and ultimately, rates of soil nutrient cycling. For example, fungal taxa harbor different numbers and types of genes coding for lignocellulolytic enzymes. If taxa harboring these genes decline in abundance or are lost completely from the community, the capacity of the community to decompose plant material will be compromised. This talk will (1) synthesize several fungal –omics datasets generated from metabarcoding, metatranscriptomics, metabolomics, and whole genome sequencing analyses, and (2) discuss how coupling –omics approaches with biogeochemical analyses has accelerated our research to understand how soil fungal communities are responding to environmental change.

MagNet: the integrated gene network of the rice blast fungus *Magnaporthe oryzae.* *H. Kim*[1], K. Cheong[1], K.T. Kim[2], J. Jeon[1], G. Choi[1], Y.H. Lee[1,2,3] 1) Interdisciplinary Program in Agricultural Genomics, Seoul National University, Seoul 08826, Korea; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea; 3) Center for Fungal Genetic Resources, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea.

Magnaporthe oryzae, the rice blast fungus, plays a role as a model organism in the area of molecular plant-microbe interaction research. Studies on pathogenic mechanism of this fungus revealed many genes in signaling pathways are essential for appressorium formation and penetration. As multi-omics data are being available, genomic-level researches have been conducted to uncover the underlying biological process during the pathogenesis of *M. oryzae*. Identifying genome-wide protein-protein interaction (PPI) network is one of omics-level approaches which can help to understand signaling and regulatory pathways. However, existing biological network resources of *M. oryzae* are not sufficient to decipher molecular mechanisms of pathogenesis. In this study, PPI network analysis platform of *M. oryzae*, MagNet, was constructed with three methods: homology-based 'Interolog' search, co-expression network construction, and domain-domain interaction based prediction. Interologs within *M. oryzae* proteins which have orthologues in model species were predicted with six PPI repositories. Co-expression networks were built with RNA sequencing data from the infection stages and those from the vegetative stage. In addition, we gathered the information of domain-domain interactions (DDIs) from two integrated DDI databases, and predicted interactions between domain-annotated proteins. With three approaches all together, the pan-network with 6,600,976 interactions was generated including highly confident 215,731 interactions found in more than three sub-networks. Experimental data on *M. oryzae* PPIs support that

our highly confident PPI network can predict PPIs with higher sensitivity (89.65%) and specificity (78.57%) compared to the previously constructed databases. MagNet would provide integrated biological network data which can help to understand the molecular mechanisms of the rice blast fungus and beyond.

Chromosome plasticity during experimental evolution of the cross-kingdom pathogen *Fusarium oxysporum*. *C Lopez-Diaz*[1], D Hazal Ayhan[2], L.J Ma[2], A Di Pietro[1] 1) Department of Genetics, University of Córdoba, Cordoba, Spain; 2) University of Massachusetts, Amherst, USA.

Filamentous plant pathogens undergo rapid evolution, leading to expansions or shifts in host range. The *Fusarium oxysporum* species complex collectively causes vascular wilt disease in more than a hundred different crops. Meanwhile, clinical isolates of the fungus cause life-threatening systemic infections in humans. Remarkably, a single strain of *F. oxysporum, Fol* 42-87, is able to kill tomato plants, immunodepressed mice and larvae of the invertebrate animal model *Galleria*. The evolutionary mechanisms underlying host range dynamics remain poorly understood. Here we followed an experimental evolution approach involving serial passages of *Fol* 42-87 through tomato plants, axenic media plates, or *Galleria* larvae. Independently evolved populations obtained after ten consecutive passages through plants or rich media displayed notable phenotypic differences with respect to the initial clonal isolate, with four of the five plate-passaged populations showing significantly reduced virulence on tomato plants. Resequencing of the evolved populations revealed segmental duplications and deletions on transposon-rich accessory regions of the genome, including loss of entire chromosomes. These findings suggest that chromosome plasticity acts as a major evolutionary driver in *F. oxysporum*, and provide new insights into the genetic mechanisms underlying host adaptation in this cross-kingdom fungal pathogen.

Multilevel selection in *Neurospora tetrasperma*. *C. Meunier*, S. Hosseini, Z. Maryush, H. Johannesson Systematic Biology, Evolution Biology Centre, Uppsala, SE.

Heterokaryosis, the coexistence of genetically divergent nuclei in the same mycelium, is a common trait in fungi. In the filamentous ascomycete *Neurospora tetrasperma*, a shift in the mating system has led to predominant heterokaryosis during the life cycle: both mating-type nuclei (*mat A* and *mat a*) are packaged together in conidia and ascospores- however, monokaryotic, haploid individuals can still be found. Such a shift can be considered as an evolutionary transition to higher complexity. During this transition, a potential threat to heterokaryons is discordant selection, when selection operating at level of haploid nuclei acts counter to selection operating at the level of the heterokaryon. Furthermore, a crucial step in the transition is the export of fitness heritability from monokaryon-level to heterokaryon-level, potentially leading to division of labor among nuclear types. In our study, we investigated pros and cons of heterokaryosis in *Neurospora tetrasperma*, studying nuclear ratios and contrasting fitness traits between the heterokaryotic mycelium and its two genetically divergent, totipotent and free ranging nuclear types. We showed that the ratio of the two nuclear types varies within a mycelium and verified that the ratio is homogeneous, but that it can deviate from a diploid-like 1:1-ratio. When measuring fitness traits of the mycelium and its component nuclear genotypes, we found that nuclear types have different fitness optima during the life cycle and that the phenotype of the heterokaryotic mycelium is reflecting the nuclear ratio in an additive manner. Thus, our data indicate subfunctionalization of the nuclear types for mycelial function, a form of division of labor. Our results also hint towards variation in nuclear ratio resulting in phenotypic flexibility of the mycelium. However, the occurrence of seemingly selfish nuclei, enjoying better replication and transmission than sister nuclei, yet being detrimental to the heterokaryotic organism, points towards ongoing discordant selection.

From genes to molecules: Assigning natural products to gene clusters by removing gene silencing. *Michael Freitag*[1], Donovon Adpressa[2], Lanelle Connolly[1], Kristina Smith[1], Sandra Loesgen[2] 1) Dept. of Biochemistry and Biophysics; 2) Dept. of Chemistry, Oregon State University, Corvallis, OR, USA.

Fungi are widely known as producers of a large variety of natural products, many of which have been developed into pharmaceuticals. Pathogenic fungi on plants, animals and humans use their arsenal of secondary metabolites to aid in infection, establishment and maintenance of disease. Aided by high-quality draft genome sequences, much progress has been made over the past decade to sort specific metabolites to corresponding gene clusters, but in many cases annotated clusters remain transcriptionally silent even when strains are grown on a wide variety of media. In previous work, we found that several *Fusarium* species maintain a repressive chromatin mark, histone H3 lysine 27 methylation (H3K27me3) on ~33% of their genome. Deleting genes responsible for H3K27 methylation resulted in overexpression of more than 25% of all genes in *F. graminearum*. This discovery gives us new opportunities to express the "cryptic genome". We have generated additional mutants that are defective in H3K27me3-dependent gene silencing and have analyzed them for secondary metabolite profiles. While some previously undetected metabolites emerge from these mutants, we found that it is not always the end product that is overproduced. In several cases intermediates of well-studied pathways accumulate or new shunts result in accumulation of compounds previously not known from *F. graminearum*. Here, we outline strategies to streamline secondary metabolite and “genes-to-molecules” approaches.

Using big data to compare evolution of fruiting body development across the Sordariomycetes. *Frances Trail*[1], Wonyong Kim[1], Zheng Wang[2], Jeffrey Townsend[2] 1) Plant Biology, Michigan State University, East Lansing; 2) Department of Biostatistics, School of Public Health, Yale University, New Haven.

Diversity in fungi is most plainly manifested in fruiting body morphology. The Sordariomycetes produce predominantly perithecia, which are designed in a niche specific way for different mechanisms of spore dispersal. Previously, we examined shifts in gene expression that underlie the evolutionary diversity of fruiting body development in two genera: *Neurospora* and *Fusarium,* and identified genes that were critical to morphological differences between the genera. Three additional genera (*Magnaporthe, Chaetomium,* and *Neocosmospora*) are being used for a systems level analysis to reveal the genetic basis of a greater scope of morphological features for fungal fruiting bodies. In addition, we are constructing transcription factor networks and comparing the roles of transcription factors in the evolution of the fruiting body development. Through these approaches, we are dissecting the process of fruiting body development and function, identifying the common and distinct features of this process across a broad range of fungi, ultimately defining the underlying genetic networks that result in the successful adaptation of fruiting body structures and functions in nature.

Thursday, March 16 3:00 PM – 6:00 PM
Fred Farr Forum
Secondary Metabolism

Genomics and evolution of secondary metabolism in *Fusarium*. *R.H. Proctor*, H.-S. Kim, D.W. Brown Mycotoxin Prevention and Applied Microbiology, USDA ARS NCAUR, Peoria, IL.

Fusarium is a species-rich genus that causes disease on virtually all plant crops and produces diverse secondary metabolites (SMs), including pigments, plant hormones, and some of the mycotoxins of greatest concern to food and feed safety. To better understand the potential SM diversity in *Fusarium* as well as the distribution and evolution of SM biosynthetic genes, we have assessed the presence and absence of known and novel SM biosynthetic genes and gene clusters in genome sequences of >200 isolates representative of 25 lineages (species complexes) of *Fusarium*. antiSMASH analysis indicates that 1.1 – 6.4% of the genes in *Fusarium* genomes are involved in secondary metabolism, and that collectively *Fusarium* species have the genetic potential to produce hundreds of structurally distinct families of SMs, including analogs of multiple SMs described in other fungi. *Fusarium* SM clusters exhibit marked variation in distribution within and among species complexes: some are present in almost all members of all species complexes; others exhibit largely continuous distributions within one or several complexes; and others exhibit discontinuous distributions within one or more complexes. Phylogenetic analyses indicate that vertical inheritance, horizontal transfer, and gene loss have been major contributors to the current distribution of SM clusters among fusaria, but that recent duplication events have contributed little to the current distribution of clusters. Further, if the ancestral *Fusarium* had a similar number (26 – 68) of SM gene clusters as extant species, hundreds of horizontal transfer and loss events would be necessary to account for the current diversity and distribution of SM gene clusters in *Fusarium*.

Evolution of secondary metabolism and host association in insect pathogens. *Kathryn E. Bushley*[1], Stephen A. Rehner[2], Joseph W. Spatafora[3] 1) Plant and Microbial Biology, University of Minnesota, Saint Paul, MN; 2) Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, MD; 3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Fungal secondary metabolite genes including nonribosomal peptide synthetases, polyketide synthases, terpenes, and alkaloids are among the most rapidly evolving of fungal genes. They respond to selective pressures in the environment, often enabling fungi to adapt to specific environments or parasitize specific hosts. We investigate population genomic variation of secondary metabolite genes and clusters in two insect pathogenic fungi, the beetle pathogen *Tolypocladium inflatum* and the wide host-range insect pathogen and biocontrol agent *Beauveria bassiana*. Using Pac Bio single molecule real time sequencing, *we* improved the resolution of the sequenced reference strain *T. inflatum* NRRL 8044 and have assembled de novo genomes of additional strains isolated from distinct environments. For the reference strain and one additional strain, we have also used a Hi-C chromosome mapping approach to provide evidence for chromosomal level assemblies. The nearly complete chromosomal assemblies produced using these methods have allowed investigation of fine-scale evolutionary genetic mechanisms contributing to the rapid evolution of secondary metabolite genes and clusters and to evaluate the relative contributions of transposition, duplication/deletion, cluster rearrangement, and horizontal transfer in cluster evolution. Similarly, using Illumina technology, we have sequenced ten strains of *B. bassiana* and several outgroup *Beauveria* species *(B. brongniartii, B. asiatica, B. australis)* to analyze the evolution of secondary metabolite clusters and other genes involved in host-specific interactions (e.g. G-protein coupled receptors, SSPs). In addition to being an insect pathogen, *B. bassiana* has recently been shown to colonize a wide-range of plant species as an endophyte. Differential expression in plant and insect infection assays examines the role(s) of specific metabolites and other genes in recognition, colonization, and pathogenesis of plant versus insect hosts. The results of our analysis shed light on the role of metabolites in shaping the interaction of these fungi with distinct hosts and in turn on the effects of host-specialization versus generalism in driving the evolution of metabolite clusters.

Tracing evolution of the *Cochliobolus heterostrophus* race T genes for T-toxin biosynthesis using phylogenetic analyses. *J.B. Gonzalez*, B.J. Condon, B.G. Turgeon Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY.

In 1970, a new race (Race T) of the Dothideomycete *Cochliobolus heterostrophus* caused the Southern Corn Leaf Blight epidemic, the most devastating epidemic in US history. Decades of research has revealed that Race T harbors ten novel genes on four scaffolds and two different chromosomes for biosynthesis of T-toxin, a host selective toxin (HST) with exquisite specificity for Texas male sterile corn (Tcms), widely planted at that time. The evolutionary origin of the *Tox1* genes enabling extreme virulence to Tcms remain a mystery, as they are not found in other Cochliobolus species, including race O, a non-aggressive version of the pathogen. Previously, a subset of the genes was discovered in *Didymella zeae-maydis*, which produces PM-toxin, an HST with the same biological specificity. More recently, the genes were found in the Dothideomycetes, *Ampelomyces quisqualis*, *Ascochyta rabiei*, *Clohesyomyces aquaticus, Corynespora cassiicola*, *Leptosphaeria maculans*, *Lindgomyces ingoldianus,* and two Eurotiomycetes, *Penicillium raistrickii*, and *Penicillium brevicompactum,* none of which are known to produce T-toxin. Notably, recently discovered gene cohorts are co-linear, an arrangement that likely reflects the ancestral organization. The identification of the cohort in Eurotiomycetes allowed us to ask if the evolutionary path to a functional T-toxin metabolite was vertical transmission of the genes from an ancestor of the two lineages, or horizontal transmission (HGT) between the two. We evaluated this question by examining phylogenetic congruence between a species tree constructed using 19 conserved proteins, individual *Tox1* protein trees, and a tree constructed using a concatenated alignment of all *Tox1* proteins. Comparison of topologies revealed incongruency between the species tree and the individual protein and full-cohort trees, with the Eurotiomycetes separate from the Dothideomycetes in the former, but clustered within the Dothideomycetes in both of the latter. These findings, plus low conservation of the Tox1 proteins among species, and that the gene cohort is thus far found predominantly in Dothideomycetes, are suggestive of an HGT event, likely ancient, from a Dothideomycete to the Eurotiomycetes. Our analyses also indicate other likely HGT events between taxa possessing *Tox1*-like genes. Acquisition of the *Tox1* genes in *C. heterostrophus* by vertical inheritance, recombination, and gene loss in intervening species versus HGT remain equally plausible.

The cereal pathogen *Fusarium pseudograminearum* produces a mimic of cytokinin plant hormones. *D.M. Gardiner*[1], J.L. Sørensen[2], A.H. Benfield[1], R.D. Wollenberg[2], K Westphal[2], R Wimmer[2], K.F. Nielsen[3], J. Carere[1], L. Covarelli[4], G. Beccari[4], J. Powell[1], T. Yamashino[5], H. Kogler[6], T.E. Sondergaard[1] 1) Agriculture and Food, CSIRO, St Lucia, Queensland, AU; 2) Department of Chemistry and Bioscience, Aalborg University, DK-9000 Aalborg, Denmark; 3) Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; 4) Department of Agricultural, Food and Environmental Sciences, University of Perugia, IT-06121 Perugia, Italy; 5) Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Furocho, Chikusa-ku, Nagoya, 464-8601 Japan; 6) Karlsruhe Institute of Technology, D-76131 Karlsruhe, Germany.

The necrotrophic pathogen *Fusarium pseudograminearum* infects a broad range of agronomically important crops including barley and wheat. During a survey of secondary metabolites produced by this fungus, a novel class of cytokinins, which are plant hormones, was identified as being produced by the pathogen during plant infection. Cytokinins are generally thought of as having growth promoting and anti-senescence activity and the production of a cytokinin mimic by a necrotrophic pathogen challenges the view that these pathogens invade by a simple barrage of lytic enzymes and toxins. Through genome mining, a gene cluster in the *F. pseudograminearum* genome for the production of these compounds was identified and the biosynthetic pathway established using gene knockouts. The *F. pseudograminearum* cytokinins can activate cytokinin signalling demonstrating their genuine hormone mimicry. *In planta* analysis of the transcriptional response to one of the *F. pseudograminearum* cytokinins suggests extensive reprogramming of the host environment by these molecules, possibly through cross talk with defence signalling pathways.

Conserved *veA*-dependent genetic elements, *rtfA* and *mtfA*, regulate secondary metabolism, morphogenesis and virulence in *Aspergillus* spp. *A.M. Calvo* Dept Biological Sci, Northern Illinois Univ, Dekalb, IL.

In the model fungus *Aspergillus nidulans* the global regulatory gene *veA* is necessary for the biosynthesis of several secondary metabolites, including the mycotoxin sterigmatocystin (ST). In order to identify additional *veA*-dependent genetic elements involved in regulating ST production, we performed a mutagenesis on a deletion *veA* strain to obtain revertant mutants (RM) that regained the capability to produce toxin. Genetic analysis and molecular characterization of two of the revertant mutants, RM3 and RM7, revealed that point mutations occurred at the coding region of the *rtfA* and *mtfA* genes respectively. *rtfA* encodes a RNA-pol II transcription elongation factor-like protein, similar to *Saccharomyces cerevisiae* Rtf1, while *mtfA* encodes a novel putative C2H2 zinc finger domain transcription factor. Both genes are conserved in *Aspergillus* spp and in other fungal genera. Further research revealed that in a *veA* wild-type background, *rtfA* controls the production of several secondary metabolites, or natural products, in *A. nidulans*, as well as in the agriculturally and medically important *A. flavus* and *A. fumigatus. mtfA* also governs the biosynthesis of natural products in *Aspergillus* spp by regulating the expression of secondary metabolite gene clusters, as demonstrated in *A. nidulans* and *A. fumigatus* functional genomics studies. Additionally, both regulators strongly influence other biological processes, including morphological development and virulence.

Mushroom polyketide synthase produces polyenes for chemical defense. *P. Brandt*[1], M. García-Altares[2], M. Nett[3], C. Hertweck[2], D. Hoffmeister[1] 1) Friedrich-Schiller-Universität, Department Pharmaceutical Microbiology at the Hans-Knöll-Institute, Winzerlaer Straße 2, 07745 Jena (Germany); 2) Leibniz Institute for Natural Product Research and Infection Biology, Department Biomolecular Chemistry, Beutenbergstraße 11a, 07745 Jena (Germany); 3) Technische Universität Dortmund, Department Biochemical and Chemical Engineering, Technical Biology, Emil-Figge-Straße 66, 44227 Dortmund (Germany).

Basidiomycetes have evolved a diverse repertoire of bioactive chemical defense compounds. After wounding of its mycelium, the taxonomically undetermined white-rotting basidiomycete BY1 produces yellow pigments *de novo*, which massively inhibit the pupation of insect larvae. These natural products were identified as the polyunsaturated fatty-acid like polyenes, 18-methyl-19-oxoicosaoctaenoic acid and 20-methyl-21-oxodocosanonaenoic acid. [1]

The objective of this study was to understand the genetic and biochemical basis of this basidiomycete defense compounds. We succeeded in identifying both alleles of a candidate gene, *PPS1*, in the genome of BY1, coding for a six-domain reducing polyketide synthase (HR-PKS). Quantitative real-time PCR showed a 9.5-fold upregulation of *PPS1* expression 48 hours past injury of the BY1 mycelium. To verify that PPS1 has polyene synthase activity, the polyene biosynthesis was heterologously reconstituted in *Aspergillus niger*. To that end, *PPS1* was placed under the control of the *terA* promoter and *trpC* terminator using plasmid SM-Xpress. [2] Combining liquid chromatography, mass spectrometry, and NMR, the structures of the PPS1 products were elucidated, and proved identical to the polyenes initially isolated from BY1. MALDI-MS imaging indicated polyene accumulation in the wounded mycelial area. Our work represents the first characterized basidiomycete HR-PKS and sets the stage for a more profound understanding of basidiomycete chemical ecology.

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[2] M. Gressler, P. Hortschansky, E. Geib, M. Brock, *Front Microbiol* **2015**, *6*, 184.

Copper-responsive isocyanide biosynthetic cluster in *Aspergillus fumigatus*. *F. Lim*[1], J. Baccile[2], T. Won[2], P. Wiemann[1], A. Lind[3], A. Rokas[3], F. Schroeder[2], N. Keller[1] 1) University of Wisconsin-Madison, Madison, WI, U.S.A; 2) Cornell University, Ithaca, NY, U.S.A; 3) Vanderbilt University, Nashville, TN, U.S.A.

Isocyanide-containing natural products are of immense interest in the biological and chemical milieu owing to its broad range of bioactivity, which is attributed by the highly reactive isocyano- functional groups tethered to structurally diverse carbon scaffolds. In contrast to the fast-growing list of naturally-occurring isocyanides, knowledge on the biosynthetic machineries that give rise to such unique chemistry is still in its infancy with only four characterized biosynthetic gene clusters in bacteria, and unprecedented in eukaryotes. A hallmark function for these isocyanide natural products is their capability for metal coordination, which is shown to impart the bioactivites for many of these naturally-occurring isocyanides and from an ecological perspective, crucial to the pathogenesis of the entomopathogenic bacterium, *Xenorhabdus nematophila* (by disabling the innate immune defense via inhibition of the cuproenzyme, phenoloxidase). Here we report on the identification of four isonitrile synthases (INS) in the genome of the human opportunistic pathogen, *Aspergillus fumigatus* and the discovery of a novel copper-responsive INS-NRPS-like hybrid enzyme (CrmA) involved in the synthesis of xanthocillin analogues, the first reported biosynthetic pathway dedicated to this family of naturally-occurring isocyanides. This work also elucidates the regulatory circuitry that bridges cellular metal homeostasis and fungal development.

A flexible expression platform for *Aspergillus* based on CRISPR mediated homologous recombination. *F.H. Kirchner*, Z.D. Jarczynska, C.D. Nødvig, U.M. Mortensen Danish Technical University, Copenhagen, DK.

Fungi produce a wide variety of secondary metabolites and enzymes. For many of these secondary metabolites, the clusters that are responsible for their production are unknown. One way to elucidate the pathway is to make gene disruptions in the producer stain, but this requires that the species can be grown in the laboratory, that it is transformable and that a genetic tool box is available. Alternatively the cluster can be transferred to a well characterized host where the genes can be heterologously expressed. To facilitate the latter method, we created a flexible expression platform with integration sites in different well characterized Aspergillus species. The integration sites contain a common integration target site that contains the colorimetric marker *lacZ* flanked by long overhangs for homologous recombination. Hence, hosts strains are blue, but if new genes are inserted correctly into the expression sites, the strains will turn white as the *lacZ* gene is eliminated as a result of the integration process. Integrations can be performed in wild-type strains using marker- free gene-targeting by using CRISPR stimulated homologous recombination. Importantly, by using our system, the same gene-targeting construct can be inserted into several different hosts; and the strain with the best product performance can then be selected for further analysis.

Thursday, March 16 3:00 PM – 6:00 PM
Kiln
RNA Biology

RNAi-dependent epimutations evoke transient antifungal drug resistance. *J. Heitman*[1], Z. Chang[1], S. Lee[1], M. Cardenas[1], S. Calo[1], F. Nicolas[2], A. Vila[2], M. Cervantes[2], S. Torres-Martinez[2], R. Ruiz-Vazquez[2] 1) Department of Molecular Genetics and Microbiology, Duke University, Durham; 2) Department of Genetics and Microbiology, University of Murcia, Spain.

Microorganisms evolve via sexual reproduction, mutators, aneuploidy, Hsp90, and prions. We discovered a new mechanism conferring antifungal drug resistance in the human fungal pathogen *Mucor.* In this species, spontaneous resistance to the antifungal drug FK506 evolves via two mechanisms. One involves Mendelian mutations conferring stable irreversible drug resistance; the other occurs via an epigenetic RNA interference (RNAi) pathway resulting in unstable, transient drug resistance. The peptidyl-prolyl isomerase FKBP12 interacts with FK506 forming a complex that inhibits the protein phosphatase calcineurin. Calcineurin inhibition by FK506 blocks the *Mucor* dimorphic transition to hyphae and enforces growth as yeast. In some FK506-resistant isolates, mutations in the *fkbA* gene encoding FKBP12 or the calcineurin *cnbR* or *cnaA* genes confer FK506 resistance restoring hyphal growth. In other isolates, no mutations occurred in these drug targets. Instead, RNAi spontaneously silenced the *fkbA* gene yielding drug-resistant epimutants that revert to drug-sensitivity in the absence of FK506. Establishment of these epimutants involves generation of abundant *fkbA* small RNAs and requires several known RNAi pathway components whereas other RNAi elements are dispensable. Surprisingly, epimutants occur at a higher frequency and are more stable in mutants lacking RNA-dependent RNA polymerase 1, revealing some RNAi components constrain epimutation. Silencing of the drug target FKBP12 appears to involve generation of a double-stranded RNA intermediate using the *fkbA* mature mRNA as template to produce antisense *fkbA* RNA. Our recent studies reveal additional components required for epimutation, including orthologs of the *Neurospora crassa* quelling inducing protein (QIP) and Sad-3 helicase (RnhA); interestingly *rnhA* is linked to the *Mucor sex* locus, suggesting possible parallels with sex induced silencing in *Cryptococcus*. We also found epimutants occur at a higher frequency and are more stable in two additional RNAi mutants lacking RNA-dependent RNA polymerase 3 or the R3B2 factor. These findings were generalized by isolating epimutations in a second *Mucor* species and identifying epimutations in the *pyrF* or *pyrG* genes conferring 5-FOA resistance. These studies reveal a novel, reversible, transient epigenetic RNAi epimutation mechanism controlling phenotypic plasticity, with possible implications for antimicrobial drug resistance and RNAi pathways in fungi and other eukaryotes.

A non-canonical RNA silencing pathway in *Mucor circinelloides* and its involvement in pathogenesis. *V. Garre*[1], F. E. Nicolás[1], J. T. Cánovas-Márquez[1], C. Pérez-Arques[1], M. I. Navarro-Mendoza[1], T. A. Trieu[1,2], J. A. Pérez-Ruiz[1], E. Navarro[1], S. Torres-Martínez[1], R. M. Ruiz-Vázquez[1] 1) Department of Genetics and Microbiology, University of Murcia, Murcia, ES; 2) Department of Genetics, Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam.

The mechanism of RNA silencing or RNA interference (RNAi) plays an important role in the maintenance of genome integrity, particularly protection against exogenous nucleic acids such as mobile elements, but also a role in the regulation of gene expression by means of regulatory endogenous small RNAs (esRNAs). *Mucor circinelloides*, an opportunistic human pathogen that is a causal agent for the rare but lethal infection mucormycosis, represents one of the most outstanding examples about the role of RNAi in gene regulation. This fungus exhibits at least three RNAi-related pathways dedicated to regulate several physiological and development processes. Besides *dicer*-dependent RNAi pathways involved in regulation, *Mucor* presents a non-canonical *dicer*-independent RNAi pathway that controls the expression of target genes by promoting the degradation of their mRNAs. This non-canonical pathway in which participate two RNA-dependent RNA polymerases (RdRP) and a putative endonuclease, named R3B2, regulates specific cellular processes, such as sexual interaction and the response to environmental stresses. However, our recent studies implicate this regulatory pathway also in virulence, since mutants for *r3b2* and *rdrp-1* show reduced virulence in a mouse model. Interestingly, R3B2, which is found exclusively in basal fungi, was suggested to play a crucial role in the biogenesis and function of regulatory esRNAs because it is also involved in the dicer-dependent RNAi pathways. *In vitro* nuclease activity assays have shown that R3B2 cleaves dsRNA producing sRNAs of a wide range of sizes, whereas recombinant human Dicer produces sRNAs of discrete size in the same analysis. Moreover, mutation of conserved residues in the RNase III catalytic domain-like of R3B2 that blocks its function in RNA silencing *in vivo* produces a loss of dsRNA cleavage activity *in vitro*. These results support an endonuclease role for R3B2 in the non-canonical RNAi pathway that controls expression of genes involved in fungal infection by Mucorales.

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Antisense transcription licenses nascent transcripts to mediate transcriptional gene silencing. Yunkun Dang[1], Jiasen Cheng[2], Xianyun Sun[3], Zhipeng Zhou[1], *Yi Liu*[1] 1) UT Southwestern Medical Center, Dallas, TX. USA; 2) Huazhong Agricultural University, Wuhan, Hubei, China; 3) Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

In eukaryotes, antisense transcription can regulate sense transcription by induction of epigenetic modifications. We previously showed that antisense transcription triggers Dicer-independent small interfering RNA (disiRNA) production and disiRNA loci DNA methylation (DLDM) in *Neurospora crassa*. Here we show that the conserved exonuclease ERI-1 is a critical component in this process. Antisense transcription and ERI-1 binding to target RNAs are necessary and sufficient to trigger DLDM. Convergent transcription causes stalling of RNA polymerase II during transcription, which permits ERI-1 to bind nascent RNAs in the nucleus and to recruit a histone methyltransferase complex that catalyzes chromatin modifications. Furthermore, we show that in the cytoplasm ERI-1 targets hundreds of transcripts from loci without antisense transcription to regulate RNA stability. Together, our results demonstrate a critical role for transcription kinetics in long non-coding RNAs-mediated epigenetic modifications and identify ERI-1 as an important regulator of co-transcriptional gene silencing and post-transcriptional RNA metabolism.

The RNAi system in the Brassicaceae smut fungus *Thecaphora thlaspeos*. *K. Boesch*[1,2], L. Frantzeskakis[1], K. Courville[1], M. Feldbrügge[1,2,3], V. Göhre[1,2,3] 1) Heinrich-Heine-University, Düsseldorf, NRW, DE; 2) Bioeconomy Science Center (BioSC), Jülich, NRW, DE; 3) Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, NRW, DE.

Smut fungi infect important crop plants, e.g. maize or potato. To develop novel plant protection strategies, the infection process has to be characterized molecularly. Genetic manipulation of the crop host plants is still difficult because of their complex genomes. For this reason, we want to characterize the interaction of the smut fungus *Thecaphora thlaspeos* with the model plant *Arabidopsis thaliana*.
T. thlaspeos infects different Brassicaceae throughout Europe, among them the perennial plant *Arabis alpina*. Its lifecycle differs in two important steps from crop smut fungi such as *Ustilago maydis*: First, teliospores only germinate in the presence of a plant-derived signal. Second, there is no dimorphism. Filaments emerge from teliospores that are directly infectious. Importantly, these filaments can be propagated as haploid cultures enabling genome sequencing and genetic manipulation.
Interestingly the genome encodes components of the RNAi machinery, which are absent in *U. maydis* but present in the smut fungus *Sporisorium reilianum*. Cross-kingdom RNAi is used by *Botrytis cinerea* to modulate *Arabidopsis thaliana*'s immune system. Based on reduced virulence of the DICER deletion mutant, it is hypothesized that sRNAs are generated in the fungus, transported into the host plant's cell and there silences expression of specific genes involved in immunity. With our new model system we will study how *T. thlaspeos* uses RNAi and ultimately investigate whether also a smut fungus utilizes cross-kingdom RNAi during infection.

Endosome-coupled mRNA transport. *M. Feldbrugge* Institute for Microbiology, Heinrich-Heine University, Düsseldorf, NRW, DE.

Active transport and local translation of mRNAs ensure the appropriate spatial organization of proteins within cells. Recent work has shown that this process is intricately connected to membrane trafficking. Here, we present new findings obtained in the model organism *Ustilago maydis*. In highly polarized cells of this fungus microtubule-dependent co-transport of mRNAs and endosomes is essential for efficient polar growth. We discuss a novel concept of endosome-coupled translation that loads shuttling endosomes with septin cargo, a process important for correct septin filamentation. Key players are RNA-binding proteins containing RNA recognition motifs for mRNA binding as well as Mademoiselle domains for protein/protein interaction. Here, new insights on protein RNA as well as protein-protein interactions will be presented. Interestingly, evidence is accumulating that RNA and membrane trafficking are also tightly interwoven in higher eukaryotes suggesting that this phenomenon is a common theme and not an exception restricted to fungi.

Exp5, a *Magnaporthe oryzae* nucleo-cytoplasmic receptor involved in non-coding RNA metabolism and plant pathogenesis. A. Illana, M. Marconi, J. Rodríguez-Romero, M. Wilkinson, *A. Sesma* Centre for Plant Biotechnology and Genomics, Technical University of Madrid, Pozuelo de Alarcon, Madrid, ES.

Following a genetic screening of a *M. oryzae* T-DNA insertional library of ca. 3,000 transformants looking for pathogenicity-deficient mutants, genes associated with RNA metabolism were selected for further characterization, including the karyopherin *EXP5*. The *M. oryzae* Exp5 protein is the orthologue of the human karyopherin exportin-5 and the *Saccharomyces cerevisiae* Msn5. Karyopherins are involved in the translocation of RNAs and/or proteins between the nucleus and the cytoplasm. To better understand the role of this karyopherin during *M. oryzae* plant infection we set out to identify its cargoes. Several t-RNA synthetases, seven subunits of the 26S proteasome and key components of signal transduction pathways such the MAPK Pmk1 and Mps1 immunoprecipitated with Exp5. An important set of mitochondrial proteins including TIM44 and TOM70 also interacted with Exp5, which suggested that mitochondrial dysfunction is possibly contributing to the strong root infection defects exhibited by Δ*exp5*. The sRNA sequencing analysis pointed out that Exp5 is potentially required for the transport of specific classes of retrotransposons, and allowed us to identify a single-stranded RNA virus present in the *M. oryzae* field isolate Guy11. Northern blots carried out with sRNAs extracted from nuclear and cytoplasmic subcellular fractions suggested deficiencies in the Δ*exp5* mutant such as accumulation of sRNAs derived from transposon elements and $tRNA^{Met}$ also supported the involvement of Exp5 in sRNA metabolism and tRNA transport. Our results suggest that Exp5 is required for the translocation of specific classes of proteins, tRNAs and sRNAs, several of which contribute to *M. oryzae* pathogenicicity.

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2. Illana A, Marconi M, Rodríguez-Romero J, Ayllón MA, Xu P, Dalmay T, Wilkinson M and Sesma A. Molecular characterization of a novel ssRNA ourmia-like virus from the rice blast fungus *Magnaporthe oryzae* (2016). Archives of Virology. DOI: 10.1007/s00705-016-3144-9

Introns regulate gene expression in *Cryptococcus neoformans*. *G. Janbon* Institut Pasteur, Paris, FR.

Cryptococcus neoformans is a basidiomycetous opportunistic pathogen leaving in the environment responsible for more than 500 000 deaths every year. Our recent work suggests that a fascinating, complex pattern of RNA molecules composes its transcriptome and this fungus is emerging for different aspects as an ideal model to study RNA metabolism in eukaryotes. It is also tempting to hypothesize that this complex RNA metabolism provides a mechanism for this yeast to respond to different environmental cues and to be an efficient pathogen. The most prominent features of this transcriptome are introns. Our recent re-annotation of the *C. neoformans* genome revealed that nearly all the expressed genes contain introns. These introns can be present within the coding sequence but also within UTR regions. We previously demonstrated that these introns are essential for gene expression. Here, we identified a large number of regulated alternative splicing events. However, alternative splicing in this yeast seems to be more a means to regulate gene expression than to diversify the proteome.

Pas3 regulates cryptococcal morphogenesis through Bre1/Npl3-Mediated alternative splicing. *Y. Zhao*, X. Lin Biology, Texas A&M University, College Station, TX.

Per-ARNT-Sim (PAS) proteins function as signal receptors, transducers, or regulators. In the human fungal pathogen *Cryptococcus neoformans*, two PAS proteins, Bwc1 and Bwc2 (white collar 1 and 2), are known to regulate mating, virulence, and tolerance of ultraviolet radiation. To comprehensively understand the regulatory role of PAS proteins in regulating morphogenesis and pathogenicity, all 9 PAS domain coding genes were analyzed in *C. neoformans*. One of the PAS domain proteins, Pas3, was found to be critical in cryptococcal filamentation and virulence. Deletion of *PAS3* in either the serotype D reference strain XL280 or the serotype A reference strain H99

severely impaired filamentation during either unisexual (a alone) or bisexual (**a**-a) reproduction. Interestingly, Pas3 was localized to the nucleus despite absence of NLS, implicating regulatory roles in addition to transducing signals. Through co-immunoprecipitation (Co-IP) coupled with mass spectrometry, we identified Bre1 and Npl3 as two potential interacting protein-partners of Pas3. Bre1, as an E3 ligase, mono-ubiquitinates histone H2B subunit, and may regulate cryptococcal morphogenesis through changing the chromatin structure at filamentation-associated genetic loci, resulting in altered gene transcription. In addition to chromatin modification effects, Bre1and Npl3 may function together in regulating alternative splicing of selective pre-mRNAs. Deletion of *BRE1* and *NPL3* individually partially recapitulated the filamentation defect of *pas3*D, indicating that Bre1 and Npl3 may function both in parallel and overlapped manners downstream of Pas3 in regulating morphogenesis. Fluorescently labeled Bre1 gave fluorescent signals all over the nucleus and also one bright non-nucleolus dot, indicating its specific role in regulating alternative splicing in addition to ubiquitination of H2B. Our forward genetic screening for non-filament mutants identified the splicing factor Cus1 as an important regulator of filamentation. This corroborates the hypothesis that alternative splicing regulates cryptococcal morphogenesis. Currently we are analyzing the alternative splicing profile in *cus1*D relative to wild type via RNA-seq. Identification of the genetic connection among Pas3, Bre1 and Npl3, and dissection of mode of action by alternative splicing in regulating fungal morphogenesis in *C. neoformans* will expand the knowledge about signal transduction and post-transcriptional regulatory network in fungal development.

Thursday, March 16 3:00 PM – 6:00 PM
Heather
Sustainable Bio-product Fermentation: From Biomass Conversion to High Value Product Formation

Substrate engineering as a viable option to complement fungal bio-engineering efforts. *J. Philipp Benz*, Manfred J. Reppke HFM, Technical University of Munich, Freising, DE.

Background
Cellulases and hemicellulases have become important industrially used enzymes, such as for feed stock processing, textile applications and the production of biofuels. The applications for these enzymes are mostly limited by the economical aspects of their production. Differently produced celluloses are commonly used as part of the fermentation media for hydrolase production by microorganisms. A wealth of potential celluloses is available, but the relevant physiochemical characteristics determining their effectiveness as inducer or substrate so far remain largely unclear. Moreover, the regulatory networks of the fungal production host strongly affect the response, but have not been fully elucidated. These factors are collectively making it difficult to rationally decide for the correct product to use.
Results
We have been analyzing different celluloses for their suitability as cellulase-inducing substrates using two filamentous ascomycetes: the genetic model fungus *Neurospora crassa* and the *Trichoderma reesei* hypercellulolytic strain Rut-C30. Performance was evaluated by quantifying gene induction, protein secretion and enzymatic activities. Results showed clear differences between the cellulosic substrates. Chemical and physical analyses identified crystallinity as a major factor affecting performance while particle morphology did not seem to have as strong of an impact as expected. Moreover, due to cross-signaling between cellulose and hemicellulose perception pathways, even small amounts of impurities were found to strongly affect the fungal response.
Conclusions
Our data demonstrate that highly pure celluloses do not necessarily perform well as substrates for the production of cellulases in filamentous fungi and indicate that certain hemicellulose impurities as well as a lower crystallinity are advantageous for their effectiveness. However, what constitutes a beneficial vs. a detrimental impurity strongly depends on the genetic background of the production host. Moreover, the fine structure of the substrate will define how bioavailable each polysaccharide is, and therefore how well it can be perceived. In collaboration with industrial fiber mills, we are now embarking on “substrate engineering” as a viable addition to fungal bio-engineering efforts. The presented results highlight key factors that need to be considered when choosing the optimal cellulosic product for a biotechnological cellulase or hemicellulase production process.

The molecular response of the white-rot fungus *Dichomitus squalens* to wood and non-woody biomass. *M.R. Mäkelä*[1], J. Rytioja[1], K. Hilden[1], M. Di Falco[2], M. Zhou[3], M.V. Aguilar-Pontes[3], O.-M. Sietiö[1], A. Tsang[2], R.P. de Vries[3] 1) Department of Food and Environmental Sciences, University of Helsinki, Helsinki, FI; 2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Utrecht, The Netherlands; 3) Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada.

Analyses of ascomycetes have shown that the genomes of the species that occupy highly defined habitats (e.g. the dung fungus *Podospora anserina* and the plant pathogen *Botrytis cinerea*) are well tailored to obtain carbon from the prevailing plant biomass substrate. In contrast, ascomycetes that thrive in a large variety of habitats (e.g. *Aspergillus niger*) possess a diverse enzymatic machinery for the degradation of a wide range of biomass.
Wood-rotting basidiomycetes are exclusively found on wood in nature, where they play a significant role in degradation of all polymeric components of wood cell walls. The white-rot basidiomycete *Dichomitus squalens* is commonly found in the northern regions of Europe, Asia and North America. It is an efficient wood degrader with a genome that possesses an extensive gene set predicted to encode diverse plant cell wall modifying enzymes.
In this study, we examined the transcriptome and exoproteome of *D. squalens* during growth on two wood substrates, aspen and spruce, and two non-woody biomasses, wheat bran and cotton seed hulls. In addition to the higher lignin composition in the wood substrates, these biomasses differ substantially in the structure of their hemicelluloses. The results showed that *D. squalens* upregulates a set of genes, and secretes their encoding enzymes, that are targeted to the degradation of plant cell walls of both wood and non-woody substrates. Moreover, the genes/enzymes that are expressed/produced display functional specificity towards the polymeric components of the substrates. These results indicate that *D. squalens* has preserved its ability to respond adequately to a variety of plant biomass, even to those which it would not be expected to encounter in nature. They also suggest that factors other than the ability to obtain carbon are responsible for its limitation to wood as a habitat.

Rewiring transcriptional control of plant cell wall degrading enzyme networks in *Aspergillus niger*. Jing Niu[1], Ebru Alazi[1], Ian Reed[2], Mark Arentshorst[1], Peter Punt[3], Jaap Visser[1], Adrian Tsang[2], *Arthur Ram*[1] 1) Institute of Biology Leiden, Molecular Microbiology and Biotechnology, Leiden, The Netherlands; 2) Centre for Structural and Functional Genomics, Concordia University, Montreal, QC, Canada ; 3) Dutch DNA Biotech, Zeist, The Netherlands.

Aspergillus niger is an important industrial fungus expressing a broad spectrum of plant cell wall degrading enzymes. The expression of genes encoding these enzymes and the metabolic pathways required for carbon utilization are tightly controlled by pathway specific transcription factors. Well known examples of such transcription factors include AmyR and XlnR, which control the expression of amylolytic and xylanolytic enzymes networks respectively. We recently identified a galacturonic acid-specific transcription factor named GaaR and showed that it acts as an activator for the expression of genes required for release and utilization of galacturonic acid from pectin (1). We performed a forward genetic screen for the isolation of mutants that constitutively express GaaR-controlled polygalacturonases. Phenotypic, genomic and transcriptomic analyses of the constitutive mutants resulted in the identification of a repressor protein, GaaX, which specifically controls expression of the genes encoding enzymes related to the galacturonate/pectin degradation pathway. The *gaaX* gene is located next to *gaaR* in the genome. Phylogenomic analysis and published reports reveal similar activator-repressor modules among fungi of the subphylum Pezizomycotina, and that they regulate gene expression involved in the utilization of quinate, galacturonate, and possibly

other carbon substrates.

(1) Alazi E, Niu J, Kowalczyk JE, Peng M, Aguilar Pontes MV, van Kan JA, Visser J, de Vries RP, Ram AF. The transcriptional activator GaaR of *Aspergillus niger* is required for release and utilization of D-galacturonic acid from pectin. FEBS Lett. 2016; 590(12):1804-15.

Sustainable and cost-effective enzyme production by deploying advanced engineering technologies in an integrated approach. *Noel van Peij*, Rene Verwaal, Herman Pel, Roel Bovenberg, Hans Roubos DSM Food Specialties, DSM Biotechnology Center, Delft, NL.

Enzymes are widely used in food and bio-based industries for various applications. The industrial production of enzymes by DSM is using fungal microorganisms such as *Aspergillus niger*. The industrial hosts have a long history of safe use and are subject to continuous improvement.

The field of molecular and engineering biology is developing rapidly. More tools become available to design strains in a rationalized and integrated way, build them with high precision in a faster way and increase the throughput of strain testing. The iterative design-build-test-learn cycle concept has significantly accelerated strain and process development and optimization in industry. A few selected examples, how recently developed tools can be used in improved, rationalized and faster strain construction for the development of high-value enzyme products, will be discussed in this presentation.

Genetic parts screening and artificial N-glycosylation motif engineering for heterologous protein production in *Aspergillus niger*. *Jinxiang Zhang*[1,3], Saori Amaike-Campen[1,2], Sam Deutsch[4], Ljiljana Pasa-Tolic[5], Erika Zink[5], Jon Jacobs[5], Blake Simmons[1,6], John Gladden[1,3], Jon Magnuson[1,2] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Pacific Northwest National Laboratory, Richland, WA; 3) Sandia National Laboratories, Livermore, CA; 4) Joint Genome Institute, Walnut Creek, CA; 5) Environmental Molecular Sciences Laboratory, PNNL, Richland, WA; 6) Lawrence Berkeley National Laboratory, Berkeley, CA.

Aspergillus niger is a genetically tractable model organism for scientific discovery and a platform organism used in industry for the production of enzymes. Expression of secreted native enzymes at tens of grams per liter have been discussed by those in industry, but high level production of heterologous enzymes remains elusive. Strategies to increase production include the use of strong promoters, protease-deficient strains, fusion proteins, multiple gene copies, etc. However, yields of heterologous proteins are still lower than desired.

We generated proteomics data from secretome samples of A. niger grown on a variety of minimal and rich media, with the goal of identifying useful genetic elements for increasing heterologous protein production. Twenty promoters, six signal sequences and four introns from the most highly secreted proteins were identified as candidate genetic elements to enhance heterologous gene expression. These candidate elements were tested for their ability to drive expression of a prokaryotic glycoside hydrolase. A vector was designed to target integration of the modified expression cassette to the native glucoamylase (glaA) gene locus by homologous recombination. Considerable diversity was seen in heterologous protein production driven by these various elements. Interestingly, a signal peptide from GPI-anchored cell wall protein showed promising results.

In addition, based on the 3D structure of heterologous protein, we generated seven individual artificial N-glycosylation motifs on the surface of the heterologous protein. Analysis of these sites for glycosylation via top-down proteomics is just beginning. Correlation of glycosylation with any changes in kinetic and thermodynamic properties of the altered enzymes is the goal of this aspect of the research.

Protease regulatory factors of *Trichoderma reesei* can be controlled to improve therapeutic protein production. Christopher Landowski[1], Ann Westerholm-Parvinen[1], Bernhard Helk[2], Juhani Saarinen[3], *Markku Saloheimo*[1] 1) VTT Technical Research Centre of Finland, Espoo, FI; 2) Novartis Pharma Ag., Basel, Switzerland; 3) Glykos FInland Oy, Helsinki, Finland.

Protease secretion limits the production of many sensitive therapeutic proteins such as hormones and cytokines that are by nature easy to degrade. There are over 40 potential proteases secreted by *Trichoderma reesei*. We looked for transcriptional regulators of these proteases with the aim to control and reduce the expression of a wide range of proteases. Protease induction studies were set up to trigger protease activity with peptide and protein substrates in liquid cultures of *T. reesei*. Genome-wide expression data was generated and clustered to find out what genes are co-regulated after different treatments. Twelve candidate transcription factors or regulatory proteins were identified. To narrow the selection, the regulator genes were located on the scaffold to see if they were physically near any protease genes. Transiently silencing *ptf1*, *prp1*, and *ptf3* with siRNA downregulated the expression of a selection of protease genes in accordance with the co-regulation observed. Treatment with both *ptf1* and *prp1* siRNAs increased the effectiveness of the knockdown and reduced protease activity. The deletion of single, double, and triple combinations of the regulators successfully reduced protease activity and increased interferon alpha 2b production. For example, the triple deletion Δ*ptf1*Δ*prp1*Δ*ptf8* lead to a 3.7-fold improvement in interferon alpha 2b production. This is the first time protease regulatory proteins have been identified in *T. reesei,* and we have demonstrated how silencing or deleting them could broadly reduce protease activity.

***Rhodosporidium toruloides*: A new platform organism for production of biofuels and bioproducts derived from depolymerized polysaccharides and lignin.** *Junko Yaegashi*[1,2], James Kirby[1,3], Masakazu Ito[4], Jian Sun[1,5], Tanmoy Dutta[1,5], Mona Mirsiaghi[6], Eric R. Sundstrom[6], Edward Baidoo[1,6], Deepti Tanjore[6], Todd Pray[6], Kenneth Sale[1,5], Seema Singh[1,5], Jay D. Keasling[1,3,6,7,8], Blake A. Simmons[1,6], Steven W. Singer[1,6], Jon K. Magnuson[1,2], Adam P. Arkin[4,6,8], Jeffrey M. Skerker[4,6], John M. Gladden[1,5] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Pacific Northwest National Laboratory, Richland, WA ; 3) California Institute of Quantitative Biosciences (QB3), University of California, Berkeley, CA ; 4) Energy Bioscience Institute, Berkeley, CA ; 5) Sandia National Laboratory, Livermore, CA ; 6) Lawrence Berkeley National Laboratory, Berkeley, CA ; 7) Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA; 8) Department of Bioengineering, University of California, Berkeley, CA.

Maximizing utilization of the different carbon sources present in raw lignocellulosic material into the desired final product is a key aspect to increasing the effectiveness of producing bioproducts from plant biomass. While conventional microbial organisms such as *E. coli* and *S. cerevisiae* are the most commonly used host microbes for bioproduct synthesis, they do not readily uptake and utilize multiple carbon sources simultaneously, especially in the presence of glucose. Other organisms that are innately able to use carbon sources other than glucose tend to lack efficient genetic tools for host engineering.

In this study we explored the utility of *Rhodosporidium toruloides* as a new platform organism for production of terpenes from deconstructed

plant biomass. Remarkably, we found that it is able to simultaneously utilize all three of the major carbon sources that are found in lignocellulosic hydrolysates: glucose, xylose, and *p*- coumaric acid. Furthermore, it is fast-growing, single-celled, and genetically tractable, making it a promising host for pathway engineering. We demonstrate the production of two heterologous terpenes, bisabolene and amorphadiene in *R. toruloides* achieved by random chromosomal integration of their respective terpene synthase expression cassettes. We show its ability to utilize each of the three carbon sources mentioned above individually as well as simultaneously for growth and production of bisabolene. Finally, we demonstrate its ability to be applied to a one-pot pretreatment, saccharification, and fermentation system of lignocellulosic feedstocks. This organism was able to tolerate the ionic liquid that was used for pretreatment and was also able to utilize all of the three major carbon sources in the hydrolysate to support growth and bisabolene production.

Cycloaspeptides: elucidation of a novel fungal cyclic peptide biosynthetic pathway. *Kate M. J. de Mattos-Shipley*[1], Claudio Greco[1], David Heard[1], Nicholas P. Mulholland[2], Jason L. Vincent[2], Thomas J. Simpson[1], Christine L. Willis[1], Russell J. Cox[1,3,4], Andrew M. Bailey[5] 1) School of Chemistry, University of Bristol, UK; 2) Syngenta, Bracknell, Berkshire, UK; 3) Institute für Organsche Chemie, Leibniz Universität Hannover, Hannover, Germany; 4) BMWZ, Leibniz Universität Hannover, Hannover, Germany; 5) School of Biological Sciences, Life Sciences Building, University of Bristol, Bristol, UK.

The cycloaspeptides are cyclic pentapeptides produced by a range of filamentous fungi that are reported to exhibit various bioactivities, including anti-malarial, anti-tumour and insecticidal. In this work, two psychrotolerant *Penicillium* species, *P. soppii* and *P. jamesonlandense*, were confirmed as producers of cycloaspeptides A and E. Genome sequencing and comparative bioinformatics identified a putative gene cluster, which was confirmed as being responsible for cycloaspeptide biosynthesis by gene knock-outs and heterologous expression. The minimal gene set consists of a 5-module non-ribosomal peptide synthetase (NRPS) and a new type of trans-acting *N*-methyltransferase. Disruption of the *N*-methyltransferase and subsequent feeding studies have uncovered a hitherto unseen trait in fungal secondary metabolism: the ability of the NRPS to adenylate and incorporate *N*-methylated tyrosine and phenylalanine directly into the cyclic peptide. This discovery has been exploited to alter the ratios of cycloaspeptides produced, including a total shift from the non-active major metabolite cycloaspeptide A to the insecticidally active minor metabolite cycloaspeptide E. It also allowed the production of cycloaspeptides B, C and G, compounds not previously detected in a *Penicillium* species. Searches for similar gene clusters in other filamentous fungi identified the known ditryptophenaline gene cluster from *A. flavus*. This pathway was shown to be homologous, in that methylated phenylalanine is also accepted by the ditryptophenaline NRPS. We have also demonstrated that novel NRP natural products can be generated by feeding synthetic amino acid analogues to these systems. This discovery, coupled with the growing field of megasynthase engineering, could potentially be used to introduce methylated amino acids and synthetic amino acids into other NRP natural products of interest.

Thursday, March 16 3:00 PM – 6:00 PM
Nautilus
Fungal-bacterial Interactions

NOD-like receptors (NLR) and non self recognition in fungi: genomic and functional insights. *M. Paoletti* Institut de Biologie et Génétique Cellulaire, CNRS, Bordeaux, FR.

Recognizing other species, be it symbiotic or pathogenic, is essential to initiate an appropriate response. In Plants and animals, if NOD Like Receptors (NLRs) were identified first in the context of innate immunity it now becomes clear (at least for animals) they also play a role in host communication with the microbiota. NLRs display a characteristic tripartite organization with an N terminal effector domain and a C terminal LRR repeat domain surrounding a central Nucleotide Binding domain. Using that tripartite organization as the main criteria we recently identified and described the NLR repertoire of about 200 fungal species. Fungal genomes encode for highly diversified and dynamic sets of NLR encoding genes. Overall diversity goes down to the individual level. Considering the huge diversity of fungal NLRs, one might hypothesize that they function as interspecific communication devices either to activate an innate immune response or to establish symbiosis, the nature of the response presumably depending on the N terminal effector domain.
In an innate immunity context plant and animal NLRs can recognize pathogens either by directly binding a pathogen marker or by recognizing altered self proteins as a consequence of the pathogen's actions. Pathogen markers and host receptors encoding genes may be engaged in an evolutionary arms race, occasionally resulting in host auto-immune diseases. In the model *Podospora anserina* the genes *het-c* encoding a Glycolipid Transfer Protein (GLTP), and *het-e* encoding a NLR were first identified as controlling vegetative incompatibility, a process of non self recognition between individuals of the same species. Their evolutionary signatures led to the idea that incompatible combinations arose as a consequence of their pathogen driven fast evolution. We now investigate this gene pair in the context of innate immunity. The GLTP is required for the proper fungal defense in confrontation with some pathogenic bacteria, and is the target of a bacterial protease known to be a pathogenicity factor. The HET-E cognate NLR may recognize these alterations of the GLTP to initiate a proper response.

Characterization of the interaction between bacteria and *Magnaporthe oryzae*. *X. Zhang*, JR Xu Botany and plant pathology, Purdue University, west lafayette, IN.

Like many other foliar pathogens, the rice blast fungus *Magnaporthe oryzae* form appressoria for plant penetration. Interestingly, bacteria attached to appressoria are often observed in in vitro assays for appressorium formation. In this study, *Pseudomonas fluorescens* strain TR3 was isolated from rice leaves and used for studying for its interaction with *M. oryzae*. TR3 could attached to conidia, germ tubes, and appressoria and caused fungal cell death. In co-cultivation assays, TR3 was inhibitory to colony growth in a contact-based manner, excluding the possibility of small antifungal metabolites. Attachment of TR3 to fungal spores was inhibitory to germination and plant infection. Antifungal activities were specifically detected in culture filtrates of TR3 grown in M9 medium together with *M. oryzae,* suggesting the involvement of the type 3 secretion system (T3SS). Mutants detected of the *hrcC* gene encoding the outer membrane pore forming protein of T3SS failed to form flagella and had reduced inhibitory activities against *M. oryzae*. In contrast, deletion of the *pilC* gene that is essential for T4SS had no impact on its antifungal potential although affected attachment to fungal hyphae or conidia. The phosphorylation level of Pmk1 MAP kinase was significantly increased after incubated with TR3 for 2 h, which was not observed in the *sho1* deletion mutant. Taken together, these results indicate that the interaction of *P. fluorescens* with *M. oryzae* is inhibitory to fungal growth, development, and pathogenesis and likely involves T3SS effectors affecting intracellular signaling pathways.

The fungal holobiont: exploring the mechanisms maintaing the *Rhizopus-Paraburkholderia* symbioses through RNA-Seq and comparative genomics. J.R. Bermúdez-Barrientos[1,2], S.J. Mondo[3], T.E. Pawlowska[3], C. Abreu-Goodger[2], *L.P. Partida-Martínez*[1] 1) CINVESTAV, Ingeniería Genética, Irapuato, Guanajuato 36821, MX; 2) CINVESTAV, Unidad de Genómica Avanzada (LANGEBIO), Irapuato, Guanajuato 36821, MX; 3) Cornell University, Plant Pathology & Plant-Microbe Biology, Ithaca, NY 14853.

Microorganisms are the most versatile and abundant living organisms on earth. They drive the biogeochemical cycles and constantly interact among themselves and with most, if not all, eukaryotes, including plants and animals. In the last years, the importance of the microbiome to the fitness of eukaryotic hosts has been recognized and new terms such as *holobiont*, *hologenome* and *symbiogenesis* had been introduced. However, these terms have been only applied to animals and plants, but not fungi. Yet, studies on the *Rhizopus-Paraburkholderia* symbioses suggest that fungi may be indeed also considered as holobionts (fungal host + symbionts).

Under the light of the holobiont concept, we will present the influence of symbiosis in the biology of *Rhizopus microsporus*. In these fungi, endofungal bacteria are the actual producers of the "mycotoxins" rhizoxin and rhizonin, and the presence of the bacterial symbionts is required for the asexual reproduction of the host. This dependency assures the vertical transmission of the endosymbionts and opens further questions about the implications of the symbiosis for the involved partners.

Strikingly, our recent RNA-Seq analyses revealed the presence of a *Narnavirus* in *R. microsporus*, suggesting a third partner in the association. Moreover, gene expression in *R. microsporus* suffers dramatic changes correlated with the presence/absence of its bacterial symbionts. We found that nuclear proteins, particularly transcription factors, are over-expressed in the absence of the endosymbiont, while fungal histone methyl-transferases are up-regulated when the endosymbiont is present, suggesting the involvement of epigenetic mechanisms in the regulation of the bacterial-fungal symbiosis.

Comparative genomics across endofungal bacteria *P. rhizoxinica* and *Ca.* Glomeribacter gigasporarum versus free-living *Paraburkholderia* revealed an enrichment of toxin-antitoxin system proteins in endofungal bacteria. We propose that these proteins may help ensure the vertical transmission of the symbionts (symbiont addiction).

Altogether these analyses have opened novel avenues for research and further support the notion that fungi can be true holobionts.

Bacteria induce pigment formation in the basidiomycete *Serpula lacrymans*. *J. Tauber*[1], V. Schroeckh[2], D. Hoffmeister[1], E. Shelest[2], A. Brakhage[2] 1) Friedrich-Schiller-University, Jena, Germany; 2) Leibniz Institute for Natural Product Research and Infection Biology– Hans Knöll Institute, Jena, Germany.

The basidiomycete *Serpula lacrymans* produces chromophoric natural products derived from the quinone precursor atromentin such as the pulvinic acid-type variegatic acid. These compounds have been shown to function in redox cycling and were induced from nutritional cues [1,2,3]. Atromentin is a widespread pigment. Hence, we addressed the question if there are other environmental cues, such as microbes, that could also cause induction of these pigments and how such induction may be regulated for Basidiomycota. *Serpula lacrymans* was co-incubated with one of three diverse terrestrial bacteria, *Bacillus subtilis*, *Pseudomonas putida*, or *Streptomyces iranensis* [4]. Analysis of pigment induction was accomplished two-fold. First, gene expression was quantified by qRT-PCR from the clustered genes encoding a quinone synthetase, an aminotransferase, and an alcohol dehydrogenase. Secondly, pigment secretion was measured chromatographically by HPLC. We showed that the atromentin gene cluster of *S. lacrymans* was up-regulated and that secreted pigments accumulated (variegatic, xerocomic, and atromentic acid) during co-incubation with each bacterium indicating induction by a diverse set of microbes. Analysis of the promoter region of each clustered gene across 12 different basidiomycetes containing putatively orthologous atromentin gene clusters indicated a common motif, and thus conserved regulation. We concluded that because a diverse set of bacteria caused pigment induction (in addition to nutritional cues), and given the widespread, conserved presence of functional atromentin-derived pigments and its regulation, these pigments are an essential aspect for basidiomycetes in nutrition uptake, protection, or both.

[1] Eastwood DC., *et al*. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science. 2011 333:762-765.

[2] Shah F., *et al*. Involutin is a Fe^{3+} reductant secreted by the ectomycorrhizal fungus *Paxillus involutus* during Fenton-based decomposition of organic matter. Appl. Environ. Microbiol. 2015 81:8427-8433.

[3] Braesel J., *et al*. Three Redundant Synthetases Secure Redox-Active Pigment Production in the Basidiomycete *Paxillus involutus*. Chem Biol. 2015 22:1325-34.

[4] Tauber, JP., *et al*. Bacteria induce pigment formation in the basidiomycete *Serpula lacrymans*. Environ Microbiol. 2016.

Bacterial biofilm formation on soil fungi: a widespread ability under controls. *Aurelie Deveau*[1], Cora Miquel Guennoc[1,2], Jessy Labbé[2] 1) INRA Université de Lorraine, Interactions Arbres-Microorganismes, UMR 1136, Champenoux, France; 2) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Although bacterial biofilms are ubiquitous, little is known about their occurrence and their involvement in bacterial/fungal interaction in soil. In order to contribute to fill this gap, we combined confocal and electronic microscopy to describe the structure, dynamic and specificity of *in vitro* biofilm formation by soil bacteria on fungal hyphae. We show that the ability to form biofilms on fungal hyphae is widely shared among soil bacteria. In contrast, some fungi, mainly belonging to the Ascomycete class, did not allow the formation of bacterial biofilms on their surfaces. The formation of biofilms was also strongly modulated by the presence of tree roots and by the development of the ectomycorrhizal symbiosis, suggesting that biofilm formation does not occur randomly in soil but that it is regulated by several biotic factors. All the biofilms formed on fungal hyphae were structured by dense networks of DNA containing filaments. Those filaments were essential for the formation and the structural maintenance of the biofilms and were produced by both gram positive and negative bacteria. Our data coupled to those produced by other groups strongly suggest that DNA is widely used by prokaryotes and eukaryotes as a structural molecule.

Ménage à trois: fluorescent rhizosphere pseudomonads pursue combined strategies to control polarity and growth of pathogenic *Verticillium* fungi and their plant hosts. K. Nesemann[1], S. A. Braus-Stromeyer[1], *R. Harting*[1], H. Kusch[1], A. Hoefer[1], C. E. Stanley[2], M. Stöckli[3], M. Landesfeind[1], A. Kaever[1], A. Ambrosio[1], C. Timpner[1], K. Hoff[4], T. Pena-Centeno[4], I. Bulla[4], M. Starke[4], A. J. deMello[2], M. Künzler[3], M. Aebi[3], G. H. Braus[1] 1) Institute of Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-Universität Göttingen, DE; 2) Institute of Chemical and Bioengineering, ETH Zürich, CH; 3) Institute of Microbiology, ETH Zürich, CH; 4) Institute of Mathematics and Computer Science, Ernst-Moritz-Arndt-Universität Greifswald, DE.

The genus Verticillium includes several root-inhabiting fungi, which can colonize plants as endophytes or phytopathogens. The impact of genes from rhizosphere Pseudomonas species for secreted metabolites including phenazines or the genes controlled by the bacterial GacA/GacS two component regulatory system on fungal growth was investigated. *Pseudomonas fluorescens* from the rhizosphere of the rapeseed *Brassica napus* cannot synthesize phenazine or 2,4-diacetylphloroglucinol mycotoxins, but caused similar growth inhibitions towards the rapeseed pathogen *Verticillium longisporum* or *V. dahliae* infecting tomato as a different host. The fungal secondary metabolism control genes *Lae1* or *Csn5* are dispensable for the fungal-bacteria interaction. Bacterial co-cultivations with different strains supports media-dependent bacterial strategies to inhibit fungal growth during agar or liquid co-cultivation. Phenazines caused Verticillium growth inhibition on high glucose, but not on pectin/amino acid agar. Co-cultivation with *P. protegens* in liquid pectin/amino acid medium alters the relative fungal transcripts ratio including 16% up-regulated genes for mitochondrial activity, detoxification or melanine biosynthesis and about half decreased gene fractions for protein synthesis or pectin degradation. Bacterial-fungal interactions at the single cell level were probed in microfluid devices in liquid channels with pectin/amino acid medium. These confrontations with physical constraints revealed that rhizosphere Pseudomonads can inhibit 80% of fungal hyphal growth independently of phenazine and 2,4-diacetylphloroglucinol by complex combinations of metabolites. 90% of *V. longisporum* hyphal tips exhibited strong polarity defects with a ‘curly’ phenotype. Co-infection experiments of host plants with fungi and bacteria revealed that only the *gacA*-controlled Pseudomonas regulatory network protects plants against Verticillium induced pathogenicity.

Antifungal activity of the human gut metabolome. *C.E. Garcia-Rangel*[1], F Tebbji[1], MC Daigneault[2], NN Liu[3], JR Ko¨hler[3], E Allen-Vercoe[2], A Sellam[1] 1) Infectious Diseases Research Centre-CRI, CHU de Québec Research Center, Quebec City, Quebec, Canada; 2) Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada; 3) Boston Children’s Hospital/Harvard Medical School, Boston, MA, USA.

The human gut contains a variety of commensal microbes which are composed of diverse organisms that belong to all three domains of life with Eukaria primarily represented by fungi. The commensal/opportunistic yeast *Candida albicans* has been reported as the most common fungus in the gut of healthy humans. Recent study has shown that commensal microbiota play a critical role in the protection of the gut against colonization by other bacterial pathogens and pathobionts. However, so far, whether *C. albicans* overgrowth or pathogenicity are controlled by other fecal microbiota is not known. In this study, we showed that the secreted human gut metabolome (HGM) exerts an

antifungal activity against different intestinal-resident yeasts including *C. albicans*, *C. tropicalis* and *C. parapsilosis.* The HGM inhibited the growth of both sensitive and drug-resistant strains of *C. albicans.* To uncover the mechanism of action of the HGM associated with its antifungal property, a genome-wide genetic screen was undertaken and identified key components of the conserved TOR (Target Of Rapamycin) pathway as required for HGM tolerance. The HGM reduced the phosphorylation state of the known TOR effector, RPS6 confirming thus that the HGM inhibit *C. albicans* growth through TOR pathway. Given the central role of TOR pathway in modulating cell growth in response to nutrients cues, these data support a model where gut microbial cohabitants control *C. albicans* nutritional competitiveness through the modulation of TOR pathway activity. Furthermore, hyphal growth, a critical virulence trait of *C. albicans*, as well as the inducibility of hyphae-specific genes were significantly reduced. In accordance with the inhibitory effect on hyphae formation, we also showed that the HGM reduced significantly the damage inflicted to the human colon epithelial cells by *C. albicans*. To identify microbial specie(s) that produce the antifungal molecule(s), individual isolates form continuous-culture chemostat of fecal extracts of two healthy human donors were screened. Our data revealed that common butyrate-producing bacteria from the two donors exhibited an apparent antifungal activity. Together, these emphasize that the novel cross-kingdom interaction mediated by butyrate-producing bacteria contribute to the control of both the growth and virulence traits of *C. albicans* and provide a new paradigm where the commensal growth of this major human pathogen in the gut is dictated by the surrounding microbiota.

'Life, Love, and Murder'- logistics of fungal bacterial interplays for soil ecosystem functioning. *L.Y. Wick* Environmental Microbiology, Helmholtz Centre for Environmental Research UFZ, Leipzig, DE.

Fungal bacterial interactions are highly diverse and contribute to many ecosystem processes. Their emergence in structurally heterogeneous soil structures or under common environmental stress scenarios, however, still remains mainly elusive. In this contribution we exemplify emerging fungal bacterial interplays by the ecosystem function of contaminant biodegradation in soil. The structural heterogeneity of soil results in the formation of a high variety of soil micro-environments and hotspots of microbial diversity and activity. In heterogeneous environments microbial activity can be considered as a 'logistic' problem, as effective biotransformation will evolve only if water, chemicals and nutrients are sufficiently available to actively degrading microbial communities. In order to cope with heterogeneous environments mycelial soil fungi have developed a unique network-based growth form. Unlike bacteria, hyphae spread efficiently in the soil, penetrate air-water interfaces and cross over air-filled pores. Here we demonstrate the role of mycelial networks for preferential bacterial colonization of subsurface interfaces and discuss its effects on contaminant degradation. In particular, we show that mycelia (i) act as hotspots for horizontal gene transfer, (ii) shape predator-prey interactions and concomitant compound turnover, and (iii) passively and actively enable the functionality of bacterial ecosystems when stressed by low nutrient availability or at low matric potentials. Given the ubiquity and length of up to 500-1000 m g^{-1} dry soil of hyphae, we conclude that transport and dispersal processes by mycelia not only play a significant role for the ecosystem service of biodegradation of chemicals but also for the nutrient and carbon turnover in soil.

Thursday, March 16 3:00 PM – 6:00 PM
Scripps
Intracellular Mobility, Traffic, and Secretion

Modelling of whole-cell endosome trafficking reveals molecular adaptation of dynein. C. Lin[2], P. Ashwin[2], M. Schuster[1], *G. Steinberg*[1] 1) School of Biosciences, University of Exeter, Exeter, GB; 2) Mathematics, University of Exeter, Exeter, GB.

Fungal early endosomes (EEs) move rapidly along microtubules, thereby organising other organelles, such as peroxisomes and lipid droplets. In *Ustilago maydis*, kinesin-3 and dynein cooperate to mediate EE motility along complex bi-polar microtubule array. Here, we develop a mathematical model of the whole-cell EE motility along various MT arrays, which is based on numerous experimental parameters, including microtubule organisation, EE numbers and motility parameters. In simulations, the model predicts EE run-length, spatial EE distribution, peroxisome distribution and dynamics in wildtype cells and kinesin-3 mutants with high accuracy. We next asked if there are alternative ways of organising organelles. In 23 simulations, we altered motor characteristics (e.g. numbers on organelle, binding rates or run-length) and rearranged the microtubule arrays (e.g. swapping polarity). While only few of these altered scenarios support even EE and PO distribution and motility, all *in silico* solutions require an extended run-length of dynein, not known from other cells. Indeed, experiments confirm that single dynein motors transport over up to 30 µm, which is due to a modified dynactin, characterised by an extended basic domain. Thus, motors have adapted to optimise long-range membrane trafficking in fungal hyphae.

Dissemination of *Cryptococcus neoformans* to the Central Nervous System is dependent on activation of phosphate uptake at alkaline pH by the transcription factor Pho4. *Julianne Djordjevic*[1,2,6], Sophie Lev[1], Keren Kaufman-Francis[1], Desmarini Desmarini[1], Pierre Juillard[1], Cecilia Li[1], Sebastian Stifter[3], Carl Feng[3], Tania Sorrell[1,2,6], Georges Grau[4], Yong-Sun Bahn[5] 1) Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, Sydney, NSW, AU; 2) Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, NSW, Australia;; 3) Immunology and Host Defense Group, Department of Infectious Diseases and Immunology, Sydney Medical School, The University of Sydney, NSW, Australia; 4) Vascular Immunology Unit, Department of Pathology, School of Medical Sciences, The University of Sydney, NSW, Australia,; 5) Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul 03 722, Republic of Korea. ; 6) The Westmead Clinical School, Sydney Medical School, The University of Sydney NSW, Australia.

Phosphate acquisition by fungi is regulated by the PHO signaling pathway. *Cryptococcus neoformans* disseminates from lung to brain and is the most common cause of fungal meningitis world-wide. To investigate the contribution of PHO signaling to cryptococcal dissemination, we identified a mutant strain (*hlh?/phoΔ*) from a transcription factor knockout library, which is defective in phosphate acquisition. Despite little similarity with other fungal Pho4 proteins, Hlh3/Pho4 functioned like a typical phosphate-responsive transcription factor in phosphate-deprived cryptococci, accumulating in nuclei and triggering expression of genes involved in phosphate acquisition. *pho4*Δ was susceptible to a number of stresses, the effect of which, except for alkaline pH, was alleviated by phosphate supplementation. Even in the presence of phosphate, the PHO pathway was activated in wild-type cryptococci at or above physiological pH and, under these conditions, phosphate uptake and growth were compromised in the *pho4*Δ mutant. *pho4Δ* was hypovirulent in a mouse inhalation model, where dissemination to the brain was reduced dramatically, and markedly hypovirulent in an intravenous dissemination model. *Pho4Δ* was not detected in blood nor did it proliferate when cultured with peripheral blood monocytes. In conclusion, dissemination of infection and the pathogenesis of meningitis are dependent on the activation of phosphate uptake and stress tolerance at alkaline pH, both of which are dependent on Pho4.

Vacuolar protein sorting VPS-27 identifies putative multivesicular bodies in *Neurospora crassa* hyphae. *M. Riquelme*[1], A. Aguilar-Romero[1], B. Bowman[2], L. Martínez-Núñez[1] 1) Department of Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE, Baja California, MX; 2) Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, California, USA.

Multivesicular bodies (MVBs) are formed when the membrane of the late endosomal compartment invaginates within its own lumen forming intraluminal vesicles (ILVs). This process requires both the ubiquitination of the cargoes destined to the ILVs and the participation of multiprotein Endosomal Sorting Required for Transport (ESCRT) complexes: ESCRT-0, I, II and III. The MVB sorting pathway participates presumably in many important cellular processes, most of which remain unknown in filamentous fungi. To study the morphology and distribution of MVBs and their role in the recycling of plasma membrane (PM) enzymes in *Neurospora crassa* we have analyzed the homologue of the yeast class E Vps27 protein. The FYVE domain of Vps27 mediates its interaction with endosomal PI(3)P. VPS-27 (NCU04015), a putative component of ESCRT-0, tagged with either GFP or mCherry, was found at fluorescent puncta all along the subapical cytoplasm excluding the apex. In further distal regions, VPS-27 was found at the membrane of pleomorphic bodies, presumably MVBs, which while advancing remained at a constant distance from the hyphal apex. VMA-1 and RAB-7, both previously identified at prevacuolar compartments (PVCs), showed partial co-localization with VPS-27 at the membrane of the putative MVBs. Different high-resolution live-imaging approaches are currently being used to investigate the fate of the MVBs.

The direct pathway connecting trans-Golgi cisternae and the plasma membrane with RabE^{RAB11} secretory vesicles. *M.A. Penalva*[1], M. Pinar[1], M. Hernández[1], H.N. Arst, Jr.[2], J. Zhang[3], X. Xiang[3], A. Pantazopoulou[1] 1) Department of Cellular and Molecular Biology, CSIC Centro de Investigaciones Biológicas, Madrid, Madrid, ES; 2) Section of Microbiology, Imperial College London, London, UK; 3) Department of Biochemistry and Molecular Biology, The Uniformed Services University of the Health Sciences, Bethesda, MD.

The trans-Golgi network of *Aspergillus nidulans* gives rise to secretory vesicles (SVs) bound to the apical plasma membrane. These SVs bud off the Golgi after a maturation process by which cisternae gradually acquire post-Golgi identity by recruiting RabE^{RAB11}. Shortly after this recruitment SVs undergo transport towards the apex using myosin-5 and kinesin motors. TRAPPII, an oligomeric GEF containing Trs120 mediates the exchange of GDP for GTP on RabE. Trs120 is notable in that the gene encoding this TRAPPII component, denoted *hypA* in *Aspergillus*, was initially identified in a screen for conditional mutations affecting hyphal morphology. HypA^{Trs120} is recruited to the TGN preceding the departure of SVs. Mutations in *rabE* that enable growth in the absence of *hypA*/TRAPPII promote spontaneous guanine nucleotide exchange on the GTPase. Transport of SVs to the Spitzenkörper involves the myosin-5 MyoE, one of the likely effectors of

RabE^{RAB11}. It also involves kinesin-1 (KinA), which is sufficient to sustain apical extension in the absence of myosin-5. Lastly SVs can also engage dynein, which mediates their retrograde transport away from the apex. Dynein loading on SVs involves the p25 subunit of dynactin.

Live-cell imaging of nuclei and filamentous actin in the basidiomycete *Schizophyllum commune*. Elke-Martina Jung[1], Erika Kothe[1], *Marjatta Raudaskoski*[2] 1) Institute of Microbiology, Microbial Communication, Friedrich Schiller University, Neugasse 25, 07734 Jena, Germany; 2) Molecular Plant Biology, Department of Biochemistry, University of Turku, Tykistökatu 6A, 20520 Turku, Finland.

The visualization of cytoskeletal structures in living hyphae of filamentous basidiomycetes can yield additional information that cannot be obtained with ascomycetes. In basidiomycetes, monokaryotic or defined dikaryotic hyphal compartments are found, and the formation of septa is tightly linked to nuclear division. In addition, some model basidiomycetes, including *Schizophyllum commune*, show nuclear division in dikaryotic hyphae linked to clamp formation. Here, we use histone H2B labeling by EGFP to allow for *in vivo* visualization of the elongated nuclei in both, mated dikaryotic and unmated monokaryotic hyphal compartments. In apical cells, the interphase nuclei moved towards the hyphal tip with a rate proportional to tip growth. In some apical cells, the nuclear content showed increased fluorescence due to chromatin condensation. The condensed chromatin gathered at the apical part of the nucleus, while the fluorescence in the rest of the nucleus remained weak and finally became separated from the bright part. The decrease in nuclear size during division has long been known in *S. commune,* but only the observation of the separation of a weakly fluorescent part observed here clearly suggests that nuclear division in filamentous basidiomycetes follows the "(late) open mitosis" principles, as previously suggested also for the yeast like growth phase of basidiomycetes including *Ustilago maydis* or *Cryptococcus neoformans*. The phenomenon was seen more clearly during nuclear division in homokaryotic than in dikaryotic hyphae. Live-cell imaging of filamentous actin with Lifeact::EGFP revealed filamentous actin after nuclear division at the formation of septa both in monokaryotic and dikaryotic hyphae. The dynamics of actin at clamp cell formation suggested a more central role for actin than recorded before. At the initial stage of clamp cell formation, seen as a small peg growing from the mother cell, the proceeding movement of nuclei halts. At the same time, an actin web below the outgrowth becomes visible. This actin web could be responsible for the cessation of nuclear movement. Associated with actin web formation is the movement of one of the nuclei into the clamp initial. Microtubules have been suggested to play a central role for the movement, but here we could show that actin filaments appear to be involved in an essential manner.

Membrane asymmetry markers and polarized growth in *Neurospora crassa*. *R.R. Mouriño.Pérez*[1], I. Murillo-Corona[1], Z. Schultzhaus[2], B.D. Shaw[2], O.A. Callejas-Negrete[1] 1) Microbiology, CICESE, Ensenada, BC, MX; 2) Department of Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, TAMU.

In fungal cells, specialized proteins gather in specific places to break cell symmetry and produce hyphae. This organization includes the orchestration of two distinct vesicle processes, endocytosis and exocytosis that take place in tandem in different areas of the apical compartment in growing hyphae. Part of the signals for endocytosis and endocytosis include the asymmetry of the plasma membrane phospholipid bilayer. We studied the flippases, DNF-1 and DRS-2 that seems to be responsible for this membrane asymmetry. The mutation of *dnf-1* and *drs-2* genes produced alteration in the maintenance and stability of the Spitzenkörper and affected the actin cytoskeleton organization in the apical compartment. Surprisingly, neither of the flippases DNF-1 and DRS-2 was present in the plasma membrane, both were localized in different layers of the Spitzenkörper, associated to different secretory vesicles. DRS-2 was associated to vesicles transporting chitin synthases. These results indicate that phospholipid flippases (P4 ATPases) may be important for polarity on secretory vesicles, Spitzenkörper integrity and thus for the localization of many tip growing proteins.

Internuclear diffusion of histone H1 within cellular compartments in *Aspergillus nidulans*. *Alexander Mela*, Michelle Momany Plant Biology, University of Georgia, Athens, GA.

Histone H1 is an evolutionarily conserved 'linker' histone protein which serves the important dynamic function of arranging and stabilizing chromatin structure. The mechanism by which histone H1 carries out its function remains unclear. One thing that is certain is the common use of fluorophore-tagged histone H1 protein in microscopy to track nuclei within cells. In time-lapse analyses, we observed stochastic exchange of photo-activated Dendra2-Histone-H1 protein between nuclei. We also observed heterogeneous mixing of histone proteins between nuclei in hyphae and conidiophores derived from heterokaryon fusions of histone H1-RFP and H1-GFP strains. Subsequent analysis of the resulting conidia that contained both RFP- and GFP-labeled histone H1 proteins, showed only parental genotypes. These data together suggest the stochastic exchange of histone H1 protein between nuclei is likely a result of diffusion rather than genetic recombination during karyogamy.

Microtubules are reversibly depolymerized in response to changing gaseous microenvironments within *Aspergillus* biofilms. *N. Shukla*[1,2], A. Osmani[2], S. Osmani[1,2] 1) The Ohio State Biochemistry Program, The Ohio State University, Columbus, Ohio 43210; 2) Department of Molecular Genetics, The Ohio State University, Columbus Ohio 43210.

Fungal persistence and drug resistance during infections occurs mainly due to their ability to form biofilms. Concentration gradients of oxygen and nutrients caused by cell crowding and potentially secreted signaling factors cause cells in different areas of the biofilm to experience different microenvironments. The goal of our study was to understand how microenvironments within different areas of a fungal biofilm impact cell physiology.

Using confocal microscopy and genetic analyses we have uncovered a new physiological response that *Aspergillus nidulans* cells in a biofilm undergo in response to their microenvironment. We found that after adhesion to a surface, cells at the base of a forming biofilm first halt their growth and later on depolymerize their microtubules (MTs) in a cell-autonomous manner. During such MT-disassembly, MT plus-end binding proteins like EB1, ClipA and Dynein transiently locate to unique bar-like structures. To our surprise, we also found that biofilm mediated MT-disassembly involves soluble gases since cells instantly repolymerize their MTs upon simple air exchange above the static biofilm media. Further experiments revealed that biofilm-mediated MT disassembly occurs as a regulated response to hypoxia. Upon further maturation, and possibly due to the formation of the extracellular matrix (ECM), cells no longer respond to simple gaseous exchange above the biofilm media. At this time, physical removal of a part of the biofilm is required to promote MT reassembly that then occurs in cells at the new biofilm edge. In experiments aimed at further understanding the volatile agent involved in the regulation of MT dynamics within biofilms

we tested the effects of the gaseous signaling agent, hydrogen sulfide (H_2S). We find that all aspects of MT depolymerization and repolymerization, including EB1, ClipA and Dynein bar formation, can be mimicked in growing cells under normoxia by H_2S addition and removal. Collectively, our study has implications in the areas of MT regulation, responses to hypoxia, gaseous signaling, and potentially fungal pathogenesis.

Friday, March 17 9:00 AM – 12:00 NOON
Merrill Hall and Chapel
Plenary Session III: Systems Biology: Genes, Genomics and Genome Structure

Sequence all the fungi: what can you do with 1000 fungal genomes? *Jason Stajich*[1], Joseph Spatafora[2], Zygolife and 1000 Fungal Genomes Consortia 1) Plant Pathology & Microbiology, Univ California-Riverside, Riverside, CA; 2) Botany & Plant Pathology, Oregon State University, Corvallis, OR.

Fungi lead the way in number and phylogenetic breadth of sequenced eukaryotic genomes. Worldwide efforts have generated many hundreds of fungal genome sequences for research ranging from biotechnology, plant and animal disease, to basic fungal and evolutionary biology. The 1000 Fungal Genomes project (1KFG; http://1000.fungalgenomes.org) is one effort to broadly sample the fungal tree of life aiming for at least 2 genomes sampled from every taxonomic family. Additionally the Zygolife project (http://zygolife.org) is an effort to increase sampling and understanding of zygomycete fungi phylogeny and evolution. These genome and organismal data are useful in a variety of questions. **Evolutionary Relationships** Using a dataset of 350+ genomes we have revised the fungal phylogeny and reclassified early branching zygomycete lineages into two new phyla. These genome data have also supported development of a broad set of conserved gene markers useful for phylogenetics and genome completeness assement. **Comparative Biology**. Genomic comparisons in protein coding gene content has revealed changes in ecological niche lifestyles and primary metabolisms. Contrasts of mycoparasite, plant and animal associated, or saprotrophic lifestyles is also revealing how evolutionary shifts in trophic associations are reflected in genomic composition and changes. **Metagenomics and classification methods.** A rich set of reference genome data is essential when analyzing gene content in metagenomic or transcriptomic datasets. We have tested these improved resolutions with rapid sequencing technologies to more quickly identify likely species classifications. Efforts to use light coverage genome sequencing coupled with reference genome resources is also proving useful for resolving phylogenetic relationships and simple gene inventories at low costs.

A guide to some of these large datasets of genomic resources for fungi will be described, how they are generated and how others can contribute species to be sampled and sequenced, how the data are disseminated and analyzed, and what ways it can be most useful to the broad community.

Epigenetic memory over geological timescales. S. Catania[1], P. Dumesic[1], C. Stoddard[1], G. Narlikar[1], C. Cuomo[2], *H. Madhani*[1] 1) Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA; 2) Broad Institute, Cambridge, MA.

It is well established that evolution operates on natural variation that is produced by variation in DNA sequence (genetic variation). An alternative form of heritable variation is epigenetic variation, defined here as heritable differences between individuals that are not due to differences in the DNA sequence. Whether such variation can be maintained accurately over a sufficient number of generations to enable evolution is not clear. Here we provide an example in which modification of DNA, cytosine methylation, can be epigenetically inherited over a time scale of millions of years. We have characterized mechanisms of establishment and maintenance of DNA methylation in the basidiomycetous yeast *Cryptococcus neoformans*, whose single predicted cytosine methyltransferase, Dnmt5, is responsible for all symmetric CG methylation. Our detailed genetic and biochemical experiments indicate that, like the mammalian Dnmt1 maintenance methylase, Dnmt5 maintains DNA methylation through two partially redundant mechanisms, which respectively involve the recognition of heterochromatin marks and of prior DNA methylation. Despite the ability of Dnmt5 to maintain cytosine methylation, it cannot perform *de novo* methylation *in vivo* or *in vitro*. Genetic experiments in which the DNMT5 gene is removed or repressed and then restored demonstrate that once lost, DNA methylation cannot be regained *in vivo*. Consistent with these results, purified full-length Dnmt5 exhibits robust cytosine methyltransferase activity on hemimethylated DNA substrates, but has no detectable activity on unmethylated substrates. By examining the genomes of species in the order of Tremellales, of which *C. neoformans* is a part, we find that ancestors of *Cryptococcus* harbored two enzymes: a Dnmt5 ortholog and second predicted cytosine DNA methyltransferase, a presumptive *de novo* methylase. Our phylogenetic analysis reveals that the gene coding for this second enzyme was lost prior to the divergence of *C. neoformans* and the genus *Kwoniella*, which is estimated to have occurred between 50 and 150 million years ago. We propose that DNA methylation has been propagated epigenetically by Dnmt5 via a “maintenance-only” mechanism since the loss of the presumptive *de novo* methylase gene.

Systems biology illuminates the mechanistic basis of host damage during fungal diseases of the human lung. *E.M. Bignell* Microbiology Section, Imperial College London, London, GB.

The human lung is continually exposed to spores of the airborne mould *Aspergillus fumigatus*. Inhaled spores are small enough to bypass mucociliary clearance mechanisms and reach the alveoli of the lung where interaction between host and pathogen cells can lead to fungal clearance, or to development of inflammatory or invasive fungal diseases. *A. fumigatus* is an accidental pathogen whose encounters with the host, although frequent, are circumstantial in nature. The capacity of this organism to cause human disease is unique amongst a cohort of several hundred related Aspergillus species and relative to closest sequenced relative there are no large scale genetic events which signify recent evolution of pathogenicity. However, multiple, recently evolved telomere-associated genes are preferentially upregulated in the host environment, including those which drive production of secondary metabolites. Clinical relevance of aspergillus species does not correlate with fungal growth rate, but is positively correlated with thermotolerance, and epithelial toxicity.

Fuelled by the results of extensive fungal transcriptome research we have developed, in recent years, a programme of research which seeks the mechanistic basis of lung damage during *A. fumigatus* infection. In order to find out why *A. fumigatus* is cytotoxic to human epithelia we have observed the interaction between host and pathogen in laboratory culture and in mouse disease, and measured epithelial decay, lytic death of host cells, host cell signalling and cytokine degradation in response to fungal challenge. This research has revealed multiple mechanisms involved in eliciting epithelial damage, occurring at different stages of the host-pathogen interaction and involving different fungal morphotypes. In this talk I will explain how systems approaches have led us to ask the following questions: Why is *A. fumigatus* cytotoxic to human cells? Does the host or the pathogen drive epithelial damage and how? How do pathogen-derived proteins shape the immune environment? How is damage driven in the whole animal host? Which elicitors of host damage are produced by *A. fumigatus*, and have they recently evolved? I will finish by introducing a new high throughout study addressing the *A. fumigatus* regulatory

network driving epithelial damage in cultured human lung tissue and in the whole animal, and examine how this knowledge might lead to novel therapeutic interventions.

How to make a fruiting body: comparative genomics and transcriptomics in ascomycetes. David Schumacher, Stefanie Traeger, Stefan Gesing, Ramona Lütkenhaus, Florian Altegoer, Daniel Schindler, *Minou Nowrousian* Department of General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany.

The development of complex multicellular structures has evolved many times in eukaryotes, and at least twice in fungi. The fruiting bodies of filamentous ascomycetes are one example, and even though many genes essential for their differentiation have been identified, a model describing the spatio-temporal regulation of this developmental process at the molecular level has yet to emerge. Our aim is to identify evolutionary trends and core processes that regulate fruiting body morphogenesis through comparative genome and transcriptome mining. We performed RNA-seq on young fruiting bodies isolated by laser microdissection from the Sordariomycete *Sordaria macrospora* and the Pezizomycetes *Pyronema confluens* and *Ascodesmis nigricans*. Comparative expression analysis of genes conserved in all three species shows structure-specific expression when looking at relative expression changes, but species-specific expression with respect to overall expression levels. Genes involved in chromatin organization and regulation of gene expression, and genes involved in the organization of endomembrane systems make up more than 40 % of genes specifically upregulated in young fruiting bodies in all three species. For further functional analysis, our focus is on genes involved in chromatin-related processes. Among the genes with conserved expression patterns identified in previous comparative studies are the histone chaperone gene *asf1* and the transcription factor gene *pro44*, both of which are essential for fruiting body development in *S. macrospora*. RNA-seq analysis of *pro44* and *asf1* mutants showed that many genes with overall weak expression are upregulated in the *asf1*, but not the *pro44* mutant, suggesting that ASF1 acts as a repressor of weakly transcribed genes. To analyze whether this transcriptional derepression might be caused by problems with nucleosome deposition in the histone chaperone mutant, we performed micrococcal nuclease sequencing (MNase-seq). Genome-wide nucleosome patterns are wild type-like in the *asf1* mutant, indicating that the transcriptional changes and developmental defects of the mutant are not caused by an overall change in nucleosome distribution. Future studies will focus on a potential role of *asf1* in nucleosome turnover and histone modifications.

Bidirectional cross-kingdom RNAi and spray-induced gene silencing for plant disease control. Ming Wang[1], Arne Weiberg[1,4], Feng-Mao Lin[2], Bart Thomma[3], Hsien-Da Huang[2], *Hailing Jin*[1] 1) University of California, Riverside, CA; 2) Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsin-Chu 300, Taiwan; 3) Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; 4) Current Address: Institute of Genetics, University of Munich Martinsried, Germany.

Small RNAs (sRNAs) are a class of short non-coding RNAs that mediate gene silencing in a sequence-specific manner. We have demonstrated that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, the fungal pathogen that causes grey mold disease on more than 1000 plant species, could be translocated into host plant cells and suppress host immunity genes for successful infection. This finding represented the first example of naturally occurring Cross-Kingdom RNAi in host – pathogen interactions. These sRNAs act as a new class of pathogen effectors that translocate into host cells to suppress host immunity.

Recently we have found that transgenic plants expressing hairpin RNAs that targeting *Botrytis* Dicer 1and Dicer 2 genes could effectively block the generation of fungal sRNA effectors and suppress grey mold disease. This strategy can be adapted to simultaneously control multiple fungal diseases. We show that Arabidopsis plants expressing hairpin RNAs that simultaneously target DCL genes of B. cinerea and V. dahliae show enhanced disease resistance to both pathogens. These results also suggest that sRNA trafficking is bi-directional and sRNAs could move from the host cells to the interacting pathogens. Most strikingly, we discovered that *Botrytis* could take up double-stranded RNAs and sRNAs from the environment. Applying sRNAs or dsRNAs that target Botrytis *Dicer* genes on the surface of fruits, vegetables and flowers significantly inhibits grey mold disease. Such pathogen gene-targeting RNAs represent a new generation of environmentally friendly fungicides.

Friday, March 17 3:00 PM – 6:00 PM
Merrill Hall
Evolution and Mechanisms of Antifungal Drug Resistance

Adaptive landscapes in fungicide resistance. *Nichola Hawkins*, Bart Fraaije Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, Hertfordshire, GB.

The use of fungicides generally exerts a strong selective pressure for resistance, and in many cases this results in the rapid spread of a single mutation conferring resistance across a mode of action. However, in other cases the evolution of resistance has been less straightforward, resulting in more durable effectiveness of some fungicides. The application of evolutionary concepts such as adaptive landscapes and fitness penalties can help to explain such differences, and to inform future resistance risk assessment and management. In the case of the QoI and MBC fungicides, the repeated parallel evolution of few, simple resistance mechanisms across many plant pathogens indicates a relatively smooth adaptive landscape with consistent functional constraints. The more complex evolution of azole resistance in *Zymoseptoria tritici* results from a more rugged adaptive landscape, with the fitness effect of each mutation varying due to epistatic interactions with other mutations and the genetic background.
The new ascomycete-active SDHI fungicides are considered to be at high risk for the emergence of resistance. Target-site mutations conferring high levels of resistance have already been reported in some key pathogens, but the rate of spread of these mutations and the effectiveness of resistance management approaches will depend upon the underlying adaptive landscape. A combination of evolutionary concepts and functional genetic tools will help to predict this.

Unveiling a novel regulatory role of the pH regulator PacC and the CCAAT-Binding-Complex in *Aspergillus fumigatus* 5-Flucytosine resistance. *F. Gsaller*, T. Furukawa, P.D. Carr, M.J. Bromley Manchester Fungal Infection Group, Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, GB.

Annually >1.5 million people die from fungal disease, a big proportion results from invasive and chronic mould infections, predominantly caused by *Aspergillus fumigatus*. Only four classes of agents, the azoles, polyenes, candins and 5-Flucytosine (5FC) are available to treat clinical infection. 5FC, a derivative of the nucleobase cytosine is rarely used as a monotherapeutic agent to treat *Aspergillus* infection as resistance develops rapidly. In addition at neutral pH (pH 7.0) the *in vitro* activity of 5FC against *A. fumigatus* is insignificant, however, 5FC is highly active at low pH (pH 5.0).
In this work we provide an explanation for the low efficacy of 5FC at pH 7.0 and uncover two transcription factors that mediate resistance to 5FC - The CCAAT binding complex (CBC) and the pH regulator PacC. Genetic inactivation of the CBC and PacC results in derepression of the purine-cytosine permease encoding gene *fcyB*, which results in 5-FC hypersusceptibility phenotypes. We show that *fcyB* expression is repressed at pH 7.0 and transcriptionally activated at pH 5.0. Disruption of *fcyB* results in hyperresistance to 5FC in wt and, moreover, significantly increases 5FC resistance in both the CBC and PacC mutant backgrounds independent of the pH. In contrast, CBC/PacC independent overexpression of *fcyB* using a conditional expression system results in 5FC hypersusceptibility. ChIP-Seq based genome-wide binding analysis using a strain expressing *gfp*-tagged *hapC* (*hapC*^{GFP}) uncovers the CBC as direct regulator of *fcyB.*
Collectively, within this study we characterised the cytosine permease encoding gene *fcyB* the expression of which is under combinatorial transcriptional control of the CBC and PacC. Furthermore, we show that both regulators act as repressors of *fcyB* during pH 7.0, which results in 5FC resistance.

Chloramphenicol inhibits appressorium formation of *Magnaporthe oryzae* via a Ser/Thr-protein phosphatase, *MoDullard*. *A. Nozaka*[1], S. Endo[1], N. Tanaka[1], M. Narukawa[1], S. Kamisuki[2], M. Nakajima[1], H. Taguchi[1], F. Sugawara[1], T. Kamakura[1] 1) App. Bio. Sci., Tokyo Univ. of Sci., Chiba, Japan; 2) Dept. Vet. Med., Azabu Univ., Tokyo, Japan.

The appressorium formation of *Magnaporthe oryzae* is essential for infection and related to a cell differentiation. Some drugs inhibiting appressorium formation will also affect the factor which is involved in eukaryotic cell differentiation in the rice blast fungus. We searched novel targets of preexisting drugs using appressorium formation of *M. oryzae* as an indicator whether the factor relates cell differentiation. As a result, antibiotic Chloramphenicol (Cm) showed remarkable inhibition of appressorium formation. Since Cm interacts to 50S ribosomal subunit in prokaryotes, Cm inhibits protein synthesis of prokaryotes specifically. However, in human Cm often causes a serious side effect aplastic anemia the target of Cm in eukaryote is unknown. If we can discover the unknown eukaryotic target of Cm, it will lead to contribute to clarifying the mechanism of the side effect caused by Cm, to develop a novel pesticide or rice blast specific fungicide, and/or to obtain the knowledge about the regulation of cell differentiation and appressorium formation.

We selected T7 phage display as a tool for searching Cm's target(s). From the candidate, we deduced that a Ser/Thr protein phosphatase Dullard (*MoDullard: MGG_03646*) was a possible binding protein of Cm. Using RTPCR, we detected the expression of *MoDullard* during appressorium formation. Then we acquired the GST-tag fused *MoDullard* expressed by *E. coli* and showed that the tag fused MoDullard protein bound to Cm. Moreover in the appressorium formation assay, *MoDullard* over expressed strains showed the lower sensitivity to Cm than WT. Each results inferred that MoDullard interacts with Cm in *M. oryzae* and affects appressorium formation of the rice blast fungus.

Now we challenge to establish *MoDullard* knockout and knockdown strain, and as soon as obtainment we are going to check the phenotype of them against Cm in appressorium formation assay. We also expect that the analysis of *MoDullard* for the mechanism of appressorium formation will shed new light on the mechanism of side effect of Cm, and contribute to development of antifungal agents.

Plasticity of the *MFS1* promoter leads to multi drug resistance in the wheat pathogen *Zymoseptoria tritici*. Selim Omrane[1], Colette Audéon[1], Amandine Ignace[1], Clémentine Duplaix[1], Lamia Aouini[2], Gert Kema[2], Anne-Sophie Walker[1], *Sabine Fillinger*[1] 1) BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, FR; 2) Wageningen University, Plant Research International, Wageningen, NL.

The ascomycete *Zymoseptoria tritici* is the causal agent of septoria leaf blotch on wheat. Disease control relies mainly on resistant wheat cultivars and on fungicide application. The fungus however displays a high potential to circumvent both methods. Resistance against all major fungicides has been observed and studied over decades. Especially the adaptation to DMIs, inhibiting sterol biosynthesis, implying modifications of the *CYP51* target gene has revealed a large spectrum of resistance mutations. Recently introduced fungicides are facing

resistance through target gene mutations as well. A different type of resistance has evolved among *Z. tritici* populations affecting multiple chemical families (Leroux & Walker, 2011). Strains considered as multi-drug resistant have been isolated since 2009.
Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs used against them. MDR is relying on an overexpressed efflux transport system that actively expulses the toxic agent outside the cell. We showed that active drug efflux was at play in *Z. tritici* MDR field strains and we identified the major-facilitator gene, *MFS1,* as principal player of this emerging resistance mechanism (Omrane et al., 2015). The *MFS1* gene was found overexpressed in all tested field strains. At the origin of the overexpression and the MDR phenotype is a 519 bp insert in the *MFS1* promoter, an LTR reminiscence of a recent retro-transposition event. The insert harbors four copies of a highly conserved transcription-factor binding-site that may explain *MFS1* overexpression. Besides this type of insert, we found a different type of promoter insert in more recent MDR strains. Interestingly, this last insert was found upstream of other genes in different *Z. tritici* strains. It also contains potential transcription factor binding sites. Finally, a 3rd type of insert was identified in two other *MFS1* overexpressing MDR strains.
Altogether, these results underline the extremely high adaptive potential of *Z. tritici* to fungicides through the plasticity of the *MFS1* promoter leading to its over-expression, to fungicide efflux and, ultimately, to MDR (Omrane et al., in prep).

Leroux & Walker, (2011) *Pest Manag Sci* **67**: 44-59.
Omrane, et al. (2015) *Env. Microbiol.* **17**: 2805-2823.

Defining the role of heteroresistance in persistent human fungal infections. *I.V. Ene*[1], K. Navarrete[1], E. Fu[1], J.M. Bliss[1,2], A.L. Colombo[3], C. Cuomo[4], R.J. Bennett[1] 1) Molecular Microbiology and Immunology, Brown University, Providence, Rhode Island, USA; 2) Women & Infants Hospital, Providence, Rhode Island, USA; 3) Universidade Federal de São Paulo, São Paulo, Brazil; 4) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

A critical problem in the clinic is the inability of available drugs to clear systemic fungal infections. In some cases, therapeutic failure is due to drug resistance, in which isolates evolve inherent resistance to the drug. However, in many cases persistent infections involve the emergence of tolerant cells (TCs) in the population, a process termed *heteroresistance*. To understand the mechanisms underlying persistence, we performed genome sequencing on a set of *Candida albicans* isolates that were drug susceptible yet persisted during antifungal therapy, and compared this set to isolates that were successfully cleared by drugs. Preliminary analyses indicate that clonal lineages often persisted during the infection, with isolates experiencing large-scale genetic changes including loss of heterozygosity (LOH) across large tracts of the genome. Persistent isolates were less susceptible to drugs and more likely to form TCs compared to isolates that were rapidly cleared from patients upon drug treatment. This implies the existence of a subset of resilient cells with distinct properties from the majority of the cells in the population. TCs formed by persistent isolates grew under much higher drug concentrations than rare TCs formed by cleared isolates. These high resistance levels were gradually lost with growth in the absence of drug, indicating that this phenotype is transient. Similar to bacterial TCs, *C. albicans* TCs have a fitness defect relative to the whole population in the absence of drug, but rapidly outcompete the rest of the population in the presence of drug. To understand how tolerance arises in a population, further genomic and genetic analyses are being performed. This includes screening of a library of all *C. albicans* transcription factors to identify genes that enable TC formation in response to different antifungals. Several gene families modulate tolerance, including telomeric and stress response genes. This study shifts the current paradigm that primary host status dictates persistence, and reveals that intrinsic *C. albicans* properties can also contribute to this important clinical phenomenon.

Functional and subcellular localization analyses of transcription factors, AtrR and SrbA, involved in azole drug resistance in *Aspergillus oryzae*. K. Sugiyama[1], A. Ohba-Tanaka[1], D. Hagiwara[2], S. Kawamoto[2], M. Tanaka[1], T. Shintani[1], *K. Gomi*[1] 1) Graduate School of Agricultural Science, Tohoku University, Sendai, JP; 2) Medical Mycology Research Center, Chiba University, Chiba, JP.

We previously demonstrated that a novel $Zn(II)_2Cys_6$ transcriptional factor, AoAtrR, regulates gene expression of the ABC transporters that would function as drug efflux pumps and contributes to the azole drug resistance in *Aspergillus oryzae, and* a disruption mutant of the *AoatrR* ortholog (*AfatrR*) in *Aspergillus fumigatus* was similarly hypersensitive to azole drugs. In addition, RNA-seq analysis in *A. fumigatus* showed that AtrR regulated several ergosterol biosynthetic genes including *erg11*. Surprisingly, most of these ergosterol biosynthetic genes regulated by AtrR were nearly identical to those regulated by the basic helix-loop-helix transcription factor, SrbA (1). Therefore, we are interested in and investigate the difference in function between AtrR and SrbA in *Aspergillus oryzae*.
The expression of ergosterol biosynthetic genes such as *erg11*, *erg24*, and *erg25*, as well as ABC transporter genes, was significantly down-regulated in the *AoatrR* disruptant. Similarly, *AosrbA* disruption resulted in remarkable down-regulation of ergosterol biosythetic genes same as in *A. fumigatus*, but not ABC transporter genes. In contrast, *AoatrR* overexpression did not resulted in up-regulation of ergosterol biosythetic genes. These results suggested that AtrR and SrbA coordinately regulate ergosterol biosynthetic genes in aspergilli. However, *AoatrR* or *AosrbA* disruption had apparently no effect on another gene expression level, suggesting their expression would be regulated independently of each other. On the other hand, the *AoatrR* disruptant was more hypersensitive to azole drugs compared to the *AosrbA* disruptant and sensitive comparable to the *AoatrR*/*AosrbA* double disruptant, suggesting that hypersensitivity of the *atrR* disruptant to azole drugs is attributed not only to lowered ergosterol levels owing to down-regulation of ergosterol biosynthetic genes, but also to reduced efflux transport of the drugs owing to down-regulation of ABC transporter genes. Subcellular localization analysis of AoAtrR and AoSrbA showed that AoAtrR was constitutively localized in the nucleus and AoSrbA was likely localized in the nuclear envelope and/or endoplasmic reticulum.
(1) Hagiwara et al., *PLoS Pathogens*, in press.

Molecular mechanisms of multidrug resistance conferred by xenobiotic detoxification in *Sclerotinia homoeocarpa*. *Geunhwa Jung*[1], Hyunkyu Sang[1], Jon Hulvey[2], Robert Green[1], Taehyun Chang[3] 1) University of Massachusetts, Stockbridge School of Agriculture, 161 Holdsworth Ways, Amherst, MA; 2) University of Massachusetts, Biology Department, 611 North Pleasant Street, Amherst, MA; 3) Kyungpook National University, School of Ecology & Environmental System, Sangju, South Korea.

Dollar spot, caused by the "sterile" ascomycete fungus *Sclerotinia homoeocarpa* is the most economically important disease on high amenity turfgrass. Fungicide resistance in *S. homoeocarpa* has developed in multiple fungicide classes through target gene mutations and

multidrug resistance (MDR) is conferred by a xenobiotic detoxification system. Multidrug resistance has been a major impediment in treating cancer patients and is an emerging problem in plant and human pathogenic fungi. In cancer cells, a single nuclear receptor, PXR (pregnane X receptor) has been known to play a pivotal role in concomitantly regulating drug-induced elevated changes in Phase I metabolizing enzymes, Phase II conjugating enzymes, and Phase III efflux transporters. We also uncovered a mechanistic analogue of PXR, single fungal-specific transcription factor (ShXDR1) in *S. homoeocarpa* that coordinately regulates Phase I cytochrome P450 (CYP450) enzymes in drug metabolism, and Phase III ATP-binding cassette (ABC) transporters in drug secretion, with a differential substrate specificity for detoxification of multiple xenobiotics. This appears to be the first report establishing a molecular mechanism for the regulation of antifungal/xenobiotic detoxification conferring multidrug resistance in filamentous plant pathogenic fungi.

Drug resistance via RNAi-dependent epimutations in *Mucor circinelloides*. *Z. Chang*[1], B. Billmyre[1], S.C. Lee[2], J. Heitman[1] 1) Duke University, Durham, NC; 2) University of Texas at San Antonio, San Antonio, TX.

The opportunistic fungal infection mucormycosis is notable for high morbidity and mortality as well as increasing incidence. Treatment is complicated by the fact that *Mucor circinelloides*, a major cause of mucormycosis, demonstrates high intrinsic resistance to the majority of antifungal agents. However, the mechanisms driving this extensive resistance remain poorly understood. Previous work demonstrated that *Mucor* is capable of developing transient resistance to the antifungal FK506 through a novel, RNA interference-dependent mechanism known as epimutation. Epimutants silence the drug target gene and can be selected by exposure to FK506; the target gene is re-expressed in these strains following passage in the absence of FK506 selective pressure. This silencing process involves the generation of small RNAs (sRNA) against the target gene via core RNAi pathway proteins including RNA-dependent RNA polymerases, Dicer, and Argonaute. To further investigate the role of epimutation in *Mucor*'s intrinsic antifungal resistance, we studied the development of resistance to a second drug, 5-fluoroorotic acid (5-FOA). We have identified epimutants that exhibit resistance to 5-FOA without mutations in either of the target genes, *pyrF* or *pyrG*. We conducted sRNA hybridization analysis to document the presence of sRNA against *pyrF* or *pyrG* in these epimutants, and demonstrated this sRNA is lost after reversion to drug sensitivity. Analysis of sRNA libraries generated from *pyrF* and *pyrG* epimutants demonstrated expression of sRNA against the *pyrF* and *pyrG* loci, respectively. From this work, we conclude that epimutation is a general mechanism through which *Mucor* can develop resistance to multiple antifungal agents. Elucidation of the role of RNAi in epimutation will afford us a fuller understanding of mucormycosis and also fungal pathogenesis and drug resistance more generally.

Friday, March 17 3:00 PM – 6:00 PM
Chapel
Cell Communication

Cell differentiation and interaction within developing yeast colonies. *Zdena Palkova*[1], Libuse Vachova[2] 1) Faculty of Science, Biocev, Charles University, Prague, Czech Republic; 2) Institute of Microbiology CAS, v.v.i., Biocev, Czech Republic.

Yeast colonies have become an excellent model for investigating various aspects of microbial multicellularity, including processes involved in cell differentiation, signaling and interaction. Two major types of *Saccharomyces cerevisiae* colonies can be classified according to their architecture: smooth colonies formed by laboratory strains and structured biofilm colonies, formed by wild strains. We have analyzed these colonies and identified several specifically localized cell subpopulations that have distinct properties and functions and even mutually interact (reviewed in *FEMS Yeast Res* 14, 96–108, 2014). Smooth *S. cerevisiae* colonies are composed of the two major cell subpopulations: U cells in upper regions that activate adaptive metabolism and gain a longevity phenotype and L cells in lower regions that behave like starved and stressed cells, activating different hydrolytic mechanisms and providing nutrients to U cells (*Mol Cell* 46:436, 2012). Using transcriptomic and confocal microscopy approaches, combined with other analytical methods and modified yeast strains, we have identified metabolic and regulatory pathways that are advantageous to the long-term vitality of these subpopulations. We have shown that differently modified mitochondria of U and L cell subpopulations are involved in the control of cellular reprogramming and the activation of specific metabolic pathways via retrograde (RTG) signaling (*Oncotarget* 7: 15299, 2016). We have identified three distinct pathways of mitochondrial RTG signaling that are all dependent on functional Rtg1p, Rtg2p and Rtg3p regulators. However, each of these pathways activates expression of specific gene targets and thus leads to divergent metabolic reprogramming and subsequent effects on the survival of specific cell subpopulations in developing colonies. These data showed that the RTG response in yeast is more complex than previously assumed, involves a number of yet to be identified regulatory elements and affects different cellular processes and the subsequent fate of differentiated yeast cells. This research was funded from the Norwegian Financial Mechanism 2009-2014 under Project Contract no. 7F14083 and by GACR 15-08225S.

Crosstalk between pheromone response pathway and STRIPAK complex regulates developmental processes in *Sordaria macrospora*. *A. Beier*, B. Ramšak, I. Teichert, U. Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE e-mail: anna.beier@rub.de.

Developmental processes are often regulated by signal transduction pathways that include phosphorylation as a signal. These pathways are not linear and require additional regulation, which is often provided by feedback loops and crosstalk. In filamentous ascomycetes, the three mitogen-activated kinase (MAPK) cascades that primarily respond to cell wall stress (CWI), pheromone exposure (PR), and osmotic stress (OS). Besides these specific functions, fungal MAPK cacades are involved in e.g. vegetative growth, cell-to-cell communication, as well as sexual and asexual development. MAPK cascades are highly conserved eukaryotic phospho-signaling modules, which are tightly regulated by feedback loops, crosstalk, and phosphatase-catalyzed signal modulations. Here, we show an interaction of the PR pathway and the striatin interacting phosphatase and kinase (STRIPAK) complex, which is a major regulator of hyphal fusion and fruiting body development in the ascomycete *S. macrospora.* Phosphorylation levels of MAK2, the MAPK of the PR pathway, in STRIPAK deletion strains suggest an association of both complexes. In yeast two-hybrid analysis, we tested the composition of the PR module with constructs encoding for MAPKKK MIK2, MAPKK MEK2, MAPK MAK2, and the putative scaffold protein, which is homologous to HAM-5 from *Neurospora crassa*. Further, yeast two-hybrid showed a physical interaction of PR components with the STRIPAK subunit PP2Ac1. This STRIPAK subunit is a catalytic subunit of the S/T protein phosphatase 2A (PP2A) and governs sexual development, hyphal fusion, and cell wall stress response in fungi (1). Deletion strains for PR pathway subunits were generated and functionally characterized. Phenotypic analysis point towards a similar function of PR and STRIPAK subunits in developmental processes. Together with previous results that revealed a connection of STRIPAK to the CWI MAPK cascade, our findings suggest a regulatory function of STRIPAK in phospho-signaling of both MAPK cascades.

1. Beier A, Teichert I, Krisp C, Wolters DA, Kück U. 2016. Catalytic subunit 1 of protein phosphatase 2A is a subunit of the STRIPAK complex and governs fungal sexual development. mBio 7(3):e00870-16. doi:10.1128/mBio.00870-16

Nod-like receptor mediated allorecognition induces germling regulated death in *Neurospora crassa*. *J. Heller*, N. L. Glass Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA.

Allorecognition-induced death in filamentous fungi has been proposed to function as a type of fungal innate immunity system, whereby recognition of non-self reduces the risk of transmission of pathogenic elements between colonies and exploitation by aggressive genotypes. Cellular fusion of vegetative cells is an essential process for fungal development and can occur between hyphal cells of an individual colony or between hyphal cells of genetically distinct colonies. Heterokaryon incompatibility (HI), a form of allorecognition, is one mechanism to restrict the propagation of fusion products between genetically dissimilar individuals by inducing a programmed cell death (PCD) response. HI and PCD have been considered to only occur after the fusion of mature hyphae while being suppressed during germling fusion.
Here, we present the identification of allorecognition mechanisms that act at the germling stage. If germlings of various wild isolates are of different specificity at so-called germling regulated death (GRD) loci, allorecognition mediates rapid cell death following membrane merger between germlings (~20 min). Flow cytometry experiments showed that death is 100% penetrant (all fused germlings of different GRD background die). By bulk segregant analysis (BSA) of progeny from a cross between two wild isolates followed by whole genome re-sequencing we identified two linked loci (*plp-1* and *sec-9*) that segregated with the GRD phenotype. The genes *plp-1* and *sec-9* have typical characteristics of allorecognition genes: they show discrete haplotypes that have a high level of polymorphism with long-term persistence through multiple speciation events (trans-species polymorphism), supporting the hypothesis that balancing selection is acting on these loci. SEC-9 is an essential SNARE protein predicted to be involved in membrane fusion and exocytosis. PLP-1 is a nod-like receptor similar to intracellular pattern recognition receptors (NLRs) that mediate recognition of microbe-associated molecular patterns, the initial step of innate immunity in animals and plants. Analyses of death frequencies in various mutants of *plp-1* and *sec-9* confirmed that non-allelic

interaction between *plp-1* and *sec-9* is required for allorecognition and GRD. By creating chimeric proteins we identified domains in SEC-9 and PLP-1 that are essential for the PCD induction and specificity. We propose a model in which PLP-1 acts as a NLR in fungal innate immunity mediating recognition of SEC-9.

Sex pheromone autocrine signaling controls vegetative fungal development. S. Vitale, A. Di Pietro, *D. Turrà* Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Cordoba, Spain,.

Mating in heterothallic ascomycetes involves paracrine signaling between two opposite mating types producing two different peptide pheromones, a and α. Inappropriate self-signaling is prevented by protease-mediated pheromone degradation and transcriptional shut-down of the cognate receptor. Here we show that in the heterothallic fungal pathogen *Fusarium oxysporum,* which lacks a known sexual cycle, pheromone signaling follows a distinct configuration in which both pheromone/receptor pairs are co-expressed, resulting in autocrine signaling. Unexpectedly, mutants lacking a given peptide pheromone or its cognate receptor displayed increased sensitivity towards the opposite pheromone, as well as defects in vegetative hyphal fusion or cell-cell agglutination. Our results support a model in which a and α pheromone coordinately control key developmental processes in *F. oxysporum*, revealing a new role for autocrine pheromone signaling in vegetative fungal development.

Fungal-plant communication in the arbuscular mycorrhizal symbiosis. *N. Requena*, R Betz, S Heidt, M Hartmann, S Walter Karlsruhe Institute of Technology, Karlsruhe, DE.

The arbuscular mycorrhiza (AM) symbiosis represents one of the most ancient and widespread symbiosis on the earth, characterized by an intimate life-long association between Glomeromycotan fungi and most plant roots. The establishment and maintenance of the symbiosis requires a complex communication exchange between symbionts and secreted fungal effector proteins might be key to facilitate colonization and nutrient exchange. Compared to other biotrophic fungi, the effectome of the AM fungus *Rhizophagus irregularis* seems to be rather small, considering the small number of proteins identified containing a secretion signal. Interestingly, *R. irregularis* is able to colonize the majority of land plants in contrast to most plant colonizing fungi that have a quite narrow host range. We thus hypothezise that the few effector candidates identified are conserved in other AM fungi and might play crucial roles in rewiring the plant physiology. We previously identified the effector SP7 (secreted protein 7) the first characterized AM effector, and a group of related proteins (SP7-like2, SP31 and SPrubi). All members of this family show the same basic protein structure with a central domain of tandem, hydrophilic and imperfect repeats and a nuclear localization signal. Although there is evidence of a positive effect on symbiosis for SP7, the molecular mechanisms underlying the symbiotic function of the SP family remain elusive. Detailed *in planta* co-localization experiments with defined cell markers together with interactomic studies revealed a direct association of the SP effectors with spliceosomal components, the nonsense mediated decay and plasmodesmata. Thereby Processing bodies (specific cytoplasmic RNA-Protein granules) could be identified as a novel plant target of fungal effectors. Our results point out towards a significant involvement of AM fungal SP effectors in the conserved plant mRNA processing machinery leading to translational control. Recent results will be presented.

More than just a funky smell: filamentous fungi use molecular vapors to communicate. *J.W. Bennett* Dept Plant Biol & Pathology, Rutgers Univ, New Brunswick, NJ.

Fungal volatile organic compounds (VOCs) are found as odiferous mixtures of alcohols, aldehydes, acids, ethers, esters, ketones, hydrocarbons, terpenes and sulfur compounds, and produced at varying concentrations depending on the species, substrate, incubation period and other environmental parameters. They are known as aroma compounds in foods and beverages and also have been used as indicators of fungal growth. There is increasing evidence that many of them serve as chemical signals influencing growth, germination, and pathogenicity. Our laboratory has used genetic models for testing the bioactivity of fungal VOCs. Certain mixtures of VOCs emitted from living cultures of molds isolated after Hurricanes Katrina and Sandy, as well as low concentrations of chemical standards of some individual VOCs, are toxic in *Drosophila melanogaster*. For example, low concentrations (0.5-2.8ppm) of “mushroom alcohol” (1-octen-3-ol) exhibit potent neurotoxicity in flies, specifically against dopaminergic neurons. When a yeast knock out library is screened for resistance to 1-octen-3-ol, ninety-one resistance genes are identified, the majority of which are involved in protein trafficking. Specifically, when either the retrograde (retromer and GARP complexes) or anterograde (ESCRT) sorting pathways are disrupted, yeast cells are resistant to 1-octen-3-ol.

Screening of twenty *Trichoderma* isolates for VOC-induced growth of *Arabidopsis* identified 9 growth promoting isolates increasing plant biomass (up to 41.6%) and chlorophyll content (>89.3%). GC-MS analysis of *Trichoderma* isolates identified over 147 compounds and several unknown terpenes. Twenty six chemical standards, representing common and uncommon fungal VOCs, were evaluated for their effects on *A. thaliana* seeds and vegetative plants growth. Several individual compounds were able to induce similar growth promoting effects at 0.5 µg and 10 ng concentrations. Due to plant growth promoting effects at relatively high and low concentrations, 1-decene was selected for plant gene expression studies, RNA sequencing data identified several candidate genes inducible by the plant hormone auxin, as well as down regulation of a large number of genes related to plant defense and stress responses.

We propose the term “mycosemiotics” to describe the study of vapor-phase signaling molecules, as well as better known hormone and secondary metabolites, that fungi use in intra- and inter-specific communication.

Fatal attraction: nematophagous fungus *A. oligospora* mimics olfactory cues of sex and food to lure nematodes. *Y. Hsueh*[1], M. Gronquist[3], E. Schwarz[4], R. Nath[2], F. Schroeder[5], P. W. Sternberg[2] 1) IMB, Academia Sinica, Taipei, TW; 2) Howard Hughes Medical Institute and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA; 3) Department of Chemistry, State University of New York at Fredonia, Fredonia, NY 14063, USA; 4) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA; 5) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA.

To study the molecular basis for predator-prey coevolution, we investigated how *Caenorhabditis elegans* responds to the predatory fungus

Arthrobotrys oligospora. *C. elegans* and other nematodes were attracted to volatile compounds produced by *A. oligospora*. Gas-chromatographic mass-spectral analyses of *A. oligospora*-derived volatile metabolites identified several odors mimicking food cues attractive to nematodes. One compound, methyl 3-methyl-2-butenoate (MMB) additionally triggered strong sex- and stage-specific attraction in several *Caenorhabditis* species; furthermore, when MMB is present, it interferes with nematode mating, suggesting that MMB might mimic sex pheromone in *Caenorhabditis* species. Forward genetic screening suggests that multiple receptors are involved in sensing MMB. Response to fungal odors involves the olfactory neuron AWCs. Single-cell RNA-seq revealed the GPCRs expressed in AWC. We propose that *A. oligospora* likely evolved the means to use olfactory mimicry to attract its nematode prey through the olfactory neurons in *C. elegans* and related species.

Membrane traffic in *Candida albicans* hyphal growth and virulence. Hayet Labbaoui[1], Stephanie Bogliolo[1], Rohan Wakade[1], Daniele Stalder[1], Norma Solis[2], Scott Filler[2], Robert Arkowitz[1], *Martine Bassilana*[1] 1) University of Côte d'Azur/ CNRS/ INSERM, Institute of Biology Valrose, Nice, France. ; 2) Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA.

Virulence of the human fungal pathogen *Candida albicans* depends, in particular, on the switch from budding to filamentous growth, which requires both polarized growth and sustained membrane traffic. Endo- and exocytosis are critical for hyphal growth (1) and the balance between these two processes is likely to be regulated by Rab (Ras-related proteins in brain) (2) and Arf (ADP-ribosylation factor) GTPases, which are key players in membrane traffic. Here, we focused on the role of Arf proteins in *C. albicans*. Previous studies have determined that the putative Arf GAP (GTPase activating protein) Age3 is required for filamentous growth, cell wall integrity and virulence, as well as hypersensitivity to antifungal drugs (3, 4). Our results indicate that of the 3 Arf proteins, Arf1-Arf3, and the 2 Arf-like proteins, Arl1 and Arl3, Arf2 is required for viability and hypersensitivity to antifungal drugs. We also show that Arf2 and Arl1 are required for invasive hyphal growth, with an *arf2* repressible mutant unable to form hyphae and the hyphae of an *arl1* deletion mutant 2-fold shorter than that of the wild-type. Both mutants are drastically reduced in virulence, with *arl1* particularly defective in oropharyngeal candidiasis. Further characterization indicated that only the *arf2* mutant is altered in hyphal-specific gene induction and Golgi integrity. In contrast, the *arl1* mutant is defective in restricting growth to a single site and exhibits increased secretion. Together, these results indicate that Arf2 and Arl1 function in different processes to regulate filamentous growth. To investigate the function of Arl1, we examined the impact on filamentous growth of a number of potential Arl1 interactors, such as that of the sole Golgin protein Imh1, the lipid flippase Drs2 and the Rab GTPase Ypt6, and are currently using non-biased approaches to probe genetic/ biochemical interactions.

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Johansen *et al.*, 2016, PLoS Biology, **14**(8): e1002534.
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Friday, March 17 3:00 PM – 6:00 PM
Fred Farr Forum
Epigenetics and Post-Transcriptional Control Mechanisms

The histone methyltransferases FfSet2 and FfAsh1 deposit H3K36 methylation at specific loci, having a major impact on vegetative growth, sporulation, secondary metabolism and virulence in *Fusarium fujikuroi*. *S. Janevska*[1], L. Baumann[1], J. Ulrich[2], J. Kaemper[2], B. Tudzynski[1] 1) Institute of Plant Biology and Biotechnology, University of Muenster, Muenster, DE; 2) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, DE.

Fusarium fujikuroi is well-studied due to its ability to produce highly bioactive plant hormones, gibberellic acids, that cause the *bakanae* disease of rice plants. In addition, *F. fujikuroi* possesses a great potential to synthesize a vast range of other secondary metabolites (SMs). The manipulation of chromatin-mediated regulation represents a powerful tool for the activation of "cryptic" SM gene clusters without yet assigned product. In the present work, we focused on the histone 3 lysine 36 methylation (H3K36me) mark which is generally associated with transcriptional activation. In budding yeast, one single methyltransferase ScSet2 confers H3K36me, while there are several enzymes dedicated to this methylation in higher eukaryotes. In filamentous fungi, only the Set2-homolog has been described as H3K36-specific methyltransferase so far.
We show that the *F. fujikuroi* Set2-homolog FfSet2 is responsible for conferring the bulk amount of H3K36me2/me3, however a significant activity remains in Δ*ffset2* mutants. Bioinformatic analysis identified a second homolog of the Set2-family in *F. fujikuroi*, FfAsh1, which is conserved among filamentous fungi. Deletion of *FfASH1* resulted in only a slight decrease of global H3K36me3. But ChIP-Seq analysis revealed that both methyltransferases deposit H3K36me3 at different and very specific loci. Δ*ffset2* and Δ*ffash1* mutants are viable, however strongly attenuated in their vegetative growth and conidiation. Microarray analysis underlined a large impact on the expression of both known as well as unknown SM gene clusters. Production of gibberellic acids is nearly fully abolished *in vitro*, going in line with a decreased pathogenicity of the deletion mutants in rice infection assays.
Furthermore, to shed more light onto the role of H3K36me in the regulatory network of gene expression, we also analyze the H3K36-specific demethylase, which counteracts the activity of FfSet2 and FfAsh1.

Regulators of histone H3K4 trimethylation influence transcription of secondary metabolite gene clusters in *Aspergillus* and *Fusarium* species. *L. Studt*[1], S. Bachleitner[1], A. Gacek-Matthews[1], Z.A. Lewis[2], J. Strauss[1] 1) BOKU University of Natural Resources and Life Science Vienna, Tulln, AT; 2) University of Georgia, Athens, GA.

Fungi produce a vast array of secondary metabolites (SMs) that contribute to the fitness of fungi in their natural environment. To prevent an unnecessary use of resources in times their beneficial properties are not required, SM gene expression is adapted to environmental and developmental signals by pathway-specific as well as wide-domain regulators operating also at the level of chromatin. One of the crucial histone posttranslational modifications (PTMs) in active chromatin is trimethylation of lysine 4 in histone H3 (H3K4me3), although this mark was also shown to be necessary for subtelomeric gene silencing. While this histone PTM was shown to be associated with active transcription also in *Aspergillus* and *Fusarium* species, it was surprising to observe that in both fungi SM gene clusters are largely devoid of this histone mark even under activated conditions[1-3]. Intriguingly, lack of CclA, a regulatory component of COMPASS (complex associated with Set1) and required for genome-wide H3K4me3, resulted in induction of subtelomeric SM gene clusters in *A. nidulans*[4]. We observed a similar phenotype also for the two plant-pathogenic fungi *Fusarium fujikuroi* and *Fusarium graminearum*. At the opposite side of the pathway, enzymes of the KDM5-family of histone demethylases remove one or two methyl groups from trimethylated H3K4 thereby functioning mainly as repressors. We studied the homologous protein designated KdmB in *A. nidulans* and showed that it indeed negatively regulates hundreds of genes but appears to be a specific activator of many SM gene clusters[1]. We started to dissect the function of KdmB domains and inactivated the highly conserved catalytic JmjC demethylase domain by point mutations. Subsequent functional characterization suggests that KdmB-mediated repression is independent of the catalytic function at selected SM gene clusters, whereas the activating role requires the integrity of the JmjC domain. Whether the KdmB-homolog Kdm5 has a similar role in SM gene regulation in *F. graminearum* is currently under investigation. Taken together, this comparative view indicates that proteins regulating the H3K4me2/me3 status play roles in the regulation of SM gene expression despite the fact that the clusters themselves are largely devoid of this histone mark.

[1]Gacek-Matthews et al. 2016, PLoS Genet. 12:e1006222
[2]Connolly et al., 2013, PLoS Genet. 9:e1003916
[3]Wiemann et al., 2013 PLoS Pathog. 9:e1003475
[4]Bok et al. 2009, Nat Chem Biol. 5:462-464

Influence of histone modifications on effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. Jessica L. Soyer[1], Colin Clairet[1], Julie Gervais[1], Marie-Hélène Balesdent[1], Lanelle R. Connolly[2], Michael Freitag[2], Thierry Rouxel[1], *Isabelle Fudal*[1] 1) INRA / AgroParisTech, UMR 1290 BIOGER, Thiverval-Grignon, France; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, USA.

Leptosphaeria maculans, the fungus responsible for stem canker of oilseed rape (*Brassica napus*), presents a particular genome structure divided into gene-rich and transposable element (TE)-rich regions. The latter are proposed to be heterochromatic domains, as they are gene-poor, TE-rich and present lower recombination rates than gene-rich regions. While overall gene-poor, TE-rich regions are enriched for putative effector genes specifically expressed during the early stages of plant infection ('early' effector genes). In contrast, effector genes expressed during the late stages of infection (endophytic growth and stem necrosis, 'late' effector genes) are located in gene-rich regions. We investigated the concerted expression of 'early' effector genes by assessing whether TE-rich regions could be targets of reversible chromatin modifications that affect the regulation of effector gene expression. We first examined the involvement of one histone modification, histone H3 lysine 9 tri-methylation (H3K9me3) in chromatin-based regulation of effector gene expression. For this purpose, we silenced expression of two key players in heterochromatin assembly and maintenance, *HP1* and *KMT1*, by RNAi. Silencing of *HP1* and

KMT1 led to an over-expression of 'early' effector genes in TE-rich regions during *in vitro* growth (but no induction of 'late' effector gene expression). The increase of expression was associated with a reduction of H3K9 tri-methylation at two effector-encoding gene loci. Our data strongly suggest that a chromatin-based control, mediated by HP1 and KMT1, represses the expression of at least part of the 'early' effector genes located in TE-rich regions during growth in axenic culture. We are currently investigating the role of another key player in heterochromatin assembly, KMT6, involved in the heterochromatic-associated histone modification H3K27me3, on the control of effector gene expression.

Mapping of *defective in silencing* (*dis*) mutants in *Fusarium graminearum* by bulk segregant analysis and high-throughput sequencing. *Kristina Smith*, Lanelle Connolly, Xiao Lan Chang, Brett Pierce, Corinne Fargo, Brian Josephson, Zackary Bango, Madison Esposito, Michael Freitag Dept. of Biochem & Biophysics, Oregon State University, Corvallis, OR.

Polycomb Group (PcG) proteins generate facultative heterochromatin in some fungi by trimethylating histone H3 lysine 27 (H3K27me3). Members of the conserved Polycomb Repressive Complex 2 (PRC2) include the H3K27 methyltransferase, KMT6, and binding partners SUZ12, EED, and CAF1-3 (MSL1). Loss of *kmt6*, *eed*, or *suz12* leads to complete loss of H3K27me3 accompanied by developmental defects and novel or increased expression of ~25% of all genes. Most genes silenced by PcG have unknown functions but are predicted to be involved in secondary metabolism and pathogenicity. To uncover suppressors of H3K27me3 silencing, and identify functional equivalents of PRC1, the complex that may initiate PRC2 action and also binds H3K27me3 in animals but does not exist in fungi, we developed a forward genetics approach utilizing UV mutagenesis. We inserted a *neo* reporter gene into the *pks2* region, which is reliably silenced by H3K27me3 in WT but de-repressed in the *kmt6* mutant. WT strains with *neo* at the heterochromatic locus were UV-irradiated, and mutants were selected on G418-containing medium. Dozens of primary mutants, which we call *defective in silencing* (*dis*), fall into several classes with distinct growth phenotypes and global gene expression patterns evidenced by transcriptome sequencing. Bulk segregants from *dis* mutants backcrossed to mapping strains with *mat* deletions to force outcrossing were sequenced and analyzed with the Genome Analysis Tool Kit to identify SNPs, insertions and deletions. The first mutant analyzed, *dis1*, carries a truncated *eed* gene, and completely phenocopies the deletion mutant we constructed. Here we will report on additional components of the PcG silencing system.

Rad51 plays a role in copy number-dependent *de novo* DNA methylation of a retrotransposon. B. Vu, Q. Nguyen, Y. Kondo, A. Iritani, T. Murata, *H. Nakayashiki* Faculty Agriculture, Kobe Univ, Kobe, JP.

Repetitive sequences are common targets for transcriptional and post-transcriptional gene silencing in eukaryotic genomes. However, molecular mechanisms responsible for sensing such repeated sequences in the genome remain largely unknown. Here we show that Rad51, which is a key enzyme in search for homologous sequences during DNA repair, is involved in copy number-dependent *de novo* DNA methylation of the retrotransposon MAGGY in the fungus *Magnaporthe oryzae.* Double knock-out (KO) of the *M. oryzae* Rad51 (Rhm51) and Ddnm1 (Deficient in De Novo Methylation 1) genes, resulted in a drastic reduction in the level of copy-number dependent-d*e novo,* but not maintenance, DNA methylation of MAGGY. A single rhm51 or ddnm1 KO mutant had a defect in DNA repair but showed only a slight reduction in the level of MAGGY DNA methylation. Co-immunoprecipitation and yeast-two-hybrid assays indicated that Ddnm1 and Rhm51physically interacted with each other. The decreased level of MAGGY DNA methylation was associated with a reduction in epigenetic marks for heterochromatin such as tri-methylation of histone H3 on lysine 9. Overall our results present a possible link between repeat-sensing machineries for DNA repair and heterochromatin formation at repetitive sequences.

Regulation of fungal effector gene expression through chromatin de-condensation. *M. Kramer*[1], D.E. Cook[1,2], M.F. Seidl[1], B.P.H.J. Thomma[1] 1) Laboratory of Phytopathology, Wageningen University & Research, Wageningen, NL; 2) Department of Plant Pathology, Kansas State University, Manhattan, KS.

Fungal plant pathogens require tight control over the expression of their effector genes which encode secreted proteins that facilitate host invasion. Failure to express such genes at the appropriate time or location during host invasion may lead to interception by the plant host, and thus failure of the infection. In many fungal plant pathogens, effector genes are not randomly distributed over the genome, but localized in distinct genomic regions that are enriched in transposable elements (TEs). TEs can transpose over the genome and thereby affect gene expression or functionality, which may negatively affect the organism. To control TE activity, TE-containing genomic regions are usually structured as heterochromatin, a highly condensed genomic structure that is not accessible to the transcription machinery. Consequently, TEs are generally silenced. Due to the close proximity of many effectors to TEs, they may be held in a co-silenced state. Consequently, upon encountering a host-plant, pathogens will require de-condensation of heterochromatin to appropriately express effector genes. To investigate whether the genomic regions containing effector genes are actively de-condensed during host colonization, we are using chromatin immunoprecipitation (ChIP) to selectively isolate heterochromatic DNA of the broad host-range fungus *Verticillium dahliae* grown *in vitro* and *in planta*. Additionally, we map the genome-wide positioning of nucleosomes to investigate differences between effector genes in TE-rich regions and genes that reside within core regions of the genome. This research will lead to a better understanding of the regulation of effector genes and reveal the importance of chromatin dynamics in this process.

The constitutive heterochromatin-defined genome organization in *Neurospora crassa* depends on subtelomeric facultative heterochromatin. *A. D. Klocko*[1], J. M. Galazka[2], T. Ormsby[1], S. Honda[3], M. Freitag[2], E. U. Selker[1] 1) Institute of Molecular Biology, University of Oregon, Eugene, OR; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR; 3) Faculty of Medical Sciences, University of Fukui, Fukui, Japan.

Eukaryotic genomes are organized into chromatin domains with three-dimensional arrangements that likely result from interactions between the chromatin constituents -proteins, DNA, and RNA- within the physical constraints of the nucleus. We used chromosome conformation capture (3C) followed by high-throughput sequencing (Hi-C) with wild-type and mutant strains of *Neurospora crassa* to understand the role of heterochromatin in genome organization and function. The average genome configuration of wild type revealed strong intra- and inter-chromosomal associations between both constitutive and facultative heterochromatic domains, yet elimination of H3K9me3 or its binding partner HP1 – both prominent features of constitutive heterochromatin – minimally alter the Hi-C pattern. It remained possible that the facultative heterochromatin mark H3K27me2/3, which is redistributed to constitutive heterochromatin upon H3K9me3 or HP1 loss, is necessary for genome organization. We found that H3K27me2/3, catalyzed by the PRC2 member SET-7, is

important for the Hi-C pattern of wild type but its redistribution upon H3K9me3 loss does not maintain genome architecture. The Hi-C pattern of a mutant defective in the PRC2 member *Neurospora* p55 (NPF), which is required for subtelomeric H3K27me2/3, was equivalent to that of the Δ*set-7* strain, suggesting that subtelomeric facultative heterochromatin is paramount for normal chromosome conformation. Cytological observations suggested loss of H3K27me2/3 partially displaces telomere clusters from the nuclear periphery. Thus, associations between heterochromatic regions are a major component of the Neurospora chromosome conformation but while two constitutive heterochromatin proteins are not necessary, H3K27me2/3 is required for the normal 3D genome organization in Neurospora. Also, the overall architecture of the genome remains largely intact even when key features of both constitutive and facultative heterochromatin are eliminated. Additional studies are needed to explore the functional significance of eukaryotic genome organization.

DNA sequence homology triggers cytosine-to-thymine mutation by a heterochromatin-related pathway. *Eugene Gladyshev*, Nancy Kleckner Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

In many eukaryotes, chromosomal regions with highly repetitive DNA are normally associated with cytosine methylation (5mC) and histone H3 lysine-9 di-/trimethylation (H3K9me2/3) in the context of heterochromatin. In the fungus *Neurospora crassa*, where the pathway of heterochromatin assembly has been characterized in great detail, H3K9me3 and 5mC are catalyzed, respectively, by a conserved SUV39 lysine methylase DIM-5 and a DNMT1-like cytosine methylase DIM-2. Here we show that DIM-2 can mediate cytosine-to-thymine (C-to-T) mutation of repetitive DNA during a process known as "RIP" (Repeat-Induced Point mutation). We further show that DIM-2-mediated mutation requires DIM-5 as well as a number of additional heterochromatin factors including HP1. The pathway can act effectively on tandem repeat arrays as well as on individual pairs of closely positioned or widely separated sequence copies. These results, when combined with our model for repeat recognition during RIP, define a new process by which heterochromatin can be nucleated specifically on repetitive DNA. Existing molecular mechanisms rely on cis-acting proteins or non-coding RNAs. We propose, instead, that formation of constitutive heterochromatin can be initiated by direct interactions between homologous double-stranded DNA segments. Given that the number of potential homologous interactions will scale with the number of repeat copies, this process could be particularly effective and important in heterochromatic silencing of large repetitive regions such as those associated with (peri)centromeric and (sub)telomeric regions. Also, irrespective of the repeat-recognition mechanism, our results implicate the DIM-5/DIM-2 pathway in the accelerated evolution of pathogenic genes that are typically found near repetitive elements in the genomes of many filamentous fungi.

Friday, March 17 3:00 PM – 6:00 PM
Kiln
Cytoskeletal Dynamics and Septa

The TOG protein Stu2 interacts with SUMO via multiple mechanisms. *Rita Miller*[1], Matt Greenlee[1], Annabel Alonso[1], Maliha Rahman[1], Savannah Morris[1], Nida Meednu[2] 1) Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, OK; 2) Department of Biology, University of Rochester, Rochester, New York.

Microtubule-associated proteins (MAPs) regulate a variety of functions for microtubule polymers. Many MAPs are regulated by phosphorylation, but little is known about how MAPs are regulated by SUMO, the Small Ubiquitin-like Modifier. SUMO can interact with proteins two ways, covalently or non-covalently. Covalent conjugation of SUMO occurs on the target protein through an isopeptide bond formed between the carboxyl group of the terminal glycine of SUMO and the epsilon amino group of the conjugated lysine residue. A non-covalent interaction occurs thorough the hydrophobic and ionic interactions of a "SUMO-Interacting Motif" within the interacting protein. The Miller lab has identified five new classes of MAPs that interact with SUMO. Here, we show that the TOG protein Stu2p interacts with SUMO by multiple mechanisms. Stu2 is the yeast homologue of the CKAP5/XMAP215 class of proteins that promotes microtubule dynamics by facilitating the loading of tubulin dimers onto microtubule ends. Although Stu2p plays a vital role in microtubule function, little is known about how it is regulated. Using two-hybrid analysis, we show that Stu2 interacts with Smt3/SUMO and several key members of the sumoylation system. Domain mapping of amino- and carboxy- terminal truncations show that the dimerization region of Stu2 is important for its interaction with SUMO. Using an *in vitro* sumoylation assay, four shifted bands of Stu2 can be observed. Higher molecular weight forms of Stu2 are seen in vivo. Pull-down assays in denaturing buffer containing urea demonstrate that an anti-SUMO reactive band co-migrates with Stu2. Inactivation of the Ulp1 SUMO protease with a temperature sensitive mutant reduces the levels of the higher molecular weight form of Stu2. Additional copies of SUMO further reduce the higher molecular weight form. Using mass spectrometry, we analyzed Stu2 for the expected signature "stub" of the wild-type SUMO motif. Stu2 also interacts with a SUMO-Targeted Ubiquitin Ligase that adds ubiquitin to SUMOylated proteins. Together, these results indicate that Stu2 is conjugated by SUMO. We hypothesize that SUMO conjugation destabilizes Stu2. To test whether Stu2 could interact non-covalently with SUMO, we analyzed Stu2 interaction with a non-conjugatable form of SUMO. Stu2 bound to the non-conjugatable SUMO but not to GST alone. Together our work suggests that Stu2 can interact with SUMO by multiple mechanisms, covalent and non-covalent.

Understanding the role of septin AspD in the early vegetative growth of *Aspergillus nidulans*. *I. Dorter*, M. Momany Plant Biology, UGA, Athens, GA.

Basic research on septin dynamics in the model organism *Aspergillus nidulans* helps us better understand the cellular and molecular mechanisms of these cytoskeletal elements and can help develop new therapeutic approaches against diseases. Under the right conditions *Aspergillus nidulans* spores emerge a germ tube to explore new growth medium. Thereby fungal hyphal tips grow into the food substrate forming a hyphal network with evenly spaced lateral branches. This basic process requires a fine and precise coordination of biochemical and cellular processes. We performed single deletions of the genes encoding for the core septins in *A. nidulans* (*AspA-D*). In the absence of AspA, AspB and AspC we could observe an abnormal increase of lateral branches, which is also known as hyperbranching. The knock-out of the fourth core septin gene *AspD* did not result in obvious phenotypic changes whereas fluorescence microscopy revealed abnormal nuclear structures that worsened with each mitotic division. In contrast to WT the average distance between two neighboring nuclei was greatly reduced in the *?aspD* mutant background, whereas the nuclei themselves were slightly elongated. Time-lapse analyses with a strain expressing AspD-GFP fusion protein showed septin bars contacting both nucleus and cell cortex. Shortly after nuclear division the septin bar located between the two newly divided nuclei suggesting that the septin AspD plays a role after mitosis. In addition, the disruption of the septins revealed an increased number of cells harboring an odd number of nuclei. This may point to an asynchronous nuclear division cycle of the multinucleated fungus in the absence of core septins. We asked the question whether septins could be part of a checkpoint that regulates nuclear division by monitoring cell cycle progression. We treated WT and septin deletion mutants with benomyl, which depolymerizes microtubules and inhibits spindle formation thereby activating the spindle assembly checkpoint. In contrast to the other cells all *ΔaspD* mutants showed changes in conidiospore density and color, a sectoring phenotype, which is the result of chromosome missegregation. This supports the idea that the SAC checkpoint did not function properly in the absence of AspD. We were able to construct a strain, which expresses both AspB-CFP and AspD-GFP and could confirm that at least two distinct septin heteropolymer populations co-exist. Future time-lapse analyses will show if these two populations play different roles during nuclear division in *A. nidulans*.

Function of the dynactin complex in dynein-mediated intracellular transport. Jun Zhang[1], Rongde Qiu[1], Xuanli Yao[1], Herb Arst[2], Miguel Penalva[3], *Xin Xiang*[1] 1) 1Department of Biochemistry and Molecular Biology, The Uniformed Services University of the Health Sciences- F. Edward Hébert School of Medicine, Bethesda, Maryland 20814, USA; 2) Microbiology Section, Department of Medicine, Imperial College London, London, SW7 2AZ, UK; 3) 3Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas CSIC, Ramiro de Maeztu 9, Madrid 28040, Spain.

Filamentous fungi are excellent model organisms for studying the mechanism of microtubule-based distribution of organelles/vesicles. In recent years, we used *Aspergillus nidulans* as a genetic system to dissect how the minus-end-directed motor dynein physically interacts with early endosomes. Our studies revealed that the dynein-binding complex, dynactin, is critical for the interaction between dynein and early endosomes. Within the vertebrate dynactin complex, multiple Arp1 (actin-related protein 1) subunits form an Arp1 mini-filament whose barbed end is occupied by capping protein and whose pointed end occupied by a pointed-end complex containing Arp11, p62, p25 and p27. In contrast to budding yeast dynactin that does not contain capping protein, p62, p25 or p27, our recent proteomic analysis combined with previous studies indicate that *A. nidulans* dynactin complex contains all these components. Our genetic screen identified the Hook protein complex that links early endosome to dynein-dynactin, and the Hook-dynein-dynactin interaction requires both dynein and the p25 subunit of dynactin. p25 is critical for dynein-mediated transport of early endosomes, and our recent data suggest that p25 is not only involved in HookA binding but also in regulating dynactin-microtubule interaction. In contrast, capping protein and p27 are not important for early endosome transport in *A. nidulans*.

Live cell imaging of the cytoskeleton in *Phytophthora* pathogens reveals unique actin and microtubule configurations. *K. Kots*[1,2], T. Ketelaar[2], D. J. Van den Hoogen[1], H. J. G. Meijer[1], F. Govers[1] 1) Laboratory of Phytopathology, Wageningen University, Wageningen, Gelderland, NL; 2) Laboratory of Cell Biology, Wageningen University, Wageningen, Gelderland, NL.

The cytoskeleton is a dynamic but well organized intracellular network that is essential for proper functioning of eukaryotic cells. We study the cytoskeleton in *Phytophthora* species, oomycete plant pathogens that cause devastating diseases worldwide. We use Lifeact-eGFP expressing *Phytophthora infestans* for live cell imaging of the actin cytoskeleton in various developmental stages. Previously we identified actin plaques as highly immobile, long-lived structures that are unique for oomycetes. Here we present two other unique actin configurations; one associated with plug deposition in germ tubes and the other with appressoria, infection structures formed prior to host cell penetration. Plugs are composed of cell wall material that is deposited in hyphae emerging from cysts to seal off the cytoplasm-depleted base after cytoplasm retraction towards the growing tip. Preceding plug formation there is a typical local actin accumulation and during plug deposition actin remains associated with the leading edge. In appressoria we observed an aster-like actin configuration that is localized at the contact point with the underlying surface. These actin configurations strongly suggest a role for the actin cytoskeleton in plug formation and plant cell penetration. For live cell imaging of the microtubule cytoskeleton we have generated a *Phytophthora palmivora* transformant expressing GFP-α-tubulin which allows us to visualize the dynamics of microtubules in oomycetes for the first time. Diseases caused by oomycetes are difficult to control and this emphases the need to find novel potential drug targets specific for oomycetes. Although actin and tubulin are conserved proteins, many structural and regulatory proteins interacting with the cytoskeleton are unique for specific phylogenetic groups. The data presented here are our first attempts to better understand the structure and functioning of the *Phytophthora* cytoskeleton. The long term goal is to uncover oomycete or *Phytophthora* specific features that might be instrumental for drug design.

Cell shape is sensed using the septin cytoskeleton. Kevin Cannon, *Amy Gladfelter* Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Cells change shape in response to diverse environmental and developmental conditions, creating topologies with micron-scale features. Although individual proteins can sense nanometer-scale membrane curvature, it is unclear if a cell could also use nanometer-scale components to sense micron-scale contours, such as the cytokinetic furrow and base of neuronal branches. Septins are filament-forming proteins that serve as signaling platforms and are frequently associated with areas of the plasma membrane where there is micron-scale curvature, including the cytokinetic furrow and the base of cell protrusions. We have found that fungal septins are able to distinguish between different degrees of micron-scale curvature. Additionally, using supported lipid bilayers on beads of different curvature, we can reconstitute and measure the intrinsic septin curvature preference. We have found that micron-scale curvature recognition is a fundamental property of the septin cytoskeleton that provides the cell with a mechanism to know its local shape. Our current focus is on identifying the molecular mechanisms that govern the preference for septins to polymerize on curved surfaces.

Two independent S-phase checkpoints regulate appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*. *M. Oses-Ruiz*, W. Sakulkoo, G. R. Littlejohn, M. Martin-Urdiroz , N. J. Talbot School of Biosciences, College of Live and Environmental Sciences University of Exeter, Exeter, EX4 4QD, United Kingdom.

The rice blast fungus *Magnaporthe oryzae* elaborates a specialised infection cell called an appressorium to infect rice leaves. The appressorium generates enormous turgor, which is focused as physical force to break the rice leaf cuticle allowing the fungus entry to rice cells. We have found that appressorium-mediated plant infection by *M. oryzae* requires two independent S-phase cell cycle checkpoints. During initial formation of appressoria on the rice leaf surface, an S-phase checkpoint operates through the DNA damage response (DDR) pathway, involving the Cds1 kinase. By contrast, during appressorium repolarization a novel, DDR-independent S-phase checkpoint is triggered by appressorium turgor generation and melanisation. This checkpoint regulates NADPH oxidase-regulated, septin-dependent, F-actin re-modeling to organise the appressorium pore and bring about entry of the fungus into rice tissue. We will report on the operation of these two checkpoints, the processes they control, and how they regulate rice infection by the *M. oryzae.*

Analysis of septins during hyphal growth of *Ustilago maydis*. *Sabrina Zander*[1], Sebastian Baumann[2], Michael Feldbrügge[1] 1) Heinrich Heine University, Düsseldorf, Institute for Microbiology, 40225 Düsseldorf, Germany; 2) CRG – Center for Genomic Regulation, 08003 Barcelona, Spain.

Septins are GTP-binding cytoskeletal proteins with functions in cell polarity, membrane remodeling, cytokinesis and cell morphology. Conserved across eukaryotes, except higher-order plants, septins assemble in nonpolar, heteromeric complexes. These further assemble end-to-end to form filaments and a variety of higher-order structures. The heterooligomeric structure of septins and their subcellular localization have already been extensively studied. However, a precise mechanism of their subcellular assembly and their intracellular transport are unknown.

The genome of the filamentous fungus *U.maydis* encodes four septin proteins: Cdc3, Cdc10, Cdc11 and Cdc12. In this study the influence of septin deletions were analyzed, during the hyphal state of *U. maydis*. All four septins are needed for efficient unipolar growth indicating a common function for septins during hyphal growth. Furthermore, the subcellular localization was analyzed with Gfp fusion proteins. All septins showed the same localization in cytoplasmic rings, at septa, in filaments and on moving early endosomes. Colocalization studies revealed that all septins localize interdependently in the same structures. Furthermore, FRET analysis showed that Cdc3 and Cdc12 interact directly *in vivo*. Endosomal transport was not only dependent on each individual septin, but also dependent on the RNA-binding protein Rrm4. RNA live imaging of all four mRNAs showed also an Rrm4-dependent transport. FRAP experiments demonstrated that recovery of Cdc3-mCherryN and Cdc12-GfpN occurs simultaneously at distinct subcellular sites and that the rate of recovery was facilitated by the endosomal RNA transport. Based on these results, a model was proposed where local translation promotes the assembly of newly synthesized septins in heteromeric structures on the surface of endosomes. This is important for the long-distance transport of septins and the efficient formation of the septin cytoskeleton.

Complex nuclear motility in a fungal hypha explained by an elegant mechanism. R. Gibeaux[1,3], A. Z. Politi[1], *P. Philippsen*[2], F. Nedelec[1] 1) Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), D-69117 Heidelberg, Germany; 2) Molecular Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland; 3) Department of Molecular and Cell Biology, University of California, CA-94720, Berkeley.

Today, biologists want to understand the mechanisms of subsystems in cells. Biochemistry, for a long time extremely successful in developing amazing in vitro systems, can no longer handle mechanistic question above a certain complexity. The only way to move forward is to build computer models to systematically assess if our current concepts are compatible – or not – with the experimental data, and to reconstruct the system in a mathematical model. This is essential to curate and assemble the information that has been gathered in the past.

Our work demonstrates this approach by studying the coordination of movements of multiple nuclei in a common cytoplasm which up to now has not been solved for any multinucleated cell. Our aim was to understand the underlying mechanism(s) of the bi-directional short- and long-range movements of nuclei including nuclear by-passing in multinucleated hyphae of *Ashbya gossypii*. Experimental data, accumulated over the past years, had pinpointed one motor (dynein), an evolved microtubule dynamic instability, yeast-like spindle pole bodies as sole microtubule organization centers, and a potential cortical dynein anchor as key elements for the complex nuclear movements. This knowledge was implemented to run for the first time realistic simulations of movements of multiple nuclei in a common cytoplasm which generated in vivo-like nuclear migration patterns. The simulations could also verify mutant phenotypes. We then exploited the simulation set up to study the influence of the number of microtubules per nucleus, an increase in cytoplasmic flow, and changes in organelle concentration on nuclear motility.

Friday, March 17 3:00 PM – 6:00 PM
Heather
Symbiosis and Commensalism

Symbiotic options for the conquest of land. *Katie Field*[1], Martin Bidartondo[2,3], Jeff Duckett[4], William Rimington[2,3], Silvia Pressel[4] 1) School of Biology, University of Leeds, Leeds, UK; 2) Department of Life Sciences, Imperial College London, London, UK; 3) Jodrell Laboratory, Royal Botanic Gardens, Kew, UK; 4) Department of Life Sciences, Natural History Museum, London, UK.

The colonisation of Earth's continental land masses by plants >475 Ma marked a turning point in the development of the terrestrial biosphere, with widespread consequences for the future of terrestrial life. It has long been hypothesised that the terrestrialisation of plants was facilitated by nutritional symbioses with Glomeromycota fungi to form arbuscular mycorrhiza-like associations. The establishment of this symbiosis would likely have enhanced access to mineral nutrients for early, rootless plants in exchange for carbon fixed through photosynthesis. Our recent discoveries that the earliest diverging clade of land plants (Haplomitriopsida liverworts) forms mutualistic symbioses with partially saprotrophic Mucoromycotina fungi challenge this long-standing paradigm. Using a combination of microscopic, molecular and isotope tracer techniques, we have shown that other lineages of early-diverging liverworts form simultaneous mutualistic associations with both Glomeromycota and Mucoromycotina fungi. Together with recent fossil evidence, these findings bring into question the identity, biology and functioning of the fungal symbionts of the earliest land plants. Here, we suggest that there were symbiotic options available to the earliest land plants and that such relationships may have been far more varied and transient than hitherto assumed.

Extensive genome diversity among isolates of a model arbuscular mycorrhizal fungus. *E.Chun-Hung. Chen*[1], S. Ndikumana[1], E. Morin[2], F. Martin[2], J. Noel[1], D. Beaudet[1], J. Giorgi[1], N. Corradi[1] 1) Biology, University of Ottawa, Ottawa, Ontario, CA; 2) UMR 1136, INRA-Nancy Université, Interactions Arbres/Microorganismes, Champenoux, FR.

Arbuscular mycorrhizal fungi (AMF) are best known for their beneficial effects on the health of many land plants and their surrounding ecosystem through establishment of the mycorrhizal symbiosis. It has been proposed that that intra-specific diversity is an essential component of a successful symbiosis with the plant host. Recent evidence of a mating-driven processes in a model AMF has revealed some of the mechanisms involved in creating intra-specific diversity in this group, but how the genome content and structure varies within one species is still poorly understood. To address this question, we compared the genome sequences of five isolates of previously proposed to be part of the *Rhizophagus irregularis* clade. Our analyses show that inter-isolate genome diversity is present at all levels, and includes large amounts of isolate-specific genes, sequence and gene copy number polymorphisms and inter-isolate rearrangements indicative of inter-isolate recombination. Plasticity affects all biological functions, leads to re-interpretations of population-based, and underscores potential problems with the species concept in these widespread plant symbionts.

Functional characterization of CRN-like effector proteins in the arbuscular mycorrhiza symbiosis. *S. Walter*[1], R. Betz[1], N. Corradi[2], N. Requena[1] 1) Karlsruhe Institute of Technology, Karlsruhe, DE; 2) University of Ottawa, Canada.

The arbuscular mycorrhiza symbiosis (AMS) between fungi of the phylum Glomeromycota and more than 80% of all living land plants is characterized by the formation of tree-like fungal structures, arbuscules, within cortical root cells, that function as the interface for the bidirectional nutrient exchange. Prior to this, a highly complex communication is necessary in order to establish the interaction. In plant-pathosystems, effector proteins are secreted during colonization that lead to modifications in the plant cell program, essentially to facilitate the infection process. Evidence is now accumulating that mutualistic fungi also use effector proteins when colonizing their host plants. Recent success in sequencing the genome of *Rhizophagus irregularis* revealed a new effector protein family, which resembles the modular structure of a protein family called Crinkler (CRN). CRNs are present in all pathogenic oomycetes and in some chytridiomycetes. Many contain a signal peptide for secretion and share a highly conserved N-terminal region, with a characteristic LFLAK domain for translocation, and a divergent C-terminal region to execute effector activity. Expression analyses of *R. irregularis* CRN-like genes showed two of them being highly expressed in mycorrhizal roots, suggesting their involvement in the symbiosis. To functionally characterize them, expression of *RiCRN1* and *RiCRN2* in *planta* was carried out. Unlike many oomycete CRNs neither RiCRN1 nor RiCRN2 induced cell death in *Nicotiana benthamiana* but both led to a strong phenotype in transgenic *Arabidopsis thaliana* that were smaller and displayed anthocyanin accumulation. Fusions with GFP and transient expression in *N. benthamiana* leaves showed nucleo-cytoplasmic localization for RiCRN1 and RiCRN2. Most interestingly preliminary experiments to downregulate these CRN-like proteins during symbiosis in *M. truncatula* using host induced gene silencing showed a severe impairment of the symbiosis. We therefore postulate that these CRN-like proteins work to promote symbiosis. Experiments are in progress to identify the *in planta* targets responsible for such a positive role.

Host specificity and effector warfare: a dissection of the signaling events during the mutualistic interaction between the ectomycorrhizal genus *Pisolithus* and their hosts. *J.M. Plett*[1], M. Freitas Pereira[2,3], K.L. Plett[1], E. Morin[2], M Dutra Costa[3], I. Grigoriev[4], A. Kohler[2], F. Martin[2], I.C. Anderson[1], Pisolithus Pan-genome Consortium 1) Hawkesbury Institute for the Environment, Western Sydney University, Richmond, AU; 2) INRA, Nancy, FR; 3) Universidade Federal de Viçosa, Viçosa, BR; 4) Joint Genomes Institute, Walnut Creek,.

In forest ecosystems the roots of most trees and shrubs are colonized by mutualistic ectomycorrhizal (ECM) fungi. Little is understood concerning the host or fungal cellular reprogramming that favors the formation of ECM colonized root tips in compatible plant:fungal interactions and what prevents the formation of ECM root tips in incompatible interactions. Using the newly available genomic and transcriptomic resources for two species within the *Pisolithus* genus, we have begun dissecting the symbiotic toolbox used by this globally dominant fungus. We have been able to define the core transcriptional responses of the plant and fungus during symbiosis and to characterize some of the proteomic modification necessary for mycorrhizal formation. As in other ECM systems, we have identified a number of effector-like small secreted proteins that are induced during symbiosis and have begun to characterize their roles in negotiating (or forcing?) symbiosis with a host tree. I will discuss some of our most recent advances in understanding ECM host specificity against the backdrop of previous findings with other mycorrhizal fungi.

Purification and characterization of a symbiosis-induced endocellulase from the ectomycorrhizal symbiont *Laccaria bicolor*. *F Zhang*[1], C Champion[2], M Haon[2], G Anasontzis[2], M Kemppainen[3], A Pardo[3], Y Daguerre[1], A Deveau[1], C Veneault-Fourrey[1], A Kohler[1], MN

Rosso[2], B Henrissat[4], JG Berrin[2], F Martin[1] 1) UMR 1136 INRA-Université de Lorraine 'Interactions Arbres/Microorganismes', Laboratoire d'Excellence ARBRE, Centre INRA-Lorraine, 54280, Champenoux, France; 2) UMR 1163 INRA-Biodiversité et Biotechnologie Fongiques, Polytech Marseille, Faculté des Sciences de Luminy, , Marseille, France; 3) Laboratorio de Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina; 4) CNRS, UMR 7257 & Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Marseille, France.

In forest soils, ectomycorrhizal fungi establish a mutualistic symbiosis with tree roots. The mutualistic fungi trade host photoassimilates against soil nitrogen and phosphorus. Differentiation of symbiotic roots induces extensive cell wall architectural modifications in the host apoplastic space. The origin of enzymes involved in these cell wall modifications has been the subject of debate for several decades. The ectomycorrhizal basidiomycete *Laccaria bicolor* has a restricted set of carbohydrate-active enzymes (CAZymes) degrading plant cell wall polysaccharides. However, several of those genes are upregulated upon symbiosis. We speculate that several of the symbiosis-induced CAZymes are involved in the remodeling of the host apoplastic space. Here, we characterize the sole GH5 endoglucanase with a cellulose-binding motif (CBM1) domain (LbGH5) identified in the genome of *L. bicolor*. We showed that the *LbGH5* gene is induced five-fold in ectomycorrhizal roots using qPCR and RNA-Seq. RNAi mutants with a decreased expression of *LbGH5* have a lower ability to form ectomycorrhizal roots. Yeast secretion trap (YST) functional screen confirmed that LbGH5 is a secreted protein. We then produced and purified the recombinant protein LbGH5 with and without its CBM1 domain in *Pichia pastoris*. The recombinant LbGH5 displayed highest actitivities towards carboxymethyl cellulose (CMC) and cellulose extracted from aspen roots. In contrast, LbGH5 displayed no activities toward *L.bicolor* cell walls or aspen hemicellulose. *In situ* localization of LbGH5 in ectomycorrhizal roots by indirect immunofluorescence confocal microscopy demonstrated that the enzyme accumulates in hyphal cell walls forming the mantle and Hartig net. These data suggest that cell wall modifications within ectomycorrhizal roots arise from cell wall–modifying enzymes of fungal origin.

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The fungal specific β-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *S. Wawra*[1], P. Fesel[1], H. Widmer[1], M. Timm[2], G. Langen[1], A. Zuccaro[1,3] 1) Univ of Cologne, Botanical Institute, Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany; 2) Univ of Würzburg, Inst of Organic Chemistry, Würzburg, Germany; 3) Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany.

β-glucans are well-known modulators of the immune system in mammals, but little is known about β-glucan triggered immunity *in planta*. We report that the secreted plant-induced protein FGB1 from the root endophyte *Serendipita indica* is a novel type of conserved β-glucan binding lectin in fungi that increases resistance to cell wall stress and is able to efficiently suppress β-glucan triggered immunity in different plant hosts. Our results highlight the importance of β-glucan as a fungal MAMP and the necessity of mutualistic and pathogenic fungi to protect β-glucan polymers from recognition during colonization of different plants. Here we will present our latest data.

Genetics-based interactions structure foliar fungal communities of *Populus trichocarpa*. *P.E. Busby*[1], Shawn Brown[1], Naupaka Zimmerman[2], Gregory Crutsinger[3], Matthew Barbour[3], Kabir Peay[4], George Newcombe[5] 1) Botany & Plant Pathology, Oregon State University, Corvallis, OR; 2) Department of Biology, University of San Francisco; 3) Department of Zoology, University of British Columbia; 4) Department of Biology, Stanford University; 5) College of Natural Resources, University of Idaho.

Fungal endophytes form diverse communities within plants, with individual endophytes playing functionally important roles in plant growth, development, and resistance to abiotic and biotic stressors. Despite the ecological importance of endophytes, little is known about the mechanisms that control endophyte community composition. In a manipulative common garden field experiment, we evaluated the impact of host genotype, endophyte inoculation (Cladosporium), and presence of herbivory on foliar fungal community composition. The twelve genotypes included in the experiment varied in tannin levels, phenological shifts in timing of bud flush, and rust disease severity. We found that community structure was most strongly influenced by host-genotype, tannin levels, and phenology (perMANOVA). The endophyte inoculation and herbivory treatments did not produce a detectable shift in endophyte community structure in our system, but these interactions were occurring naturally across all plants and could occlude observable treatment effects. Host-genotype was by far the most influential factor in structuring fungal endophytes (explaining 21.81% of community variation) based on our variance partitioning analyses (PVCA). The relatively weak effect of tannins and phenology on endophyte communities was confirmed using mixed model ANOVAs of NMDS axes loading scores. Additionally, using LEfSe analyses, we identified OTUs that are strongly associated with particular genotypes, providing targets for mapping genetic controls of community members. Taken together, our results indicate that unmeasured host genetic effects are far more important in determining endophyte communities than the traits measured in this study. To further investigate and disentangle host genetics/gene expression from environmental constraints on endophytic communities, we are using both a Genome Wide Association Study (GWAS) and RNA-Seq analysis of endophyte-inoculated leaves.

Comparative genomics of lichen mycobionts. *A. Kuo*[1], D. Culley[2], O. Mueller[3], P. Dyer[4], J. Magnuson[2], F. Lutzoni[3], I. Grigoriev[1] 1) DOE JGI, Walnut Creek, CA; 2) Pacific Northwest National Lab, Richland, WA; 3) Duke Univ, Durham, NC; 4) Univ of Nottingham, Nottingham, UK.

Lichens are mutualistic symbioses usually between an alga (the photobiont) and a fungus (the mycobiont). The photobiont contributes fixed carbon to the partnership, while the mycobiont provides a physical structure that encloses the photobiont, roots the partners to a substrate, and acquires nutrients often of air-borne origin. Lichens are an ancient (possibly 600 Mya) and highly successful adaptation, covering 8% of terrestrial ecosystems, and comprising as much as 20% of fungal species diversity. The lichen lifestyle has evolved multiple times and occurs in at least 5 fungal classes. As part of an ongoing effort to represent the full phylogenetic and ecological diversity of fungi in the Joint Genome Institute MycoCosm and the 1000 Fungal Genomes Project, we have partnered with members of the lichen research community to sequence, assemble, and annotate several lichen mycobionts and photobionts. Initial comparison of 5 publicly available mycobiont genomes from 3 distinct lichen-forming clades with their nearest non-lichenizing relatives has revealed varied combinations of secondary metabolite synthase, transporter, and protease gains and losses. The diversity of gains and losses suggest that the independent evolutionary transitions to the lichen lifestyle may have involved different gene-level adaptations. We expect to expand on these findings by examining additional functional characters, and by sequencing more genomes, as well as importing more genomes from our partners and from the public domain.

Friday, March 17 3:00 PM – 6:00 PM
Nautilus
Effectors and Small Secreted Cysteine-Rich Proteins

SnTox1, a cysteine rich small secreted protein provides *in planta* protection from wheat chitinases. *Timothy L. Friesen*[1,2], Yuanyuan Gao[2], Zhaohui Liu[2], Jonathan Richards[2], Justin D. Faris[1] 1) USDA-ARS, Northern Crop Science Lab, Cereal Crops Research Unit, Fargo, ND, 58102, U.S.A; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND, 58108, U.S.A.

Parastagonospora nodorum is a necrotrophic fungal pathogen and the causal agent of Septoria nodorum blotch (SNB) on wheat. *P. nodorum* produces necrotrophic effectors (NEs) that are critical to the virulence of the pathogen. The NEs produced by *P. nodorum* are recognized directly or indirectly by dominant sensitivity genes contributing to susceptibility in wheat. Nine NE-host dominant sensitivity gene interactions have been identified, and three NE genes have been cloned, including *SnToxA*, *SnTox1* and *SnTox*3. SnTox1 is a cysteine rich small secreted protein that interacts directly with the wheat protein Snn1. In the presence of Snn1, SnTox1 triggers programmed cell death (PCD) involving hallmarks of the defense response including an oxidative burst and up regulation of wheat pathogenesis related (PR) proteins including chitinases. The triggering of PCD involves membrane disruption and electrolyte leakage, and unlike a biotrophic interaction, provides a nutrient source for the pathogen. However, a pathogen with this lifestyle must also be able to protect itself from the negative effects of this defense response including the accumulation of chitinases in its growth environment. In addition to triggering PCD, we have shown that SnTox1 binds chitin, providing protection to the growing fungus in the apoplast during infection. Several additional genes encoding small, secreted, cysteine-rich proteins are present in the *P. nodorum* genome and are predicted to be localized to the apoplast during infection. Some of these proteins could play a role in protecting this pathogen from the host defense response.

***Ustilago maydis* effectors targeting the auxin signaling pathway.** *A. Djamei*, F. Navarrete, M. Gallei, J. Bindics, S. Uhse Gregor Mendel Institute of Molecular Plant Biology, Vienna, AT.

Biotrophic plant pathogenic fungi employ a battery of small secreted molecules, so called effectors, to suppress host defense responses and to redirect the host metabolism in favor of the invader. Although effector proteins are shaping the interaction between the pathogen and the host, it is challenging to elucidate their function as they largely show no sequence homology to proteins with known functional domains. The maize infecting tumor inducing fungus *Ustilago maydis* became in the past decades a model to study biotrophic interactions. Hormone signaling pathways in plants are prime targets for biotrophic pathogens in order to suppress defense responses, reshape host metabolism and host development systemically. In a systematic approach, we study the involvement of *U. maydis* effectors in hormone signaling pathways. Here we report about the identification of several auxin signaling inducing effectors of *U. maydis* and their host sided plant interaction partner. The plant target identified is highly conserved across plant species which opens new applications as a tool for plant biologists.

Towards understanding the mechanism of cytoplasmic effector translocation during biotrophic development of *Magnaporthe oryzae*. *E. Oliveira Garcia*, B. Valent Plant Pathology, Kansas State University, Manhattan, KS , KS.

Rice blast caused by *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*), a hemibiotroph and facultative pathogen, is the most destructive disease of rice worldwide. During the infection process, *M. oryzae* secretes various effectors, which are hypothesized to be involved in effective host infection. Effectors are classified by their destinations in the interaction court, with apoplastic effectors residing in the extracellular plant compartment and cytoplasmic effectors translocating into the cytoplasm of living plant cells. Notably, cytoplasmic effectors of *M. oryzae* are associated with a specialized interfacial structure, the biotrophic interfacial complex (BIC). To date, little is known about the mechanisms of effector uptake into plant cells during fungal infection. Here we show evidence for translocation of the cytoplasmic effectors Bas1, Pwl1 and Pwl2 in vesicles from BICs to rice cytoplasm during biotrophic development. Using fluorescent protein tagging, we found that cytoplasmic effectors Bas1, Pwl1 and Pwl2 are sorted into different vesicles in BICs formed on primary hyphae (PH), revealing new levels of functional complexity for this biotrophic structure. In contrast, most of the vesicles from BICs on mature bulbous hyphae showed colocalization of the cytoplasmic effectors. Whereas BICs on primary hyphae deliver effectors in micro-vesicles, BICs on mature bulbous hypha deliver effectors in macro-vesicles, at times reaching diameter sizes over 3 µm. Furthermore, we demonstrate that endocytosis inhibitors Cantharidin, Triclosan and Wortmannin induce abnormally-shaped and swollen BICs as well as the accumulation of cytoplasmic effectors in the BICs. Moreover, Cantharidin, Triclosan and Wortamannin treatment induced the accumulation of the cytoplasmic effectors under penetration pores, suggesting that effector uptake begins even before host penetration. Based on these results, it appears that cytoplasmic effector translocation is mediated by vesicle formation and may be characteristic of appressoria as well as biotrophic invasive hyphae. Our results also suggest a potential role of *M. oryzae* effectors for manipulation of the host cell endocytosis process.

Functional characterization of a protein complex formed by four *Ustilago maydis* effectors essential for virulence. *N. Ludwig*[1], L. Liang[1], K. Schipper[1,2], S. Reißmann[1], D. Aßmann[1], T. Glatter[1], J. Altmüller[3], R. Kahmann[1] 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Heinrich Heine University Düsseldorf, Düsseldorf, Germany; 3) Cologne Center for Genomics (CCG), Cologne University, Cologne, Germany.

The fungus *U. maydis* causes smut disease in maize. *U.maydis* is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection *U.maydis* needs to suppress plant defense responses and manipulate host physiology for its own benefit. To accomplish this, *U.maydis* secretes a cocktail of about 300 effector proteins. The majority of these proteins lack a functional annotation and their function remains to be uncovered. Our current work focuses on effectors expressed early during infection. Systematic deletion of the most highly expressed effector genes in this class resulted in the discovery of three mutants unable to cause disease. Their mutant phenotype resembled previously identified *stp1* (stop after penetration) mutants, and the newly identified genes were designated *stp2*, *stp3* and *stp4*. A similar phenotype was also observed for mutants lacking the essential effector *pep1* (Döhlemann *et al.*,2009). *stp1-4* deletion strains were able to form appressoria that penetrate maize epidermal cells, but their growth was arrested in epidermal tissue. Their growth arrest was accompanied with the elicitation of plant defense responses and plant cell death. Co-IP with individually tagged effectors

followed by mass-spectroscopic analysis revealed that Stp1, Stp3, Stp4 and Pep1 form a complex, while Stp2 is not part of the complex. We will discuss our current efforts to localize the complex and to functionally characterize its components as well as Stp2. Furthermore, we are testing possible theories for the existence of the complex, such as a function in translocation or the shielding of Avr proteins.

An antimicrobial and phytotoxic ribonuclease secreted by the wheat pathogen *Zymoseptoria tritici*. *G.J. Kettles*, C. Bayon, C.A. Sparks, K. Kanyuka, J.J. Rudd Plant Biology & Crop Science, Rothamsted Research, Harpenden, Hertfordshire, UK.

The dothidiomycete fungus *Zymoseptoria tritici* (*Z. tritici*) is amongst the most prevalent and damaging pathogens of wheat throughout the world. During early (symptomless) colonisation of wheat leaves, there is minimal activation of the host immune system. Later, there is a switch to necrotrophy, accompanied by host defence gene induction and extensive cell death in infected tissues. We recently identified a secreted ribonuclease (Zt6) as a candidate effector protein based on transcriptomic analysis of *Z. tritici* infection of susceptible wheat. Zt6 displays an unusual double-peak expression pattern during wheat infection, with maximal expression both shortly after spore germination on the leaf surface (1 dpi) and after the transition to necrotrophy (14 dpi). Expression at transition (9 dpi) was comparatively low, in contrast to the majority of putative secreted protein effectors identified to-date.

Agrobacterium-mediated transient expression of Zt6 in the non-host model plant *Nicotiana benthamiana* revealed this effector protein to be a highly potent inducer of cell death. Both full-length protein and its mature form (lacking signal peptide) were equally potent in cell death induction. Particle co-bombardment of GFP- and Zt6-expressing constructs into wheat leaves resulted in no GFP expression, indicating Zt6 phytotoxicity against the natural host. Further co-bombardment experiments utilising a 22-amino acid N-terminal loop deletion (?1-22) and catalytic mutation (H52A) revealed both regions of Zt6 to be essential for full functionality and suggested a role for the N-terminal loop in Zt6 translocation into the host cytoplasm. Interestingly, recombinant Zt6 protein showed a ribotoxin-like activity by cleaving native rRNA semi-specifically, with production of distinct cleavage fragments. Finally, microbial toxicity assays revealed Zt6 to be highly toxic to both bacteria and yeast but not to more complex filamentous fungi, including *Z. tritici* itself. Together, our data reveal a new multi-functional cytoplasmic ribotoxin-like effector that is toxic to both host and non-host plants, and also to microbial competitors.

Learning from nature: establishment of a library of natural and rationally designed hydrophobins from *Trichoderma* spp. with diverse surface activity. *Gunseli Bayram Akcapinar*[1,2], Agnieszka Przylucka[1,3], Cai Feng[4], Thiago Machado Mello De Sousa[5], Hinrich Grothe[6], Erik Reimhult[7], Astrid R. Mach-Aigner[5], Qirong Shen[4], Irina S. Druzhinina[1] 1) Microbiology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; 2) Department of Medical Informatics and Biostatistics, Acibadem University, Kayisdagi Cad. No:32, 34752 Istanbul, Turkey; 3) Austrian Centre of Industrial Biotechnology (ACIB) GmbH c/o Institute of Chemical Engineering, University of Technology of Vienna, Vienna, Austria; 4) Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjing, China; 5) Gene Technology Group, Research Area Biotechnology and Microbiology, Institute of Chemical Engineering, Vienna University of Technology, 1060 Vienna, Austria; 6) Research Division Physical Chemistry, Institute of Materials Chemistry, Vienna University of Technology, 1060 Vienna, Austria; 7) Department of Nanobiotechnology, Institute for Biologically Inspired Materials, University of Natural Resources and Life Sciences Vienna , Muthgasse 11, 1190 Vienna, Austria.

Hydrophobins (HFBs) are the small, secreted cysteine rich, amphiphilic proteins which exhibit high surface activities. They are mainly produced by fungi. Their ability to self-assemble at hydrophobic/hydrophilic interfaces makes them ideal candidates for a diversity of industrial applications. In this study, two complementary approaches were used for the construction of a library of HFB4 proteins from a diverse array of *Trichoderma* species. In the first approach, positive evolution, structure guided protein engineering was employed to generate five mutants of HFB4 from *T. virens* ($HFB4_{vir}$) ($HFB4_{vir\text{-}V19I}$, $HFB4_{vir\text{-}V19Q}$, $HFB4_{vir\text{-}V19R}$, $HFB4_{vir\text{-}L64Q}$, $HFB4_{vir\text{-}L64R}$) using a newly identified repertoire of 160 orthologous genes encoding a novel HFB4 in different species of *Trichoderma*. Molecular Dynamics simulations were performed to decipher the functionally important residues and to study the effects of proposed mutations. In the second approach, eight different natural HFB4 orthologues exhibiting diverse properties were selected from the newly identified repertoire of *Trichoderma* species based on pI and hydrophobicity using bioinformatics methods. Natural and tailor-made HFB4s were heterologously expressed in *Pichia pastoris*. A collection of biochemical and biophysical techniques were employed to verify the expression and surface activity of these proteins and to analyse the differences in the surface activity. Three mutants of $HFB4_{vir}$ ($HFB4_{vir\text{-}V19I}$, $HFB4_{vir\text{-}V19R}$, $HFB4_{vir\text{-}L64R}$) were found to exhibit improved surface activity on glass. All HFB4 orthologues were shown to increase the water contact angle (WCA) of the glass to different extents. Two HFB4 orthologues, $HFB4_{kon}$ and $HFB4_{est}$, exhibited the highest surface activity on glass by increasing the WCA by almost 70° and 40°, respectively.

Functionalization of surfaces using hydrophobin-fused laccase from *Aspergillus nidulans*. *O. Fokina*, L. Winandy, R. Fischer Inst. for Appl. Biosciences / Dep. of Microbiology, Karlsruhe Institute of Technology - KIT, Karlsruhe, Baden-Wuerttemberg, DE.

Hydrophobins are small amphiphilic proteins that can self-assemble into monolayers on hydrophilic and hydrophobic surfaces and change their properties. They are secreted by fungi to reduce surface tension at the medium-air interface during hyphal growth and are also responsible for the hydrophobicity of the conidiospore surface. Hydrophobins are divided into two classes, depending on their structural characteristics. Especially class I hydrophobins are interesting for biotechnology, because their layers are stable at high temperatures and can only be removed with strong acids.

Here we we developed a system to functionalize surfaces with enzymes using hydrophobin as anchor protein or "glue". To this end *Aspergillus nidulans* laccase C (LccC) was fused to the class I hydrophobins DewA or DewB and used for specific functionalization of hydrophobic/hydrophilic polystyrene and glass surfaces. Fusion proteins were secreted into the culture medium, which was directly used for coatings without additional purification steps. Protein immobilization using hydrophobins provides a stable binding of the fused enzyme to the surface without additional chemical treatments that could potentially be harmful to the enzyme or the surface. Also the spatial orientation of the enzyme is predetermined by the design of the fusion construct. This study presents an easy-to-use alternative to classical enzyme immobilization techniques and can probably be applied not only for laccases, but also for other biotechnologically relevant enzymes.

Recruitment of esterase by fungal hydrophobins bound on polyesters and subsequent promotion of polyester degradation. *K. Abe*[1,2], T. Tanaka[1], Y. Terauchi[1], T. Takahashi[1], F Hasegawa[2] 1) Dept Microbial Biotechnology, Grad. Sch. Agricult. Sci., Tohoku Univ., Sendai, Miyagi, JP; 2) New Industry Creation Hatchery Center, Tohoku University.

Hydrophobins are amphipathic proteins and are ubiquitous among filamentous fungi [1]. When the industrial fungus *Aspergillus oryzae* is grown in liquid media containing the polyester polybutylene succinate co-adipate (PBSA) as a sole carbon source, *A. oryzae* produces RolA, a hydrophobin, and CutL1, a PBSA-degrading cutinase (polyesterase) [2]. We previously discovered that RolA promotes the CutL1-dependent hydrolysis of PBSA by attaching to the surface of PBSA and interacting with CutL1 to concentrate CutL1 on the PBSA surface [2]. To elucidate the underlying mechanism of RolA-CutL1 interaction, we analyzed kinetic profiles of the interactions between RolA variants and CutL1 variants by using a Quartz Crystal Microbalance (QCM). The QCM analyses revealed that D142, D171 and E31, located on the hydrophilic molecular surface of CutL1, and H32 and K34, located in the N-terminus of RolA, play crucial roles in the RolA–CutL1 interaction via ionic interactions [3]. Phylogenetic and alignment analyses showed that the N-terminal regions of several RolA/RodA orthologs contained positively charged residues and that the corresponding negatively charged residues on the molecular surface of CutL1 that were essential for the RolA–CutL1 interaction were conserved in several CutL1 orthologs [4]. A PBSA degradation assay, a pull-down assay using Teflon particles, and a kinetic analysis using a QCM revealed that recombinant *Aspergillus nidulans* RodA interacted via ionic interactions with two recombinant *A. nidulans* cutinases. Recently, hydrophobin-dependent promotion of polymer degradation by cutinases has been reported in other fungi [5, 6]. Together, these imply that ionic interactions between hydrophobins and cutinases may be common among aspergilli and other filamentous fungi.

1) Wösten H. A. *Annu Rev Microbiol.* 55: 625 (2001)
2) Takahashi T. et al., *Mol. Microbiol.* 57: 1780 (2005)
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4) Tanaka T. et al., *Appl Microbiol Biotechnol.* In press
5) Espino-Rammer L. et al., *Appl Environ Microbiol.* 79:4230 (2013)
6) Pham C.L. et al., *Sci Rep*. 6:25288 (2016)

Friday, March 17 3:00 PM – 6:00 PM
Scripps
Fungi in Society

Old collection, new discoveries. *Kyria Boundy-Mills*[1], Luis A. Garay[1], Irnayuli R. Sitepu[1,2], Tomas Cajka[3], Oliver Fiehn[3] 1) Food Science, University of California Davis, One Shields Ave, Davis, CA USA 95616; 2) Department of Biotechnology, Indonesia International Institute for Life Sciences, Jalan Pulo Mas Barat Kav. 88, East Jakarta, DKI 10 Jakarta, Indonesia; 3) UC Davis Genome Center-Metabolomics, University of California, Davis, 451 Health Sciences Drive, Davis, California 95616 USA.

The Phaff Yeast Culture Collection at the University of California Davis is the fourth largest public collection of its kind in the world, containing over 7,500 strains belonging to over 800 different species. The yeasts were gathered, characterized and preserved over the last century by University of California personnel, primarily by Herman Phaff (1913-2001), from environmental sources such as plants, insects, and foods and beverages. The collection is expanding through rescue of over 3,000 yeasts from retiring professors W. T. Starmer (Syracuse University) and P. Ganter (Tennessee State University). Ribosomal sequences (partial 26S and ITS) have been generated for thousands of yeast strains. The yeasts are utilized by academic, government agency and industrial researchers around the world in a wide range of areas such as functional genomics, metabolomics, biogeography, ecology, food fermentations, and metabolomics. The yeast strains and associated data are available to the scientific community through the collection website, http://phaffcollection.ucdavis.edu. The broad diversity enables in-house research including studies of stress tolerance, yeast lipid accumulation, synthesis and secretion of glycolipids, and development of starter cultures for olive fermentations. Several yeast species were identified that are able to synthesize and secrete significant quantities of a class of glycolipids called polyol esters of fatty acids (PEFA), consisting of a 3-hydroxy fatty acid and either mannitol or arabitol, with varying degrees of acetylation. Because the molecule is amphipathic, it has biosurfactant activity. These discoveries are possible thanks to the accumulation, characterization and preservation of yeasts by forward-thinking scientists over many decades.

Feeding fellow biologists their fungal vegetables. *C. Adams* Plant and Microbial Biology, UC Berkeley, Oakland, CA.

Among eukaryotes, Kingdom Fungi is extremely diverse in terms of estimated species numbers, as well as ecologies, genetic systems, and more. Yet despite their comparable importance with, for example, animals and plants, the U. S. public knows very little about fungi. Though much remains to be learned about fungi, many aspects are well-understood, such as their importance in nutrient cycling and decomposition, heterothallic mating systems, and production of socially important compounds such as antibiotics. Then why are fungi relatively absent from primary education, and often even college-level biology? This illiteracy may be partially due to the fact biologists have historically understudied fungi, which were long mistaken for “lower plants” of little value or importance. This knowledge gap among biologists themselves hinders not only teaching mycology to the public, but conducting interdisciplinary research. In this talk, I will create a dialog to discuss the aspects of fungal biology that mycologists consider critical for other biologists to know. I will also present strategies that mycologists can use to better communicate with other biologists across a range of social media platforms.

Diversity of spalting fungi in the Peruvian amazon. Sarath M. Vega Gutierrez, *Sara C. Robinson* Wood Science & Engineering, Oregon State University, Corvallis, OR.

Most of the research related to fungi of the Peruvian Amazon is focused on edible mushrooms and pathogens. Other important fungi, such as spalting fungi, are not broadly studied because most of them do not produce fruiting bodies, are difficult to locate, and do not present an immediate value to the forager. Finding spalting fungi involves a complete sampling of downed trees and branches in a given area through the opening of the xylem to identify potential pigmenting and zone line producing fungi. This methodoly was employed in the district of Las Piedras, Madre de Dios, Peru. Fungi suspected of causing internal pigment and zone lines were collected, cultured, isolated, and sequenced. The species found belonged to the orders of the Helotiales, Xylariales, Hypocreales, Russulales, Polyporales, Botryosphaeriales and two specimens of the class Leotiomycetes. Most of the fungi produced pigments or zone lines in wild conditions and all of them were part of the wood decomposing fungi. Findings from this research will benefit Amazonian communities as well as Peru as whole, through identification and location of value-added wood products that can widen the commercial wood market currently focused on dark, old growth trees. This also gives more inherent value to the forest, potentially saving the land from being converted to plantation or pasture.

Improving STEM student retention via early research engagement: a pilot. *Michael Watters*, Patrice Bouyer Dept Biol, Valparaiso Univ, Valparaiso, IN.

We describe here the results of a pilot study, the goal of which was to expose freshman to an ongoing research project during the academic year to promote student growth and improve retention in the STEM disciplines. Freshmen worked with a faculty mentor and were also chaperoned by a more senior student researcher in order that they learn lab techniques and the capacity to work independently. Participants were fully engaged in a research project (performing experiments, analyzing and discussing results), not a classic classroom projects, but discovery based projects. By bringing students into the research lab at this early stage, our aim was to improve retention by allowing science students to actually *act* as scientists, providing an enhanced experience over the usual freshman survey course content. Of the 6 students who joined the program as freshmen, 5 are still in their major, 4 are still actively engaged in research with a faculty member and have co-authored 11 different papers and conference presentations as of their junior year.

Genetic diversity of 100+ *Aspergillus* species - the aspMine analysis resource. *T. C. Vesth*[1], J. L. Nybo[1], S. THEOBALD[1], R. P. DE VRIES[4], I. V. GRIGORIEV[3], S. E. BAKER[2], M. R. ANDERSEN[1] 1) Department of Bioingineering, Technical University of Denmark, Lyngby, Denmark; 2) Joint Bioenergy Institute, Berkeley, CA, USA, Berkeley, CA, USA; 3) Joint Genome Institute, Walnut Creek, CA, USA, Walnut Creek, CA, USA; 4) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, Utrecht, The Netherlands.

The filamentous fungal species of the Aspergillus genus are of broad interest to the scientific community including applied, medical and basic research. These fungi are prolific producers of native and heterologous proteins, organic acids, and secondary metabolites (including

bioactives and toxins such as ochratoxin A). Because of these abilities, they represent a substantial economic interests in pharmaceutical, biotechnology, and bioenergy applications. In a project collaboration with the US Joint Genome Institute and JBEI we are de novo sequencing 300 different species of Aspergillus and establishing an online analysis platform for the scientific community, aspMine. The goal of this project is to develop a targeted tool to expand and improve our knowledge and expertise about this versatile group of fungi. At time of writing, 200 genomes are in various stages of sequencing and a bioinformatic pipeline has been established to analyze and store the data. This project covers a wide range of biologically interesting ideas surrounding the concept of speciation, such as genetic diversity, primary and secondary metabolism and proteome diversity. Complementary to the tools offered by FungiDB and JGI, the aspMine analysis resource offers tools for tracking genes and functions across species, allowing for investigation of shared genes and clusters across the genus as well as species- and clade-specific genes. The online platform also offers comparative analysis of secondary metabolism gene clusters with focus on synteny and functional conservation across species. The aspMine is implemented as a number of web applications created in R shiny, a graphical interface for analysis. The different tools are collected on a webpage which also includes method descriptions and relevant literature. The webpage is available from the beginning of 2016 and will be continually expanded. It is our goal to provide a comprehensive analysis platform for the community for comparative analysis of Aspergillus species.

PHI-base - the Pathogen-Host Interactions database. *Kim Hammond-Kosack*[1], Alayne Cuzick[1], Kim Rutherford[2], Helder Pedro[3], Martin Urban[1] 1) Dept of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; 2) Cambridge Systems Biology and Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge, Cambridgeshire CB2 1GA, UK; 3) The European Molecular Biology Laboratory, The European Bioinformatics Institute, Hinxton, Cambridgeshire, CB10 1SD, UK.

The pathogen-host interactions database PHI-base (www.phi-base.org) is a knowledge database. It contains expertly curated molecular and biological information on genes proven to affect the outcome of pathogen-host interactions reported in peer reviewed research articles. Genes not affecting the disease interaction phenotype are also curated. Viruses are not included. Here we describe a revised PHI-base Version 4 data platform with improved search, filtering and extended data display functions. Also a BLAST search function is now provided. The database links to PHI-Canto, a new multi-species author self-curation tool adapted from PomBase-Canto. The recent release of PHI-base version 4 has an increased data content containing information from >2000 manually curated references. The data provide information on 4460 genes from 264 pathogens tested on 176 hosts in 8046 interactions. Pro- and eukaryotic pathogens are represented in almost equal numbers. Host species belong ~70% to plants and 30% to other species of medical and/or environmental importance. Additional data types included into PHI-base 4 are the direct targets of pathogen effector proteins in experimental and natural host organisms. The different use types and the future directions of PHI-base as a community database are discussed.

Urban et al., (2017) PHI-base: A new interface and further additions for the multi-species pathogen-host interactions database. *Nucleic Acids Research* (database issue Jan 2017)

This work is supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC) (BB/I/001077/1, BB/K020056/1). PHI-base receives additional support from the BBSRC as a National Capability (BB/J/004383/1).

Advances in chemical genetic tools and impact on the research and development of novel crop protection products. Siân Deller, Helen Carter, Helen Clake, Eileen Scott, *Michael Csukai* Syngenta, Jealott's Hill, GB.

Chemical control agents are likely to remain a part of the mixture of control strategies open to farmers for the foreseeable future. In order to support the discovery and development of new active ingredients a wide range of chemical genetic tools are utilised and are under constant development. Historically many of these tools used the model organism *Saccharomyces cerevisiae,* but improvements in genomic information and tools make it possible to perform research directly in field-relevant plant pathogens. Some of the wide range of tools used in support of new fungicide discovery will be outlined, including:

- Forward genetic tools, which have long been the mainstay of mode of action diagnosis, provide an unbiased approach for the identification of the molecular target of a chemical inhibitor. If resistant mutants can be generated, the technique can now be applied to any pathogen, providing a reference genome is available and single nucleus cells/spores can be isolated.
- Libraries of engineered model organism strains. Chemistry with lower resistance risk is often associated with the inability to isolate resistant mutants in the plant pathogen of interest. In this case unbiased genetic tools are still of value and model organisms are employed. Haploinsufficiency or overexpression-induced resistance within a yeast library can provide information on proteins or protein families targeted by novel chemistry. Hypotheses can then be based on the mutations seen, and compared to information from additional phenotypes such as biochemical pathway inhibition and microscopic observations.

The improvements in transformation procedures, genetic engineering techniques and availability of multiple selectable markers make it possible to test hypotheses in fungal pathogens of economic importance such as *Zymoseptoria*. Additionally, genes from any species can be tested in a uniform host cell background, for instance, by heterologous expression. Furthermore, these tools can be used to assess the lifetime of a new fungicide product. Mutations induced in the lab or discovered in the field can be assessed for their importance in different genetic backgrounds and an assessment made of the impact on sensitivity and fitness. Advancing molecular biology tools and understanding of phytopathogens are helping to inform and support the development of ever safer and more effective chemical crop protection strategies. How these tools have been used to study Zymoseptoria will be shown.

Evolutionary and population genetics of culture collections. *K. McCluskey* Fungal Genetics Stock Center, Kansas State University, Manhattan, KS.

Living microbe collections are established for a number of reasons including biodiversity, genetics, genomics, industrial and agricultural applications, and in support of patents. The Fungal Genetics Stock Center has holdings in support of multiple areas including genetics, genomics, and biodiversity. Having developed to support researchers using Neurospora and Aspergillus, the FGSC is more of a research resource repository and this is reflected in its narrow taxonomic scope. Similar collections include the Coli Genetic Stock Center, the Bacillus Stock Center, and the Chlamydomonas Center. Other collections, such as the ATCC and the USDA NRRL have much broader

taxonomic scope and are traditional culture collections.
While collections of each type are vulnerable to market effects, the recent change in collection funding by the US National Science Foundation means that collections with narrow focus may not have critical mass sufficient to support curation, quality control, and distribution. As model organisms solve the questions they were developed to address, and as modern questions are directly accessible in key health, industrial, or agriculturally important organisms, the value of the model systems decreases. Overall these effects combine and model organisms and the collections that preserve and distribute them pass into the area of science history. Fungal genetics has seen such areas come and go. Early work with Allomyces, Ustilago, Schiziphyllum, Coprinopsis, and Sordaria generated strains that are held at the FGSC, but are not highly valued in the modern research context.

Saturday, March 18 9:00 AM – 12:00 NOON
Merrill Hall and Chapel
Plenary Session IV: Sensing and Signaling: Perception of the Complex World in which Fungi Thrive or Survive

Synthetic biology and optogenetics: developing biotechnological solutions and pushing the boundaries between science and art. *L.F. Larrondo* Departamento de Genética Molecular y Microbiología, Millennium Nucleus for Fungal Integrative and Synthetic Biology, P. Universidad Católica de Chile.

The filamentous fungus *Neurospora crassa* has been one of the main models for the study of photobiology, providing great insights on how microorganisms perceive and respond to light. This ascomycete responds specifically to blue light (but not to other wavelengths) through a transcriptional heterocomplex named White Collar Complex (WCC). One of its components, WC-1, possesses a LOV (Light Oxygen Voltage) domain capable of detecting blue light, which promotes a conformational change that leads to dimerization that results in strong transcriptional activation, in a light-intensity dependent manner. In order to design and improve optogenetic switches that can be utilized in other organisms as orthogonal controllers, we have been exploring the dynamics of light responses in this fungus. Thus, through the development of Neurospora-based optogenetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, in yeast, now we can efficiently induce gene expression up to 4000-fold and control biotechnological relevant phenotypes such as flocculation by switching on/off the lights.
On the other hand, we have adopted optogenetic approaches to further delve into Neurospora's circadian and light-responses. In doing so, we were able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a Neurospora carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image. Thus, we have established a live canvas in which images are genetically processed and reconstituted with real-time dynamics. Such technology not only allows studying light-responses with great resolution, but is also provides a powerful artistic substrate. Remarkably, since the live canvas circuit is integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase responses, but it also allows for the opportunity to ponder on concepts such as vision and memory. MN-FISB120043, FONDECYT 1131030.

Fear the titans: when bad yeast get worse. *K. Nielsen* Department of Microbiology and Immunology, University of Minnesota, Minneapolis, MN.

The human and animal pathogenic yeast *Cryptococcus neoformans* is a major cause of fungal morbidity and mortality worldwide, particularly in immunocompromised individuals. Upon inhalation into the lungs, a subset of *C. neoformans* cells transform into enlarged titan cells. While typical *C. neoformans* cells are 5-7 µm in diameter, the titan cells can be up to 100 µm in diameter. Along with their large size, titan cells also exhibit alterations in their cell wall and capsule structure. These morphological changes impact how the host immune system perceives and responds to the *C. neoformans* infection and ultimately leads to increased disease. In addition to morphological changes, titan cells are also polyploid. Typical *C. neoformans* cells are haploid (1C) but titan cells are tetraploid (4C), octoploid (8C), 16C, etc. with ploidies as high as 312C readily observed. Surprisingly, the polyploid titan cells appear to have a single nucleus and divide by budding to produce haploid or aneuploid daughter cells that can have novel traits such as increased drug resistance and tolerance to various stresses. Current studies aim to understand how *C. neoformans* senses the host environment to trigger titan cell formation as well as the molecular processes underpinning the unique morphological and genomic changes observed in titan cells.

Developmental consequences of inter-microbial chemical communication. *Nancy Keller*[1], Saima Khalid[1], Philipp Wiemann[1], Joshua Baccile[2], Frank Schroeder[2], Joseph Spraker[1] 1) University of Wisconsin- Madison; 2) Boyce Thompson Institute - Cornell University.

Filamentous fungi and bacteria are renown for the production of a diverse array of secondary metabolites (SMs). These natural products are valued for their bioactive properties stemming from their functions in microbial biology, including protection from abiotic and biotic stress and establishment of a secure niche. The rhizosphere provides a rich milieu for fungal/bacterial interactions, one that may be adapted to agricultural practices worldwide. Here we illustrate an unexpected role for SMs in microbial crosstalk among soil-borne pathogens: the bacterium *Ralstonia solanacearum* and the fungi *Aspergillus flavus* and *Fusarium* spp. Ralsolamycin, a lipopeptide inducing chlamydospore formation in fungi (Spraker et al 2016), differentially regulates expression of the bikaverin pathway in *Fusarium fujikuroi* and the imizoquin pathway in *A. flavus.* On one hand, bikaverin specifically accumulates in the chlamydospores and retards *R. solanacearum* proliferation whereas imizoquin, suppressed by ralsolamcyin, promotes *Aspergillus* germination via a ROS protective mechanism. We speculate on a role for agricultural in fueling a SMs arms race affecting microbial ecology and disease development.

Spraker JE, Sanchez LM, Lowe TM, Dorrestein PC, Keller NP (2016) *Ralstonia solanacearum* lipopeptide induces chlamydospore development in fungi and facilitates bacterial entry into fungal tissues. ISME J. 10(9):2317-30.

Complex fungal communities in the lung. *Deborah Hogan*[1], Elora Demers[1], Amy Biermann[1], Jason Stajich[2] 1) Dept Microbiology/Immunology, Geisel School of Medicine at Dartmouth , Hanover, NH; 2) Department of Plant Pathology and Microbiology, UC Riverside.

Chronic fungal infections are often recalcitrant to treatment, and the recalcitrance of chronic infections is not well understood. Recent studies in different systems, from bacterial infections to human tumors have found that even within seemingly uniform populations, there is genetic and physiologically-relevant heterogeneity that likely impacts the course of disease and the response to treatment. Heterogeneity within clinical populations of fungal pathogens have not been as well studied. We have identified chronic high burden *C. lusitaniae* lung infections in three unrelated subjects with cystic fibrosis (CF). In all three subjects, *C. lusitaniae* isolates within each population showed high variance in antifungal sensitivity, measured as the minimum inhibitory concentration (MIC), with some isolates stably resistant. In the studies presented here, we will discuss bacterial adaptation to the lung, the repeated selection for genes involved in drug resistance even in

the absence of antifungal therapy, and the roles of antifungal resistance genes in complex populations, microbial communities that contain bacteria and fungi, and in the host environment.

Novel interkingdom sensing and signaling in rice blast. *N.I. NAQVI*[1], Y KOU[2], R.N PATKAR[3], Y LIU[1], Z QU[1], F YANG[1] 1) Temasek Life Sciences Laboratory, Singapore, SG; 2) China National Rice Research Institute, China; 3) Maharaja Sayajirao Univ. of Baroda, India.

Rice Blast represents a model pathosystem, wherein the causal fungus *Magnaporthe oryzae* perceives and responds to host cues to initiate pathogenic development. Recently, we gained insight into the role of the CFEM motif in surface sensing/response function of the Pth11 receptor, which upon activation utilizes the late endosomal compartment as a scaffold for sustained G-protein/cyclic AMP signaling to induce appressorium formation. Highly regulated redox homeostasis was found to be important for Pth11-mediated appressorium formation in *M. oryzae*. Swapping of the CFEM motifs and *in vitro* binding assays provided further insight into the molecular signatures that are likely recognized by the individual CFEM domains.

Our data suggest that a key step in the appressorium initiation process is cessation of vegetative growth at the germ tube tip, which is achieved through the timely synthesis and action of a novel signaling moiety, Jasmonic acid (JA; fungal oxylipin), in *M. oryzae.* Such intrinsically produced JA is an important signal for appressorium formation and acts in concert with the MAPK cascade in *M. oryzae.* Interestingly, the cAMP signaling itself is necessary for the synthesis of JA in the blast fungus, which subsequently utilizes the ABM monooxygenase to produce a novel metabolite effector ie 12-hydroxy jasmonic acid. Typically, a biotrophic phase precedes necrotrophy during Magnaporthe-Rice interaction and disease development. The blast fungus secretes 12OH-JA into the host tissue and directly inhibits the phytohormone signaling involved in pathogen recognition and defense in rice. We propose a critical role for intrinsic jasmonates in pathogenic differentiation in *M. oryzae*, and a highly intricate chemical communication system during biotrophic interactions in Rice Blast.

Saturday, March 18 2:00 PM – 5:00 PM
Merrill Hall
Signal Transduction and Gene Regulatory Networks

Roles for predicted G protein coupled receptors in plant cell wall sensing in *Neurospora crassa*. *K.A. Borkovich*, L. Collier, A. Ghosh, I.E. Cabrera Plant Pathology & Microbiology, University of California, Riverside, CA.

G protein coupled receptors (GPCRs) regulate facets of growth, development and environmental sensing in eukaryotes, including filamentous fungi. The largest predicted GPCR class in these organisms is the Pth11-related, with members similar to a protein required for disease in the plant pathogen *Magnaporthe oryzae*. Largely through the work of undergraduate students in a research-based course at UC Riverside, we have demonstrated that Pth11-related GPCRs are required for normal growth and asexual development on cellulose and other plant-derived carbon sources in the filamentous fungus *Neurospora crassa*. Our work revealed that 3 Pth11-related mutants grew relatively better than wild type on medium containing crystalline cellulose as an alternative to sucrose. We were particularly interested in this result, as this may correlate with sensing of the plant surface for colonization. The most prevalent developmental phenotype for Pth11-related mutants on sucrose was a defect in aerial hyphae height (8 mutants). We are further exploring aerial hyphae height on cellulose and have identified 5 mutants with phenotypes. Our preliminary analysis of conidia production revealed 6 mutants with defects. Based on the cellulose growth and aerial hyphae/conidiation defects, we reasoned that Pth11-related genes may be required for normal aerial hyphae and conidia production on plant-derived carbon sources. Mining of publically available transcriptional profiling data showed that 14/22 detected Pth11 genes were expressed to higher levels on cellulose than sucrose, including the 3 genes that produced a growth phenotype. These results suggest that the other 19 highly expressed genes may regulate growth on cellulose, but that gene redundancy is masking phenotypes in single mutants. Importantly, these findings also support roles for Pth11-related proteins in sensing plant-derived carbohydrates during growth and development in *N. crassa*.

A role for the velvet protein VE-1 in light sensing and conidiation in *Neurospora crassa*. María del Mar Gil-Sánchez, Eva M. Luque, *Luis M. Corrochano* Department of Genetics, University of Seville, Seville, ES.

The *N. crassa ve-1* gene is the homolog of *veA* in *Aspergillus nidulans*. In *A. nidulans* the VeA protein forms protein complexes with blue and red photoreceptors and with other regulators to control development and secondary metabolism. The *N. crassa ve-1* mutant has defects in aerial hyphal growth and increased conidiation, and we have shown that the *ve-1* mutant has an altered accumulation of carotenoids after light exposure. How does VE-1 regulate pigment biosynthesis and development in *N. crassa*?
We have observed a ten-fold reduction in the sensitivity to light in the *ve-1* mutant, an indication for a role of VE-1 in light sensing. The mutation in *ve-1* results in decreased light-dependent accumulation of the mRNAs of several genes, including the carotenogenesis genes (*al-1*, *al- 2*, *al-3*, *cao-2*). However, the absence of any change in the amount of the photoreceptor WC-1 suggests that the reduction of sensitivity to light in the *ve-1* mutant is due to a direct effect of VE-1 on the regulation of transcription. Accordingly, we observed that VE-1 is preferentially located in the nucleus, consistent with a role as a transcriptional regulator.
We have detected the presence of VE-1 during conidial development but the absence of VE-1 in aerial hyphae kept in the dark was puzling given the presence of *ve-1* mRNA. We have assayed the stability of VE-1 in vegetative mycelia and we have found that the degradation of VE-1 is modulated by the photoreceptor WC-1, and requires the activity of the CSN signalosome and the F-box protein FWD-1. These results suggests that VE-1 is subjected to ubiquitination prior to degradation. We propose that the presence of VE-1 in aerial hyphae and the regulation of its stability by light are key steps in the regulation of conidial development in *N. crassa*.

Signaling through Lrg1, Rho1 and Pkc1 governs *Candida albicans* morphogenesis in response to diverse cues. Jinglin L. Xie[1], Nora Grahl[2], Trevor Sless[1], Michelle D. Leach[1,3], Sang H. Kim[1], Deborah A. Hogan[2], Nicole Robbins[1], *Leah Cowen*[1] 1) Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada; 2) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, 03755, USA; 3) Aberdeen Fungal Group, School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, United Kingdom.

The capacity to transition between distinct morphological forms is a key virulence trait for diverse fungal pathogens. A poignant example of a leading opportunistic fungal pathogen of humans for which an environmentally responsive developmental program underpins virulence is *Candida albicans*. *C. albicans* mutants that are defective in the transition between yeast and filamentous forms typically have reduced virulence. Although many positive regulators of *C. albicans* filamentation have been defined, there are fewer negative regulators that have been implicated in repression of filamentation in the absence of inducing cues. To discover novel negative regulators of filamentation, we screened a collection of 1,248 *C. albicans* homozygous transposon insertion mutants to identify those that were filamentous in the absence of inducing cues. We identified the Rho1 GAP Lrg1, which represses filamentous growth by stimulating Rho1 GTPase activity and converting Rho1 to its inactive, GDP-bound form. Deletion of *LRG1* or introduction of a *RHO1* mutation that locks Rho1 in constitutively active, GTP-bound state, leads to filamentation in the absence of inducing cues. Deletion of the Rho1 downstream effector *PKC1* results in defective filamentation in response to diverse host-relevant inducing cues, including serum. We further established that Pkc1 is not required to sense filament-inducing cues, but its kinase activity is critical for the initiation of filamentous growth. Our genetic analyses revealed that Pkc1 regulates filamentation independent of the canonical MAP kinase cascade. Further, although Ras1 activation is not impaired in a *pkc1Δ/pkc1Δ* mutant, adenylyl cyclase activity is reduced, consistent with a model in which Pkc1 functions in parallel with Ras1 in regulating Cyr1 activation. Thus, our findings delineate a signaling pathway comprised of Lrg1, Rho1 and Pkc1 with a core role in *C. albicans* morphogenesis, and illuminate functional relationships that govern activation of a central transducer of signals that control environmental response and virulence programs.

Intracellular pH acts as a second messenger for MAPK signaling in fungi. *Tânia Ribeiro Fernandes*[1], Antonio Serrano Salces[1], Teresa Fernández-Acero[2], David Turrà[1], María Molina[2], Antonio Di Pietro[1] 1) Department of Genetics, University of Cordoba, Cordoba, Cordoba, ES; 2) Departamento de Microbiología II, Universidad Complutense de Madrid, 28040 Madrid, Spain.

Ambient pH controls fundamental processes in fungi such as growth, development, metabolism and pathogenicity. We previously found

that extracellular pH governs infectious growth in the plant pathogen *Fusarium oxysporum* by reprogramming phosphorylation levels of mitogen-activated protein kinases (MAPKs). The molecular events underlying the pH response are currently unknown. Here we identify intracellular pH (pHi) as a new second messenger regulating MAPK activity in *F. oxysporum*. Using the ratiometric GFP-based pH sensor pHluorin, we found that *F. oxysporum* responds to extracellular alkalinisation and acidification with a transitory shift in pHi. Exogenous application of diethylstilbestrol (DES), a specific inhibitor of the plasma membrane P-type H^+-ATPase, induced a rapid and sustained decrease of pHi accompanied by rapid and transitory changes in MAPK phosphorylation, supporting the idea that pHi acts as a key switch controlling MAPK activity. To search for fungal proteins involved in pHi-mediated MAPK regulation, we screened a subset of acid-sensitive mutants from the yeast deletion library for loss of DES-triggered MAPK phosphorylation. This identified a number of candidates functioning in conserved cellular processes such as glycosylation, endocytosis or vacuolar ATPase function, many of which have predicted orthologues in Fusarium. Understanding how pHi regulates MAPK signaling may reveal new ways to control fungal growth, development and pathogenicity.

Investigating how a thermally dimorphic fungal pathogen, *Histoplasma capsulatum,* senses and responds to its host environment. *Lauren Rodriguez*, Sarah Gilmore, Sinem Beyhan, Anthony Myint, Mark Voorhies, Anita Sil Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, Ca.

Thermally dimorphic fungal pathogens utilize temperature as a signal to distinguish between growth in the environment and growth within a mammalian host. Growth at 37ºC triggers alterations in morphology and gene expression. We study *Histoplasma capsulatum,* which exists in a multicellular hyphal phase in the soil that transitions to a unicellular yeast phase after inhalation by a mammalian host. Here we describe the identification of insertion mutants that are locked in the yeast phase independent of temperature. These mutants are either defective in sensing temperature or transducing the temperature signal. One insertion mutation disrupts the regulation of the *MSB2* gene, whose ortholog encodes a putative osmosensor in *Saccharomyces cerevisiae*. Gene expression profiling of the *MSB2* mutant revealed that, unlike wild-type cells, the transcription factor Efg1 fails to be induced at room temperature in the yeast-locked mutant. Since Efg1 promotes filamentation in *C. albicans*, we explored the role of Efg1 in filamentation in *H. capsulatum*. We determined that overexpression of Efg1 is sufficient to promote filamentation even at 37ºC. Taken together, these data suggest that Msb2 may be required for Efg1 induction to promote filamentation in response to temperature. We are currently characterizing the role of *MSB2* in temperature response pathways in *H. capsulatum*.

Functional analyses of the RNAi pathway required for sexual development in the cereal pathogen *Fusarium graminearum*. *Da-Woon Kim*, Hee-Kyoung Kim, Sung-Hwan Yun Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam, KR.

Fusarium graminearum, the causal agent of Fusarium head blight in cereal crops, produces sexual progeny (ascospore) as an important overwintering and dissemination strategy for completing the disease cycle. This homothallic ascomycetous species carries two opposite mating-type (*MAT*) loci in a single nucleus to control sexual development. Recently, we have identified putative target genes or regulatory pathways controlled by the MAT proteins, among which a RNA interference (RNAi) pathway has been suggested to control a late stage of sexual development. Using several molecular strategies, we have determined the functions of several genes involved in the putative RNAi pathway such as *FgSAD-1*, *FgSAD-3*, *FgDCL-1*, *FgDCL-2*, *FgRecQ-2*, *FgQIP*, *FgSMS-2*, *FgQDE-3*, *FgQDE-2* and *FgQDE-1*. In particular, we have focused on the role of *FgSMS-2* encoding an Argonaute-like protein, which is a part of the RNA-induced silencing complex for specific cleavage of target mRNAs, in *F. graminearum*. Both gene deletion- and gene overexpression-strains of *FgSMS-2* were defective in ascospore/asci maturation. A GFP-tagging analysis showed that FgSMS-2 was specifically localized on perinuclear regions inside the immature asci. A BiFC analysis revealed that FgSMS-2 was able to bind to a Dicer-like protein, FgDCL-1 in cytoplasmic region during the early stage of sexual development. However, three isoforms of the AGO2 protein from *Drosophila melanogaster* were not able to complement the defects caused by *FgSMS-2* in *F. graminearum*, indicating an evolutionary divergence of the AGO-2 like proteins between these two eukaryotes. In addition, we identified a putative target gene (*TAG1*) of FgSMS-2, which showed an unknown protein similarity. The overexpression of *TAG1* led at least 12 h-delay of ascospore formation in *F. graminearum*. Overall, it is likely that the a RNAi pathway plays an important role during the sexual development in *F. graminearum*, particularly the Argonaute-like protein, FgSMS-2 protein, controls a set of mRNAs that might be unnecessary during the meiotic event in *F. gramineaerum*.

A conserved regulator of meiosis has been rewired to control chlamydosporulation in the fungal pathogen of humans *Candida albicans*. A. Hernandez-Cervantes[1], S. Znaidi[1,2], V. Basso[1], J. Ropars[1], N. Sertour[1], D. Sullivan[3], G. Moran[3], F. Vincent[4], F. Dalle[4], M.E. Bougnoux[1], S. Bachellier-Bassi[1], *C. d'Enfert*[1] 1) Fungal Biology and Pathogenicity, Institut Pasteur, INRA, Paris, FR; 2) Laboratoire de Microbiologie Moléculaire, Vaccinologie et Développement Biotechnologique, Institut Pasteur de Tunis, Tunis, TUN; 3) Trinity College, Dublin, IRL; 4) UMR 1347, Université de Bourgogne, Dijon, FR.

Chlamydospores are large, spherical, thick-walled cells produced by several fungi, including the fungal pathogens of humans *Candida albicans* and *Candida dubliniensis*. Little is known regarding the function of these cells and the pathway controlling chlamydosporulation. While investigating the function of the transcription factor Rme1 in the regulation of morphogenesis in *C. albicans*, we have observed that it binds and positively regulates genes that have been previously associated to chlamydospore formation (1). Consistently, inactivation of *RME1* abolished chlamydospore formation in *C. albicans* and *C. dubliniensis* while its over-expression resulted in profuse chlamydosporulation. *RME1* overexpression could also bypass the requirement for other chlamydospore formation regulators such as Efg1 and Hog1. Finally, the expression levels of *RME1* and its targets were highly correlated to the varying ability of *C. albicans* clinical isolates to produce chlamydospores. Thus, Rme1 plays a central role in the control of chlamydosporulation in *C. albicans* and *C. dubliniensis.*

Consistent with this role, *RME1* expression increased in the course of chlamydosporulation. Chromatin-immunoprecipitation experiments, experiments using conditional *RME1* expression and gene fusion experiments with a luciferase reporter allowed to demonstrate that *RME1* expression is subject to a dual regulation whereby (i) elevation of *RME1* mRNA levels in response to chlamydospore-inducing conditions is independent of Rme1 and (ii) sustained expression of *RME1* during chlamydosporulation is subject to autoregulation. Taken together, our results establish Rme1 as a central regulator of the chlamydospore developmental pathway in *C. albicans.* Surprisingly, in *S. cerevisiae* and other yeasts, Rme1 plays an important role in the control of meiosis, and thus ascospore formation. Therefore, our

results indicate that Rme1 can be used alternatively to control sexual or asexual development in Saccharomycotina.
(1) Palige *et al*. PLoS One 8:e61940, 2013

Subcellular dynamics of the MAP kinase MAK-2 correlate with its activity during germling fusion, thereby mediating a conserved cell-cell signaling mechanism. *A. Serrano*[1], M. Weichert[1], R. Marschall[2], J. Illgen[1], U. Brandt[1], N. Thieme[1], A. Lichius[3], N. Read[3], P. Tudzynski[2], A. Fleissner[1] 1) Department of Genetics, TU Braunschweig, Braunschweig, DE; 2) Institute of Molecular Biology and Biotechnology of Fungi, Schlossplatz 8, Münster, DE; 3) Institute of Inflammation and Repair, University of Manchester, CTF Building 46, UK.

Cell-cell fusion is essential for the development of most eukaryotic organisms. However, the molecular basis of this process is poorly understood. In *Neurospora crassa*, fusion occurs between germinating vegetative spores so-called germlings. This process employs an unusual mode of communication, in which the two fusion partners coordinately alternate between signal sending and receiving in a “cell dialog”. It includes the alternating membrane recruitment of the SO protein (signal sending) and the MAP kinase MAK-2 (signal receiving). To analyze the relationship between the localization and activity of MAK-2, we permanently tethered the kinase to the plasma membrane by using a –CaaX motif. This mislocalization resulted in a fusion-defective phenotype in the Δ*mak-2* background, indicating that the dynamics of MAK-2 are essential for its function. Western blot analyses revealed a hyper-phosphorylation of the membrane-bound MAK-2 by its upstream MAP kinases. In addition, membrane tethering of activated MAK-2 in WT background disrupts the dynamic localization of SO and reduces the number of tropic interactions.
Similar to *N. crassa*, the grey mold *Botrytis cinerea* undergo cell fusion during colony formation. Localization of the MAK-2 homolog, BMP-1, shows a comparable subcellular dynamic localization during *B. cinerea* cell interactions. Surprisingly, we found that *N. crassa* and *B. cinerea* germlings undergo mutual attraction and establish physical contact, although complete fusion events were not detected.
Together, these data suggest that the “cell dialog” mode of communication is conserved in filamentous ascomycete fungi and that divergent fungal species share a common language

Saturday, March 18 2:00 PM – 5:00 PM
Chapel
Fungal Multicellular Development

ChIP-Seq analysis identifies components of the cell wall integrity, NADPH oxidase and pheromone signaling integrity pathways as target genes of the fungal developmental regulator PRO1. *U. Kück*, S. Steffens, K. Becker, S. Krevet, I. Teichert Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE.

Fruiting body formation is a fundamental step during the life cycle of filamentous fungi that requires tight spatiotemporal regulation. Working with the model fungus *Sordaria macrospora*, we have previously demonstrated that this major process in the sexual life cycle is controlled by the $Zn(II)_2Cys_6$ zinc finger transcription factor PRO1.
Here, we further investigated the genome-wide regulatory network controlled by PRO1 by employing chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq) to identify binding sites for PRO1 (Steffens et al. 2016). We identified several target regions that occur in the promoter regions of genes encoding components of diverse signaling pathways. Furthermore, we identified a conserved DNA-binding motif that is bound specifically by PRO1 in vitro. In addition, PRO1 controls in vivo the expression of a DsRed reporter gene under the control of the esdC target gene promoter. Our ChIP-seq data suggest that PRO1 also controls target genes previously shown to be involved in regulating the pathways controlling cell wall integrity, NADPH oxidase and pheromone signaling. Our data point to PRO1 acting as a master regulator of genes for signaling components that comprise a developmental cascade controlling fruiting body formation.

Steffens E, Becker K, Krevet S, Teichert I, Kück U (2016) Mol Microbiol (in press)

Findings on the origin and genetic background of fungal multicellularity. *K. Krizsan*[1], B. Balint[2], A. Prasanna N.[1], B. Kiss[1], E. Almasi[1], Gy. Sipos[3], I. Nagy[1], L. Nagy G.[2] 1) Biochemistry Inst, BRC, Szeged, Hungary; 2) Seqomics Ltd, 6782 Mórahalom, Hungary; 3) Institute of Silviculture and Forest Protection, University of West-Hungary, 9400 Sopron, Hungary.

The independent emergence of complex fruiting bodies was a major step in fungal evolution. Despite decades-long interest in fruiting body development, its genetic bases and the origins of the involved genes are still incompletely known. Here, we set out to identify structural and regulator genes underlying the development of 3 mushroom-forming fungi and to infer a core set of developmentally regulated genes required to build an agaricoid fruiting body. We obtained expression profiles for equivalent developmental stages (vegetative mycelium, early and late primordium, young and mature fruiting body) and tissue types (cap, stipe, lamellae) of *Coprinopsis cinerea* AmutBmut*, Schizophyllum commune* H4-8*, Armillaria ostoyae* C-18 using RNA-Seq. Our results demonstrate that fruiting body formation requires a drastic reorganization of gene expression patterns, with the most dramatic changes observed at the transition from vegetative mycelium to primordia and from young to mature fruiting bodies. Based on global expression profiles in both *Coprinopsis* and *Armillaria*, early primordia grouped with samples derived from stipes, while cap and gill tissues comprised a distinct similarity group, suggesting that two separate developmental pathways exist during the formation of agaricoid fruiting body types. Mining of the genomes of related mushroom-forming fungi indicated a high conservation for most structural genes across the Agaricomycetes, whereas, regulators of gene expression showed more restricted taxonomic distributions. These results will contribute to understanding the genetic background and conserved and lineage-specific mechanisms of fruiting body formation in the Agaricomycetes.

***IDC2* and *IDC3*, two genes involved in cell non-autonomous signaling of fruiting body development in the model fungus *Podospora anserina*.** *Herve Lalucque*[1], Fabienne Malagnac[1,2], Kimberly Green[3], Valérie Gautier[1], Pierre Grognet[1,2], Laetitia Chan Ho Tong[1,4], Barry Scott[3], Philippe Silar[1] 1) Univ Paris Diderot, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, Case courrier 7040 Lamarck, 75205 Paris, France; 2) Present adress : Institut de Biologie Intégrative de la Cellule, UMR 9198 CEA CNRS Univ Paris Sud, 91405 Orsay, France; 3) Institute of Fundamental Sciences, Massey University, Palmerston North, 4442, New Zealand; 4) Present adress : Institut Français du Pétrole Energies Nouvelles 1-4 Avenue du Bois Préau, 92852 Rueil-Malmaison, France.

Filamentous ascomycetes produce complex multicellular structures during sexual reproduction. Little is known about the genetic pathways enabling the construction of such structures. Here, with a combination of classical and reverse genetic methods, as well as genetic mosaic and graft analyses, we identify and provide evidence for key roles for two genes during the formation of perithecia, the sexual fruiting bodies, of the filamentous fungus *Podospora anserina*. Data indicate that the proteins coded by these two genes function cell-non-autonomously and that their activity depends upon conserved cysteines, making them good candidate for being involved in the transmission of a reactive oxygen species (ROS) signal generated by the PaNox1 NADPH oxidase inside the maturing fruiting body towards the PaMpk1 MAP kinase, which is located inside the underlying mycelium, in which nutrients are stored. These data provide important new insights to our understanding of how fungi build multicellular structures.

Origins of novel sexual development phenotypes in the evolution of the transcriptome in five fungi. *Jeffrey P. Townsend*[1], Zheng Wang[1], Kayla Stefanko[2], Caitlyn Cubba[2], Frances Trail[2] 1) Yale University, New Haven, CT; 2) Michigan State University, East Lansing, MI.

Changes in gene expression have been hypothesized to play an important role in the evolution of divergent morphologies. To test this hypothesis, we examined developmental differences in fruiting body morphology of filamentous fungi, culturing five species in a common garden environment and profiling genome-wide gene expression at five stages. We reconstructed ancestral gene expression, identifying genes with the largest evolved increases in gene expression across development. Conducting knockouts and performing phenotypic analysis in two divergent species typically demonstrated altered fruiting body development in the species that had evolved increased expression. Our evolutionary approach to finding relevant genes proved far more efficient than other gene deletion studies targeting whole genomes or gene families. Combining gene expression measurements with knockout phenotypes facilitated the refinement of Bayesian models of the gene network underlying fruiting body development, regulation of which is one of the least understood processes of multicellular development.

Role of blue light for fruiting body induction and development in basidiomycetous fungi. *Y. Sakamoto*[1], H. Muraguchi[2] 1) Department of Bioresource Sciences, Iwate Biotechnology Research Center, Kitakami, JP; 2) Department of Biotechnology, Akita Prefectural University, Akita, JP.

Light is one of crucial environmental factor for sexual development in fungi. Especially basidomycetous fungi form lager fruiting bodies compared with other filamentous fungi. Therefore relationship between light and fruiting body development in basidiomycetous fungi has been investigated, but molecular mechanism of fruiting body development under light has not been well understood yet compared with ascomycetous fungi, like *Neurospora crassa*. Several basidiomycetous species can form fruiting body under complete darkness. In *Flammulina velutipes* and *Coprinopsis cinerea*, fruiting bodies can be formed under dark, but cap can not mature under darkness, called pinhead fruiting body, or dark stipe. Light can induce cap development on pinhead fruiting bodies by light stimulation in *F. velutipes*. In *Lentinula edodes*, fruiting body formed under dark is whiter compared with normal fruiting body formed under light, and light can stimulate pigmentation toward white fruiting bodies formed under dark. These suggest that light stimulates fruiting body morphogenesis in basidiomycetous fungi. Blue light receptors were identified in *C. cinerea*, dst-1 and dst-2, from dark stipe mutant. The dst-1 encodes WC-1, a homologue of White Collar-1 in *N. crassa*, and dst-2 encodes a protein that has putative flavin adenine dinucleotide (FAD)-binding-4 domain. Homologue of WC-2, a partner of WC-1, in basidiomycetous fungi was first found in *L. edodes*, and disruption of WC-2 in *C. cinerea* resulted dark stipe.

Light also promotes fruiting body induction in several basidomycetous fungi. In *C. cinerea* AmutBmut strain #326, hyphal knot, initial of fruiting body, can be induced concurrently on concentric circle like fairy ring by blue light stimulation. Concurrent hyphal knot induction can be induced in WC-2 mutant. This suggests that WC-1/WC-2 complex will not be involved in hyphal knot induction in *C. cinerea*. Gene expression after blue light stimulation for concurrent hyphal knot induction will be discussed.

RNA editing: posttranscriptional control of multicellular development in *Sordaria macrospora*. *I. Teichert*, T. Dahlmann, U. Kück, M. Nowrousian Ruhr-University Bochum, Allgemeine und Molekulare Botanik, Bochum, DE.

The filamentous ascomycete *Sordaria macrospora* has a long history as a model system for studying fungal fruiting body formation. *S. macrospora* generates mature perithecia within seven days without a mating partner and does not produce any conidia, allowing easy observation of sexual structures. The genetic analysis of sterile mutants has enabled the identification of many developmental factors, such as kinases and phosphatases, divers signaling proteins, and transcription factors [1, 2]. Further, transcriptomic analysis of mutants and different developmental stages revealed a large number of potential regulators of fruiting body formation [3, 4]. Studying *S. macrospora* RNA-seq data in more detail, we detected an increase of putative A-to-I RNA editing events in wild type protoperithecia compared to sexually developing mycelia. These data are consistent with a recent study in *Fusarium graminearum*, which identified fruiting body-specific A-to-I RNA editing [5]. Strikingly, the above-mentioned increase of putative editing events in *S. macrospora* wild type was not observed in RNA-seq data of protoperithecia from sterile mutants pro1 and Dnox1, lacking genes for a transcription factor and an NADPH oxidase, respectively. These data indicate that A-to-I RNA editing is linked to fruiting body formation in *S. macrospora*. Putative editing sites in five genes were verified from independent cDNA samples by Sanger sequencing. The same sites were not edited in cDNA from sterile mutant pro1. The effect of RNA editing on protein function is under investigation.

[1] Teichert et al. 2014 Adv Genet 87:199
[2] Teichert et al. 2014 PLoS Genet 10:e1004582
[3] Teichert et al. 2012 BMC Genomics 13:511
[4] Dirschnabel et al. 2014 Genetics 196:729
[5] Liu et al. 2016 Genome Res 26:499

Cag1, one of the Tup1 paralogues in *Coprinopsis cinerea*, is required for gill formation in fruiting body development. *H. Muraguchi*, R. Masuda, N. Iguchi, K. Tsukuta, T. Nagoshi, K. Kemuriyama Dept Biotechnology, Akita Prefectural Univ, Akita, JP.

The pileus (cap) of the fruiting body in homobasidiomycete fungi bears the hymenium, a layer of cells that includes the basidia where nuclear fusion, meiosis and sporulation occur. *Coprinopsis cinerea* is a model system for studying fruiting body development. The hymenium of *C. cinerea* forms at the surface of the gills in the pileus. We have mutagenized a homokaryotic fruiting strain, #326, and isolated a mutant that fails to enlarge the cap tissue on the primordial shaft in fruiting. Genetic analysis of this mutant, *cap-growthless*, indicated that the mutant phenotype was brought about by a single gene, designated as *cag1*. The *cag1* locus was mapped on chromosome IX by linkage analysis using RAPD markers that were mapped to each chromosome. The *cag1* gene was identified by transformation experiments using BAC DNAs and their subclones derived from chromosome IX, and found to encode a homologue of *Saccharomyces cerevisiae* Tup1. The *C. cinerea* genome contains another Tup1 homologue called *Cc.tupA*. We examined whether the expression levels of *cag1* and *Cc.tupA* are developmentally regulated during fruiting. In the vegetative mycelium, *Cc.tupA* were expressed more than *cag1*. In contrast, in the growing pileus, the *cag1* expression levels were higher than those of *Cc.tupA.* To examine changes in the spatial and temporal expression of Cag1 and Cc.TupA in the pileus, we reciprocally fused fluorescent tags, i.e. EGFP and mCherry, to these proteins. Compared with Cc.TupA, Cag1 was preferentially expressed in the gill trama tissue cells, suggesting that the function of Cag1 is required for gill trama tissue differentiation and maintenance. Because it is known that *S. cerevisiae* Tup1 forms homotetramer, we examined whether Cag1 interacts with itself and with Cc.TupA using yeast two-hybrid (Y2H) system. Y2H analysis reveals that Cag1 can interact with itself and with Cc.TupA through their N-terminal regions. Like Tup1, which interacts with Cyc8, the N-terminal region of Cag1 also interacts with the N-terminal region of Cc.Cyc8, which contains tetratricopeptide repeats. Y2H analysis and co-localization of Cag1 and Cc.TupA suggested that Cag1 interacts with Cc.TupA in the nuclei of certain cells.

Feeding developing button mushrooms. *A. Sonnenberg*, J. Baars Plant Breeding, Wageningen University and Research Centre, Wageningen, NL.

The number and sizes of fruiting bodies of saprotrophic fungi heavily depend on the amount and quality of substrate available for these fungi. Is thus not surprising that substrate for the production of edible mushrooms is one of the main research areas to improve yield and quality and as a result the profitability of commercial production systems. For button mushrooms (*Agaricus bisporus*), next to substrate, a

nutritionally poor casing layer on top of the substrate is a prerequisite to induce the vegetative mycelium to form fruiting initials. The quality of this layer also has a prominent influence on the quality of mushrooms. Quality is reduced when mushrooms mature, i.e. when the cap is opening and sporulation starts. Previous research on button mushrooms has shown that there is a signalling between the top layer where fruiting bodies are formed and the bottom layer where substrate is degraded and where nutrients are transported to the developing mushrooms. The induction of fruiting changes the physiology and gene expression of the vegetative mycelium in the compost towards feeding the developing mushrooms. Changing the nutritional quality and the total amount of nutrients in the substrate layer influences the yield and quality but not always in a logical way. Many observations have been done in the past in mainly empirical and applied research that have generated some understanding.

We intend to give an overview on research done in the past and more recently on the cultivation of button mushrooms (*Agaricus bisporus*) and evaluate what this has learned us so far. We intend to focus also on the type of research is needed to generate the knowledge necessary to understand the production system and to use this to either improve the present cultivation system or even to develop an entirely new one.

Saturday, March 18 2:00 PM – 5:00 PM
Fred Farr Forum
Synthetic Biology, Fungal Tools, and Fungi as Tools

JGI fungal single cell genomics pipeline. *Doina Ciobanu*[1], Alicia Clum[1], Asaf Salamov[1], Wiliam Andreopoulos[1], Steven Ahrendt[1,2], Alisha Quandt[3], Igor Grigoriev[1,2], Timothy James[3], Jan-Fang Cheng[1] 1) Genomics, Joint Genome Institute, Walnut Creek, CA; 2) Plant and Microbial Biology, University of California, Berkeley, CA; 3) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

We developed a single cell genomics pipeline for noncultured fungal species and used it for sequencing seven early diverging fungi: *Blyttiomyces helicus* [Chytridiomycota], *Caulochytrium protostelioides* [Chytridiomycota], *Rozella allomycis* [Cryptomycota], *Syncephalis pseudoplumigaleata* [Zoopagomycotina], *Thamnocephalis sphaerospora* [Zoopagomycotina], *Piptocephalis cylindrospora* [Zoopagomycotina], *Dimargaris cristalligena* [Kickxellomycotina], and one Dikarya: *Metschnikowia bicuspidata* [Ascomycota]. The established pipeline for fungal single-cell genome recovery consists of seven steps: environmental sample collection and analysis, single cell isolation, single cell lysis and whole genome amplification, single cell amplified genome (SCAG) quality screening, best SCAG genome deep sequencing and assembly and finally best SCAG or combined SCAG (C-SCAG) annotation and functional analysis. Each of these steps can have an impact on the quality of the resulting genome and annotation. We validated each step of the pipeline using a set of quality check criteria such as start genome amplification (SGA), fold genome amplification (FGA), random twenty-mer uniqueness (RTU), genome GC content, assembled genome size plus a set of metrics for genome assembly quality and evaluated each using criteria for its predictability power in relationship to genome completeness, which was estimated using CEGMA (CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. G. Parra, K.Bradnam, I.Korf. Bioinformatics, V23, Iss 9, pp1061-1067). We examined all the factors that have a critical impact for fungal single cell genome recovery, the most important of which is amplification bias, which can be characterized by the combination of SGA, FGA, RTU and genome GC content. We offer a suite of protocols for a large scale workflow that can be applied to a wider range of organisms than fungi.

A global co-expression network approach for connecting genes to specialized metabolic pathways. *Jennifer Wisecaver*, Abigail Lind, Antonis Rokas Vanderbilt University, Nashville, TN.

Both fungi and plants produce a tremendous diversity of specialized metabolites (SMs) to interact with and manage their environment. A major challenge hindering efforts to tap this seemingly boundless source of pharmacopeia is the identification of SM pathways and their constituent genes. To address this challenge, much attention has been paid to fungal SM gene clusters, but the number of SM pathways in fungi that are non-clustered or only partially clustered is completely unknown. The genome of *Aspergillus fumigatus,* an opportunistic human pathogen and one of the leading causes of fungal-related human deaths, provides a stark example; 21 of its 37 SM gene clusters are missing either transporters, transcription factors or both, suggesting these genes may be located elsewhere in the genome. Given the well-established observation that the genes comprising a SM pathway are co-regulated in response to specific environmental conditions, we hypothesized that genes from a given SM pathway would form tight associations (modules) with each other in gene co-expression networks, facilitating their identification even without knowledge of the genome sequence. To evaluate this hypothesis, we first used 10 global co-expression datasets—each a meta-analysis of hundreds to thousands of expression experiments—across eight plant model organisms to identify hundreds of modules of co-expressed genes for each species. In support of our hypothesis, 15.3-52.6% of modules contained two or more known SM biosynthetic genes (e.g., cytochrome P450s, terpene synthases, and polyketide synthases), and module genes were enriched in SM functions. Moreover, modules recovered many experimentally validated SM pathways in these species, including all those known to form SM gene clusters. For each clustered SM pathway, the module analysis expanded the known genetic repertoire of the pathway by identifying additional unclustered enzymatic genes as well as unclustered genes involved in pathway regulation and product transport. We are currently expanding our network analysis to fungal taxa including *Aspergillus* and submit that global gene co-expression is a rich, but largely untapped, data source for discovering the genetic basis and architecture of natural products.

Universal expression system for eukaryotic microorganisms. *A. Rantasalo*, C. Landowski, J. Kuivanen, J. Jäntti, D. Mojzita VTT Technical Research Centre of Finland Ltd.

We have developed a novel orthogonal expression system that functions in a wide spectrum of eukaryotic microorganisms. The expression system is based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by an engineered, universal core promoter that provides a low, but sufficient expression level of the sTF. The sTF expression is constitutive and thus present in the cells in all growth conditions and in all developmental and growth stages. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter functional in diverse species. This core promoter can be further modified by inserting synthetic repressor (sR) binding sites, through which an additional expression control is gained upon expression of the sR. The combination of multiple sTF-binding sites and the core promoters (including the repressible versions) enables specific adjustment over a wide range of target gene expression levels, from very low to very high. This expression system provides robust, stable, and tuneable expression levels of the target genes in a broad spectrum of host organisms. Further it simplifies the genetic tools needed for the construction of new protein expression hosts, including those with undeveloped know-how. The method for selecting the universal core promoters, constructions of the expression systems, and demonstrations of their performance in diverse hosts, including yeasts and filamentous fungi, will be presented.

A CRISPR/Cas9 based toolbox for efficient genome editing of filamentous fungi. *U.H. Mortensen*, C. S. Nødvig, Z.D. Jarczynska, M.L. Nielsen, F. H. Kirchner, J.B. Hoof Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, DK.

Large numbers of fungi are currently being fully sequenced and will contribute dramatically to our understanding of fungal biology. However, the fact that gene targeting is inefficient in most fungal species hampers exploitation of the genome sequences. This problem has been significantly reduced after efficient CRISPR/Cas9 gene editing has been introduced in several different fungal species. A challenge of the CRISPR/Cas9 system is to deliver gRNAs to form the Cas9 ribonuclease. Several approaches have been presented in the literature including methods where gRNAs are co-transformed into the cells along with the gene editing DNA substrates, methods where the gRNA is

produced by RNA polymerase III, and methods where the gRNA is liberated by ribozymes from a larger transcript produced by RNA polymerase II. Since the different methods have different advantages/disadvantages, we envision that they may work with different efficiencies in different fungal species. We have therefore developed a flexible CRISPR/Cas9 toolbox adapted for filamentous fungi to facilitate genome editing. Our toolbox includes bio-bricks containing e.g. different genetic markers and polymerase promoter types allowing for a rapid and efficient vector assembly and bricks that allow for quick insertion of new genes into strong expression sites for heterologous expression. Moreover, it includes bricks to facilitate trouble shooting including a *cas9-RFP* reporter gene to evaluate Cas9 levels in new hosts and a system allowing the efficiency of individual gRNA species to be tested in vivo. Using our toolbox, we have successfully edited the genomes of more than 10 species and used it to make a different range of genetic alterations including site specific mutations by using oligonucleotides as repair templates and deletions. In this way we have linked secondary metabolites to genes in species that have not previously been genetically engineered. For strains where we plan to do extensive gene targeting, we typically use CRISPR to mutate the *pyrG* gene, hence, producing a marker than can be selected/counter selected. Next, we mutate a gene in the NHEJ pathway to produce a strain where gene targeting is very efficient. We will show how this strategy can be used investigate the biosynthetic pathway of gene clusters. Lastly, we will show how markers and mutations in NHEJ genes can be easily reverted to wild-type if a wild-type background is desirable in the subsequent analyses.

Synthetic biology as powerful discovery tool to understand and rewire the cell factory *Aspergillus niger*. *V. Meyer* Applied and Molecular Microbiology, TU Berlin, Berlin.

Synthetic Biology interrogates the complexity of biological systems on a molecular level. It takes a constructive approach to design novel biological parts and circuits with non-natural function and to reengineer biological networks. Obviously, systems biology and synthetic biology disciplines – systems analysis and design – ideally complement each other and a multitude of methodological crosslinks exist. Our focus is to pioneer genetic tools and molecular gene switches for the industrial strain *Aspergillus niger* and to explore its capabilities as producer of secreted proteins and natural products. By engineering titratable and tightly regulated conditional mono- and polycistronic gene expression systems and targeting them to specific genomic loci, we rewire the metabolism of *A. niger*. On the one hand, this enables us to improve this cell factory as expression host for proteins and secondary metabolites with product yields high enough for commercial exploitation. On the other hand, this approach helps us to unravel the function of complex gene networks *in vivo*.

Measuring intracellular pH as a well-controlled physiological parameter responding to extracellular cues. *G. Smits* Dept Molecular Biol, SILS, Univ Amsterdam, Amsterdam, NL.

In baker's yeast, intracellular pH can be monitored in a time-resolved, organelle specific manner in living and growing cells, using the genetically encoded pH responsive GFP variant pHluorin. We assessed intracellular pH, and found that it dynamically responds to changes in the cellular environment, and cytoplasmic pH can vary between 4.5 and 7.4. In Candida glabrata, pH responds to cues that are associated with pathogenicity. Because pH also changes in response to the influx of weak organic acids, such as those used as food preservatives or produced as industrial precursors using yeast or filamentous fungi. We have established online intracellular pH measurement as a valid proxy measurement of acid influx, allowing the establishment of kinetic parameters.
In a genome-wide analysis using the yeast deletion collection, we identified genes responsible for pH homeostasis. We established that intracellular pH is a second messenger, controlling yeast's maximal cell division rate.

Engineering of *Ustilago maydis* for efficient organic acid production. E. Geiser[1,2], S.K. Przybilla[1], M. Bölker[3], T. den Hartog[4], L.M. Blank[1], *N. Wierckx*[1] 1) Institute of Applied Microbiology (iAMB), RWTH Aachen University, Aachen, DE; 2) BioSC, c/o Forschungszentrum Jülich, Jülich, DE; 3) Department of Biology, Philipps-University Marburg, Marburg, DE; 4) Institute for Technical and Macromolecular Chemistry (ITMC), RWTH Aachen University, Aachen, DE.

The family of Ustilaginaceae contains promising biocatalysts for a variety of industrially relevant chemicals. One promising product is itaconic acid, a platform chemical for the production of pharmaceuticals, adhesives and polymers. We recently characterized the itaconate biosynthesis pathway and related gene cluster in *U. maydis* MB215 (1). The *U. maydis* itaconate gene cluster is remarkably similar to that of *Aspergillus terreus* (2). However, in contrast to the known biosynthesis pathway of *A. terreus*, itaconate production in *Ustilago* proceeds via the unusual intermediate *trans*-aconitate. Two additional genes in the cluster, *cyp3* (cytochrome P450 monooxygenase) and *rdo1* (ring-cleaving dioxygenase) are not directly involved in itaconate production. Instead, Cyp3 has a role in the further oxidation of itaconate into 2-hydroxyparaconate (3). We will present the application of this new biochemical knowledge to enhance the overall product titer and yield up to 4.5-fold, but also to control product specificity.

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***Kluyveromyces marxianus* as a robust systems and synthetic biology platform.** *R. Estrela*[1,3], P. Cernak[1,3], C. Zhang[1,3], V. Yu[1,3], J. Cate[1,2,3] 1) Molecular and Cell Biology Department, UC Berkeley, Berkeley, CA; 2) Department of Chemistry, UC Berkeley, Berkeley, CA; 3) Energy Biosciences Institute, Berkeley, CA.

Among "non-standard" yeast, there is increasing interest in studying *Klyuveromyces marxianus (Km)* given its combination of industry-appealing traits: broad range of carbon source utilization, ability to grow at temperatures up to 52ºC and ferment at 45ºC and one of the highest growth rates among eukaryotes. It is not surprising, however, that most of the studies published on *Km* up to date exploits its general biotechnological potential rather than studying the basic biology underlying its interesting traits. The lack of genetic tools has hampered in-depth genetics and biochemistry focused studies in *Km* and in many other non-model organisms. Our goal is to tackle this problem and develop *K. marxianus* into a robust platform for systems biology studies and synthetic biology applications in industrial

microbiology. We also established CRISPR-Cas9 genome editing in *Km* and used this system to engineer several strains to use in classical genetics. We have been using these synthetic biology tools to probe the genetic basis for thermotolerance in *Km* and also to use *Km* to produce value-added chemicals. We are successfully using CRISPR-Cas9 to knockout genes of interest and identify ones that might have an important role in thermotolerance. Our synthetic biology tools are also being used to explore the potential of *Km* to produce renewable fuels and chemicals.

Saturday, March 18 2:00 PM – 5:00 PM
Kiln
Chromosome Dynamics

What drives speciation? Examination into the evolutionary events of more than 100 *Aspergillus* species. *J.L. Nybo*[1], T.C. Vesth[1], S. Theobald[1], I. Kjaerboelling[1], J.C. Frisvad[1], T.O. Larsen[1], R. Riley[2], A. Salamov[2], I.V. Grigoriev[2], S.E. Baker[3], M.R. Andersen[1] 1) Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, DK; 2) Joint Genome Institute, Walnut Creek, CA, USA; 3) Joint Bioenergy Institute, Berkeley, CA, USA.

The study of speciation - how new species arise, diverge and remain separate, has a central role in evolutionary biology. Partly because it embraces so many disciplines, including population genetics, behavioral sciences, comparative genomics, evolutionary biology, biodiversity, biogeography and ecology. It also remains one of the most fascinating questions in evolution.

Speciation is nearly impossible to study and in most cases, we know very little about the genetic basis of species formation. But in this project we aim to identify evolutionary events that can drive speciation, such as gene duplications, creations and losses, and horizontal gene transfers between closely or distantly related species within the genus of the filamentous fungi Aspergillus. This diverse genus holds species relevant to both plant and human pathology, food biotechnology, enzyme and bulk chemical production, model organisms, and it even contains some extremophiles.

To identify these events, we have developed a homologous protein prediction software that has been used to generate a high-resolution pan-genomic map. From where, we have identified genes specific to species, clades and core that allows for guilt-by-association-based mapping of genotype-to-phenotype.

Our results illustrate a highly diverse genus where 500-2000 genes are unique to each species. These genes are predominantly within regulation or compound biosynthesis, supporting the notion of natural selection. A conservative estimate of the number of protein families shared by all Aspergillus species is surprisingly low, only about 2600 core families, suggesting high environmental adaptation within this genus.

Transitions between tetrapolar and bipolar fungal mating type driven by chromosomal translocations involving intercentromeric recombination. *Sheng Sun*[1], Vikas Yadav[2], R. Blake Billmyre[1], Christina A. Cuomo[3], Minou Nowrousian[4], Jean-Luc Souciet[5], Teun Boekhout[6], Betina Porcel[7], Patrick Wincker[7], Joshua A. Granek[1], Liuyang Wang[1], Kaustuv Sanyal[2], Joseph Heitman[1] 1) Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC; 2) Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 3) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; 4) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum, Germany; 5) Université de Strasbourg, CNRS UMR7156, Strasbourg, 67000, France; 6) CBS-KNAW Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands; Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands; 7) Commissariat à l'Energie Atomique (CEA), Institut de Génomique (IG), Genoscope, Evry, France, Université d'Evry, UMR 8030, Evry, France, Centre National de Recherche Scientifique (CNRS), UMR 8030, Evry, France.

Species within the human pathogenic *Cryptococcus* species complex are major threats to public health, causing more than one million infections globally each year. *Cryptococcus amylolentus* is the most closely known related species of the pathogenic *Cryptococcus* species complex, and it is non-pathogenic. Additionally, while pathogenic *Cryptococcus* species have bipolar mating systems with a single large *MAT* locus that represents a derived state in Basidiomycetes, *C. amylolentus* has a tetrapolar mating system with two *MAT* loci (*P/R* and *HD*) located on different chromosomes. Thus, studying *C. amylolentus* could shed light on the origin and evolution of pathogenesis, as well as the transition from tetrapolar to bipolar mating systems in the pathogenic *Cryptococcus* species. In this study, we sequenced, assembled, and annotated the genomes of two *C. amylolentus* isolates, CBS6039 and CBS6273, which are interfertile. Genome comparison between the two *C. amylolentus* isolates identified the boundaries and the complete gene contents of the *P/R* and *HD* loci. Also, bioinformatics and ChIP-seq analyses showed that *C. amylolentus* has regional centromeres that are enriched with species-specific transposable and repetitive elements, similar to the centromeric structures in the pathogenic *Cryptococcus* species. Additionally, we found that while neither of the *P/R* and *HD* loci in *C. amylolentus* is physically linked to its centromere, both *MAT* loci showed centromere linkage in meiosis, suggesting the presence of recombination repressors and/or epistatic gene interactions in the inter *MAT*-CEN regions. Furthermore, genomic comparison between *C. amylolentus* and pathogenic *Cryptococcus* species provided evidence that chromosomal rearrangements mediated by intercentromeric recombination have occurred after the two lineages split from their common ancestor. We propose a model in which the evolution of the bipolar mating system was initiated by an ectopic recombination event mediated by repetitive elements located within the centromeric regions and shared between chromosomes. This translocation brought the *P/R* and *HD* loci onto the same chromosome, and was followed by chromosomal rearrangements that resulted in the two *MAT* loci becoming physically linked and eventually fused to form the single contiguous *MAT* locus that is now extant in the pathogenic *Cryptococcus* species.

Genome plasticity impacts adaptive genome evolution in the vascular wilt pathogen *Verticillium*. *M.F. Seidl*, L. Faino, D.E. Cook, M. Kramer, X. Shi-Kunne, G.C.M. van den Berg, B.P.H.J. Thomma Laboratory of Phytopathology, Wageningen University & Research, Wageningen, NL.

Genome plasticity enables organisms to adapt to environmental changes and to occupy novel niches. This is established by mechanisms ranging from single-nucleotide polymorphisms to large-scale chromosomal variations, all of which contribute to differences in chromosomal size, organization and gene content. While these mechanisms operate in all organisms, they are particularly relevant for plant pathogens that engage in a co-evolutionary arms race with their hosts. Plant pathogens secrete so-called effectors that contribute to host colonization and counteract host immunity. Effector genes often cluster in highly plastic, transposon-rich genomic regions. However, mechanistic understanding of the evolution of these plastic genomic regions remains scarce. We study these molecular mechanisms in the fungal genus

Verticillium that contains economically and ecologically important plant pathogens, among which *Verticillium dahliae* is the most notorious pathogen that causes vascular wilt disease on >200 plant species. Using long-read sequencing technology, we completely assembled two *V. dahliae* strains. By comparative genomics, we established that transposable elements play important roles in shaping the genome of *V. dahliae*. Plastic genomic regions in *V. dahliae* that contain all known effectors evolve by extensive genomic rearrangements that are mediated by erroneous double-strand breaks, often over transposons. Extensive genomic rearrangements are not only restricted to *V. dahliae*, but also occur in related *Verticillium* species. Furthermore, recent segmental duplications are enhanced in the plastic regions. These regions, in contrast to the core genome, are also enriched in active transposons that further contribute to local plasticity. In fungi, transposons are located in tightly condensed chromatin, so called heterochromatin, that is supposed to suppress transposon activity and repress structural variations. In contrast, many fungal pathogens have highly plastic transposon-rich regions. Therefore, research into chromatin opens new avenues to link genome organization, genome plasticity and adaptive genome evolution in fungal pathogens.

The adaptive potential of *Candida albicans* in response to *in vivo* stress. G. Cromie[1], E. Jeffery[1], S. Filler[2], J. Berman[3], A. Dudley[1], *A. Forche*[4] 1) PNRI, Seattle, WA USA; 2) UCLA, Los Angeles, CA USA; 3) Tel-Aviv University, Tel-Aviv, Israel; 4) Bowdoin College, Brunswick, ME USA.

To understand the adaptive potential of *C. albicans* to its host, we characterized the genomes of ~1000 isolates recovered after single passage through mice from an oropharyngeal Candidiasis model (OPC) and a systemic model of Candidiasis (BSI). We used flow cytometry to determine ploidy and ddRADseq to assess whole genome karyotypes. Our analysis revealed high levels of aneuploidy and loss of heterozygosity (LOH) among isolates from both models. There was no significant difference in the overall frequencies of aneuploidy and LOH. However, chromosome (Chr) 6 trisomy was significantly higher in the OPC population. Intriguingly, Chr6 is particularly enriched for genes from multiple virulence gene families. Segmental aneuploidies and LOH occurred at similar frequencies in both model. Interestingly, all but one aneuploidy breakpoint (at the rDNA locus) were unique to each model. The majority of aneuploidy and LOH breakpoints were found near repetitive DNA and tRNAs. In addition, many LOH breakpoints were located near proposed origins of replication. This finding suggests that *in vivo* environments may trigger replication stress-induced genome instability, which could be due partially to the resolution of arrested replication forks. In contrast to similar overall frequencies of genome changes in the two models, genetic diversity within each mouse host was significantly higher for the OPC model, suggesting that the oral cavity represents a much more dynamic and diverse niche.

Processing of replication forks by homologous recombination factors to avoid mitotic abnormalities. A. Ait-saada, A. Da silva, K. Fréon, *S.A.E. Lambert* Institut Curie, CNRS, University Paris Sud, Paris-Saclay, University PSL Research University, UMR3348 F-91405, Orsay, France.

The completion of eukaryotic DNA replication requires the sequential activation of replication origins and the merging of converging forks. The completion of DNA replication is continuously threatened by a broad spectrum of unavoidable replication fork barriers (RFBs) that are caused by intrinsic chromosomal features (such as DNA sequence and chromatin), endogenous stress linked to cellular metabolism (such as transcription) and environmental factors including DNA damage (1). RFBs interrupt replication fork elongation, often causing multiple temporary pauses to a single replisome and occasionally causing terminal fork arrest. To avoid these perturbations creating chromosomal aberrations additional replication-based pathways have evolved to ensure the completion of DNA replication and thus help maintain genome stability. Terminally-arrested forks can be reactivated by DNA repair pathways such as homologous recombination (HR), and maintaining replication fidelity relies on close links between the replication machinery and HR (2). To investigate the consequences of terminally arrested forks on genome stability, we have developed conditional and genetically encoded RFBs which allow the block of replication forks at specific loci, in the fission yeast S. pombe (3 and 4). Our recent data on understanding how dysfunctional forks are processed and restarted by the HR pathway will be presented and discussed. More precisely, our data reveal a new role for Rad51-mediated fork-protection that ensures the rescue of arrested forks by an incoming converging fork to avoid sister chromatid bridging at site of replication stress.

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The role of histone-methyltransferases KMT1 and KMT6 in genome and chromatin organization of the wheat pathogen *Zymoseptoria tritici*. *M. Moeller*[1,2], K. Schotanus[1,2,4], M. Freitag[3], Eva H. Stukenbrock[1,2] 1) Christian-Albrechts-University, Kiel, Germany; 2) Max Planck Institute for Evolutionary Biology, Plön, Germany; 3) Oregon State University, Corvallis, OR, United States of America; 4) Duke University Medical Center, Durham, NC, United States of America.

Zymoseptoria tritici is a plant pathogenic fungus specialized to infect wheat (*Triticum aestivum*). The genome of the sequenced reference isolate comprises 21 chromosomes of which eight are accessory chromosomes. These chromosomes are highly instable during meiosis and mitosis, transcriptionally repressed and show enrichment of repetitive elements and of heterochromatic histone marks. To elucidate the role of heterochromatin-associated histone modifications on genome stability and transcriptional regulation in *Z. tritici,* we created deletion mutants of the methyltransferases KMT6 and KMT1 that are responsible for H3K27me3 and H3K9me3, respectively. We combined experimental evolution, genetic and high-resolution microscopic analyses to follow the impact of these deletions on chromosome and genome stability. We used ChIP-seq, whole genome sequencing and RNA-seq to compare changes in chromatin and genome structure and differences in gene expression between mutant and wild type strains. Analyses of genome and ChIP-seq data from the Δ*kmt1* mutants reveal dramatic chromatin reorganization, genome rearrangements and formation of “neochromosomes”. Transcriptome analysis revealed transposable element activation. The Δ*kmt6* mutant, however, displays an increased stability of the accessory chromosomes compared to

the wild type under normal growth conditions *in vitro*. Based on these results we conclude a strong impact of H3K9me3 in chromatin and genome organization and an important role of H3K27me3 for the stability of accessory chromosomes.

Epigenetic suppression of transposons co-opted as global regulator of virulence gene expression in a fungal phytopathogen. *Adam P. Taranto*, Megan C. McDonald, Peter S. Solomon Plant Sciences Division, Research School of Biology, The Australian National University, Canberra, Australia.

Many fungal plant-pathogens display a rapid transcriptomic switch in gene expression upon infection of a susceptible host. We have explored the role of epigenetic suppression in regulation of this shift towards virulence in the wheat-pathogen *Parastagonospora nodorum*. We have characterised the methylome of *P. nodorum* via whole genome bisulfite sequencing and assessed the contribution of DNA-methylation to gene expression by RNA-seq. Genomic regions subject to epigenetic control were enriched in transposable elements, effector-like genes, and putatively horizontally acquired genes. Key necrotrophic effectors, *ToxA*, *Tox1* and *Tox3* occur in close proximity to transposons. Molecular disruption of epigenetic silencing mechanisms active in these regions (H3K9me3, H3K27me3, and 5mC DNA methylation) allowed for partial recapitulation of the early-infection transcriptomic profile of *P. nodorum*. Similar epigenetic reprogramming of pathogen gene expression was achieved through application of chemical inhibitors of epigenetic pathways.

Dynamics of accessory chromosomes in *Fusarium oxysporum*. *M. Rep*, P. van Dam, P.M. Houterman, H.C. van der Does, S. Shahi, S. Schmidt, I. Vlaardingerbroek, S. Widinugraheni, J. Li, M. Constantin, N. Tintor, M. de Sain, B.V. Chellappan, B.J.C. Cornelissen, L. Fokkens Molecular Plant Pathology, University of Amsterdam, Amsterdam, NL.

Genomes of the *Fusarium oxysporum* (Fo) species complex contain eleven 'core' chromosomes and a variable number of 'accessory' chromosomes. The three smallest of the core chromosomes are different from the eight larger ones in that they have a higher SNP frequency between strains, a higher fraction of *in planta*-induced genes and, similar to accessory chromosomes and subtelomeric regions, a high incidence of histone H3 lysine 27 trimethylation. They do not, however, have the very high repeat-content characteristic of accessory chromosomes. Surprisingly, one of the three small core chromosomes is dispensable for saprophytic growth and pathogenicity.
Also among the accessory chromosomes some stand out: in plant-pathogenic strains of Fo, effector genes are present on specific accessory chromosomes that we call 'pathogenicity chromosomes'. Expression of the effector genes is very low *in vitro* and highly induced *in planta*. We have shown horizontal transfer of two pathogenicity chromosomes, one for tomato- and one for cucurbit-infection, in both cases conferring disease-causing ability to the recipient strain. SMRT sequencing has allowed us to assemble repeat-rich pathogenicity chromosomes and identify centromeres in all chromosomes. Comparison of pathogenicity chromosomes between strains reveals a high rate of intra-chromosomal recombination. In several cases, we could localize recombination to repetitive elements, suggesting that the repeat-rich nature of accessory chromosomes is one factor underlying their highly dynamic nature.

Saturday, March 18 2:00 PM – 5:00 PM
Heather
Early-diverging Fungi

Evolutionary genomics of the zygomycete fungi. *J.W. Spatafora*[1], Y. Chang[1], J.E. Stajich[2], ZyGoLife Research Consortium 1) Dept Botany & Plant Pathology, Oregon State Univ, Corvallis, OR; 2) Department of Plant Pathology and Microbiology and Institute for Integrative Genome Biology, University of California, Riverside, Riverside CA.

Zygomycetes comprise two separate phyla, Zoopagomycota and Mucoromycota, of Kingdom fungi. They represent early diverging lineages of nonflagellated fungi that arose after the zoosporic lineages of chytrids (Chytridiomycota and Blastocladiomycota) and prior to Dikyara (Ascomycota and Basidiomycota). As such, they are thought to represent lineages that colonized early terrestrial landscapes and associated with some of the earliest forms of terrestrial life. Zoopagomycota is the earliest diverging lineage and includes three subphyla, Entomophthoromycotina, Kickxellomycotina and Zoopagomycotina, which are primarily parasites and pathogens of animals, mycoparasites, and soil saprobes. Mucoromycota is sister group to Dikarya and includes Glomeromycotina, Mortierellomycotina and Mucoromycotina, which are primarily symbionts of plants (e.g., arbuscular mycorrhizae, root endophytes), saprobes of plant material, and soil fungi, although animal pathogens and mycoparasites are known. Although nonflagellated, Zoopagomycota possess a centriole-like organelle, while Mucoromycota possesses a spindle pole body typical of nonflagellated fungi. Based on this foundation of ecology and cell biology, phylogenomic analyses of these taxa stand to provide critical insights into the evolution of fungi and the origin of fungal associations and interactions with other terrestrial life forms. Here we will emphasize the use of genome scale data to resolve evolutionary relationships, and comparative genomics to understand transitions associated with the loss of flagellum and evolution of nutritional modes reliant on animal, fungal and plant-based carbon sources among early diverging fungi.

The genome of an uncultured nematode-destroying fungus and its role in resolving the zygomycete tree of life. *K.R. Amses*, T.Y. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Phylogenomic analyses of the artificial Zygomycota *s.l.* show support for its reorganization into two segregate phyla, each containing three subphyla. Of these six subphyla of zygomycetes, many are represented by only one or a few draft genomes, leading to some ambiguity in analyses. The sampling of more taxa from these poorly represented groups will bolster the strength of phylogenomic hypotheses formed at these deep nodes, strengthening the lines delineating the proposed taxonomic groupings. The Zoopagomycotina, a subphylum comprised of obligate parasites of other zygomycetes and small soil animals, is represented by a single draft genome in the most recent analyses. Establishment of axenic cultures has proven a central obstacle to the genome-enabled study of these cryptic soil fungi as acquisition of sufficient material for traditional whole genome sequencing is difficult. Single cell genomics approaches offer a solution to these complications, allowing for the sequencing and assembly of whole genome data sets from miniscule amounts of input material summing to only one or a few cells. Leveraging these techniques in order to address the underrepresentation of Zoopagomycotina in phylogenomic analyses, we have sequenced the genome of *Stylopage hadra*, a cryptic but ubiquitous fungus that captures and consumes nematodes in natural and agricultural soils. Our single cell genomics approach generated four sequencing libraries with an average cumulative scaffold size of 22.81 Mbp and genome completeness scores of 56.65%, suggesting a *S. hadra* genome size of approximately 40 Mbp. Preliminary phylogenetic analyses based on concatenated RPB1 and RPB2 sequences support *S. hadra*'s placement in the Zoopagomycotina. Sequencing libraries contain a putative endosymbiont of *S. hadra* belonging to the beta-proteobacterian genus *Glomeribacter* with cumulative scaffold sizes indicating a genome size of approximately 1.80 Mbp. Known from the cytoplasm of arbuscular mycorrhizal fungi and species of *Mortierella*, species of *Glomeribacter* comprise a group of opportunistic soil-borne endosymbionts that tend to reduce the growth rates of their associated fungi, indicating a weakly parasitic interaction.

E2F and SBF transcription factors in early-diverging fungi bind overlapping and unique cis-regulatory sites. A.M. Augustus[1,2], E.M. Medina[1,2], R. Gordan[2,3,4], *N.E. Buchler*[1,2] 1) Department of Biology, Duke University, Durham, NC; 2) Center for Genomic & Computational Biology, Duke University, Durham, NC; 3) Department of Biostatistics & Bioinformatics, Duke University, Durham, NC; 4) Department of Computer Science, Duke University, Durham, NC.

Although cell cycle control is a conserved and essential process, some core animal and fungal cell cycle regulators are not homologous (e.g. E2F and SBF). Using eukaryote-wide phylogenetic analyses, we have shown that evolution along the fungal lineage was punctuated by the early acquisition and entrainment of the SBF transcription factor, a protein with homology to the KilA-N domain of DNA viruses. Ancestral SBF may have hijacked cell cycle control by binding cis-regulatory elements targeted by E2F and activated transcription of G1/S genes to drive cell proliferation. Cell cycle evolution in the fungal ancestor proceeded through a hybrid network containing both SBF and its ancestral animal counterpart E2F, which is still maintained in early-diverging fungi such as *Spizellomyces punctatus* (a Chytrid). The hybrid network had parallel E2F and SBF pathways that regulate similar target genes, thus providing redundant control which enabled the eventual loss of E2F and its replacement by the SBF pathway in the ancestor of Dikarya.

To address the question of redundancy and specificity of E2F and SBF, we measured the binding specificity of E2F and SBF in chytrids and zygomycetes using a high-throughput protein-binding microarray (PBM) assay. PBM assays measure, in a single experiment, the binding of recombinant proteins to tens of thousands of synthetic DNA sequences, guaranteed to cover all possible 10-bp DNA sequences in a maximally compact representation. Our data show that chytrid and zygomycete E2F and SBF have nearly identical DNA-binding specificity to their human and yeast counterparts. We further show that E2F and SBF can bind a common set of motifs, which supports the hijacking hypothesis and binding redundancy between E2F and SBF. Last, we show that there are specific motifs that can be bound only by E2F or only by SBF. This suggests that certain classes of genes could be under E2F-only or SBF-only control, which may explain why both transcription factors are still maintained in some species, such as *Spizellomcyes punctatus*.

Pervasive adenine N6-methylation of active genes in fungi. *S.J. Mondo*[1], R.O. Dannebaum[1], R.C. Kuo[1], A. Gryganskyi[2], J. Magnuson[3], T.Y. James[4], M.A. O'Malley[5], J.E. Stajich[6], J.W. Spatafora[7], A. Visel[1], I.V. Grigoriev[1] 1) Fungal Genomics, Joint Genome Institute, Walnut Creek, CA; 2) L. F. Lambert Spawn Co, Coatesville, PA; 3) Pacific Northwest National Laboratory, Richland, WA; 4) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 5) Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA; 6) Department of Plant Pathology and Microbiology, University of California, Riverside, California; 7) Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Epigenetics plays a critical role in regulation and expression of genetic information. Many important epigenetic marks are encoded as DNA-based modifications, for example 5-methylcytosine (5mC) or N6-methyldeoxyadenine (6mA). 6mA is a non-canonical eukaryotic DNA base modification reported genome-wide in only four species: an alga, *Chlamydomonas reinhardtii* and three animals, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Mus musculus*. However, the genome-wide abundance of 6mA in these organisms ranges from low (0.4% of all A's in *C. reinhardtii*) to extremely low (0.001%-0.07% in *D. melanogaster*). Despite low levels, 6mA is critical for proper development, as it plays an important role in regulation of gene expression. Regrettably, exploration of 6mA thus far has been limited to single model eukaryotes and therefore its relevance in many major branches of Eukaryota is still unknown. Here we conduct the first kingdom-wide exploration of both 6mA and 5mC, where we interrogate the Fungi, an ancient and extremely diverse eukaryotic lineage. We found heavy utilization of 6mA in early-diverging fungi where up to 2.8% of all adenines were methylated, vastly exceeding the levels observed in other eukaryotes and more derived fungi, whereas 5mC was primarily in the CpG context and restricted to repeats. Interesting, with one exception 6mA and 5mC were mutually exclusive (i.e. lineages with high 6mA had no 5mC and vice versa). 6mA occurred symmetrically at ApT dinucleotides, where it concentrated in dense 6mA 'islands' at gene promoters. Islands were positioned nearby promoter thymine-blocks and ≈95% of methylated genes were expressed. Our results demonstrate the importance of 6mA as a broadly conserved epigenomic mark in eukaryotes as well as identify key components involved in positioning of genomic 6mA. Furthermore, our results implicate 6mA as an epigenomic mark transmissible across nuclear division and suggest its involvement in nucleosome organization. We anticipate that our study will provide a foundation for deeper exploration of 6mA not only in fungi, but across all Eukaryota.

Bacterial endosymbionts as a tool to unravel the basic biology of Mucoromycotina. Olga Lastovetsky[1], Stephen Mondo[2], Maria Gaspar[3], Colin Barber[2], Susan Henry[3], *Teresa Pawlowska*[2] 1) Graduate Field of Microbiology, Cornell University, Ithaca, NY 14853; 2) School of Integrative Plant Science, Plant Pathology & Plant Microbe-Biology, Cornell University, Ithaca, NY 14853; 3) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853.

Despite their ecological significance and formative role in unraveling the principles of fungal reproduction, Mucoromycotina remain one of the least understood lineages of filamentous fungi. This knowledge gap is largely caused by limited genetic tractability that bedevils these organisms. To overcome this impediment, we rely on the *Rhizopus microsporus-Burkholderia* symbiosis, in which the endobacteria can be manipulated and used as a tool to perturb various processed in the fungus. Through transcriptional profiling, pharmacological manipulation, identification of genes that display altered expression patterns due to endobacteria, and mapping them to well-characterized regulatory and metabolic networks in other fungi, we identified candidate genes responsible for regulation of several processes in Mucoromycotina, including reproductive biology, lipid metabolism, and defense against antagonistic bacteria. These findings create a foundation for functional characterization of key biological processes in Mucoromycotina.

Resolving the Mortierellaceae phylogeny through Multi-Locus Sequence Typing (MLST) and phylogenomics. *Natalie Vande Pol*[1], Jason Stajich[2], Kerry O'Donnell[3], Alessandro Desiro[1], Gregory Bonito[1] 1) Michigan State University, East Lansing, MI; 2) University of California at Riverside, Riverside, CA; 3) Mycotoxin Prevention & Applied Microbiology Research Unit, NCAUR-ARS-USDA, Peoria, IL.

The Mortierellaceae (Mortierellomycotina) are a diverse family of fungi that are of evolutionary and ecological relevance. They are the closest lineage to the arbuscular mycorrhizae (Glomeromycotina) and include some of the first species to evolve fruiting body production. The Mortierellaceae are estimated to contain at least 100 species classified within six polyphyletic genera that cannot be resolved with ribosomal markers. With advances in DNA sequencing technology, it is now feasible to generate sequence data from many loci in parallel (MLST), or to perform low-coverage genome (LCG) sequencing to identify phylogenetically informative loci. In collaboration with the ZyGo Life consortium and the Joint Genome Institute, we sequenced 68 LCGs representing 50 unique species of Mortierellaceae. From these, we identified 400 informative loci and used RaxML to build a concatenated tree. The resulting phylogeny has very strong bootstrap support and a very different structure from existing ribosomal trees. In parallel, we analyzed three *de novo* sequenced *Mortierella* genomes and extracted 13 informative loci. For each locus, we designed PCR primers for multiplexed PCR amplification across 333 Mortierellaceae isolates, which included the 68 LCG isolates. We will discuss the strengths and limitations of these two approaches.

An Omics-based approach to understanding Host-Pathogen interactions during Mucormycosis. *V.M. Bruno*, T.N. Watkins, M.C. Chibucos, S. Soliman, T. Ghebremarimam , A.S. Ibrahim Dept of Microbiology & Immunology, University of Maryland Sch of Med, Baltimore, MD.

Mucormycosis is a deadly invasive infection caused by several fungal organisms belonging to the subphylum Mucormycotina, order Mucorales. The major risk factors include uncontrolled diabetes mellitus that results in hyperglycemia and ketoacidosis (DKA), other forms of acidosis, treatment with corticosteroids, solid organ or bone marrow transplantation, neutropenia, trauma and burns (e.g., wounded soldiers in Iraq and Afghanistan), malignant haematological disorders and deferoxamine therapy in patients receiving haemodialysis. The infection is generally acquired by inhalation of spores that are ubiquitous in nature and cause either rhino-orbital (almost exclusively in DKA patients) or pulmonary (mainly in neutropenic leukemic patients) disease. There are currently no vaccines and only two antifungal agents approved by the USA FDA to treat this disease. In the absence of surgical removal of the infected focus (such as excision of the eye in patients with rhinocerebral mucormycosis), antifungal therapy alone is rarely curative. Even when surgical debridement is combined with high-dose antifungal therapy, the mortality associated with mucormycosis is >50%. In patients with prolonged neutropenia or disseminated disease, mortality is 90-100%**.** The high mortality rate, limited options for therapy and the extreme morbidity of highly disfiguring surgical therapy make it imperative to look for alternative strategies to treat and prevent mucormycosis. In this work, we have undertaken an unbiased, systematic approach to identify and characterize fungal and host factors that contribute to disease progression with the goal of developing novel therapeutic targets.

RNAi-based functional genomics identifies new virulence determinants in mucormycosis. *M.I. Navarro-Mendoza*[1], T.A. Trieu[1,2], C. Pérez-Arques[1], M. Sanchis[3], J. Capilla[3], P. Navarro-Rodríguez[3], L. López-Fernández[3], S. Torres-Martínez[1], V. Garre[1], R. Ruiz-Vázquez[1], F.E. Nicolás[1] 1) Department of Genetics and Microbiology, University of Murcia, Murcia, Spain; 2) Current address: Department of Genetics, Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam; 3) Microbiology Unit. University Rovira i Virgili. IISPV. Tarragona, Spain.

Mucorales are an emerging group of human pathogens that are responsible for the lethal disease mucormycosis. Unfortunately, functional studies on the genetic factors behind the virulence of these organisms are hampered by their limited genetic tractability, since they are reluctant to classical genetic tools like transposable elements or gene mapping. In this work, we describe an RNAi-based functional genomic platform that allows the identification of new virulence factors through a forward genetic approach firstly described in Mucorales. This platform contains a whole-genome collection of *Mucor circinelloides* silenced transformants that presented a broad assortment of phenotypes related to the main physiological processes in fungi, including virulence, hyphae morphology, mycelial and yeast growth, carotenogenesis and asexual sporulation. Selection of transformants with reduced virulence allowed the identification of *mcplD*, which encodes a Phospholipase D, and *mcmyo5*, encoding a probably essential cargo transporter of the Myosin V family, as required for a fully virulent phenotype of *M. circinelloides*. Knock-out mutants for those genes showed reduced virulence in both *Galleria mellonella* and *Mus musculus* models, probably due to a delayed germination and polarized growth within macrophages. This study provides a robust approach to study virulence in Mucorales and as a proof of concept identified new virulence determinants in *M. circinelloides* that could represent promising targets for future antifungal therapies.

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Saturday, March 18 2:00 PM – 5:00 PM
Nautilus
Disease Epidemiology

Azole resistance in *Aspergillus fumigatus*: the silent tsunami. *Paul Verweij*[1,2] 1) Radboud University Medical Center, Nijmegen, NL; 2) Center of Expertise in Mycology Radboudumc/CWZ.

Aspergillus fumigatus is an important opportunistic fungus that causes a spectrum of diseases in humans ranging from allergic syndromes to acute invasive disease. Since nearly two decades acquired resistance to medical azoles has emerged and the application of azole compounds in the environment appears to play an important role in resistance selection. Resistance mutations associated with the Cyp51A-gene continue to emerge, which confer a pan-azole resistant phenotype. As *A. fumigatus* is not a phytopathogen, growth of the fungus in organic waste, such as compost, which contains azole fungicide residues appears to be a condition that facilitates the selection of resistance mutations. Although these mutations confer resistance to azole fungicides, the isolates are cross resistant to medical triazoles due to molecule similarity. Over the years three evironmental mutations have been identified consisting of a tandem repeat in the gene promoter region often combined with point mutations in the Cyp51A-gene (TR_{34}/L98H; TR_{53}; TR_{46}/Y121F/T289A). Recently another two mutations were found in patients and the environment, indicating that the current situation is not sustainable and unless measures are taken the class of azoles may be lost for treatment of fungal diseases.
The clinical significance of azole resistance is substantial in patients at risk of invasive aspergillosis. A recent Dutch studied showed that within a five-year cohort of patients with invasive aspergillosis the mortality rate was 36% in patients infected with an azole-susceptible isolate, compared with 86% in those with azole-resistant aspergillosis. Early diagnosis of resistance is difficult and delay of treatment with an effective drug contributes to the poor outcome. Furthermore, due to lack of effective alternative agents, certain manifestations of invasive aspergillosis, such as central nervous system aspergillosis, are virtually untreatable. In the Netherlands very high resistance rates of 25% to 32% have been reported in critically-ill patients and those with hematological malignancy, prompting changes in initial treatment strategies. Unlike antibacterial resistance, there appears to be little awareness of resistance in *A. fumigatus* and associated problems in the management of aspergillus diseases. This silent tsunami will continue to spread unless research is initiated that investigates the dynamics of environmental resistance selection and enables effective measures to be taken.

Genome-wide genetic dissection of trait variation between species. Carly Weiss[1], Rylee Hackley[1,2], Jeremy Roop[1], Jeffrey Skerker[3], *Rachel Brem*[1,2] 1) Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Buck Institute for Research on Aging, Novato, CA; 3) Energy Biosciences Institute, UC Berkeley, Berkeley, CA.

Geneticists since Mendel have sought to explain how and why traits vary among wild individuals. The industry standard toward this end is to test for DNA sequence variants that correlate with phenotype across a population, but such methods cannot be applied across reproductive barriers. To fill this analysis gap, we developed a high-throughput, interspecific version of the reciprocal hemizygote test. In a viable hybrid formed from the mating of two species, we introduce disrupting mutations at each allele of each gene in turn via transposon mutagenesis, and we quantify phenotypes of the resulting hemizygote mutants in a pooled format via Tn-seq. As a testbed for our approach, we focused on thermotolerance in yeasts. At 39°C, *Saccharomyces cerevisiae* grows faster and accumulates more biomass than its sister species *S. paradoxus*, to a degree far exceeding the variation among isolates of each species. Culture and sequencing of *S. cerevisiae* x *S. paradoxus* hemizygotes revealed a battery of loci at which the species' alleles conferred differences in thermotolerance. Few of these genes had known roles in resistance to heat shock or other stresses. At most mapped loci, the *S. cerevisiae* allele was associated with improved thermotolerance relative to the *S. paradoxus* allele. Independent transgenesis experiments verified the benefit at high temperature of *S. cerevisiae* versions of the housekeeping genes *AFG2*, *CEP3*, and *NIP100*. Together, these results elucidate the complex genetics of *S. cerevisiae* thermotolerance, and they pioneer a genetic mapping method that will be applicable across the tree of life.

Population structure and drivers of genetic diversity in *Candida glabrata*. *E. Shor*[1], K. Healey[1], C. Jimenez-Ortigosa[1], S. Lockhart[2], V. Loparev[2], D. Perlin[1] 1) Public Health Research Institute, Rutgers University, Newark, NJ; 2) Centers for Disease Control, Atlanta, GA.

The prevalence of *Candida glabrata* infections has been rising for several decades, and it now accounts for approximately 25% of all Candida bloodstream infections in the U.S. and can in some settings predominate as the principal bloodstream fungal pathogen. Furthermore, *C. glabrata* exhibits elevated intrinsic resistance to triazoles and readily acquires resistance to echinocandins. It also becoming increasingly apparent that *C. glabrata* has a highly genetically diverse population structure. However, how this diversity arises and whether it contributes to the virulence and drug resistance of *C. glabrata* is unknown. A multi-locus sequence typing (MLST) scheme has thus far identified over 80 different *C. glabrata* sequence types (STs); however, our preliminary analysis indicates that this number is likely much higher. We also found that different STs carry different alleles of DNA repair gene *MSH2*, some of which cause elevated rates of drug resistance *in vitro*, indicating that different STs have different propensity towards mutability and acquiring drug resistant gene variants. *C. glabrata* also displays a high degree of variability at the level of chromosomal architecture. For instance, it has been reported that clinical *C. glabrata* isolates show great diversity in terms of chromosomal number and structure, displaying a high number of chromosomal rearrangements relative to the reference laboratory strain. This observation has led to the hypothesis that emergence of new chromosomes is a virulence mechanism in *C. glabrata* and may underlie emergence of drug resistance. However, we find that strains of the same ST have similar chromosomal patterns, suggesting that specific chromosomal configurations may pre-exist emergence of virulence and may be a feature of commensal *C. glabrata* populations. To identify new determinants of virulence and/or drug resistance in *C. glabrata*, we are performing optical mapping and long read genome sequencing (PacBio) of several STs that are prevalent in the U.S. We are also investigating the importance of the DNA replication checkpoint in facilitating chromosomal rearrangements in *C. glabrata*.

Epidemiology of *Cryptococcus* spp. using comparative genomics. *D.M. Engelthaler*[1], C.C. Roe[1], G.R. Thompson[3], W. Meyer[2], S. Lockhart[4] 1) TGen North, Translational Genomics Research, Flagstaff, AZ; 2) University of Sydney; 3) University of California Davis; 4) Centers for Disease Control and Prevention.

Background: Fungi present a number of problems for molecular epidemiology due to their reproductive, morphologic, ecologic, and

genomic complexities. While numerous methods of genotyping can infer genetic relationships among isolates, they provide limited empirical estimates of genetic relatedness among strains. The combination of advances in whole genome sequencing and bioinformatics analyses along with the recent emergences of *Cryptococcus* in previously non-endemic regions has allowed for the use of advanced genomic analyses to understand the dispersal and epidemiology of *Cryptococcus* and other environmental fungi. Prototypical examples are the emergence of novel populations of *C. gattii* in the temperate North American Pacific Northwest, and the recent genomic understanding of distinct lineages Southeastern U.S.
Methods: Using Illumina next generation sequencing technology, we sequenced >250 *C. gattii* isolates, representing the known global diversity, and applied several bioinformatics approaches to establish genomic and genetic relatedness and distance. These analyses also included genomes from sequenced isolates of *C. neoformans var. neoformans* and *C. n. var. grubii*.
Results: WGS-based analysis was able to confirm outbreak sources, elucidate the population structure of the various subtypes and establish phylogeographic understanding of the various species and subtypes. These analyses describe the highly diversity of global populations of major subtypes and established likely source of dispersal events (e.g., VGII emanating out of South America); additionally they also established recent emerging subtypes as being completely clonal but with continuing their local evolution in newly endemic regions. Numerous epidemiologically significant gene content differences were identifiable between and among subtypes, including genes potentially related to habitat adaptation, virulence and clinical differentiation. Various statistical analyses have established likely age estimates for dispersal and emergence events.
Discussion: The next generation of molecular epidemiology of fungal pathogens must include WGS analyses. The use of WGS and appropriate bioinformatics enables investigations to be informed with empirical knowledge of strain and subtype relatedness rather than reliance on epidemiological inference based on genetic similarity. Additionally, the continued use of WGS in epidemiology allows for the creation of public archives of sequence data for future research and investigations, further improving overall biological, public health and clinical understanding. More specific, the findings here provide greater understanding of dispersal, emergence and epidemiology of *Cryptococcus* species.

Genomic diversity of *Malassezia* yeasts: implications for epidemiology and the clinic. *Teun Boekhout*[1,2], Anna Kolecka[1], Bart Theelen[1], Claudia Cafarchia[3], Roberta Iatti[3], Thomas Dawson[4], the Malassezia Genome Consortium 1) CBS Fungal Biodiversity Centre, Utrecht, NL; 2) Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, NL ; 3) Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, 70010 Valenzano, Bari, Italy ; 4) A*STAR Institute of Medical Biology (IMB), Singapore.
Malassezia is a genus of lipid-dependent basidiomycetous yeast species belonging to Ustilaginomycotina. *Malassezia* spp. are a major component of the human skin mycobiome and may be implicated in several skin disorders, such as pityriasis versicolor, seborrheic dermatitis and atopic dermatitis. Some species may also causes sepsis, especially in neonates. Here we present results from a comparative genomics study of all species of the genus with the aim to contribute to our understanding of the biodiversity, disease potential and genetic processes such as hybridization.

Methods.
The genomes of all species were sequenced by Illumina technology, and for some species more than one isolate using Illumina and Pacific BioSciences technology. Bioinformatics analysis included de novo assembly, annotation and gene family analysis. Additional analysis also included MultiLocus sequencing, MALDI-TOF MS and Amplified Fragment length Polymorphism (AFLP) applied to a greater set of isolates, including outbreak isolates.

Results.
Malassezia have among the smallest free-living eukaryotic genomes, ranging between 7-9 Mb, with some exceptions having ca. 14Mb genomes. Three phylogenetic clusters can be discerned, largely confirming previous insight obtained by the analysis of rDNA. The compact genomes differ from those of related Ustilaginomycotina by major gene loss and limited gene gain processes. Interestingly, the larger genomes all belonged to the *M. furfur* complex with three distinct clades, with one representing potential aneuploids as hypothesized based on previous MLST, AFLP and PFGE data. Strains belonging to one of the potential *M. furfur* parental clades seem to be more prevalent in causing invasive infection. Detailed comparative genomics analysis of the *M. furfur* strains may shed light on the molecular mechanisms. In addition, MLST revealed a significant genetic heterogeneity of outbreak isolates suggesting that patients can be infected by multiple isolates.

Conclusions: All 17 hitherto described species of *Malassezia* belong to three major clades that differ in gene loss/gain patterns. The genomes of all species are small, except for those isolates that seem to represent hybrids in the *M. furfur* complex. Invasive isolates of *M. furfur* mainly belong to a specific parental group but also show considerable diversity. It remains to be seem how this relates to the *Malassezia* diversity present on skin of neonates, parents and hospital staff.

Emergence of bat white-nose syndrome in North America. *D. Blehert* US Geological Survey, National Wildlife Health Center, Madison, WI.
White-nose syndrome (WNS) is an emergent wildlife disease that has spread rapidly and caused unprecedented mortality among bats of eastern North America. Since first detected in New York during winter of 2006-2007, WNS has spread to 29 US states and five Canadian provinces, and the disease is estimated to have killed over five million bats. Prior to emergence of WNS, such massive population declines of mammalian species due to an infectious disease were unprecedented. White-nose syndrome is caused by the psychrophilic fungus *Pseudogymnoascus destructans*. The fungus grows optimally at temperatures consistent with bat hibernation (approximately 2 to 12°C), causing a lethal skin infection that disrupts hibernation physiology and behavior. Additionally, the fungus persists throughout the year in soil of underground bat hibernation sites, including during summer months, establishing environmental reservoirs of the pathogen. A growing body of evidence suggests that *P. destructans* was introduced to North America, likely from Eurasia where bats seem to co-exist with the fungus, suggesting that WNS in North America is the result of a novel pathogen introduced into a population of naïve host species.

Naturally occurring mismatch repair mutants mediate rapid phenotypic change and drug resistance in the Pacific Northwest *Cryptococcus gattii* outbreak. *R. Blake Billmyre*, Shelly Clancey, Shelby Priest, Joseph Heitman Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Pathogenic microbes confront a constant evolutionary conflict between the pressure to maintain genome stability and the need to adapt in response to mounting external stresses. Over the past fifteen years, an ongoing outbreak of the human fungal pathogen *Cryptococcus deuterogattii* has occurred in the Pacific Northwest of the United States and Canada. Whole genome resequencing of outbreak strains identified one lineage of the outbreak that harbors a nonsense mutation in *MSH2* and exhibits a hypermutator phenotype. Genetic analysis of progeny as well as independent deletions of *MSH2* demonstrate linkage of the hypermutator phenotype to *MSH2* mutation. This defect in mismatch repair destabilizes homopolymer runs throughout the *Cryptococcus* genome, resulting in inactivation of genes with coding homopolymer runs. As a result, resistance to the clinically-used immunosuppressive drugs FK506 and rapamycin is rapidly generated, as well as resistance to 5-fluorocytosine (5-FC), a frontline drug often used to treat Cryptococcal infections and for which spontaneous drug resistance occurs rapidly, necessitating use only in combination with amphotericin B. One environmental isolate with an *MSH2* mutation even exhibited an unselected FK506/rapamycin drug resistance phenotype attributable to a homopolymer run shift. Competition experiments demonstrate this hypermutator state is deleterious in rich growth conditions, but allows more rapid adaptation to stressful conditions. Ongoing work has identified strains with elevated mutation rate present throughout the population of the sister species of *Cryptococcus neoformans* and representing multiple independent origins of hypermutation. Hypermutator states may represent a general mechanism by which fungi adapt to changing environmental conditions or drug challenges.

Latest insights in the epidemiology and diversity of *Fusarium oxysporum* f.sp. *cubense*, the causal agent of Panama disease in banana. *Gert H.J. Kema*[1,3], Fernando Garcia[2,3], Nadia Ordóñez[1,3], Maricar Salacinas[1,3], Michael F Seidl[1], Bart P.H.J. Thomma[1], Harold JG Meijer[3] 1) Wageningen University and Research, Laboratory for Phytopathology; 2) Wageningen University and Research, Laboratory for Plant Breeding; 3) Wageningen Plant Research.

Panama disease or Fusarium wilt of banana draws global attention. The currently developing epidemic of the so-called Tropical Race 4 (TR4) is caused by a single clone of *Fusarium oxysporum* f.sp. *cubense* (Foc); vegetative compatibility group 01213. It is reminiscent of the previous epidemic that wiped out “Gros Michel” bananas in Central America, which pushed the banana industry into bankruptcy. The epidemic was eventually quenched by cultivating “Cavendish” bananas, which are resistant to the so-called Foc Race 1 strains that caused the epidemic in “Gros Michel”. The industry revived and thrives by the success of “Cavendish” that has developed into a global monoculture. The emergence of TR4 caused havoc and wipes out “Cavendish” plantations in South East Asia, from where the disease now has spread into the Near and Middle East and Africa[a]. Banana production in many regions is at stake and there are no sustainable solutions available. Our research focuses on the international complexity and addresses mostly genetic diversity in host and pathogen as well as epidemiological aspects embedded in multidisciplinary programs. We have used genotyping by sequencing technologies to describe global and regional diversity in the causal agent Foc and have phenotyped hundreds of banana accessions with various Foc genotypes. Methods to rapidly detect - particularly TR4 - and manage the disease have been developed to slow down the epidemic. This provides the necessary time for developing durable solutions that also contribute to break the hegemony of the global “Cavendish” monoculture by introducing a diversified panel of banana cultivars. The latest developments will be presented and discussed.

[a] Ordonez Román, N.I., Seidl, M.F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B.P.H.J., Ploetz, R.C., and Kema, G.H.J., 2015. Worse comes to worst: Bananas and Panama disease - when plant and pathogen clones meet. PLoS Pathogens, DOI: 10.1371/journal.ppat.1005197.

Saturday, March 18 2:00 PM – 5:00 PM
Scripps
Cell Walls and Polysaccharides

***Aspergillus* biofilm exopolysaccharide – from virulence factor to therapeutic target.** *Don Sheppard*[1,2] 1) Microbiology and Immunology, McGill University, Montreal; 2) Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montreal.

Aspergillus fumigatus is the most common cause of invasive mold infection in humans. Reverse-genetic studies from our group have discovered that *A. fumigatus* virulence is enhanced by the production of galactosaminogalactan (GAG), an exopolysaccharide composed of galactose and N-acetyl-galactosamine (GalNAc) that is required for fungal biofilm formation, adherence to and invasion of host cells as well as evasion of host immune defenses.

Using molecular and biochemical approaches we have elucidated the biosynthetic pathways governing the synthesis of GAG. These studies have revealed parallels between *Aspergillus* and bacterial exopolysaccharides, most notably the requirement for post-synthetic deacetylation, which renders the final polymer cationic and able to mediate adhesion. Within the GAG biosynthetic complex, we have identified two enzymes, Sph3 and Ega3, which contain glycoside hydrolase domains that can degrade pre-formed GAG. Recombinant hydrolase domains from both of these proteins are able to disrupt pre-formed biofilms of *A. fumigatus* at nanomolar concentrations. Treatment of *A. fumigatus* with recombinant hydrolases increases susceptibility to antifungal agents, and prevents invasion and damage of pulmonary epithelial cells in vitro. Recombinant Sph3 is non-toxic when administered intratracheally to mice, and treatment of neutropenic mice with Sph3 dramatically attenuates virulence.

Collectively these studies have revealed that GAG is a key virulence factor of the pathogenic fungus *A. fumigatus*, and reveal a promising new therapeutic strategy to target this virulence factor.

Some like it on the rocks: recurring stresses select for rock-inhabiting fungi with manifold protective pigments. *Nicole Knabe*[1], Romy Breitenbach[1,2], Polina Dementyeva[1], Felix Heeger[3], Camila Mazzoni[3], Anna A. Gorbushina[1,2] 1) Federal Institute for Materials Research and Testing (BAM), Department Materials and the Environment, Berlin, Germany; 2) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy & Department of Earth Sciences, Berlin, Germany; 3) Berlin Center for Genomics in Biodiversity Research, Berlin, Germany.

Black ascomycetous microcolonial fungi (MCF) are persistent inhabitants of rock surfaces in deserts as well as ubiquitous in other wide-spread terrestrial ecosystems including man-made materials such as solar panels. The ability of MCF to cope with multiple, rapidly fluctuating stresses makes the group an interesting subject in the study of stress resistance. Also, applied research is necessary to find ways of preventing MCF from colonising and degrading solar panels and historic monuments.

Carotenoids and melanin are amongst the protective pigments that contribute to the robustness of MCF. We studied their role in stress resistance of the non-pathogenic rock-inhabiting fungus *Knufia petricola* (Chaetothyriales) strain A95. Several knock-out mutants have been produced in melanin- (A95ΔSDH and A95ΔPKS) as well carotenoid- (A95ΔPDG) synthesis and a double mutant (A95ΔPKS/ΔPDG) also exists. Disruption of melanin synthesis affects not only oxidative-stress resistance but also the extracellular polysaccharide (EPS) matrix and lipid composition of the cell membrane. Exposure to ≤ 30 mM H_2O_2 did not affect the growth rate of A95ΔPKS as compared to wild type cells. Almost twice as much water-soluble EPS was present in cultures of the A95ΔPKS mutant compared to the wild type and A95ΔPDG strains. Comparative gene expression analyses of the wild type and the A95ΔPKS mutant identified genes regulated under oxidative stress conditions. These data will help elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

Fungal surface amyloids function in host-microbe interactions. *Melissa Garcia-Sherman*[1], Safraz Hamid[2], Desmond Jackson[1], James Thomas[1], Peter Lipke[1] 1) Dept of Biology, Brooklyn College CUNY, Brooklyn, NY; 2) Dept of Biology, Princeton University, Princeton, NJ.

Previously we have shown that Als5p, a member of *Candida albicans* cell wall adhesins, contains amyloid forming sequences which are conserved in other Als family members. These sequences are critical for amyloid fibril formation by soluble peptides and for the intact Als5 protein. The Als5p amyloid sequence is also critical for cell adhesion and biofilm formation (Garcia *et al*, PLoS ONE e17632). I will present data that demonstrate an amyloid forming peptide that contains a sequence of Als5p and binds to the surface of non-albicans species of *Candida*. Additionally, *Candida tropicalis* aggregates stained positive with the amyloid binding dye thioflavin T, and an Als5p amyloid-inhibiting peptide blocked cell aggregation. The amyloid inhibiting peptide also blocked *C. albicans*, *C. tropicalis*, and *Candida parapsilosis* adhesion to monolayers of FaDu epithelial-derived cells, monolayers. These data demonstrates a functional consequence of amyloid inhibition and is the first evidence that amyloid-like interactions function in adhesion to human cells by opportunistic fungi. I will also present data that show that fungal surface amyloids are present in autopsy tissue sections from patients inflicted with several fungal infections. The fungi present in these patients test positive with amyloid binding dyes, such as thioflavin T, thioflavin S, and Congo red. In the infected tissue and *in vitro*, amyloid-expressing fungi were coated with human serum amyloid P component (SAP), a amyloid-binding, pattern recognition receptor with anti-inflammatory function.

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Comparative analysis of the function of α-1,3-glucan synthases, AgsA and AgsB, in *Aspergillus nidulans*. *K. Miyazawa*[1], A. Yoshimi[2], S. Yano[3], S. Kasahara[4], F. Hasegawa[2], K. Abe[1,2] 1) Grad. Sch. Agric. Sci., Tohoku Univ., Sendai, JP; 2) NICHe, Tohoku Univ., Sendai, JP; 3) Yamagata Univ., Yonezawa, JP; 4) Miyagi Univ., Sendai, JP.

Although α-1,3-glucan (AG) is one of major polysaccharides in the cell wall of *Aspergillus* species, the biological function of AG remains unclear, except for the role as a virulence factor in some pathogenic fungi. Previously, we carried out functional analysis of two α-1,3-glucan synthase (AGS) genes (i.e. *agsA* and *agsB*) in the model filamentous fungus *Aspergillus nidulans*. The *agsB*? strain lost most cell wall AG, suggesting that a main AGS in this fungus is AgsB. Interestingly, the hyphae of the *agsB*? strain was dispersed under liquid culture

conditions, whereas the wild-type strain formed hyphal pellets under same culture conditions. These results suggest that AG has the role as an adhesive factor for hyphal cells. On the other hand, because the expression of *agsA* gene was scarcely detected under normal growth conditions and the *agsA*? strain did not show phenotypic defects, the role of *agsA* remains unclear. In this study, in order to investigate the roles of AgsA and AgsB in cell wall AG synthesis, we comparatively analyzed cell wall polysaccharides synthesized by AgsA and AgsB. First, we constructed *agsA* or *agsB* gene overexpression (O/E) strain by replacing the promoter region of *agsA* or *agsB* with *tef1* promoter under the genetic background of the other *AGS* gene disruption, and confirmed the high expression of either of the *AGS* genes in the O/E strains. The O/E of *agsA* restored the growth characteristics of the *agsB*? strain under liquid culture conditions: the O/E *agsA* strain formed the hyphal pellets. This suggests that the *agsA* gene encodes a functional AGS. To elucidate the differences of cell wall structure between these two strains, we performed the alkaline-fractionation of cell wall and analyzed the sugar composition of the fractions. The carbohydrate analyses revealed that the sugar compositions of the AS2 fraction were similar in these two strains, but the texture of the AS2 fraction derived from these two strains was markedly different from each other, suggesting that the detailed chemical structure of AG obtained from the O/E *agsA* differs from that of AG derived from the O/E *agsB* strains.

Mannan molecular sub-structures control nanoscale glucan exposure in *Candida*. Matthew S. Graus[1,2], Michael J. Wester[3], David L. Williams[4], Michael D. Kruppa[4], Douglas W. Lowman[4,5], Jesse M. Young[1], Harry C. Pappas[2], Keith A. Lidke[6], *Aaron K. Neumann*[1] 1) Department of Pathology, University of New Mexico, Albuquerque, NM 87131; 2) Department of Nanoscience & Microsystems Engineering, University of New Mexico, Albuquerque, NM 87131; 3) Department of Mathematics and Statistics, University of New Mexico, Albuquerque, NM 87131; 4) Department of Surgery, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37684; 5) AppRidge International, LLC, Telford, TN 37690; 6) Department of Physics and Astronomy, University of New Mexico, Albuquerque, NM 87131.

N-linked mannans (N-mannans) in the cell wall of *Candida albicans* are thought to mask β-(1,3)-glucan from recognition by Dectin-1, contributing to innate immune evasion. Lateral cell wall exposures of glucan on *C. albicans* are predominantly single receptor-ligand interaction sites and are restricted to nanoscale geometries. *Candida* species exhibit a range of basal glucan exposures and their mannans also vary in size and complexity at the molecular level. We used super resolution fluorescence imaging and a series of protein mannosylation mutants in *C. albicans* and *C. glabrata* to investigate the role of specific N-mannan features in regulating the nanoscale geometry of glucan exposure. Decreasing acid labile mannan abundance and α-(1,6)-mannan backbone length correlated most strongly with increased density and nanoscopic size of glucan exposures in *C. albicans* and *C. glabrata*, respectively. Furthermore, a *C. albicans* clinical isolate with high glucan exposure produced similarly perturbed N-mannan structures and exhibited similar changes to nanoscopic glucan exposure geometry. We conclude that acid labile N-mannan controls glucan exposure geometry at the nanoscale. Furthermore, variations in glucan nanoexposure characteristics are clinically relevant and are likely to impact the nature of the pathogenic surface presented to innate immunocytes at dimensions relevant to receptor engagement, aggregation and signaling.

Regulation of hyphal guidance by Pxl1 and Cst20 in *C. albicans*. *M.C. Almeida*[1], E.M. Morrison[1], J. Craven[2], N.A.R. Gow[1], A.C. Brand[1] 1) MRC Centre for Medical Mycology, University of Aberdeen, Aberdeen, Aberdeen, GB; 2) Department of Molecular Biology and Biotechnology, The Krebs Institute, University of Sheffield, Sheffield, UK.

Candida albicans lives as a commensal yeast in humans but can cause life-threatening bloodstream infections in susceptible patient groups. The formation of invasive hyphae is a characteristic virulence trait of *C. albicans* and we have shown that the penetration of host tissue depends on the ability of hyphae to steer as they grow. We are investigating the regulatory mechanisms of how hyphae sense guidance cues in the environment and translate these signals to re-orient the direction of growth. We have identified two types of 'steering-locked' mutants and the proteins associated with them. A wavy hyphal phenotype is generated by deletion of Pxl1, a Paxillin-like homologue, and its putative regulatory kinase, Ptk2 (homologue of Focal Adhesion Kinase). In contrast, truncation of Cst20 ($Cst20^{1-549}$) or hyper-activation of the small GTPase, Rho1, cause hyphae to grow uni-directionally. Both the wavy and unidirectional phenotypes correlate with an inability to respond normally to directional cues. Pxl1-GFP localises to bud tip and bud neck in yeast and to the growing tip in hyphae, but not to the septa, suggesting that Pxl1 is involved in the transport or localisation of a specific subset of cell-wall remodelling proteins. RFP-Ptk2 localises throughout the cytoplasm. Although the wavy and unidirectional phenotypes are strikingly different, the Pxl1 and $Cst20^{1-549}$ strains exhibit other phenotypes in common. In yeast, TEM analysis showed both mutants have cell separation defects. In hyphae, they exhibit aberrant intracellular structures. These shared phenotypes suggest that Pxl1 and Cst20 are involved in the same pathway that governs cell growth. We hypothesise that Cst20, a homolog of PAK that negatively regulates Paxillin in humans, may have a similar function in *C. albicans*, suggesting that constant turnover of the Pxl1 complex is required for hyphal steering responses.

Functional genomic analysis reveals fungal modulators of host immune responses. *Teresa O'Meara*, Kwamaa Duah, Leah Cowen Molecular Genetics, University of Toronto, Toronto, Ontario, CA.

Candida albicans is a leading human fungal pathogen that causes life-threatening systemic infections. The first line of defense against this pathogen is the innate immune response. Macrophages readily engulf *C. albicans*, and the engulfed *C. albicans* cells subsequently undergo a morphogenetic switch from yeast to a filamentous growth. This change in morphology is temporally coupled to the induction of macrophage pyroptosis, an inflammatory host cell death program mediated via activation of NLRP3 and caspase-1. Recently, we discovered that it is the remodeling of the fungal cell wall in response to the macrophage environment that exposes the trigger of host cell death, not the filament itself. Further, we found that heat-killed previously phagocytized *C. albicans* cells could drive macrophage lysis, unlike their counterparts that had not been phagocytized prior to killing, suggesting that this fungal cell wall remodeling is sufficient to drive macrophage lysis, and that the remodeling occurs specifically in response to the macrophage phagosome. However, the specific trigger and mechanisms involved remain enigmatic.

To address this question, we developed a high-content imaging platform to monitor interactions between *C. albicans* and macrophages, and have leveraged this platform to identify *C. albicans* mutants that are defective in pyroptosis. Using murine macrophages expressing an ASC-fluorescent protein (FP) reporter, we quantified ASC-FP paranuclear specks, which are indicative of NLRP3 inflammasome activation. Our primary screen allows us to simultaneously quantify not only pyroptosis, but also *C. albicans* filamentation within macrophages. Genes required for filamentation in the macrophage cannot be predicted *a priori* given that distinct genes regulate filamentation in response to

distinct cues. Our initial analysis of 96 filamentation-competent conditional expression strains that enable transcriptional repression of cell wall genes identified 34 genes that are important for activation of pyroptosis. This included genes that encode glucan biogenesis enzymes, mannosyltransferases, and GPI-anchored proteins, and their potential regulators. Together, we have identified novel circuitry regulating fungal induction of host immune cell death.

Capsule synthesis and regulation in the fungal pathogen *Cryptococcus neoformans*. *T.L. Doering*[1], L.X. Li[1], S.R. Gish[1], E.J. Maier[2,3], M.R. Brent[2,3,4] 1) Department of Molecular Microbiology, Washington University Medical School, Saint Louis, MO; 2) Department of Computer Science & Engineering, Washington University , Saint Louis, MO; 3) Center for Genome Sciences, Washington University Medical School, Saint Louis, MO; 4) Department of Genetics, Washington University Medical School, Saint Louis, MO.

Cryptococcus neoformans is a pathogenic fungus that kills hundreds of thousands of people a year, mainly immunocompromised individuals in developing areas of the world. This yeast bears a protective polysaccharide capsule that is required for virulence; capsule polysaccharides are also shed from the cell and impair the host immune response to infection. Capsule thickness varies dramatically with environmental conditions, becoming significantly greater during infection. We are dissecting this regulation, with the dual goals of understanding how environmental signals are integrated to determine capsule size and defining the downstream capsule synthetic machinery.

We have developed computational approaches[1] to map the transcriptional regulatory network of *C. neoformans* and model interactions between transcription factors (TFs) and their target genes.[2,3] We used these network maps to predict which TFs were mostly likely to impact capsule regulation and tesetd our predictions by deleting the corresponding genes. The resulting mutants produced abnormal capsules, validating our predictions.[3] Many also showed reduced virulence, in one case completely altering the course of the disease.[3,4] We further used our network maps to examine the target genes of capsule-implicated TFs and understand how these regulators influence capsule thickness. We are continuing to probe capsule regulation and investigate the roles of the affected gene products, with particular interest in proteins involved in the glycan synthetic pathways that produce capsule.

1 Haynes, B. C. *et al.* Mapping functional transcription factor networks from gene expression data. *Genome Res* **23**, 1319-1328 (2013).
2 Haynes, B. C. *et al.* Toward an integrated model of capsule regulation in *Cryptococcus neoformans*. *PLoS Pathog* **7**, e1002411 (2011).
3 Maier, E. J. *et al.* Model-driven mapping of transcriptional networks reveals the circuitry and dynamics of virulence regulation. *Genome Res* **25**, 690-700 (2015).
4 Gish, S. R. *et al.* Computational Analysis Reveals a Key Regulator of Cryptococcal Virulence and Determinant of Host Response. *mBio* **7** (2016).

Biochemistry and Metabolism

1W A global co-expression network approach for connecting genes to specialized metabolic pathways. *Jennifer Wisecaver*, Abigail Lind, Antonis Rokas Vanderbilt University, Nashville, TN.

Both fungi and plants produce a tremendous diversity of specialized metabolites (SMs) to interact with and manage their environment. A major challenge hindering efforts to tap this seemingly boundless source of pharmacopeia is the identification of SM pathways and their constituent genes. To address this challenge, much attention has been paid to fungal SM gene clusters, but the number of SM pathways in fungi that are non-clustered or only partially clustered is completely unknown. The genome of *Aspergillus fumigatus,* an opportunistic human pathogen and one of the leading causes of fungal-related human deaths, provides a stark example; 21 of its 37 SM gene clusters are missing either transporters, transcription factors or both, suggesting these genes may be located elsewhere in the genome. Given the well-established observation that the genes comprising a SM pathway are co-regulated in response to specific environmental conditions, we hypothesized that genes from a given SM pathway would form tight associations (modules) with each other in gene co-expression networks, facilitating their identification even without knowledge of the genome sequence. To evaluate this hypothesis, we first used 10 global co-expression datasets—each a meta-analysis of hundreds to thousands of expression experiments—across eight plant model organisms to identify hundreds of modules of co-expressed genes for each species. In support of our hypothesis, 15.3-52.6% of modules contained two or more known SM biosynthetic genes (e.g., cytochrome P450s, terpene synthases, and polyketide synthases), and module genes were enriched in SM functions. Moreover, modules recovered many experimentally validated SM pathways in these species, including all those known to form SM gene clusters. For each clustered SM pathway, the module analysis expanded the known genetic repertoire of the pathway by identifying additional unclustered enzymatic genes as well as unclustered genes involved in pathway regulation and product transport. We are currently expanding our network analysis to fungal taxa including *Aspergillus* and submit that global gene co-expression is a rich, but largely untapped, data source for discovering the genetic basis and architecture of natural products.

2T A novel integral membrane protein (Imp1) mediates TOR signaling in *Magnaporthe oryzae* and is required for deterministic (non-random) appressorium formation and biotrophic growth in rice cells. *G. Sun*[1], R. Wilson[1,2] 1) Department of Plant Pathology, University of Nebraska Lincoln, Lincoln, NE; 2) Redox Biology Center, University of Nebraska–Lincoln, Lincoln, Nebraska.

The devastating rice blast fungus *Magnaporthe oryzae* accesses rice plants by forming appressorial cells that disrupt the rice leave cuticle using internal turgor acting on a thin penetration peg. We recently reported that Target of Rapamycin (TOR), a conserved regulator of cell growth in response to nutrient availability, drives appressorium formation when inactivated on the nutrient-free leaf surface and, when activated, promotes biotrophic growth in rice cells following host penetration. We sought to identify and characterize additional components of this critical signaling pathway and here we report that a novel integral membrane protein, Imp1, mediates TOR signaling to ensure normal appressoria formation and *in planta* development by *M. oryzae*. We discovered *IMP1* by using *Agrobacterium tumefaciens*-mediated mutagenesis to select for rapamycin resistant *M. oryzae* strains. Following targeted deletion of *IMP1* by homologous gene recombination, we found that the resulting Δ*imp1* mutant strain was impaired for appressorium formation. Appressorium formation in Δ*imp1* deletants was stochastic such that some germinating spores of Δ*imp1* produced appressoria, but most formed undifferentiated swellings at germ tube tips. These latter germinating spores demonstrated increased mitosis and loss of autophagy, consistent with constitutive TOR activation. Appressorium formation rates in Δ*imp1* deletants were not remediated by rapamycin (or cAMP) treatment, suggesting Imp1 is required either for TOR inactivation or transduction of the inactive TOR signaling cascade. Those spores that formed appressoria could penetrate leaf cuticles but elaborated morphologically abnormal invasive hyphae that failed to conduct cell-to-cell movement in rice cells.

Imp1 localized to surface of vacuoles and is likely involved in autophagosome-vacuole fusion and vacuole function in response to TOR activity status. We thus propose that Imp1 is a downstream component of the TOR signaling pathway such that in response to TOR inactivation, Imp1 mediates autophagosome-vacuole fusion resulting in autophagy and, in germinating spores, mitotic arrest and deterministic appressorium development. Our results thus reveal new insights on both the mediation of TOR signaling and the molecular mechanisms underlying appressorium formation by the rice blast fungus *M. oryzae*.

3F A trehalose-regulatory subunit moonlights to regulate cell wall homeostasis through modulation of chitin synthase activity in *Aspergillus fumigatus*. *Arsa Thammahong*, Alayna Caffrey, Sourabh Dhingra, Josh Obar, Robert Cramer Microbiology and Immunology, Dartmouth College, Hanover, NH.

Purpose: Trehalose biosynthesis is a metabolic pathway found in fungi but not humans. Proteins involved in trehalose biosynthesis are essential for human and plant fungal pathogen virulence. Loss of canonical trehalose biosynthesis genes in the human pathogen *Aspergillus fumigatus* alters virulence and cell wall integrity through undefined mechanisms. Here we characterize additional genes, herein called *tslA* and *tslB,* which encode proteins that contain similar protein domains as OrlA (a trehalose-6-phosphate phosphatase), but lack critical catalytic residues for phosphatase activity.
Methods: We utilized a genetics approach to generate null mutants of *tslA* and *tslB*. To observe the phenotypes of these mutants, we used trehalose assays and cell wall perturbing agents. Furthermore, LC-MS/MS and co-immunoprecipitation were performed to define protein-protein interactions of TslA . To further characterize the phenotype of *tslA* null mutant, chitin synthase activity assay and spinning-disk confocal microscope were used to study the chitin content and the localization of CsmA. A chemotherapeutic murine model was utilized to study the host-pathogen interaction.
Results: Loss of *tslA* reduced trehalose content in both conidia and mycelia, impaired cell wall integrity, and significantly altered cell wall structure. Unexpectedly, immunoprecipitation assays coupled with LC-MS/MS revealed a protein interaction between TslA and CsmA, a type V chitin synthase enzyme. TslA regulates not only chitin synthase activity but also CsmA localization. Loss of TslA directly affected the host immune response to *A. fumigatus* characterized by an increase in murine mortality likely due to enhanced immune cell recruitment.
Conclusion: Our data provide a mechanistic model whereby proteins in the trehalose pathway play critical roles in fungal cell wall homeostasis that alters fungal-host interactions. Future studies are underway to elucidate the mechanism(s) through which OrlA, TslA, TslB, and their interacting partners control fungal cell wall homeostasis and virulence.

4W Disruption of *mgr2* gene in *Aspergillus fumigatus* alters redox balance, cell wall structure, spore germination and susceptibility to macrophage killing. E.S. Santos[1], *L.L.L. Balico*[1], A.C. Silva[2], L.O. Sousa[1], S.A. Uyemura[1] 1) FCFRP - USP, Ribeirão Preto, BR; 2) FMRP - USP, Ribeirão Preto, BR.

Aspergillus fumigatus is a filamentous saprophytic fungus and a major pathogen in immunosuppressed patients. *mgr2* (mitochondrial genome maintenance protein) gene encodes a protein of 108 amino acids whose functions have not yet been elucidated. In humans, ROMO1 (MGR2 orthologous) regulates ROS balance, proliferation, cell growth and death, and mitochondrial fission/fusion. In *S. cerevisiae*, MGR2 is part of TIM23 complex, a translocase of the mitochondrial inner membrane. The aim of this study was to understand MGR2 function in *A. fumigatus* by generating a knockout mutant (*Δmgr2*) and to phenotypically characterize the mutant in comparison to wild type strain (KU80) and to *Δmgr2* complemented with *mgr2* gene (Δ*mgr2*::*mgr2*$^{+}$). Our results show that MGR2 deletion increases spore germination and ROS formation (analyzed by carbonylated protein assay), alters cell wall composition as observed by confocal microscopy after Congo Red and Calcofluor White staining, and alters sensitivity to enzymatic cell wall degradation. Interestingly, *Δmgr2* also showed a lower susceptibility to macrophage killing of conidia. New studies are necessary to further understand MGR2 function in *A. fumigatus*.

5T Mechanism for Subcellular Compartmentalization of Melanization in *Aspergillus fumigatus*. Srijana Upadhyay, Xinping Xu, *Xiaorong Lin* Dept Biol, Texas A&M University, College Station, TX.

Melanins are biopolymers that confer coloration and protection to the host organism against biotic or abiotic insults. The level of protection offered by melanin depends on its biosynthesis and its subcellular localization. Previously we discovered that *Aspergillus fumigatus* compartmentalizes melanization in endosomes by recruiting all melanin enzymes to the secretory pathway. Surprisingly, although two laccases involved in the late steps of melanization are conventional secretory proteins, the four enzymes involved in the early steps of melanization lack a signal peptide or a transmembrane domain and are thus considered “atypical” secretory proteins. Here, we found interactions among melanin enzymes and all melanin enzymes formed protein complexes. Surprisingly, forming protein complexes by melanin enzymes was not critical for their trafficking to the endosomal system. By palmitoylation profiling and biochemical analyses, we discovered that all four early melanin enzymes were strongly palmitoylated during conidiation. This post-translational lipid modification correlates the endosomal localization of all early melanin enzymes. Furthermore, treatment with palmitoylation inhibitor not only drastically reduced the palmitoylation of the PKS enzyme Alb1, but also weakened its endosomal localization. Intriguingly, bioinformatic analyses predict palmitoylation a common mechanism for potential membrane association of other polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) in *A. fumigatus*. Our findings indicate that protein-protein interactions facilitate melanization by metabolic channeling while post-translational lipid modifications help recruit the “atypical” enzymes to the secretory pathway, which is critical for compartmentalization of secondary metabolism.

6F Copper-responsive isocyanide biosynthetic cluster in *Aspergillus fumigatus*. *F. Lim*[1], J. Baccile[2], T. Won[2], P. Wiemann[1], A. Lind[3], A. Rokas[3], F. Schroeder[2], N. Keller[1] 1) University of Wisconsin-Madison, Madison, WI, U.S.A; 2) Cornell University, Ithaca, NY, U.S.A; 3) Vanderbilt University, Nashville, TN, U.S.A.

Isocyanide-containing natural products are of immense interest in the biological and chemical milieu owing to its broad range of bioactivity, which is attributed by the highly reactive isocyano- functional groups tethered to structurally diverse carbon scaffolds. In contrast to the fast-growing list of naturally-occurring isocyanides, knowledge on the biosynthetic machineries that give rise to such unique chemistry is still in its infancy with only four characterized biosynthetic gene clusters in bacteria, and unprecedented in eukaryotes. A hallmark function for these isocyanide natural products is their capability for metal coordination, which is shown to impart the bioactivites for many of these naturally-occurring isocyanides and from an ecological perspective, crucial to the pathogenesis of the entomopathogenic bacterium,

Xenorhabdus nematophila (by disabling the innate immune defense via inhibition of the cuproenzyme, phenoloxidase). Here we report on the identification of four isonitrile synthases (INS) in the genome of the human opportunistic pathogen, *Aspergillus fumigatus* and the discovery of a novel copper-responsive INS-NRPS-like hybrid enzyme (CrmA) involved in the synthesis of xanthocillin analogues, the first reported biosynthetic pathway dedicated to this family of naturally-occurring isocyanides. This work also elucidates the regulatory circuitry that bridges cellular metal homeostasis and fungal development.

7W Biosynthesis of dihydrolysergic acid by heterologous expression of *Claviceps* spp. genes in *Neosartorya fumigata*. Stephanie Arnold, Paige Bragg, *Daniel Panaccione* West Virginia University, Morgantown, WV.

Ergot alkaloids derived from lysergic acid (LA) and dihydrolysergic acid (DHLA) can be used to treat dementia, migraines, hyperprolactinemia, and other disorders. LA and DHLA are structurally similar fungal secondary metabolites, but they have different activities related to cognitive function and other clinical applications. LA has been produced by genetic modification of the model fungus *Neosartorya fumigata* (synonym *Aspergillus fumigatus*), but the biosynthetic pathway to DHLA has not been established. Previous studies showed that the enzyme CloA from the LA-producing fungus *Epichloë typhina* x *festucae* oxidized the substrate agroclavine to LA. We transformed *cloA* from *E. typhina* x *festucae* into a *N. fumigata* strain that accumulates festuclavine, a precursor to DHLA, but it failed to oxidize festuclavine to DHLA. We hypothesized that CloA from *Claviceps africana*, a DHLA-producing fungus, would oxidize festuclavine to DHLA. A genomic clone of *C. africana* cloA was not processed correctly in *N. fumigata*, so coding sequence only versions of *cloA* alleles from *C. africana* and closely related *C. gigantea* were synthesized and expressed in the festuclavine-accumulating mutant background. HPLC and mass spectrometry analyses demonstrated that transformants expressing CloA from either of these *Claviceps* spp. produced DHLA. Substrate feeding studies indicated interesting differences in specificity of CloA from LA producing and DHLA producing fungi. Our results show that production of DHLA requires specialized alleles of two ergot alkaloid pathway genes compared to those found in the LA pathway.

8T Role of the urea cycle in the synthesis of nitric oxide. *D. Canovas*[1,2], A.T. Marcos[1], J.F. Marcos[3], T. Schinko[2], J. Strauss[2] 1) Department of Genetics, University of Sevilla, Sevilla, Spain; 2) Division of Microbial Genetics and Pathogen Interactions, Department of Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Sciences Vienna, Austria; 3) Department of Food Science, Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain.

Nitric oxide (NO), and its role in signalling, has been extensively studied in mammals and to some extent in plants. However, little is known about the role of NO in fungi and how it is synthesized in these organisms. Recently, we reported that NO production in *A. nidulans* is coupled to conidiation and requires a functional nitrate reductase (NR) gene (*niaD*) that is upregulated under these conditions even in the presence of the repressing nitrogen source ammonium. NO levels influence the balance between conidiation and sexual reproduction. Here we report that NO levels are also modulated by light, a general environmental cue and a regulator of fungal development. The light-dependent modulation of nitric oxide levels involves NO catabolism by the mitochondrial flavohemoglobin *fhbB*, and *agaA*, an arginase that controls the intracellular concentration of the NO precursor arginine. Addition of arginine to the cultures provokes a transient increase of the production of NO. However, analogues of arginine did not affect the production of NO. Mutants in the urea cycle genes show differences in NO levels compared to the wild type strain. Taken together our findings indicate that light-dependent developmental processes in *A. nidulans* interfere with nitric oxide metabolism which – in addition to nitrate reduction - is modulated by enzymes of the urea cycle.

Reference:

Marcos AT, Ramos MS, Marcos JF, Carmona L, Strauss J, Cánovas D. Nitric oxide synthesis by nitrate reductase is regulated during development in *Aspergillus*. Mol Microbiol (2016) 99:15-33.

9F COP9 signalosome and Cand mediated Cullin-RING ligase control and *Aspergillus nidulans* development. *A. M. Koehler*, G. H. Braus Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany.

Cullin RING ligases (CRLs) are the specificity factors which mark target substrates for degradation by the ubiquitin 26S proteasome system. CRLs are activated by linkage of the ubiquitin-like modifier NeddH/Nedd8 to a conserved lysine residue within the cullin core module of this enzyme complex. *Aspergillus nidulans* posseses approximately 70 F-box proteins as receptors for different substrates, which are linked through the adaptor SkpA to cullins. Fungal development requires the controlled degradation of various substrates and therefore exchanges of F-box proteins within CRLs. This exchange depends on the inactivation of CRLs by removing NeddH and subsequent reassembly. At the molecular level this process is catalysed by the two deneddylases COP9 signalosome and DenA [1, 2], and the Cand complex [3]. In fungi a seven-subunit pre-COP9 signalosome is first assembled and activated by the integration of the eighth CsnE NeddH-specific isopeptidase subunit [4]. Cand binds to deneddylated cullins at the SkpA binding and the neddylation site and its function is a prerequisite for CRL reassembly. Defects in COP9 or Cand subunits result in impaired fungal development and a dysregulated secondary metabolism. Cand represents a single polypeptide in numerous filamentous fungi and mammals, but is split in *A. nidulans* into at least two polypeptides (34kDa CandAN blocks neddylation site; 114 kDa CandAC blocks SkpA binding site), which form a cullin binding heterodimeric complex and are presumably the result of a genomic rearrangement event [3]. The gene locus of the two *A. nidulans cand* genes was analysed in more detail and revealed that the fungal Cand complex consists apparently of even three proteins, which all are required for fungal development and coordinated secondary metabolism. The interplay between Cand subunits and the two deneddylases as well as their molecular function and consequences for fungal differentiation are presented.

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[2] Schinke J *et al.,* 2016.**PLoS Genetics** 12, e1005949.
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10W Growth-phase sterigmatocystin formation on lactose is mediated via low specific growth rates in Aspergillus nidulans. *L. Karaffa*[1], Z. Németh[1], Á.P. Molnár[1], B. Fejes[1], L. Novák[2], N.P. Keller[3,4], E. Fekete[1] 1) Department of Biochemical Engineering, University of Debrecen, Debrecen, HU; 2) Department of Physical Chemistry, Faculty of Science and Technology, University of Debrecen, HU; 3) Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, USA; 4) Department of Bacteriology, University of Wisconsin, Madison, USA.

Seed contamination with polyketide mycotoxins such as aflatoxin (AF) and sterigmatocystin (ST) produced by *Aspergillus spp*., is an agricultural, economic, and medical issue worldwide. ST is the penultimate intermediate in the biosynthesis of AF, and in several fungi including the model fungus *A. nidulans*, it is the end product of the AF pathway. This biosynthetic pathway is well-characterized in *A. nidulans*, but many of the regulatory aspects related to the carbon source available for the fungus are still enigmatic. This is particularly true for the heterodisaccharide lactose (milk sugar; 1,4-*O*-beta-D-galactopyranosyl-D-glucose), inasmuch as some ST production mutant strains still synthesize ST on lactose but not on other carbon substrates including the customary D-glucose. Here, kinetic data from well-controlled single-carbon substrate submerged fermentations revealed that on D-glucose, ST forms only after the sugar is depleted from the medium, while on lactose, ST appears when the majority of the carbon source is still available. Maximal biomass-specified ST production in lactose medium was significantly higher than on D-glucose. These data suggested that ST formation may either be mediated by a carbon catabolite regulatory mechanism prominent on D-glucose, or induced by low specific growth rates attainable on lactose. These hypotheses were tested by constant-mass chemostat-type continuous fermentations on D-glucose as a sole carbon source at two different dilution rates (D = 0.090 h^{-1} and D = 0.020 h^{-1}), representing a state of carbon catabolite repression and derepression, respectively. ST production under such conditions negatively correlated with the dilution rate, i.e., no ST formed at high growth rate, while low growth rate led to the formation of 0.4 mg L^{-1} ST. Essentially identical results were obtained with a CreA mutant strain, indicating that CreA does not regulate the formation of ST during growth on D-glucose. We concluded that low specific growth rates may be the primary cause of sustained, mid-growth ST formation on the slowly assimilating lactose in *A. nidulans*, and that carbon utilization rates likely play a general regulatory role during biosynthesis.

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11T Discovery of novel aromatic processing enzymes from ferulic acid-tolerant *Aspergillus niger* evolution mutant. *April J. M. Liwanag*[1,2], Adiphol Dilokpimol[1,2], Isabelle Benoit-Gelber [1,2], Ronald de Vries[1,2] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, NL; 2) Fungal Molecular Physiology, Utrecht University, Utrecht, NL.

The biological degradation of lignin generates a complex mixture of aromatic compounds in the environments, but the metabolic pathways by which fungi may utilize these are poorly understood. A putative aromatic pathway has been described in *Aspergillus japonicus*[1]. However, most enzymes involved in aromatic conversions have not been identified, despite the potential in using such enzymes for the conversion of lignin-derived aromatic compounds.

Aspergillus niger is widely used for industrial applications (e.g., enzyme and metabolite production) and is a model organism for strain improvement. Benzoate-para-hydroxylase (BphA, EC 1.14.13.12.) is the only aromatic processing enzyme that has been characterized so far in *A. niger*, which converts hydroxybenzoate into protocatechuate[2]. To identify enzymes involved in the aromatic metabolism of fungi and strains capable of efficient aromatic conversion, we have developed an *A. niger* adaptive evolution mutant by growing *A. niger* N402 on incrementally increasing concentrations of ferulic acid. This mutant has a growth threshold on 0.1% ferulic acid, in the absence of any sugar source, and it tolerates a 10 fold-higher concentration of ferulic acid compared to the parental strain.

Transcriptomics was performed to investigate the difference in the expression level of genes between the mutant and parental strain grown on ferulic acid. The results revealed candidate genes encoding enzymes that are potentially involved in aromatic metabolism. The selected genes were then analyzed by qPCR and cloned for biochemical characterization of the corresponding enzymes.

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12F Analysis of aspirochlorine (ACL) productivity and ACL cluster sequence in Aspergillus oryzae strain. *S. Ryota*[1], O. Tami[1], U. Miyuki[1], O. Ken[1], I. Kazuhiro[1,2] 1) Nstional Research Institution of Brewing, Hiroshima, Hiroshima, Japan; 2) Graduate School of Advanced Science of Matter, Hiroshima, Hiroshima, Japan.

Aspergillus oryzae is GRAS (generally recognized as safe) and various type of strains are used in the industry. The *A*. *oryzae* genome sequence analysis conducted in 2005, revealed the presence of many conserved secondary metabolism clusters in *A*. *oryzae*. In these studies, a cluster similar to the gliotoxin synthesis cluster was identified in the *A. oryzae* genome. Recently, the product of this cluster was identified as aspirochlorine (ACL), and this cluster was designated as the ACL cluster. ACL is antibiotics A30641, which inhibits fungal protein synthesis and represses the growth of *Candida albicans.* Although, some *A*. *oryzae* strains are known to produce ACL, the ACL productivity in several *A*. *oryzae* strains remain unclear. In this study, we investigated the physiological and molecular biological features of ACL productivity among different *A*. *oryzae* strains. First, we investigated ACL productivity in 3 different media (WATM, CYA, YES) in agar plate condition and rice koji condition by using *A*. *oryzae* RIB40. We observed that the amount of ACL was different in each plate culture medium. However, in rice koji conditions, RIB40 did not produce ACL even after 8 days of cultivation. We then examined the ACL production of 13 *A*. *oryzae* strains, one from each of the 13 phylogenetic clusters of *A. oryzae*. We examined the ACL productivity of these strains in YES agar medium, as RIB40 showed the maximum ACL production; ACL productivities among these strains were considerably different. In particular, RIB301, RIB430 and RIB1172 did not produce ACL. Furthermore, we examined the ACL production by these strains in rice koji conditions and found that none of the strains produced ACL. This difference in ACL productivity likely be based on the difference between the ACL cluster genes sequences. Hence, we examined the genome sequence of ACL clusters in these 13 strains. The ACL cluster was found to be conserved in all the 13 strains and many mutations were found in each strain. Some strain-specific mutations were also observed; in particular, in the strains that did not produce ACL.

In this study, it is clear that ACL productivity is affected by nutrient conditions. Interestingly, in the rice koji condition, no strains produced

ACL, and it supposed that Japanese sake does not contain ACL. Hence, we investigated the ACL concentration in Japanese sake. We collected 11 types of sake from 20 prefectures, and did not find ACL.

13W Characterization of small proteins secreted by lignolytic fungi. *N. Valette*[1,2], Eric Gelhaye[1,2], Mélanie Morel-Rouhier[1,2] 1) UMR 1136 Tree-Microbe interactions, Lorraine University, Vandoeuvre les Nancy, FR; 2) INRA, Interactions Arbres–Microorganismes, UMR1136, F-54280 Champenoux, France.

Saprophytic fungi play an important biological and ecological role in forest ecosystems. Indeed, thanks to many extracellular enzymes, they are able to degrade all wood components, a key step for carbon recycling. During this process, wood releases various molecules, called extractives, which can be toxic for fungal cells. This is the reason why these microorganisms have developed extensive detoxification systems. Interestingly, a transcriptomic analysis revealed that numerous up-regulated genes involved in extractive response in *Phanerochaete chrysosporium* are still of unknown function. Among them, one group called small secreted proteins (SSP) has been highlighted. A SSP from *P. chrysosporium* has been produced as a recombinant protein in *Escherichia coli* and purified for functional characterization. Our results show that this SSP has several interesting structural features. Indeed, it can form a kind of hydrogel and forms fibers depending on pH. Moreover, the protein is highly stable being insensitive to temperature, denaturating agents or reductants. Since it can interact with wood extractives, this protein can be involved in the process by which fungi protect themselves against toxic molecules.

14T Powdery mildew conidiophore initiation but not spore fitness is correlated with host plant lipid precursor availability. *A. McRae**[1], J. Jaenisch*[1], M.Y. Lee[1], C. Hirai[1], L. Silva[2], T. Northen[2], M.C. Wildermuth[1] 1) Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA; 2) Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Walnut Creek, CA.

Powdery mildews are widespread plant pathogens that infect agriculturally important crops. These obligate biotrophs depend on host metabolism for nutrients to complete their life cycle. Powdery mildews grow epiphytically and develop specialized feeding structures called haustoria in the epidermal cells of its host. These feeding structures assimilate nutrients to fuel the metabolically demanding process of asexual reproduction. Asexual reproductive structures (conidiophores) consist of chains of newly formed spores which contain many lipid bodies. Others previously demonstrated that lipid storage bodies in germinating spores decrease over time, suggesting these energy rich molecules are consumed to support development prepenetration in *Blumeria graminis* f sp *hordei* (*Bgh*). cDNA microarray analysis of *Bgh* showed lipid degradation pathways are upregulated prepenetration, consistent with this function (Plant Cell **17**:2107-22). *Golovinomyces orontii* MGH1 infects the model plant, *Arabidopsis thaliana*. Infection site transcriptomics revealed that select metabolic genes/pathways are up regulated at 5 days post inoculation (dpi), concurrent with the production of asexual reproductive structures. This includes genes involved in the pyruvate dehydrogenase (PDH) bypass. The PDH bypass can facilitate increased production of Acetyl-CoA, which can be utilized as precursors for lipid biosynthesis. ^{13}C-ethanol feeding to detached infected leaves to provide labelled lipid precursor via the host PDH bypass showed isotopic enrichment in plant lipids and fungal spore lipids. Furthermore, powdery mildew colonies on plant mutants in PDH bypass genes have reduced numbers of conidiophores 5 dpi. However, the number of newly formed spores per conidiophore remains unchanged. The lipid content of spores was assessed using flow cytometry with Nile Red and BODIPY 505/515 lipid stains. Flow cytometry analysis found the spores from powdery mildew grown on these plants versus wild-type plants have the same lipid content. Furthermore, these two sets of spores also exhibit similar germination rates. Taken together, this suggests that not only does the induced host PDH bypass provide lipid precursors for fungal spore formation, but that a PDH bypass-derived lipid precursor acts as signal to regulate conidiophore formation while maintaining the fitness of those spores that are produced.

*presenting authors

15F Powdery Mildew Manipulation of Plant Host Metabolism Fuels Asexual Reproduction. Mi Yeon Lee[1], Chihiro Hirai[1], Johan Jaenisch[1], Amanda McRae[1], Leslie Silva[2], Trent Northen[2], *Mary Wildermuth*[1] 1) University of California, Berkeley, CA; 2) Lawrence Berkeley National Laboratory, Walnut Creek, CA.

Powdery mildew fungi are obligate biotrophs that alter plant cellular architecture and metabolism to acquire their nutrients from the plant, while limiting plant defense. Using site-specific analyses, we found that the powdery mildew *Golovinomyces orontii* induces endoreduplication in plant mesophyll cells underlying the fungal feeding structure concomitant with fungal proliferation (PNAS 107:460-5). Multiple rounds of DNA replication without mitosis result in enhanced cell ploidy with ~16-fold elevated DNA content. Increased endoploidy has long been associated with enhanced metabolism, but the detailed mechanisms underlying this relationship are not well understood. We identified a conserved eukaryotic transcription factor and plant-specific factors that control the extent of induced ploidy and found the extent of fungal proliferation correlates with DNA ploidy levels (MPMI 26:537-45). This suggests ploidy-dependent enhanced host metabolism is needed to support the metabolically demanding proliferation phase of the powdery mildew life cycle. Using transcriptome, genetic, and biochemical analyses we have identified specific ploidy-dependent alterations in Arabidopsis primary metabolism that are utilized by the powdery mildew to fuel its asexual reproduction. Specifically, use of the plant pyruvate dehydrogenase (PDH) bypass which can increase Acetyl-CoA and precursors for lipid synthesis is correlated with the number of fungal asexual reproductive structures. However, the number of new spores per reproductive structure remains unchanged. Powdery mildew spores contain lipid bodies which appear to be utilized to fuel germination and pre-penetration events (Plant Cell 17:2107). 13C-ethanol feeding to detached infected leaves to provide labelled lipid precursor via the plant PDH bypass showed isotopic enrichment in plant lipids and fungal spore lipids. FACS analysis of lipid stained spores from powdery mildew grown on PDH bypass mutant plants versus wild-type plants show they have similar lipid content. Furthermore, these two sets of spores also exhibit similar germination rates. Taken together, this suggests that not only does the induced ploidy-associated use of the host PDH bypass provide lipid precursors for fungal spore formation, but that a PDH bypass-derived lipid precursor acts as signal to regulate conidiophore formation while maintaining the fitness of spores that are produced.

16W Resistance to anilinopyrimidine fungicides in field populations of *Botrytis cinerea* is caused by independent mutations in two nuclear-encoded mitochondrial proteins. *A. Mosbach*[1], D. Edel[1], S. Widdison[2], A. Farmer[3], R. Dietrich[4], A. Corran[2], G. Scalliet[1] 1) Syngenta Crop Protection AG, 4332 Stein, Switzerland; 2) Syngenta Jealott's Hill Int. Research Cen, Bracknell RG42 6EY, UK; 3) National Center for Genome Resources, Santa Fe NM 87505, USA; 4) Syngenta Crop Protection LLC, Research Triangle Park, USA.

Anilinopyrimidine (AP) fungicides were first introduced in Europe more than 20 years ago and still display a good efficacy for the control of

a range of ascomycetes in various crops worldwide. Although resistant field isolates occurring at low to moderate frequency in most of these pathogens are known for many years, the underlying resistance mechanisms have not been deciphered.
The grey mold *Botrytis cinerea* is a high risk pathogen for the development of fungicide resistance, and isolates showing reduced sensitivity to APs are frequently found in populations from grapes, strawberries and other crops. To characterize the resistance mechanisms in *B. cinerea*, two complementary approaches were undertaken: The induction of resistance-conferring mutations by *in vitro* random mutagenesis followed by next generation sequencing, and the characterization of mapping populations from crosses between resistant field isolates and reference strains. These approaches led to the identification of nine different nuclear genes conferring resistance, all coding for proteins associated with the mitochondria. Such diversity of possible fungicide resistance mechanisms is so far unique.
Some of these genes have been reported to play roles in mitochondrial protein maturation and quality control, or the defense against oxidative damage. One of the nine genes was found both *in vitro* and in the field, another one exclusively in nature. Based on our monitoring, these two genes account for all of the resistance observed in the field. By targeted gene knock-out we could demonstrate that some of the resistance-conferring mutations most likely lead to a partial loss of function of the encoded proteins, as the deletion mutants displayed increased tolerance to cyprodinil. These results, in addition to AP treatment-related metabolomics and transcriptomics information enable us to progress our understanding of the mode of action for this class of fungicides.

17T The fungal cell wall as an antifungal drug target. *Carol Munro*, Louise Walker, Sami Alawfi, Giuseppe Buda de Cesare, Chibuike Ibe MRC Centre for Medical Mycology, University of Aberdeen, Aberdeen, GB.

The cell wall of the pathogenic yeast *Candida albicans* is a dynamic organelle, primarily composed of chitin, β-1,3-glucan, β-1,6-glucan and mannoproteins. The polysaccharides give the wall its mechanical strength and the mannoproteins include important virulence factors as well as carbohydrate active enzymes that contribute to maintaining cellular integrity. The majority of cell wall components are fungal-specific and attractive targets for the development of much needed therapies. This potential has already been realised by the development of the echinocandins; antifungal drugs that target the cell wall by inhibiting β-1,3-glucan synthesis. The echinocandins generally provide effective therapy but sporadic resistant *Candida* isolates have emerged through acquisition of point mutations in the *FKS* target genes. *C. albicans,* and other fungal species, respond to sub-MIC echinocandins by up-regulating chitin biosynthesis and modulating the cell wall glycoproteome. The changes are brought about by activation of cell wall salvage mechanisms that involve PKC and calcium/calcineurin signalling. Modifications to the cell wall such as elevated chitin reduce susceptibility to echinocandins *in vitro,* as well as in infection models. Several predicted carbohydrate-active (CAZy) enzymes involved in modulating and cross-linking chitin and glucan (Phr1, Phr2, Pga4, Crh11, Utr2) are among the cell wall proteins (CWPs) positively regulated in response to cell wall damage. Overexpression or deletion of specific CWP genes results in altered caspofungin susceptibility, paradoxical growth and influences host-pathogen interactions. Changes in environmental conditions and the application of cell envelope stresses can substantially change the cell wall, which should be seen as a moving target.

18F Managing Plant Diseases Through Disruption of Fungal Nicotinamide Adenine Dinucleotide Cycle. *Shay Covo*, Daniel Waiger, Vinay Bary, Nuria Vital Plant pathology and Microbiology, Hebrew University, Rehovot, IL.

Nicotinamide adenine dinucleotide (NAD) is a common and important metabolite in eukaryotic cells. NAD is crucial for proper redox balance and it is a cofactor of DNA repair enzymes and histone deacetylases from the sirtuins superfamily. During histone deacetylation, sirtuins convert NAD to nicotinamide (NAM). It was previously shown that NAM can reduce fungal growth in mice infected with *Candida albicans* due to inhibition of the ascomycete-specific sirtuin HST4. Here we tested the ability of NAM and other compounds in the NAD pathway to restrict the growth of fungal plant pathogens. NAM was able to inhibit both hyphal growth and conidial germination of *Fusarium oxysporum* and *Botrytis cinerea* in vitro (IC50 - 4 mM). NAM was able to reduce significantly fungal growth on slices of tomatoes.We are looking for ways to reduce the effective dose of NAM to manage plant disease. One direction is to inhibit the enzyme that further metabolites NAM in the cell. PNC1 is a fungal-specific nictinamidase that was shown to be inhibited in vitro by nicotinaldehyde (NA). We were able to show that NA inhibits the growth of *Fusarium* and *Botrytis* using even lower concentrations than NAM. Surprisingly, as determined by RNAseq the mode of action of NAM and NA is completely different. Genes that are upregulated due to NAM exposure are mainly transcription factors as expected from histone deacetylase inhibitor. Some of the GO terms enriched for genes that are downregulated by NAM belong to the redox balance. In contrast, genes that are up-regulated by NA belong to the redox- balance. These results suggested to us that while NAM inhibits chromatin biology NA disrupts NAD biosynthesis. This is in agreement of the role of PNC1 in NAD salvage biosynthesis pathway. Indeed we were able to show that NA toxicity can be bypassed by addition of niacin the next compound in NAD biosynthesis cycle. Our preliminary results show that NA indeed reduces NAD cellular concentrations. Finally, we were able to show that NA is synthetic lethal with Boscalid a SDHI. We propose that by developing better chemistry to inhibit PNC1 and HST4 a novel class of fungicides that have a broad effect on cellular functions will be obtained.

19W Elucidating the structural basis of effector induced susceptibility in the *Parastagonospora nodorum* - wheat interaction. M.A Outram[1], X Zhao[1], S Breen[2], P.S Solomon[2], B Kobe[1], *S.J Williams*[1,2] 1) School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, AU; 2) Research School of Biology, Australian National University, Canberra, ACT, AU.

Parastagonospora nodorum, the causal agent of Septoria Nodorum Blotch, is a major necrotrophic fungal pathogen of wheat worldwide. *P. nodorum* secretes small cysteine-rich proteinaceous effectors (ToxA, Tox1 and Tox3) that interact with corresponding dominant host sensitivity gene products, promoting cell death and rendering the plant susceptible to disease. Whilst it is now understood that these effectors are required for disease, there is little understanding of their function. Recently Tox3 from *P. nodorum* has been shown by yeast two-hybrid and co-immunoprecipitation assays to interact with basic and acidic wheat pathogenesis-related 1 (PR1) proteins. We have developed a protein expression and purification system that produces Tox3 and wheat PR1 proteins and the recombinant Tox3 protein was determined to be functional based on wheat infiltration experiments. We present here the first crystal structure of an acidic wheat PR1 protein and have obtained crystals of Tox3. These data provide a platform to understand the molecular details of the Tox3 and wheat PR1 interaction.

20T Biosynthesis and detoxification of trichosetin is regulated by two cluster-specific transcription factors in *Fusarium fujikuroi*. *S. Janevska*[1], B. Arndt[2], L. Baumann[1], L. Apken[1], H.-U. Humpf[2], B. Tudzynski[1] 1) Institute of Plant Biology and Biotechnology, University of Muenster, Muenster, DE; 2) Institute of Food Chemistry, University of Muenster, Muenster, DE.

The PKS-NRPS-derived tetramic acid equisetin and its *N*-desmethyl derivative trichosetin exhibit significant toxic properties against a variety of organisms, including plants and bacteria (methicillin-resistant *Staphylococcus aureus*). The first equisetin biosynthetic gene cluster was described for *Fusarium heterosporum* (Kakule *et al*., 2013), a species distantly related to the notorious rice pathogen *Fusarium fujikuroi*.

Here we present the identification and activation of a homologous but silent gene cluster in *F. fujikuroi*. Bioinformatic analysis revealed that this cluster does not harbor the equisetin *N*-methyltransferase gene *eqxD* and accordingly, trichosetin was isolated as final product. Indeed, heterologous expression of *F. heterosporum eqxD* in a trichosetin-producing *F. fujikuroi* strain resulted in the accumulation of equisetin. Constitutive overexpression of one of the two cluster-specific transcription factor (TF) genes, designated *TF22*, led to induced expression of the three biosynthetic cluster genes, including the PKS-NRPS key gene. Accumulation of trichosetin strongly inhibited growth of the producing fungus. Therefore, we adapted the inducible bacterial-fungal hybrid promoter system Tet-on (Meyer *et al*., 2011) for *F. fujikuroi*, to achieve a controlled overproduction of trichosetin and a functional characterization of each cluster gene. In contrast, overexpression of the second cluster-specific TF gene, designated *TF23*, did not activate the expression of the other cluster genes. Instead, *TF23* was induced by the final product trichosetin itself *via* a yet unknown mechanism. TF23 in turn was shown to be essential for expression of the cluster gene *MFS-T*, encoding a transporter of the major facilitator superfamily. Our evidence suggests that TF23 and MFS-T contribute to detoxification of trichosetin and thus, self-protection of the producing fungus.

Kakule TB *et al*. (2013) ACS Chem Biol 8: 1549–57

Meyer V *et al*. (2011) Appl Environ Microbiol 77: 2975-83

21F Cell wall biosynthesis in Fusarium graminearum. *Rohan Lowe*[1], Neil Shirley[2], Alan Little[2], Mark Bleackley[1], Vincent Bulone[2], Marilyn Anderson[1] 1) Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, VIC, Australia; 2) ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, The University of Adelaide, SA, Australia.

Fungal cell walls contain several polysaccharides that are not encountered in plants and/or animals and thus represent an ideal target for control of fungal diseases. However, a better understanding of cell wall structure and biosynthesis in pathogenic fungi is needed for the rational design of new efficient cell wall inhibitors. Fungal cell walls have been understudied during the past decades due to the general assumption that they are generally homogeneous in structure throughout the kingdom Fungi. Instead, the fungal cell wall is a highly dynamic structure whose composition constantly changes as a response to the environment and developmental cues. Using the cereal pathogen Fusarium graminearum as our model fungus, genes encoding carbohydrate active enzymes (CAZy) were tracked by RNA-Seq during growth in a corn stalk rot time course and in vitro. In total, 516 CAZy genes were identified in the Fusarium genome and 494 were detected as expressed in either in planta or in vitro. Twelve of these genes were identified as potentially encoding chitin synthases. Eight were expressed both in vitro and in the corn stalk, with differential expression levels observed as infection progressed. An analytical carbohydrate analysis was performed to determine the basal F. graminearum cell wall composition during in vitro growth. In addition to major wall components, subtle structural features in cell walls can play an essential role in wall stability and be targeted by specific inhibitors for disease control. Future work will aim to deploy specific defensin plant innate immunity proteins to disrupt the biosynthesis of fungal cell wall carbohydrates, such as chitin.

22W Brown rot fungi do the 'two-step' to deploy an ephemeral reactive oxygen species (ROS) pretreatment in wood. *J. Schilling*[1], J. Zhang[1], G. Presley[1], C. Hunt[2], K. Hammel[2,3], E. Panisko[4], M. Figueroa[1] 1) University of Minnesota, Saint Paul, MN; 2) U.S. Forest Products Laboratory, Madison, WI; 3) Unviersity of Wisconsin, Madison, WI; 4) Pacific Northwest National Laboratory, Richland, WA.

We recently showed that brown rot wood-degrading fungi differentially express genes for lignocellulose oxidation (LOX) ahead of those associated with traditional cell wall hydrolysis, including glycoside hydrolases (GHs). This window of oxidative upregulation was very brief when colonizing wood (<48 hrs). Here, I will present the thin wafer system being used to map these fine-scale temporal dynamics, and share the results of two studies harnessing this set-up.

For the first study, we have been able to address the question 'What cues the gene expression transition from oxidative to hydrolytic during brown rot?' To do this, we have matched one effort to quanitify soluble wood carbon fractions, spatially, as the model brown rot fungus colonizes and degrades wood with another effort to test carbon source regulation of gene expression. This paired effort, using qPCR to target genes that we previously showed via whole transcriptome profiling are differentially-expressed, indicates that key soluble sugars play a dual role in repression of LOX and simultaneous upregulation of GHs. Notably, cellulase production in brown rot fungi that has long been characterized as 'constitutive' given the lack of glucose repression we demonstrated instead is inducible but not repressed, later. This has been overlooked given the brief window prior to induction of GHs, lost in the noise of whole-block decay trials. Concerning the transition, coordinating reguation is a logical approach to segregating pretreatment from saccharification, given that ROS generated from oxidative pathways will denature enzymes. This also offers insight into the control of this unique mechanism, one that consolidates harsh oxidative pretreatments with enzymatic hydrolysis without requiring seperate 'reactor' volumes.

Second, we have begun to address the question 'How representative is the staggered two-step mechanism in *P. placenta* of other brown rot fungi, and how does it compare with its white rot ancestors?' To do this, we have use whole transcriptome sequencing (RNAseq) to overlay wafer data among two brown rot species (*P. placenta* one of them) from distinct clades and two white rot species. Data has revealed surprsingly more similarities in mechanisms than expected, but some key distinctions in the analyses that are ongoing. These similarities and distinctions will help target genes unique to the brown rot mechanism as well as those common among these important carbon-cycling decomposers.

23T Mapping of *defective in silencing* (*dis*) mutants in *Fusarium graminearum* by bulk segregant analysis and high-throughput sequencing. *Kristina Smith*, Lanelle Connolly, Xiao Lan Chang, Brett Pierce, Corinne Fargo, Brian Josephson, Zackary Bango, Madison Esposito, Michael Freitag Dept. of Biochem & Biophysics, Oregon State University, Corvallis, OR.

Polycomb Group (PcG) proteins generate facultative heterochromatin in some fungi by trimethylating histone H3 lysine 27 (H3K27me3). Members of the conserved Polycomb Repressive Complex 2 (PRC2) include the H3K27 methyltransferase, KMT6, and binding partners SUZ12, EED, and CAF1-3 (MSL1). Loss of *kmt6*, *eed*, or *suz12* leads to complete loss of H3K27me3 accompanied by developmental defects and novel or increased expression of ~25% of all genes. Most genes silenced by PcG have unknown functions but are predicted to be involved in secondary metabolism and pathogenicity. To uncover suppressors of H3K27me3 silencing, and identify functional equivalents of PRC1, the complex that may initiate PRC2 action and also binds H3K27me3 in animals but does not exist in fungi, we developed a forward genetics approach utilizing UV mutagenesis. We inserted a *neo* reporter gene into the *pks2* region, which is reliably silenced by H3K27me3 in WT but de-repressed in the *kmt6* mutant. WT strains with *neo* at the heterochromatic locus were UV-irradiated, and mutants were selected on G418-containing medium. Dozens of primary mutants, which we call *defective in silencing* (*dis*), fall into several classes with distinct growth phenotypes and global gene expression patterns evidenced by transcriptome sequencing. Bulk segregants from *dis* mutants backcrossed to mapping strains with *mat* deletions to force outcrossing were sequenced and analyzed with the Genome Analysis Tool Kit to identify SNPs, insertions and deletions. The first mutant analyzed, *dis1*, carries a truncated *eed* gene, and completely phenocopies the deletion mutant we constructed. Here we will report on additional components of the PcG silencing system.

24F Roles of polyamine metabolism in appressorium function. *Raquel Rocha*, Richard Wilson Plant Pathology, University of Nebraska - Lincoln, Lincoln, NE.

Polyamines are a group of ubiquitous and essential metabolites found in all living organisms. In fungi, the most abundant molecules - putrescine, spermidine, and spermine – have roles in conidiation and spore germination in *Aspergillus*, *Mucor,* and *Phycomyces* species. Little is known about how polyamine metabolism affects the development of fungal infection structures. Here, we report that a spermine synthase - encoded by *SMT1* –is required by the rice blast fungus *Magnaporthe oryzae* to generate spermine from spermidine and is essential for developing functional infection cells (appressoria) on rice leaf surfaces. *M. oryzae*, the most important pathogen of cultivated rice, elaborates appressoria at the tips of germ tubes emerging from spores on the leaf surface. The melanin-rich wall of the appressorium allows solutes such as glycerol to accumulate and generate enormous internal turgor pressure. This pressure is translated into mechanical force acting on the penetration peg emerging from a pore at the base of the appressorium, forcing it through the plant cuticle. In contrast, incipient cytorrhysis experiments determined that the appressoria of Δ*smt1* mutant strains generated turgor pressures that were 1.8 higher than wild type (WT), but no penetration pegs were observed breaching rice leaf cuticles by confocal microscopy. These developmental defects were not due to misregulated lipid mobilization or cell cycle progression, which occurred like WT in the D*smt1* mutant strains. Rather, by calculating the ratio of appressoria plasmolysis to cytorrhysis following PEG treatment, we determined that appressorial cell wall porosity was increased 3.5 in Δ*smt1* mutant strains compared to WT. We conclude that polyamine metabolism in *M. oryzae* appressoria, like in *Arabidopsis*, might control cell wall porosity. Perturbing porosity in Δ*smt1* appressoria could affect turgor and/or the rigidity of the cell wall. In turn, these structural defects might affect the polarized growth of the penetration peg from the appressorial pore. Ongoing studies of D*smt1* will, compared to WT, examine the structure of the appressorial cell wall, examine the pore at the base of the appressorium, and determine the status of penetration peg formation. Taken together, our results reveal novel roles for polyamine metabolism in appressorium function that enrich our understanding of the metabolic pathways intrinsic to rice cell colonization by *M. oryzae*.

25W Production and composition characteristics of *Tremella fuciformis* exopolysaccharide. *Ji Young Kang*[1], Jeesun Chun[2], Baek Rock Oh[1] 1) Industrial Microbiology and Bioprocess Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup-si, 580-185, Republic of Korea; 2) Department of Molecular Biology, Department of Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea.

An edible mushroom *Tremella fuciformis* is known to produce exopolysaccharides. Yeast-like cells (blastospore) were isolated from the fruiting body of the mushroom, and designated to the strain *T. fuciformis* TFC6. The exopolysaccharides of the strain are most highly produced in submerged culture at 4 culture day (2g/l). Moreover, TFCUV-5 obtained by UV mutagenesis to increase the exopolysaccharide productivity shown that the exopolysaccharide production reached 3.6g/l in flask culture which is an increase of 80% compared with the corresponding values of wild type TFC6. The optimum culture condition for maximum exopolysaccharide productivity was 25°C and pH7. The exopolysaccharides derived by the strain TFCUV-5 contained fucose, glucose, xylose, mannose and glucuronic acid about 17.1%, 1.3%, 22%, 40.3% and 19.3%, respectively, so called a glucuronoxylomannan. The mutant enhanced the exopolysaccharide production might be applicable to several filed such as cosmetic and dietary supplements industries.

26T Expression data integration in an *Aspergillus niger* genome-scale metabolic model. *M. V. Aguilar-Pontes*[1], E. McDonnell[2], K. Strasser[2], D. Fulton[2], A. Tsang[2], R. P. de Vries[1] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Utrecht University, Utrecht, NL; 2) CSFG-Concordia University, Montreal, Canada.

Filamentous fungi include important species used in industrial applications. One of the main representatives is *Aspergillus niger*, an industrial workhorse used for enzyme and metabolite production. In order for *A. niger* to achieve its full potential as a cell factory, deeper knowledge of its metabolism is needed. We propose a new metabolic network based on a manually curated annotation of the *Aspergillus niger* genome, including literature references, as well as information from curated and automated protein, metabolite and reaction databases and RNA-seq data. Reconstruction of the model and manual curation of the network based on different expression data sets was performed using Pathway Tools. In order to assess and improve our model, a comparison with previously publish genome scale models was performed (Andersen *et al.*, 2008; Lu *et al.,* 2016). Experimental results will be used to validate predictions of essential genes involved in growth as well as mutant behavior.

Our aim is to create a model that will give us new insights into the carbon metabolic pathways of *A. niger* and obtain leads to improve industrial processes. This model will also enable us and other researchers to study carbon utilization by fungi in more detail.

27F Physiological effects of hemicellulosic pentose sugars on glucose metabolism in the maize pathogen *Bipolaris maydis*. *Hiroshi Yoshida*, Chihiro Tanaka Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

Fungi secrete extracellular hydrolytic enzymes to degrade various environmental substrates, some of which can serve as nutrients or signaling molecules for fungal growth. The filamentous fungus *Bipolaris maydis*, also known as *Cochliobolus heterostrophus*, is the causal agent of southern leaf blight of maize. On *B. maydis*-infected maize leaves, there is observed early development of water-soaked lesions, probably relating to plant cell wall degrading enzymes produced by this fungus. Since arabinoxylan, a hemicellulosic polysaccharide comprising xylose polymer backbone and arabinose side chains, is a major component of maize cell wall, ability of *B. maydis* to degrade this saccharide is likely to be highly involved in fungal nutrient acquisition or physiological regulation during infection. We sought to elucidate the physiological effects of arabinoxylan and its component pentose sugars in *B. maydis* to reveal its post-invasion pathogenic lifestyle. A quantitative RT-PCR (qPCR) analysis and GFP-reporter assay of a β-xylosidase gene of *B. maydis* (*BmXyp1*) showed that it was upregulated immediately after appressorial penetration, suggesting that xylan degradation and enzymatic release of xylose were activated during early infection. Our qPCR analysis also demonstrated that xylose metabolic genes were simultaneously induced with *BmXyp1*. These results possibly indicate the importance of xylan degradation for fungal nutrition in host tissue. However, xylulokinase gene-disrupted strains (Δ*XkiA*), which lacked the ability to grow on xylose, arabinose or xylan, still showed a certain level of virulence on maize, indicating that the fungus did not mainly depend on these sugars for nutrient sources.
Next, we focused on a notable effect of xylose on Δ*XkiA*: "unmetabolizable" xylose strongly inhibited growth, even if in the presence of glucose. A qPCR analysis of glucose metabolic genes demonstrated that a hexokinase gene *BmHxkA* and a glucose-6-phosphate dehydrogenase gene *BmZwf1* were remarkably upregulated in Δ*XkiA* on xylose but not on glucose, compared to in the absence of any sugars. Our results suggest a possible role for hemicellulosic pentose sugars in *B. maydis* physiology, i.e., a part as a signal to induce NADPH production through the hexose monophosphate shunt.

28W Assessing the role of γ-aminobutyric acid during asexual reproduction and pathogenesis in the wheat pathogen, *Parastagonospora nodorum*. *O.L. Mead*, S.A. Breen, P.S. Solomon Plant Sciences, Australian National University, Canberra, ACT, AU.

Parastagonospora nodorum is the causal agent of the Septoria Nodorum Blotch disease on wheat. This disease is prevalent in the U.S.A, Europe, the Middle East and Australia causing significant economic losses to wheat industries globally. Sporulation is critical to disease formation as this necrotrophic fungus relies on multiple cycles of infection, reproduction and re-infection of the host to reduce grain yields. We have previously demonstrated that the non-proteinaceous amino acid, γ-aminobutyric acid (GABA), induces an *in vitro* state of hyper-sporulation in *P. nodorum* under conditions normally producing vegetative growth (Mead *et al.* 2013). GABA is ubiquitous across Kingdoms and is a product of primary metabolism. The anabolic pathway to GABA circumvents a key ATP-producing reaction in the Krebs Cycle and thus imparts a metabolic cost. Pertinently, GABA is produced by plants in response to biotic and abiotic stress. In fungi, the function of this molecule and its associated metabolic pathway are yet to be determined. We show that disruption of key enzymatic steps in GABA metabolism reduces pathogenicity. A transcriptomics approach to identifying mechanisms of GABA-induced sporulation has revealed that the canonical core sporulation pathways in *Neurospora crassa* and *Aspergillus nidulans* are not activated during sporulation in this *Dothideomycete*. We describe a reverse genetics investigation into mechanisms of sporulation in the wheat pathogen, *P. nodorum.*

29T Antifungal activity of wood extractives. *M. Morel-Rouhier*[1,2], Nicolas Valette[1,2], Thomas Perrot[1,2], Sormani Rodnay[1,2], Gelhaye Eric[1,2] 1) UMR 1136 Tree-Microbe iinteractions, Lorraine University, Vandoeuvre les Nancy, FR; 2) INRA, Interactions Arbres–Microorganismes, UMR1136, F-54280 Champenoux, France.

Extractives are non-structural wood molecules that represent a minor fraction in wood. However, they are source of diverse molecules putatively bioactive. Inhibition of fungal growth is one of the most interesting properties of wood extractives in a context of wood preservation, crop protection or medical treatments. The antifungal effect of molecules isolated from wood extractives has been mainly attributed to various mechanisms such as metal and free radical scavenging activity, direct interaction with enzymes, disruption of membrane integrity and perturbation of ionic homeostasis. Lignolytic fungi, which are microorganisms adapted to wood substrates, have developed various strategies to protect themselves against this toxicity. Comparative genomics clearly show that detoxification systems are evolutionary correlated with white rot wood decay. We have shown at the functional level that the neofunctionalization of some enzymes in lignolytic fungi could explain their high resistance capacity to wood extractives.

30F The cereal pathogen *Fusarium pseudograminearum* produces a mimic of cytokinin plant hormones. *D.M. Gardiner*[1], J.L. Sørensen[2], A.H. Benfield[1], R.D. Wollenberg[2], K Westphal[2], R Wimmer[2], K.F. Nielsen[3], J. Carere[1], L. Covarelli[4], G. Beccari[4], J. Powell[1], T. Yamashino[5], H. Kogler[6], T.E. Sondergaard[1] 1) Agriculture and Food, CSIRO, St Lucia, Queensland, AU; 2) Department of Chemistry and Bioscience, Aalborg University, DK-9000 Aalborg, Denmark; 3) Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; 4) Department of Agricultural, Food and Environmental Sciences, University of Perugia, IT-06121 Perugia, Italy; 5) Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Furocho, Chikusa-ku, Nagoya, 464-8601 Japan; 6) Karlsruhe Institute of Technology, D-76131 Karlsruhe, Germany.

The necrotrophic pathogen *Fusarium pseudograminearum* infects a broad range of agronomically important crops including barley and wheat. During a survey of secondary metabolites produced by this fungus, a novel class of cytokinins, which are plant hormones, was identified as being produced by the pathogen during plant infection. Cytokinins are generally thought of as having growth promoting and anti-senescence activity and the production of a cytokinin mimic by a necrotrophic pathogen challenges the view that these pathogens invade by a simple barrage of lytic enzymes and toxins. Through genome mining, a gene cluster in the *F. pseudograminearum* genome for the production of these compounds was identified and the biosynthetic pathway established using gene knockouts. The *F. pseudograminearum* cytokinins can activate cytokinin signalling demonstrating their genuine hormone mimicry. *In planta* analysis of the transcriptional response to one of the *F. pseudograminearum* cytokinins suggests extensive reprogramming of the host environment by these molecules, possibly through cross talk with defence signalling pathways.

31W Clade-specific variations in wood decay strategies among brown rot fungi indicate decay mechanism heterogeneity among brown rot fungi. *G.N. Presley*, J. S. Schilling Bioproducts and Biosystems Engineering , University of Minnesota, St. Paul, MN.

Brown rot fungi are a polyphyletic group of wood degrading fungi that employ both oxidative and hydrolytic mechanisms to degrade wood. These mechanisms are incompatible in discrete space, and differential gene expression has been implicated in *Postia placenta* to segregate these reactions. It is unclear, however, how this 'two-step' varies in other brown rot clades with different gene options and distinct niches in nature. Using proteomics, we analyzed the temporal progression of spruce wood decay by brown rot fungi *Serpula lacrymans* (Boletales) and *Gloeophyllum trabeum* (Gloeophyllum clade), using a space-for-time wood wafer design. Both fungi produced greater oxidoreductase diversity upon wood colonization and greater glycoside hydrolase activity later, consistent with a two-step mechanism. Each fungus, however, utilized a different wood colonization strategy - *S. lacrymans* invested more in fungal biomass while *G. trabeum* invested more in protein production, with ergosterol/protein ratios 7x higher in *S. lacrymans* than in *G. trabeum*. In line with their native substrate associations, hemicellulase specific activity was dominated by mannanase in *S. lacrymans* and by xylanase in *G. trabeum*. Despite containing a GH 6 cellobiohydrolase (CBH) in its genome, a distinguishing feature of brown rot members of the Boletales, *S. lacrymans* notably did not produce CBH on spruce wafers. This work highlights similarities in a two-step brown rot decay mechanism among distantly related fungi but identifies unique niche-specific variations. It also supports targeting gene discovery efforts on *S. lacrymans*, given the efficacy of its secretome despite its reduced diversity.

32T Mushroom polyketide synthase produces polyenes for chemical defense. *P. Brandt*[1], M. García-Altares[2], M. Nett[3], C. Hertweck[2], D. Hoffmeister[1] 1) Friedrich-Schiller-Universität, Department Pharmaceutical Microbiology at the Hans-Knöll-Institute, Winzerlaer Straße 2, 07745 Jena (Germany); 2) Leibniz Institute for Natural Product Research and Infection Biology, Department Biomolecular Chemistry, Beutenbergstraße 11a, 07745 Jena (Germany); 3) Technische Universität Dortmund, Department Biochemical and Chemical Engineering, Technical Biology, Emil-Figge-Straße 66, 44227 Dortmund (Germany).

Basidiomycetes have evolved a diverse repertoire of bioactive chemical defense compounds. After wounding of its mycelium, the taxonomically undetermined white-rotting basidiomycete BY1 produces yellow pigments *de novo*, which massively inhibit the pupation of insect larvae. These natural products were identified as the polyunsaturated fatty-acid like polyenes, 18-methyl-19-oxoicosaoctaenoic acid and 20-methyl-21-oxodocosanonaenoic acid. [1]

The objective of this study was to understand the genetic and biochemical basis of this basidiomycete defense compounds. We succeeded in identifying both alleles of a candidate gene, *PPS1*, in the genome of BY1, coding for a six-domain reducing polyketide synthase (HR-PKS). Quantitative real-time PCR showed a 9.5-fold upregulation of *PPS1* expression 48 hours past injury of the BY1 mycelium. To verify that PPS1 has polyene synthase activity, the polyene biosynthesis was heterologously reconstituted in *Aspergillus niger*. To that end, *PPS1* was placed under the control of the *terA* promoter and *trpC* terminator using plasmid SM-Xpress. [2] Combining liquid chromatography, mass spectrometry, and NMR, the structures of the PPS1 products were elucidated, and proved identical to the polyenes initially isolated from BY1. MALDI-MS imaging indicated polyene accumulation in the wounded mycelial area. Our work represents the first characterized basidiomycete HR-PKS and sets the stage for a more profound understanding of basidiomycete chemical ecology.

[1] D. Schwenk, M. Nett, H.-M. Dahse, U. Horn, R. A. Blanchette, D. Hoffmeister, *J Nat Prod* **2014**, *77*, 2658-2663.
[2] M. Gressler, P. Hortschansky, E. Geib, M. Brock, *Front Microbiol* **2015**, *6*, 184.

33F Biosynthesis of acurin A and B in *Aspergillus aculeatus*. M.L. Nielsen, P.P. Wolff, L.M. Petersen, L.N. Andersen, T.I. Petersen, D.K. Holm, U.H. Mortensen, C.S. Nødvig, T.O. Larsen, *J.B. Hoof* Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark.

Aspergillus aculeatus is known for the commercial utilization in production of several enzymes. We have identified two stereoisomeric compounds of mixed polyketide-nonribosomal peptide origin in the extracts of *A. aculeatus* that we named acurin A and acurin B. The structures of the compounds strongly resemble the structure of the mycotoxin fusarin C produced by several *Fusarium* species. CRISPR-Cas9 was used to construct a non-homologous end-joining deficient strain of *A. aculeatus*, which enabled efficient gene deletions in the acurin gene cluster. Using RT-qPCR in combination with metabolite profiling of gene deletion strains, the acurin producing gene cluster was delineated, which allowed us to propose a biosynthetic pathway for formation of acurin. Our results show that acurin, in contrast to fusarin C, is biosynthesized by an individual polyketide synthase and non-ribosomal synthetase. At least six other enzymatic activities are required for the biosynthesis of acurin. This study shows how we exploit the CRISPR-Cas9 system for the rapid construction of fungal host strains that can be readily engineered to generate valuable knowledge.

34W Analysis of secondary metabolite gene clusters in *Alternaria alternata*. *B. Voss*, R. Fischer Department of Microbiology, Institute for Applied Bioscience, Karlsruhe, Baden-Württembe, DE.

The filamentous fungus *Alternaria alternata* is an ascomycete that causes huge economic losses every year because of plant infection and food and feed spoilage. *A. alternata* produces more than 60 different secondary metabolites, and more are to be expected because many secondary metabolite gene clusters are not expressed under laboratory conditions. In order to discover new metabolites and/or identify the corresponding genes, different physiological conditions were tested for the induction of certain gene cluster. Using quantative Real-Time PCR we found that the four non-ribosomal peptide synthases (NRPS) found in the genome, were expressed under glucose-starvation conditions. Nitrate starvation induced two genes (AAT_PG02707, AAT_PG03557), whereas no significant difference occurred during phosphate starvation. When *A. alternata* was exposed to osmotic stress, two genes (AAT_PG02707, AAT_PG04268) were induced. In order to identify the corresponding metabolites, CRISPR Cas technology was used to inactivate all four NRPS genes (1). The metabolite profile of the mutant strains will be compared to the profile of corresponding wild-type strains by HPLC. In addition, the NRPS genes along with other putative tailoring enzyme encoding genes found in the vicinity of the NRPS genes, will be expressed heterologously in *Aspergillus oryzae* (2). One NRPS (AAT_PG04268) was already successfully introduced into *A. oryzae* and the metabolite analysis is under way.

(1) Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH (2015) A CRISPR-Cas9 System for Genetic Engineering of Filamentous Fungi. PLoS ONE 10(7): e0133085.

(2) Pahirulzaman KAK, Williams K, Lazarus CM (2012) A Toolkit for Heterologous Expression of Metabolic Pathways in *Aspergillus oryzae*. Methods in Enzymology, 517: 241-260

35T Analysis of the alternariol producing gene cluster in *Alternaria alternata*. *M. Wenderoth*, R. Fischer Department of Microbiology, Institute for Applied Bioscience, Karlsruhe, BW, DE.

The filamentous fungus *Alternaria alternata* is an ascomycete that colonizes many different organic substrates and is pathogenic for many plants. It causes tremendous economic damage every year due to plant infections and post-harvest food and feed spoilage. *A. alternata* produces a variety of different secondary metabolites but only little is known about their biosynthetic pathways and the regulation of the corresponding genes. One of the most prominent toxins is alternariol (AOH). Although the toxin itself is well studied, the way of synthesis and the corresponding gene cluster remained unknown. In the ascomycete *Parastogonospora nodorum* the corresponding PKS was identified recently (1). However, other genes found in the vicinity of the PKS were not studied.
In *A. alternata* ten *pks* gene clusters were identified (2). In order to assign a function to PksI and inactivate the gene we adapted the recently developed CRISPR-Cas9 system for *A. alternata* (3). *pksI* is located in a gene cluster consisting of PksI, three tailoring enzymes and a putative transcription factor. To further understand the mechanisms of AOH and alternariol-monomethyl ether (AME) biosynthesis we expressed *pksI* in combination with three tailoring enzymes in *Aspergillus oryzae* as a heterologous host using a multi expression vector (4). Single expression of *pksI,* lead to AOH production. By co-expression of the O-methyl transferase located in this cluster the production of (AME) was observed. Through multiple co-expression of the remaining tailoring enzymes a new compound was identified. Using a laccase based reporter gene assay, we confirmed that the transcription factor induces *pksI* expression.
(1) Chooi *et al.* (2015). Appl Environ Microbiol. 81(16):5309-17
(2) Saha *et al.* (2012). PLoS ONE. 7(7): e40564
(3) Nødvig *et al.* (2015). PLoS ONE. 10(7): e0133085
(4) Pahirulzaman *et al.* (2012). *Methods Enzymol*. 517: 241-260

36F Gene cluster diversity for indole-diterpene biosynthesis in the grass endophyte *Epichloë festucae*. *T.A. Miller*[1,2], W.J Mace[1], B Scott[2], R.D Johnson [1] 1) Grasslands, AgResearch, Palmerston North, Manawatu, NZ; 2) Institute of fundamental science, Massey University, Palmerston North, Manawatu, NZ.

Indole-diterpenes (IDT) are an important class of bioprotective metabolites synthesized by *Epichloë festucae* in association with *Festuca* and *Lolium spp*. The most abundant product in *L. perenne* infected with strain Fl1 is lolitrem B, a potent tremorgenic mycotoxin responsible for the mammalian syndrome 'ryegrass staggers'. Molecular cloning and functional analysis has shown that the genes responsible for lolitrem B biosynthesis are organized in three sub-telomeric gene clusters interspersed with transposon relics. These 11 *idt* genes comprise a set of core genes for the biosynthesis of the first stable intermediate, paspaline, and additional genes that encode products for further elaboration of this core molecule. Genome sequence and PCR analysis has shown that there is remarkable diversity in the *idt* gene profile within *E. festucae*, resulting in a diversity of IDT products synthesized *in planta*. The aim of this work is to analyse the *idt* gene cluster structure in an agriculturally important *E. festucae var loli* (asexual) strain PN3136 and determine whether this explains the observed IDT chemical phenotype. The genome of PN3136 was sequenced using Illumina technology and the *idt* gene cluster analysed. PN3136 was found to have all 11 *idt* genes with conserved synteny to strain Fl1. However, *idtP* and *idtQ* (cytochrome P450 monooxygenases) had a lower than expected sequence identity when compared with Fl1. In addition *idtF* (encoding a DMAT synthase) and *idtK* (encoding a P450 monooxygenase) have SNPs that introduce frame-shift mutations and premature termination of translation. The *in planta* LC-MS/MS detection of metabolites consistent with IDT products lacking the additional prenylated cyclic ring as found in lolitrem B and E, is consistent with IdtF and IdtK being non-functional. Interestingly, this IDT chemical phenotype is strikingly similar to that previously determined in an *E. festucae* (sexual) strain Fg1. We therefore sequenced the genome of Fg1 and found that the *idt* gene profile was identical to PN3136 except for *idtK*, which had a different SNP that results in earlier termination of translation. PN3136 and Fg1 are therefore two closely related asexual and sexual *E. festucae* strains that have a novel IDT chemical profile.

37W Transcriptional co-regulation and spatial coordination of mevalonate and sesquiterpene biosynthetic pathway enzymes of *Fusarium graminearum* based on *in vitro* RNAseq and organellar proteomics. Karen Broz[1], *Marike Boenisch*[2], H. Corby Kistler[1,2] 1) USDA ARS Cereal Disease Laboratory, Saint Paul, MN, USA; 2) University of Minnesota, St. Paul, MN, USA.

Farnesyl pyrophosphate (FPP), a product of the primary metabolic mevalonate pathway, gives rise to both primary and secondary metabolite terpenes. The phytopathogenic fungus *F. graminearum* produces the sesquiterpene mycotoxin deoxynivalenol (DON) during infection of wheat or when induced in culture. Under conditions conducive to DON production, FPP utilization may shift towards secondary metabolites, requiring primary and secondary metabolism pathway coordination. Transcriptome analysis (RNAseq) of *F. graminearum* grown in DON inducing and non-inducing medium over time shows that mevalonate- and DON biosynthetic pathway genes are transcriptionally upregulated during DON induction. At the same time, genes for synthesis of ergosterol, a diterpene primary metabolite, are down regulated, suggesting coordination of terpene pathways and a shift in precursors towards DON production. When reporter strains with fluorescently labeled enzymes are grown in DON induction medium, the mevalonate pathway enzyme HMG CoA reductase (Hmr1) strictly co-localizes with membrane-associated DON biosynthetic cytochrome P450s Tri1 and Tri4 in membrane accumulations of the endoplasmic reticulum (ER). Mevalonate and sesquiterpene biosynthetic pathway enzymes are spatially coincident suggesting that the enzymes may be part of a multi-enzyme complex. To identify further proteins of a potential multi-enzyme complex, fluorescence-activated cell sorting (FACS) was used to enrich for Tri4::RFP labeled ER membranes for proteomic analysis. Proteomics of the RFP-fluorescent ER fraction revealed additional proteins involved in DON synthesis (Tri1, Tri11 and Tri14), as well as Hmr1, and a number of conserved ER proteins. RNAseq data further indicate that genes corresponding to enriched proteins in the ER proteome are transcriptionally upregulated upon DON induction. Transcriptomics and ER proteomics suggest that DON biosynthesis may require not only coordinate expression of genes for specific primary and secondary metabolic pathways, but also co-localization of biosynthetic enzymes to specific organelles.

38T Thinking outside the box:NADPH oxidases as signaling hubs are more than just simple ROS producer. *R. Marschall*, P. Tudzynski Institute of Plant Biology and Biotechnology, Münster, DE.

The filamentous fungus *Botrytis cinerea* is one of the most devastating plant pathogens. Molecules, which are directly involved in the communication between host and pathogen are reactive oxygen species (ROS). They are produced as mere by-products of the respiratory chain or in highly conserved processes by e.g. NADPH oxidase complexes. In *B. cinerea* two different Nox complexes do exist, both regulating a huge variety of developmental processes. While for the NoxA complex the elucidation of the complex composition and interacting proteins (BcIqg1, BcNoxD) is already sophisticated[1/2], there are hardly any information about proteins that directly interact with the second catalytic subunit BcNoxB or the overall regulator BcNoxR.

Most recently, we identified a large set of proteins in a pulldown screening with the catalytic subunit NoxB and the regulator NoxR. While potential interaction partner that were previously postulated as putative Nox complex members could not be verified, other target proteins were fished in the pulldown approach and verified by Y2H experiments.

Surprisingly, in four independent experiments proteins were pulled down with BcNoxB that all belong to a predicted PKS5-NRPS7 cluster. Subsequent Y2H analyses revealed that indeed proteins of the cluster interact directly with BcNoxB, which might indicate a role for BcNoxB in the secondary metabolism. Moreover, different cluster proteins seem to build up a complex since some of them are interacting with each other.

However, not only BcNoxB seems to be more than just a simple ROS producer. The first catalytic subunit BcNoxA was shown to interact with the cytochrome C peroxidase. Thus, for the first time in fungi a direct link came apparent between mitochondria, the respiratory chain and the NoxA complex. Since BcNoxA is additionally associated with the protein disulfide isomerase BcPDI it can be speculated that BcNoxA is contributing to ROS levels, but is also working as a signaling hub, connecting essential intracellular processes such as protein folding, redox homeostasis, respiration and ROS production.

[1)]Marschall et al., 2016: Update on Nox function, site of action and regulation in *Botrytis cinerea*. Fungal Biology and Biotechnology20163:8
[2)]Marschall and Tudzynski, 2016: Reactive oxygen species in development and infection processes. Semin Cell Dev Biol. 2016 Sep;57:138-46.

39F Identification and characterisation of polyketide synthases in the barley pathogen *Ramularia collo-cygni*. *F. Dussart*[1,2], P.N. Hoebe[1], S.H. Spoel[2], N.D. Havis[1], G.R.D. McGrann[1] 1) Crop and Soil Research Department, SRUC, Edinburgh, GB; 2) School of Biological Sciences, University of Edinburgh, Edinburgh, GB.

The filamentous fungus *Ramularia collo-cygni* (Rcc) causes the late season barley disease Ramularia Leaf Spot (RLS). In the past twenty years it has become an important disease in northern Europe and other temperate regions of the world. RLS can quickly cause substantial yield losses and reduce grain quality. Limited options to control RLS have given this disease a higher profile and attracted scientific attention enhancing the understanding of the Rcc life cycle which indicated that this fungus is an endophyte with a necrotrophic phase.

Rcc produces a series of anthraquinone toxins called rubellins, thought to be involved in RLS development. Anthraquinones are known to be synthesised through the polyketide pathway. In this study, ten putative polyketide synthases (PKS) were identified in the genome of Rcc. Using *in silico* genome walking eight secondary metabolite-related gene clusters were identified near core PKS genes. Clusters with no PKS core genes were also identified. Gene expression of six core PKS genes was assessed during disease development in barley seedlings. Expression of most PKS transcripts declined during disease development. Co-regulation of secondary metabolism-related genes that clustered with core PKS genes was also observed. Together these data imply that, if produced; polyketide-derived secondary metabolites in Rcc might not be associated with disease symptom development. Instead, secondary metabolites may act as antifungal agents, as we found that Rcc inhibited the growth of several major barley fungal pathogens *in vitro*, suggesting these compounds may be important for Rcc niche exploitation.

40W Elucidating the biosynthetic pathway of the anticancer secondary metabolite calbistrin in *Penicillium decumbens*. *Sietske Grijseels*[1], Carsten Pohl[2], Zahida Wasil[1], Jens Christian Nielsen[3], Yvonne Nygård[2], Jens Nielsen[3], Jens C. Frisvad[1], Kristian Fog Nielsen[1], Mhairi Workman[1], Thomas Ostenfeld Larsen[1], Arnold Driessen[2], Rasmus John Normand Frandsen[1] 1) Department of Biotechnology and Biomedicine, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark; 2) Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands; 3) Department of Biology and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden.

Filamentous fungi are important producers of secondary metabolites, low molecular weight molecules that often have bioactive properties. One interesting secondary metabolite is calbistrin, a compound recently found to have bioactivity against leukemia cells. This compound consists of two polyketides linked by an ester bond; a decalin containing polyketide similar to lovastatin, and a linear 12 carbon dioic acid structure. Calbistrin is known to be produced by several uniseriate black Aspergilli, *Aspergillus versicolor*-related species, and several Penicillia. Among the Penicillia, the recently genome sequenced *P. decumbens* is interesting as it produces several putative intermediates of the calbistrin pathway, such as decumbenone A and B and versiol. In this study, the molecular and enzymatic mechanisms underlying the biosynthesis of calbistrin are elucidated using a combinatorial approach of bioinformatics, molecular biology and analytical chemistry. Comparative studies of the polyketide synthase (PKS) sequences from the three genome sequenced species *A. versicolor*, *A. aculeatus* and *P. decumbens* resulted in the identification of a putative gene cluster for production of the decalin part of calbistrin. Implementation of CRISPR/Cas9 technologies in *P. decumbens* facilitated the deletion of the putative PKS in this species. Subsequent UHPLC-MS analysis of extract metabolites revealed that calbistrin and putative intermediate compounds were absent, proving the involvement of the PKS in calbistrin production. Further characterization of the predicted gene cluster is achieved by targeted deletion of the individual biosynthetic genes in the cluster.

41T Mechanisms of capsaicin tolerance in fungal seed pathogens of wild chili peppers. *C.A. Adams*[1,2], K. Zimmerman[2,4], K. Fenstermacher[3], D. Geiser[3], A. Pringle[1,5] 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge MA; 3) Department of Plant Pathology and Environmental Microbiology, Penn State University, University Park, PA ; 4) Gingko Bioworks; 5) Department of Botany and Bacteriology, University of Wisconsin-Madison,

Madison, WI.

To deter herbivores and pathogens, many plants produce secondary metabolites. However, herbivores and pathogens that commonly associate with plants can evolve resistance to defensive compounds. Such co-evolution is evident in a wild chili pepper, *Capsicum chacoense*. Though plant-produced capsaicinoids slow fungal growth, fungi that infect *C. chacoense* seeds possess much higher capsaicinoid tolerance than other tested microbes. Previous researchers have proposed two separate mechanisms of capsaicin inhibition. A small number of studies propose that capsaicinoids can cause cell membrane disruption, while a separate, larger body of literature has demonstrated that capsaicinoids like capsaicin and dihydrocapsaicin bind to the NADH dehydrogenase (Complex I) of the Electron Transport Chain (ETC), crippling Oxidative Phosphorylation (OXPHOS) and subsequent energy production (ATP). Here, we investigated how fungal seed pathogens of wild *Capsicum chacoense* evolved tolerance to capsaicin. We measured fungal growth rate in the presence of different ETC inhibitors to determine 1) if these fungi possess alternative respiratory enzymes, and 2) if capsaicinoids' effects on the ETC fully explained the effect of these inhibitors. We selected 16 fungal isolates from four *Ascomycete* genera (*Alternaria, Colletotrichum, Fusarium* and *Phomopsis)*. In all isolates, we found evidence for a Complex I alternative NADH dehydrogenase. Unexpectedly, we also found evidence for a Complex III alternative cytochrome oxidase in many isolates. These fungi may achieve additional capsaicin tolerance by degrading capsaicin. Inhibition of OXPHOS appears to weakly explain the mechanism by which dihydrocapsaicin slows fungal growth, but not capsaicin. We hypothesize capsaicin disrupts cell and mitochondrial membranes in addition to inhibiting OXPHOS. Our research also suggests alternative respiratory enzymes are more prevalent in plant pathogens than was previously appreciated.

42F Identification and characterization of the *Aspergillus flavus* aspergillic acid gene cluster. *J. Cary*[1], M. Lebar[1], C. Carter-Wientjes[1], B. Mack[1], R. Majumdar[1], J. Diana Di Mavungu[2], S. De Saeger[2] 1) USDA-ARS-SRRC, New Orleans, LA; 2) Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium.

Aspergillus flavus produces toxic and carcinogenic aflatoxins. *In silico* analysis of the *A. flavus* genome revealed 56 gene clusters encoding for secondary metabolites (SMs). Although about 20 SMs have been identified from *A. flavus* cultures, studies by our group and others have experimentally assigned metabolites to just ten *A. flavus* gene clusters. We are interested in how these SMs affect fungal development, survival, and virulence. We are particularly interested in *A. flavus* metabolites that are produced during infection of corn seed. RNA-Seq analysis of all predicted *A. flavus* secondary metabolic gene cluster 'backbone' genes during corn kernel infection showed that in addition to the aflatoxin cluster polyketide synthase (PKS) gene, *aflC*, one of the earliest genes expressed was the uncharacterized Cluster 11 nonribosomal peptide synthetase (NRPS) gene, *asaB* (AFLA_023020). SMURF analysis indicated that Cluster 11 may be composed of as many as 12 genes, however we focused on five genes encoding the putative NRPS, desaturase, P450 oxidoreductase, MFS transporter and C6 transcription factor. LC-MS analysis of extracts from knockout mutants of these genes showed that they were responsible for the synthesis of the previously characterized pyrazinone, deoxyaspergillic acid (deoxy-AA), and the antimicrobial hydroxamic acid-containing mycotoxins aspergillic acid (AA) and hydroxy-AA (OH-AA). From the LC-MS data we have proposed a biosynthetic pathway for the production of these metabolites. In addition, we found that AA can form a trimer with one atom of iron to generate ferriaspergillin, perhaps indicating that this cluster is involved in regulation of iron homeostasis in *A. flavus*.

43W *Trichoderma reesei* forms surface attachment structures and senses cellulosic biomass using class XIII GPCRs. Eva Stappler[1], Guofen Li[1], David Turrà[2], Stefanie Kindel[1], Ursula Sauer[1], Antonio Di Pietro[2], *Monika Schmoll*[1] 1) Health and Bioresources, AIT Austrian Institute of Technology GmbH, Tulln, Lower Austria, AT; 2) Department of Genetics, University of Cordoba, Campus de Excelencia Internacional Agroalimentario, Córdoba, Spain.

Trichoderma reesei (syn. *Hypocrea jecorina*) is one of the most prolific producers of plant cell wall degrading enzymes. Production of these enzymes is induced in response to the presence of carbon sources related to degradation production of plant biomass.
Here we tested chemotropic growth of *T. reesei* germ tubes towards different compounds, and observed a robust response to glucose, the organic nitrogen source glutamate and the presence of a mating partner. However, no chemotropic response was observed for the cellulase-inducing disaccharide sophorose or to the non-fermentable sugar lactose.
Recently, comparison of transcript data from different inducing carbon sources indicated, that the class XIII G protein-coupled receptors (GPCRs) CSG1 and CSG2 are involved in transcriptional activation of cellulases by the substrate cellulose. Mutants lacking CSG1 or CSG2 were impaired in the chemotropic response to glucose. Moreover, whereas the wild-type strain formed broadened appressoria-like structures at the hyphal tips during growth on a natural cellulosic substrate (maple leaves), the two mutants failed to differentiate these structures. Contact angle analysis suggested that regulation of hydrophobin expression did not play a role in this difference. Our results suggest that *T. reesei* can chemotropically sense cellulosic plant biomass through its degradation product glucose, and that this sensing requires the GPCRs CSG1 and CSG2. Transcriptome data of ?*csg1* reveal the molecular pathways involved in the response to cellulose.

44T Sensing and responding to hypersaline conditions: same signal but different mechanisms of activation of HOG pathway in halotolerant and halophilic fungi, isolated from hypersaline environments. *A. Plemenitas*[1], N. Gunde-Cimerman[2], M. Lenassi[1], T. Konte[1] 1) Institute of Biochemistry, University of Ljubljana, Faculty of Medicine, Ljubljana, SI; 2) Biology Department, University of Ljubljana, Biotechnical Faculty,Ljubljana, SI.

The main signaling pathway in fungi responsible for cellular responses to increased NaCl concentrations is high osmolarity glycerol (HOG) pathway. Here we present the architecture of HOG signal transduction pathways for extremely halotolerant *H.wernecki,* moderately halotolerant *A.pullulans* and for halophilic *W.ichthyophaga,* which were all isolated from the environments, characterized by extremely high NaCl content, like solar salters. Our studies were mostly focused on extremely halotolerant *H.werneckii* and halophilic *W.ichthyophaga* which both tolerate NaCl up to 5M concentration. Moderately halotolerant *A.pullulans* was included for comparative studies. With the availability of the genomes of these fungi, the presence of known components of HOG pathway, as well as novel HOG components were confirmed through homology searches. We show that although there are many similarities between halotolerant and halophilic fungi, important differences were found in components of HOG pathway as well as in phosphorylation of MAPK Hog1 that might also explain the different halotolerant/ halophilic characters of the studied fungi. At the protein level, we observed in *H. werneckii* a phosphorylation pattern similar to that of *S. cerevisiae*, with the exception, that the HwHog1 kinase was fully phosphorylated only when *H. werneckii* cells were exposed to high salt concentrations, 3 M NaCl or higher. In *W. ichthyophaga*, a completely opposite phosphorylation pattern was observed:

WiHog1 kinase is dephosphorylated after hypo-osmotic or hyperosmotic shock, and is constitutively phosphorylated only under optimal salinity concentration (3.4 M NaCl). We also demonstrated that HwHog1 is activated via both, SHO and SLN branches of HOG pathway, in *W.ichthyophaga*, on the other hand, it appears that only SLN branch is involved in the activation of WiHog1. Importance of HOG pathway in adaptation to high NaCl concentrations in halotolerant/halophilic fungi was confirmed by applying specific inhibitor of MAPK Hog1.

45F Hemolytic activity of Mannosylerythritollipids is based on mono-acetylated variants. T. Deinzer[1], U. Linne[2], M. Bölker[1], *B. Sandrock*[1] 1) Department of Biology - Genetics, Philipps-University Marburg, Marburg, DE; 2) Department of Chemistry, Philipps-University Marburg, Marburg, DE.

Mannosylerythritollipids (MELs) are secondary metabolites produced by fungi. MELs are used as biosurfactants in industrial processes for the production of e.g. detergents or pharmaceuticals. In the phytopathogenic fungus *Ustilago maydis* biosynthesis of these glycolipids can be studied under nitrogen starvation conditions. A gene cluster composed of five genes has been identified and is necessary for MEL biosynthesis. The present work was initiated by characterisation of the major facilitator transporter protein Mmf1 and its function during MEL production. Cells carrying a deletion of *mmf1* produced only di-acetylated MEL variants, which are less hemolytically active. The transporter could be functionally replaced by Mmf1 of *Ustilago hordei* but not by Mfs1, a closely related major facilitator transporter encoded in the genome of *U. maydis*. Strains capable of producing predominantly either non-, mono- or di-acetylated MEL isoforms were constructed demonstrating that mono-actylated MELs contain the highest degree of hemolytical activity. Therefore we suggest that active transport of these MEL variants by Mmf1 is necessary to prevent self destruction by cell lysis.

46W Trichothecene mycotoxin transport in the phytopathogen *Fusarium graminearum*. *Sean P. O'Mara*[1], Karen Broz[2], Yanhong Dong[3], H. Corby Kistler[2,3] 1) Department of Plant Biology, University of Minnesota, St. Paul, MN, USA; 2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA; 3) Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA.

Fusarium graminearum is a necrotrophic pathogen and the causal agent of Fusarium Head Blight disease of small grain cereals. *F. graminearum* infection results in the contamination of grain with potent trichothecene mycotoxins. These mycotoxins are protein synthesis inhibitors and when ingested, trichothecene toxicosis can lead to ribotoxic stress, cellular apoptosis, and immuno-modulation. Accumulation of the trichothecene deoxynivalenol (DON) by *F. graminearum* during infection is essential for full virulence to wheat (*Triticum aestivum*). While the biosynthetic pathway of DON production has been well studied, potential modes of DON transport and cellular sequestration have not. Previous evidence indicated that the major facilitator superfamily (MFS) transporter, Tri12, whose gene is located within the core trichothecene biosynthetic cluster of *F. graminearum*, is not the sole transporter of DON. Thus, we aim to elucidate other modes of DON transport and cellular sequestration in *F. graminearum* utilizing both *in vitro* and *in vivo* methods. Genes for six proteins predicted to be involved in DON transport or sequestration (three ATP binding cassette (ABC) transporters, one MFS transporter, one t-SNARE protein, and one Rab GTPase) were deleted using the split-marker homologous recombination technique. These mutants were then tested for DON accumulation *in vitro* using toxin-inducing medium, and *in planta* using a 14-day wheat point-inoculation bioassay. Our results indicate that the ABC transporter *Abc1* as well as *Rab7*, a Rab GTPase essential for vacuolar biogenesis, are crucial proteins allowing for DON accumulation. Individually, these two mutants showed significantly lowered virulence *in planta* ($p<0.05$). The virulence on wheat of *abc1* mutants was only ~28% of wild-type *F. graminearum* PH1, and *rab7* mutants were completely incapable of spreading beyond the inoculated floret. Future work aims to generate double mutants of DON transporter proteins to analyze their relative contribution to DON export as well as the potential redundant or synergistic function of these multiple modes of transport.

Biotechnology

47T Development of CRISPR-Cas9 for targeted gene disruption in the multinucleate filamentous pathogen *Sclerotinia sclerotiorum*. *Jingtao Li*[1,2], Yanhua Zhang[1,2], Hongyu Pan[1], Jeffrey A. Rollins[2] 1) College of Plant Science, Jilin University, Changchun, Jilin, China; 2) Department of Plant Pathology, University of Florida, Gainesville, Florida, USA.

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal plant pathogen of Arabidopsis and many agriculture crops. With the development of complete genome sequencing and annotation many potentially important genes for pathogenicity and development have been discovered. Conventionally, knockout or disruption mutants of target genes have been created by homologous recombination, knockdowns by RNAi, and random insertion mutations introduced by T-DNA. These approaches, while utilized effectively, have been of low efficiency. The emerging technologies of CRISPR-Cas9 represent efficient, convenient, timely, and cost-saving approaches for gene manipulation. In this research, we developed a CRISPR-Cas9 tool based on modification of existing plasmids. This vector was used to explore the efficiency of target gene mutation using the oxalate biosynthesis *oah1* gene as a test target. We found that transformation with a circular plasmid resulted in a high frequency (40%) of targeted gene mutation among transformants. Unexpectedly, 100% of these mutants integrated large segments of the transforming plasmid at the target site. This result was confirmed among multiple target sites within the *oah1* gene and among three independent isolates of *S. sclerotiorum*. The efficiency of creating CRISPR-Cas9 insertion mutants has allowed us to confirm the requirement of *oah1* for oxalic acid biosynthesis in multiple isolates of *S. sclerotiorum* and to begin characterization of shared phenotypes among isolates. One such phenotype not previously reported is the overproduction of compound appressoria. These mutants are capable of infecting healthy host tissue allowing us to explore the early compatibility events and factors associated with host infection independent of oxalic acid. Comparative transcriptomic analysis between *oah1* mutants and wild type is being pursued to identify these factors.

48F Functionalization of surfaces using hydrophobin-fused laccase from *Aspergillus nidulans*. *O. Fokina*, L. Winandy, R. Fischer Inst. for Appl. Biosciences / Dep. of Microbiology, Karlsruhe Institute of Technology - KIT, Karlsruhe, Baden-Wuerttemberg, DE.

Hydrophobins are small amphiphilic proteins that can self-assemble into monolayers on hydrophilic and hydrophobic surfaces and change their properties. They are secreted by fungi to reduce surface tension at the medium-air interface during hyphal growth and are also responsible for the hydrophobicity of the conidiospore surface. Hydrophobins are divided into two classes, depending on their structural characteristics. Especially class I hydrophobins are interesting for biotechnology, because their layers are stable at high temperatures and can only be removed with strong acids.

Here we we developed a system to functionalize surfaces with enzymes using hydrophobin as anchor protein or "glue". To this end *Aspergillus nidulans* laccase C (LccC) was fused to the class I hydrophobins DewA or DewB and used for specific functionalization of hydrophobic/hydrophilic polystyrene and glass surfaces. Fusion proteins were secreted into the culture medium, which was directly used for coatings without additional purification steps. Protein immobilization using hydrophobins provides a stable binding of the fused enzyme to the surface without additional chemical treatments that could potentially be harmful to the enzyme or the surface. Also the spatial orientation of the enzyme is predetermined by the design of the fusion construct. This study presents an easy-to-use alternative to classical enzyme immobilization techniques and can probably be applied not only for laccases, but also for other biotechnologically relevant enzymes.

49W Fast and highly efficient gene replacement in Aspergillus niger using CRISPR/Cas9. *Jean Paul Ouedraogo*, Yun Zheng, Tricia John, Letian Song, Adrian Tsang Concordia University, CSFG, 7141 Sherbrooke St.W.Montreal, QC, H4B1R6.

The CRISPR (clustered regularly interspaced short palindromic repeat)–Cas9 (CRISPR-associated nuclease 9) system is transforming biology by providing a simple and efficient method to precisely edit the genome of any organism. As an important bio industrial workhorse, *Aspergillus niger* has attracted widespread interest for exploring broaden application in the sectors of industrial enzymes and organic acids. The availability of its genome sequence enables the redesign and engineering novel stains of *A. niger* based on CRISPR/Cas9 tools. In this study, we have extended CRISPR/Cas9 system for gene editing in *A. niger*. The endogenous tRNA gene as RNA polymerase III promoter was employed to lead the expression of guide-RNA (gRNA) cassette to delete and replace gene by homologous recombination in *Aspergillus niger.* The combination of CRISPR/Cas9 system and non-homologous end joining deficient strain allows fast and highly efficiency gene replacement in an organic acids producer *A. niger* strain with 100 % of transformants showing correct gene replacement.

50T Genetic parts screening and artificial N-glycosylation motif engineering for heterologous protein production in *Aspergillus niger*. *Jinxiang Zhang*[1,3], Saori Amaike-Campen[1,2], Sam Deutsch[4], Ljiljana Pasa-Tolic[5], Erika Zink[5], Jon Jacobs[5], Blake Simmons[1,6], John Gladden[1,3], Jon Magnuson[1,2] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Pacific Northwest National Laboratory, Richland, WA; 3) Sandia National Laboratories, Livermore, CA; 4) Joint Genome Institute, Walnut Creek, CA; 5) Environmental Molecular Sciences Laboratory, PNNL, Richland, WA; 6) Lawrence Berkeley National Laboratory, Berkeley, CA.

Aspergillus niger is a genetically tractable model organism for scientific discovery and a platform organism used in industry for the production of enzymes. Expression of secreted native enzymes at tens of grams per liter have been discussed by those in industry, but high level production of heterologous enzymes remains elusive. Strategies to increase production include the use of strong promoters, protease-deficient strains, fusion proteins, multiple gene copies, etc. However, yields of heterologous proteins are still lower than desired.

We generated proteomics data from secretome samples of A. niger grown on a variety of minimal and rich media, with the goal of identifying useful genetic elements for increasing heterologous protein production. Twenty promoters, six signal sequences and four introns from the most highly secreted proteins were identified as candidate genetic elements to enhance heterologous gene expression. These candidate elements were tested for their ability to drive expression of a prokaryotic glycoside hydrolase. A vector was designed to target integration of the modified expression cassette to the native glucoamylase (glaA) gene locus by homologous recombination. Considerable diversity was seen in heterologous protein production driven by these various elements. Interestingly, a signal peptide from GPI-anchored cell wall protein showed promising results.

In addition, based on the 3D structure of heterologous protein, we generated seven individual artificial N-glycosylation motifs on the surface of the heterologous protein. Analysis of these sites for glycosylation via top-down proteomics is just beginning. Correlation of glycosylation with any changes in kinetic and thermodynamic properties of the altered enzymes is the goal of this aspect of the research.

51F Genomic and transcriptomic analysis of *Aspergillus niger* producing thermophilic bacterial cellulases. *J. Kim*[1,2], S. A. Campen[1,2,5], B. A. Simmons[1,3], S. E. Baker[1,2], J. M. Gladden[1,4], J. K. Magnuson[1,2] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Pacific Northwest National Laboratory, Richland, WA; 3) Lawrence Berkeley National Laboratory, Berkeley, CA ; 4) Sandia National Laboratories, Livermore, CA; 5) J. Craig Venter Institute, La Jolla, CA (current affiliation).

Enzymatic hydrolysis of pretreated lignocellulosic biomass is important in efficient conversion of lignocellulosic biomass to biofuel. Thermophilic bacterial cellulase enzymes have advantages in industrially relevant conditions due to their stability in high temperature and tolerance to inhibitory compounds generated during biomass pretreatment. *Aspergillus niger* is a filamentous fungus known for its ability to produce high levels of enzymes, and it has been used for the industrial production of many useful enzymes. In this work, we employ genomics and transcriptomics approaches to understand and improve the production of thermophilic bacterial cellulases in *A. niger*.

Comparative transcriptomic analysis of the *A. niger* ATCC 11414 parent strain and an engineered strain expressing a thermophilic bacterial cellulase using RNA-Seq shows that more than two-thirds of the genes are differentially expressed. We also investigated differential RNA polymerase binding and histone modification using ChIP-Seq, in order to examine these potential causes of differential expression. Using whole-genome resequencing, we identified the genetic variation in the engineered *A. niger* strain as well as the copy number and integration locus of the introduced bacterial cellulase gene. These genetic changes could be responsible for the epigenetic changes and subsequent differential gene expression in heterologous enzyme producing *A. niger* strain.

52W Milk-clotting enzymes from *Aspergillus oryzae* and *Aspergillus luchuensis*. Yoko Takyu[1], Taro Asamura[1], Ayako Okamoto[1], Hiroshi Maeda[1], Michio Takeuchi[1], Ken-Ichi Kusumoto[2], Hitoshi Amano[3], Hiroki Ishida[4], *Youhei Yamagata*[1] 1) Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, JP; 2) NFRI, Ibaraki, JP; 3) Amano Enzyme Inc., Gifu, JP; 4) Research Institute, Gekkeikan Sake Company Ltd., Kyoto, JP.

Milk-clotting enzymes were used for the cheese production. Chymosin is an aspartic endopeptidase functioned as a key enzyme for curdling process. The enzyme is found in rennet made from the abomasum of calves. Chymosin specifically cleaves κ-casein between Phe[105]-Met[106]. The scission causes collapse of the casein micelles in the milk and curd formation. Although the clotting enzyme from *A. oryzae* was reported, the enzyme was not identified. We searched aspartic endopeptidases with milk-clotting activity in *A. oryzae*. The genome project of *A. oryzae* revealed that the fungus has 134 proteolytic enzyme genes, and the aspartic endopeptidase genes are 11. We overexpressed the enzymes in *A. nidulans* as a host and found two enzymes showing the milk-clotting activity. One enzyme showed a

high curdling activity and another showed a weak activity. The high-activity enzyme (AOchymosin) was purified and characterized. On the other hand, we also found the homologous enzyme genes in *Aspergillus luchuensis* (formerly known as *A. awamori*) used for production of *awamori*, a kind of *shochu*. The similarity of amino acid sequence between the AOchymosin and the *A. luchuensis* counterpart (ALchymosin) was 95%. ALchymosin was also overexpressed in *A. nidulans* and purified as a single band in SDS-PAGE. The molecular masses of the both purified fungal chymosin were about 40 kDa. The enzymes showed maximum milk-clotting and proteolytic activities at pH 5.5 and 3.0. respectively. The peptide analysis of the digested κ-casein showed that the cleavage sites were coincide, Phe^{105}-Met^{106} and Lys^{24}-Tyr^{25}. We concluded the both enzymes would be able to use for cheese production.
This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

53T Expression of an AT-biased *Rhizopus* glucoamylase gene in *Aspergillus oryzae* using codon optimization. *D. Zawil*, M. Tanaka, T. Shintani, K. Gomi Grad. Sch. Agric. Sci, Tohoku Univeristy, Sendai shi, Miyagi, JP.

Glucoamylase from *Rhizopus oryzae* is of great importance in biotechnological applications because of its strong hydrolyzing activity toward raw starch compared to other glucoamylases. However, despite such an advantage of *Rhizopus* glucoamylase, *R. oryzae* produces low amounts of glucoamylase in submerged cultures. To overcome some of the obstacles of mass production of *Rhizopus* glucoamylase, we try to produce it at high levels using *Aspergillus oryzae* as a host.
Working with two organisms of different GC content, however, codon optimization is a must to be able to achieve highly efficient production of the glucoamylase, according to our previous studies (1, 2) suggesting that AT-biased heterologous gene transcripts tend to be polyadenylated prematurely and be degraded rapidly. Hence, we firstly compare the production level of *Rhizopus* glucoamylase overexpressed in *A. oryzae* and in the yeast *Saccharomyces cerevisiae*. So far we have succeeded in transforming the *Rhizopus* glucoamylase in both yeast and *A. oryzae*. Proceedingly we are checking for the glucoamylase activity and its transcript level by northern blot analysis in the transformants.
1) Tokuoka et al, *Appl. Environ. Microbiol.*, **74**, 6538?6546 (2008)
2) Tanaka et al, *Appl. Microbiol. Biotechnol.*, **96**, 1275?1282 (2012)

54F Adsorption kinetics and self assembled structures of *Aspergillus oryzae* hydrophobin RolA on chemically modified solid surfaces. Y. Terauchi[1], M. Nagayama[1], T. Tanaka[1], H. Tanabe[1], T. Arita[2], H. Higuchi[2], F. Hasegawa[3], *K. Abe*[1,3] 1) Dept. Microbial biotechnology, Grad. Sch. Agricult. Sci., Tohoku Univ., Sendai, Miyagi, JP; 2) IMRAM, Tohoku University; 3) NICHe, Tohoku University.

Hydrophobins are amphipathic proteins that are ubiquitous among filamentous fungi, and required for the formation of fungal aerial structures (1). Hydrophobins also play an important role in the infection of pathogenic fungi because hydrophobins are not recognized by pattern recognition receptors of animals and plants (2, 3). The industrial fungus *Aspergillus oryzae* produces a hydrophobin RolA (4). RolA attaches and self-assembles on solid surfaces such as polyesters, and recruits an esterase CutL1, which results in stimulation of the hydrolysis of polyester by CutL1 (4). RolA also evades from recognition by mouse immune system (unpublished results). Because of these properties, RolA is expected to be applicable to industrial and medical fields. Therefore, kinetic properties and self-assembled properties of RolA-adsorption on solid surfaces are important. To investigate kinetic properties and self-assembled structures of RolA on solid surfaces, we constructed self-assembled monolayers (SAM) of 1-undcanethiol (hydrophobic), 11-amino-1-undecanethiol (cationic), and 10-carboxy-1-undecanethiol (anionic) on the electrodes of a Quartz Crystal Microbalance (QCM). We analyzed kinetics of RolA-adsorption on SAM-electrodes at various pH, and then observed self assembled structures of RolA by an AFM. Affinities between RolA and all SAM-electrodes were highest at pH 4 and lowest at pH 10. The forms of self-assembled structure of RolA depended on the pH values, amounts of adsorbed RolA, and functional groups of SAMs. Rod-like structures of self-assembled RolA were observed on all SAM-electrodes at pH 4. In addition, surface tensions of SAM-electrodes were modified by RolA adsorption. Overall, these results suggest that hydrophobic and electrostatic interactions between RolA molecules adsorbed on SAM-electrodes and soluble RolA molecules in water-phase depend on pH values and mainly contributed to the kinetics of RolA adsorption and self-assembly of RolA molecules on solid surfaces.
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2) Aimanianda V. et al., Nature. **460**:1117 (2009)
3) Martellini F. et al., Mol Biotechnol. 55: 27 (2013)
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55W A novel constitutive promoter for engineering *Trichoderma* to control biological contaminants in industrial processes and as a biostimulant in the Phosphite metabolizing Crops Platform. *N. Carreras-Villaseñor*[1], G. Rico-Ruiz[1], M. Leyva-González[1], A. Herrera-Estrella[2], D. López-Arredondo[1], L. Herrera-Estrella[2] 1) StelaGenomics Mexico, Irapuato, Guanajuato, MX; 2) LANGEBIO-UGA Irapuato, Guanajuato, MX.

Members of the genus *Trichoderma* are used in the industry as producer of homologous and heterologous enzymes due to its high secretory capacity. Achieving high expression of heterologous genes requires a strong promoter to drive robust transcription, in this regard, the use of constitutive promoters for expression of recombinant proteins has advantages, such as, the avoidance of the use of inducers and the complex gene regulation. Based in transcriptional data of *T. atroviride* subjected to many growth conditions, we selected the promoter of one of the most expressed gene in any of the tested conditions. This promoter is called Pta1. To evaluate the strength of Pta1 promoter, we used it to express the coding sequence of the *ptxD* gene in *T. atroviride*. PtxD is a bacterial Phosphite dehydrogenase that oxidizes Phosphite (Phi) into Phosphate (Pi). *Trichoderma* transgenic strains using the Pta1 promoter have expression levels of *ptxD* comparable with those observed when *ptxD* was placed under control of Ppki, one of the strongest promoters available for expression of native or foreign proteins in this fungus. *T. atroviride* is unable to metabolize Phi and use it as a sole phosphorus source. By contrast transgenic strains expressing PtxD under control of Pta1 and Ppki promoters could grow in Phi as a sole source of P. Expression of *ptxD* did not alter the native characteristics of *T. atroviride*, as the transgenic strains preserve their abilities as biocontrol agent and plant growth promoting organisms. Therefore, Pta1 is a good candidate as constitutive promoter for the expression of homologous and heterologous proteins with industrial importance. In this poster, to produce proteins or spores in a system that does not require sterilization to prevent biological contaminations and thus significantly reducing the cost of production. We will also report on the progress of using *Trichoderma*-Phi metabolizing strains as a biostimulant and biocontrol agent for crop production on a Phi-based fertilizer system.

56T Selective cleavage of β-*O*-4 lignin bond by novel β-etherase of the white-rot fungus *Dichomitus squalens*. *M. Marinovic*[1], M.R. Mäkelä[1], P. Nousiainen[4], A. Dilokpimol[2], R. Moore[4], J. Sipilä[4], R.P. de Vries[2,3], K. Hildén[1] 1) Department of Food and Environmental Sciences, Division of Microbiology and Biotechnology, University of Helsinki, Finland; 2) Fungal Physiology, CBS-KNAW, Utrecht, The Netherlands; 3) Fungal Molecular Physiology, Utrecht University, The Netherlands; 4) Department of Chemistry, Laboratory of Organic Chemistry, University of Helsinki, Finland.

Lignin is a recalcitrant aromatic polymer present in woody plant cell walls. It is depolymerized by classical lignin-modifying oxidoreductases (laccases and class II heme peroxidases) produced by wood decaying fungi, which catalyze non-selective cleavage of different types of bonds present in lignin. Novel enzymes catalyzing aromatic conversions are needed to unlock the potential of lignin as a major renewable source of bulk and fine chemicals.

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) constitute a complex and diverse superfamily of multifunctional enzymes with essential roles in cellular detoxification. These proteins are widespread in animals, plants, bacteria and fungi. Some of the bacterial GSTs possess β-etherase activity that can catalyze selective cleavage of β-*O*-4 aryl ether linkages present in lignin. However, only few fungal GSTs have been characterized so far, and little is known about these enzymes. In this work, putative GST-encoding genes from the lignocellulose degrading basidiomycete *Dichomitus squalens* were cloned and heterologously expressed in *Escherichia coli*. One of the enzymes that showed a weak amino acid level homology to bacterial β-etherase ligE was shown to cleave β-*O*-4 bond in α-oxo erol lignin model compound. To our knowledge, this is the first report of eukaryotic β-etherase.

57F Expanding feruloyl esterase gene family of *Aspergillus niger*: characterization of a new feruloyl esterase, FaeC. *A. Dilokpimol*[1,2], M. R. Mäkelä[1,2,3], S. Mansouri[3], O. Belova[1,2], M. Waterstraat[4], M Bunzel[4], R. P. de Vries[1,2,3], K. S. Hildén[3] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; 2) Fungal Molecular Physiology, Utrecht University, Utrecht, the Netherlands; 3) Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki, Finland ; 4) Department of Food Chemistry and Phytochemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Germany.

Ferulic acid (FA) and to a lesser extent other hydroxycinnamic acids (e.g. *p*-coumaric acid) are ester-linked to plant cell wall polymers, mainly to (hetero)xylans and pectins, forming polysaccharide-polysaccharide and polysaccharide-lignin cross-links. Phenolic cross-links increase the physical strength and integrity of plant cell walls and reduce their biodegradability by microorganisms. Feruloyl esterases (FAEs) [E.C. 3.1.1.73] are able to release FA and other phenolic acids from natural plant sources and agro-industrial by-products and are therefore widely used in food, feed, pulp-paper, bioethanol and pharmaceutical industries.

Two FAEs from *Aspergillus niger* (FaeA and FaeB) have been previously characterized and are among the best-studied FAEs. Phylogenetic analysis of fungal FAEs revealed numerous FAE candidates, including one from *A. niger* (FaeC).[1,2] Recombinantly produced FaeC was most active at pH 7.0 and 50°C and showed broad substrate specificity. The enzyme released both ferulic acid and *p*-coumaric acid from wheat arabinoxylans and sugar beet pectins, and acted synergistically with a commercial xylanase. The expression profile of *faeC* on a set of phenolic compounds differed from those of *faeA* and *faeB*, indicating that these FAE isoenzymes may target different substrates in a complementary manner. FaeC showed promising potential for applications, particularly because of its broad substrate profile and its maximum activity at neutral pH.[3]

Acknowledgement
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58W Disruption of the glucose repressor gene *cre1* in *Trichoderma harzianum* CFAM-422 and its effect on plant cell wall degrading enzymes production. *M.S. Tamietti*[1], G.E.O. Midorikawa[1], T.D. Mendes[1], M.C.T. Damaso[1], E.P.S. Bon[2], A.S. Silva[3], L.M.F. Gottschalk[4], E.F. Noronha[5], L.C.L. Fávaro[1] 1) Embrapa Agroenergia, Brazilian Agricultural Research Corporation (Embrapa), Brasília, DF, BR; 2) Biochemistry, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, BR; 3) Catalysis and Biochemical Processes, National Institute of Technology (INT), Rio de Janeiro, RJ, BR; 4) Food Agroindustry, Brazilian Agricultural Research Corporation (Embrapa), Rio de Janeiro, RJ, BR; 5) Molecular Biology, University of Brasilia (UnB), Brasilia, DF, BR.

The Carbon Catabolite Repression (CCR) is a mechanism by which saprophytic fungi and bacteria can utilize preferrably highly energetic compounds over compounds of difficult degradation. For T*richoderma reesei*, the protein that acts as the repressor in the presence of glucose is CRE1. In this project, we aim to disrupt the *cre1* gene in the strain CFAM-T422 of *Trichoderma harzianum*, obtaining mutants with enhanced plant cell wall degrating enzymes production. The disruption of *cre1* in *T. harzianum* CFAM-422 was performed by transforming protoplast cells with a cassette containing a hygromycin B phosphotransferase gene (*hph*) flanked by *cre1* gene sequence, favoring its integration in the fungal genome by homologous recombination. A stability test was performed by successive transferences to nonselective medium (without hygromicin B) for ten generations, the last one being transferred to medium containing hygromicin B (200 µg/mL). Mutants genetically stable and the parental strain were tested for enzyme production in both inducing and repressing conditions with four different carbon sources (carboxymethylcellulose, pectin, starch and xylan). Their enzymatic indexes (e.i.), the ratio between the degradation halo diameter over the colony diameter, were determined. The glucose content for the test under repressing condition was determinded as the minnimal concentration that triggers CCR in CFAM-T422. The totality of the stable transformants showed increased enzymatic index in all carbon sources tested. For the xylan containing media, which showed the most expressive differences between wild and mutant strains, the i.e. for the parental was 1 and transformants varied from 2,0 to 2,9 regardless of the presence of glucose. These

findings indicate that the *cre1* gene in *T. harzianum* regulates the expression of carbohidrate active enzymes and its deletion can result into mutant strains with enhanced cellulase, hemicellulase and pectinase production with lower glucose inhibition.

59T Comparing Fungal Capacity for Bioremediation of Human Solid Waste. Alexander Mehreteab, Victor Manzanilla, *Michael Watters* Dept Biol, Valparaiso Univ, Valparaiso, IN.

Neurospora crassa can be used to reduce human solid waste while converting it into fungal biomass which has the potential to be used as a dietary supplement. Typically portrayed as an environmental hazard and source of undesirable odor, solid waste represents an untapped resource. Filamentous fungi are natural decomposers with the ability to use this resource and reduce its environmental impact.

We report here on experiments to determine the conditions which maximize yield of fungal biomass. We compared the effect of the length of incubation and alternative methods of aeration as well as choice of fungal species on fungal growth. Rates of conversion were variable, with Neurospora producing the highest average rate of conversion (grams of dry fungal biomass produced from grams of dry waste in the media) of fungi tested. Additionally fungal growth reduced the characteristic odor of the media. Although this project was initially proposed to address problems inherent to long-term space flight: food storage & waste management, it holds potential benefit in diverse situations including livestock confinement operations.

60F Genome analysis and gene silencing of the citrus pathogen *Colletotrichum abscissum*. *Eduardo Goulin*[1,2], Marco Takita[1], Marcos Machado[1] 1) Biotechnology, Centro de Citricultura "Sylvio Moreira", Cordeirópolis, Cordeirópolis, BR; 2) State University of Campinas.

The citrus crop is a worldwide important crop. But, it is constantly affected by several pathogens, and fungi is an economically relevant class of citrus pathogens. New technologies are being applied every year to better understand the pathogens biology, and it can contribute to plant diseases control. The whole genome sequencing become a popular technology making possible and fast many knowledge advances. Therewith, we used NGS to investigate the RNA interference canonical machinery genes in the Post-Bloom Fruit Drop causal agent, *Colletotrichum abscissum*, as well as targets to RNAi silencing. The genome sequencing was performed in Illumina HiSeq platform, followed by *de novo* assembly using CLC genomics workbench software, and ORFs prediction by Augustus. The Local Blast were used for gene sequences investigation. The genome of *C. abscissum* is well coverage (169 times), which allowed the gene prediction, checked by RNAi machinery proteins search and confirmed by sanger sequencing of Argonaute, Dicer and RdRp. The RNAi functionality was proved after RT-qPCR of several genes and also transforming the fungus with a hairpin construction to induce the gene silencing. The fungus had a previously inserted report gene silenced after transformation attesting the machinery functionality. The genome provided a range of genes that can become a target to RNAi. Therefore, the genome of *Colletotrichum abscissum* was efficiently sequenced, assembly and used to a relevant investigation, providing information about the fungus biology, and also a tool to gene function studies and pathogen control.

61W Parasitism of *Trichoderma* on closely related fungi (adelphoparasitism) is linked to intensive interfungal DNA exchange. *Irina S. Druzhinina* Institute of Chemical Engineering, TU Wien, Vienna, Austria.

The remarkable cellulolytic activity of the initially fungicolous fungus *Trichoderma* (Hypocreales, Pezizomycotina, Ascomycota) arose as a result of unprecedented intensive lateral transfer of genes (LGT) that are required for the degradation of plant biomass. The evolutionary analysis of all lignocellulose degrading carbohydrate active enzymes (lclCAZymes) of *Trichoderma* showed that one half of more than 120 genes was obtained through LGT from herbivore Pezizomycotina fungi (such as Eurotiomycetes, Sordariomycetes incl. Hypocreales, Leotiomycetes and others) but none from other fungi or prokaryotes. Here we review the ecology of *Trichoderma* and present details on ultrastructure of its parasitism on different fungi. We provide evidence that the high frequency of LGT of lclCAZymes is linked to the unique ability of *Trichoderma* to parasitize on genetically closely related fungi that is extended up to adelphoparasitism in its strict sense. The data suggests that the taxonomically and functionally biased genome enrichment of lclCAZome by LGT allowed *Trichoderma* to expand its diet from plesiomorphic fungivory/carnivory up to apomorphic phytosaprotrophy, thus resulting in the emergence of its outstanding ecological versatility referred as environmental opportunism.

The case study on *Trichoderma* allows us to assume that intracellular adelphoparasitism may be a prerequisite for the lateral transfer of genes also in other organisms. Although such parasitic interactions are rare in nature, potential systems in other low eukaryotes will be discussed.

62T Marker recycling through CRIME. *A.P. Mitchell*, M. Huang Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA.

We describe here a new approach to marker recycling, a controlled sequence of manipulations in which a genetic marker is selected, then lost, then selected again. Our work was carried out with the fungal pathogen *Candida albicans*, which is typically a diploid and has no complete sexual cycle. The approach makes use of the RNA-guided DNA cleavage activity of CRISPR-Cas9, which was recently engineered for use in *C. albicans* by Valmik Vyas and colleagues (PMID: 25977940). We used the high-speed adaptation for gene deletion described by Kayden Min in our lab (PMID: 27340698) for the specific studies that will be presented. Because the CRISPR-Cas9 system has been implemented in diverse fungi, we believe that CRIME may be applicable to diverse fungi.

To use CRIME, we first created marker cassettes flanked by direct repeats. We used one repeat-flanked marker to create a homozygous mutation in our favorite gene, *OFG1*, by CRISPR-Cas9 transformation. Then, in the *ofg1*/*ofg1* strain, we made a homozygous mutation in a second gene, *OFG2*, by use of a different repeat-flanked marker. In that second transformation, we also included a guide RNA gene to direct cleavage of the marker that lay in the *ofg1* mutant alleles. The idea was that either single-strand annealing or homology-directed repair would then excise the marker we cleaved and leave behind only a copy of the flanking repeats. The idea proved correct: we recovered *ofg1*/*ofg1 ofg2*/*ofg2* double mutants from the transformation that had only the marker in the *ofg2* alleles; they had lost both copies of the marker that had been in the *ofg1* alleles. In fact, we have been able to create successive homozygous mutations in three genes by use of two markers, and still ended up with one marker available for further selection in the triple homozygote. That sequence of steps and analyses required 3 weeks total. Our findings illustrate that CRIME pays, at least in this one context.

We are now modifying our approach to enable wild-type allele reconstruction through a process that we call TIME. We feel that this capability will be critical for geneticists of the future, because CRISPR-Cas9 systems can have off-target effects. Our hope is that geneticists of the future will always remember that, if they do the CRIME, they must do the TIME.

63F A flexible expression platform for *Aspergillus* based on CRISPR mediated homologous recombination. *F.H. Kirchner*, Z.D. Jarczynska, C.D. Nødvig, U.M. Mortensen Danish Technical University, Copenhagen, DK.

Fungi produce a wide variety of secondary metabolites and enzymes. For many of these secondary metabolites, the clusters that are responsible for their production are unknown. One way to elucidate the pathway is to make gene disruptions in the producer stain, but this requires that the species can be grown in the laboratory, that it is transformable and that a genetic tool box is available. Alternatively the cluster can be transferred to a well characterized host where the genes can be heterologously expressed. To facilitate the latter method, we created a flexible expression platform with integration sites in different well characterized Aspergillus species. The integration sites contain a common integration target site that contains the colorimetric marker *lacZ* flanked by long overhangs for homologous recombination. Hence, hosts strains are blue, but if new genes are inserted correctly into the expression sites, the strains will turn white as the *lacZ* gene is eliminated as a result of the integration process. Integrations can be performed in wild-type strains using marker- free gene-targeting by using CRISPR stimulated homologous recombination. Importantly, by using our system, the same gene-targeting construct can be inserted into several different hosts; and the strain with the best product performance can then be selected for further analysis.

64W Protease regulatory factors of *Trichoderma reesei* can be controlled to improve therapeutic protein production. Christopher Landowski[1], Ann Westerholm-Parvinen[1], Bernhard Helk[2], Juhani Saarinen[3], *Markku Saloheimo*[1] 1) VTT Technical Research Centre of Finland, Espoo, FI; 2) Novartis Pharma Ag., Basel, Switzerland; 3) Glykos FInland Oy, Helsinki, Finland.

Protease secretion limits the production of many sensitive therapeutic proteins such as hormones and cytokines that are by nature easy to degrade. There are over 40 potential proteases secreted by *Trichoderma reesei*. We looked for transcriptional regulators of these proteases with the aim to control and reduce the expression of a wide range of proteases. Protease induction studies were set up to trigger protease activity with peptide and protein substrates in liquid cultures of *T. reesei*. Genome-wide expression data was generated and clustered to find out what genes are co-regulated after different treatments. Twelve candidate transcription factors or regulatory proteins were identified. To narrow the selection, the regulator genes were located on the scaffold to see if they were physically near any protease genes. Transiently silencing *ptf1*, *prp1*, and *ptf3* with siRNA downregulated the expression of a selection of protease genes in accordance with the co-regulation observed. Treatment with both *ptf1* and *prp1* siRNAs increased the effectiveness of the knockdown and reduced protease activity. The deletion of single, double, and triple combinations of the regulators successfully reduced protease activity and increased interferon alpha 2b production. For example, the triple deletion Δ*ptf1*Δ*prp1*Δ*ptf8* lead to a 3.7-fold improvement in interferon alpha 2b production. This is the first time protease regulatory proteins have been identified in *T. reesei,* and we have demonstrated how silencing or deleting them could broadly reduce protease activity.

65T Identification and demonstration of a single nucleotide substitution with significant impact on specific productivity in *Trichoderma reesei*. R. Rabinovich, *A. Virag*, E. Bodie, Z. Chen, M. Ward DuPont Industrial Biosciences, Palo Alto , CA.

Within our perpetual quest to increase amounts of secreted protein produced by our strains, we chemically mutagenized *Trichoderma reesei* strain RL-P37 and obtained a daughter *T. reesei* strain with improved specific productivity. To find the gene(s) responsible for the improved specific productivity, the genomes of the daughter strain and its parent strain RL-P37 were sequenced, and a large number of intragenic and intergenic mutations were identified. A subset of non-synonymous substitutions were individually introduced into the parent RL-P37 strain and assessed for specific productivity. Introduction of a single nucleotide substitution in a histidine kinase gene (hk^{mutant}) into strain RL-P37 brought specific productivity up to levels typical for the daughter strain. Besides increasing specific productivity, introduction of the hk^{mutant} mutation caused sensitivity to high osmolarity, which is in line with the function of histidine kinase homologs as osmosensors in other filamentous fungi. Deletion of the native hk^{wt} allele in strain RL-P37 significantly reduced specific productivity, indicating that the hk^{mutant} allele contains a gain of function mutation. Replacing the mutant hk^{mutant} allele with the wild type hk^{wt} allele in the daughter strain significantly reduced specific productivity, demonstrating that the mutated *hk* gene is the main gene responsible for the specific productivity improvement in the daughter strain. To demonstrate that we can get the same benefit in other host backgrounds and strains overexpressing proteins of interest, we replaced the native hk^{wt} allele with the hk^{mutant} allele in a *T. reesei* strain over-expressing Cbh1. The increase in specific productivity that we observed confirmed that we can combine the hk^{mutant} allele with other useful host modifications. The use of the discovered hk^{mutant} allele, alone or combined with other beneficial mutations, will significantly improve secreted protein production.

66F *Rhodosporidium toruloides*: A new platform organism for production of biofuels and bioproducts derived from depolymerized polysaccharides and lignin. *Junko Yaegashi*[1,2], James Kirby[1,3], Masakazu Ito[4], Jian Sun[1,5], Tanmoy Dutta[1,5], Mona Mirsiaghi[6], Eric R. Sundstrom[6], Edward Baidoo[1,6], Deepti Tanjore[6], Todd Pray[6], Kenneth Sale[1,5], Seema Singh[1,5], Jay D. Keasling[1,3,6,7,8], Blake A. Simmons[1,6], Steven W. Singer[1,6], Jon K. Magnuson[1,2], Adam P. Arkin[4,6,8], Jeffrey M. Skerker[4,6], John M. Gladden[1,5] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Pacific Northwest National Laboratory, Richland, WA ; 3) California Institute of Quantitative Biosciences (QB3), University of California, Berkeley, CA ; 4) Energy Bioscience Institute, Berkeley, CA ; 5) Sandia National Laboratory, Livermore, CA ; 6) Lawrence Berkeley National Laboratory, Berkeley, CA ; 7) Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA; 8) Department of Bioengineering, University of California, Berkeley, CA.

Maximizing utilization of the different carbon sources present in raw lignocellulosic material into the desired final product is a key aspect to increasing the effectiveness of producing bioproducts from plant biomass. While conventional microbial organisms such as *E. coli* and *S. cerevisiae* are the most commonly used host microbes for bioproduct synthesis, they do not readily uptake and utilize multiple carbon sources simultaneously, especially in the presence of glucose. Other organisms that are innately able to use carbon sources other than glucose tend to lack efficient genetic tools for host engineering.

In this study we explored the utility of *Rhodosporidium toruloides* as a new platform organism for production of terpenes from deconstructed plant biomass. Remarkably, we found that it is able to simultaneously utilize all three of the major carbon sources that are found in lignocellulosic hydrolysates: glucose, xylose, and *p*- coumaric acid. Furthermore, it is fast-growing, single-celled, and genetically tractable,

making it a promising host for pathway engineering. We demonstrate the production of two heterologous terpenes, bisabolene and amorphadiene in *R. toruloides* achieved by random chromosomal integration of their respective terpene synthase expression cassettes. We show its ability to utilize each of the three carbon sources mentioned above individually as well as simultaneously for growth and production of bisabolene. Finally, we demonstrate its ability to be applied to a one-pot pretreatment, saccharification, and fermentation system of lignocellulosic feedstocks. This organism was able to tolerate the ionic liquid that was used for pretreatment and was also able to utilize all of the three major carbon sources in the hydrolysate to support growth and bisabolene production.

67W Universal expression system for eukaryotic microorganisms. *A. Rantasalo*, C. Landowski, J. Kuivanen, J. Jäntti, D. Mojzita VTT Technical Research Centre of Finland Ltd.

We have developed a novel orthogonal expression system that functions in a wide spectrum of eukaryotic microorganisms. The expression system is based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The sTF expression is driven by an engineered, universal core promoter that provides a low, but sufficient expression level of the sTF. The sTF expression is constitutive and thus present in the cells in all growth conditions and in all developmental and growth stages. The sTF-dependent promoter regulating the expression of the target gene also contains a similar type of universal core promoter functional in diverse species. This core promoter can be further modified by inserting synthetic repressor (sR) binding sites, through which an additional expression control is gained upon expression of the sR. The combination of multiple sTF-binding sites and the core promoters (including the repressible versions) enables specific adjustment over a wide range of target gene expression levels, from very low to very high. This expression system provides robust, stable, and tuneable expression levels of the target genes in a broad spectrum of host organisms. Further it simplifies the genetic tools needed for the construction of new protein expression hosts, including those with undeveloped know-how. The method for selecting the universal core promoters, constructions of the expression systems, and demonstrations of their performance in diverse hosts, including yeasts and filamentous fungi, will be presented.

68T Transcriptome analysis of two unrelated fungal β-lactam producers *Acremonium chrysogenum* and *Penicillium chrysogenum*: Velvet-regulated genes are major targets during conventional strain improvement programs. *D. Terfehr*, T.A. Dahlmann, U. Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE.

Acremonium chrysogenum and *Penicillium chrysogenum* are the industrial producers of the β-lactam antibiotics cephalosporin C and penicillin G, which are used worldwide for the treatment of bacterial infections in human and veterinary medicine. Beside the ability to generate anti-infectives, both species do not have much in common and are members of distinct taxonomic groups within the Ascomycota. Previously, wild-type isolates from *A. chrysogenum* ATCC11550 and *P. chrysogenum* NRRL1951 were randomly mutated during several rounds of conventional mutagenesis to reach economically relevant titers of the corresponding β-lactam antibiotics. Although, previous research has shed light on the biosynthesis pathway as well as parts of the regulatory processes involved in β-lactam production, there is still a lack of knowledge how the increased β-lactam production was achieved during conventional strain improvement programs. Here, we performed RNA-seq analysis of the two wild-type strains and industrial strains of both species (*A. chrysogenum* A3/2 and *P. chrysogenum* P2niaD18) to address the question whether both fungi have undergone similar expressional changes during strain improvement. Furthermore, our analysis includes the investigation of two mutants (ΔAcveA and ΔPcvelA) that lack Velvet, a global regulator of fungal secondary metabolism. Within the set of differential expressed genes during strain improvement, both fungi show an intersection of genes associated directly or indirectly to industrial β-lactam production. Furthermore, we will demonstrate that genes assigned to specific functional categories are also regulated by Velvet. Interestingly, Velvet regulated genes seem to be one of the primary targets of classical strain improvements programs in both fungi. This assigns Velvet an important role in strain improvement of industrial β-lactam producers, although components of the Velvet complex might be different in both fungi. Our results will contribute to develop alternative strategies for strain improvements of these β-lactam antibiotic producers.

69F Cycloaspeptides: elucidation of a novel fungal cyclic peptide biosynthetic pathway. *Kate M. J. de Mattos-Shipley*[1], Claudio Greco[1], David Heard[1], Nicholas P. Mulholland[2], Jason L. Vincent[2], Thomas J. Simpson[1], Christine L. Willis[1], Russell J. Cox[1,3,4], Andrew M. Bailey[5] 1) School of Chemistry, University of Bristol, UK; 2) Syngenta, Bracknell, Berkshire, UK; 3) Institute für Organsche Chemie, Leibniz Universität Hannover, Hannover, Germany; 4) BMWZ, Leibniz Universität Hannover, Hannover, Germany; 5) School of Biological Sciences, Life Sciences Building, University of Bristol, Bristol, UK.

The cycloaspeptides are cyclic pentapeptides produced by a range of filamentous fungi that are reported to exhibit various bioactivities, including anti-malarial, anti-tumour and insecticidal. In this work, two psychrotolerant *Penicillium* species, *P. soppii* and *P. jamesonlandense*, were confirmed as producers of cycloaspeptides A and E. Genome sequencing and comparative bioinformatics identified a putative gene cluster, which was confirmed as being responsible for cycloaspeptide biosynthesis by gene knock-outs and heterologous expression. The minimal gene set consists of a 5-module non-ribosomal peptide synthetase (NRPS) and a new type of trans-acting *N*-methyltransferase. Disruption of the *N*-methyltransferase and subsequent feeding studies have uncovered a hitherto unseen trait in fungal secondary metabolism: the ability of the NRPS to adenylate and incorporate *N*-methylated tyrosine and phenylalanine directly into the cyclic peptide. This discovery has been exploited to alter the ratios of cycloaspeptides produced, including a total shift from the non-active major metabolite cycloaspeptide A to the insecticidally active minor metabolite cycloaspeptide E. It also allowed the production of cycloaspeptides B, C and G, compounds not previously detected in a *Penicillium* species. Searches for similar gene clusters in other filamentous fungi identified the known ditryptophenaline gene cluster from *A. flavus*. This pathway was shown to be homologous, in that methylated phenylalanine is also accepted by the ditryptophenaline NRPS. We have also demonstrated that novel NRP natural products can be generated by feeding synthetic amino acid analogues to these systems. This discovery, coupled with the growing field of megasynthase engineering, could potentially be used to introduce methylated amino acids and synthetic amino acids into other NRP natural products of interest.

70W Resistance Gene-Guided Genome Mining: Serial Promoter Exchanges in Aspergillus nidulans Reveal the Biosynthetic Pathway for Fellutamide B, a Proteasome Inhibitor. Hsu-Hua Yeh[1], Manmeet Ahuja[2], Yi-Ming Chiang[2,3], C. Elizabeth Oakley[2], Shauna Moore[2], Olivia Yoon[2], Heather Hajovsky[2], Jin-Woo Bok[4], Nancy P. Keller[4], *Clay C. C. Wang*[1,5], Berl R. Oakley[2] 1) Department of

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Fungal genome projects are revealing thousands of cryptic secondary metabolism (SM) biosynthetic gene clusters that encode pathways that potentially produce valuable compounds. Heterologous expression systems should allow these clusters to be expressed and their products obtained, but approaches are needed to identify the most valuable target clusters. The inp cluster of Aspergillus nidulans contains a gene, inpE, that encodes a proteasome subunit, leading us to hypothesize that the inp cluster produces a proteasome inhibitor and inpE confers resistance to this compound. Previous efforts to express this cluster have failed, but by sequentially replacing the promoters of the genes of the cluster with a regulatable promotor, we have expressed them successfully. Expression reveals that the product of the inp cluster is the proteasome inhibitor fellutamide B, and our data allow us to propose a biosynthetic pathway for the compound. By deleting inpE and activating expression of the inp cluster, we demonstrate that inpE is required for resistance to internally produced fellutamide B. These data provide experimental validation for the hypothesis that some fungal SM clusters contain genes that encode resistant forms of the enzymes targeted by the compound produced by the cluster.

71T The chromatin remodeler suberoyl bis-hydroxamic acid (SBHA) induces changes in the pattern of metabolites produced by *Acremonium chrysogenum*. A. Barron Gutierrez[1], *W. Perez Perez*[1], J. Mendoza-Espinoza[2,3], F. Fierro Fierro[1], F. Fernandez Perrino[1] 1) Departamento de Biotecnologia, Universidad Autonoma Metropolitana - Iztapalapa, Mexico city, Mexico city, MX; 2) Departamento de Ciencias de la Salud, Universidad Autonoma Metropolitana - Iztapalapa, Mexico city, Mexico city, MX; 3) Universidad Autónoma de la Ciudad de México, Mexico city, Mexico city, MX.

Introduction. Many new fungal metabolites are being discovered by genome mining approaches. One strategy to find new secondary metabolites is the use of chemical chromatin remodelers, which would result in the activation of silenced gene clusters and the production of the "cryptic" metabolites. One of these chromatin remodelers is the histone deacetylase inhibitor suberoyl bis-hydroxamic acid (SBHA). Inhibition of histone deacetylase activity would maintain an acetylated state of histones, which is associated with a less condensed chromatin state and higher transcriptional activity.

Acremonium chrysogenum is used in the industry to produce the antibiotic cephalosporin, the sequencing of its genome revealed the presence of 42 biosynthetic clusters for secondary metabolites. The objective of this work was to study the changes in the chemical pattern, as observed by HPLC, of the metabolites produced by *Acremonium chrysogenum* in cultures added with SBHA.

Methodology. The wild type strain of *A. chrysogenum*, ATCC 11550, was used in this study. To obtain the Ultraviolet-Visible (UV) spectra, 10 mL of the extract were taken and read in a GenWay spectrometer. HPLC was performed with a CLAR equipment (Agilent 1260), using an elution gradient of water-acetonitrile on a C-18 column.

Results. The UV absorption spectra showed that the absorption values were between 200 and 480 nm. These spectra are considered as a chemical fingerprint of the extract and allow to fix the sweep of wavelengths for the HPLC analysis. CLAR analysis at 215 nm revealed changes in the chemical profile related to the time of culture and concentration of SBHA.

Conclusions. The use of suberoyl bis-hydroxamic acid (SBHA) revealed the feasibility to elicit the production of "cryptic" secondary metabolites in *A. chrysogenum*, and can be considered as a genome mining strategy applicable to this fungus.

72F *Aspergillus niger versus Aspergillus oryzae*: Expression platforms for heterologous secondary metabolite production. *E. Geib*, M. Brock Fungal Genetics and Biology, University of Nottingham, Nottingham, GB.

Filamentous fungi are treasure chests for novel secondary metabolites and genome mining has uncovered a multitude of yet unexplored secondary metabolite biosynthesis gene clusters. Their identification and characterisation is crucial for the development of drugs that combat various kinds of diseases. Unfortunately, most of these gene clusters appear silent under laboratory cultivation conditions, which requires heterologous gene expression in well-characterised expression systems. Previously, we developed such an expression system in *Aspergillus niger*, which bases on regulatory elements from the *Aspergillus terreus* terrein biosynthetic gene cluster. We used this platform to produce polyketides (e.g. lecanoric acid), non-ribosomal peptide synthetase-like products (e.g. aspulvinone E) and reconstituted the Asp-melanin biosynthesis pathway from *A. terreus*.

The latter studies led to an interest in understanding the chemistry of NRPS-like enzymes that produce metabolites with antifungal, cytotoxic, antitumorigenic and antiviral activity. Enzymes of this class may accept the same substrate, but form different products depending on their thioesterase domain. To study these domains, we compared the aspulvinone E synthetase MelA from *A. terreus* with the atromentin synthetase InvA5 from *Paxillus involutus*. While recombinant expression of *melA* in *A. niger* resulted in aspulvinone E production, expression of *invA5* led to a range of yet unknown products, but failed to produce atromentin. In contrast, recombinant and purified InvA5 produced atromentin *in vitro*. We therefore speculated that the physiology of *A. niger* might lead to a modification of the InvA5-derived metabolite. Consequently, the expression system was transferred to the alternative host *Aspergillus oryzae*. Indeed, *A. oryzae* produced aspulvinone E from MelA and, even more, atromentin from InvA5.

In conclusion, our recombinant expression system is perfectly suited for heterologous production of secondary metabolites. However, the metabolic physiology of *A. niger* and *A. oryzae* differs and at least two different expression platforms should be selected when aiming in the characterisation of novel secondary metabolite biosynthesis genes.

73W Development of a T. reesei "Sexual reproduction toolkit" enabling BSA-seq technology and industrial strain improvement. *L. Chan Ho Tong*[1], D. Naquin[2], P. Silar[3], A. Margeot[1], F. Bidard[1] 1) IFP Energies nouvelles, Biotechnology Department, France; 2) Institute for Integrative Biology of the Cell, High throughput sequencing platform, France; 3) Paris-Diderot University, Paris Interdisciplinary Energy Research Institute, France.

The *Sordariomycetes Trichoderma reesei* is a mesophilic filamentous fungus currently used to produce enzymes hydrolysing lignocellulosic biomass to glucose to be subsequently fermented to ethanol.

T. reesei, originally described from a single wild type isolate called QM6a, was believed to be devoid of a sexual cycle whereas its teleomorph, *Hypocrea jecorina*, undergoes an heterothallic sexual cycle involving MAT1-1 and MAT1-2 loci. However, after identification of a MAT1-2 loci in QM6a, a sexual reproduction has been obtained successfully with a MAT1-1 natural isolate. Further experiments have shown that QM6a and its derivatives are female-sterile but male-fertile.

Sexual reproduction in industrial strains is of a great economical and fundamental importance, as it makes a valuable genetic tool. In order to apply sexual reproduction as a tool in *T. reesei*, we developed a toolkit : ascospores isolation and conservation, optimization of nutritional and environmental factors to get a precise control of stromata production, characterization and sequencing of a wild type MAT1-1 strain sexually compatible with QM6a and its derivatives. This toolkit enables exploration of new genetic strategies.

Two examples of applications have been implemented and are described here. First, we successfully carried out a Bulk Segregant Analysis associated with high-throughput sequencing (BSA-seq) to identify the known causative mutation of the cellulase negative QM9136 strain. Secondly, we generated genetic diversity by crossing the RutC30 industrial strain with A2 wild type isolate. Improved progenies in cellulase production were obtained.

Materials and methods developed in our toolkit are available for the *T. reesei* community.

74T Functional dynamics of wood-decay through genome-wide analysis of the white rot Polyporales species *Phlebia radiata*. *Mari Mäkinen*[1], Jaana Kuuskeri[1], Netta Risulainen[1], Pia Laine[2], Olli-Pekka Smolander[2], Lars Paulin[2], Markku Varjosalo[3], Petri Auvinen[2], Taina Lundell[1] 1) Microbiology and Biotechnology, Department of Food and Environmental Sciences, University of Helsinki; 2) DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki; 3) Proteomics Unit, Institute of Biotechnology, University of Helsinki.

White rot Agaricomycetes of Basidiomycota secrete carbohydrate-active enzymes and auxiliary oxidoreductases to decompose wood lignocellulose. With genome sequencing projects and accumulating omics data, fungal metabolism and degradation processes are under intensive research. *P. radiata* is an ecologically important fungus able to degrade efficiently the main components of plant cell wall, presenting high applicability in biotechnological processes, and has a systematic importance as type species of phlebioid clade of Polyporales (1). The aim of the study was to identify main activities and proteins for wood-decay in *P. radiata* cultivated on Norway spruce and to study gene expression and enzyme activities in the presence of lignocellulosic waste material. Next-generation sequencing of the isolate 79 resulted first in construction of the mitogenome (2), then assembling and gene annotating the nuclear genome (3).

Time-scale analysis of transcriptome by RNA-seq and proteomics analysis upon a six weeks' cultivation period on spruce wood presented a set of genes that were up-regulated during the fungal colonization of wood and expressed as proteins (3). According to the proteome analyses, especially the lignin-modifying class-II peroxidases together with glyoxal and alcohol oxidases were abundantly produced on wood. Dynamic changes e.g. enhanced production of LiPs and MnPs on week 2 of growth on wood were detected. Comparative analysis of transcriptome and proteome data showed that majority of the CAZy encoding transcripts upregulated on spruce wood represented activities against plant-cell wall and included the main activities of white rot decay. Our study indicates an initial strong oxidative attack mainly against lignin then followed by longer-term expression of hydrolytic CAZymes. Among others, the wood transcriptome revealed expression of AA9 LPMOs thus indicating their importance in oxidative cleavage of carbohydrate polymers.

(1) Kuuskeri J (2015) BMC Microbiol, 15:217
(2) Salavirta H (2014) PLoS ONE, 9(5): e97141
(3) Kuuskeri J (2016) Biotech Biofuels, 9:192

75F Overexpression of a C_4-dicarboxylate transporter is the key for converting citric acid production to C_4-dicarboxylic acid production in *Aspergillus carbonarius*. Lei Yang, Eleni Christakou, Jesper Vang, Mette Luebeck, *Peter Luebeck* Chemistry and Bioscience, Aalborg University, Aalborg, DK.

C_4-dicarboxylic acids, including malic acid, fumaric acid and succinic acid, are valuable organic acids that can be produced and secreted by a number of microorganisms. *Aspergillus carbonarius* is capable of producing high amounts of citric acid, however, all attempts to change the citric acid production into C_4-dicarboxylic production by pathway engineering have been with very limited success.
In this study, a glucose oxidase deficient strain of *A. carbonarius* was used as the parental strain to overexpress a native C_4-dicarboxylate transporter and the *frd* gene encoding fumarate reductase from *Trypanosoma brucei* individually and in combination to investigate their impacts on organic acid production. Overexpression of the C_4-dicarboxylate transporter alone and in combination with the *frd* gene significantly increased the production of C_4-dicarboxylic acids and reduced the accumulation of citric acid, whereas expression of the *frd* gene alone did not result in any significant change of the organic acid production profile.
This study demonstrates that the key to change the citric acid production into production of C_4-dicarboxylic acids in *A. carbonarius* is the C_4-dicarboxylate transporter. Furthermore it shows that the C_4-dicarboxylic acid production in *A. carbonarius* can be further increased via metabolic engineering.

76W Engineering the smut fungus *Ustilago maydis* for the degradation of pectin. P. Stoffels[1,3], E. Geiser[2,3], L.M. Blank[2,3], M. Feldbrügge[1,3], N. Wierckx[2,3], *K. Schipper*[1,3] 1) Institute for Microbiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany ; 2) iAMB – Institute of Applied Microbiology, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany ; 3) Bioeconomy Science Center (BioSC), c/o Forschungszentrum Jülich, 52425 Jülich, Germany.

The microbial conversion of plant biomass components to valuable products in a consolidated bioprocess would greatly increase the ecologic and economic impact of a biorefinery. Plant-pathogenic fungi are promising candidates for biomass valorization, because they contain a vast repertoire of hydrolytic enzymes to sustain their lifestyle. However, expression of the corresponding genes is usually tightly regulated and mostly restricted to the pathogenic phase. We use the biotrophic smut fungus *Ustilago maydis* for the degradation of plant

cell wall components by activating its intrinsic enzyme potential during axenic growth. This fungal model organism is equipped with a potent set of hydrolytic enzymes, and moreover, it naturally produces value-added substances such as organic acids and biosurfactants. To achieve the deregulated expression of hydrolytic enzymes during the industrially relevant yeast-like growth in axenic culture, the native promoters of the respective genes were replaced by constitutively active synthetic promoters. This led to an enhanced conversion of biomass components like xylan, cellobiose, and polygalacturonic acid to fermentable sugars. Currently, the intrinsic fungal repertoire is activated and in parallel supplemented with potent heterologous enzymes for the degradation of pectin. Importantly, this can be achieved using both conventional secretion for enzymes of eukaryotic and unconventional secretion for enzymes of bacterial origin. In summary, our results demonstrate the potential applicability of activating the expression of native hydrolytic enzymes from phytopathogens in a biocatalytic process.

77T Upscaling of cellulase enzyme production using *Thermoascus aurantiacus*. *Timo Schuerg*[1,2], Raphael Gabriel[1,2], Jan-Philip Prahl[1,2], Simon Harth[1,2], Ziyu Dai[3], Beth Hofstad[3], Jon Magnuson[3], Steven Singer[1,2] 1) Lawrence Berkeley National Laboratory, Berkeley, CA; 2) Joint BioEnergy Institute (JBEI), Emeryville, CA; 3) Pacific Northwest National Laboratory, Richland, WA.

Thermophilic filamentous fungi are a valuable source of thermostable cellulases. *Thermoascus aurantiacus*, often isolated from self-heating compost piles, is a true thermophile with a growth optimum at 50°C. Here we report successful scale-up of enzyme production by *T. aurantiacus* to 20 L scale. We demonstrated that MXP (Methyl beta-D-xylopyranoside), a non-metabolizable xylose analog, strongly induces cellulases in batch cultivations, whereas metabolizable xylose is much less potent. Based on our observations we hypothesized that carbon catabolite repression (CCR) might be responsible for the inhibited induction of xylose. To test our hypothesis, we established an easily deployable fed-batch system for the 50 mL shake flask scale, which allowed us to circumvent CCR by feeding only small amounts of xylose over time. When continuously feeding only 78 mg xylose h^{-1} L^{-1}, a 5x increase in cellulase induction was achieved as compared to feeding the same amount of xylose in one pulse. The system proved to be successful in optimizing fed-batch cultivation conditions at 50 mL scale and was successfully transferred to 2 L and 20 L pilot scales with crude enzyme titers ranging from 1.1 - 3.2 g/L. In parallel, we established a transformation system and successfully engineered a *Dku70* strain, which will facilitate strain engineering strategies based on homology directed repair. Current efforts focus on genetically disrupting CCR, overexpressing the two major activators of cellulase genes, *clr1* and *clr2*, and disrupting unfavorable protease activity. Furthermore, we established procedures for classical mutagenesis and screening and developed a system for obtaining recombinant progeny from sexual crosses. Establishing CRISPR/Cas9 genome editing technology is ongoing. In summary, *T. aurantiacus* has great potential to become an attractive production platform for thermostable cellulases.

78F Expression platforms impact the surface modulating properties of *Trichoderma* class II hydrophobins. Agnes Przylucka[1,2], *Gunseli Bayram Akcapinar*[1,7], Klaus Bonazza[3,8], Thiago M. Mello-de-Sousa[4], Astrid R. Mach-Aigner[4], Erik Reimhult[5], Hinrich Grothe[6], Christian P. Kubicek[1,2,9], Irina S. Druzhinina[1,2] 1) Microbiology Group, Research Area Biochemical Technology, Institute of Chemical Engineering, TU Wien, Vienna, AT; 2) ACIB GmbH, c/o Institute of Chemical Engineering, TU Wien, Vienna, AT; 3) klaus.bonazza@tuwien.ac.at, TU Wien, Vienna, AT; 4) Synthetic Biology and Gene Technology Group, Research Area Biochemical Technology, Institute of Chemical Engineering, TU Wien, Vienna, AT; 5) Institute for Biologically Inspired Materials, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, AT; 6) Institute of Materials Chemistry, TU Wien, Vienna, AT; 7) present address: Department of Statistics and Medical Informatics, School of Medicine, Acibadem University, Istanbul, TR; 8) present address: Program in Cellular and Molecular Medicine and Division of Hematology, Department of Medicine, Boston Children's Hospital, and Department of Biological Chemistry and Pharmacology, Harvard Medical School, 3 Blackfan Circle, 02215 Boston, M; 9) present address: Steinschötelgasse 7, 1100 Vienna, AT.

Hydrophobins are small proteins secreted by filamentous fungi that are characterized by eight conserved cysteine residues forming four intramolecular disulfide bonds; they show an extremely high surface activity and spontaneous formation of amphipathic layers that convert the surface from hydrophilic to hydrophobic and vice versa. These remarkable properties have consequently been investigated for many industrial and medical applications. Despite this interest, few studies have been conducted towards production of hydrophobins. In this paper, we have compared the heterologous production of two class II hydrophobins from *Trichoderma virens* (HFB4 and HFB7) in two canonical hosts, *Escherichia coli* and *Pichia pastoris*. While the production in both systems was rather similar (0.25 – 0.4 g/L), the ability to bind to hydrophilic and hydrophobic surfaces differed depending on the host used. Structural analysis of the produced proteins in solution by circular dichroism and dynamic light scattering, as well as analysis of surface binding by atomic force spectroscopy and quartz crystal microbalance with dissipation analysis revealed significant differences between the proteins produced in *E. coli* and *P. pastoris*. Our data show that the expression platform significantly influences the structure of hydrophobins, their surface modulating properties and behaviour in solutions; however, the impact is also protein-specific. This effect should be considered when hydrophobins are produced for particular applications.

79W Lichen symbiosis between *Caloplaca flavorubescens* and *Trebouxia gelatinosa*. S.Y. Park[1], J.A. Kim[1], M.H. Jeong[1], C. H. Park[1], J. J[2], Y.H. Lee[2], *J.S. Hur*[1] 1) Korean Lichen Research Institute, Sunchon National University, Suncheon, KR; 2) Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea.

Lichens are symbiotic organisms, composed of a fungal partner (the mycobiont) and at least one eukaryotic algal or cyanobacterial species (the photobiont). As demonstrated by the world-wide distribution of lichens in various kinds of habitats from the tropics to the Polar region, lichen symbiosis seems to be a highly successful adaptation to a diverse range of environmental conditions. For establishment of successful lichen symbiosis, it requires a mycobiont to associate with an appropriate free-living photobiont or arrest lichenized photobiont cells. In order to understand the symbiosis, a total of five lichen-forming fungal isolates and their partner algal isolates were selected to analyze in ultrastructural and molecular levels using our developed resynthesis method. A typical secondary metabolites of *Caloplaca flavorubescens* were produced and their morphological developments showed soredia-like clusters after 4 to 6 weeks co-culturing combination in *C. flavorubescens*. Thus, we monitored the lichenization processes by light microscopes, scanning electron microscope, and transmission electron microscope. RNA sequencing analysis was used to investigate gene expression profiles during resynthesis of *C. flavorubescens*. We then analyzed expression patterns of effector-type small secreted proteins (SSPs). We reveal that the six most highly

expressed SSPs with unknown function are consistently expressed during resynthesis. Using antibody of the six SSPs, we provide evidence for the six genes involved in resynthesis of *C. flavorubescens* with their partner alga. This finding will enhance our understanding of the adaptive evolution of the lichen-forming fungi with the algae to their ecological niches.

80T Growing *Trichoderma reesei* on a mix of carbon sources reveals links between development and cellulase production. *A. Pirayre*, D. Ivanoff, E. Jourdier, A. Margeot, L. Duval, Frederique Bidard Biotechnology, IFP Energies nouvelles, Rueil-Malmaison, FR.

The degradation of cellulose and hemi-cellulose molecules into simpler sugars such as glucose takes part of the biofuel production process. Hydrolysis of lignocellulosic substrates is naturally performed by enzymes produced and secreted e.g. by fungus *Trichoderma reesei*. For decades, numerous strains of *T. reesei* have been developed in order to improve its enzyme production level. Here, we focus on the hyper-producer strain RutC30, which notably differs from the wild type QM6a by three rounds of mutagenesis leading to many genetics alterations (SNV, chromosomal translocations, deletions). It is important to note that one of the key mutations is the truncation of Cre1, which is responsible of the carbon catabolite repression.

In this work, we studied the impact of the carbon source on the cellulase production. For this purpose, different mixtures of glucose and lactose were used as a substrate for the cellulase production phase in fed-batch mode (after an initial growth phase on glucose in batch mode), while keeping the global amount of carbon source constant. RNA extraction was performed 24h and 48h after production start, and a transcriptomic analysis was carried out. We found 650 genes differentially expressed in at least one of the studied condition, where the 100% glucose was taken as reference. A clustering (K-means algorithm) allows us to separate the 650 genes into 5 classes. In addition, the gene regulatory network (GRN) built using the BRANE Cut software reveals three kinds of sub-networks linked to i) cellulase mechanism, ii) beta-glucosidase mechanism and iii) development. These results improve our understanding regarding the regulation of cellulase production in *Trichoderma reesei*.

Cell Biology and Development

81F Shrink, sputter and fade away: How azoles kill *Aspergillus fumigatus*. Bernadette Geißel[1], Laura Sturm[1], Zhaojun Zhu[1], Cees A. M. J. J. van den Hondel[2], *Johannes Wagener*[1] 1) Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universität München, Munich, Germany; 2) Molecular Microbiology and Biotechnology, Institute of Biology Leiden, Leiden University, Leiden, Netherlands.

Aspergillus fumigatus is an airborne opportunistic pathogen. In immunocompromised patients this mold causes severe systemic infections, a disease that is called invasive aspergillosis. Azole antifungals such as voriconazole are currently recommended as first-line treatment of invasive aspergillosis. This drug class exerts a fungicidal activity on *Aspergillus* species. In *Candida* species, however, azoles are primarily fungistatic. Azoles disrupt the ergosterol biosynthesis pathway by inhibition of the lanosterol 14-α-demethylase. While this mechanism is well characterized, the exact physiological consequences remain largely unknown. In order to understand the increased azole resistance of mitochondrial dynamics mutants (Neubauer *et al.*, Mol Microbiol. 2015 Dec;98(5):930-45) we studied the effect of the azole voriconazole on the fungal physiology of *A. fumigatus*. By exploiting new reporter systems we identified different variants of fungal death caused by the exposure to voriconazole. In addition, we observed an irregular deposition of cell wall polymers which indicates a defect in cell wall biogenesis and could contribute to the fungicidal activity. All the death variants are ultimately linked to the specific inhibition of the lanosterol 14-α-demethylase because conditional downregulation of this enzyme invokes very similar phenotypes. Furthermore, our results indicate that the increased azole resistance of the mitochondrial dynamics mutants is probably not directly linked to dysfunctional mitochondrial fission or fusion processes. In parallel, we performed an RNA-Seq analysis of the mitochondrial dynamics mutant transcriptome. Our results indicated the upregulation of various efflux pumps (approx. 8 ABC and 18 MFS transporters) as well as differential regulation of multiple transcription factors and thereby suggest the activation of a multidrug resistance network. To further dissect this possible network we constructed a number of conditional ABC transporter and transcription factor mutants to characterize their roles in drug resistance. We could identify several genes whose expression influences the azole resistance of *A. fumigatus*.

82W An oxylipin signal mediates hyphal branching in pathogenic Aspergilli. *M. Niu*[1], G. Fischer [1], N. Keller[1,2] 1) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, USA; 2) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA.

Oxylipins are a group of diverse oxygenated polyunsaturated fatty acids found in all eukaryotes that modulate growth, development, and cellular communication. Three oxylipin generating oxygenases, PpoA (Psi producing oxygenase A), PpoB, and PpoC, mediate development and stress responses in pathogenic *Aspergillus* species. However, the cellular targets of these oxylipin metabolites, their cellular functions, and signal transduction pathway(s) transmitting the signal are yet to be investigated. Our laboratory has recently identified that exogenous treatment of 5(S),8(R)-dihydroxide octadecadienoic acid (5,8-diHODE), the final oxylipin product of PpoA, resulted in stunted apical growth, increased lateral growth or hyper-branching, and decreased septal distance in the human fungal pathogen *A. fumigatus* and plant pathogen *A. flavus*. Our results suggested that the observed hyperbranching phenotype is specific to C18 diol-oxylipin acids with specific structural features yet to be identified. In addition, the branching phenotype by 5,8-diHODE treatment was remediated by high amount of Ca^{2+} to the wildtype level, suggesting that Ca^{2+} is involved in oxylipin signal transduction in the pathogenic Aspergilli species.

83T Understanding cell tropisms in *Aspergillus fumigatus* hyphae. *P. Geranios*[1], N. Al-Furaji[1], K. Lord[2], M. Bromley[1], N. Read[1] 1) Manchester Fungal Infection Group, University of Manchester, Manchester, GB; 2) University of Edinburgh, Edinburgh, GB.

Fungal cell tropisms involve directional changes of growing fungal cells or hyphae in response to an external stimulus. Negative cell tropisms are ubiquitous in filamentous fungi. Two clear examples are: (1) avoidance of adjacent germ tubes during colony initiation; and (2) avoidance of adjacent vegetative hyphae and branches at the colony periphery. Both tropisms have been proposed to be important in reducing the competition of neighbouring germ tubes/hyphae for nutrients. Despite the widespread occurrence of negative tropisms in fungi, little is known about the signalling processes governing the phenomenon. The aim of our work is to understand the mechanistic basis of negative cell tropism (self-avoidance) during spore germination and hyphal growth in the human pathogen Aspergillus fumigatus. To this

end, confocal live-cell imaging and quantitative image analysis was employed to image and measure the angles formed between germlings when visualized in both 2D and 3D as the conidial germlings invade the agar substrata of different hardnesses. The angles formed between germlings were found to be dependent on the number of germlings in a given group. Our time-lapse imaging data indicated that re-arrangement of the growth axis occurs almost immediately when hyphae approach each other. Furthermore, the avoiding hyphae also exhibited a propensity to invade their agar substratum and this was influenced by its hardness. Mutants blocked in secondary metabolite synthesis or in the presence of a NO scavenger or CO_2 indicated that the avoidance signal was probably not a secondary metabolite or NO. A kinase knockout library of 96 mutant strains of A. fumigatus has been screened with the aim of identifying strains defective in hyphal self-avoidance. The initial screening has involved classifying the mutants into three categories based on their growth phenotype: i) no obvious effect on polarized growth, ii) possibly defective in self-avoidance, and iii) major growth defects. During the kinase mutant screen we have found out that the best way to visualise negative tropisms in a screen like this is to image leading hyphae at the periphery of approaching colonies.

The chemical signal responsible for these negative tropisms has not yet been identified but is suspected to be likely a volatile because it occurs on cellophane overlying growth medium. Currently, the volatiles released by the developing colony are being identified and analysed.

84F Components of the *Aspergillus fumigatus* Ras Post-translational modification pathway regulate conidial viability, hyphal growth, and virulence. *Q. Al Abdallah*, TA Norton, JR Fortwendel Department of Clinical Pharmacy, UTHSC, Memphis, TN.

The Ras superfamily is comprised of highly conserved, membrane-associated GTPase proteins and activate several downstream signaling cascades. Localization of Ras proteins to the plasma membrane (PM) is an essential step for fungal growth and pathogenesis. In order to be properly localized, Ras proteins must undergo multiple steps of post-translational modifications (PTMs) at the conserved C-terminal CAAX box. In yeast and mammalian cells, these steps include farnesylation, followed by protease-mediated cleavage of the -AAX amino acids, carboxymethylation, and palmitoylation. Our *in silico* analysis suggests that this pathway is conserved in *Aspergillus fumigatus*. Additionally, our previous work shows that palmitoylation of *A. fumigatus* RasA is required for PM localization and for full virulence. The purpose of this study is to investigate the roles of the sole farnesyltransferase complex, as well as the CAAX protease and the methyltransferase in the growth of *A. fumigatus*. To achieve this, we deleted the genes that encode for RasA PTM homologues in *A. fumigatus* and characterized the deletion mutants for their growth rates, RasA localization and activation, and fungal virulence. Conidial germination, overall growth rate, and virulence were all reduced in the farnesylation-deficient mutant, although hyphal morphology appeared normal. Conidial viability was also decreased in the farnesylation mutant and was associated with formation of anuclear conidia. As expected, loss of farnesylation led to decreased RasA PM association. Surprisingly, fungal growth and development was not affected by either single or double deletion of the enzymes contributing to CAAX proteolysis. In addition, RasA localization to the PM and steady-state activation of RasA in the double mutant was unperturbed. However, loss of one of the CAAX proteolysis homologs, *rce1*, is associated with increased sensitivity to cell wall disruption. Together, our results suggest that CAAX proteolysis is not an essential step for fungal growth or regulation of RasA in *A. fumigatus*, whereas farnesylation contributes heavily to these processes.

85W A palmitoylated protein ArkA and its putative targeted proteins in *Aspergillus*. *Yuanwei Zhang*[1,2], Congcong Sun[1], Ling Lu[1] 1) Nanjing Normal University, Nanjing, China; 2) University of Dundee, Dundee, GB.

Finely tuned changes in cytosolic free calcium ($[Ca^{2+}]_c$) mediate numerous intracellular functions resulting in the activation or inactivation of a series of target proteins. Palmitoylation involves the reversible posttranslational modification that addition of palmitate to cysteines and promotes protein binding and subcellular localization, yet the relationship between palmitoylation and calcium signaling is poorly understood. Here, we present proof that the yeast palmitoyl transferase ScAkr1p homolog, AkrA in *Aspergillus nidulans*, regulates $[Ca^{2+}]_c$ homeostasis and plays an important role in hyphal growth and conidiation under low calcium conditions. We utilized calcium reporter aequorin expression system and showed that the $[Ca^{2+}]_c$ responses in *akrA* mutants to high extracellular calcium or ER/plasma membrane stress were defective. Furthermore, we demonstrated that all of these effects on the $[Ca^{2+}]_c$ responses mediated by AkrA were tightly related to the cysteine of the DHHC motif which is required for AkrA auto-palmitoylation. When auto-palmitoylation of AkrA was inhibited, by either mutation of cysteine or truncated the DHHC motif, the susceptibility of the mutants to azole drugs was increased. By acyl-biotin exchange assay, we identified potential substrates palmitoylated by AkrA including calcium-related proteins and ergosterol biosynthetic proteins which involved in azole drug resistance in *A. nidulans*. In addition, deletion of AkrA homolog in *A. fumigatus* displays a similar phenotype of the colony growth defect to that in *A. nidulans*, indicating function of AkrA in *Aspergillus* or in fungi might be conserved. Thus, this study provides new insights into the relationship among palmitoylation, calcium homeostasis and azole resistance, which has implications for the development of novel antifungal drugs and therapeutic methods. Further details about the cellular function for putative proteins targeted by AkrA in *A. fumigatus* are ongoing.

86T Comparative analysis of the function of α-1,3-glucan synthases, AgsA and AgsB, in *Aspergillus nidulans*. *K. Miyazawa*[1], A. Yoshimi[2], S. Yano[3], S. Kasahara[4], F. Hasegawa[2], K. Abe[1,2] 1) Grad. Sch. Agric. Sci., Tohoku Univ., Sendai, JP; 2) NICHe, Tohoku Univ., Sendai, JP; 3) Yamagata Univ., Yonezawa, JP; 4) Miyagi Univ., Sendai, JP.

Although α-1,3-glucan (AG) is one of major polysaccharides in the cell wall of *Aspergillus* species, the biological function of AG remains unclear, except for the role as a virulence factor in some pathogenic fungi. Previously, we carried out functional analysis of two α-1,3-glucan synthase (AGS) genes (i.e. *agsA* and *agsB*) in the model filamentous fungus *Aspergillus nidulans*. The *agsB*? strain lost most cell wall AG, suggesting that a main AGS in this fungus is AgsB. Interestingly, the hyphae of the *agsB*? strain was dispersed under liquid culture conditions, whereas the wild-type strain formed hyphal pellets under same culture conditions. These results suggest that AG has the role as an adhesive factor for hyphal cells. On the other hand, because the expression of *agsA* gene was scarcely detected under normal growth conditions and the *agsA*? strain did not show phenotypic defects, the role of *agsA* remains unclear. In this study, in order to investigate the roles of AgsA and AgsB in cell wall AG synthesis, we comparatively analyzed cell wall polysaccharides synthesized by AgsA and AgsB. First, we constructed *agsA* or *agsB* gene overexpression (O/E) strain by replacing the promoter region of *agsA* or *agsB* with *tef1* promoter under the genetic background of the other *AGS* gene disruption, and confirmed the high expression of either of the *AGS* genes in the O/E strains. The O/E of *agsA* restored the growth characteristics of the *agsB*? strain under liquid culture conditions: the O/E *agsA* strain formed the hyphal pellets. This suggests that the *agsA* gene encodes a functional AGS. To elucidate the differences of cell wall structure between

these two strains, we performed the alkaline-fractionation of cell wall and analyzed the sugar composition of the fractions. The carbohydrate analyses revealed that the sugar compositions of the AS2 fraction were similar in these two strains, but the texture of the AS2 fraction derived from these two strains was markedly different from each other, suggesting that the detailed chemical structure of AG obtained from the O/E *agsA* differs from that of AG derived from the O/E *agsB* strains.

87F Understanding the role of septin AspD in the early vegetative growth of *Aspergillus nidulans*. *I. Dorter*, M. Momany Plant Biology, UGA, Athens, GA.

Basic research on septin dynamics in the model organism *Aspergillus nidulans* helps us better understand the cellular and molecular mechanisms of these cytoskeletal elements and can help develop new therapeutic approaches against diseases. Under the right conditions *Aspergillus nidulans* spores emerge a germ tube to explore new growth medium. Thereby fungal hyphal tips grow into the food substrate forming a hyphal network with evenly spaced lateral branches. This basic process requires a fine and precise coordination of biochemical and cellular processes. We performed single deletions of the genes encoding for the core septins in *A. nidulans* (*AspA-D*). In the absence of AspA, AspB and AspC we could observe an abnormal increase of lateral branches, which is also known as hyperbranching. The knock-out of the fourth core septin gene *AspD* did not result in obvious phenotypic changes whereas fluorescence microscopy revealed abnormal nuclear structures that worsened with each mitotic division. In contrast to WT the average distance between two neighboring nuclei was greatly reduced in the *?aspD* mutant background, whereas the nuclei themselves were slightly elongated. Time-lapse analyses with a strain expressing AspD-GFP fusion protein showed septin bars contacting both nucleus and cell cortex. Shortly after nuclear division the septin bar located between the two newly divided nuclei suggesting that the septin AspD plays a role after mitosis. In addition, the disruption of the septins revealed an increased number of cells harboring an odd number of nuclei. This may point to an asynchronous nuclear division cycle of the multinucleated fungus in the absence of core septins. We asked the question whether septins could be part of a checkpoint that regulates nuclear division by monitoring cell cycle progression. We treated WT and septin deletion mutants with benomyl, which depolymerizes microtubules and inhibits spindle formation thereby activating the spindle assembly checkpoint. In contrast to the other cells all *ΔaspD* mutants showed changes in conidiospore density and color, a sectoring phenotype, which is the result of chromosome missegregation. This supports the idea that the SAC checkpoint did not function properly in the absence of AspD. We were able to construct a strain, which expresses both AspB-CFP and AspD-GFP and could confirm that at least two distinct septin heteropolymer populations co-exist. Future time-lapse analyses will show if these two populations play different roles during nuclear division in *A. nidulans*.

88W ZtfA is a novel regulator of asexual development, secondary metabolism and oxidative stress defense in *Aspergillus nidulans*. K.G. Thieme, *J. Gerke*, C. Sasse, S. Thieme, O. Valerius, G.H. Braus Institute of Microbiology and Genetics, Georg-August University, Göttingen, DE.

In the filamentous fungus *Aspergillus nidulans*, asexual and sexual development are tightly interconnected with secondary metabolism, orchestrated by the velvet protein transcriptional network. Here, we analyze the new $Zn(II)_2$-Cys_6 transcription factor ZtfA, which regulates asexual development, secondary metabolite production and oxidative stress response downstream of the velvet factor VosA. A *ztfA* deletion strain produces drastically diminished numbers of conidiospores, whereas a *ztfA* overexpression strain forms conidiophores even in vegetative growth, where normally only vegetative mycelia is formed. Moreover, conidiospores show a rapid loss in viability in ?*ztfA*. Genetic analyses indicate that ZtfA activates the conidiation pathway via the major regulator encoding *brlA*. In the absence of *ztfA* the fungus is not able to produce the secondary metabolites austinol and dehydroaustinol, whereas the biosynthesis of sterigmatocystin in ?*ztfA* as well as in the overexpression of *ztfA* is increased. In addition, ZtfA is involved in the oxidative stress response system. In the absence of *ztfA*, the fungus shows an increased tolerance towards H_2O_2 compared to the wild type.

89T Control of growth and development by lipid asymmetry in *Aspergillus nidulans*. *Z.S. Schultzhaus*[1], W. Zheng[2], G. Cunningham[1], Z. Wang[2], R. Mouriño-Pérez[3], B. D. Shaw[1] 1) Plant Pathology and Microbilogy, Texas A&M University, College Station, TX; 2) State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, China; 3) Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, México.

The constituents of the plasma membrane, primarily lipids, are continuously in flux through vesicle fusion, budding, diffusion, chemical alterations, and transport between leaflets of the bilayer. The mechanisms that maintain order in fungal plasma membranes, particularly at the actively growing hyphal tip, are not well understood. One group of proteins that regulates eukaryotic plasma membranes is the phospholipid flippases, which are a distinct class of P-type ATPases (a superfamily of transporters which includes Na^+/K^+ pumps and calcium pumps) that move phospholipids toward the cytosolic plane of membranes (e.g. Golgi, endosomes, and the plasma membrane). Two of the four *Aspergillus nidulans* flippases, DnfA and DnfB, were previously found to in different regions of the hyphal tip and to share a function essential for hyphal growth. Here, we report on the function and localization of DnfA and DnfB, and another *A. nidulans* flippase, DnfD, in more detail. DnfA and DnfB exhibited different recovery rates within the Spitzenkörper, in agreement with them being transported on different populations of vesicles. Deletion of *dnfB* alone does not affect Spitzenkörper dynamics, but deleting *dnfA* results in a Spitzenkörper that is unstable and diminished. Only a simultaneous downregulation of both (through repression of *cdc50*), however, results in a dramatic mislocalization of secretory and endocytic proteins, suggesting that their roles in the secretory and endocytic pathways overlap substantially. Next, we looked at the localization and function of another flippase, DnfD. DnfD localizes earlier in the secretory pathway (almost overlapping exclusively to the TGN/late Golgi), but appears to play no role in hyphal growth. Rather, a *dnfD* deletion exhibits severe defects in both production of conidia (asexual reproduction) and conidiophore morphology. Combinatorial analysis of flippase mutants, additionally, suggests unique relationships are shared between these proteins in fungal growth and development.

90F Proteins TaoA and EcoA localize to the apex and sub-apical collar of growing hyphae in *Aspergillus nidulans*. *B. Commer*, Z. Schultzhaus, B. Shaw Plant Pathology & Microbiology, Texas A&M University, College Station, TX.

Filamentous fungi produce polarized cells called hyphae. Polarization of hyphae is dependent on a balance between endocytosis and exocytosis that may be maintained through apical recycling. This balance requires the Spitzenkörper (SPK), a secretory body found only in growing hyphae, and a sub-apical collar 1-5µm immediately distal to the SPK that is enriched for endocytosis. Evidence of this relationship is observed through the investigation of landmark proteins along the plasma membrane that mark areas of growth. These proteins are

displaced along the membrane as growth occurs and new membrane is added. Here, an informatics approach was used to identify proteins in *Aspergillus nidulans* that contain an endocytic NPFxD motif. The long-term goal of this project is to observe the kinetic localization and examine the predicted endocytic association of these NPFxD motif-containing proteins during hyphal growth using fluorescent markers and live-cell imaging. Two of the 42 proteins, TaoA and EcoA, were selected based on previous studies of orthologs in yeast. The TaoA ortholog, Tao3, is a component of the RAM signaling network, which is involved in cell morphology and localizes to polarization sites. The EcoA ortholog, Pal1, is of unknown function and is found to localize to endocytic sites in yeast. Here, strains expressing either TaoA::GFP or EcoA::GFP were constructed and visualized to document dynamic localization during hyphal growth. TaoA::GFP localization was observed at the apical crescent, a cortical area found at the extreme apex in growing hyphae, proximal to the SPK. TaoA::GFP localization terminated in an area that corresponds to the sub-apical collar, suggesting that it may be cargo for endocytic uptake. EcoA::GFP localized to the endocytic collar, and was not observed in the apical crescent. Continuing work for both proteins will include verification of the endocytic association and co-localization with known tip-localized endocytic and exocytic machinery.

91W Developmental regulation by constitutive activation and inactivation of MpkB MAPK pathway in *Aspergillus nidulans*. Sang-Cheol Jun[1], Kwang-Yeop Jahng[2], Jong-Hwa Kim[3], *Kap-Hoon Han*[3] 1) Institute for Genetic Engineering, Chonbuk National University, Jeonju, South Korea; 2) Department of Life Sciences, Chonbuk National University, Jeonju, South Korea; 3) Department of Pharmaceutical Engineering, Woosuk Univ, Wanju, Jeonbuk, South Korea.

The *mpkB* gene is a member of the multiple MAPK pathways in *Aspergillus nidulans*. The MpkB has been known to play a key role in the asexual and sexual development and secondary metabolite production. The MAPK kinase MkkB can be phosphorylated at Ser218 and Thr222 residues within the activation loop site by SteC MAPKK kinase and consequently, activates MpkB by phosphorylating at threonine and tyrosine residues of MpkB in *A. nidulans*. By introducing negatively charged (aspartic acid) or non-polar (proline and lysine) residues that may mimic the effect of phosphorylation at positions 218 and 222 in MkkB, we constructed mutants which constitutively phosphorylate MpkB MAPK and constitutively non-phosphorylate MpkB MAPK. Expression of the constitutively activated MkkB in *A. nidulans* led to the hyper-activation of MpkB MAPK and resulted in a great elevation of sexual development, while asexual sporulation was remarkably reduced. In contrast, constitutively inactivated MkkB mutant could not produce sexual organs, while asexual sporulation and sterigmatocystin production were normally processed. Differential expression pattern of proteins among these mutants were analyzed by using the 2D proteomic analysis, which revealed that expression level of 375 spots are significantly different.

92T An intrinsically disordered domain of the VelB velvet domain provides specificity for heterodimer formation in *Aspergillus nidulans*. *S. Thieme*, J. Gerke, C. Sasse, O. Valerius, K. G. Thieme, G. H. Braus Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany.

Genetic networks are often controlled by DNA binding proteins, which act as homo- or heterodimers. The velvet domain represents a conserved fungal DNA binding and dimerization domain of approximately 150 amino acids, which is reminiscent to the *rel* domain of NF-kB in the mammalian immune and infection response [1]. Aspergilli possess four members of velvet family proteins (VeA, VelB, VelC and VosA), which can form homo- or heterodimers and are required to control development coordinated with the appropriate interconnected secondary metabolism and spore viability [2]. VeA interacts with several epigenetic methyltransferases and shuttles VelB as a VeA-VelB heterodimer into the nucleus [3, 4]. VelB is also part of the VelB-VosA complex supporting reduced and delayed asexual spore formation and promoting spore viability. Only the velvet domain of VelB is interrupted by an insertion of additional amino acids in ascomycetes. The *A. nidulans* insert consists of 99 amino acids and is conserved among Aspergilli. We compared a VelB with or without insert and found that the insert is required for an accurate light control which normally promotes asexual development and reduces sexual development as well as secondary metabolite production [5]. This is reflected on the molecular level by the finding that the VelB interaction partners with insert differ to the VelB interaction partners without insert. This suggests that the insertion changes the potential of velvet domains and provides a surface for altered protein-protein interactions.

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93F Molecular characterization of microtubule organizing centers(MTOCs) in the filamentous fungus *Aspergillus nidulans*. *Xiaolei Gao*, Ying Zhang, Marjorie Schmid, Reinhard Fischer Department of Microbiology, Institute for Applied Bioscience, Karlsruhe, DE.

Microtubule polymerization starts in fungal and animal cells from large multi-subunit protein complexes, the microtubule-organizing centers (MTOCs).In the model fungus *Aspergillus nidulans* septum-associated MTOCs(sMTOCs) together with the spindle pole bodies nucleate microtubules. We focused on the structure and composition of sMTOCs and how they are formed during septation. We show that in *A. nidulans* several SPB outer plaque proteins are conserved at sMTOCs, such as the gamma-tubulin ring complex (γ-TuRC), the centrosomin-domain (CM) containing protein ApsB and the disordered protein Spa18. The latter two proteins were required for recruiting the γ-TuRC component GcpC to sMTOCs and are necessary for microtubule formation from septa. Another intrinsically disordered protein, Spa10, colocalized with tropomyosin (TpmA) at the beginning of septation, while ApsB and Spa18 were only found at mature septa. Spa10 was necessary for ApsB and Spa18 recruitment and thus essential for septal microtubule nucleation. Whereas Spa10 formed a central disk in the septal pore, Spa18 and ApsB formed a discontinuous ring structure with several punctate foci attached to the rim of the Spa10 disk. Different from eMTOCs in *Schizosaccharomyces pombe*, which are only transiently formed during septation, sMTOCs in *A. nidulans* are assembled after septum formation and represent stable structures in hyphal compartments.

94W Identification and functional characterization of a Bcl-2 associated athanogene in *Aspergillus nidulans*. *Sachin Jain*[1], Nancy Keller[2], Mehdi Kabbage[1] 1) Plant Pathology, University of Wisconsin-Madison, Madison, WI; 2) Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI.

The Bag (Bcl-2 associated athanogene) family is a multifunctional group of proteins that is involved in multiple cellular processes ranging from apoptosis to tumorigenesis. As evolutionarily conserved proteins, they are distinguished by a common conserved region known as the Bag domain. In animals and plants, Bag genes are believed to function as adapter proteins forming complexes with signaling molecules and molecular chaperones. In this study, we focus on the model filamentous fungus *Aspergillus nidulans* to study the function of Bags in fungi. The *A. nidulans bagA* gene expression was strongly induced during sexual development and apoptosis inducing insults. In order to characterize this gene, we generated over-expression and knock-out strains of *bagA* in *A. nidulans*. Our results indicate that *ΔbagA* strains are negatively impacted in their sexual development and had an altered secondary metabolite (SM) profile in both sexual and vegetative growth phases when compared to wild type strain. Interestingly, over-expression of BagA leads to increase in sexual/asexual spore ratio. LC-MS analysis suggests that *bagA* may be a negative regulator of secondary metabolism since several SMs were enriched in the *ΔbagA* strain. SMs from microorganisms play a role in niche securement and those produced by fungi have been particularly rich source of useful natural products. Thus, our results suggest that the disruption of cell death pathways could alter the SM profile of fungi and might help uncover novel bioactive compounds, including antimicrobials and pharmaceutical drugs.

95T Citrate overflow by Aspergillus niger is associated with extended pre-autolytic growth. *Baljinder Kaur*, Narayan S Punekar Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra, India-400076.

The filamentous fungus *Aspergillus niger* is commercially important for its production of enzymes, metabolites and organic acids. It has been exploited at industrial scale for the production of citric acid. The citric acid is produced by *A. niger* under a set of stringent fermentation conditions optimized over a century but the mechanisms responsible for this metabolic overflow remain tentative. The two key features of the media for fermentation are limiting nitrogen and trace metal deficiency. Both of them affect the gross morphology of the fungus. In order to better understand the physiological relevance of *A. niger* mycelial morphology on acidogenic growth a detailed microscopic analysis was carried out. This was assisted by using a strain expressing EGFP in the cytoplasm. The major cellular compartments namely, cytoplasm, mitochondria, vacuole and nucleus were monitored under normal and acidogenic growth conditions. While the intracellular morphology remained unaltered throughout the idiophase of fermentation, it corresponded to the pre-autolysis growth phase of mycelia frown on normal media. The hyphae were thin and heavily loaded with vacuoles but were maintained away from immediate autolysis. The continuance of mycelia in the pre-autolysis stage could be a feature responsible for citrate overflow. Possible involvement of autophagy growth during acidogenesis by *A. niger* is explored using fluorescence microscopy and suitable gene deletion strains.

96F A metalloprotease, AdmB, involves cell wall construction and carbon starvation stress response in *Aspergillus oryzae*. *T. Kobayashi*, T. Oiwa, H. Maeda, M. Takeuchi, Y. Yamagata The United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, JP.

Aspergillus oryzae is a mold necessary for Japanese food and brewing. The fungi sense external environment during growing. Genome sequencing and analysis of *A. oryzae* was reported in 2005. As a result, 126 genes predicted to encoding proteases in a total of about 12,000 genes. We found two "a disintegrin and metalloproteinases (ADAMs)" in *A. oryzae* and named them AdmA and AdmB. The ADAMs are membrane proteins and contain two characteristic domains, metalloprotease and disintegrin domain. In mammals, ADAMs play important roles in cell adhesion, protein processing and signaling. Especially, they are able to cleavage and release a ligand for signaling on the cell surface and so are called "sheddase". However, the function of fungal ADAMs has been unknown. We aimed to elucidate biological functions of AdmA and AdmB in *A. oryzae*.

Firstly, to analyze AdmB localization, we constructed an *admB*-enhanced green fluorescence protein (EGFP) strain that highly expressed the AdmB-EGFP fusion protein. Fluorescence microscopy analysis showed that AdmB-EGFP localized to the plasma membrane and some vesicles. Next, we examined *admA* and *admB* expression kinetics by quantitative RT-PCR. As a result, we revealed that the transcription of *admB* was increased in a late growth stage and induced by a carbon starvation stress. Finally, we produced single- and double-gene deletion strains, *?admA*, *?admB* and *?admA?admB* using *pyrG* marker recycling. These strains showed normal growth like a control strain, while two strains, *?admB* and *?admA?admB* were sensitive to cell wall perturbing agents, calcofluor-white and congo red. Moreover, *?admB* showed MpkA phosphorylation at lower concentration of congo red stimulation than the control strain. Taken together, these results suggest that AdmB involves in not only the carbon starvation stress response but also cell wall construction and its integrity. Incidentally, AdmB may play roles in these biological processes by acting like mammalian ADAMs.

97W Identification and characterization of a vegetative incompatibility locus in *Botrytis cinerea*. *Saadiah Arshed*[1,2], Ross Beever[3], Murray Cox[4], James Lee[1,2], Michael Pearson[2], Joanna Bowen[1], Matthew Templeton[1,2] 1) The New Zealand Institute for Plant & Food Research, NZ; 2) The University of Auckland, New Zealand; 3) Landcare Research, New Zealand; 4) Massey University, New Zealand.

*V*egetative *i*ncompatibility (VI) is a fungal non-self recognition system characterized by the inability of genetically distinct conspecific fungal strains to form a viable heterokaryon, and is controlled by multiple polymorphic loci termed *vic* (*v*egetative *i*ncompatibility) or *het* (*het*erokaryon incompatibility). We report here the first *vic* locus that has been genetically identified and characterized in the economically important plant pathogenic, necrotrophic fungus *Botrytis cinerea*. We generated near isogenic lines of interacting compatible and incompatible strains that differed at a single *vic* locus and employed a bulk segregant analysis approach coupled with whole genome Illumina sequencing to map a 55-kb genomic region containing the *vic* locus. Within that locus, we identified two adjacent highly polymorphic open reading frames encoding predicted proteins that contain domain architectures implicated in VI in other filamentous fungi, *Bcvic1* and *Bcvic2*. Deletion of *Bcvic1* and *Bcvic2* individually did not have any effect on vegetative incompatibility. However, deletion of the region containing both *Bcvic1* and *Bcvic2* resulted in gene deletion lines that were severely restricted in growth and also showed loss of VI. Complementation of these mutants by ectopic expression restored the growth and vegetative incompatibility phenotype indicating that *Bcvic1* and *Bcvic2* are interacting and controlling VI at this *vic* locus.

98T Pharmacological and transcriptomic analyses of a *B. cinerea* flavohemoglobin deficient mutant demonstrate that nitric oxide affects germination, DNA replication and cell cycle. Francisco Anta[1], Daniela Santander[1,4], Wilson Acosta[1], Rodrigo Santamaría[2], Pedro San Segundo[3], *José María Díaz-Mínguez*[1], Ernesto P. Benito[1] 1) CIALE-UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN; 2) DPTO. DE INFORMÁTICA Y AUTOMÁTICA, FAC. DE CIENCIAS, UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN; 3) INSTITUTO DE BIOLOGÍA FUNCIONAL Y GENÓMICA, CSIC-UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN; 4) FAC. DE CIENCIAS AGROPECUARIAS Y AMBIENTALES, UNIVERSIDAD TÉCNICA DEL NORTE, IBARRA, ECUADOR.

Nitric oxide (NO) is a highly reactive molecule with fundamental roles in the biology of all living systems. The participation of NO in developmental processes has been described in several fungal species. However, the nature of the mechanisms and factors being affected by NO in fungi is poorly characterized. *Botrytis cinerea* is a plant pathogenic fungus which has attracted much attention given its wide host range and its necrotrophic life style. The production of NO by the fungus has been demonstrated as well as its detoxification by means of a flavohemoglobin enzyme encoded by gene *Bcfhg*1. It has been suggested that the physiological functions of the flavohemoglobin could be related to its involvement in the modulation of endogenous NO levels produced by the fungus during specific developmental stages. Pharmacological studies in which germinating spores and mature mycelium were exposed either to NO donors or to NO scavengers indicate that NO affects germination. From the data obtained, in combination with the results derived from the analysis of the response of *ΔBcfhg*1 germinating spores to NO, it can be concluded that NO exerts and inmediate and transitoy effect on germination efficiency, on germ tube elongation and on nuclear division rate. Global expression analysis of *ΔBcfhg*1 in these conditions detected major changes in the expression pattern with about one third of the genes predicted in the *B. cinerea* genome responding to exposition to NO. Functional enrichment analysis allowed to identify links between exposition to NO, growth arrest and down-regulation of "DNA replication", "nucleolus" and "cell cycle" genes.

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99F Regulation of hyphal guidance by Pxl1 and Cst20 in *C. albicans.* *M.C. Almeida*[1], E.M. Morrison[1], J. Craven[2], N.A.R. Gow[1], A.C. Brand[1] 1) MRC Centre for Medical Mycology, University of Aberdeen, Aberdeen, Aberdeen, GB; 2) Department of Molecular Biology and Biotechnology, The Krebs Institute, University of Sheffield, Sheffield, UK.

Candida albicans lives as a commensal yeast in humans but can cause life-threatening bloodstream infections in susceptible patient groups. The formation of invasive hyphae is a characteristic virulence trait of *C. albicans* and we have shown that the penetration of host tissue depends on the ability of hyphae to steer as they grow. We are investigating the regulatory mechanisms of how hyphae sense guidance cues in the environment and translate these signals to re-orient the direction of growth. We have identified two types of 'steering-locked' mutants and the proteins associated with them. A wavy hyphal phenotype is generated by deletion of Pxl1, a Paxillin-like homologue, and its putative regulatory kinase, Ptk2 (homologue of Focal Adhesion Kinase). In contrast, truncation of Cst20 ($Cst20^{1-549}$) or hyper-activation of the small GTPase, Rho1, cause hyphae to grow uni-directionally. Both the wavy and unidirectional phenotypes correlate with an inability to respond normally to directional cues. Pxl1-GFP localises to bud tip and bud neck in yeast and to the growing tip in hyphae, but not to the septa, suggesting that Pxl1 is involved in the transport or localisation of a specific subset of cell-wall remodelling proteins. RFP-Ptk2 localises throughout the cytoplasm. Although the wavy and unidirectional phenotypes are strikingly different, the Pxl1 and $Cst20^{1-549}$ strains exhibit other phenotypes in common. In yeast, TEM analysis showed both mutants have cell separation defects. In hyphae, they exhibit aberrant intracellular structures. These shared phenotypes suggest that Pxl1 and Cst20 are involved in the same pathway that governs cell growth. We hypothesise that Cst20, a homolog of PAK that negatively regulates Paxillin in humans, may have a similar function in *C. albicans*, suggesting that constant turnover of the Pxl1 complex is required for hyphal steering responses.

100W Rsr1 GTPase regulation is required for hyphal steering and differential effector binding in *Candida albicans* yeast and hyphae. *T. Bedekovic*, A. Brand University of Aberdeen, Aberdeen, GB.

Candida albicans is an opportunistic fungal pathogen that can cause systemic infections in immunocompromised patients, where the formation of invasive hyphal filaments contributes to fatal levels of sepsis and organ failure. Hypha-mediated tissue damage depends on directional growth responses that are regulated by the small N-Ras-like GTPase, Rsr1. In *S. cerevisiae*, Rsr1 is K-Ras-like and specifies the yeast bud site where it is activated by its guanine-nucleotide exchange factor (GEF), Bud5, and de-activated by its GTPase activating protein (GAP), Bud2. However, its role in *C. albicans* hyphae is less understood. To elucidate the role of Rsr1 in hyphae, we generated mutant strains expressing a single copy of Rsr1 in a GDP-locked ($Rsr1^{K16N}$) or GTP-locked ($Rsr1^{G12V}$) form and compared their phenotypes with those of the *rsr1*Δ, *bud5*Δ and *bud2*Δ null strains. Deletion or dysregulation of Rsr1 caused large, rounded yeast cells and shorter hyphae that were unable to steer round obstacles or penetrate agar. Deletion of Rsr1 and Bud2 caused yeast and hyphae to become multinucleate. Strikingly, these defects were seen in the strain expressing $Rsr1^{K16N}$ but not in the *bud5*Δ null, suggesting that Rsr1 may be regulated by a second GEF that functions in hyphal steering responses. The site of Rsr1 activity is thought to be regulated by the localization of the Bud5 and Bud2 regulators at the tip and subapical region of hyphae, respectively. Here we show that YFP-$Rsr1^{K16N}$ and YFP-$Rsr1^{G12V}$ were differentially localised. YFP-$Rsr1^{K16N}$ appeared as patches on the plasma membrane whereas YFP-$Rsr1^{G12V}$ was uniformly localised to the plasma membrane, similar to WT. Early proteomics results using GST-tagged versions of these proteins expressed in yeast and hyphae show that activity state and cell morphology differentially determines the binding partners of Rsr1. These findings suggest a more complex role for the N-Ras Rsr1 GTPase in *C. albicans* than has been described for the K-Ras Rsr1 GTPase in the yeast, *S. cerevisiae.*

101T Two celll wall glycosyl hydrolases are required for the maturation of the N. crassa conidial cell wall. Jie Ao, *Stephen Free* Dept Biological Sci, SUNY Univ, Buffalo, Buffalo, NY.

Using a proteomic analysis, we identified 35 cell wall proteins in mature conidia. A comparison with the proteome of the vegetative hyphae showed that 16 cell wall proteins were shared. These shared proteins were cell wall biosynthetic proteins or cell wall structural proteins. We analyzed deletion mutants for 34 of the genes for phenotypes indicative of conidial cell wall defects. We found that mutants for two cell wall glycosyl hydrolases, the beta-1,3-glucanase CGL-1 (NCU07523) and the exochitinase NAG-1 (NCU10852), have a conidial separation phenotype. These two enzymes function in remodeling the cell wall between adjacent conidia to facilitate conidia formation and dissemination. We generated promoter::RFP or promoter::GFP constructs for the 19 genes encoding what we thought might be conidia-

specific proteins. We demonstrated that the promoters for 15 of the conidia-specific cell wall genes, including cgl-1 and nag-1, provided for conidia-specific gene expression or for a significant increase in their expression during conidiation.

102F Very high expression and incorporation of specific cell wall proteinssecures fast surface expansion during apical growth of *Ashbya gossypii* . R. Rischatsch[1], S. Voegeli[1], M. Finlayson[1], A. Martinez[3], Q. Yuan Yin[3], C. de Koster[3], F. M. Klis[3], *P. Philippsen*[1], P. W. J. de Groot[2,3] 1) Molecular Microbiology, Biozentrum, University of Basel, 4056 Basel, Switzerland; 2) Regional Center for Biomedical Research, University of Castilla–La Mancha, Spain; 3) Swammerdam Institute for Life Sciences, University of Amsterdam, 1018 WV Amsterdam, The Netherlands.

The phytopathogen *Ashbya gossypii* and the yeast *Saccharomyces cerevisiae* are phylogenetically closely related. It is not known how *A. gossypii* has evolved an exclusively hyphal growth mode with very rapid apical extension requiring cell wall expansion rates that are up to 40-fold faster compared to *S. cerevisiae*.

The *A. gossypii* genome codes for 44 putative GPI proteins, 10 without a homolog in *S. cerevisiae*. It also reveales amplification of several cell wall protein-encoding genes, notably *CWP1*. Transcriptome studies show that one third of the CWP-encoding genes are expressed at higher levels than ribosomal protein genes. Mass spectrometric analysis of protein extracts from purified walls of rapidly growing hyphae resulted in the identification of 14 covalently bound cell wall proteins (CWPs). Some CWPs that are common in hemiascomycetes are missing in *A. gossypii*. On the other hand, the chitin deacetylase Cda1/Cda2 was identified in addition to three novel proteins (Agp1, Awp1, and Sod6), all without homologs in baker's yeast (NOHBYs). Phenotypic analysis confirmed the importance of these NOHBYs for cell wall integrity. Interestingly, hyphal walls of *A. gossypii* contain very little chitin, and orthologs of genes required for cell wall remodeling and degradation of septa during cell division in *S. cerevisiae* show low expression or are absent. We conclude that loss of distinct cell wall genes, acquisition of novel genes, and amplification as well as increased expression of evolutionary conserved fungal cell wall genes led to the evolution of fast polar surface expansion of *A. gossypii* hyphae.

103W Some like it on the rocks: recurring stresses select for rock-inhabiting fungi with manifold protective pigments. *Nicole Knabe*[1], Romy Breitenbach[1,2], Polina Dementyeva[1], Felix Heeger[3], Camila Mazzoni[3], Anna A. Gorbushina[1,2] 1) Federal Institute for Materials Research and Testing (BAM), Department Materials and the Environment, Berlin, Germany; 2) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy & Department of Earth Sciences, Berlin, Germany; 3) Berlin Center for Genomics in Biodiversity Research, Berlin, Germany.

Black ascomycetous microcolonial fungi (MCF) are persistent inhabitants of rock surfaces in deserts as well as ubiquitous in other wide-spread terrestrial ecosystems including man-made materials such as solar panels. The ability of MCF to cope with multiple, rapidly fluctuating stresses makes the group an interesting subject in the study of stress resistance. Also, applied research is necessary to find ways of preventing MCF from colonising and degrading solar panels and historic monuments.

Carotenoids and melanin are amongst the protective pigments that contribute to the robustness of MCF. We studied their role in stress resistance of the non-pathogenic rock-inhabiting fungus *Knufia petricola* (Chaetothyriales) strain A95. Several knock-out mutants have been produced in melanin- (A95ΔSDH and A95ΔPKS) as well carotenoid- (A95ΔPDG) synthesis and a double mutant (A95ΔPKS/ΔPDG) also exists. Disruption of melanin synthesis affects not only oxidative-stress resistance but also the extracellular polysaccharide (EPS) matrix and lipid composition of the cell membrane. Exposure to ≤ 30 mM H_2O_2 did not affect the growth rate of A95ΔPKS as compared to wild type cells. Almost twice as much water-soluble EPS was present in cultures of the A95ΔPKS mutant compared to the wild type and A95ΔPDG strains. Comparative gene expression analyses of the wild type and the A95ΔPKS mutant identified genes regulated under oxidative stress conditions. These data will help elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

104T Spatiotemporal regulation of clock associated mRNA in *Neurospora crassa*. *Bradley M. Bartholomai*[1], Samantha E.R. Dundon[2], Amy S. Gladfelter[3], Jennifer J. Loros[4], Jay C. Dunlap[1] 1) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT; 3) Department of Biology, University of North Carolina, Chapel Hill, NC; 4) Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Neurospora crassa has long been an important model for circadian biology. However, the spatiotemporal dynamics of the *N. crassa* clock components at the level of individual hyphae have remained elusive. The core oscillator in the *Neurospora* clock is a negative feedback loop in which WC-1 and WC-2 acting together as a transcription factor drive expression of *frequency (frq)* whose product, the Intrinsically Disordered Protein FRQ, dimerizes, complexes with the DEAD-Box Helicase FRH and with CK1, and depresses the activity of WC-1/WC-2. We set out to investigate whether *frq* mRNA is non-randomly positioned in the cytoplasm. We hypothesized that *frq* mRNA translation may be spatially controlled through the association with an RNA-binding protein. To examine if *frq* transcript is spatially patterned, we employed single molecule RNA FISH coupled with quantitative image processing. The image analysis suggested that there is non-random clustering of *frq*, indicating that there is spatial regulation of mRNA position. We are currently using bacteriophage stem-loop and coat protein based techniques to discover *frq* mRNA binding proteins that participate in regulating localization and to visualize clock related mRNA in living cells.

105F A novel signaling network regulates circadian adaptation in Rice Blast. *N.I. NAQVI*[1], Y.Z DENG[2], Z QU[1] 1) Temasek Life Sciences Laboratory, Singapore; 2) South China Agricultural University, China.

Phototropic response and circadian rhythms are critical for growth, differentiation and environmental adaptation in fungi. However, the precise regulators of the intracellular metabolic response during such dark-light rhythms have not been identified/characterized yet. The blast fungus, *Magnaporthe oryzae*, initiates conidiation in response to phototropic and nutritional cues. We found that autophagy is induced specifically upon exposure to light and is essential for asexual development in *M. oryzae.* Comparative RNAseq analyses provided insights into novel signal transduction cascades underlying such phototropic induction of conidiation and in adaptation to the host milieu. We identified a histone acetyltransferase that negatively regulates light- and nitrogen-starvation induced autophagy during conidiation *in planta*. Furthermore, we identified a novel circadian-regulated *Twilight* (*TWL*) function essential for phototropic response and pathogenesis in *M. oryzae.* Expression of *TWL* showed a distinct circadian rhythm, and its transcript level peaked at subjective twilight. Mutually exclusive

phosphorylation acetylation cycles governed the intracellular dynamics and regulation of the Twl protein. Acetylated GFP-Twl remained cytosolic in the dark, whereas the light-induced phosphorylated (via Snf1 kinase) version translocated to the nucleus. Twilight function was required for regulating the mRNA levels of several important transcription/repair factors, including *TFB5*. Overexpression of *Tfb5* significantly suppressed the conidiation defects associated with the *twl*Δ mutant. Tfb5-GFP translocated to the nucleus during the phototropic response and under redox stress, whereas it failed to do so in the *twl*Δ mutant. Lastly, we provide mechanistic insight into Twl-based regulation of nutrient and redox homeostasis in the adaptation of the pathogen to the host milieu; and propose a regulatory mechanism that underlies the induction of autophagy by important environmental clues such as light and nutrients in the rice blast pathosystem.

106W Analysis of predicted essential genes in the *MAT* type of *Cryptococcus neoformans* and insights into the mechanisms of mitochondrial uniparental inheritance. *Giuseppe Ianiri*[1], Shen Sun[1], Alex Idnurm[2], Joseph Heitman[1] 1) Molecular Genetics and Microbiology, Duke University , Durham, NC; 2) School of BioSciences, University of Melbourne, VIC 3010, Australia.

The *MAT* locus of *Cryptococcus neoformans* has a bipolar system and it is characterized by an unusually large structure, spanning over 100 kb. The majority of the *MAT* genes have been characterized by functional genetics as being involved in sexual reproduction, virulence and mitochondrial uniparental inheritance (UPI). However, classical gene replacement failed to achieve mutants for five *MAT* genes (*RPL22, RPO41, MYO2, PRT1, RPL39*) of *C. neoformans*, indicating that they are likely playing an essential role. In order to infer their function, targeted gene replacement was performed in a diploid background for both the α and **a** alleles of these genes. Homologous recombination (HR) was achieved using a *NAT*-based split marker approach coupled with the use of W7 hydrochloride, a chemical that increases HR by inhibiting the non-homologous end joining pathway. While the study of *RPO41***a/**α and *PRT1***a/**α is in progress**,** Mendelian analysis of haploid progeny failed to recover the *NAT* marker from heterozygous mutants *RPL22***a/***rpl22*αΔ, *RPL22*α/*rpl22***a**Δ, *RPL39***a**/*rpl39*αΔ, and *RPL39*α/*rpl39***a**Δ**,** consistent with an essential function. Strikingly, while *myo2***a**Δ is inviable, *NAT* resistant progeny was obtained for *MYO2***a**/*myo2*αΔ. Ongoing studies seek to test the hypotheses that *MYO2*α is either a non-essential gene or it is an evolvable essential gene, the last being a phenomenon recently described in *Saccharomyces cerevisiae* and that consists in mechanisms of adaptive evolution that overcome the loss of gene function by changes in the ploidy status of the cell. Moreover, in yeast Myo2 is a myosin motor involved in actin-based transport of cargos, including mitochondria. To function, it is bound by Ypt11 and it requires components of the ERMES complex Mmm1, Mdm10, Mdm12, and Mdm34. Mutants for genes encoding these proteins are tested for mitochondrial UPI through genetic crosses, and mitochondrial distribution through mitotracker staining and Hem15-GFP localization. Our study will contribute to elucidate the functions of the *MAT* locus in *C. neoformans* and how it influences mitochondrial UPI.

107T Role of microfilament motor, MYO5 in the D-amino acid utilization. *Ami Khanal Lamichhane*, Yun Chang, Kwon-Chung June National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD.

The ability to utilize D-proline as sole Nitrogen source has been used as a diagnostic test to distinguish *Cryptococcus gattii* from its sister species, *C. neoformans*. *MYO5* gene was identified as one of the gene required for utilization of D-proline in *C. gattii* strain by screening insertional mutants library. Deletion of *MYO5* gene in *Cryptococcus gattii* compromised its growth in D-proline and complementation of gene was able to reconstitute the phenotype as wild type. Increasing concentrations of D-proline inhibited its growth suggesting the toxic effect of increasing amount of D-proline with deletion of *MYO5* gene. As previous studies suggested D-amino acid oxidase gene 2 (*RDAO2*) is a major gene involved in utilization of many D-amino acids, we checked if deletion of *MYO5* gene affects the expression of D-amino acid oxidase gene. Deletion of *MYO5* gene resulted in increased expression of RDAO2 in D-proline and D-alanine. *MYO5* gene was tagged with mNG marker and compared its localization with actin using lifeact-RFP marker. MYO5 was seen to be colocalized with most of actin structures such as cortical actin patches and ring structures. Our ongoing study suggest possible role of microfilament motor protein, MYO5 in utilization of D-amino acids through vesicles trafficking of D-amino acid oxidase.

108F Elucidating fluconazole based aneuploidy in *Cryptococcus neoformans*. *D.Sophie. Altamirano*, D. Fang, C. Simmons, L. Kozubowski Genetics and Biochemistry, Clemson University, Clemson, SC.

Cryptococcus neoformans is a pathogenic yeast that causes lethal cryptoccocal meningitis in immunocompromised patients. One of the challenges in treating cryptococcosis is the development of resistance to azole antifungals. Previous studies linked the azole resistance to the elevated copies of critical resistance genes in aneuploid cells. However, how aneuploidy is formed in the presence of azole drugs remains unclear. We found that treatment with inhibitory concentrations of an azole drug, fluconazole, led to a gradual and non-uniform depletion of ergosterol from the plasma membrane with concomitant progressive inhibition of budding and failure of cytokinesis. On the other hand, fluconazole had relatively less inhibitory effect on the progression through the cell cycle, as indicated by the premature formation of the mitotic spindle and continued nuclear division. To investigate which part of cytokinesis fails in the presence of fluconazole, we monitored the dynamics of two cytokinetic components: 1) non-muscle myosin II, Myo1, which is an essential constituent of the actomyosin ring (AMR) and 2) the septin Cdc10, which is one of the four septins that assemble into a higher order complex at the mother-bud neck. Our data show heterogeneous responses to fluconazole in terms of timing and rate of constriction of the AMR. Following the constriction of the AMR, septum was usually formed between the mother and daughter cells. Septin Cdc10 localization and dynamics were not significantly perturbed. Our findings suggest that in the presence of inhibitory concentrations of fluconazole, the AMR does constrict and septin assembly is normal. However, a final degradation of the septum between mother and daughter is affected, resulting in a lack of complete separation between the cells. Despite a defect in daughter cell separation, cell cycle continues leading to an increase in ploidy, potentially contributing to the formation of aneuploidy and fluconazole resistance.

109W Elucidating septin-based mechanisms of cytokinesis in *Cryptococcus neoformans*. *P. Stempinski*, E. Huey, E. McCormack, A. Rubin, J. Zielinski, L. Kozubowski Genetics and Biochemistry, Clemson University, Clemson, SC.

Cryptococcus neoformans is a basidiomycetous yeast, able to infect immunocompromised individuals due to its ability to grow at host temperature. Septins are conserved filament forming GTP-binding proteins that assemble as a complex at the site of cell division and contribute to cytokinesis. In *C. neoformans*, septins are not required for cytokinesis at 24°C but are essential at the host temperature of 37°C. Moreover, septins become essential at 24°C when the calcium-dependent protein phosphatase calcineurin is inhibited. While the role

of septins in cytokinesis is conserved in most eukaryotes, their exact contribution to this process remains elusive. Additionally, mechanisms of cytokinesis in *C. neoformans* are largely unknown. To define the roles of the septin complex and to identify other proteins involved in cytokinesis in *C. neoformans*, we performed a screen for genes necessary for growth at 37°C based on a collection of ~4,500 strains containing single deletions of non-essential genes. We searched for strains that at 37°C or in the presence of calcineurin inhibitor exhibited elongated bud morphology, a phenotype characteristic of septin mutants and indicative of cytokinesis failure. Our screen revealed 21 candidate genes, including 4 that were previously associated with cytokinesis and/or stress response (non-muscle myosin II, chitin synthase regulator, RAM pathway component, and the kinase PDK1), and 4 that encode hypothetical proteins. We are currently testing candidate genes identified in the screen for synthetic interaction with the septin deletion to probe for the potential involvement in septin pathway. In addition, we are testing if the candidate genes are involved in septin complex assembly and dynamics by introducing fluorescently tagged septin to the identified deletion strains. Our research will provide a better understanding of cytokinesis in *C. neoformans* and may lead to identification of novel antifungal drug targets.

110T The role of mitochondrial fission in cyclophilin D-dependent mitophagy in the filamentous ascomycete *Podospora anserina*. *A. Hamann*, P. Kramer, A. Jung, H.D. Osiewacz Molecular Biosciences, J.W. Goethe University, Frankfurt am Main, DE.

During aging of the filamentous ascomycete *Podospora anserina*, the mitochondrial peptidyl prolyl-cis, trans-isomerase cyclophilin D, PaCYPD, accumulates. This protein regulates the opening of the mitochondrial permeability transition pore (mPTP) which plays a key role in programmed cell death and induction of autophagy. Concordantly, we found that overexpression of *PaCypD* leads to increased general autophagy and mitophagy, while deletion prevents the previously demonstrated age-related induction of autophagy. Moreover, PaCYPD is required for a beneficial, so-called mitohormetic response to mild oxidative stress which results in lifespan extension. From these data we conclude that during aging, PaCYPD exerts a pro-survival function via induction of autophagy and mitophagy. However, excessive autophagy and mitophagy as it is observed upon constitutive strong *PaCypD* overexpression is detrimental and accelerates aging. To investigate the underlying mechanism, we analyzed whether mitochondrial fission, a prerequisite for the selective sequestration of damaged or dysfunctional mitochondria, is required for PaCYPD-dependent mitophagy. To this end, we used a *PaDnm1* deletion mutant which lacks the mitochondrial fission protein PaDNM1 resulting in impaired mitochondrial fission. Interestingly, although basal mitophagy does not depend on PaDNM1, preliminary data suggest that PaCYPD-induced mitophagy is PaDNM1-dependent. Thus, fission-dependent and -independent forms of mitophagy seem to exist.

111F Microtubules are reversibly depolymerized in response to changing gaseous microenvironments within *Aspergillus* biofilms. *N. Shukla*[1,2], A. Osmani[2], S. Osmani[1,2] 1) The Ohio State Biochemistry Program, The Ohio State University, Columbus, Ohio 43210; 2) Department of Molecular Genetics, The Ohio State University, Columbus Ohio 43210.

Fungal persistence and drug resistance during infections occurs mainly due to their ability to form biofilms. Concentration gradients of oxygen and nutrients caused by cell crowding and potentially secreted signaling factors cause cells in different areas of the biofilm to experience different microenvironments. The goal of our study was to understand how microenvironments within different areas of a fungal biofilm impact cell physiology.

Using confocal microscopy and genetic analyses we have uncovered a new physiological response that *Aspergillus nidulans* cells in a biofilm undergo in response to their microenvironment. We found that after adhesion to a surface, cells at the base of a forming biofilm first halt their growth and later on depolymerize their microtubules (MTs) in a cell-autonomous manner. During such MT-disassembly, MT plus-end binding proteins like EB1, ClipA and Dynein transiently locate to unique bar-like structures. To our surprise, we also found that biofilm mediated MT-disassembly involves soluble gases since cells instantly repolymerize their MTs upon simple air exchange above the static biofilm media. Further experiments revealed that biofilm-mediated MT disassembly occurs as a regulated response to hypoxia. Upon further maturation, and possibly due to the formation of the extracellular matrix (ECM), cells no longer respond to simple gaseous exchange above the biofilm media. At this time, physical removal of a part of the biofilm is required to promote MT reassembly that then occurs in cells at the new biofilm edge. In experiments aimed at further understanding the volatile agent involved in the regulation of MT dynamics within biofilms we tested the effects of the gaseous signaling agent, hydrogen sulfide (H_2S). We find that all aspects of MT depolymerization and repolymerization, including EB1, ClipA and Dynein bar formation, can be mimicked in growing cells under normoxia by H_2S addition and removal. Collectively, our study has implications in the areas of MT regulation, responses to hypoxia, gaseous signaling, and potentially fungal pathogenesis.

112W Complex nuclear motility in a fungal hypha explained by an elegant mechanism. R. Gibeaux[1,3], A. Z. Politi[1], *P. Philippsen*[2], F. Nedelec[1] 1) Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), D-69117 Heidelberg, Germany; 2) Molecular Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland; 3) Department of Molecular and Cell Biology, University of California, CA-94720, Berkeley.

Today, biologists want to understand the mechanisms of subsystems in cells. Biochemistry, for a long time extremely successful in developing amazing in vitro systems, can no longer handle mechanistic question above a certain complexity. The only way to move forward is to build computer models to systematically assess if our current concepts are compatible – or not – with the experimental data, and to reconstruct the system in a mathematical model. This is essential to curate and assemble the information that has been gathered in the past.

Our work demonstrates this approach by studying the coordination of movements of multiple nuclei in a common cytoplasm which up to now has not been solved for any multinucleated cell. Our aim was to understand the underlying mechanism(s) of the bi-directional short- and long-range movements of nuclei including nuclear by-passing in multinucleated hyphae of *Ashbya gossypii*. Experimental data, accumulated over the past years, had pinpointed one motor (dynein), an evolved microtubule dynamic instability, yeast-like spindle pole bodies as sole microtubule organization centers, and a potential cortical dynein anchor as key elements for the complex nuclear movements. This knowledge was implemented to run for the first time realistic simulations of movements of multiple nuclei in a common cytoplasm which generated in vivo-like nuclear migration patterns. The simulations could also verify mutant phenotypes. We then exploited

the simulation set up to study the influence of the number of microtubules per nucleus, an increase in cytoplasmic flow, and changes in organelle concentration on nuclear motility.

113T Adaptation of endocytosis to fast hyphal surface growth. D. Nordmann[1], K. Masai[3], H. P. Helfer[3], R. Kurre[2], *P. Philippsen*[3], H. P. Schmitz[1] 1) AG Genetik University of Osnabrück, 49090 Osnabrück, Germany; 2) Center for Advanced Light Microscopy University of Osnabrück, 49090 Osnabrück, Germany; 3) Molecular Microbiology, Biozentrum, University of Basel, 4056 Basel, Switzerland.

Intracellular traffic is influenced by the efficiency of endocytosis. This process is best characterized in budding yeast. Over 50 proteins cooperate in 1-2 minutes lasting multistep reactions from initiating membrane internalization to scission of the actin-coated vesicles. Endocytosis is enriched in growth zones e.g. yeast buds where polarity factors, exocyst components and excess of membranes have to be internalized to maintain polar surface expansion. We found a much accelerated endocytic process in growth zones of *Ashbya gossypii*, which is phylogenetically closely related to budding yeast but exerts up to 40x higher rates of polar surface expansion. Orthologs for all yeast endocytic proteins, except Sla2, are encoded in the *A. gossypii* genome, yet several of them fulfill their function in much less time than the budding yeast orthologs. On average, it takes less than 30 sec from the selection of an endocytic site to the abscission of an actin-coated vesicle. These vesicles have a fairly homogeneous size as shown by electron tomography (Gibeaux et al. Euk Cell 2013). Remarkably, Clathrin is not involved at any step of endocytosis. We also could demonstrate that the density of endocytic events at the growth zone increases approximately proportionally to the increase in polar surface expansion rate.

114F Developmental genetics of a fungal monstrosity: comparative analyses of the secotioid and agaricoid forms of Lentinus trigrinus. *A. Knudson*[1], Z. Xu[2], B. Wu[1], N. Chen[2], K. Krizsán[3], R. Riley[4], K. LaButti[4], A. Lipzen[4], W. Schakwitz[4], C. Pennachio[4], R. A. Ohm[5], A. Carlson[1], I. V. Grigoriev[4], L. G. Nagy[3], D. Hibbett[1] 1) Dept of Biology, Clark University, Worcester, MA; 2) Institute of Applied Mycology, Huazhong Agricultural University Wuhan, 430070 China; 3) Synthetic and Systems Biology Unit, Institute of Biochemistry, BRC-HAS, Szeged 6726, Hungary; 4) US Department of Energy (DOE) Joint Genome Institute, Walnut Creek CA 94598, United States; 5) Microbiology, Department of Biology, Utrecht University, Utrecht 3584 CH, The Netherlands.

Lentinus tigrinus is a wood-decaying basidiomycete that occurs in two distinct forms: an "agaricoid" form, which has exposed gills that release spores into the air, and a "secotioid" form, which has its gills enclosed by a layer of tissue that traps the spores, much like a puffball. Prior genetic studies suggested that the secotioid form is conferred by a recessive allele at a single locus (sec). In an attempt to identify the genetic basis of the secotioid form, we produced draft genome sequences of two monokaryons, one sec+ and one sec-. We performed a cross between the monokaryons and generated progeny that we pooled by genotype (sec- vs. sec+), and subjected to Illumina sequencing to identify regions that segregate with the secotioid phenotype. We also produced six dikaryons, three homozygous sec- and three homozygous sec+, and obtained transcriptomes from developing fruiting bodies at four developmental stages (mycelium, primordium, and young and mature fruiting bodies). Preliminary analyses identify approximately 20 SNPs that co-segregate with the secotioid phenotype, and 27 genes that have differential expression in homozygous agaricoid vs. secotioid dikaryons.

115W Functional analysis of class II hydrophobin HFB4 in *Trichoderma harzianum* and its sibling species *T. guizhouense*. Feng Cai[1,2], Mohammad Rahimi[2], Gunseli B. Ackapinar[1], Civan Yagtu[2], Agnieszka Przylucka[2], Guan Pang[1], Qirong Shen[1], *Irina S. Druzhinina*[2] 1) Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, China; 2) Institute of Chemical Engineering, TU Wien, Vienna, Austria.

Hydrophobins are the small secreted cysteine-rich proteins common in filamentous fungi. They have a distinct ability to self-assemble into stable amphiphilic layers at hydrophobic-hydrophilic interfaces and/or attach to various surfaces modulating their hydrophobicity. Thus, they can be involved in a diversity of functions related to the development of the fungus, its resistance to biotic and abiotic stress factors and in interactions with other organisms in the environment. The genome of the mycoparasitic filamentous fungus *Trichoderma* (Ascomycota, Dikarya) encodes a particularly large diversity of hydrophobins ranging from 6 up to 12 genes. In this work we have studied the function of hydrophobin 4 (HFB4) that is universally present in all *Trichoderma* groups. For this purpose, we have constructed libraries of HFB4 deletion and overexpressing mutants for *T. harzianum* CBS 226.95 and its sibling species *T. guizhouense* NJAU 4742 and also heterologously expressed the protein in *Pichia pastoris* cell factory. Results confirmed the involvement of HFB4 in hyphal growth in liquid medium. However, the impact was different in two *Trichoderma* species. *T. guizhouense* deletion mutants were not able to overcome the liquid surface tension and did not form aerial mycelia on liquid media, while *T. harzianum* deletion mutants did not express such deficiencies. More remarkably, HFB4 deletion mutants of *T. guizhouense* had essentially increased capacity to colonize tomato roots but their antagonistic potential in interactions with other fungi was reduced. Contrary, HFB4 deletion mutants of *T. harzianum* have shown reverse phenotypes. The results will be presented in a context of the analysis of the complete hydrophobome expression for both species.

116T Knockdown of *PaMus10* coding for a putative mitochondrial E3 ligase of *Podospora anserina*: impact on mitochondria and aging. *A.T. Jung*, H.D. Osiewacz Institute for Mol. Biosciences, J. W. Goethe University, Frankfurt am Main, DE.

Podospora anserina is a filamentous fungus used as a model organism to unravel molecular mechanisms of organismal aging. In this system, aging has a strong mitochondrial etiology. Pathways involved in respiration, ROS generation and scavenging, and in particular in surveillance of a population of “healthy” mitochondria (e.g. mitochondrial biogenesis, dynamics and proteostasis) strongly affect aging and lifespan. In order to analyze the contribution of the ubiquitin proteasome system (UPS) to mitochondrial quality control, we examined the role of the putative mitochondrial ubiquitin E3 ligase PaMUS10, which is a possible functional homolog of the human E3 ligase PARKIN. We found that homokaryotic *?PaMus10* ascospores display mitochondrial impairments. The developing mycelia show a hyper-branching phenotype and stop growth soon after spore germination. Since these severe impairments do not allow to efficiently investigate the function of MUS10 in *P. anserina*, we developed an approach allowing to knockdown the expression of *PaMus10* in cultures originating from heterokaryotic *?PaMus10/PaMus10* spores. Cultivation on medium supplemented with hygromycin resulted in a gradual depletion of nuclei containing *PaMus10* and leads to reduced fertility and a strong reduction in lifespan. This phenotype correlates with a pronounced alteration of the mitochondrial morphology from long reticular networks to fragmented mitochondria. In addition, we found that the amount of the mitochondrial outer membrane protein PaPORIN strongly depends on the PaMUS10 level indicating that PaPORIN is a potential substrate of this E3 ligase. Since the human homolog of PaPORIN, VDAC, is a substrate of PARKIN this finding supports the assumption that

PaMUS10 is the functional homolog of PARKIN. Taken together, the results from our analysis provide first evidence for a role of ubiquitination of mitochondrial proteins in *P. anserina* and a role of the UPS in lifespan control.
We would like to thank the LOEWE excellence initiative of the state Hesse (*Ub-Net*) for support.

117F Neutral lipid metabolism in the dimorphic fungus *Blastomyces dermatitidis* influences temperature-dependent morphologic development. Isabelle Cooperstein, Amber Marty, *Gregory Gauthier* Department of Medicine, University of Wisconsin, Madison, WI.

Worldwide, the thermally dimorphic fungi cause several million human infections each year. The temperature-dependent morphologic switch between mycelia (22^{o}C) and yeast (37^{o}C) defines the biology of these fungi and is critical for pathogenesis. Knowledge about the mechanisms underlying temperature adaptation remain poorly understood and represent a significant gap in knowledge. Using *Blastomyces dermatitidis*, the etiologic agent of blastomycosis, as a model system, we identified a GATA transcription factor encoded by *SREB* (sidereophore biosynthesis repressor in *Blastomyces*) that governs the transition from yeast to mycelia following a drop in temperature from 37^{o}C to 22^{o}C. *SREB* null mutants fail to complete the conversion to mycelia, cannot properly regulate iron assimilation, and have reduced biosynthesis of neutral lipids (triacylglycerol, ergosterol) including lipid droplets at 22^{o}C. Altering exogenous iron concentrations did not affect the morphologic defect in *SREB* null mutants. In contrast, treatment with saturated fatty acids (16:0, 18:0) accelerated the morphologic switch and transiently restored lipid droplet abundance in *SREB* null mutants at 22^{o}C. Gene expression microarray and qRT-PCR analyses demonstrated reduced transcription of neutral lipid biosynthetic genes including *ARE*, which is involved with triacylglycerol biosynthesis and ergosterol esterification. Overexpression of *ARE* in *SREB* null mutants accelerated the conversion to hyphae at 22^{o}C and increased lipid droplet formation. To assess the influence of *ARE* on the morphologic switch in wild-type *B. dermatitidis* we targeted it for gene editing using CRISPR-cas9. Edited strains exhibited impaired conversion to mycelia at 22^{o}C but did not have reduced lipid droplet formation. Collectively, these data suggest that neutral lipids including genes involved with neutral lipid biosynthesis influence the temperature-dependent switch to mycelia.

118W Live-cell imaging of nuclei and filamentous actin in the basidiomycete *Schizophyllum commune*. Elke-Martina Jung[1], Erika Kothe[1], *Marjatta Raudaskoski*[2] 1) Institute of Microbiology, Microbial Communication, Friedrich Schiller University, Neugasse 25, 07734 Jena, Germany; 2) Molecular Plant Biology, Department of Biochemistry, University of Turku, Tykistökatu 6A, 20520 Turku, Finland.

The visualization of cytoskeletal structures in living hyphae of filamentous basidiomycetes can yield additional information that cannot be obtained with ascomycetes. In basidiomycetes, monokaryotic or defined dikaryotic hyphal compartments are found, and the formation of septa is tightly linked to nuclear division. In addition, some model basidiomycetes, including *Schizophyllum commune*, show nuclear division in dikaryotic hyphae linked to clamp formation. Here, we use histone H2B labeling by EGFP to allow for *in vivo* visualization of the elongated nuclei in both, mated dikaryotic and unmated monokaryotic hyphal compartments. In apical cells, the interphase nuclei moved towards the hyphal tip with a rate proportional to tip growth. In some apical cells, the nuclear content showed increased fluorescence due to chromatin condensation. The condensed chromatin gathered at the apical part of the nucleus, while the fluorescence in the rest of the nucleus remained weak and finally became separated from the bright part. The decrease in nuclear size during division has long been known in *S. commune,* but only the observation of the separation of a weakly fluorescent part observed here clearly suggests that nuclear division in filamentous basidiomycetes follows the “(late) open mitosis” principles, as previously suggested also for the yeast like growth phase of basidiomycetes including *Ustilago maydis* or *Cryptococcus neoformans*. The phenomenon was seen more clearly during nuclear division in homokaryotic than in dikaryotic hyphae. Live-cell imaging of filamentous actin with Lifeact::EGFP revealed filamentous actin after nuclear division at the formation of septa both in monokaryotic and dikaryotic hyphae. The dynamics of actin at clamp cell formation suggested a more central role for actin than recorded before. At the initial stage of clamp cell formation, seen as a small peg growing from the mother cell, the proceeding movement of nuclei halts. At the same time, an actin web below the outgrowth becomes visible. This actin web could be responsible for the cessation of nuclear movement. Associated with actin web formation is the movement of one of the nuclei into the clamp initial. Microtubules have been suggested to play a central role for the movement, but here we could show that actin filaments appear to be involved in an essential manner.

119T Comparative analysis of the Bud Site Selection System and the Septation Inititation Network in the Taxonomically Unrelated ß-lactam Producers *Acremonium chrysogenum* and *Penicillium chrysogenum*. *J. Kluge*, U. Kueck General and Molecular Botany, Ruhr-University Bochum, Bochum, NRW, DE.

Beta-lactam antibiotics are of major biotechnological and medical relevance and have a huge world market value. Whereas the filamentous fungus *Acremonium chrysogenum* is the primordial producer of cephalosporin C, *Penicillium chrysogenum* is the industrial source of penicillin. A typical morphological feature of *A. chrysogenum* is the fragmentation of vegetative mycelium into arthrospores. It is assumed that the constriction of these uni-or binuclear cells occurs from septa. Due to the known correlation of cephalosporin C production and arthrospore formation, we are interested in the identification of specific regulatory factors affecting both, cephalosporin C biosynthesis and morphological development. To expand the current topic regarding the septation process within arthrospore formation in *A. chrysogenum* and conidiospore development in *P. chrysogenum*, we performed a comparative analysis of both fungi. Here, we present functional characterizations of Axl2, a component of the bud site selection system and the germinal center kinase Mst1, which act as a connecting protein of the septation initiation network and the morphogenesis network (Heilig et al. 2014). In microscopic analysis and quantitative evaluations, we determined a time-delayed arthrospore development together with an altered conidiophore phenotype. In both fungi, *axl2* and *mst1* deletion mutants show growth defects in response to osmotic and cell wall stress conditions. The observation of the dynamic actin cytoskeleton and associated development of septa was performed by the establishment of the live cell marker Lifeact. The deletion of *mst1* in *A. chrysogenum* leads to an abnormal localization of actin within the hyphae. Our results highlight the similar function of developmental networks in two taxonomically unrelated ascomycetes.

Heilig Y, Dettmann A, Mourino-Perez RR, Schmitt K, Valerius O, Seiler S (2014) Proper Actin Ring Formation and Septum Constriction Requires Coordinated Regulation of SIN and MOR Pathways through the Germinal Centre Kinase MST-1. Plos Genet 10 (4):e1004306

120F Crosstalk between pheromone response pathway and STRIPAK complex regulates developmental processes in *Sordaria macrospora*. *A. Beier*, B. Ramšak, I. Teichert, U. Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE e-mail: anna.beier@rub.de.

Developmental processes are often regulated by signal transduction pathways that include phosphorylation as a signal. These pathways are not linear and require additional regulation, which is often provided by feedback loops and crosstalk. In filamentous ascomycetes, the three mitogen-activated kinase (MAPK) cascades that primarily respond to cell wall stress (CWI), pheromone exposure (PR), and osmotic stress (OS). Besides these specific functions, fungal MAPK cacades are involved in e.g. vegetative growth, cell-to-cell communication, as well as sexual and asexual development. MAPK cascades are highly conserved eukaryotic phospho-signaling modules, which are tightly regulated by feedback loops, crosstalk, and phosphatase-catalyzed signal modulations. Here, we show an interaction of the PR pathway and the striatin interacting phosphatase and kinase (STRIPAK) complex, which is a major regulator of hyphal fusion and fruiting body development in the ascomycete *S. macrospora.* Phosphorylation levels of MAK2, the MAPK of the PR pathway, in STRIPAK deletion strains suggest an association of both complexes. In yeast two-hybrid analysis, we tested the composition of the PR module with constructs encoding for MAPKKK MIK2, MAPKK MEK2, MAPK MAK2, and the putative scaffold protein, which is homologous to HAM-5 from *Neurospora crassa*. Further, yeast two-hybrid showed a physical interaction of PR components with the STRIPAK subunit PP2Ac1. This STRIPAK subunit is a catalytic subunit of the S/T protein phosphatase 2A (PP2A) and governs sexual development, hyphal fusion, and cell wall stress response in fungi (1). Deletion strains for PR pathway subunits were generated and functionally characterized. Phenotypic analysis point towards a similar function of PR and STRIPAK subunits in developmental processes. Together with previous results that revealed a connection of STRIPAK to the CWI MAPK cascade, our findings suggest a regulatory function of STRIPAK in phospho-signaling of both MAPK cascades.

1. Beier A, Teichert I, Krisp C, Wolters DA, Kück U. 2016. Catalytic subunit 1 of protein phosphatase 2A is a subunit of the STRIPAK complex and governs fungal sexual development. mBio 7(3):e00870-16. doi:10.1128/mBio.00870-16

121W Screening for genes important to cold acclimatization in Neurospora crassa. Victor Manzanilla, Holly Howell, Alexander Mehreteab, Eric Rose, Nichole Walters, Nicholas Seitz, Jacob Nava, *Michael Watters* Dept Biol, Valparaiso Univ, Valparaiso, IN.

The environmental conditions that life must contend with can vary widely. Organisms have evolved a wide range of mechanisms for contending with these changing conditions. For relatively simple, sessile organisms (such as fungi), they must adapt themselves to the extremes of the environment. When subjected to rapid drops of temperature (cold shock), Neurospora responds with a dramatic, but temporary shift in its branching pattern. The fungal response, seems to parallel the physiological response to similar cold shock which has been observed in E. coli in which a set of genes is activated transiently in order to adjust the cell for growth in the cold. While the Neurospora response has been described morphologically, it has yet to be examined to any serious extent genetically. This project aims to begin the genetic characterization of the cold shock response and the associated acclimatization to cold environments. We report here the results of two screens of mutants from the Neurospora knockout library for their response to cold shock. In the first, strains with knockouts in genes previously suspected to be involved in hyphal development as well as knockouts resulting in morphological changes are tested for their response to cold shock. In the second, strains with knockouts of genes homologous to E. coli genes known to alter their expression in response to cold shock are examined for their morphological response. Several were identified with altered responses. The genes impacted in these mutants are listed and discussed.

122T A downy mildew effector evades recognition by polymorphism of expression and subcellular localization. *S. Asai*[1], V. Cevik[2], O.J. Furzer[2], D.S. Kim[2], N Ishaque[2], K Shirasu[1], J.D.G. Jones[2] 1) RIKEN CSRS, Yokohama, JP; 2) Sainsbury Laboratory, Norwich, UK.

Hyaloperonospora arabidopsidis (*Hpa*; formerly *Peronospora parasitica*) is an obligate biotrophic oomycete that causes downy mildew in *Arabidopsis thaliana*. The Arabidopsis-*Hpa* pathosystem has been extensively used to study host/pathogen co-evolution, and has enabled identification of cognate host resistance and pathogen avirulence genes. Arabidopsis Col-0 is resistant to *Hpa* isolates Emoy2 and Emwa1 through recognition by a resistance gene *RPP4*, but its cognate avirulence effector(s) were not previously identified. Comparative genomics and transcriptomics among different isolates of *Hpa* including Emoy2 and Emwa1 uncovered an *Hpa* effector recognized by *RPP4*. Here, we report how its unrecognised alleles evade immune recognition by *RPP4*.

123F The potential role of an RLXR effector protein in *Phytophthora infestans* sexual reproduction. *George Tzelepis*, Anna Åsman, Ramesh Vetukkuri, Johan Fogelqvist, Christina Dixelius Department of Plant Biology, Uppsala Biocenter, Swedish University of Agricultural Sciences, Linnean Center for Plant Biology, P.O. Box 7080, S-75007, Uppsala, Sweden.

Phytophthora infestans is a notorious fungal-like plant pathogen, which belongs to the class Oomycota within the Stramenopiles kingdom, responsible for major economic losses in potato and tomato cultivations worldwide. In order to establish a successful infection, this pathogen deploys an arsenal of small-secreted proteins, termed effectors. Among them, effectors harboring the RXLR motif are the best-studied ones. They are highly induced during early biotrophic stage of infection and are able to manipulate plant defense mechanisms operating inside the host cell. *Phytophthora infestans* is a heterothallic species, with two distinguished mating types (A1 and A2), and sexual reproduction plays a crucial role in epidemiology.

In this study we investigated the transcription profiles of different *P. infestans* isolates (originated from different parts in Europe) during the mating process, since mating frequencies are known to differ among European countries. Our results revealed that an RXLR effector-encoding gene is highly induced during mating in most of our crossings. Further analysis showed that oospores production is significantly reduced when this effector has been silenced. Moreover, we observed that this protein is accumulated in hyphal tips during mating, while infection of *N. bethamiana* plants with the GFP-tagged strain and transient expression in potato leaves confirmed the cytoplasmic localization of this effector upon infection. In this study, we could also detect the RXLR protein in the extracellular environment, when *P. infestans* was grown in pea broth medium without forming haustoria, indicating that this cytoplasmic RXLR effector can also be secreted from the hyphal tips. Furthermore, co-immunoprecipitation assays revealed that this RXLR protein putatively binds to a nuclease and to a nucleolus protein during mating. Interestingly, silencing lines displayed reduced virulence compared to wild type.

In conclusion, this study revealed a potential multifunctional role of an RXLR effector. Interestingly, the presence of this effector only in heterothallic closely related to *P. infestans* species, supports its potential role in sexual reproduction. We can speculate that certain effectors might be involved in other aspects of *P. infestans* biology than in oomycete-plant interactions. If this function reflects evolutionary adaptation remains to be clarified.

124W Origins of novel sexual development phenotypes in the evolution of the transcriptome in five fungi. *Jeffrey P. Townsend*[1], Zheng Wang[1], Kayla Stefanko[2], Caitlyn Cubba[2], Frances Trail[2] 1) Yale University, New Haven, CT; 2) Michigan State University, East Lansing, MI.

Changes in gene expression have been hypothesized to play an important role in the evolution of divergent morphologies. To test this hypothesis, we examined developmental differences in fruiting body morphology of filamentous fungi, culturing five species in a common garden environment and profiling genome-wide gene expression at five stages. We reconstructed ancestral gene expression, identifying genes with the largest evolved increases in gene expression across development. Conducting knockouts and performing phenotypic analysis in two divergent species typically demonstrated altered fruiting body development in the species that had evolved increased expression. Our evolutionary approach to finding relevant genes proved far more efficient than other gene deletion studies targeting whole genomes or gene families. Combining gene expression measurements with knockout phenotypes facilitated the refinement of Bayesian models of the gene network underlying fruiting body development, regulation of which is one of the least understood processes of multicellular development.

125T Cag1, one of the Tup1 paralogues in *Coprinopsis cinerea*, is required for gill formation in fruiting body development. *H. Muraguchi*, R. Masuda, N. Iguchi, K. Tsukuta, T. Nagoshi, K. Kemuriyama Dept Biotechnology, Akita Prefectural Univ, Akita, JP.

The pileus (cap) of the fruiting body in homobasidiomycete fungi bears the hymenium, a layer of cells that includes the basidia where nuclear fusion, meiosis and sporulation occur. *Coprinopsis cinerea* is a model system for studying fruiting body development. The hymenium of *C. cinerea* forms at the surface of the gills in the pileus. We have mutagenized a homokaryotic fruiting strain, #326, and isolated a mutant that fails to enlarge the cap tissue on the primordial shaft in fruiting. Genetic analysis of this mutant, *cap-growthless*, indicated that the mutant phenotype was brought about by a single gene, designated as *cag1*. The *cag1* locus was mapped on chromosome IX by linkage analysis using RAPD markers that were mapped to each chromosome. The *cag1* gene was identified by transformation experiments using BAC DNAs and their subclones derived from chromosome IX, and found to encode a homologue of *Saccharomyces cerevisiae* Tup1. The *C. cinerea* genome contains another Tup1 homologue called *Cc.tupA*. We examined whether the expression levels of *cag1* and *Cc.tupA* are developmentally regulated during fruiting. In the vegetative mycelium, *Cc.tupA* were expressed more than *cag1*. In contrast, in the growing pileus, the *cag1* expression levels were higher than those of *Cc.tupA.* To examine changes in the spatial and temporal expression of Cag1 and Cc.TupA in the pileus, we reciprocally fused fluorescent tags, i.e. EGFP and mCherry, to these proteins. Compared with Cc.TupA, Cag1 was preferentially expressed in the gill trama tissue cells, suggesting that the function of Cag1 is required for gill trama tissue differentiation and maintenance. Because it is known that *S. cerevisiae* Tup1 forms homotetramer, we examined whether Cag1 interacts with itself and with Cc.TupA using yeast two-hybrid (Y2H) system. Y2H analysis reveals that Cag1 can interact with itself and with Cc.TupA through their N-terminal regions. Like Tup1, which interacts with Cyc8, the N-terminal region of Cag1 also interacts with the N-terminal region of Cc.Cyc8, which contains tetratricopeptide repeats. Y2H analysis and co-localization of Cag1 and Cc.TupA suggested that Cag1 interacts with Cc.TupA in the nuclei of certain cells.

126F Environmental and genetic control of the coordinated process of fruiting body development in *Coprinopsis cinerea*. *Shanta Subba*, Ursula Kües Molecular Wood Biotechnology, University of Göttingen, Göttingen, DE.

Fruiting body development in *Coprinopsis cinerea* follows a conserved scheme defined by day and night phases, with well predictable distinct stages over the time. Starting with hundreds of structures, every day some will be given up in development with only a few to mature. Fruiting starts with primary hyphal knot (PHK) formation in the dark, followed by light-induced compact aggregates, secondary hyphal knots (SHKs) in which stipe and cap tissues differentiate. Primordium development (P1 to P5) takes five days to culminate on day 6 of development in karyogamy (K) and meiosis (M) within the basidia and subsequent basidiospore production which parallels fruiting body maturation (stipe elongation and cap expansion). Mature fruiting bodies autolyse on day 7 to release the spores in liquid droplets. The developmental pathway is regulated by factors such as light, temperature, humidity and nutrients. Light signals induce formation of SHKs, the differentiation of tissues within the growing primordia and karyogamy within the basidia. Without light so-called 'dark stipes' are formed from SHKs with elongated stipes and underdeveloped caps. Aeration is a new key factor we show here to influence the morphological development of the fungus. Inhibiting passage of air into cultures can alter normal behaviour of the fungus. Hindering air entry before light-induced formation of SHKs fully blocks fruiting and results in the formation of fluffy mycelial growth. When SHKs have been formed blockage of air development arrests. Block in aeration at P1 to P3 leads in light to outgrowth of elongated stipes that are similar to the structures observed in aerated cultures in the dark. When plates are air-sealed at the P4 or the P5 stage fruiting body maturation happens but caps are colourless by reduced spore numbers. Mutants in light reception can form dark stipes in light while the phenotype in others may arise from defects in regulation by aeration. Most mutants in fruiting are however blocked at specific steps in development. In a collection of about 1500 strains, mutations do not evenly distribute over the complete pathway. High numbers of mutants are available from the early developmental stages up to stage P1, comparably few in the subsequent steps up to P3. Larger sets of mutants exist for P4 and P5 when karyogamy and meiosis have to occur in the basidia and fruiting body maturation to be initiated. Mutant numbers may reflect the complexity of specific steps in fruiting.

127W *IDC2* and *IDC3*, two genes involved in cell non-autonomous signaling of fruiting body development in the model fungus *Podospora anserina*. *Herve Lalucque*[1], Fabienne Malagnac[1,2], Kimberly Green[3], Valérie Gautier[1], Pierre Grognet[1,2], Laetitia Chan Ho Tong[1,4], Barry Scott[3], Philippe Silar[1] 1) Univ Paris Diderot, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, Case courrier 7040 Lamarck, 75205 Paris, France; 2) Present adress : Institut de Biologie Intégrative de la Cellule, UMR 9198 CEA CNRS Univ Paris Sud, 91405 Orsay, France; 3) Institute of Fundamental Sciences, Massey University, Palmerston North, 4442, New Zealand; 4) Present adress : Institut Français du Pétrole Energies Nouvelles 1-4 Avenue du Bois Préau, 92852 Rueil-Malmaison, France.

Filamentous ascomycetes produce complex multicellular structures during sexual reproduction. Little is known about the genetic pathways enabling the construction of such structures. Here, with a combination of classical and reverse genetic methods, as well as genetic mosaic and graft analyses, we identify and provide evidence for key roles for two genes during the formation of perithecia, the sexual fruiting bodies, of the filamentous fungus *Podospora anserina*. Data indicate that the proteins coded by these two genes function cell-non-autonomously and that their activity depends upon conserved cysteines, making them good candidate for being involved in the transmission of a reactive oxygen species (ROS) signal generated by the PaNox1 NADPH oxidase inside the maturing fruiting body towards the PaMpk1 MAP kinase, which is located inside the underlying mycelium, in which nutrients are stored. These data provide important new insights to our understanding of how fungi build multicellular structures.

128T Leveraging the diversity in the hypervariable species *Schizophyllum commune* to understand mushroom development. Ioana Marian, Jordi Pelkmans, Luis Lugones, Han Wösten, *Robin Ohm* Microbiology, Utrecht University, Utrecht, NL.

Schizophyllum commune is used as a model system to study mushroom development. A large number of wild isolates of *S. commune* have been isolated from all over the world. These strains show a large diversity in mushroom development phenotypes. For example, some strains develop mushrooms in high CO_2 concentrations, which usually inhibit mushroom development. Moreover, mushroom shape and development varies when grown under lab conditions. Many of these traits show mendelian inheritance, allowing it to be mapped by genome sequencing and bulk segregant analysis.

Novel regulators of mushroom development were identified. The previously identified homeodomain transcription factor Hom2 is involved in the CO_2 sensing pathway as well as subsequent mushroom development. Several subdomains were shown to be indispensable for the correct function of the protein.

129F Dsciphering the roles of the Phox-homolog domain proteins during the development and pathogenesis of *Fusarium graminearum*. *Y. Lou* College of Plant protection, Fujian A & F University, fuzhou, Fujian, CN.

The previous study indicates that retrograde trafficking from endosome to trans-Golgi network mediated by retromer is very important for fungal development and pathogenicity in *Fusarium graminearum*. The Vps5 and Vps17 subunits of the retromer complex which was localized to the endoplasmic reticulum membrane can bind with PtdInsP (phosphoinositides) lipids, indicating that differences in membrane composition may be involved in the morphogenesis and host invasion. The Phox homolog domain protein is localized to different organelles through binding with different types of phosphoinositides, and is involved in cell trafficking, endocytosis, exocytosis and cell morphogenesis. In *Fusarium graminearum*, thirteen Phox-homolog domain proteins have been identified. We currently characterized homologs of *BEM1*, *SPO*14, *GRD19*, *SNX4*, *YPT35*, *MVP1*, *YKR078*, *YPR097*, *SNX14*, *MDM1*, *VPS5* and *VPS17* and determined their functions as well as contribution to the development and pathogenesis in *F. graminearum*. The deletion mutants of Phox homolog domain proteins, besides *YPR097,* showed defect in the pathogenesis. Interestingly, the deletion mutants of *BEM1* may be involved in the regulation of ROS homeostasis. Progress in understanding the roles of Phox homolog domain in cell trafficking and protein interaction will be presented.

130W Chemical genetics to unravel *Fusarium* development and pathogenesis. *G. Subramaniam*[1,2,3], C Mogg[3], C Bonner[2], D Desveaux[3] 1) Ottawa Research and Development, Agriculture Canada, Ottawa, Ontario, Canada; 2) Carleton University, Ottawa, Ontario, Canada; 3) University of Toronto, Toronto, Onatrio, Canada.

Chemical genetics provides a powerful tool to identify targets and genetic pathways involved in all aspects of cellular development. Unlike classical forward genetics, chemical genetic have an added advantage to identify essential genes involved in cellular pathways. We used chemical genetics to identify genes involved in *Fusarium graminearum* development. One chemical, Antofine, previously characterized to inhibit fungal growth, including *F. graminearum* was used in a haploid insufficiency (HIP) screen in the yeast *S. cerevisiae* to identify targets. We identified genes associated with the target of rapamycin (TOR) pathway as targets of Antofine, implicating this important pathway in *F. graminearum* development. The results of the HIP screen and characterization of the TOR pathway in *F. graminearum* will be presented.

131T Characterization and functional roles of the flavohemoglobin genes of the plant pathogen *Fusarium oxysporum*. Eduardo Argotti, Serenella Sukno, *José María Díaz-Mínguez*, Ernesto P. Benito CIALE-UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN.

Nitric oxide (NO) is a highy reactive molecule playing important roles in essential developmental processes and stress responses. Among them, it is a crucial component of the plant immune response. This poses an interesting challenge for phytopathogenic fungi: they may use NO as a signalling molecule to modulate development but, concurrently, they have to counteract the plant defensive responses primed by NO. Fungi have evolved several mechanisms against nitrosative stress, among them the detoxification of NO radicals by the enzymes flavohemoglobins is a prominent one.

Genome analysis of several *Fusarium oxysporum* isolates showed that this fungus has four genes encoding flavohemoglobins *(FHG1-4). FHG1, FHG2* and *FHG3,* encode fungal cytoplasmatic flavohemoglobins, while *FHG4* encodes a mitochondrial flavohemoglobin with a probable bacterial origin. Gene expression analyses performed with weakly virulent (WV) and highly virulent (HV) *F. oxysporum* f. sp. *phaseoli* strains showed that *FHG1* and *FHG2* responded transcriptionally to exogenous NO during spore germination. *FHG3* showed a complex regulation while *FHG4* did not show any transcriptional response in the assayed conditions. Functional analysis of mutants deleted in the *FHG2* gene and silenced in the *FHG1* gene in both strains, confirmed that *FHG1* plays a major role and *FHG2* a minor role in NO detoxification, which confers WV and HV virulent strains resistance to nitrosative stress and prevents the delay in spore germination produced by high levels of NO. Plant inoculation assays performed either with single *FHG2* deleted mutants or double D*FHG2-FHG1*RNAi mutants, showed that these genes are not pathogenicity or virulence factors. However, *FHG1* upregulation during the first week after infection suggests that this flavohemoglobin is required for plant colonization. Finally, it cannot be precluded that flavohemoglobin FHG4 plays a role in the detoxification of the NO produced during denitrification under low oxygen concentrations.

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132F Sex pheromone autocrine signaling controls vegetative fungal development. S. Vitale, A. Di Pietro, *D. Turrà* Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Cordoba, Spain,.

Mating in heterothallic ascomycetes involves paracrine signaling between two opposite mating types producing two different peptide

pheromones, a and α. Inappropriate self-signaling is prevented by protease-mediated pheromone degradation and transcriptional shut-down of the cognate receptor. Here we show that in the heterothallic fungal pathogen *Fusarium oxysporum,* which lacks a known sexual cycle, pheromone signaling follows a distinct configuration in which both pheromone/receptor pairs are co-expressed, resulting in autocrine signaling. Unexpectedly, mutants lacking a given peptide pheromone or its cognate receptor displayed increased sensitivity towards the opposite pheromone, as well as defects in vegetative hyphal fusion or cell-cell agglutination. Our results support a model in which a and α pheromone coordinately control key developmental processes in *F. oxysporum*, revealing a new role for autocrine pheromone signaling in vegetative fungal development.

133W Structure-activity relationship of α mating pheromone from the fungal pathogen *Fusarium oxysporum* . *S. Vitale*[1], A. Partida-Hanon[2], S. Serrano[2], A. Martínez-del-Pozo[3], A. Di Pietro[1], M. Bruix[2], D. Turrà[1] 1) Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Cordoba, Spain,; 2) Department of Biological Physical Chemistry, Institute of Physical Chemistry Rocasolano, CSIC, Madrid, Spain; 3) Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Complutense University, Madrid, Spain.

During sexual development, ascomycetes produce two types of peptide pheromones termed a and α. The α pheromone from the budding yeast *Saccharomyces cerevisiae,* a thirteen residue peptide which elicits cell cycle arrest and chemotropic growth, has served as paradigm for the interaction of small peptides with their cognate G protein-coupled receptors. However, no structural information is currently available for α pheromones from filamentous ascomycetes, which are significantly shorter and share almost no sequence similarity with the *S. cerevisiae* homolog. High-resolution structure of synthetic α-pheromone from the plant pathogenic ascomycete *Fusarium oxysporum* revealed the presence of a central β-turn resembling that of its yeast counterpart. Disruption of the fold by D-alanine substitution of the conserved central Gly_6-Gln_7 residues or by random sequence scrambling demonstrated a crucial role for this structural determinant in chemoattractant activity. Unexpectedly, the growth inhibitory effect of *F. oxysporum* α-pheromone was independent of the central β-turn but instead required two conserved Trp_1-Cys_2 residues at the N-terminus. These results indicate that, in spite of their reduced size, fungal α-pheromones contain discrete functional regions with a defined secondary structure that regulate diverse biological processes such as polarity reorientation and cell division.

134T Structure-function studies on the components of the Polycomb Repressive Complex 2. *Brian Josephson*, Zackary Bango, Allyson Erlendson, Brett Pierce, Lanelle Connolly, Michael Freitag Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

Polycomb Repressive Complex 2 (PRC2) is a histone methyltransferase responsible for trimethylating histone 3 on lysine 27 (H3K27me3). Mutations within its three primary subunits, Kmt6, Eed, and Suz12, result in inherited diseases and sporadic cancers. In the fungus *Fusarium graminearum*, deletion of the gene encoding the catalytic subunit, *kmt6*, results in derepression of ~25% of the genome as well as distinct phenotypes. While previous ChIP-seq and RNA-seq has revealed genomic targets for PRC2, little is known about how it localizes to specific areas of the genome and spreads the H3K27me3 mark along the chromosome. We addressed these questions by engineering a set of *kmt6*, *eed*, and *suz12* mutants, for which we used the copper responsive *tcu1* promoter, resulting in tunable control of PRC2 expression. We characterized these mutants by fluorescent microscopy, ChIP-seq, and RNA-seq. Our studies were further guided by a partial crystal structure for PRC2 from the fungus *Chaetomium thermophilum*. We generated several Kmt6 mutants, all tagged with Green Fluorescent Protein (GFP), to evaluate if structure-function relationships proposed *in vitro* are supported by *in vivo* studies. By making minor changes in the primary sequence of Kmt6 important Kmt6-Eed-Suz12 interphases were disturbed and resulted in loss of function and phenotypes similar to that of our kmt6 deletion strain. Cytology showed mislocalization of Kmt6-GFP that is similar to that of Eed-GFP in kmt6 deletion mutants. Further assays will test for PRC2 activity as well as H3K27me3 enrichment by chromatin immunoprecipitation (ChIP).

135F De-regulation of the tryptophan biosynthesis pathway affects fungal viability and sexual reproduction. Bastian Dörnte, *Ursula Kües* Molecular Wood Biotechnology, University of Göttingen, Göttingen, DE.

Aromatic amino acid biosynthesis begins with chorismate as a common precursor. Tryptophan is produced from chorismate in six consecutive steps which are mediated in *Coprinopsis cinerea* by four different enzymes. Tryptophan synthase encoded by the *trp1*$^+$ gene is a bifunctional enzyme which catalyzes the final two reactions in the biosynthetic pathway to tryptophan. It consists of two distinct functional domains separated by a central linker region, unlike the situation in bacteria where the two domains are encoded by separate genes. The N-terminal A-domain (TrpA) of Trp1 is responsible for the conversion of indole-3-glycerol-phosphate (IGP) into indole and the C-terminal B-domain (TrpB) for subsequent production of tryptophan from serine and indole. The *trp1*$^+$ gene is often used in *C. cinerea* as a marker gene in protoplast transformation of *trp1-1,1-6* mutants. DNA integrates in transformation at multiple ectopic sites into the *C. cinerea* chromosomes. This allows cotransformation with two or more vectors. Surprisingly, numbers of transformants are doubled in cotransformation of *a trp1*$^+$-vector with a non-*trp1*$^+$-vector as compared to single *trp1*$^+$-vector transformations. This paradoxical phenomenon results from loss of clones in single vector transformations by tryptophan feedback inhibition and de-regulation of cross-pathway control of aromatic amino acid biosyntheses by insertion of multiple *trp1*$^+$ copies into nuclei of *C. cinerea* (Dörnte and Kües 2016, Appl Microbiol Biotechnol 100:8789-8807). Similar lethal effects were recently reported for *Schizophyllum commune* when the fungus transformed with its *trp1*$^+$ gene (orthologous to *trp2*$^+$ of *C. cinerea*). This gene encodes a trifunctional enzyme with glutamine amidotransferase (GATase), phosphoribosylanthranilate isomerase (PRAI) and indol-3-glycerol-phosphate synthase (IGPS) activities. Transformation with only the *IGPS* DNA region induced fruiting body development in *S. commune* several days earlier than normal which indicates a link between the tryptophan biosynthesis pathway and sexual development (Sen et al. 2016, Biosci Biotechnol Biochem 80:2033-2044). Moreover, in *C. cinerea* sexual development is affected by *trp1*$^-$ defects in irregular pigment synthesis of basidiospores. Studies on effects on viability and sexual development by gene *trp2*$^+$ as well as by the *trp3*$^+$ and *trp4*$^+$ genes of *C. cinerea* are underway.

136W Uni-directional mating type switching in *Davidsoniella virescens*. *P. Markus Wilken*[1], Emma T. Steenkamp[2], Michael J. Wingfield[1], Brenda D. Wingfield[1] 1) Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa ; 2) Department of Microbiology & Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of

Pretoria, South Africa.

Uni-directional mating type switching is a form of secondary homothallism where self-fertile isolates are able to produce both self-fertile and self-sterile offspring. Although this process has been reported in species in *Ceratocystis*, *Endoconidiophora*, *Davidsoniella*, *Sclerotinia*, *Glomerella* and *Chromocrea,* the *MAT1* locus of only *Ceratocystis* and *Sclerotinia* has been extensively studied. The gene complement of self-fertile strains contains both *MAT1-1* and *MAT1-2* related genes. Two copies of a direct repeat mediate the deletion of part of the *MAT1* locus, resulting in the loss of the *MAT1-2* genes. This renders the isolate self-sterile. In the present study, we examined the *MAT1* locus in *Davisoniella virescens* that is also capable of uni-directional mating type switching. The self-fertile *MAT1* locus in this species was found to include three mating genes in the order *MAT1-2-1*, *MAT1-1-1* and *MAT1-1-2*. Two copies of a 517 bp direct repeat flank the *MAT1-2-1* gene, with one complete copy present as part of the *MAT1-1-1* gene. The switching process results in the deletion of 4.6 kb of the *MAT1* locus and includes the full *MAT1-2-1* gene and flanking regions as well as one copy of the repeat. This is similar to what has been found in *Ceratocystis* and *Sclerotinia*, although the switching process in *D. virescens* produced a truncated *MAT1-1-1* gene that shortens the predicted protein from 322 to 273 amino acids. The effect of this truncation on the sexual cycle of *D. virescens* is unknown, and will form the basis of future studies.

137T Sensing and signalling intercalary growth in *Epichloë* endophytes: A transcriptomics approach to mechanosensing. *A. Ozturk*[1], S. Ariyawansa[1], R. E. Bradshaw[2], P. Maclean[1], J. Koolaard[1], P. Dupont[2], C. J. Eaton[2], N. D. Read[3], N. A. Gow[4], C. R. Voisey[1] 1) AgResearch, Grasslands Research Centre, Private Bag 11-008, Palmerston North 4442, New Zealand; 2) Bio-Protection Research Centre, Institute of Fundamental Sciences, Massey University, Palmerston North 4474, New Zealand; 3) Manchester Fungal Infection Group, Division of Infection, Immunity and Respiratory Medicine, University of Manchester, Manchester M13 9NT, United Kingdom; 4) The Aberdeen Fungal Group, School of Medicine, Medical Science and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB252ZD, UK.

Although fungal hyphae normally extend at apical tips, exceptions to polar growth characterise the ecology of many important species. We are investigating the physiology of intercalary growth in *Epichloë festucae*, a seed-transmitted symbiont that colonises the aerial parts of grasses. Developing leaves are initially invaded by hyphal tips, however continued hyphal colonisation occurs through expansion of intercalary compartments. We recently tested the hypothesis that intercalary growth is stimulated by mechanical forces imposed on hyphae through their attachment to expanding host cells. *E. festucae* hyphae were grown *in vitro* on silicon membranes and stretching forces were shown to induce intercalary compartment extension and division, as observed in developing leaves.

The aim of this study was to identify the cellular responses that induce intercalary hyphal growth after stretch. *E. festucae* cultures were grown on silicon membranes fitted to a custom-designed stretching frame, stretched by 8%, and then harvested either 5 min or 3 h later. The transcriptomes of stretched hyphae were compared against controls grown on the same devices with no stretch applied. Over 100 genes were differentially expressed in cultures harvested 5 min after stretch, whereas after 3 h over 400 genes were differentially regulated. The results suggest that reprogramming of primary metabolism and plasma membrane organisation occurs almost immediately in response to mechanical stress, and mobilisation of cell wall enzymes and secondary metabolism is observed in the cultures harvested later. These findings will assist in determining the role of mechanical stress in regulating *E. festucae* physiology and growth in plants.

138F Sterol-rich membrane domains of the basidiomycete *Schizophyllum commune*. *J. Poetschner*, K. Krause, E. Kothe Department of Microbiology, Microbial Communication, Friedrich Schiller University, Jena, Germany.

The highly polarized growth of filamentous fungi requires continuous supply of proteins and lipids to the hyphal tip. This transport is managed by a constant flow of secretion vesicles trafficking via the actin and microtubule cytoskeleton. Special microdomains, called lipid rafts are involved in the transport of proteins from the cell body to the growing hyphal tip. The main compounds of these membrane structures are glycosphingolipids and sterol. Apical sterol-rich plasma membrane domains (SRDs) are gaining attention for their important role in polarized growth of filamentous fungi and as receptor platform for signal recognition and forwarding. Although the importance of SRDs is becoming clear due to investigations on ascomycetes, their exact roles and formation mechanisms remain rather unclear.

Here we investigated lipid rafts of the basidiomycete *Schizophyllum commune*, which has been in the focus of research for many decades. Due to its fast passage over the entire life cycle under laboratory conditions, it became a model organism for physiological and genetic studies. We used fluorescent dyes like filipin to visualize sterol-rich membrane domains in this saprotrophic white-rot fungus. Furthermore, detection and identification of lipid raft localized signal proteins should give insights into the formation and localization of lipid rafts in basidiomycetes.

139W SIP-1 and BRO-1 interact with the SO protein and are essential for germling communication in *Neurospora crassa*. *M.R. Schumann*[1], A. Oostlander[1], H. Haj Hammadeh[1], J. Wissing[2], L. Jänsch[2], A. Fleissner[1] 1) Department of Genetics, TU Braunschweig, Braunschweig, DE; 2) Cellular Proteome Research, Helmholtz Centre for Infection Research, Braunschweig, DE.

Cell fusion is an essential process for the growth and development of eukaryotic organism. In the ascomycete fungus, *Neurospora crassa* germinating conidia sense each other and fuse. As a result, a network of germlings is formed, which further develops into the mycelial colony.

In order to establish physical contact, fusing germlings employ an unusual mode of communication. In a kind of “cell dialog”, the two fusion partners coordinately switch between two physiological stages probably related to signal sending and receiving. This involves the alternating recruitment of the MAK-2 MAP kinase module and the SO protein to the plasma membrane of fusion tips.

We identified SIP-1 and BRO-1 as new interaction partners of the SO protein. A deletion of the *sip-1* or *bro-1* gene results in a Δ*so*-like phenotype, resulting in the loss of chemotropic interactions and subsequent fusion. Subcellular localization and live-cell imaging revealed that SIP-1 and BRO-1 are recruited to the tips of interacting germlings in the same oscillating manner as SO and co-localizes with it. Surprisingly, in contrast to other known switching proteins, SIP-1 also accumulates in the described oscillating fashion at the membrane of isolated, non-interacting germlings. This surprising finding suggests, that already the individual germlings switch between two physiological stages, and that during cell-cell interaction these switches become coordinated.

We hypothesize that SIP-1 contributes to fusion competence and the initiation of cell-cell communication. Future characterization of SIP-1 functions will further our insight into the unique communication mechanism described as the “cell dialog”.

140T Subcellular dynamics of the MAP kinase MAK-2 correlate with its activity during germling fusion, thereby mediating a conserved cell-cell signaling mechanism. *A. Serrano*[1], M. Weichert[1], R. Marschall[2], J. Illgen[1], U. Brandt[1], N. Thieme[1], A. Lichius[3], N. Read[3], P. Tudzynski[2], A. Fleissner[1] 1) Department of Genetics, TU Braunschweig, Braunschweig, DE; 2) Institute of Molecular Biology and Biotechnology of Fungi, Schlossplatz 8, Münster, DE; 3) Institute of Inflammation and Repair, University of Manchester, CTF Building 46, UK.

Cell-cell fusion is essential for the development of most eukaryotic organisms. However, the molecular basis of this process is poorly understood. In *Neurospora crassa*, fusion occurs between germinating vegetative spores so-called germlings. This process employs an unusual mode of communication, in which the two fusion partners coordinately alternate between signal sending and receiving in a "cell dialog". It includes the alternating membrane recruitment of the SO protein (signal sending) and the MAP kinase MAK-2 (signal receiving). To analyze the relationship between the localization and activity of MAK-2, we permanently tethered the kinase to the plasma membrane by using a –CaaX motif. This mislocalization resulted in a fusion-defective phenotype in the Δ*mak-2* background, indicating that the dynamics of MAK-2 are essential for its function. Western blot analyses revealed a hyper-phosphorylation of the membrane-bound MAK-2 by its upstream MAP kinases. In addition, membrane tethering of activated MAK-2 in WT background disrupts the dynamic localization of SO and reduces the number of tropic interactions.

Similar to *N. crassa*, the grey mold *Botrytis cinerea* undergo cell fusion during colony formation. Localization of the MAK-2 homolog, BMP-1, shows a comparable subcellular dynamic localization during *B. cinerea* cell interactions. Surprisingly, we found that *N. crassa* and *B. cinerea* germlings undergo mutual attraction and establish physical contact, although complete fusion events were not detected. Together, these data suggest that the "cell dialog" mode of communication is conserved in filamentous ascomycete fungi and that divergent fungal species share a common language

141F The gray mold's take on color: Role of the eleven photoreceptors of Botrytis cinerea in photomorphogenesis. *K C Cohrs*, L Pape, J Schumacher IBBP, WWU Münster, Münster, DE.

Light is an important environmental signal for fungi to regulate processes such as morphogenetic programs, pigmentation, stress responses and the circadian clock. The plant pathogen *Botrytis cinerea* produces conidia when exposed to light, and sclerotia, strongly melanized enduring structures, in the dark. Induction of conidiation by full-spectrum light is mediated by the light-responsive transcription factor (TF) BcLTF2. The formation of conidia is regulated by at least four different light qualities: exposure to blue light inhibits conidiation and leads to the formation of sterile aerial hyphae, an effect that can be reverted by near-UV (NUV) or far-red light, but not by red light. *B. cinerea* possesses a total of eleven putative photoreceptors (PRs) – more than most other filamentous ascomycetes: two NUV-sensing cryptochromes (CRYs), four blue light-sensing LOV (light, oxygen, voltage domain) proteins, two green light-sensing opsins, and three red/ far-red light-sensing phytochromes (PHYs). The role of the PRs in photomorphogenesis was studied by gene deletion and overexpression approaches.

The White Collar Complex (WCC), containing the blue light receptor/ GATA-TF BcWCL1, negatively regulates conidiation through repression of *bcltf2* and acts as a primary sensor of light as it induces the expression of other PR-encoding genes. For example, expression of *bcvvd1*, encoding a putative blue light receptor, depends on the WCC. BcVVD1 in turn acts as a negative regulator of WCC activity, creating a negative feedback loop and allowing for the fine tuning of conidiation. Additionally, the putative NUV PR BcCRY2 acts as a negative regulator of conidiation in the light through a yet unknown mechanism. The role of the red/ far-red light-sensing PHYs in the light response system is still unclear as single deletions did not affect conidia formation. Because the three PHYs may have redundant functions, the generation of double and triple mutants has been initiated.

142W Chitin-deacetylase activity has important roles in inducing appressorium formation. *M. Kuroki*[1], Y. Shiga[1], K. Okauchi[1], K. Saitoh[2], T. Teraoka[2], M. Narukawa[1], T. Kamakura[1] 1) Tokyo University of Science, Noda, Chiba, JP; 2) Tokyo University of Agriculture and Technology, Fuchu, Tokyo, JP.

Magnaporthe oryzae (*Pyricularia oryzae*) is a filamentous plant pathogen and causes rice blast. *M. oryzae* differentiates appressorium in order to infect plant cell. *M. oryzae* seems to perceive environmental signals, but induction mechanism of appressorium has remained unrevealed. We focused on *CBP1* (MGG_12939) that have two chitin-binding domains and chitin-deacetylase (CDA) homologous domain. Appressorium formation is delayed in *CBP1* deletion mutant compared with wild type on hydrophobic substrate, but there is no difference between *CBP1* deletion mutants and wild type when cutin monomer was added. We suspect Cbp1 is an important component for induction of appressorium formation.

We previously observed appressorium formation of CDA inactive Cbp1 mutants is low level and chitosan amount is lower in *CBP1* deletion mutant. These data suggest Cbp1 has CDA activity and it has an important role in appressorium formation. In this study, we observed expression patterns of other *CDA*s in *M. oryzae*. *M. oryzae* has 6 *CDA* homologous genes except for*CBP1*. These 6 *CDA*s were not up-regulated in *CBP1* deletion mutant, but some of them were up-regulated more than wild type by adding cutin monomer. We estimated that CDA activity is required to form appressorium in its early stage and 6 CDAs highly express to compensate for deletion of *CBP1* when be added cutin monomer.

In wild type, chitosan was accumulated in tips of germ tubes before it started to differentiate appressorium. On the other hand, in *CBP1* deletion mutant, chitosan amount in tips of germ tubes seemed much less than wild type. In addition, amino acid sequence suggested Cbp1 was GPI-anchored protein and locates cell surface, but other 6 CDAs were free secreted proteins. We presumed location of Cbp1 could explain Cbp1's importance in appressorium formation in contrast to other CDAs. We are going to observe Cbp1's location in detail and verify whether Cbp1 is GPI-anchored protein.

CDAs generally convert chitin into chitosan and release acetic acid together. We focused on acetic acid as inducer of appressorium formation. We are trying to observe appressorium formation under existence of acetic acid.

143T Chloramphenicol inhibits appressorium formation of *Magnaporthe oryzae* via a Ser/Thr-protein phosphatase, *MoDullard*. *A. Nozaka*[1], S. Endo[1], N. Tanaka[1], M. Narukawa[1], S. Kamisuki[2], M. Nakajima[1], H. Taguchi[1], F. Sugawara[1], T. Kamakura[1] 1) App. Bio. Sci., Tokyo Univ. of Sci., Chiba, Japan; 2) Dept. Vet. Med., Azabu Univ., Tokyo, Japan.

The appressorium formation of *Magnaporthe oryzae* is essential for infection and related to a cell differentiation. Some drugs inhibiting appressorium formation will also affect the factor which is involved in eukaryotic cell differentiation in the rice blast fungus. We searched novel targets of preexisting drugs using appressorium formation of *M. oryzae* as an indicator whether the factor relates cell differentiation. As a result, antibiotic Chloramphenicol (Cm) showed remarkable inhibition of appressorium formation. Since Cm interacts to 50S ribosomal subunit in prokaryotes, Cm inhibits protein synthesis of prokaryotes specifically. However, in human Cm often causes a serious side effect aplastic anemia the target of Cm in eukaryote is unknown. If we can discover the unknown eukaryotic target of Cm, it will lead to contribute to clarifying the mechanism of the side effect caused by Cm, to develop a novel pesticide or rice blast specific fungicide, and/or to obtain the knowledge about the regulation of cell differentiation and appressorium formation.

We selected T7 phage display as a tool for searching Cm's target(s). From the candidate, we deduced that a Ser/Thr protein phosphatase Dullard (*MoDullard: MGG_03646*) was a possible binding protein of Cm. Using RTPCR, we detected the expression of *MoDullard* during appressorium formation. Then we acquired the GST-tag fused *MoDullard* expressed by *E. coli* and showed that the tag fused MoDullard protein bound to Cm. Moreover in the appressorium formation assay, *MoDullard* over expressed strains showed the lower sensitivity to Cm than WT. Each results inferred that MoDullard interacts with Cm in *M. oryzae* and affects appressorium formation of the rice blast fungus.

Now we challenge to establish *MoDullard* knockout and knockdown strain, and as soon as obtainment we are going to check the phenotype of them against Cm in appressorium formation assay. We also expect that the analysis of *MoDullard* for the mechanism of appressorium formation will shed new light on the mechanism of side effect of Cm, and contribute to development of antifungal agents.

144F Two independent S-phase checkpoints regulate appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*. *M. Oses-Ruiz*, W. Sakulkoo, G. R. Littlejohn, M. Martin-Urdiroz , N. J. Talbot School of Biosciences, College of Live and Environmental Sciences University of Exeter, Exeter, EX4 4QD, United Kingdom.

The rice blast fungus *Magnaporthe oryzae* elaborates a specialised infection cell called an appressorium to infect rice leaves. The appressorium generates enormous turgor, which is focused as physical force to break the rice leaf cuticle allowing the fungus entry to rice cells. We have found that appressorium-mediated plant infection by *M. oryzae* requires two independent S-phase cell cycle checkpoints. During initial formation of appressoria on the rice leaf surface, an S-phase checkpoint operates through the DNA damage response (DDR) pathway, involving the Cds1 kinase. By contrast, during appressorium repolarization a novel, DDR-independent S-phase checkpoint is triggered by appressorium turgor generation and melanisation. This checkpoint regulates NADPH oxidase-regulated, septin-dependent, F-actin re-modeling to organise the appressorium pore and bring about entry of the fungus into rice tissue. We will report on the operation of these two checkpoints, the processes they control, and how they regulate rice infection by the *M. oryzae.*

145W Phenotypic and genotypic analysis of a new set of *Aspergillus nidulans* mutants defective in hyphal morphogenesis and secretion. Laure Ries[1], Thaila dos Reis[1], Karthik Boppidi[2], Anna Lipzen[3], Kerrie Barry[3], Igor Grigoriev[3], Gustavo Goldman[1], Mark Marten[2], *Steven Harris*[4] 1) Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Brazil; 2) Department of Chemical, Biochemical and Environmental Engineering, University of Maryland-Baltimore County, Baltimore, MD; 3) JGI-DOE, Walnut Creek, CA; 4) Center Plant Sci Innovation, Univ Nebraska, Lincoln, NE.

Investigation of the mechanisms that coordinate hyphal morphogenesis with vesicle trafficking and secretion is central to understanding how filamentous fungi grow. Reverse genetic approaches that characterize homologues of well-known yeast gene products involved in morphogenesis and vesicle trafficking proteins have yielded significant insight into these mechanisms. Nevertheless, morphogenesis and secretion are clearly more complex in hyphae than in yeast cells. For example, hyphae can simultaneously sustain multiple polarity axes, and there is increasing evidence for the existence of multiple routes of vesicle delivery to the cell surface. To account for the greater complexity of these processes in filamentous fungi, we contend that unbiased forward genetic screens represent an attractive alternative approach that is not constrained by existing knowledge of other systems. This is particularly important given that ~50% of annotated genes have no known function in a typical filamentous fungus, some of which undoubtedly play key roles in morphogenesis and secretion. Here, we present the preliminary characterization of a new set of *Aspergillus nidulans* morphological mutants with associated secretion defects. Mutants were derived from strain FGSC A4 using a filtration-based enrichment followed by secondary plate-based screens for secretion defects. We report here on the phenotypes and genome sequences of 39 mutants. A small number of these mutants exhibit an apparent hyper-secretion phenotype, and possess mutations predicted to impact regulation of the GTPases Cdc42 and Rac1, myosin-based transport, and function of the protein kinase PakB. Other mutants possess mutations in a number of genes not previously implicated in hyphal morphogenesis or vesicle transport. We anticipate that this collection of mutants and genome sequences will prove to be a valuable community resource.

146T Extensive transcriptional differentiation during acquisition of a plastic developmental competence state in *Aspergillus nidulans*. Luke Noble[1,2], Linda Holland[1,3], *Alex Andrianopoulos*[1] 1) School of BioSciences, University of Melbourne, Parkville, Victoria, Australia; 2) Department of Biology, New York University, New York, USA; 3) School of Biotechnology, Dublin City University, Dublin, Ireland.

Development requires precise temporal and spatial timing of gene expression. At the cellular level where differentiation occurs, this is controlled by the presence of inductive and repressive cues, such as hormones and cell-cell contacts but must also be buffered against intrinsic biological noise that can lead to sporadic induction of differentiation. A central mechanism to buffer biological noise is to control when cells become competent to receive developmental cues. We have undertaken a broad analysis of the environmental and genetic basis of 'developmental competence' in the filamentous eukaryote *Aspergillus nidulans*. Density and pH are critical parameters, and we identify 21 genes with effects on competence timing through genetic screens and candidate mutagenesis, from fungal-specific transcription factors to the conserved unfolded protein response regulator HacA, GTPase RasB and ambient pH sensor PalH. Transcriptional profiling and cross-species comparison demonstrates substantial, concordant genome-wide activity, in the absence of morphological differentiation, including a wave of gene expression around centromeres indicative of chromatin remodeling. Species-specific endogenous hormones and

metabolic capacity likely determine competence timing. An understanding of reproductive competence in fungi is of relevance to all biology associated with the 'adult' form – asexual and sexual reproduction, primary and secondary metabolism, and pathogenesis – and provides a new model of nutrient-limited life history phases.

147F A conserved regulator of meiosis has been rewired to control chlamydosporulation in the fungal pathogen of humans *Candida albicans*. A. Hernandez-Cervantes[1], S. Znaidi[1,2], V. Basso[1], J. Ropars[1], N. Sertour[1], D. Sullivan[3], G. Moran[3], F. Vincent[4], F. Dalle[4], M.E. Bougnoux[1], S. Bachellier-Bassi[1], *C. d'Enfert*[1] 1) Fungal Biology and Pathogenicity, Institut Pasteur, INRA, Paris, FR; 2) Laboratoire de Microbiologie Moléculaire, Vaccinologie et Développement Biotechnologique, Institut Pasteur de Tunis, Tunis, TUN; 3) Trinity College, Dublin, IRL; 4) UMR 1347, Université de Bourgogne, Dijon, FR.

Chlamydospores are large, spherical, thick-walled cells produced by several fungi, including the fungal pathogens of humans *Candida albicans* and *Candida dubliniensis*. Little is known regarding the function of these cells and the pathway controlling chlamydosporulation. While investigating the function of the transcription factor Rme1 in the regulation of morphogenesis in *C. albicans*, we have observed that it binds and positively regulates genes that have been previously associated to chlamydospore formation (1). Consistently, inactivation of *RME1* abolished chlamydospore formation in *C. albicans* and *C. dubliniensis* while its over-expression resulted in profuse chlamydosporulation. *RME1* overexpression could also bypass the requirement for other chlamydospore formation regulators such as Efg1 and Hog1. Finally, the expression levels of *RME1* and its targets were highly correlated to the varying ability of *C. albicans* clinical isolates to produce chlamydospores. Thus, Rme1 plays a central role in the control of chlamydosporulation in *C. albicans* and *C. dubliniensis.*

Consistent with this role, *RME1* expression increased in the course of chlamydosporulation. Chromatin-immunoprecipitation experiments, experiments using conditional *RME1* expression and gene fusion experiments with a luciferase reporter allowed to demonstrate that *RME1* expression is subject to a dual regulation whereby (i) elevation of *RME1* mRNA levels in response to chlamydospore-inducing conditions is independent of Rme1 and (ii) sustained expression of *RME1* during chlamydosporulation is subject to autoregulation. Taken together, our results establish Rme1 as a central regulator of the chlamydospore developmental pathway in *C. albicans.* Surprisingly, in *S. cerevisiae* and other yeasts, Rme1 plays an important role in the control of meiosis, and thus ascospore formation. Therefore, our results indicate that Rme1 can be used alternatively to control sexual or asexual development in Saccharomycotina.

(1) Palige *et al*. PLoS One 8:e61940, 2013

148W Regulation, localization and stability of the velvet protein VE-1 in Neurospora crassaM. del Mar Gil-Sánchez, Eva M. Luque, Luis M. Corrochano. *M. del Mar Gil Sanchez*, Eva M. Luque Fobelo, Luis M. Corrochano Pelaez Genetics, University of Seville, Seville, Seville, ES.

In *Neurospora crassa* light is perceived through the WC complex, a light-dependent transcription factor complex that regulates the transcription of a number of genes. The *Neurospora* genome contains a homolog of the *Aspergillus nidulans veA* gene, *ve-1*. In *A. nidulans* mutations in *veA* results in constitutive conidiation that is independent of light, and the VeA protein forms a complex with photoreceptors. VE-1 is a protein with a nuclear localization signal and a velvet factor domain that is highly conserved in fungi. The *N. crassa ve-1* mutant has defects in aerial hyphal growth and increased conidiation.

We have characterized the light-dependent accumulation of carotenoids in a strain with a deletion in *ve-1* and in the wild-type. A ten-fold reduction in sensitivity was observed in the Δ*ve-1* strain, an indication for a role of VE-1 in light sensing in *N. crassa.*

We have created a strain with a tagged version of VE-1 to detect the protein. We observed a minor increase in the accumulation of *ve-1* mRNA after light exposure in vegetative mycelia (30 min), which did not lead to changes in VE-1 accumulation. The mutation in *ve-1* results in decreased light-dependent accumulation of mRNA for several genes, including the carotenogenesis genes, *wc-1*, *vvd*, and *frq*. We have characterized the cellular localization of VE-1 under different light conditions and we have observed that VE-1 is preferentialy located in the nucleus under all conditions, but VE-1 was also detected in the cytoplasm. VE-1 was not present during conidial development in the dark, but VE-1 was detected in vegetative mycelia. In the light, however, VE-1 was detected in aerial hyphae and vegetative mycelia and it is localized mostly in the nucleus. Our results suggest that the presence of VE-1 in aerial hyphae could depend on light, given that VE-1 is not detected in light neither in the dark in the ?*wc1* mutant despite the detection of the mRNA for *ve-1*. This could indicate a posttranslational protein regulation. Therefore, we studied the degradation of VE-1 through the signalosome CSN and the FWD-1 pathway. The accumulation of VE-1 is higher in the *fwd-1* mutant than in the wt. In addition, we have observed that light regulates the stability of VE-1 and the protein is more stable in the absence of CSN-5 and FWD-1. The regulation by light of the degradation of VE-1 through the CSN and after interactions with FWD-1 could be key in the regulation of conidial development in *N. crassa*.

149T Cell-to-cell communication in *Neurospora crassa*: Transcriptional regulation and the interplay between self and non-self recognition. *M.S. Fischer*, N.L. Glass Plant & Microbial Biology, UC Berkeley, Berkeley, CA.

Self/non-self recognition and communication between genetically identical cells is an essential developmental process across the tree of life. We use the model filamentous Ascomycete *Neurospora crassa* to investigate the genetic and molecular mechanisms of cell-to-cell communication. As *N. crassa* conidia germinate into germlings, they begin to communicate with other nearby germlings. If they recognize that nearby germlings are genetically identical ("self"), then they will fuse and work together to establish a new colony. Several genetic screens and experiments have identified over 60 genes that are required for normal chemotropic growth and fusion between germlings. Among these 60 genes are two transcription factors, ADV1 and PP1. We identified that in germlings PP1 is required for transcription of ADV1, which controls the expression of at least 30% of the known communication and fusion genes (identified via RNAseq). Furthermore, overexpression of ADV1 is sufficient to suppress the pleiotropic phenotype of *Δpp1*, but the inverse is not true. Many of the genes regulated by ADV1 and PP1 are poorly characterized or simply annotated as hypothetical proteins. We hypothesize that some of these uninvestigated genes are involved in germling communication. *ham11*, *doc1*, and *doc2* are all in the PP1/ADV1 transcriptional regulon, and until recently, these genes were also uninvestigated hypothetical proteins. Several germling communication assays with *Δham11*, *Δdoc1*, *Δdoc2* and all possible double and triple mutants led us to the conclusion that HAM11 and DOC1/DOC2 function in parallel to mediate germling communication. HAM11 mediates self-communication by positively reinforcing communication between genetically identical germlings. In contrast, DOC1 and DOC2 strongly repress communication between genetically dissimilar germlings. We are currently working toward a

deeper understand of the interplay between the self and non-self recognition pathways during germling communication and colony establishment.

150F *Neurospora* MSH1 protein functions to maintain the mitochondrial DNA. *T. Yokoi*, Y. Kojima, S. Tanaka, S. Hatakeyama Saitama University, Saitama, Saitama, JP.

The *nd* (*natural death*) strain of *Neurospora* was originally isolated which showed serious growth defect even on complete medium and restrictive temperature. This mutant shows sensitivity to alkylating agent and ceases hyphal growth in approximately two weeks along with large deletion of mitochondrial DNA (mtDNA). Previously, we identified that the *nd* strain harbored the nonsense mutation in *msh1 (MutS homolog 1)* gene and wild type *msh1* gene could complement phenotypes of the *nd* strain. MSH proteins, MSH1 to MSH6, have identified as eukaryotic homologs of *E. coli* MutS protein, which recognizes the mis-incorporated nucleotide in the newly synthesized DNA strand. Among these, MSH1 is conserved exclusively in fungi, so this protein is implied to have unique role in fungal cell. Knock out of the *msh1* gene in *Neurospora* caused similar phenotypes of the *nd* strain. The predicted amino acid sequence of *Neurospora* MSH1 reveals that mitochondrial localization signal (MLS) and DNA binding motif are well conserved in the N-terminus. Since deletion of the MLS resulted in showing similar phenotypes to those of the *msh1* KO and *nd* strains, MSH1 might be functioned in the mitochondria. Further, in these mutants, not only large size deletion of mtDNA was detected, but also aberrant branched DNA in mitochondria was accumulated. Expression of the fusion protein of wild type MSH1 with GFP showed its localization in mitochondria, but interestingly, MLS deletion version of MSH1 protein also associated to this organelle. From these results, we are forecasting that the MSH1 protein may function in the maintenance of mtDNA, which is indispensable to sustain mitochondrial function one of which correlates to energy supply for the elongation of hyphal tip.

151W CSE-7, a predicted ER-chaperone, plays an important role in the delivery of CHS-4 to hyphal tips and septa in *Neurospora crassa*. *A. Rico Ramirez*, M. Riquelme Microbiology, CICESE, Ensenada, Baja California, Mexico.

Chitin synthesis is catalyzed by chitin synthases (CHS), a family of plasma membrane (PM) embedded enzymes. In *Saccharomyces cerevisiae*, exit of Chs3p from the endoplasmic reticulum (ER) requires the help of chaperone Chs7p, while at the Golgi, it requires the exomer complex components Chs5p and Chs5p-Arf1p binding proteins or ChAPs (Chs6p, Bch2p, Bch1p and Bud7p). In addition, the localization and activation of Chs3p at the PM depends on its interaction with Chs4p. Corresponding homologues for the yeast proteins involved in Chs3p transport have been identified *in silico* in the *Neurospora crassa* genome: CSE-7 (Chs7p), CBS-5 (Chs5p), BUD-7 (as homologue of the four Chs5p-Arf1p binding proteins or ChAPs, Chs6p, Bch1p, Bch2p and Bud7p), and three homologues of Chs4p (CSA-1, CSA-2 and CSR-3).

N. crassa CHS-4 (orthologue of the yeast Chs3p) tagged with GFP was located at the core of the Spitzenkörper (Spk) and in septa. CSE-7-GFP was revealed at the Spk core, at developing septa, and additionally, at an extensive subapical endomembranous tubular network, resembling the fluorescence pattern observed for mCherry containing the ER retaining signal HVEL. Total Internal Reflection Fluorescent Microscopy revealed highly dynamic vesicles at hyphal subapical regions for CSE-7-GFP but not for mCherry-HVEL. In addition, fixed hyphae of *N. crassa* expressing CSE-7-mChFP and exposed to ER-Tracker Blue-White-DPX revealed a partial co-localization, confirming that CSE-7 is indeed at the ER. In a *N. crassa Δcse-7* mutant CHS-4-GFP no longer accumulated at the Spk and septa; instead, fluorescence was observed in the subapical region lighting up tubular structures similar to those observed for CSE-7-GFP. In the complemented strain *Pchs4::chs-4-gfp::nat^R;Δcse-7::hph$^+$;his3$^-$;Δmus51::bar$^+$;Pccg-1::cse-7::mchfp::his-3$^+$* the localization of CHS-4-GFP at the Spk and septa was restored, providing evidence that CSE-7 is necessary for ER exit of CHS-4. Co-immunoprecipitation (Co-IP) studies are undergoing to confirm interaction between CSE-7 and CHS-4, and also to identify other physiologically relevant interactors.

152T A HAD family phosphatase PSR-1 regulates circadian output pathway in *Neurospora crassa*. *X. Zhou*[1], B. Wang[1], J. Emerson[1], C. Ringelberg[1], S. Scott[1,3], J Loros[2], J Dunlap[1] 1) Molecular and System Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 3) Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Hanover, NH.

Circadian clocks are ubiquitous in eukaryotic organisms where they are used to anticipate regularly occurring diurnal and seasonal environmental changes. Although the mechanism of the core oscillator is becoming understood, little is known regarding pathways connecting the core clock to its output pathways. Here, we demonstrate the HAD family phosphatase PSR-1 is involved in circadian clock output but not in the core oscillator. The Δ*psr-1* deletion mutant has a circadian output defect on race tubes under free running conditions such that constant conidiation is observed instead of typical clock-controlled rhythmic banding. However, further analysis indicates that the FRQ-WCC (FREQUENCY-WHITE COLLAR COMPLEX) Oscillator functions normally in Δ*psr*-1 strains albeit with a minor 3.5-hour phase delay. PSR-1 physically interacts with WHI-2, the Δ*whi-2* mutant has phenotypes similar to those observed in Δ*psr-1*, and analysis of the Δ*psr-1*, Δ*whi-2* double mutant reveals PSR-1 plays the major catalytic role in the PSR-1/WHI-2 complex. PSR-1 primarily localizes to the nucleus and directly interacts with WC-1, it is essential for maintaining WC-1 protein and phosphorylation levels. PSR-1 is also important for the interaction between VVD and WC-1. Double mutant Δ*psr-1,* Δ*vvd* strains showed further reduced WC-1 protein amounts and phase delays compared to either single mutant suggesting that the reduced amount of WC-1 may factor in determining the phase delay. Consistent with this, when WC-1 protein level is increased by use of a *qa-2*-induced promoter in the Δ*psr-1* mutant, the phase delay phenotype is partially rescued although not the defect in rhythmic banding. Loss of overt banding but not of core clock function in ?*psr*-1 suggested a discrete loss of circadian control of transcriptional output from the clock, and screening of candidate clock-controlled transcription factors identified loss of control of ADV-1, a direct target of the WCC, as responsible for the loss of overt rhythmicity. The PSR-1/WHI-2 complex participates in *Neurospora* clock output, regulating the level and phosphorylation status of WC-1 and thereby promoting proper transcriptional/translational activation of *adv-1*/ADV-1. These data provide a precedent for an essential role of post-translational regulation in the early steps of circadian output.

153F Is auxin biosynthesis in *N. crassa* connected to the eisosomal localized BEM46 protein? *F. Kempken*, K. Kollath-Leiß, P. Sardar, Q. Yang Institute of Botany, Christian-Albrechts University, Kiel, DE.

We previously showed tryptophan-dependent auxin (IAA) production in *Neurospora crassa* by HPLC and TLC analyzes (1). Further we identified the complete IAA-biosynthesis network by bioinformatical tools. These pathways were confirmed by quantitative and qualitative determination of the produced indolic compounds in newly generated double and triple mutants. BEM46 (2), a protein that influences the expression of genes involved in the IAA-biosynthesis network and also of the *mtr* gene encoding a tryptophane transporter (1) is a conserved αβ-hydrolase in *N. crassa* (3). Over-expression of *bem46* led to loss of ascospore germination and a clear reduction of hyphal elongation. RNAseq data have recently been obtained are being analysed. *bem-46*-knock-out strains show a reduced ascospore germination and a slightly but significant higher elongation of vegetative hyphae. Y2H and split-YFP experiments demonstrated the interaction of BEM46 with anthranylate synthase, which is involved in the tryptophan biosynthesis of the fungus (1).

BEM46::eGFP was found to be localized to the ER (2) and to eisosomes (1). The latter are static, plasma membrane associated protein complexes with a hypothetical function of protecting particular proteins from endocytosis (4). For a better understanding of the eisosomal composition in a filamentous fungus, GFP and RFP reporter constructs of various transport proteins have been constructed and protein localization in different developmental stages is being analyzed. Most interestingly, MTR, a tryptophane transporter is also localized in the eisosome.

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154W Characterization of the mutant *dnf-4,* a phospholipid flippase in the filamentous fungus *Neurospora crassa.* *O.A Callejas-Negrete*[1], I Murillo-Corona[1], B.D Shaw[2], R.R Mouriño-Pérez[1] 1) Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada B. C. México. ; 2) Department Plant Pathology and Microbiology. Texas A&M University College Station, TX, US.

Phospholipid flippases are transmembrane proteins that are conserved among eukaryotic organisms; they translocate specific population of phospholipids from the luminal leaflet to the cytosolic leaflet and have been associated with apoptosis signaling, protein sorting, vesicular traffic and secretion. The *dnf-4* gene of *Neurospora crassa* has a high identity with the *neo-1* gene of *Saccharomyces cerevisiae* that is an essential gene in budding yeast and belongs to a highly conserved subfamily of P-type ATPase genes that encode phospholipid flippases. We studied the dynamics of DNF-4 and the effect of the mutation of the *dnf-4* gene in *Neurospora crassa*. The Δ*dnf-4* mutant had a decrease of 41% in cell growth, 30% in biomass production and 76% in conidia production ($p<0.05$). Branching rate was three-fold higher in the Δ*dnf-4* mutant ($p<0.05$). Mature hyphae showed a Spk smaller and less stable and also presented a distorted morphology, presenting bulging sites. DNF-4-GFP was present as bright spot surrounding the endoplasmic reticulum and the Golgi, it was not present in the apex or the plasma membrane. The mutation *dnf*-4 gene in *N. crassa* is not essential but affects growth rate and morphology and severely reduced the production of conidia. It is a phospholipids translocator of the ER and Golgi membrane.

155T Nod-like receptor mediated allorecognition induces germling regulated death in *Neurospora crassa*. *J. Heller*, N. L. Glass Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA.

Allorecognition-induced death in filamentous fungi has been proposed to function as a type of fungal innate immunity system, whereby recognition of non-self reduces the risk of transmission of pathogenic elements between colonies and exploitation by aggressive genotypes. Cellular fusion of vegetative cells is an essential process for fungal development and can occur between hyphal cells of an individual colony or between hyphal cells of genetically distinct colonies. Heterokaryon incompatibility (HI), a form of allorecognition, is one mechanism to restrict the propagation of fusion products between genetically dissimilar individuals by inducing a programmed cell death (PCD) response. HI and PCD have been considered to only occur after the fusion of mature hyphae while being suppressed during germling fusion. Here, we present the identification of allorecognition mechanisms that act at the germling stage. If germlings of various wild isolates are of different specificity at so-called germling regulated death (GRD) loci, allorecognition mediates rapid cell death following membrane merger between germlings (~20 min). Flow cytometry experiments showed that death is 100% penetrant (all fused germlings of different GRD background die). By bulk segregant analysis (BSA) of progeny from a cross between two wild isolates followed by whole genome re-sequencing we identified two linked loci (*plp-1* and *sec-9*) that segregated with the GRD phenotype. The genes *plp-1* and *sec-9* have typical characteristics of allorecognition genes: they show discrete haplotypes that have a high level of polymorphism with long-term persistence through multiple speciation events (trans-species polymorphism), supporting the hypothesis that balancing selection is acting on these loci. SEC-9 is an essential SNARE protein predicted to be involved in membrane fusion and exocytosis. PLP-1 is a nod-like receptor similar to intracellular pattern recognition receptors (NLRs) that mediate recognition of microbe-associated molecular patterns, the initial step of innate immunity in animals and plants. Analyses of death frequencies in various mutants of *plp-1* and *sec-9* confirmed that non-allelic interaction between *plp-1* and *sec-9* is required for allorecognition and GRD. By creating chimeric proteins we identified domains in SEC-9 and PLP-1 that are essential for the PCD induction and specificity. We propose a model in which PLP-1 acts as a NLR in fungal innate immunity mediating recognition of SEC-9.

156F Role of class II myosin in apical growth and morphogenesis of *Neurospora crassa*. Fernando Nuñez-Moreno, *Salomon Bartnicki-Garcia*, Olga Callejas-Negrete, Rosa. Mouriño-Pérez Dept Microbiology, CICESE, Ensenada, MX.

Evidence continuous to mount for the central role of the actin cytoskeleton and its binding proteins (ABP) in the growth of fungal

hyphae. Here we present evidence for the participation of MYO-2, a type II myosin, in the actin cytoskeleton of *Neurospora crassa,* and its role in growth and morphogenesis. We generated a strain expressing MYO-2 fused to green fluorescent protein (GFP) and a *myo-2* deletion mutant (*Δmyo-2*). MYO-2-GFP was present in hyphal tips, in septa and in microconidia. By confocal microscopy, we discovered that MYO-2-GFP colocalizes sharply with the FM4-64-stained Spitzenkörper (Spk). FRAP analysis of the MYO-2-GFP fluorescent signal in the Spk yielded a 50% recovery after 50 sec and a total recovery after 130 sec, about twice as long compared to the known recovery times of CHS-GFP signals in the Spk of *N. crassa.* By blocking the binding of ATP to MYO-2-GFP with blebistatin, the fluorescence in the Spk became dispersed showing that Spk localization was dependent on ATPase activity. *Myo*-2 deletion caused severe loses: biomass was decreased by 57%, while colony radial growth was reduced by 75%; this was accompanied by a 45% decrease in hyphal diameter and only about half as many branches were produced per hypha. Hyphal septa disappeared in the *Δmyo-2* mutant together with an almost complete inhibition of aerial mycelium formation (97%), and no conidiation was observed. In the absence of MYO-2, the Spk appeared normal though smaller in size in concordance with the smaller hyphal diameter. Mutant hyphae showed an increased tendency for meandering. Although similar findings have been reported for *myo-2* deletion causing severe reduction in growth and conidiation of other fungi (*Aspergillus nidulans, Penicillium. marneffei* and *Fusarium graminearum*) this is the first report on the localization of MYO-2 in the Spk of a fungus. The exact function of this myosin in the Spk is uncertain but MYO-2 seems not to be essential for hyphal growth though its presence may contribute to Spk stability thus assuring full hyphal growth potential. In contrast, MYO-2 does play an essential role in septation and conidiation as a component of the septal actomyosin constricting ring as was previously demonstrated.

157W The penta-EF-hand Protein PEF-1 is part of the membrane damage response during cell fusion- induced lysis in *Neurospora crassa*. M. R. Schumann, L. Hartung, *A. Fleissner* TU Braunschweig, Braunschweig, DE.

During colony establishment, germinating vegetative spores of *Neurospora crassa* fuse with each other to form a supracellular network, which further develops into the mycelial colony. Fusion pore formation is mediated by a controlled cell wall breakdown and plasma membrane merger. These steps bear the risk of cell lysis and death by membrane rupture. Earlier studies indicated that in the plasma membrane fusion mutant Δ*Prm1*, lysis of fusion pairs occurs more frequently. In the absence of calcium, lysis rates increased even further, suggesting the presence of Ca^{2+}-mediated membrane repair mechanisms.

We identified the Ca^{2+}-binding penta-EF-hand protein PEF-1 as a potential part of this proposed repair mechanism. Subcellular localization and live-cell imaging revealed that PEF-1 is recruited to the fusion point of lysing germling pairs and accumulates at the plasma membrane after treatment with the anti-fungal and membrane destabilizing drug nystatin. Deletion of the *pef-1* gene in the D*Prm-1* mutant had a comparable effect to the depletion of calcium and resulted in an increased lysis rate. In contrast to recent publications on Pef-1 functions in the budding yeast *Saccharomyces cerevisiae*, a deletion of *pef-1* in *N. crassa* does not result in reduced stress tolerance. Interestingly, a deletion of *Pef-1* in the important human fungal pathogen *Candida albicans* results in reduced growth in the presence of cation-chelating agents EGTA and membrane- destabilizing SDS. The mammalian PEF-1 homolog ALG-2 is thought to link calcium-dependent signaling with vesicle trafficking. In addition, vesicles commonly contribute to membrane repair, for example by the formation of wound patches in response to a calcium influx.

Based on these observations, we hypothesize that in *N. crassa*, membrane damage results in the influx of calcium, which activates PEF-1, which in turn mediates vesicle-based plasma membrane repair. Further studies aim to fully characterize this repair mechanism, which is also activated in response to the important anti-fungal drug nystatin.

158T The chitin synthase regulator CSR-3 and the protein SO function together in cell-cell fusion and stress-induced cell wall remodeling in *Neurospora crassa*. *S. Herzog*, T. Sedlacek, A. Hirsch, U. Brandt, A. Fleissner Department Of Genetics, TU Braunschweig, Braunschweig, Niedersachsen, DE.

The filamentous ascomycete fungus *Neurospora crassa* is a widely used model system to study cell-cell fusion. Genetically identical germinating spores fuse with each other and form a subcellular network resulting in an interconnected mycelium. The interacting cells establish an unusual mode of communication by switching between signal sending and receiving. During this 'cell dialog' the MAK-2 MAP kinase module and the protein SO are recruited to the interacting cell tips in an alternating manner. After cell-cell contact, the MAK-1 MAP kinase module which is part of the cell wall integrity (CWI) pathway localizes together with MAK-2 and SO to the forming fusion pore. Despite the described function of SO as a scaffold for the CWI protein kinases in *S. macrospora,* its molecular function during these processes is still unknown.

In an Y2H screen we identified in addition to MEK-1 and MIK-1, the up-stream MAP kinases of MAK-1, the chitin synthase regulator CSR-3 as potential interaction partner of SO. CSR-3 transiently co-localizes with these proteins to the prospective fusion site after cell-cell contact and remains there until pore formation is completed. The lack of this regulator increase lyses of fusing cell pairs suggesting that CSR-3 stabilizes fusion pore formation. The protein exhibits three potential phosphorylation sites whose potential role in this process will be analyzed via targeted mutagenesis. In addition, CSR-3 co-aggregates with SO and MEK-1 in complexes which form at the cell periphery in response to cell wall stress. In hyphae, CSR-3 and its potential target the chitin synthase CHS-2 are also involved in septa formation, a well described process that requires new synthesis of cell wall compounds.

Together, these findings suggest that cell wall reconstruction during cell fusion is based on a fine-tuned equilibrium between cell wall synthesis and degradation. Both fusion pore formation related cell wall remodeling and the response to cell wall stress share common molecular signaling networks, in which SO might mediate the interaction of the MAK-1 MAP kinase module with its targets.

159F Membrane asymmetry markers and polarized growth in *Neurospora crassa*. *R.R. Mouriño.Pérez*[1], I. Murillo-Corona[1], Z. Schultzhaus[2], B.D. Shaw[2], O.A. Callejas-Negrete[1] 1) Microbiology, CICESE, Ensenada, BC, MX; 2) Department of Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, TAMU.

In fungal cells, specialized proteins gather in specific places to break cell symmetry and produce hyphae. This organization includes the orchestration of two distinct vesicle processes, endocytosis and exocytosis that take place in tandem in different areas of the apical compartment in growing hyphae. Part of the signals for endocytosis and endocytosis include the asymmetry of the plasma membrane phospholipid bilayer. We studied the flippases, DNF-1 and DRS-2 that seems to be responsible for this membrane asymmetry. The mutation of *dnf-1* and *drs-2* genes produced alteration in the maintenance and stability of the Spitzenkörper and affected the actin

cytoskeleton organization in the apical compartment. Surprisingly, neither of the flippases DNF-1 and DRS-2 was present in the plasma membrane, both were localized in different layers of the Spitzenkörper, associated to different secretory vesicles. DRS-2 was associated to vesicles transporting chitin synthases. These results indicate that phospholipid flippases (P4 ATPases) may be important for polarity on secretory vesicles, Spitzenkörper integrity and thus for the localization of many tip growing proteins.

160W The protein disulfide isomerase of *Botrytis cinerea*: An ER protein involved in protein folding and redox homeostasis mediates NADPH oxidase signaling processes. *R. Marschall*, P. Tudzynski Institute of Plant Biology and Biotechnology, Münster, DE.

One of the most devastating pathogens, which is responsible for tremendous crop losses of edible fruits and vegetables, is *Botrytis cinerea*, causative agent of the grey mold disease. The fungus has a necrotrophic lifestyle and benefits from humid air moisture and moderate temperatures. Central molecules within pathogenic processes are reactive oxygen species (ROS). ROS are ubiquitous present in all cells that depend on molecular oxygen and are known to work as signaling molecules in appropriate concentrations while they are harmful to macromolecules in elevated amounts. While ROS result from the activity of the respiratory chain as mere byproducts, there are enzymes such as the NADPH oxidase (Nox) complexes, which actively forming superoxide (O_2^-) by transporting electrons across the lipid bilayer onto molecular oxygen. In *B. cinerea*, Nox complexes are contributing to a wide range of vegetative and pathogenic processes such as the formation of infection structures and the sporulation. However, despite of major advances regarding the composition and function of fungal Nox complexes[1], there are still open questions concerning their integration in the complex signaling network.
Recently, we identified the protein disulfide isomerase (PDI) BcPdi as protein, which directly interacts with the catalytic subunit BcNoxA in Y2H and co-IP assays. PDIs are highly conserved among different species. They are involved in essential processes such as the protein folding and the UPR and are responsible for the maintenance of the redox homeostasis. By characterizing deletion mutants we found out that Δ*bcpdi* and Δ*bcnoxA* exhibit a quite similar phenotype. Moreover, we elucidate that upon calcium stimulation BcNoxA, BcPdi and the second catalytic subunit BcNoxB work closely together, resulting in ROS production and an oxidative burst inside the cytosol. The activity of the three proteins might be regulated by the phosphatase calcineurin, which came up as an additional interaction partner of BcPdi.
Taken together, we postulate an essential role for BcPdi in the Nox signaling machinery and speculate that Nox driven developmental processes are regulated by PDI. Further on, we highlight an additional linkage between ROS and calcium dependent signaling pathways, which supports the hypothesis that both pathways are interconnected.

[1)] Marschall and Tudzynski, 2016: Reactive oxygen species in development and infection processes. Semin Cell Dev Biol. 2016 Sep;57:138-46.

161T LaeA/1-velvet interplay in *Aspergillus* and *Trichoderma*: Regulation of secondary metabolites and cellulases. *R. Karimi Aghcheh*[1], C. P. Kubicek[2], J. Strauss[3], G. Braus[4] 1) Department of Molecular Microbiology and Genetics, Georg August University Goettingen, Goettingen, DE; 2) Institute of Chemical Engineering, Vienna University of Technology, Gumpendorfer Strasse 1a A-1060 Vienn, Austria; 3) Fungal Genetics and Genomics Unit, Department of Applied Genetics and Cell Biology, BOKU University Vienna, Austria; 4) Department of Molecular Microbiology and Genetics, Georg August University Goettingen, Goettingen, DE.

Filamentous fungi are of considerable economic and social significance for human health, nutrition and
in white biotechnology. These organisms are dominant producers of a range of primary metabolites such as citric acid, microbial lipids (biodiesel) and higher unsaturated fatty acids (HUFAs). In particular, they produce also important but structurally complex secondary metabolites with enormous therapeutic applications in pharmaceutical industry, for example: cephalosporin, penicillin, taxol, zeranol and ergot alkaloids. Several fungal secondary metabolites, which are significantly relevant to human health do not only include antibiotics, but also e.g. lovastatin, a well-known antihypercholesterolemic agent produced by *Aspergillus. terreus*, or aflatoxin, a carcinogen produced by *A*. flavus. In addition to their roles for human health and agriculture, some fungi are industrially and commercially important: Species of the ascomycete genus *Hypocrea spp.* (teleomorph of *Trichoderma*) have been demonstrated as efficient producer of highly active cellulolytic enzymes. This trait makes them effective in disrupting and depolymerization of lignocellulosic materials and thus applicable tools in number of biotechnological areas as diverse as clothes-washing detergent, animal feed, and pulp and fuel productions.
Fungal LaeA/LAE1 (**L**oss of ***a****flR* **E**xpression **A**) homologs their gene products act at the interphase between secondary metabolisms, cellulase production and development. Lack of the corresponding genes results in significant physiological changes including loss of secondary metabolite and lignocellulose degrading enzymes production. At the molecular level the encoded proteins are presumably methyltransferases or demethylases which act directly or indirectly at heterochromatin and interact with velvet domain proteins. Velvet proteins bind to DNA and affect expression of secondary metabolites (SMs) genes and cellulases. The dynamic interplay between LaeA/LAE1, velvet proteins and additional interaction partners is the key for an understanding of the coordination of metabolic and morphological functions of fungi and is required for a biotechnological control of the formation of desired bioactive products. *Aspergilli* and *Trichoderma* represent different biotechnologically significant species with significant differences in the LaeA/LAE1-Velvet protein machinery and their target proteins. We therefore performed a comparative study of the interaction partners of this machinery and the dynamics of the various protein-protein interactions using our robust proteomic and mass spectrometry techniques. This enhances our knowledge about the fungal coordination of secondary metabolism, cellulase production and development and thereby will certainly improve recombinant fungal strain construction for the production of industrial secondary metabolite or lignocellulose hydrolytic enzymes.

162F Internuclear diffusion of histone H1 within cellular compartments in *Aspergillus nidulans*. *Alexander Mela*, Michelle Momany Plant Biology, University of Georgia, Athens, GA.

Histone H1 is an evolutionarily conserved 'linker' histone protein which serves the important dynamic function of arranging and stabilizing chromatin structure. The mechanism by which histone H1 carries out its function remains unclear. One thing that is certain is the common use of fluorophore-tagged histone H1 protein in microscopy to track nuclei within cells. In time-lapse analyses, we observed stochastic exchange of photo-activated Dendra2-Histone-H1 protein between nuclei. We also observed heterogeneous mixing of histone proteins between nuclei in hyphae and conidiophores derived from heterokaryon fusions of histone H1-RFP and H1-GFP strains. Subsequent analysis of the resulting conidia that contained both RFP- and GFP-labeled histone H1 proteins, showed only parental genotypes. These data together suggest the stochastic exchange of histone H1 protein between nuclei is likely a result of diffusion rather than genetic recombination during karyogamy.

163W Genes that modulate unconventional secretion of GAPDH. *M.J. Cohen*[1,2], A Chichester[1], P Lipke[1,2] 1) Biology, Brooklyn College, Brooklyn, NY; 2) Molecular, Cell, and Development, CUNY Graduate Center, New York, NY.

Extracellular proteins with a signal sequence exit yeast through a well characterized classical secretory system. Many cell wall proteins in *Saccharomyces cerevisiae* lack a signal sequence, and reach the extracellular environment by uncharacterized mechanisms. We screened *S. cerevisiae* with deletions in vesicular transport genes for changes in activity of the unconventionally secreted cell wall protein GAPDH (glyceraldehyde-3-phosphate dehydrogenase, encoded by the genes *TDH1, TDH2* and *TDH3*). Deletions of *VPS21* and *YPT7* Rab GTPases involved in endosomal transport to the vacuole, the GTP Exchange Factor *VAM6* (which encodes a subunit of the HOPS endocytic tethering complex), and *BRO1* (a homolog of mammalian ALIX which may be involved in sorting proteins into the multivesicular body) decreased extracellular GAPDH activity. S. cerevisiae lacking the GEFS Vps9, Muk1 or the ESCRT-II and III components Snf7 and Snf8 had increased extracellular GAPDH activity.

Additionally, S. cerevisiae lacking autophagy genes *ATG5, ATG7*, and *ATG12* have normal levels of GAPDH activity in the cell wall. These genes were previously reported to be required for unconventional secretion of Acb1. This result suggests that GAPDH and Acb1 reach the cell surface of S. cerevisiae by different mechanisms. Thus, GAPDH reaches the cell wall by a process that is positively and negatively impacted by different elements of the vacuolar protein sorting pathway.

164T Nanoscale clustering of the cytosolic enzyme trichodiene synthase to a trichothecene biosynthetic enzyme complex at the endoplasmic reticulum resolved by superresolution microscopy and FRET. *Marike Boenisch*[1], Karen Broz[2], Ailisa Blum[3,4], Donald Gardiner[3], H. Corby Kistler[1,2] 1) University of Minnesota, St. Paul, MN, USA; 2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA; 3) CSIRO Agriculture & Food, St. Lucia, Brisbane, Queensland, Australia; 4) SAFS, University of Queensland, St. Lucia, Brisbane, Queensland, Australia.

The phytopathogenic fungus *Fusarium graminearum* produces the trichothecene mycotoxin deoxynivalenol (DON) during infection of wheat and when induced *in vitro*. Enzymes in the primary mevalonate pathway and in the secondary DON pathway catalyze key steps for fungal DON production and are transcriptionally co-regulated. Mevalonate pathway enzyme HMG CoA reductase (Hmr1) and the DON biosynthetic pathway enzymes trichodiene oxygenase (Tri4) and calonectrin oxygenase (Tri1) are membrane associated proteins. When labeled with fluorescent proteins and grown in DON induction medium, Hmr1, Tri4, and Tri1 strictly co-localize at the endoplasmic reticulum (ER), suggesting they may be part of a multi-enzyme complex within the ER membrane. This hypothesis is supported by positive fluorescence resonance energy transfer (FRET) between either Hmr1 or Tri1 and Tri4, suggesting they are less than 10 nm apart. However, GFP tagged mevalonate pathway enzyme HMG CoA synthase (Hms1) and DON pathway enzyme trichodiene synthase (Tri5) are localized in the cytosol under DON inducing conditions. To determine if these enzymes were located proximal to the ER, 3D SIM superresolution microscopy was applied to a Tri5-GFP/Tri4-RFP or a Hms1-GFP/Tri4-RFP dual tagged strain grown in DON inducing medium. Measuring mean GFP intensity (n=91) in 250 nm cytosolic subregions, Tri5-GFP intensity was significantly higher ($P<0.001$) surrounding ER membranes (visualized by Tri4-RFP) than elsewhere in the cytosol, while the opposite effect was detected with Hms1-GFP. Tri5 thus may preferentially accumulate at and surrounding the ER membranes that harbor the postulated DON biosynthetic enzyme complex, allowing pathway intermediates to be spatially concentrated at particular regions of the cell. Compartmentalization of toxic pathway intermediates to particular organelles and cytosolic subregions might facilitate DON biosynthesis and self-protection of the fungus.

165F Distinctive nuclear localization signals in the oomycete *Phytophthora sojae*. *Y. Fang*[1,2,5], H. Sang[3], G. Watson[4], D. Wellapilli[2], B.M. Tyler[1,2] 1) Interdisciplinary Ph.D. Program in Genetics, Bioinformatics & Computational Biology, Virginia Tech, Blacksburg, VA ; 2) Center for Genome Research & Biocomputing and Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR ; 3) Department of Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR; 4) Biological & Population Health Sciences, Oregon State University, Corvallis, OR; 5) Current address: Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC.

Nuclear localization signals (NLSs) are stretches of residues that direct proteins from the cytoplasm into the nucleus in eukaryotic cells. Despite their critical roles in protein regulation, NLSs in oomycetes have not been defined. Here, we use the soybean pathogen *Phytophthora sojae* as a model to investigate these sequences in oomycetes. By establishing a reliable *in vivo* NLS assay based on confocal microscopy, we found that *P. sojae* uses NLS for translocation of proteins into the nucleus that differs from conventional well-characterized NLSs. Most tested classical NLSs and proline-tyrosine NLSs (PY-NLSs) from model organisms showed poor nuclear import activities in *P. sojae*. In comparison, functional nuclear localization sequences from *P. sojae* nuclear proteins resembled conventional NLSs but required additional basic residues, either within the NLS motif or nearby. In addition, multiple weak NLSs could also work collaboratively for the efficient nuclear import of proteins. To learn more about the features of NLS-mediated transport of nuclear proteins in *P. sojae*, we further characterized in depth the nuclear import mechanism of a *P. sojae* basic leucine zipper transcription factor, PsbZIP1. We found that the nuclear translocation of PsbZIP1 was determined by a central conserved region. Mutational analysis of this region indicated its nuclear translocation was mediated by four distinct motifs but was independent of conserved DNA-binding residues. Three motifs showed autonomous NLS activity and the fourth motif served as a nuclear localization enhancer. Sequence comparison and mutational analysis of these nuclear localization motifs revealed a new form of bipartite NLS consisting of a triplet of basic residues followed by a tail of scattered basic amino acids.

Manuscripts under revision:

Distinctive Nuclear Localization Signals in the Oomycete *Phytophthora sojae*. *Frontiers in Microbiology.*

Nuclear localization of a putative *Phytophthora sojae* bZIP1 transcription factor is mediated by multiple targeting motifs. *Molecular Microbiology.*

166W Phosphoproteomic analysis of *Penicillium chrysogenum* reveals 383 phosphorylated proteins. U. Carrasco, *J. E. Zuniga*, F. J. Fernandez, F. Fierro Universidad Autonoma Metropolitana, UAM-I, Iztapalapa, DF.

Introduction*: Penicillium chrysogenum* is used for the industrial production of the b-lactam antibiotic penicillin. We have recently performed a proteomic analysis of the Pga1-mediated heterotrimeric G protein signaling pathway of this fungus (**1**), finding two phosphorylated proteins as effectors of the pathway. To advance in the characterization of this signaling pathway we have performed a phosphoproteomic analysis. Here we describe the identification of a total of 383 phosphorylated proteins and their functional classification. **Methods**: After trypsin digestion, phosphopeptide enrichment was carried out by metal oxide affinity chromatography with TiO_2, then the phosphopeptides were submitted to LC-MS/MS for identification, Validation of protein identification from MS/MS was carried out with the program Scaffold v4.0.5 (Proteome Software Inc.), Confirmed identification of a protein was considered when the probability was above 95 % and at least one peptides from the protein were positively identified. **Results**: 383 phosphorylated proteins were identified from 8343 total spectra. The classification of the proteins according to their biological function showed that 28% of the proteins are involved in the primary metabolism. and 1.96% are proteins related to signal transduction Approximately 20 % of the proteins are described as located in the nucleus. Fifteen probable transcription factors and chromatin remodeling proteins were identified. It is interesting to note that several phosphorylated proteins are described as involved in polarized growth and morphogenesis. **Conclusions**. We have performed the first analysis of the *P. chrysogenum* phosphoproteome, identifying 383 phosphorylated proteins. **References**: 1) Microb Cell Fact. 2016;15(1):173.

167T Unpacking the molecular mechanism behind unisexual reproduction in *Huntiella moniliformis*. *A. Wilson*[1], M. van der Nest[1], P.M. Wilken[1], M.J. Wingfield[2], B.D. Wingfield[1] 1) Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa ; 2) Department of Microbiology & Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Unisexual reproduction is a reproductive strategy where an individual fungus is able to produce sexual offspring despite possessing genes that represent a single *MAT* idiomorph. This form of reproduction has been described in a few species of fungi, but was most recently observed in MAT2 individuals of the filamentous ascomycete; *Huntiella moniliformis* (Wilson *et al*. 2015. Fun. Genet. Biol. 80:1-9). This fungus is a member of the family Ceratocystidaceae, a group including well-known pathogenic fungi that infect a wide variety of economically important plants. The underlying molecular mechanisms responsible for unisexuality in filamentous fungi are poorly understood and this study aimed to elucidate these mechanisms using a comparative transcriptomics approach. By sequencing the mRNA from vegetative and sexually-competent cultures of *H. omanensis*, a strictly heterothallic relative of *H. moniliformis*, we were able to identify some of the genes that are important for sexual reproduction in these fungi. By comparing these data to similar data obtained from cultures of *H. moniliformis*, we were able to detect significant differences in gene expression between the heterosexual and unisexual pathways. Most notable was the mating type-independent expression of both the α- and a-factor pheromone genes in the unisexual *H. moniliformis*. This was in contrast to *H. omanensis*, were MAT1 individuals produce the α-factor pheromone and MAT2 individuals the a-factor pheromone in a manner similar to other heterothallic species such as *Neurospora crassa*. *H. moniliformis* cultures also expressed both pheromone receptors at a constitutive level during vegetative growth and sexual reproduction compared to the potentially mating type-specific expression observed in *H. omanensis*. The results suggest that mating type-independent expression of these pheromones plays an important role in the unisexual capabilities of *H. moniliformis*. This can be compared to a unisexual pathway in *C. albicans* where endogenous pheromone production allows for self-activation.

168F Yeast colony life style is affected by the environment. *L. Vachova*[1], Z. Palkova[2] 1) Institute of Microbiology CAS, v.v.i. - Biocev, Prague, Czech Republic; 2) Faculty of Science, Biocev, Charles University, Prague, Czech Republic.

Wild strains of *Saccharomyces cerevisiae*, which are adapted to hostile natural environment, are able to form structured biofilm colonies, composed of aerial part and of pseudohyphae that invade the agar and attach the colony to the substratum. These colonies evolve a number of protective strategies, such as production of Flo11p adhesin, production of extracellular matrix by cells localized to the colony interior and activation of multidrug resistance pumps in cells localized to the colony surface (*J Cell Biol* 194:679, 2011). Rich nutrient supplies induce the process of domestication in wild strains leading to formation of cells that start to form smooth colonies similar to colonies of laboratory strains. This domestication is accompanied by switching off of most protective mechanisms (*Fungal Genet Biol* 47:1012, 2010). Domestication can be reversed under adverse conditions when feral subclones start to appear that reacquire the ability to form biofilm colonies with wild type-like biofilm colony architecture (*BMC Genomics*. 15:136, 2014). We compared various features of colony cell populations formed by wild, domesticated and feral strains by transcriptomic and confocal microscopy approaches, combined with other analytical methods. Colonies formed by the feral strain resemble biofilm colonies of the wild strain in several ways, including restoration of the major features that are switched off during domestication, such as formation of extracellular matrix, production of Flo11p adhesin and ability to absorb high amount of water. The main functional groups of genes, typical of colonies with a “structured” morphotype, include genes linked to cell wall remodeling and plasma membrane functions as well as genes involved in signaling cascades. This research was funded from the Norwegian Financial Mechanism 2009-2014 under Project Contract no. 7F14083 and by GACR 13-08605S.

169W Structural reorganization of the endoplasmic reticulum upon induction of mycotoxin biosynthesis in *Fusarium graminearum*. *Marike Boenisch*[1], Karen Broz[2], H. Corby Kistler[1,2] 1) University of Minnesota, St. Paul, MN, USA; 2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA.

The ascomycete fungus *Fusarium graminearum* causes disease on wheat and barley and contaminates grains with trichothecene (TRI) mycotoxins. To understand how toxin biosynthesis is spatially regulated in the fungal cell and to explore potential self-protection mechanisms of the fungus, the subcellular localization of TRI biosynthetic enzymes was determined and cellular changes during toxigenesis were studied. Using fluorescence tagged proteins and the blue fluorescent dye ER-Tracker, enzymes, catalyzing early and late steps in trichothecene biosynthesis, hydroxymethylglutaryl CoA reductase (Hmr1), trichodiene oxygenase (Tri4), and calonectrin oxygenase (Tri1), were observed to co-localize with the endoplasmic reticulum (ER) when TRI is induced artificially *in vitro* and naturally *in planta*. In addition to these enzymes, a protein of unknown function, Tri14, encoded by a gene within the trichothecene gene cluster, also co-localizes with Tri4 at the ER during TRI production. Interestingly the trichodiene synthase Tri5, while localized in the cytosol, appears to cluster at the

ER. Applying 3D SIM superresolution microscopy to the wild type strain, we demonstrate that, upon TRI induction, the ER stained with ER-Tracker shifts from being reticulate to being thickened with pronounced perinuclear and peripheral ER. This reorganization of the ER was confirmed with reporter strains containing the ER resident protein Sec22-GFP, as well as with strains having GFP linked to the ER retrieval sequence (HDEL) and expressed in the fungal ER lumen. Transmission electron microscopy revealed that organized smooth ER membranes (OSER) are formed in TRI producing cells, but not in non-TRI producing cells. OSER are lamellar or concentric stacks of smooth perinuclear or peripheral ER membranes, with a ~10 nm cytoplasmic space between each ER sheet. Since enzyme active sites for Tri1, Tri4, and Hmr1 are predicted to be on the cytoplasmic side of the ER membrane, we conclude that TRI is likely synthesized in the cytoplasmic spaces within OSER. Thus, TRI metabolites may be sequestered from targets of TRI inhibition e.g. ribosomes and mitochondria and might facilitate TRI biosynthesis and self-protection of the fungus.

170T Naming mating genes: A review of Pezizomycotina MAT gene nomenclature. Markus Wilken[1], Emma Steenkamp[2], Michael Wingfield[1], *Brenda Wingfield*[1] 1) Dept Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Department of Microbiology & Plant Pathology, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

The sub-phylum Pezizomycotina (Ascomycetes) includes fungi with a diverse range of sexual strategies, all regulated by the mating (*MAT*) genes. Naming these genes is dictated by a nomenclature system that has been applied for more than 15 years. Contemporary techniques including whole genome sequencing have resulted in the description of numerous novel *MAT* genes, which in many cases have been erroneously named. Reasons for this include the low levels of sequence similarity between putative homologs, the misidentification of genes in public sequence repositories and misinterpretations of the nomenclature rules. This situation emphasises the need to re-evaluate and revise the manner in which the nomenclatural system for *MAT* genes is applied. In this study, we examined all genes that have been described from the *MAT1* locus in the Pezizomycotina. Our results showed that the 15 known mating genes described in the Pezizomycotina *MAT1* locus represent at least 24 distinct genes, some of which have known homologs outside of the *MAT1* locus. To address this discrepancy, we propose a revision of the nomenclature system that would involve renaming some of these genes. This revision would result in the addition of two *MAT1-1* and four *MAT1-2* gene names, as well as the removal of four currently used *MAT* gene names. The overall outcome would bring the total number of unique mating genes to 18. This study is being prepared as a review with firm recommendations on changes to the current names of these genes. Our hope is that such a revision will clarify existing problems and provide clear guidelines as well as reference sequences to be used when these important genes are named in the future.

171F Characterization of two ferroxidases and one ascorbate oxidase belonging to the multicopper oxidase family in the filamentous fungus *Podospora anserina*: a new role in fungal development. N. Xie[1,2], G. Ruprich-Robert[3], P. Silar[2], E. Herbert[2], R. Ferrari[2], *F. Chapeland-Leclerc*[3] 1) Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China; 2) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, 75205 Paris, France; 3) Univ Paris Descartes, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, 75205 Paris, France.

Our previous phylogenetic analyses showed that the *Podospora anserina* genome contains a large family of 15 MCOs, including two genes encoding FET3-like proteins and one gene encoding an AO-like protein. FET3-1, FET3-2 and AO1 are involved in global laccase activity, since deletion of the relevant genes led to a decrease of activity. However, contrary to the *P. anserina* MCO proteins previously characterized, none of these three MCOs seemed to be involved in lignocellulose degradation and in resistance to phenolic compounds and H_2O_2. Deletion of the *ao1* gene led to an almost total loss of AO activity. No direct involvement of AO1 in fungal developmental process in *P. anserina* was evidenced, except in a *fet3-1*$^{\Delta}$ background. We showed that the bulk of FET3 activity was clearly due to FET3-1, and only in minor part to FET3-2, although FET3-1 does not contain all the residues typical of FET3 proteins. Moreover, we showed that FET3-1, closely related to the *Aspergillus fumigatus* ABR1 protein, was clearly and specifically involved in pigmentation of ascospores. Surprisingly, phenotypes were more severe in mutants lacking both *fet3-1* and *ao1*. Overall, unlike other previously characterized MCOs, we thus evidence a clear involvement FET3-1 protein in fungal development.

172W Functions of *PRM1* and *KAR5* in cell-cell fusion and karyogamy drive distinct bisexual and unisexual cycles in the *Cryptococcus* pathogenic species complex. *Ci Fu*, Joseph Heitman Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Cryptococcus neoformans and *Cryptococcus deneoformans* are two sibling species belong to the opportunistic human fungal pathogen *Cryptococcus* species complex. *C. neoformans* mainly undergoes bisexual reproduction, whereas *C. deneoformans* undergoes both unisexual and bisexual reproduction. During both sexual cycles, a dramatic yeast-to-hyphal morphological transition takes place and generates either monokaryotic or dikaryotic hyphae, and this process is regulated by a common set of genetic circuits. The unisexual cycle can also generate genotypic and phenotypic diversity *de novo*. Despite the similarities between unisexual and bisexual cycles, there are still genetic and morphological differences, such as an absence of an opposite mating partner and monokaryotic instead of dikaryotic hyphae. The natural populations for both species are mainly α mating type; however, the unisexual cycle is well established in *C. deneoformans* but not in *C. neoformans*, suggesting differences in mating mechanisms may contribute to this. To understand what distinguishes the different modes of sexual cycles, we focused on two cellular processes involved in sexual reproduction: cell-cell fusion and nuclear fusion. We identified orthologs of the plasma membrane fusion protein Prm1 and the nuclear membrane fusion protein Kar5 in both *Cryptococcus* species, and showed that they play conserved roles in cell fusion and karyogamy during sexual reproduction. The involvement of Prm1 and Kar5 indicates that there are fundamental differences between unisexual and bisexual reproduction, and between bisexual reproduction of the two sibling species. Cell fusion and karyogamy are largely dispensable for unisexual reproduction, and the unisexual cycle achieves diploidization early during hyphal development, likely through endoreplication. During the bisexual cycles, in *C. deneoformans*, karyogamy occurs early during the hyphal development whereas it occurred inside the basidium in *C. neoformans*.

173T A genetic screen uncovers inositol-phosphate signaling as essential for sexual reproduction in *Podospora anserina*. N. Xie[1,2], *G. Ruprich-Robert*[3], F. Chapeland-Leclerc[3], H. Lalucque[2], E. Coppin[4,5], S. Brun[2], R. Debuchy[5], P. Silar[2] 1) Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China; 2) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, 75205 Paris, France; 3) Univ Paris Descartes, Sorbonne Paris Cité, Laboratoire Interdisciplinaire des Energies de Demain (LIED), UMR 8236, 75205 Paris, France; 4) CNRS, Institut de Génétique et Microbiologie UMR8621, Orsay, France; 5) Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris Sud, UMR 9198, 91405 Orsay, France.

The molecular pathways involved in the development of multicellular fruiting bodies in fungi are not yet well known. Especially, the interplay between the mycelium, the female tissues and the zygotic tissues of the fruiting bodies is poorly documented. Here, we describe *PM154*, a new strain of the model ascomycetes *Podospora anserina* that mimics a homothallic and that enabled the easy recovery of new mutants affected in fruiting body development. By complete genome sequencing of *spod1*, one of the new mutants, we identified an inositol phosphate polykinase gene that plays a critical role for fruiting body development and growth. A factor present in the wild type and diffusible into mutant hyphae was able to induce the development of the maternal tissues of the fruiting body in *spod1*, but failed to promote complete development of the zygotic ones. Addition of myo-inositol in the growth medium was able to increase the number of developing fruiting bodies in the wild type, but not in *spod1*. Overall, the data indicated that inositol polyphosphates were involved in promoting fruiting body maturation, but also in regulating the number of fruiting bodies that developed after fertilization. Key role of inositol signaling during fruiting body maturation is conserved during the evolution of *Sordariales* fungi.

174F Are RIP and heterochromatin involved in *Podospora anserina* sexual development? *F. Carlier*, V. Contamine, F. Malagnac Institute for Integrative Biology of the Cell (I2BC, UMR 9198), CEA, CNRS, Universite´ Paris Sud, 91400 Orsay CEDEX, France.

In Pezizomycotina, DNA repeats are subjected to a silencing mechanism called RIP (Repeat Induced Point mutation). RIP introduces mutations, preferentially C/G to A/T transitions and sometimes, in species such as *Neurospora crassa*, RIPed sequences are *de novo* methylated. The putative DNA-methyltransferase Rid protein of *N. crassa* is essential for RIP. Besides, when methylated, the RIPed sequences are marked as constitutive heterochromatin (H3K9me3) by histone methyltransferase DIM5 and recognized by the chromodomain containing protein 1 (HP1). In *Podospora anserina*, although no significant levels of DNA methylation have been detected yet, repeats are subjected to RIP during sexual reproduction. Unlike the *N. crassa rid* mutants, the *P. anserina rid* mutants are unable to complete sexual development and thus to produce ascospores. Moreover, fertility of *P. anserina rid* mutants is restored only if PaRID harbors a functional DNA methyltransferase catalytic domain.

To better understand the relations between RIP, chromatin remodeling and *P. anserina* sexual development, we constructed the Δ*PaDim5* and Δ*PaHp1* null mutants. Although H3K9me3 can be detected by Western-blot analysis in wild-type *P. anserina* strains, the single mutant strains, as well as the corresponding double mutant strain, are not affected in sexual reproduction and present mild growth defects. As expected, in a wild-type genetic background, PaDim5-mCherry and PaHp1-GFP proteins are nuclear. The PaHp1-GFP proteins form foci, a pattern that is disrupted in a Δ*PaDim5* genetic background but not in the Δ*PaRid* background. These results suggest that the epigenetic component of the *P. anserina* developmental pathway is PaDim5 independent. Then, we explored the role of the facultative heterochromatin mark H3K27me3 set up by the PaKmt6 histone methyltransferase. The Δ*PaKmt6* null mutant causes a significant growth defect and is impaired in sexual reproduction which suggests that H3K27me3 plays a central role into *P. anserina* sexual development. Identification of the PaRid target during sexual reproduction might contribute to complete our understanding of this crucial developmental pathway.

175W Forward genetics identifies developmental genes in the filamentous fungus *Sordaria macrospora*. *I. Teichert*[1], M. Lutomski[1], A. Hamann[2], R. Märker[1], M. Nowrousian[1], H.D. Osiewacz[2], U. Kück[1] 1) Ruhr-University Bochum, Allgemeine und Molekulare Botanik, Bochum, DE; 2) Molecular Developmental Biology, Faculty of Biosciences and Cluster of Excellence 'Macromolecular Complexes', Johann Wolfgang Goethe University, Frankfurt am Main.

The filamentous ascomycete *Sordaria macrospora* is a model system for studying fungal sexual development. *S. macrospora* forms perithecia within one week in the laboratory. A large collection of sterile mutants is available for this fungus, with mutants blocked at different stages of fungal development. Focusing on mutants of the “pro” type, which generate only immature spherical prefruiting bodies (protoperithecia) already identified quite a number of developmental factors. However, many sterile mutants have not been characterized yet, and functional characterization of many developmental factors is still not complete [1, 2].

Here, we analyzed two further mutants, spadix (spd) and pro34, to identify the underlying genotype. Pooled DNA from progeny of crosses of the mutants to wild type was sequenced by Illumina sequencing. Bioinformatics analysis identified the mutations underlying the mutant phenotypes. The sterile phenotype of mutant spd is caused by deletion of the *spd4* gene. SPD4 is required for fruiting body formation, but not vegetative cell fusion in *S. macrospora*, although both processes have long been thought to be linked. SPD4 displays a nuclear localization and is restricted to ascogonial tissues during sexual development [3]. The sterile phenotype of pro34 is caused by a deletion in the *cia84* (complex I assembly) gene. PRO34 displays a mitochondrial localization, and the mutant shows a respiratory defect. Like SPD4, PRO34 is not required for cell fusion in *S. macrospora*. Thus, sequencing the genomes of two sterile mutants identified additional genes involved in sexual development in a model fungus. Both, SPD4 and PRO34, are required for fruiting body formation, but not cell fusion. These two proteins therefore provide specific insights into the regulation of sexual development in fungi.

[1] Teichert et al. 2014 Adv Genet 87:199
[2] Teichert et al. 2014 PLoS Genet 10:e1004582
[3] Teichert et al. 2016 Mol Genet Genomics, in press

176T Courtship ritual of male and female nuclei during fertilization in *Neurospora crassa*. *S. Brun*[1], Hsiao-Che Kuo[3], Chris Jeffree[4], Nick Read[2] 1) LIED-UMR 8236, Univ Paris-Diderot, Paris, FR; 2) MFIG, manchester University, UK; 3) Fungal Cell Biology Group, University of Edinburgh, UK; 4) Institute of Plant Sciences, University of Edinburgh, UK.

Sexual reproduction is a key event in the life cycle of fungi. It relies on very elaborated genetic regulation systems as well as complex cytological events. Fertilization starts by the recognition of “male” and “female” haploid cells, namely the conidium and the trichogyne

emitted by the protoperithecium, respectively. Then fusion of these cells (plasmogamy) follows. However, the fusion of the two parental nuclei (karyogamy), giving rise to the diploid nucleus undergoing meiosis, will take place within the protoperithecium.
In laboratory conditions, fertilization in the heterothallic model fungus *Neurospora crassa* can be observed few hours after inoculation of petri dishes bearing protoperithecia by conidial suspensions of opposite mating type (Bistis, 1981). Using parental strains in which nuclei were tagged with either the Green Fluorescent Protein or the Red Fluorescent Protein, we achieved for the first time live imaging of male and female nuclei during fertilization. Our study reveals that the parental nuclei behave totally differently and that the movement of male nuclei down through the trichogyne is a complex process. Indeed, while male nuclei migrate down through the trichogyne changing of shape from rounded to stretched in an inchworm-like movement, female nuclei "freeze" just prior the entry of male nuclei into the trichogyne and remain totally immobilized throughout the process. Furthermore, protoperithecia usually emit several trichogynes that are often branched. Hence, multi-branched trichogynes can fuse to several macroconidia allowing the discharge of multiple male nuclei into a same network connected to a single protoperithecium. In this labyrinth, we could trace male nuclei and follow their "orienteering race", changing direction at junctions and probably competing with each other to fertilize the protoperithecium.
Our pioneering study based on live imaging of nuclei during sexual reproduction in *N. crassa* addresses fundamental questions regarding the identity of male and female nuclei as well as which components of the cytoskeleton are involved in nuclei movements during fertilization. It also raises brand new questions about i) the signal guiding male nuclei to the protoperithecium and ii) a possible signal emitted by the fertilized perithecium that inhibits polyspermy.
Bistis, G.N. (1981). Chemotropic Interactions between Trichogynes and Conidia of Opposite Mating-Type in Neurospora crassa. Mycologia *73*, 959–975.

177F ChIP-Seq analysis identifies components of the cell wall integrity, NADPH oxidase and pheromone signaling integrity pathways as target genes of the fungal developmental regulator PRO1. *U. Kück*, S. Steffens, K. Becker, S. Krevet, I. Teichert Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE.

Fruiting body formation is a fundamental step during the life cycle of filamentous fungi that requires tight spatiotemporal regulation. Working with the model fungus *Sordaria macrospora*, we have previously demonstrated that this major process in the sexual life cycle is controlled by the $Zn(II)_2Cys_6$ zinc finger transcription factor PRO1.
Here, we further investigated the genome-wide regulatory network controlled by PRO1 by employing chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq) to identify binding sites for PRO1 (Steffens et al. 2016). We identified several target regions that occur in the promoter regions of genes encoding components of diverse signaling pathways. Furthermore, we identified a conserved DNA-binding motif that is bound specifically by PRO1 in vitro. In addition, PRO1 controls in vivo the expression of a DsRed reporter gene under the control of the esdC target gene promoter. Our ChIP-seq data suggest that PRO1 also controls target genes previously shown to be involved in regulating the pathways controlling cell wall integrity, NADPH oxidase and pheromone signaling. Our data point to PRO1 acting as a master regulator of genes for signaling components that comprise a developmental cascade controlling fruiting body formation.
Steffens E, Becker K, Krevet S, Teichert I, Kück U (2016) Mol Microbiol (in press)

178W Sex prepares the plant pathogenic fungus *Botrytis cinerea* for host invasion. Alexander Y. Rodenburg[1], Razak Terhem[1,2], *Jan A.L. van Kan*[1] 1) Phytopathology, Wageningen University, Wageningen, the Netherlands; 2) Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia, Serdang, Malaysia.

Botrytis cinerea is a plant pathogenic ascomycete producing apothecia as sexual fruiting bodies. We used RNAseq to analyse expression profiles in sclerotia, three stages of apothecium development (primordia, full grown stipe, apothecial disk), and mature ascospores. Very pure tissue samples of the five developmental stages could be isolated and transcriptional changes between successive developmental stages were analysed. Changes were observed in the expression levels of as many as 15 secondary metabolite gene clusters, in different stages. The most profound overall changes in transcript profiles were at the initiation of sexual development (transition from sclerotia to primordia, >2500 differentially expressed genes) and even more so, at the completion of sexual reproduction in the mature ascospores: 1424 genes were upregulated and 2485 genes were downregulated in ascospores as compared to mature apothecial disks. Among the genes upregulated in ascospores were many genes encoding virulence factors (plant cell wall hydrolases, proteinases, multidrug efflux ABC transporters, superoxide dismutase, phytoalexin-oxidising laccase, an oxalic acid biosynthetic enzyme), several hexose transporters and a number of signal transduction components known to be involved in virulence. These observations clearly suggest that ascospores are transcriptionally primed for infection already prior to their arrival on host plants.
Strikingly, the large transcriptional changes at the initiation and the completion of the sexual cycle often affected numerous clusters of genes (not only genes in secondary metabolite clusters), rather than genes randomly dispersed through the genome. Sexual development also coincided with changes in the expression of several genes potentially involved in chromatin organization (C-5 cytosine methylase, several histone acetyltransferases, dicer-like protein), suggesting an epigenetic regulation of gene expression during the sexual cycle.

179T Deciphering the *Teratosphaeria destructans* mating system: a genomics approach. *C.T. Tatham*, M.A. van der Nest, P.M Wilken, M.J. Wingfield, B.D. Wingfield Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Fungal pathogens are increasingly threatening global plantations of intensively propagated non-native *Eucalyptus* spp. In this regard, one of the most destructive shoot and leaf pathogens is *Teratosphaeria destructans*, a fungus of unknown origin that has devastated plantations particularly in southeast Asia. Despite this, virtually nothing is known regarding its biology or genetics. In this study, we sought to explore the details of the mating strategy of *T. destructans*. The genome of a *T. destructans* isolate was sequenced and assembled. The draft genome sequence was used to identify and characterize the mating locus. This isolate contained only the *MAT1-2-1* gene, and was used to describe this isolate as a MAT 2 isolate containing a *MAT1-2* idiomorph. Other genes such as DNA lyase, APC5 and cytochrome oxidase C that are often linked to the MAT1 locus in fungi were present in the vicinity of the *T. destructans MAT1* locus. PCR analysis of 21 *T. destructans* isolates showed that the MAT1-2-1 gene was present in only about half of the isolates. This suggests that *T. destructans* reproduces in a heterothallic manner, and that the remaining isolates are likely to harbour the *MAT1-1* idiomorph. Collectively, the results

suggest that *T. destructans* would be capable of reproducing sexually in regions where isolates of opposite mating types are present. This could explain why the pathogen has commonly adapted to infect clones selected for resistance.

180F The phosducin-like protein PhnA of *Botrytis cinerea* is involved in fungal development and pathogenicity. *J. Kilani*[1,2], M. Miazzi[3], C. Audeon[1], S. Fillinger[1] 1) UMR BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; 2) Univ. Paris-Sud, Université Paris-Saclay, France; 3) Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.

Botrytis cinerea is a necrotrophic, polyphageous plant pathogen, that causes gray mold disease and can infect over 1000 plant species including several agronomically important crops (grapevine, strawberry, tomatoes …). Fungicides remain the most effective means to combat this disease. However *B. cinerea* rapidly adapts to fungicides. Presently, the phenylpyrrole fludioxonil is one of the most efficient fungicides against *B. cinerea*. Therefore deciphering the response to fludioxonil in *B. cinerea* is crucial.

Fungi rapidly adapt to their environment involving to signalling pathways like those of mitogen activated protein kinases (MAPKs). In *Botrytis cinerea*, the fungicide fludioxonil activates the Sak1 and Bmp3 MAPKs, which are respectively involved in osmoregulation, cell wall integrity, development and pathogenicity. In order to trace the transduction of fludioxonil to the MAPK pathways, we have started a phosphoproteomic approach and the functional analysis of key genes.
A phosphoproteomic pilot project of the wild-type strain subjected to fludioxonil, lead to the identification of "fludioxonil" candidate signalling proteins such as the phosducin-like protein PhnA. In eukaryotes, phosducin allows the dimerization between the subunits Gβ and Gγ in the G-protein signalling pathway. In the phytopathogenic fungi *Cryphonectria parasitica* and *Fusarium graminearum*, phosducin is involved in pigmentation, sporulation, and pathogenicity while in *Aspergillus nidulans* this protein is involved in growth, development and mycotoxin production.
We have initiated the functional analysis of *phnA* in *B. cinerea*. Its deletion revealed that *phnA* is involved in vegetative growth, pathogenicity, and in development. This functional analysis of *phnA* highlighted it as a new pathogenicity factor in *B. cinerea*. The role of PhnA-phosphorylation for its activity and the link to fludioxonil, G-protein, and cAMP signalling is under investigation by site-directed mutagenesis of the identified phosphorylation site.

181W Plant sensing enhances sexual development and overrides nutrient sensing in *Trichoderma reesei*. *G. Li*[1], D. Turrà[2], A. Di Pietro[2], M. Schmoll[1] 1) Austrian Institute of Technology GmbH, Tulln, AT; 2) University of Cordoba, Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Córdoba, Spain.

The natural environment of the potent cellulase producer *Trichoderma reesei* (syn. *Hypocrea jecorina*) is a tropical forest, where it degrades cellulosic plant biomass. However, related *Trichoderma* spp. are also known as efficient plant protection agents, antagonizing fungal pathogens.
In the plant pathogen *Fusarium oxysporum*, plant sensing is accomplished by pheromone receptors responding to secreted plant peroxidases. Here we investigated the relationship between plant sensing, sexual development and nutrient detection in *T. reesei*.
We found that *T. reesei* is able to sense plant root exudates and peroxidases via pheromone receptors, suggesting that this mechanism is conserved between *Trichoderma* and *Fusarium*. Moreover, *T. reesei* also showed a chemotropic response to glucose. Interestingly, a nutrient-rich medium such as malt extract (ME) resulted in bipolar and multipolar germination without discernible chemotropism, whereas exudates from soybean roots or from a compatible mating partner in the presence of ME caused unipolar germination with a robust chemotropic response. Hence, signals related to sexual development and plant sensing override nutrient regulated germination.
Accordingly, we found that the presence of soybean germlings promotes sexual development of *T. reesei* as do compounds releasing nitric oxide, a signaling molecule, which is produced by plants to counteract oxidative stress and involved in plant pathogen interaction. Also reacting oxygen species were found to play a role in sexual development, which may explain the relevance of sensing of a peroxidase by pheromone receptors.
We propose that *T. reesei* is attracted to plants by a mechanism that integrates directed hyphal growth and sexual development for efficient adaptation to the host and potentially also for optimized antagonism of competitors and plant pathogens. Thereby, association with a plant has higher priority than availability of nutrients.

182T Investigation and visualisation of signal transduction elements in *Schizophyllum commune*. *Elke-Martina Jung*, Sophia Wirth, Lisa-Marija Ahrens , Erika Kothe Department of Microbiology, Friedrich Schiller University, Jena, DE.

The white rot basidiomycete *Schizophyllum commune* is an early colonizer of tree wounds and after forest fires with competitive abilities based on the production of specific extracellular metabolites and a strategy of fast growth. In addition, recognition of extracellular signals is essential for mating, directed growth, and fruiting body production. G-protein coupled receptors like Bar2 were visualized by GFP-tagging and indirect immunofluorescence in the cell membranes of clamp cells. Downstream signalling is mediated by heterotrimeric G-proteins. An RGS (regulator of G-protein signaling) protein, Thn1, acts as GTPase activating protein (GAP) inhibiting Gα signalling. The interaction of this RGS protein and the pheromone receptor is shown using bimolecular fluorescent complementation (BIFC), establishing this technique for the first time in fruiting body forming, filamentous basidiomycete. Additional steps in signal transduction are mediated by Small GTPases from the Ras superfamily. The *S. commune* genome encodes more than 30 putative Ras GTPases, containing the Ras, Rho, Rab, Ran and Arf subfamilies. The function of the *S. commune* small G-proteins Ras1 and Ras2 have been postulated in MAPK signalling during pheromone response, in morphology, asexual development and mating. Constitutively active Ras1 mutant strains show phenotypes like reduced growth rates, hyperbranching and abnormal growth pattern. Furthermore, the mutants produce abnormal fruiting bodies with weakly developed subhymenium and aberrant spore formation. Thus, both Ras GTPases are major regulators of cellular development.

183F Deciphering the HET domain-mediated programmed cell death in *Neurospora crassa*. *A.I. DASKALOV*, N. L. GLASS Plnat and Microbial Biology, UC Berkeley, Berkeley, CA.

Programmed cell death (PCD) plays a critical role in homeostasis and immunity in Eukaryotes. In Fungi, PCD has been described in the phenomenon of Heterokaryon Incompatibility (HI) – a cell death reaction occurring between somatic fusions of genetically distinct conspecific strains. Currently, there is a relatively good description of the genetic control of HI and a number of incompatibility factors have

been characterized at molecular level. However, the molecular mechanisms and the signal-transducing pathways remain, overall, poorly understood.
Several of the allorecognition determinants share a common domain, termed HET (~150 aa), instrumental for the triggering of PCD during HI. Intriguingly, the HET domain is ubiquitous in fungal genomes and although its cellular function remains unknown, it has been shown *in silico* to bear structural similarity to the Toll/interleukin-1 receptor (TIR) domain – an important effector domain in plant innate immunity. For those reasons, we decided to better characterize the PCD reaction mediated by the HET domain in *Neurospora crassa*.
First, we analyzed ~65 HET domain-containing proteins, encoded in the genome of *N. crassa*, using the online MEME suite tool and identified 25 significantly conserved (e-value <2.3e-002) amino acid motifs outside of the HET domain. Then, we used the patterns of these motifs to defined three major protein families (A, B and C) and to identify additional putative domains, systematically associated with the HET domain. Here, of particular interest is a putative domain in family A (~40 members) consisting of Zn-finger-like motifs (CxxC—CxxxHxxC) situated at the N-terminus of the proteins, that we termed ZHAD (Zn-finger-like HET-associated domain). We investigated the involvement in HI of the newly identified and widespread ZHAD domain using the mating-type HI determinant TOL. We showed – using site-directed mutagenesis – that the ZHAD domain of TOL is important for the PCD reaction, which was also dependent, unsurprisingly, on the HET domain of TOL. Further analyses will define the precise molecular function of the ZHAD domain and will help address some long-standing questions regarding the molecular mechanism of HET domain-mediated PCD in fungi.

184W The plant phenolic ferulic acid modulates gene expression and the phosphoproteome of *Cochliobolus heterostrophus*. Samer Shalaby, Hiba Simaan, Orit Goldshmidt-Tran, *Benjamin Horwitz* Faculty of Biology, Technion - IIT, Haifa, IL.
Ferulic acid (FA) belongs to a group of phenolics widespread in plants, which are perceived by the necrotrophic maize pathogen *C. heterostrophus* as a stress signal inducing programmed cell death (PCD) (Shalaby et al. 2016, Environ. Microbiol). The mechanisms of signaling and toxicity are unknown. We used RNAseq to study the transcriptomic signature of the early (30 min) response to exposure to a 0.5-2 mM range of concentration of FA. Genes related to metabolism of phenolics, major facilitator family transporters and acetyl CoA metabolism were prominent among the large number of differentially expressed genes. Following the intriguing earlier observation that FA promotes nuclear retention of the redox-sensitive transcription factor ChAP1 without up-regulating genes for oxidant tolerance, we are using the RNASeq dataset to identify specific ChAP1-dependent targets of FA. The time-dependent phosphoproteome was determined for the 5 to 30 min time points at 2 mM FA, the highest concentration tested in the transcriptome study. Of a little over 5000 phosphopeptides detected, about 400 showed differential regulation by FA in two experiments. Integration of these large datasets should generate an initial picture of the events downstream from exposure to FA.
Supported by the Israel Science Foundation, ISF & INCPM 332/13; proteomics by Rina Zuchman & Tamar Ziv, Smoler Protein Center; RNASeq by Olga Karinski, Maor Hatoel, Anastasia Diviatis, Avital Polsky & Tal Katz-Ezov, Technion Genome Center.

185T Lipid biosensors: a novel tool for investigating the composition of fungal membranes. *A.V. Candy*[1,2], A. Ram[1], PY. Dupont[1,2], B. Scott[1,2], C.J Eaton[1,2] 1) Institute of Fundamental Sciences, Massey University, Palmerston North, Manawatu-Wanganui, NZ; 2) Bio-Protection Research Centre, Massey University, NZ.
In plant-associated fungi, the production of reactive oxygen species (ROS) by the NADPH oxidase (Nox) complex plays an integral role in controlling the growth of both beneficial and pathogenic fungi within their plant host. Therefore, specific regulation of the Nox complex is essential to maintaining a successful symbiotic relationship. Whilst much is known about the importance of ROS in plant-fungal interactions, comparatively little is known about how its production is regulated. In plant and mammalian systems, production of ROS by the Nox complex is regulated via the recruitment of its cytosolic components. This recruitment is controlled by lipid signalling, with cytosolic components containing sequences which target them to specific phospholipids enriched at certain locations within in the plasma membrane. It is hypothesised that similar regulation also occurs in fungi.

Very little is known about the lipid composition of fungal membranes, so the aim of this project is to comprehensively analyse membrane lipid composition in the beneficial fungal symbiont of ryegrass, *Epichloe festucae,* using a suite of biosensors. These biosensors consist of highly specific mammalian lipid binding domains fused to a fluorophore, enabling live cell imaging of phospholipid localisation via fluorescence microscopy. This approach also allows for analysis of membrane lipid composition of hyphae growing *in planta*. Using a phospholipase C-eGFP biosensor, phosphatidylinositol 4,5-bisphosphate (PI[4,5]P_2) was detected in the plasma membrane and at septa of hyphae grown in culture and *in planta*. Similar to yeast and mammalian systems, phosphatidylinositol 4-phosphate **(**PI[4]P), detected with mCherry-Plekha3 and Pleckha3-eGFP, was found to be localised to golgi vesicles in culture grown hyphae. Biosensors to other key modified phospholipids, namely phosphatidylinositol 3-phosphate **(**PI[3]P), phosphatidylinositol 3,4-bisphosphate (PI[3,4]P_2), and phosphatidylinositol 3,4,5-triphosphate (PI[3,4,5]P_3), localised to the cytoplasm of hyphae in culture and *in planta*, suggesting that these phospholipids are absent under these growth conditions in *E. festucae*.

186F Are dormant spores really dormant? A perspective from the transcription level. *Fang Wang*, Liguo Dong, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau, Macau SAR, China.
Fungal spores are specialized reproductive cells of filamentous fungi with properties for dispersal as well as for survival under harsh conditions and over a long period of time. It has been shown in *Aspergillus niger* that the resting spores have little metabolic activities and consume minimal energy. Previous studies have detected mRNA transcripts in dormant spores of some filamentous fungi, but it is not clear whether the transcripts were carried over from the process of conidiation or were actively synthesized in the resting spores. Moreover, a previous study in *A. niger* has been shown that genes involved in protein biosynthesis, RNA degradation, respiration and nitrogen metabolism are induced within an hour upon shifting to favorable growth conditions, suggesting that spores are constantly sensing and readily responding to the environment for growth. In this work, we aim to find out 1) whether resting spores of *Aspergillus nidulans* have any active transcription, 2) whether dormant spores can respond to stresses during dormancy and 3) whether resting spores of different ages and spores kept under different stresses behave differently during the break of dormancy. To address these, we have used Chromatin Immuno-precipitation followed by Next Generation Sequencing (ChIPseq) against RNA polymerase II (Pol II) to map active transcription events in spores kept under different conditions (e.g. fresh spores at 37? , spores aged at RT or 4? with or without oxygen) and during the

germination process. Our preliminary results show that Pol II is engaged on many genes genome-wide in resting spores. Gene Ontology analysis shows that these genes are enriched with stress response, ATP generation and the conidiation process. Unexpectedly, we found a significant level of Pol II accumulation near the 5' end of many genes in resting spores but absent in germinated spores and mycelia. The accumulation pattern is similar to the promoter-proximal pausing phenomenon in higher eukaryotes. We are in the process of confirming and understanding the functional significance of this phenomenon. We speculate that Pol II may be preloaded to specific promoters during the spore maturation process such to reduce the steps and energy needed by activation of genes for growth and survival in energetically dormant spores. Therefore, this Pol II "pausing" phenomenon might have important implications in the physiology and durability of spores.

187W White collar proteins are involved in basidiocarp development, and other responses in *Ustilago maydis*. *J. Sanchez-Arreguin*[1,2], J. Cabrera-Ponce[1], C. León-Ramírez[1], E. Arechiga-Carvajal[2], J. Ruiz-Herrera[1] 1) Genetics engeniering, CINVESTAV - CIE6010281U2, Irapuato, Guanajuato, México; 2) Universidad Autonoma de Nuevo León. San Nicolas de Los Garza, Nuevo León, México.

Description of fruiting body development in *Ustilago maydis*, was a report out of the ordinary, since the Class Ustilaginomycetes (Subphylum Ustilaginomycotina) has been described as a non-forming basidiocarps group. Based on the observation that illumination was required for the formation of fruiting bodies (basidiocarps) of *U. maydis* under laboratory conditions, in this study we explored whether the homologues of the White Collar (WC) photoreceptors played a role in this process. Accordingly, we obtained mutants in each one of its three WC homologue genes, *WC1a, WC1b*, and *WC2*, and analyzed the phenotype of the corresponding mutants. It was observed that the *wc1b* and *wc2* mutants were severely affected in basidiocarp formation when illuminated with white, blue or red light, whereas *wc1a* mutants formed basidiocarps in the three conditions; a result that suggests that Wc1b acts as a photoreceptor or/and that Wc1a and Wc2 are involved in a photoreaction dependent on a phytochrome. Wc1b was also involved in virulence to maize. In addition, Wc1a and Wc2, in a light independent mode, were involved in H_2O_2 protection, and in the control of maximal growth of the fungus.

188T The trascription factor Ztf1 (UMAG_02301) is involved in the pathogenesis and stress response of Ustilago maydis. *D. Martinez-Soto*[1], J. Velez-Haro[2], J. Ruiz-Herrera[1], J. Velez-Haro 1) Genetics engeniering, CINVESTAV - CIE6010281U2, Irapuato, Guanajuato, MX; 2) Departamento de Ingenieria Bioquìmica, Instituto Tecnologico de Celaya.

Gene UMAG_02301 from *Ustilago maydis*, encoding a transcription factor (named Ztf1) was found to be differentially regulated during the infection of *U. maydis* in the experimental host *Arabidopsis thaliana*. Further on, we found that the gene was also differentially regulated during *U. maydis* infection of the natural host *U. maydis*.

With these data we proceeded to delete the gene in FB1 and FB2 strains, and analyze the phenotypic characteristics of the mutants obtained. We observed that mutants devoid of the transcription factor were more sensitive than the wild type strain to different kinds of stress, among them: ionic stress, osmotic stress, oxidative stress, acid stress, stress by compounds that affect the membrane or the cell wall. It was also observed that the mutant strain was affected in the yeast-to mycelium dimorphic process induced by growth in the presence of an fatty acid as carbon source. It was also observed that the haploid mutant strains were not affected in mating, but they showed a significant reduction in their virulence during the infection of *Zea may*s and *A. thali*ana.

These results and the previous transcriptomic analyses, demonstrate that this transcription factor is important in different aspects of the physiology of *U. maydis* being involved at least in dimorphism, stress resistance, and in the advanced stages of the pathogenic process specifically after the fungus has penetrated and has become established within of the host tissues.

189F The peroxisomal import machinery enables contact sites between peroxisomes and different organelles. *T. Stehlik*[1], M. Boelker[1], J. Freitag[2] 1) Biology - Genetics, University of Marburg, Marburg, Hessia, DE; 2) Department of Molecular and Cellular Biology, Howard Hughes Medical Institute, University of California, Berkeley, USA.

Peroxisomes are nearly ubiquitous and dynamic organelles that harbor diverse metabolic pathways such as β-oxidation of fatty acids and partial reactions of the glyoxylate cycle. They are surrounded by a single membrane and the lack of a genome necessitates the import of all proteins that are destined for the peroxisomal matrix. The import is facilitated by the coordinated action of a membrane localized protein complex and a cytosolic receptor that binds and translocates newly synthesized cargos. In most cases cargo recognition depends on the presence of a C-terminal peroxisomal targeting signal type 1 (PTS1) with the prototypical sequence Ser-Lys-Leu which is recognized by the import receptor Pex5p. Recently, we found that several organisms possess membrane proteins with a potential PTS1 signal and asked whether such proteins might facilitate the formation of contact sites between peroxisomes and other organelles. Here we show that in both *Ustilago maydis* and *Saccharomyces cerevisiae* the protein phosphatase Ptc5p with the afore-metioned architecture tethers peroxisomes to mitochondria *in vivo*. Further, we demonstrate that tethering depends on (i) a functional PTS1 signal and (ii) the presence of the import receptor Pex5p and (iii) that this mechanism does not require specific protein domains by generating a synthetic ER-peroxisome tether.

190W Analysis of septins during hyphal growth of *Ustilago maydis*. *Sabrina Zander*[1], Sebastian Baumann[2], Michael Feldbrügge[1] 1) Heinrich Heine University, Düsseldorf, Institute for Microbiology, 40225 Düsseldorf, Germany; 2) CRG – Center for Genomic Regulation, 08003 Barcelona, Spain.

Septins are GTP-binding cytoskeletal proteins with functions in cell polarity, membrane remodeling, cytokinesis and cell morphology. Conserved across eukaryotes, except higher-order plants, septins assemble in nonpolar, heteromeric complexes. These further assemble end-to-end to form filaments and a variety of higher-order structures. The heterooligomeric structure of septins and their subcellular localization have already been extensively studied. However, a precise mechanism of their subcellular assembly and their intracellular transport are unknown.

The genome of the filamentous fungus *U.maydis* encodes four septin proteins: Cdc3, Cdc10, Cdc11 and Cdc12. In this study the influence of septin deletions were analyzed, during the hyphal state of *U. maydis*. All four septins are needed for efficient unipolar growth indicating a common function for septins during hyphal growth. Furthermore, the subcellular localization was analyzed with Gfp fusion proteins. All septins showed the same localization in cytoplasmic rings, at septa, in filaments and on moving early endosomes. Colocalization studies revealed that all septins localize interdependently in the same structures. Furthermore, FRET analysis showed that Cdc3 and Cdc12 interact directly *in vivo*. Endosomal transport was not only dependent on each individual septin, but also dependent on the RNA-binding protein Rrm4. RNA live imaging of all four mRNAs showed also an Rrm4-dependent transport. FRAP experiments demonstrated that

recovery of Cdc3-mCherryN and Cdc12-GfpN occurs simultaneously at distinct subcellular sites and that the rate of recovery was facilitated by the endosomal RNA transport. Based on these results, a model was proposed where local translation promotes the assembly of newly synthesized septins in heteromeric structures on the surface of endosomes. This is important for the long-distance transport of septins and the efficient formation of the septin cytoskeleton.

191T Role of polyamines in Ustilago maydis mating. *F. Perez-Rodriguez*, J. Ruiz-Herrera Genetics engeniering, CINVESTAV - CIE6010281U2, Irapuato, Guanajuato, MX.

Polyamines are low molecular weight molecules possessing several amino groups, and which are indispensable for the normal growth of living beings. At physiological pH polyamines interact with negatively charged macromolecules such as nucleic acids, proteins, etc.; being involved in a variety of cellular functions such as regulation of macromolecule synthesis, proliferative phenomena, and cell differentiation. Despite these roles, the precise mechanism by which polyamines are involved in these phenomena remains rather unknown. In order to investigate this problem, we proceeded to utilize *Ustilago maydis* mating as a simple model. The mating process in *U. maydis* involves two unlinked mating *loci,* denominated *a* and *b*; the first one with 2 alleles, and the second one with about 27. Successful mating between two partners occurs when they have different *a* and *b* alleles. To test the role of polyamines in the process we utilized two *odc*- (ornithine decarboxylase) polyamine auxotrophic strains, one *a1b1* (LG1), and the other *a2b2* (LG4). Mating was determined by the Fuz reaction on solid MM with activated charcoal, and by microscopic observation in liquid MM, both containing low (0.1 mM) or high (5mM) putrescine (the auxotrophs are unable to grow in the absence of polyamines). It was observed that with low putrescine, Fuz reaction was almost negative, and in liquid medium the number and length of the conjugation tubes were highly reduced. When mating involved the LG4 strain and a w.t. strain labeled with mCherry fluorescent protein, the number and length of germination tubes were lower in the mutant, compared with the w.t. fluorescent strain. Expression of *mfa, pra*, *bW* and *bE* genes of LG1 and LG2 mating partners at low or high putrescine levels was measured by semi-quantitative PCR. The results showed that expression of all genes was reduced at low levels of polyamines; but levels of *maf1* and *maf2* genes expression were the most affected ones. These results show that *U. maydis* mating requires polyamines, and that their role occurs at the level of selective gene expression.

192F The Nma1 protein of *Ustilago maydis*: a novel player in vesicular transport processes. T. Obhof[1], K. Schneider[1], N. Pinter[1,2], N. Kellner[1], O. Valerius[2], G. Braus[2], *J. Kämper*[1] 1) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, Germany; 2) Institute of Microbiology and Genetics, Georg-August-Universität Göttingen, Germany.

We have identified the Nma1 protein in *Ustilago maydis* as an interactor for Num1 (Kellner et al., 2014), a protein that functions both as a component of the NineteenComplex splicing apparatus and in cytoplasmic transport of early endosomes (EEs). Co-IP and MS analysis revealed that Nma1 interacts with Vps3, Vps11 and Vps18, core components of the CORVET complex involved in tethering and fusion of EEs. Concomitantly, Nma1 co-localizes to Rab5 and Yup1(tSNARE) positive EEs. Deletion of Rab5, the EE-localized Rab-GTPase tethering the CORVET complex to EEs, or of Vps3, the Rab5 interacting subunit of CORVET, abolishes the localization of Nma1 to EEs. Deletion of Nma1 affects the movement of EEs and recycling endosomes (REs), but not on secretory vesicles or late endosomes, implicating a functional role in transport of a defined set of vesicles. Interestingly, during mitosis, Nma1 localization is restricted to spindle pole bodies (SPBs); together with Nma1, we observed also the localization of Rab5 marked EEs at SPBs, implicating a role for EEs (or associated proteins) during mitosis, as recently reported for mammalian cells (Das et al., 2015). As Nma1 interacts with the vesicle population localizing to the SPB, we envision a functional connection with the so far poorly understood role of membrane trafficking during mitosis.

Das *et al.*, 2015, Small GTPases, 6:1, 11-15
Kellner *et al.*, 2014, PLoS Genet 10(1): e1004046

193W The role of Pep4 in autophagy and programed cell death in *Ustilago maydis* . *C. Soberanes-Gutierrez*, J Ruiz-Herrera Genetics engeniering, CINVESTAV - CIE6010281U2, Irapuato, Guanajuato, MX.

Saccharomyces cerevisiae is currently a well-established eukaryotic model organism used in the elucidation of the molecular mechanisms underlying necrosis, autophagy, and apoptotic pathways. Autophagy is a complex degradative process in which cytosolic material is randomly sequestered within autophagosomes, which are targeted to the vacuole. After degradation by resident hydrolases, the contents are recycled.

In order to determine whether the *U. maydis pep4* gene, that encodes the acid vacuolar proteinase A, ortholog to human Cathepsin D, was important for autophagy in *U. maydis*, we compared the capacity of the wild-type, and *Dpep4* strains to accumulate autophagic bodies within the vacuoles when the cells were exposed to carbon stress conditions. Wild type and *Dpep4* cells were incubated for 5 h in minimal medium lacking a carbon source (MM-C), and processed for TEM. Our observations showed the accumulation of autophagic bodies within the vacuole of *Dpep4* cells, in contrast to the absence of these structures into vacuoles of wild-type cells, demonstrating that the proteinase A deficient mutant is affected in its capacity to lyse the autophagic bodies, and all the cytosolic material is accumulated in the autophagosomes present into the vacuole.

In addition we must cite that in several systems it has been concluded that the acidic protease vacuolar is involved in the programmed cell death. Accordingly, it was decided to determine cell death in *U. maydis* FB2 strain and the *Δpep4* mutant. To analyze cell viability the cells were taken to the stationary stage, and subsequently they were washed and resuspended in minimal synthetic medium pH 7, and followed their viability at different incubation times at 28^{o}C. We observed that the *Δpep4* mutant conserve its viability for a longer time than the wild type strain. This was consistent with the observation of cell staining with berberine sulphate, a non-permeating fluorescent compound. It was also observed that the *Dpep4* mutant strain was more resistant to H_2O_2 and acetic acid treatment than the wild type strain. All these data are evidence that proteinase A is involved in programed cell death of *U. maydis*.

194T Morphological characterization, transcription profiling and metabolic expression during ascospore formation within fertilized sclerotia of *Aspergillus flavus.* *J.M. Luis*[1], I. Carbone[1], G.A. Payne[1], D. Bhatnagar[2], J. Cary[2], M. Lebar[2], G.G. Moore[2], P.S. Ojiambo[1] 1) Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, 27695; 2) USDA/ARS/SRRC, New Orleans, LA 70124.

Sclerotia of *Aspergillus flavus* can give rise to new hyphae, asexual conidia, or stroma containing sexual spores after fertilization by conidia of the opposite mating type. The transition from asexual to sexual reproduction can have broad implications on the survival and genetic diversity of the fungus, but this process is not well understood. Shifts between sexual and asexual reproduction affect the quantity and diversity of secondary metabolites produced by the fungus, including the carcinogen aflatoxin, which accumulates in field and tree crops infected with *A. flavus*. As asexual structures, the thick-walled and darkly pigmented sclerotia of *A. flavus* are important for survival in the soil. Upon fertilization with conidia of the opposite mating type, the sclerotia can become ascospore-bearing ascocarps. While there is information on the genetic aspects of sexual development in *A. flavus*, there is little to no information on biochemical, transcriptional, or morphological changes that occur between fertilization of sclerotia and the formation of ascopores. This study takes advantage of differences in female fertility between reciprocal crosses to examine morphological, biochemical, and transcriptional differences during sexual reproduction. Specific objectives were to: (i) identify differentially expressed genes between mating pairs with high and low levels of sexual fertility, and (ii) characterize metabolite production when sclerotia are fertilized. These changes were observed every two weeks from the time of fertilization of sclerotia for a total of eight weeks. We hypothesize that genes involved in mating, sexual reproduction, ascocarp development and secondary metabolism will show increased expression in highly fertile sclerotia at the time of ascocarp development, and that there are marked differences in the metabolic profiles of fertilized sclerotia over time. Results from the study will facilitate our understanding of the transitional phase of asexual to sexual reproduction in *A. flavus* and this information could be useful in screening of atoxigenic strains of *A. flavus* with high sexual fertility.

195F Clampless mutants of the self-fertile *Coprinopsis cinerea* homokaryon AmutBmut. Weeradej Khonsuntia, Ulrich van Campe, *Ursula Kües* Molecular Wood Biotechnology, University of Göttingen, Göttingen, DE.

A clampless and fruiting-defective mutant E3 was originally obtained by REMI mutagenesis of homokaryon AmutBmut which is self-compatible by mutations in the mating type loci. E3 grew initially with a normal fluffy aerial mycelium and produced oidia in light. In aged cultures, mycelia glued together into rubber-like films that could be lifted off from the agar. The original E3 strain was however lost while two morphological different lines, JE3 and J2E3, remained from sub-culturing E3. Both lines were clampless, did not initiate fruiting but had lost the ability to form rubber-like films. JE3 grew faster with aerial mycelium (4.3 mm/day) than J2E3 (2.7 mm/day) that had barely any aerial mycelium and showed invasive growth into agar. JE3 produced large amounts of oidia in dark ($2.6x10^9$ per plate) and even more in light ($9.6x10^{10}$). JE3 and J2E3 were also not stable. Spontaneous sectoring was observed in cultures of both strains. Sectorial isolate JE3-1 had a denser mycelium compared to the parental JE3. It lacked clamp cells, produced massive amounts of oidia in dark and formed large swollen cells on thick-walled swollen hyphae. J2E3-1 from a J2E3 sector produced fluffy mycelia, had fused clamp connections at hyphal septa and fruited. Comparable to typical dikaryons, J2E3-1 produced nearly no oidia in dark ($1.3x10^5$ per plate) but some in light ($3.3x10^6$ per plate). Recurrence of clamp connections and an ability to initiate fruiting body development indicates that the mutations in the *A* and *B* mating type loci from strain AmutBmut are still extant. To test the situation in JE3-1, crosses were performed with monokaryon PG78. All progeny of the cross was clampless, suggesting that possibly the *A* mutation to self-fertility had been reversed to self-sterility. Progeny was sorted into categories thick and thin mycelium and into evenly and irregular growing. Both types of categories split 1:1. Thick mycelium and evenly growing were linked (25 cases of 69) and thin mycelium and irregular shape (26 cases of 69). Furthermore, 13 colonies with thick mycelium formed rubber-like films in aging cultures. The results suggest that the mutation from the original E3 mutant was still present. The repeated changes in morphology and other phenotypes in sub-culturing of E3 and mycelial descendants possibly indicate stress situations caused by genetic defect(s) in complex regulatory networks. Sectoring into new growth types might help to relieve pressure from such stress.

196W Developmental regulators in the mushroom *Coprinopsis cinerea*. Weeradej Khonsuntia, Bastian Dörnte , *Ursula Kües* Molecular Wood Biotechnology, University of Göttingen, Göttingen, DE.

Four putative regulatory genes involved in developmental processes in *Coprinopsis cinerea* are being investigated, the duplicated genes *FLU1-II.1* and *FLU1-II.2* as homologs of *Aspergillus nidulans fluG* for a regulatory protein with a C-terminal glutamine synthase I (GSI)-like domain, *crg1* as homolog of *A. nidulans flbA* for a regulator of G-protein signaling, and *NWD2* which encodes a NACHT-NTPase and suppresses a defect in primary hyphal knot formation (*pkn1*) in *C. cinerea* mutant Proto159. All four genes were subcloned for overexpression behind the *gpdII* promotor of *Agaricus bisporus* into a plasmod with a *pab1*$^+$ selection marker and transformed into different strains of *C. cinerea*, i.e. into the self-compatible homokaryon AmutBmut, its fruiting-defective mutant Proto159 and the normal monokaryon FA2222. A *pab*$^+$ plasmid pYSK7 habouring the laccase gene *lcc1* was used as a control for effects of random DNA integration in transformation. Transformation of all four genes changed mycelial morphologies of homokaryon AmutBmut on a minimal medium used for selection as compared to the control transformants. Transformation of *Flu1-II.1*, *Flu1-II.2* and *crg1* lead to extended aerial hyphal growth, transformants of *NWD2* produced less dense mycelium and shorter aerial hyphae. On YMG/T complete medium, further phenotypes were observed such as in mycelial color, in increased sclerotia production and in massive secretion of yellow liquid droplets. Effects of the genes on fruiting are in test. Transformants of Proto159 of all four genes and of the laccase gene *lcc1* had an enhanced aerial mycelium on YMG/T complete medium as compared to Proto159. Mutant Proto159 produces a strong pigmentation of the YMGT agar medium during aging. The majority of transformants of genes *crg1* (87%), *Flu1-II.2* (85%), Flu1-II.1 (82%), and *NWD2* (73%) but also of the control vector (75%) delayed the pigmentation as compared to Proto159. We conclude that the *pab1*$^+$ selection marker possibly strengthen the strain by complementing its *pab1* auxotrophy. Transformants of monokaryon FA2222 were also obtained for all four genes and await their morphological characterization.

197T Identification of recessive lethal alleles linked to unidirectional loss-of-heterozygosity events in *Candida albicans*. *Timea Marton*[1,2], Adeline Feri[1,2], Pierre-Henri Commere[3], Corinne Maufrais[4], Natacha Sertour[1], Gavin Sherlock[5], Marie-Elisabeth Bougnoux[1], Christophe d'Enfert[1], Melanie Legrand[1] 1) Institut Pasteur, INRA, Labex IBEID, Unité Biologie et Pathogénicité Fongiques, Paris, France; 2) Univ. Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, rue du Docteur Roux, Paris, France; 3) Institut Pasteur, Imagopole, Plate-Forme de Cytométrie, Paris, France; 4) Institut Pasteur, Centre d'Informatique pour la Biologie, Paris, France; 5) Department of Genetics, Stanford University, Stanford, California, USA.

Candida albicans is a frequent human commensal yeast responsible for both mucosal and the majority of life-threatening nosocomial fungal infections. Its heterozygous diploid genome is highly plastic, with frequent aneuploidies and loss-of-heterozygosity (LOH) events

commonly associated with acquired antifungal resistance. Although LOH events can be seen on all 8 chromosome pairs, a chromosome homozygosis bias is observed for certain chromosomes regarding the retained homolog in the homozygous state. This suggests the occurrence of recessive lethal allele(s) (RLA) that would prevent large-scale LOH events from being stably maintained.
To further explore the impact of LOH on *C. albicans* biology, we took advantage of the *Saccharomyces cerevisiae* I-*Sce*I endonuclease and fluorescent markers (GFP/BFP) to establish a system that allows 1) the induction of a DNA double-strand break (DSB) at a specific location in the genome and 2) the detection of the occurrence of long-tract homozygosis resulting from repair of the induced DNA-DSB.
This approach was applied to identify RLAs on Chr7, where haplotype B (HapB) is never found in the homozygous state, unlike haplotype A, suggesting the presence of RLA on Chr7B. I-*Sce*I successfully targeted and induced a DNA-DSB on both Chr7 homologs as illustrated by an increase in LOH. However, our inability to recover cells homozygous for the right arm of Chr7B confirmed the existence of RLA(s). Mining of genome sequencing data for 154 *C. albicans* isolates allowed identifying a nonsense-generating SNP within the HapB allele of C7_03400c, whose ortholog in *S. cerevisiae ScMTR4* encodes for an essential RNA helicase involved in nuclear RNA processing. As well, an RLA and a deleterious recessive allele on another chromosome (Chr4B) have been found explaining why Chr4A cannot be lost. This methodology also allowed highlighting that repeated regions, such as major repeat sequences, are hotspots for homologous recombination between homologs and play a role in LOH dynamics by generating new allele combinations upon haplotype swapping. Overall, our work uncovers regions whose homozygosis might impact *C. albicans*'s ability to persist as a commensal or to cause infections.

198F Mauriceville plasmid-induced senescence impacts nuclear genome stability. *Amani Alharthi*, Natasha Nazir, Kala Chinnaswarmy, Charles D. Baudo, John C. Kennell Biology , Saint Louis University , saint louis , MO.

Strains of *Neurospora crassa* containing the Mauriceville plasmid are prone to senesce due to plasmid integration into the mitochondrial genome or as a result of plasmid overeplication. Next-generation sequencing (NGS) of pre-senescent cultures revealed an unexpected high number of single nucleotide polymorphisms (SNPs) in the nuclear genome. The number of SNPs in senescent cultures was found to be significantly greater than those associated with plasmid-free strains, suggesting that Mauriceville plasmid leads to increased rates of nuclear mutation. To assess this hypothesis, the viability of multi-nucleate macroconidia versus uni-nucleate microconidia was compared. The results showed that as cultures were transferred, the viability of microconidia decreased much sooner than macroconidia, which may reflect the lack of additional nuclei to complement mutations in essential genes. In addition, rates of reversion of a nuclear auxotrophic mutation in pre-senescent cultures were also determined. The plasmid-containing strains were found to have up to a 40-fold increase in reversion rates in transfers close to senescence suggesting that mitochondrial dysfunction influences the frequency of nuclear mutation. Together, these findings suggest that mitochondrial dysfunction associate with plasmid-induced senescence impacts nuclear genome stability.

199W Mitochondria-mediated nuclear genome instability associated with plasmid-induced senescence in *Neurospora crassa*. *C. D. Baudo*[1], S. Ryu[1], N. M. Dutken[1], K. McCluskey[2], S. E. Baker[3], J. C. Kennell[1] 1) Department of Biology, Saint Louis University, Saint Louis, MO; 2) Fungal Genetics Stock Center, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri; 3) Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington.

Senescence in *Neurospora crassa* is associated with dysfunctional mitochondria and most commonly observed in strains harboring specific mitochondrial plasmids. Variant forms of the Mauriceville retroplasmid (pM) trigger senescence by integrating into the mitochondrial genome disrupting essential mitochondrial genes or by over-replicating and interfering with protein synthesis. The transcriptomes of Mauriceville cultures undergoing senescence and wild-type plasmid-free cultures exposed to two mitochondrial inhibitors were analyzed by RNA-sequencing. Approximately 10% of nuclear encoded genes were regulated similarly under plasmid-induced senescence, inhibition of the electron transport chain, and inhibition of mitochondrial protein synthesis. Critical pathways involved in antioxidant defense, DNA damage response, and mitochondrial homeostasis were upregulated and validated using quantitative PCR. In addition, anomalous SNP accumulation was observed in pre-senescent cultures. Transcriptomic profiling and examination for signatures of oxidative DNA damage indicate that these SNPs do not result from ROS-mediated mutagenesis until stages of growth immediately prior to senescence. The rate of SNP formation in senescent cultures was compared with plasmid-free strains and to a long-lived (LL) derivative of Mauriceville capable of escaping senescence. Surprisingly, the rate of SNP formation in pre-senescent cultures was up to 50 fold greater than plasmid-free and LL strains indicating that plasmid-related mitochondrial stress mediates mutations in the nuclear genome. The mechanisms that allow the LL culture to tolerate and withstand SNPs, despite having abundant levels of plasmid variants, remain to be identified. Further characterization of longevity mutants should help to elucidate mitochondrial-nuclear communication pathways that respond to mitochondrial dysfunction and potentially lead to new therapies for human mt diseases.

Comparative and Functional Genomics

200T Exploring fungal dark matter using single-cell genomics. *S. Ahrendt*[1,2], C. A. Quandt[3], D. Ciobanu[2], A. Clum[2], A. Salamov[2], J-F. Cheng[2], T. Woyke[2], T. James[3], I. Grigoriev[1,2] 1) Department of Plant and Microbial Biology, University of California, Berkeley, CA; 2) DOE Joint Genome Insititute, Walnut Creek, CA; 3) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Current estimates suggest that only approximately 100,000 of the estimated 5 million fungal species worldwide have been described, and the overwhelming majority of those fall within the Dikarya. Thus, the diversity of the non-Dikarya lineages has been poorly explored. Our lacking insight into the metabolic potential of these fungi is a reflection of their absence from sequence databases, limiting our ability for meaningful comparative analyses and lifestyle predictions. Exacerbating this issue is the fact that a substantial fraction of these organisms is largely uncultivated, challenging genomic exploration, despite being detected in environmental PCR surveys as a significant component of a community. The difficulties inherent in exploring the genetic make-up of this "fungal dark matter" can be overcome using single-cell sequencing to reconstruct genomes of uncultivated organisms directly isolated from the environment. Environmental fungi, particularly among the zoosporic and other early-diverging fungal lineages, make exceptional targets for single-cell genomic techniques; however, as much of the current single-cell genomic work focuses on mammalian, bacterial, and archaeal systems, there is a pressing need to adopt these protocols for fungi. Here we developed and tested these single-cell methods to sequence the genomes of seven species which cannot be grown in pure culture, including the first representatives of the previously unsequenced Zoopagomycotina subphylum. We show

that although there is a large variation in gene space recovery from each single cell (ranging from 6 - 88%), combining multiple cell libraries can increase this recovery to around 90%. Phylogenomic analyses allowed us to place previously unsampled lineages within the fungal tree of life, even when considering partially complete genomes derived from single individual cells. Additionally we explored gene family expansions to identify patterns consistent with lifestyle aspects of these biotrophic organisms.

201F PHI-base - the Pathogen-Host Interactions database. *Kim Hammond-Kosack*[1], Alayne Cuzick[1], Kim Rutherford[2], Helder Pedro[3], Martin Urban[1] 1) Dept of Plant Biology and Crop Sciences, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; 2) Cambridge Systems Biology and Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge, Cambridgeshire CB2 1GA, UK; 3) The European Molecular Biology Laboratory, The European Bioinformatics Institute, Hinxton, Cambridgeshire, CB10 1SD, UK.

The pathogen-host interactions database PHI-base (www.phi-base.org) is a knowledge database. It contains expertly curated molecular and biological information on genes proven to affect the outcome of pathogen-host interactions reported in peer reviewed research articles. Genes not affecting the disease interaction phenotype are also curated. Viruses are not included. Here we describe a revised PHI-base Version 4 data platform with improved search, filtering and extended data display functions. Also a BLAST search function is now provided. The database links to PHI-Canto, a new multi-species author self-curation tool adapted from PomBase-Canto. The recent release of PHI-base version 4 has an increased data content containing information from >2000 manually curated references. The data provide information on 4460 genes from 264 pathogens tested on 176 hosts in 8046 interactions. Pro- and eukaryotic pathogens are represented in almost equal numbers. Host species belong ~70% to plants and 30% to other species of medical and/or environmental importance. Additional data types included into PHI-base 4 are the direct targets of pathogen effector proteins in experimental and natural host organisms. The different use types and the future directions of PHI-base as a community database are discussed.

Urban et al., (2017) PHI-base: A new interface and further additions for the multi-species pathogen-host interactions database. *Nucleic Acids Research* (database issue Jan 2017)

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202W Developing a new genome annotation for improved gene prediction of *Cryphonectria parasitica*. *Di Ren*, Angus Dawe Biological Sciences, Mississippi State University, Mississipi State, MS.

Cryphonectria parasitica, causal agent of chestnut blight, devastated the American Chestnut population in the early 20th century. Hypoviruses that reduce the severity of infection offer potential for biological control. However, their spread is hampered by a diverse nonself-recognition system, vegetative incompatibility (VI), among strains of *C. parasitica*. CpVIB-1 was identified as a likely transcription regulator with an important role in mediating programmed cell death in response to allelic variation at the *vic*4 locus. However, challenges for obtaining further insights into the VI mechanism by identifying the targets of CpVIB-1 through transcriptome comparison arose when performing the analysis using the currently available genome annotation provided by the Joint Genome Institute (JGI). This JGI version (v2.0, released in 2010; http://genome.jgi.doe.gov/Crypa2/Crypa2.home.html) has been found to include some incorrect predictions and is also limited in gene structural annotation details when compared to the information that can be provided by more recent annotation pipelines. A configurable genome annotation and curation pipeline, Two-pass MAKER2, was applied to annotate and mask repetitive elements in the genome, align protein and RNA evidence in a splice-aware manner, and accurately identify genes. This pipeline improves gene predictions by modifying gene models from GeneMakr-ES, SNAP and Augustus gene predictors with evidence from RNA-seq transcriptome data, and protein information from the Uniprot/SwissProt, OrthoMCL, and *Magnaporthe oryzae* protein databases. The reference genome used was the 2010 v2.0 *C. parasitica* genome assembly from JGI. Gene numbers predicted varied between 11704 and 11792 (~ 0.75 %) using the three gene predictors. 91% had Annotation Edit Distance (AED) values < 1, indicating strong support, with the OrthoMCL data. Uniprot/Swissprot and *M. oryzae* data yielded 86.7% and 88.9% of predicted genes with AED values < 1, respectively. The new output included gene structural features such as mRNA, exons, and untranslated regions (UTRs), along with a quality metric system for each prediction and the detailed evidence used to support the prediction. New unique IDs for each prediction have also been linked to InterPro ID by blastp. Using this new annotation has more accurately predicted certain known genes and will provide the basis for improved transcriptional profiling to address questions related to VI and resulting cell death.

203T FungiDB: An integrated functional genomics database for fungi and oomycetes. *Evelina Basenko*[1], Omar Harb[2], Achchuthan Shanmugasundram[1], David Roos[2] 1) Centre for Genomic Research, Functional and Comparative Genomics, University of Liverpool, Liverpool, UK; 2) Penn Center for Bioinformatics and Department of Biology, University of Pennsylvania, Philadelphia, PA.

FungiDB (http://FungiDB.org) is a free online resource for data-mining and functional genomics analysis. As part of the Eukaryotic Pathogen Bioinformatics Resource Center (http://EuPathDB.org), FungiDB provides an easy to use and interactive interface to explore genomes, annotation, functional data (transcriptomics or proteomics), metabolic pathways and results from numerous genome wide analyses (ie. InterPro scan, signal peptide and transmembrane domain predictions, orthology, etc..). FungiDB contains an expanding number of genomes from species spanning the Ascomycota*,* Basidiomycota*,* zygomycete, and chytrid fungi; including pathogenic species from the *Cryptococcus, Histoplasma*, and *Coccidiodes* genera.

The graphical user interface in FungiDB allows users to conduct *in silico* experiments that leverage the available data and analyses. For example, a search in FungiDB can identify all genes in *Candida albicans* that do not have orthologs in mammals, have a predicted signal peptide, are annotated as a kinase and are expressed under conditions of high oxygen stress. Results from any search can be further analyzed visually using a companion genome browser or through analysis tools such as genome ontology and metabolic pathway enrichment. All results and searches can be saved in a user's profile or downloaded in multiple formats.

Users of FungiDB can also privately analyze their own data (ie. RNAseq or genomic sequence) via the EuPathDB galaxy implementation. All genomes in EuPathDB are preloaded into this galaxy instance allowing more streamlined data analysis. Results from galaxy workflows can be downloaded or analyzed within FungiDB, such as through visualization of RNAseq read coverage plots in the genome browser.

Input from the community (images, files, PubMed records, *etc*) can be easily added to FungiDB records (ie. gene pages) via user

comments; these comments are attributed to the submitted and become immediately visible and searchable.

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Authors are individuals present at this meeting and representing the EuPathDB team.

204F Parallel fitness using barcode sequencing in A. fumigatus: Assessment of the role of Aspergillus Kinases in azole resistance and as Targets for Antifungal Drug Discovery. *N. ALFURAIJI*, J. Mabey , M. Bromley , P. Bowyer Manchester Fungal Infection Group,Division of Immunity, Infection and Respiratory Medicine,Faculty of Biology, Medicine and Health Sciences, University of Manchester.

Fungi cause a wide range of infections including invasive and life threatening disease, superficial infections of the skin and mucosal membranes, as well as allergic disorders. The mortality rate due to fungal diseases remains unacceptably high, and is thought to exceed one million patients annually. Currently, only four groups of antifungals are available to treat systemic fungal infections: the polyenes, flucytosine, triazoles and echinocandins. The emergence of resistance to the available antifungal drugs and toxicity associated with some classes necessitates the exploration of novel pharmacologically effective antifungal drugs. Protein phosphorylation by protein kinases (PK) impacts all areas of cellular activity. Their critical roles in a wide range of cellular functions, along with the relative ease in which drug inhibitor assays can be developed have highlighted this class of enzymes as potential drug targets.

A bioinformatic analysis of the genomes of 9 Aspergillus species has revealed, in contrast to a previous study, that the number of protein kinases in each species is relatively consistent ranging from a minimum of 140 in *A. nidulans* up to 175 in *A. niger*. The kinases are distributed over 11 sub-groups including genes which apparently encode tyrosine like kinases and tyrosine kinases. We present comparative analysis which suggests that there are a sub-group of kinases that are specific to filamentous fungi.

To assess the role of these kinases in growth, virulence and drug tolerance, we have generated a library of 90 genetically barcoded knockout mutants. We have validated a barcode-sequencing approach to assess in pooled cultures, the fitness defect in each null mutant when in standard culture conditions and in the presence of the antifungal drug itraconazole. We show that 2 genes, the mitogen-activated protein kinase *mpkB* (AFUB_078810) and a serine/threonine protein kinase *ssn3* (AFUB_035220) are critical for azole tolerance. In addition, we have identified 25 genes that encode kinases that are essential for viability. Our assessment of virulence of these strains is currently in progress.

205W Comparison of a commercial Aspergillus oryzae strain and its degenerated strain by transcriptome analysis. *Y. Zhong*, W. Nong, L. Xing, X. Lu, H. Kwan The Chinese University of Hong Kong, N.T., HK.

With a great capacity of enzyme production for macronutrient hydrolysis, *Aspergillus oryzae* is important in Koji-making process in soy sauce fermentation to contribute to subsequent fermentation and the characteristic aroma and flavor. Chinese commercial strain *A. oryzae* RD2 can produce soy sauce with high quality. The strain TS2 was isolated after repetitive use of RD2, which has limited enzyme production and produces poor-quality soy sauce. We aimed to explain the phenotypic differences between two strains at the molecular level by transcriptome analysis. We first cultivated RD2 and TS2 in the mixture of steamed soy beans and wheat flour and sampled in duplicate in three Koji-fermentation stages: mycelium expansion (ME), early sporulation (ES) and mature sporulation (MS). Then we sequenced the extracted RNA in Illumina Hiseq 2000 Platform. Differentially expressed genes (DEGs) were identified: 1589 genes in ME, 686 genes in ES and 225 genes in MS. Among them, we mainly focused on those related to hydrolytic process including amylases and proteinases. The result shows that the active strain RD2 has higher expression level in both types of hydrolases throughout all stages, which explains the better performance of RD2 in Koji fermentation. Interestingly, in the degenerated strain TS2, more genes related to glycolysis and amino acid catabolism are expressed at a higher level compared to RD2. So the off-flavor of soy sauce produced by TS2 is probably due to the poorer hydrolase expression and the overuse of flavor compounds for energy, and RD2 could not compete with TS2. In conclusion, the metabolic saccharolytic and proteolytic activities make RD2 a good fermentative strain in soy sauce production due to its capacity of releasing compounds for flavor formation and subsequent fermentation, while the spontaneous degeneration would cause loss of the industrially favorable phenotypes but gain of growth dominance. Based on the transcriptome comparison, this study provides insights into the identification of molecular markers related to good soy sauce fermentation, which informs the selection and breeding of superior strains. We will explore to find more molecular markers of superior soy sauce production strains, and investigate the metabolism and functions needed for good soy sauce production.

206T Spatially-resolved gene regulation in wood-degrading fungi highlights carbohydrate- selective machinery in brown rot fungi. *J. Zhang*[1], K. Silverstein[2], M. Figueroa[3], J. Schilling[1] 1) Bioproducts and Biosystems Engineering, University of Minnesota, saint paul, MN; 2) Minnesota Supercomputing Institute, University of Minnesota, minneapolis, MN; 3) Department of Plant Pathology, University of Minnesota, saint paul, MN.

Brown and white rot are two typical woody plant cell wall degradation strategies among *Agaricomycotina*. Compared to the simultaneous extraction of all carbon sources of white rot, brown rot selectively utilizes carbohydrates by leaving most of the lignin, potentially a more efficient carbohydrate-converting system. Recent genomic comparisons have noted that losses of ligninolytic genes have occurred in brown rot fungal evolution, corresponding to a surprising increase in decay efficiency. To approach this conundrum, we compared these two wood-decaying strategies, tracking temporal gene regulation at a whole genome-level among fungi from disparate phylogenetic clades. These data show that brown rot fungi up-regulate transcription of reactive oxygen species (ROS) at the advancing hyphal front, with an inducible glycoside hydrolase expression at later stages. In contrast, data showed the white rot fungi expressing laccase and several class II peroxidases at the hyphal front, presumably targeting lignin as a pretreatment. In later stages, white rot fungi coupled the transcriptional up-regulation of lignocellulose-oxidizing genes with glycoside hydrolase genes. In addition, we observed 115 genes up-regulated (2-fold change) only in brown rot fungi in early decay, narrowing perhaps the candidate list of lignocellulose pretreatment genes in these unique, carbohydrate-selective fungi.

207F Supersize fungi: Rust in the genomics era! Cecile Lorrain[1], Stephen J. Mondo[2], Emmanuelle Morin[1], Kurt LaButti[2], Anna Lipzen[2], Pascal Frey[1], Joseph W. Spatafora[3], Igor V. Grigoriev[2], *Sebastien Duplessis*[1] 1) INRA, UMR 1136 Interactions Arbres-Microorganismes, INRA/Université de Lorraine, 54270 Champenoux, France; 2) US Department of Energy Joint Genome Institute, Walnut

Creek, CA, USA; 3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA.

Rust disease fungi cause consequent losses in plantations of major crops and trees. Despite their importance as plant pathogens, limited progress has been made in understanding molecular bases of host infection. Indeed, rust fungi exhibits the most complex life cycles described among fungi (e.g. heteroecious and macrocyclic) and they are true obligate biotrophs with no culture or manipulation possible in the lab, making their study a real challenge. So far, only a handful of virulence effectors have been described in a limited number of species and no clear characterization of their possible role was established. Rust fungi also exhibit the largest genomes known for fungi so far, full of transposable elements and with many expanded and specific gene families of unknown function. Rust genome sequencing is also most often based on dikaryotic spores, which adds to the difficulty to obtain complete and neat assembly. The 336 Mb genome of the poplar rust fungus *Melampsora allii-populina* recently sequenced in the frame of the US DoE Joint Genome Institute 1KFG project [1] will be introduced to illustrate how comparative genomics within the Melampsoraceae and the Pucciniales allow to explore host specificity in rust gene repertoires, particularly for rust candidate effectors.

[1] Mycocosm: http://jgi.doe.gov/fungi

Pucciniomycotina: *Melampsora allii-populina* 12AY07 v1.0

208W Genetic analysis of natural variation in *Botrytis cinerea* field isolates identifies a single gene with a major effect on pathogenicity and sporulation. Wilson Acosta[1], Francisco Anta[1], *José María Díaz Mínguez*[1], Michael Thon[1], Jan A.L. van Kan[2], Ernesto P. Benito[1] 1) CIALE-UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN; 2) WAGENINGEN UNIVERSITY, WAGENINGEN, THE NETHERLANDS.

Natural populations of *Botrytis cinerea* from vineyards of Castilla y León, Spain, are highly diverse both genetically and physiologically. Evaluation of aggressiveness in a representative sample of field isolates collected from different *Vitis* varieties made it posible to identify isolates displaying large differences in their ability to cause infection. In order to perform a genetic analysis of these differences, crosses between a highly aggressive isolate (448) and a non-pathogenic isolate (371) were performed. In the progeny, a 1:1 segregation was observed for the phenotype “ability to infect”. Remarkably, the “ability to infect” strictly cosegregated with the “capacity to sporulate”. Within the pathogenic progeny large differences in aggressiveness were found, suggesting a key role for a “major pathogenicity gene” essential for the expression of a number of pathogenicity factors which segregate in the progeny. In an attempt to identify the gene responsible, a strategy based on “bulk segregant analysis (BSA)” was designed. First, the sequence of the genomes of both parental strains was determined and a list of SNPs and of small indels was elaborated. Then, two pools of genomic DNA were prepared and sequenced: a first one (P1) from a group of isolates resembling the aggressive parental strain 448, and a second one (P2) from a group of non-aggressive isolates, like parental strain 371. Finally, the distribution in both pools of the polymorphisms found between isolates 448 and 371 was analysed searching for isolate 371 specific variants exclusively found in pool P2 but absent in pool P1 and for isolate 448 specific variants found in pool P1 but absent in pool P2. A candidate region for the major pathogenicity gene to be located in has been identified which is currently being characterized.

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209T Defining the role of heteroresistance in persistent human fungal infections. *I.V. Ene*[1], K. Navarrete[1], E. Fu[1], J.M. Bliss[1,2], A.L. Colombo[3], C. Cuomo[4], R.J. Bennett[1] 1) Molecular Microbiology and Immunology, Brown University, Providence, Rhode Island, USA; 2) Women & Infants Hospital, Providence, Rhode Island, USA; 3) Universidade Federal de São Paulo, São Paulo, Brazil; 4) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

A critical problem in the clinic is the inability of available drugs to clear systemic fungal infections. In some cases, therapeutic failure is due to drug resistance, in which isolates evolve inherent resistance to the drug. However, in many cases persistent infections involve the emergence of tolerant cells (TCs) in the population, a process termed *heteroresistance*. To understand the mechanisms underlying persistence, we performed genome sequencing on a set of *Candida albicans* isolates that were drug susceptible yet persisted during antifungal therapy, and compared this set to isolates that were successfully cleared by drugs. Preliminary analyses indicate that clonal lineages often persisted during the infection, with isolates experiencing large-scale genetic changes including loss of heterozygosity (LOH) across large tracts of the genome. Persistent isolates were less susceptible to drugs and more likely to form TCs compared to isolates that were rapidly cleared from patients upon drug treatment. This implies the existence of a subset of resilient cells with distinct properties from the majority of the cells in the population. TCs formed by persistent isolates grew under much higher drug concentrations than rare TCs formed by cleared isolates. These high resistance levels were gradually lost with growth in the absence of drug, indicating that this phenotype is transient. Similar to bacterial TCs, *C. albicans* TCs have a fitness defect relative to the whole population in the absence of drug, but rapidly outcompete the rest of the population in the presence of drug. To understand how tolerance arises in a population, further genomic and genetic analyses are being performed. This includes screening of a library of all *C. albicans* transcription factors to identify genes that enable TC formation in response to different antifungals. Several gene families modulate tolerance, including telomeric and stress response genes. This study shifts the current paradigm that primary host status dictates persistence, and reveals that intrinsic *C. albicans* properties can also contribute to this important clinical phenomenon.

210F Systematic genetic analysis in the opportunistic yeast *Candida albicans* reveals evolutionary plasticity of cell size homeostasis mechanisms in eukaryotes. *J. Chaillot*, A. Sellam Infectious Diseases Research Centre (CRI), CHU de Québec Research Center (CHUQ), University Laval, Quebec, QC, CANADA.

The basis for commitment to cell division in late G1 phase, called Start in yeast and the Restriction Point in metazoans, is a critical but still poorly understood aspect of eukaryotic cell proliferation. All eukaryotic cells must grow to a critical cell size before commitment to division occurs. This size threshold couples cell growth to division and thereby establishes long-term size homeostasis. To probe the conservation of the extensive size homeostasis network, we performed a quantitative genome-wide analysis of a systematic collection of gene deletion strains in the pathogenic yeast *Candida albicans*. Analysis of the size phenome uncovered 195 genes that markedly altered cell size. Our results revealed an unexpected high degree of divergence between genes that affect size in *C. albicans* versus the yeast model *Saccharomyces cerevisiae*. In addition to conserved size regulators previously identified in *S. cerevisiae* and metazoans, we uncovered previously undocumented regulatory circuits that govern critical cell size at Start. A comprehensive genetic connectivity, using suppressive-

dosage and epistasis genetic interactions, were used to order the size regulatory network in *C. albicans*. This comprehensive analysis revealed a complex network of novel regulator of Start and cell size the most potent of which were the transcription factors Ahr1, Sfp1, Dot6, the AGC kinase Sch9 and the MAPK Hog1. In particular, we delineate a novel stress-independent function of the p38/HOG MAPK network in coupling cell growth to cell division. Our genetic and biochemical analysis suggests that the HOG module directly and interacts with central components of both the cell growth and cell division machineries. Furthermore, we identify a novel size network where the helix-loop-helix transcription factors Dot6 act as a major effector of the TOR pathway to modulate cell size at Start through control of both cytoplasmic and mitochondrial translation. We also showed that the transcription factor Ahr1 that modulates adhesion genes and virulence in *C. albicans*, control cell size of both yeast and filamentous forms by connecting amino acid metabolism with cell cycle commitment decision. This work establishes the first systematic characterization of the mechanisms underlying regulation of growth and division in a pathogenic fungus and illuminate the evolutionary plasticity of the size control network in eukaryotes.

211W Functional characterization of chestnut blight fungus, *Cryphonectria parasitica septin gene*, *CpSep1*. Myeongjin Jo, Yo-Han Ko, Kum-Kang So, Jyotiranjan Bal, *Jeesun Chun*, Dae-Hyuk Kim Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea.

Chestnut blight fungus, *Cryphonectria parasitica*, and its hypovirus present a useful model system for investigating the mechanisms of hypoviral infection. Pathoproteomics analysis revealed a protein spot, further identified as a septin, showing up-regulation in the presence of *Cryphonectria* hypoviruses (CHV1) and tannic acid supplementation. Blast search revealed its homology towards *AspA* gene of *Aspergillus nidulans* (an ortholog of *Saccharomyces cerevisiae CDC11*). In order to analyze the functional role of this septin gene (*CpSep1*), we attempted to construct its null mutant and a total of eight *CpSep1*-null mutants out of 137 transformants were obtained. All *CpSep1*-null mutants showed retarded growth with less aerial mycelia and intense pigmentation on PDAmb plate. When the marginal hyphae were examined, hyperbranching was observed compared to that of the wild-type. However, the inhibition of colonial growth was partially recovered when the *Cpsp1*-null mutants were cultured in the presence of an osmostabilizer. Conidia production of the *CpSep1*-null mutants was significantly i.e., at least ten-fold more increased and electron microscopy suggested abnormal conidia-bearing structure. Interestingly, conidial morphology of the *CpSep1*-null mutants changed to be circular in comparison to the rod-shaped spores of the wild-type indicating a role of septin in spore morphology of *C. parasitica*. However, no difference in the germination process in terms of germ tube number, polarity, and timing was observed. Mycelial growth of the *Cpsep1*-null mutants on the excised chestnut tree bark was severely affected suggesting that the virulence wsas affected due to the fungal growth defect in the host tissue. This study indicated that the *CpSep1* gene is required for an appropriate mycelial growth and pigmentation, osmotic stabilizing, spore morphology, and fungal growth in the host plant, which is consistent with the results of up-regulation by the hypoviral infection and tannic acid supplementation.

212T *Colletotrichum acutatum* species complex genomes reveal signatures of host adaptation in the casual agent of lupin anthracnose. *R. Baroncelli*[1], F. Pensec[1], S.A. Sukno[2], B. Henrissat[3], S. Haridas[4], W. Schackwitz[4], I.V. Grigoriev[4], S. Sreenivasaprasad[5], M.R. Thon[2], G. Le Floch[1] 1) Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (LUBEM), Université de Brest, EA 3882, Brest, France; 2) Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), University of Salamanca, Spain; 3) Aix-Marseille Université, UMR 7257, Marseille, France; 4) US Department of Energy, Joint Genome Institute, Walnut Creek, USA; 5) Institute of Biomedical and Environmental Science and Technology (iBEST), University of Bedfordshire, United Kingdom.

Many species belonging to the genus *Colletotrichum* are associated with plant diseases, commonly referred to as anthracnose. In addition to its economic impact, *Colletotrichum* is one of the most studied genera of plant pathogenic fungi. Closely related species belonging to *Colletotrichum acutatum* species complex (CAsc) can be pathogens of a wide range of hosts or can show a high level of host specialization. *C. lupini* is a species within the CAsc and it is the major threat of its only host *Lupinus* spp. We sequenced and annotated the genomes of two *C. lupini* reference genomes and resequenced three isolates along with twelve closely related CA species characterized by a polyphagous lifestyle. The genome sequences were used to perform a comparative analysis with the aim of exploring genomic signatures associated with host preference in *C. lupini*. Whole genome alignments revealed that only 1.766% of *C. lupini* nuclear genome is species specific (SS) and 0.035% is isolate specific. 117 genes make up the SS portion and only 7 of those have been predicted to be secreted. Manual inspection revealed that among the seven predicted secreted proteins, four do not have similarity with any another sequence in databases, one has similarity with other *Colletotrichum* proteins with unknown functions, one is a metalloproteinase and one is a necrosis and ethylene inducing protein.

Hierarchical clustering of gene family and functional domain assignments revealed expansions of pathogenicity related genes such as necrosis and ethylene-inducing proteins (NEPs) and proteins characterized by CFEM domain (fungal specific cysteine rich domain) and other families with unknown function. We also found contractions in the CAZy repertoire (mainly glycoside hydrolases such as GH43 and GH3), transcription factors and in several clusters conserved within the genus of unkown functions and lacking conserved functional domains. We provide a first look at the adaptations in gene content that are associated with host specialization in *Colletotrichum* spp. This study also illustrates the plasticity of *Colletotrichum* genomes, and shows that relatively recent changes in gene content are associated with major changes in host range. This study also demonstrates the need for higher resolution taxonomic sampling in order to better understand the role of gene gain and loss in the evolution of fungal genomes and the possible association with fungal adaptation.

213F *Cryptococcus neoformans* genomics influence human survival and immunologic responses. *Aleeza Gerstein*[1], David Boulware[1], David Meya[2,3], Kirsten Nielsen[1] 1) Department of Microbiology & Immunology, University of Minnesota, Minneapolis, MN; 2) Infectious Diseases Institute, Makerere University, Kampala, Uganda; 3) Makerere University College of Health Sciences, School of Medicine, Department of Medicine, Kampala, Uganda.

Patient outcomes are due to a complex interplay between the quality of medical care, the host immunity factors, and the infecting pathogen's characteristics. Pathogen burden is clearly associated with mortality risk, but other pathogen characteristics, such as genotype and phenotype, have not been fully explored. A number of studies have recently suggested that pathogen genotype may directly influence host survival, even with tremendous variability of other host factors. To further probe this connection, we examined *Cryptococcus neoformans* genotypes from Ugandan clinical isolates collected as part of the Cryptococcal Optimal ART Timing (COAT) trial to examine whether there was a genomic signature in the pathogen associated with patient survival and/or immune response. We focused on

ST93, the most frequently observed sequence type (ST) among the isolates, and performed whole-genome sequencing on 41 ST93 isolates, acquired from 22 survivors and 19 from patients who died. Sixteen other isolates from a diversity of ST groups were also sequenced for comparison. We identified 131754 SNPs and 16924 small indels among all isolates, with ~40% in at least one ST93 genome. We used the variants to construct a phylogenetic tree, and surprisingly found that ST93 isolates grouped into two separate clades (clade A and clade B). We focused our analyses on the 219 SNPs and 58 indels that were variable among the ST93 genomes, were unassociated with a centromere, and were predicted to have a fitness effect. Approximately half of these variants deliniated ST93 clade A isolates (123 SNPs and 23 indels). Importantly, a number of these variants were significantly associated with decreased 26-week survival (clade A: 29% vs clade B: 63%, 17 SNPs), decreased CD4 T cell counts nadir (a two base pair deletion), and altered CSF cytokines: a six base pair insertion associated with increased levels of pro-inflammatory interleukin (IL)-6, and a ten base pair deletion associated with increased protective Th1 response of IL-12 and increased protective Th2 response of IL-13. Additional non-clade specific SNPs (n=26) were also each significantly associated with at least two immunologic trait responses. This work demonstrates that fungal genotype may significantly influence human immune responses and survival and provides important candidate genes for future studies on virulence-associated traits in *Cryptococcus*.

214W Genome of the obligately alkaliphilic fungus *Sodiomyces alkalinus* reveals its adaptations to high pH. *A.A. Grum-Grzhimaylo*[1], D.L. Falkoski[2], J. van den Heuvel[1], B. Min[3,4], I-G. Choi[3,4], B. Henrissat[5,6], H.J. Franssen[7], E.N. Bilanenko[8], R.P. de Vries[2,9], J.A.L. van Kan[10], I.V. Grigoriev[3], A.J.M. Debets[1] 1) Laboratory of Genetics, Wageningen University, Wageningen, NL; 2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, NL; 3) DOE Joint Genome Institute, Walnut Creek, CA, USA; 4) Computational & Synthetic Biology Laboratory, College of Life Sciences & Biotechnology, Korea University, Anam-dong, Seongbuk-gu, Seoul, South Korea; 5) CNRS, Aix-Marseille Université, AFMB, Marseille, France; Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Marseille, France; 6) Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; 7) Laboratory of Molecular Biology, Wageningen University, Wageningen, NL; 8) Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia; 9) Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, NL; 10) Laboratory of Phytopathology, Wageningen University, Wageningen, NL.

Alkaliphilic fungi, i.e. fungi that grow optimally at high pH, are exceptional with only a handful of species described to date. Here, we sequenced the obligate alkaliphilic ascomycete *S. alkalinus* isolated from alkaline soda soils, and explored the features responsible for its unusual life-style. We found that PacC, the major regulator of alkaline-related genes, is up-regulated at higher pH values than PacC orthologues of neutrophilic species. To assess how this fungus can degrade carbohydrate sources under extremely alkalic conditions, we searched for the CAZymes encoded in the genome of *S. alkalinus* and performed enzyme assays. We revealed many cellulases, but their overall activity was low presumably because of slower induction. Although cellulolytic and hemicellulolytic activity was optimal at pH 6, there still was some activity at pH 10, at which pH it was completely absent in the neutrophilic *A. oryzae*. Excellent *in vitro* growth on xylan indicates that grasses are the preferred nutritional source for *S. alkalinus* in nature. We detected potent proteolytic activity at alkaline pH, compared to the neutrophilic *A. oryzae*, which may reflect the need for obtaining extra nitrogen, as this essential element becomes limiting at alkaline conditions. Proteins of bacterial cells, which are present in bulk at soda lakes, seem the likely source of nitrogen. In support of this hypothesis, we found several instances of horizontal transfer of prokaryotic genes into the genome of *S. alkalinus*, encoding enzymes that degrade bacterial cell walls. The genome of *S. alkalinus* will provide a valuable source to further study the biology and evolution of alkaliphilic trait in fungi with respect to neutrophilic species. In addition, it may provide alkaline-active metabolites of commercial interest.

215T Investigating outcomes of fungal interactions during wood decay. G. Powell[1], L. Boddy[2], E. Dudley[3], J. Hiscox[2], S.C. Moody[1], M. Savoury[2], *D.C. Eastwood*[1] 1) Department of Biosciences, Swansea University, Swansea. GB; 2) School of Biosciences, Cardiff University, Cardiff. GB; 3) School of Medicine, Swansea University, Swansea. GB.

Wood decomposition in natural habitats is achieved by a succession of interacting decay species. Species occupy niches which may be loosely defined as primary, secondary, tertiary and quaternary decay depending upon the amount of decomposition of the substrate. Evidence of priority effects have been described, but the process of succession within natural communities is not well understood. Interactions between species within or between decay niches can vary from replacement to deadlock, or mutual replacement, and outcomes can be affected by the nature of the substrate, temperature and water and nutrient availability. Here we report ongoing research on the competitive interaction between wood decay fungi from different decay niches using wood block-based experiments. Transcriptomic and proteomic data is used to analyse the outcomes of different decay scenarios to gain insight into the mechanisms regulating competitive interactions and ultimately start to understand how natural communities develop over time.

216F Fungal gene clusters adapted to plant secondary metabolites. *E. Gluck-Thaler*, V. Vijayakumar, J. Slot Department Plant Pathology, The Ohio State University, Columbus, OH.

In order to successfully colonize plant hosts, fungal saprophytes, symbionts and pathogens often require metabolic capabilities to degrade plant secondary metabolites and/or mitigate their fungitoxic effects. Degradative metabolic pathways are occasionally maintained in gene clusters encoding all of the necessary enzymes, transporters and regulators required for the metabolic pathway to function. We recently identified a type of fungal gene cluster that appears to encode the degradation of stilbenes, a diverse group of plant secondary metabolites that play an important role in the constitutive and induced chemical defenses of plants. This cluster contains a gene encoding stilbene dioxygenase (*sdo*), which is known to degrade stilbene compounds, along with other uncharacterized genes. Using a novel algorithm that detects unexpectedly conserved synteny, we further explored the diversity of *sdo*-containing clusters and identified three new and differently composed cluster types distributed across 78 diverse species. In order to determine whether metabolic pathways encoded by these different clusters are adapted to specific plant metabolites, we simultaneously investigated the substrate specificity of different *sdo* enzymes using *in vitro* assays as well as the evolution and ecological associations of the various cluster types. Given the ecological distribution of *sdo* cluster types, we hypothesized that each cluster type is specialized for the degradation of ecology-specific stilbene molecules. The substrate specificity of four different *sdo* enzymes from the plant pathogens *Magnaporthe oryzae* and *Sphaeropsis sapinea*, the dung decayer *Podospora anserina* and the saprotroph *Penicilium roquefortii* is conserved, providing no evidence of enzyme specialization according to cluster type or ecology. Ancestral state reconstructions of cluster composition, on the other hand, suggest that shifts in fungal

ecology are associated with the overall composition of clusters and the acquisition of clusters by horizontal gene transfer. The association of distinct cluster types but not *sdo* substrate specificity with ecological transitions suggests that processes driving fungal adaptations to plant chemical defenses may act at the level of genome organization, rather than at the level of individual gene function.

217W Mycorrhizosphere interactions: From genes to proteome to volatilome. *E. Kothe*, C Henke, D Sammer, K Wagner, K Krause Dept Microbiol, Microbial Communication, Friedrich Schiller University, Jena, DE.

Ectomycorrhiza establishes a mutual symbiosis that stabilized forest ecosystems. The mycorrhizosphere community can modulate this interaction by interfering with the signaling between both partners. We investigated the association of *Tricholoma vaccinum* with its specific host, *Pinus sylvestris*, addressing the fungal secretome and volatilome specifically. The genome sequence of *Tricholoma* revealed 11,981 genes coding for proteins, 297 of which with prediction to be excreted. Of these, 26 could be verified by 2D gel electrophoresis, with seven specifically up-regulated after addition of spruce root exudates. The fungal signaling components revealed formation of the plant hormone indole acetic acid (IAA) that induced short root formation in the tree and increased branching in the fungus, thereby allowing for the formation of Hartig' net for nutrient exchange. The influence of the mycorrhizosphere was dissected from microbiome analyses, and a zygomycete-derived compound was found to induce the specific transport of IAA from *Tricholoma* cells. All in all, the tripartite interaction between tree, ectomycorrhizal fungus and the mycorrhizosphere community could be shown to regulate ectomycorrhizal formation through specific exchange of signal molecules. Transformants over-expressing a gene involved in fungal IAA synthesis verified the hypothesis of increased Hartig' net formation. A change in the volatilome of ectomycorrhizal, stressed trees was observed compared to non-stressed trees. This supports the role of mycorrhiza in stabilizing the ecosystem.

218T Rise to the bait: Towards identifying the *Puccinia graminis* effector Avr4/5 "baited in" by the Rpg5 protein kinase integrated decoy domain. *R. Sharma Poudel*, S. Solanki, S. Shrestha, J. Richards, R. Brueggeman Department of Plant Pathology, North Dakota State University, Fargo, ND.

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a threat to wheat and barley production with virulent races posing a threat to world food security. The barley *rpg4*/*Rpg5* stem rust resistance locus (*rpg4/5*) locus confers resistance against the majority of the prevalent *Pgt* races, including the recently emerged highly virulent race TTKSK (A.K.A Ug99) and its lineage. The positional cloning of rpg4/5 locus showed that three tightly linked genes, two NBS-LRR (NLR) resistance-like genes, *Rpg5* and *HvRga1*, and the actin depolymerization factor *HvAdf3* are required together to confer resistance. *Rpg5* is the functional resistance gene at this locus encoding a typical NLR with a predicted C-terminal protein kinase (PK) decoy domain. The *rpg4/5* genomic architecture and predicted protein domain function support the integrated decoy domain model. However, the experimental validation of this hypothesis is hampered without identification of the corresponding *Pgt* Avr4/5 effector. This research aims to identify and validate *Pgt Avr4/5* by utilizing diverse *Pgt* isolates collected in North Dakota since 1970. Thirty-seven *Pgt* isolates showing a differential response on barley genotype with *rpg4/5* were genotyped utilizing Restriction site Associated DNA-Genotyping by Sequencing to identify 24 diverse isolates (9 avrRpg4/5 and 15 AvrRpg4/5). *In planta* RNAseq was conducted by collecting infected tissue from the susceptible barley variety Harrington, 5 DPI (days post inoculation) infected with the 24 *Pgt* isolates. Association mapping conducted using SNPs identified from the RNAseq showed no significant association. However, eleven candidate secreted effector proteins (CSEPs) showing differential presence/absence polymorphism correlating with avrrpg4/5 and AvrRpg4/5 isolates were identified. These eleven *Avr4/5* CSEPs are being transformed into *Pichia pastoris* for protein expression and infiltration in barley *rpg4/5*+ and *rpg4/5*- genotypes. These CSEPs are also being tested in Yeast two hybrid interaction with the Rpg5 PK domain as bait. A mutation approach involving gamma irradiation (1000Gray) of seven AvrRpg4/5 isolates to induce virulence by mutating the *Avr4/5* gene in the avirulent isolates is also being utilized towards Avr4/5 identification.

219F Mining effectors in *Phyllosticta citricarpa*. *Paulo Santos*[1,2], Carol Rodrigues[2], Tiago Oliveira[1,2], Marco Takita[2], Chirlei Glienke[3], Marcos Machado[2] 1) Universidade Estadual de Campinas, Campinas, São Paulo, Brasil; 2) Centro de Citricultura Sylvio Moreira, Cordeirópolis, São Paulo, Brasil; 3) Universidade Federal Do Paraná, Curitiba, Paraná, Brasil.

Citrus black spot (CBS) is one of the most devastating disease of citrus. Caused by the *Phyllosticta citricarpa* (McAlp. Van de Aa) the disease affects all citrus species of economic importance, and its symptoms are marked by dark spots on the rinds of fruits, rendering to their depreciation, early fruit drop and reduced crop yield. The molecular mechanisms under the interaction between *P. citricarpa* and citrus remain elusive. We hypothesize citrus plants might recognize effector proteins of *P. citricarpa,* leading to hypersensitivity responses on infected tissues. In order to gain insight into the molecular basis of CBS pathosystem, we obtained the genome sequence using Illumina platform HiSeq2000 and further *de novo* assembly using CLC Genomics Workbench software. For predicting ORFs we used Augustus software. Annotation and categorization of ORFs were performed automatically in Blast2GO. Data from the sequenced genome generated 179,880,616 reads resulting in 19,143 contigs after assembly with 16,267 ORFs and an estimated genome size of 32 Mb. In order to predict candidate effectors in the genome of *P. citricarpa,* we developed a bioinformatics pipeline*,* taking into account common features found in pathogen effectors. Among the criteria established in our pipeline included: presence of secretion signal, absence of transmembrane anchors, functional domains, presence of nuclear localization signals, and other general structural features. We found 334 candidates secreted effector proteins (CSEP) out of 16.217 total evaluated proteins and selected 15 for further analysis. In order to evaluate the gene expression of CSEP, we established contrasting fungal growth conditions, by supplementing M1 medium with either phenolic compounds or albedo extract of sweet orange (susceptible) or albedo extract of Tahiti lime (resistant), compared to the control; only the medium. We observed that seven genes had their expression induced in the supplemented treatments. Considering these findings, we believe that compounds present in the supplements trigger the upregulation of CSEP genes, which might be recognized by the plant.
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220W Functional suppression of B. napus R genes; a new trick by L. maculans to avoid host defense responses. K. Ghanbarnia[1,2], N. Larkan[3], M. Lisong[1], P. Haddadi[1], W.G.D. Fernando[2], *M.H. Borhan*[1] 1) Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada; 2) Dept. of Plant Science, 222 Agriculture Building, 66 Dafoe Road, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada; 3) Armatus Genetics Inc., Saskatoon, SK, Canada.

The ascomycete fungus Leptosphaeria maculans (Lm) is the causal agent of economically important blackleg disease of canola (Brassica

napus). B. napus immunity to Lm is largely governed by race-specific resistance (R) genes that mediate the recognition of corresponding Lm effector proteins encoded by Lm avirulence (Avr) genes. Two B. napus R genes, LepR3 and Rlm2 and seven Lm Avr genes have been cloned including, AvrLm1 and AvrLm2 that convey avirulence towards LepR3 and Rlm2, respectively. The majority of predicted and all cloned Lm Avr genes are located in repeat-rich regions of the genome, which promotes virulence via mutation or complete deletion of Avr genes. Another mechanism for evolving virulence is by suppression of host R gene response, as was recently reported for AvrLm3, in which its recognition by B. napus Rlm3 is masked in the presence of another Lm effector, AvrLm4-7. Here we provide an additional example of this epistatic suppression of Avr recognition. We took a map based cloning approach to identify the Lm avirulence gene AvrLm9, which encodes a typical small cysteine-rich secreted effector protein. However, when AvrLm4-7 is present Lm is able to avoid Rlm9-mediated recognition of AvrLm9. Hypotheses will be presented for the possible mechanism of suppression of the Rlm3 and Rlm9 defense responses by AvrLm4-7.

221T Comparative evolutionary histories of fungal proteases reveal gene gains in mycoparasitic and nematode-parasitizing fungi. *M. Iqbal*[1], M. Dubey[1], M. Viketoft[2], D.F. Jensen[1], M. Karlsson[1] 1) Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-75007, Uppsala, Sweden; 2) Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, SE-75007, Uppsala, Sweden.

Clonostachys rosea [Bionectriaceae (Hypocreales, Ascomycota)] is a biological control fungus that can control several important plant diseases caused by plant pathogenic fungi and nematodes. In this study, we analysed the evolutionary history of protease gene families in 13 hypocrealean fungi, and *Neurospora crassa*. Proteases were classified into subgroups based on the MEROPS peptidase database. The highest number of protease genes (601) was found in *Fusarium solani*, followed by *C. rosea* with 589 genes. A computational analysis for gene family evolution (using CAFE version 3) identified the five serine protease gene families S08A, S09X, S10, S12 and S33 to be accelerated in gene gain or loss. S09A serine protease gene numbers were specifically increased in *C. rosea*, *Trichoderma virens* and *Hirsutella minnesotensis*, all reported to have the ability to parasitize nematodes. The mycoparasitic species *C. rosea*, *T. atroviride* and *T. virens* contained high gene numbers of S08A and S33 serine proteases. These results suggest that serine proteases contribute to the broad environmental opportunism displayed by *C. rosea* and certain *Trichoderma* spp.

222F *In silico* characterisation of Endo-polygalacturonase in members of the Agaricales. L. Potgieter, B.D. Wingfield, M.A. van der Nest, *M.P.A. Coetzee* Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Endo-polygalacturonases (endo-PGs) are cell wall degrading enzymes responsible for the degradation of homogalacturonan chains in pectin. These enzymes are important in fungal host infection as they cause tissue maceration that enables the fungi to infect and subsequently colonise plant hosts. Very little is known regarding their characteristics in member species of the Agaricales. The aim of this study was thus, to identify, characterize and study the distribution and evolution of putative endo-PGs from 54 species of the Agaricales for which whole genome sequences are publicly available. Using amino acid similarity and keyword searches, endo-PG genes were identified in most of the genomes. Many of the genes are present as multi copies. In general, saprotrophs, dung decomposing, litter decaying fungi, and mycorrhizal fungi harbored fewer gene copies than pathogens and wood rot fungi. This is expected, as these genes play an important role in host infection and colonizing. Functional prediction showed that the genomes harbor endo-PG isoforms active at different pH, ranging between 3.55 and 9.72 (average: 5.37). All the genomes in which endo-PGs genes were identified, contained endo-PGs predicted to be optimally active at an acidic pH, while genes predicted to encode basic endo-PGs were not present in all the genomes. Endo-PG isoforms active at different pH allow for activity and substrate access at variable ambient pH created by oxalic acid during pathogenesis. The number of introns varied greatly among the endo-PG genes identified in the different species. In comparison to species from other orders, endo-PG genes within the Agaricales contained, on average, four times more introns and the introns are at relatively conserved positions. This suggests that the ancestral endoPG gene in the Agaricales contained several introns prior to gene duplications. Overall, results of this study suggest that gene duplication followed by gene loss and/or gene expansion played an important role in the distribution and evolution of endo-PGs in the Agaricales.

223W Comparative Genomic Analyses of Two Pyrophilous Fungi. *A. Carver*[1,3], K. Stillman[1], H. Liu[1], S. Branco[2], A. Kuo[3], T. Bruns[1], I. Grigoriev[1,3] 1) Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Laboratoire Ecologie, Systématique et Evolution, Un Paris Sud, CNRS-UPS-AgroParisTech, Orsay France; 3) Joint Genome Institute, Lawrence Berkeley National Laboratory, Walnut Creek, CA.

Pyrophilous fungi are filamentous fungi that have historically been found fruiting exclusively on burned soil. Forest soil changes greatly after fire, with an enrichment of dead biomass, partially pyrolyzed carbon compounds and increased hydrophobicity. The fungal species that inhabit such soil comprise a diverse guild, though most are Ascomycota in the order Pezizales and most of these are in the family Pyronemataceae. To better understand the role of pyrophilous fungi in post-fire soil, we compare the genomes of two pyrophilous fungi of the Pyronemataceae family, *Pyronema omphalodes* and *Wilcoxina mikolae,* to 12 non-pyrophilous Pezizales species. Although *P. omphalodes* and *W. mikolae* have otherwise distinct lifestyles as saprotrophs or as ectomycorrhizae respectively, both are dominant in forest soil communities after fires. They share an expansion in a novel chitin-binding gene family that is unique amongst sequenced fungi and may be horizontally transferred, and both genomes contain glycoside hydrolases that are absent in other sequenced Pezizomycetes species. In addition, *P. omphalodes* has an expansion in heat shock proteins over other sequenced Ascomycetes, which may assist in its colonization of burned forest soil. The information from this study will increase our understanding of the lifestyles and metabolic processes of pyrophilous fungi and their role in post-fire carbon cycling.

224T Patterns of genome evolution across the fungal kingdom. Michiel Konings, *Robin Ohm* Microbiology, Utrecht University, Utrecht, NL.

A comparative genomics analysis of over 200 fungal genomes has revealed large differences in genome evolution across the fungal kingdom. Driving forces behind these differences are variable rates of interchromosomal rearrangements and variable inversion lengths in intrachromosomal rearrangements. Each class of fungi follows its own pattern of genome evolution.

For example, it was previously shown in the class Dothideomycetes (phylum Ascomycota) that a low rate of interchromosomal

rearrangements and high rate of intrachromosomal rearrangements leads to mesosyntheny, where gene content but not gene order is conserved. In the class Saccharomycetes (phyum Ascomycota), in contrast, interchromosomal rearrangements occur frequently, quickly degrading conserved syntheny. A similar pattern of genome evolution occurs in the class Agaricomycetes (phylum Basidiomycota) as in Dothideomycetes, with the important difference that the intrachromosomal inversion length is much smaller. This results in a distinct pattern only found in this class. Moreover, in Agaricomycetes several conserved ancestral chromosomes could be identified with varying patterns of genome evolution, possibly due to their difference in sequence composition. On gene level, conserved gene clusters were identified despite the observed intrachromosomal inversions. Notably, certain transcription factors were over-represented in these conserved gene clusters.

225F Early origins of fungal multicellularity-related genes in eukaryotic evolution. *E. Kiss*, A. Prasanna N, K. Krizsan, L. G. Nagy Synthetic and Systems Biology Unit, Biological Research Centre, Szeged, HU.

The emergence of multicellular organisms from single-celled ancestors is considered one of the major transitions in the history of life. It has been suggested that genes underlying several multicellularity-related traits, like cell adhesion, cell-cell communication and programmed cell death already existed in unicellular organisms. These traits might have predisposed them for evolving multicellularity, consistent with the multiple origins of multicellularity in many groups, including fungi. However, so far there have not been detailed studies on the evolution of multicellularity-related gene families in Fungi, where multicellularity has different physiological bases from that of other groups.
We obtained information on the genetic background of fungal multicellularity by collecting known and putative multicellularity-related genes from the literature. We identified 995 such genes, involved in signal transduction, gene expression regulation, adhesion and hyphal morphogenesis. The evolutionary origins of these genes were examined using complete genomes of 77 unicellular and multicellular eukaryotes. Through similarity based clustering we found that the 996 genes grouped into 580 clusters of homologues. We next performed phylostratigraphic analysis on these gene clusters using a custom pipeline to uncover the evolutionary origins of their gene families and reconstructed gene duplication/loss histories in 525 clusters using reconciled gene trees, which gave a high-resolution view on the dynamics of these putatively multicellularity-related gene families over time.
The analyses of gene families revealed that part of the genetic toolkit behind fungal multicellularity was already present in ancestral unicellular eukaryotes and we found that several multicellularity related gene families started diversifying before the emergence of multicellular fungi. These observations might explain the convergent evolution of hyphae in early-diverging groups. Further, our results suggest a role of gene regulatory network rewiring in addition to *de novo* gene family birth and gene duplications. Taken together, these results suggest that unicellular fungal ancestors were partially predisposed for evolving multicellularity, which, probably combined with lineage-specific genetic innovations, gave rise to extant filamentous fungi.

226W *Microbotryum*-mediated sex conversion of *Silene latifolia*. *A. Harkess*[1], P. Baldrich[1], L. Delph[2], M. Perlin[3], B. Meyers[1] 1) Donald Danforth Plant Science Center, St. Louis MO 63132; 2) Indiana University, Bloomington IN 47405; 3) University of Louisville, Louisville KY 40292.

More than a century ago, Strasburger (1900) observed that *Microbotryum lychnidis-dioicae* (formerly *Ustilago violacea*) was able to infect *Silene latifolia* flowers and lead to sexual conversions. This peculiar fungal parasitism can cause *S. latifolia* XX females to produce rudimentary anthers inside which the fungus propagates, but interestingly the infection of XY male plants does not produce female organs. Given that our understanding of the molecular basis of sex determination on the *S. latifolia* X and Y chromosomes is incomplete, this parasitism could be a key to identifying the master sex determination genes on the *Silene* XY sex chromosome pair. To understand the molecular mechanisms of this sexual transition, we are sequencing mRNA, small RNA, and PARE ("degradome") libraries from pre- and post-infection *S. latifolia* flowers, as well as attempting genetic transformation of both species for Cas9-based genome editing. Given that there may exist species co-diversification of both fungus and host, we hypothesize that the transition from hermaphroditism (self-compatibility) to dioecy (outcrossing) in *S. latifolia* may have been spurred by a plant-fungus evolutionary "arms race".

227T Flip/flop mating-type switching in the methylotrophic yeast Ogataea polymorpha is regulated by an Efg1-Rme1-Ste12 pathway. *S.J. Hanson*[1,2], K.P. Byrne[2], K.H. Wolfe[2] 1) Molecular Biology, Colorado College, Colorado Springs, CO; 2) UCD Conway Institute, School of Medicine, University College Dublin, Dublin 4, Ireland.

In *Ogataea (Hansenula) polymorpha* an environmental signal, nitrogen starvation, induces a reversible change in the physical structure of a chromosome. This process, mating-type switching, involves inverting a 19-kb DNA region to place either *MAT*a or *MAT*alpha genes under centromeric repression of transcription, depending on the orientation of the region. Here, we investigated the genetic pathway that controls switching. We first characterized the transcriptomes of haploid and diploid *O. polymorpha* by RNAseq in rich and nitrogen-deficient media, and found that there are no constitutively a-specific or alpha-specific genes other than the *MAT* genes themselves. We identified a switching defect in a related species (*O. parapolymorpha* strain DL-1) and mapped the defect by interspecies bulk segregant analysis to a frameshift in the transcription factor *EFG1*, which in *Candida albicans* regulates filamentous growth and white/opaque switching. By gene knockout, overexpression and ChIPseq experiments we established that *EFG1* regulates *RME1*, which in turn regulates *STE12*. All three genes are necessary for mating-type switching in response to nitrogen starvation, as well as for mating. Overexpression of *RME1* or *STE12* alone is sufficient to induce switching without a nitrogen signal. Our results show that the pathway controlling switching in *O. polymorpha* is substantially different from that in *S. cerevisiae*, which does not involve an environmental signal, and that it shares some components with mating-type switching in *Kluyveromyces lactis* and with white/opaque phenotypic switching in *C. albicans.* The downstream mechanism by which the chromosomal inversion occurs remains unidentified.

228F Decoding the pathogenicity of Armillaria species through phylogenomics and transcriptomics. *A. Prasanna N*[1], G. Sipos[3], B. Kiss[1], B. Bálint[2], K. Krizsán[1], I. Nagy[2], L. G. Nagy[1] 1) Synthetic and Systems Biology Unit, BRC, Szeged, Hungary; 2) Seqomics Ltd, Mórahalom, Hungary; 3) Institute of Silviculture and Forest Protection, University of West-Hungary, Sopron, Hungary.

The genus *Armillaria* is a group of pathogenic white rot fungi, imparting significant economic and environmental losses worldwide. They cause root rot disease and propagate as rhizomorphs and infect plants through direct root contact, a unique dispersal mechanism only found in *Armillaria* spp. Here, our aim is to understand genome evolution in Armillaria spp and identify the factors involved in their pathogenicity. For this purpose, we reconstructed genome evolution across 5 *Armillaria* and 22 brown rot, white rot, litter decomposer and

ECM fungi, and analyzed transcriptomes from rhizomorphs, different developmental stages of *Armillaria ostoyae C18*.

We detected a significant genome expansion in the *Armillaria* genus, which comprised 2012 gene duplications affecting several families with pathogen-specific Pfam signatures. Next, we performed a domain based copy number analysis of plant cell wall degrading enzymes (PCWDE) and putative pathogenicity-related genes. Compared to non-pathogens, pathogenic *Armillaria* spp showed higher copy numbers of several putative pathogenicity factors and PCWDE's including expansins, hydrophobins, Trp-halogenases, secondary metabolite genes, genes with chitin bidning ability (LysM), chitosanase (GH75), pectin lyases, pectin methyltransferase, class II peroxidases and cellular transport and detoxification pathway components (p450, MFS_1).

Transcriptome analyses indicated that expression patterns of rhizomorphs are intermediate between fruiting bodies and vegetative mycelia, suggesting a link between fruiting body and rhizomorph development. Several putative pathogenicity genes, mannosyltransferases, hydrophobins, and pore-forming toxin genes were upregulated, indicating exploratory, nutrient uptake, defense and fruiting body-like functionalities for rhizomorphs. Taken together, our results suggest that pathogenic Armillaria species have significantly expanded their putative pathogenicity and PCWDE gene artillery through lineage-specific genetic innovations over the course of evolution, which might explain their saprotrophic and pathogenic potentials worldwide.

229W Evolutionary strata on young mating-type chromosomes despite lack of sexual antagonism. *S. Branco*[1], H. Badouin[1,2], J. Gouzy[2], R. Rodriguez de la Vega[1], G. Aguiletta[1], S. Siguenza[2], J.-T. Brandenbourg[1], M. Coelho[3], M. Hood[4], T. Giraud[1] 1) Laboratoire Ecologie, Systématique et Evolution, Universite de Paris Sud, Orsay, FR; 2) LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, FR; 3) UCIBIO-REQUIMTE, Dept. Ciências Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, PT; 4) Department of Biology, Amherst College, Amherst, MS.

Sex chromosomes often show exceptional features such as successive steps of suppressed recombination (called "evolutionary strata"), structural dimorphism and degeneration. The current theory for explaining these patterns is a successive linkage of multiple sexually-antagonistic genes to sex-determining genes. However, little evidence supports this explanation and alternative hypotheses have been proposed. Fungi are interesting models to test this theory as there is no male/female role associated with their mating-type chromosomes while they can display extensive suppressed recombination. Here we report the first compelling evidence for multiple evolutionary strata in fungal mating-type chromosomes, by comparing nearly finished genome assemblies of closely related anther-smut fungi. The existence of evolutionary strata in these fungi despite the lack of sexual antagonism calls for a unified theory of sex-related chromosome evolution, incorporating more general hypotheses, such as the sheltering of deleterious alleles or balancing selection maintaining rearrangements across longer evolutionary times than on autosomes.

230T What drives speciation? Examination into the evolutionary events of more than 100 *Aspergillus* species. *J.L. Nybo*[1], T.C. Vesth[1], S. Theobald[1], I. Kjaerboelling[1], J.C. Frisvad[1], T.O. Larsen[1], R. Riley[2], A. Salamov[2], I.V. Grigoriev[2], S.E. Baker[3], M.R. Andersen[1] 1) Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, DK; 2) Joint Genome Institute, Walnut Creek, CA, USA; 3) Joint Bioenergy Institute, Berkeley, CA, USA.

The study of speciation - how new species arise, diverge and remain separate, has a central role in evolutionary biology. Partly because it embraces so many disciplines, including population genetics, behavioral sciences, comparative genomics, evolutionary biology, biodiversity, biogeography and ecology. It also remains one of the most fascinating questions in evolution.

Speciation is nearly impossible to study and in most cases, we know very little about the genetic basis of species formation. But in this project we aim to identify evolutionary events that can drive speciation, such as gene duplications, creations and losses, and horizontal gene transfers between closely or distantly related species within the genus of the filamentous fungi Aspergillus. This diverse genus holds species relevant to both plant and human pathology, food biotechnology, enzyme and bulk chemical production, model organisms, and it even contains some extremophiles.

To identify these events, we have developed a homologous protein prediction software that has been used to generate a high-resolution pan-genomic map. From where, we have identified genes specific to species, clades and core that allows for guilt-by-association-based mapping of genotype-to-phenotype.

Our results illustrate a highly diverse genus where 500-2000 genes are unique to each species. These genes are predominantly within regulation or compound biosynthesis, supporting the notion of natural selection. A conservative estimate of the number of protein families shared by all Aspergillus species is surprisingly low, only about 2600 core families, suggesting high environmental adaptation within this genus.

231F Phylogenomic analyses of the genetic bases of ballistospory. *M. Sanchez-Garcia*[1], L. Nagy[2], D. Hibbett[1] 1) Biology Department, Clark University, Worcester, MA; 2) Synthetic and Systems Biology Unit, Biological Research Center, HAS, Szeged, Hungary.

Agaricomycetes (mushrooms) with an exposed hymenium have a complex mechanism of forcible spore discharge, called ballistospory. Structural features associated with this mechanism include short and curved sterigmata, spores with a hilar appendage, and production of a droplet of fluid at the base of the spore. Gasteroid forms (such as puffballs) produce spores internally and in most cases have basidia that lack such features and that do not discharge spores forcibly. The mechanism of ballistospory evolved once in the phylum Basidiomycota and has been lost repeatedly during the evolutionary history of this group. It has been suggested that the loss of ballistospory is irreversible. We analyzed 60 genomes of Agaricomycetes including 12 gasteroid forms in order to infer the genetic bases of ballistospory by identifying parallel losses of genes potentially involved in this mechanism. We used a recently developed pipeline called COMPARE (Comparative Phylogenomic Analysis of Trait Evolution) to map gene duplications and losses onto a species tree and to correlate them with the presence of ballistospory.

232W Predicting dispensable chromosomes(scaffolds) in newly sequenced Fusaorium species. *A. Salamov*[1], W. Andreopoulos[1], L-J Ma[2], J Coleman[3], I Grigoriev[1] 1) DOE Joint Genome Insitute, Walnut Creek, CA; 2) Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA, 01003, USA; 3) Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, 36849, USA.

Many Fusarium species have dispensable (or lineage-specific) chromosomes which are not required for normal growth, but may control host-specific pathogenicity of these species. For newly sequenced species from *F.solani* and *F.oxysporum* species complexes we investigate the possibility of predicting dispensable chromosomes (scaffolds) based on genomes sequence features and similarity to two reference genomes of *F.oxysporum f.sp lycopersici* and *Nectria haematococca* with experimentally determined dispensable chromosomes. We have found that when new strains are close enough to the reference genomes, dispensable chromosomes can be reliably predicted based on combination of genome alignments and mapping of proteins from reference genomes to new sequences. We also built Hidden Markov Models for gene families, specific for dispensable chromosomes of reference genomes, which in combination with other features, such as repeat content, sparsity of genes with known functional domains, codon usage, and GC content can be used for prediction of dispensable chromosomes in more distant species.

233T Genomic comparison of a dispensable chromosome within the *Fusarium fujikuroi* complex. L. De Vos[1], *E.T. Steenkamp*[2], M.A. van der Nest[1], W-Y. Chan[2], S. van Wyk[2], B.D. Wingfiled[1] 1) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Species within the *Fusarium fujikuroi* complex (FFC) are considered important pathogens of commercially planted plants and trees. Members of this complex typically have 12 chromosomes, of which the 12th chromosome is the smallest and considered to be part of the accessory genome. This hypothesis is based on low sequence similarity among the corresponding chromosome from different species, and the apparently dispensable nature of this chromosome within the FFC. In this study, we investigated this hypothesis by making use of a comparative genomic approach. For this purpose, whole genome sequence data available for representatives of the FFC (i.e., *F. circinatum*, *F. subglutinans*, *F. nygamai, F. fujikuroi* and *F. temperatum*) were used. From these data, sequences for chromosome 12 of *F. fujikuroi* and *F. temperatum* were used to assemble the corresponding chromosome in the other three species. Chromosome 12 comparisons within the FFC showed that the density and average size of genes encoded on this molecule were lower when compared to whole-genome data. Assignment of gene functions revealed genes primarily involved in oxidation-reduction and macromolecule metabolic processes, cation and nucleoside phosphate binding, and as intrinsic components of membranes. Phylogenetic analyses also showed that the genes of chromosome 12 had diverse and non-orthologous origins, with only a small proportion apparently sharing an evolutionary trajectory similar to those of the species harbouring them. Taken together, these findings therefore suggest that the ancestor of the FFC likely harboured a version of chromosome 12. Also, consistent with being part of the accessory genome, the evolution of chromosome 12 involved the acquisition of genes from sources outside the FFC. Future research should seek to determine the processes encoded by chromosome 12 and the biological properties this molecule might confer to the species harbouring it.

234F Genome mining, pathogenicity and secondary metabolism of three strains of *Fusairum fujikuroi*, the causal agent of bakanae disease on rice. *E. Piombo*[1,2], H. Banani[1,2], I. Siciliano[1], P. Abbruscato[3], A. Acquadro[2], M.L. Gullino[1,2], D. Spadaro[1,2] 1) Universita degli Studi di Torino, Centro AGROINNOVA; 2) Universita degli Studi di Torino, DISAFA; 3) Bioeconomy Unit, Parco Tecnologico Padano.

Bakanae is an important seedborne disease of rice, caused by *Fusarium fujikuroi*. This pathogen can produce a wide range of secondary metabolites, including fumonisins and gibberellins. In order to gain insight into secondary metabolites (SM) synthesis in *F. fujikuroi,* the genomes of the 3 strains *Augusto2*, *CSV1* and *I1.3* were sequenced to identify the allelic variants in the genes responsible for SM production*,* and compare the virulence on rice and the SM production *in vitro* and on rice. Sequence analysis was conducted by *de novo* and *reference guided* genome assembly. Three genomes of 42.8 Mb on average were obtained. The gene clusters responsible for fumonisin and gibberellin production, formed respectiveley by 15 and 7 genes, were analyzed and aminoacidic differences were predicted for *fum1, fum13* and *fum21*. The 3 strains also exhibited distinct differences in colony morphology and growth kinetics. The virulence and fumonisin production of the three strains on cultivar "Galileo" of rice were compared*.* At 3 weeks post germination *I1.3* showed statistically higher virulence compared to *Augusto2* and *CSV1*. *Augusto2* was the major producer of fumonisins both *in vitro* and *in vivo*, followed by *CSV1*, while *I1.3* was unable to produce fumonisins either *in vivo* or *in vitro*. The lack of fumonisin production in *I1.3* is probably caused by a number of identified mutations in the sequence of key genes (*fum1* and *fum21*) in the fumonisin gene cluster, while the difference in the production between *Augusto2* and *CSV1* might be due to a mutation in the gene *VeA*, regulator of SM biosynthesis, including fumonisins. *CSV1* was unable to produce gibberellins *in vivo* and *in vitro* on Petri dish, confirming the different symptomatology of *CSV1* on rice, characterized by dwarfing and chlorosis, but lack of stem elongation. This study showed how *F. fujikuroi* can present a great deal of intra-species variation, both in the induced symptoms and in SM production.

235W Quantitative mapping of pathogenicity factors in the *Fusarium fujikuroi* complex with an interspecific genetic cross. Wei Yue[1], Christopher Toomajian[1], Nik Mohd Izham Mohamed Nor[1,2], *John F. Leslie* [1] 1) Dept Plant Pathology, Kansas State Univ, Manhattan, KS, USA; 2) School of Biological Sciences, Science University of Malaysia, Penang, Malaysia.

The *Fusarium fujikuroi* species complex contains agronomically important plant pathogens that produce secondary metabolites such as mycotoxins and gibberellic acids (GAs) that are involved in pathogen virulence. Two members of this species complex, *F. proliferatum* (*Fp*) and *F. fujikuroi* (*Ff*), are closely related phylogenetically but have different host specificity and produce different mycotoxins. Our long-term aim is to explore the genetic bases behind these differences, and we have begun by conducting QTL analyses of progeny from an interspecific cross. Using the R/qtl software package, we constructed a high density genetic map with data from 6,381 high quality Genotyping-by-Sequencing (GBS) markers and 253 progeny from the cross. The final genetic map consisted of 12 linkage groups, which correspond to the 12 chromosomes present in each species, and had a length of over 1900 cM with only 4 gaps greater than 20 cM. In parallel, we generated next generation sequence data from the *Fp* parent of this cross and performed a draft genome assembly. Our sequence-based markers allowed us to anchor 97.8% (42.2 Mb) of the total assembly onto the 12 linkage groups. Not surprisingly for an

interspecific cross, we observed several from expectations in terms of marker segregation and recombination patterns. Many markers display significantly skewed segregation towards the *Fp* allele, although we also detected a significant skew towards the *Ff* allele in other regions. Using our GBS markers mapped to our *Fp* genome assembly and reference *Ff* genomes, we detected heterogeneity in recombination rates. Though some of this heterogeneity is consistent with recombination patterns observed in other Fusarium species, certain recombination hotspots appeared to be due to shared recombination events among all progeny collected from the same perithecium, while the absence of recombination in other regions helped to confirm inferred chromosomal inversions between the species. We performed QTL mapping of two traits relevant to the pathogenicity of each parent: GA level and pathogenicity to onion. The major peak for onion pathogenicity co-localizes with a known secondary metabolite cluster. In addition to mapping the biosynthetic gene cluster for GA production, we also identified an unlinked modifier locus that interacts epistatically with it. This map provides valuable information for understanding the genetic bases underlying differences between these two closely related *Fusarium* species.

236T Comparative transcriptomics analysis of two different pathotype strains of Fusarium fujikuroi obtained from rice in Korea. Hee-Kyoung Kim, *Da-Woon Kim*, Sung-Hwan Yun Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam, KR.

Fusarium fujikuroi is known to be commonly associated with rice, not only causing bakanae disease, but also producing a group of secondary metabolites, such as gibberellins, bikaverin, fumonisins (FB), and fusarin C. Here, we have focused on two *F. fujikuroi* isolates (B20 and B14) obtained from rice grains in Korea, which show different pathogenesis mechanisms on rice seedling. The B20 strain is able to cause a typical stem elongation in rice seedling, while B14 causes no change in the seedling height; rather it causes a discoloration in root of rice seedling. To characterize the difference in pathogenicity between these two pathotype strains, we performed a RNA-seq analysis using total RNA samples obtained from rice seedlings inoculated with these strains. Compared to those expressed during the vegetative growth on PDA, genes encoding cell wall degrading enzymes, fusaric acid biosynthesis proteins, and proteases were up-expressed on rice (at 3 dpi and 6 dpi stages) from both strains. However, the expression patterns of several gene clusters for secondary metabolites (SM) of these strains were different from each other on rice. The expressions of most gibberellin gene cluster members in B14 were highly induced on rice, whereas those in B20 seemed to be constitutively expressed on both PDA and rice. Interestingly, the entire fumonisin gene cluster members in B14 were highly expressed on both PDA and rice, while six genes in the cluster were missing in B20; furthermore the expression level of the rest members on rice were almost undetectable. In addition, the apicidin F cluster, which showed a rice-specific expression pattern in B20, was not physically present in the genome of B14. Further functional analyses including a targeted gene deletion will elucidate if variations in the expression of SM gene clusters would be responsible for the different pathogenesis mechanisms between these two *F. fujikuroi* strains.

237F Comparative population genomics of *Fusarium graminearum* reveals adaptive divergence among cereal head blight pathogens. *A.C. Kelly*, Todd J. Ward ARS, USDA, Peoria.

In this study we sequenced the genomes of 60 *Fusarium graminearum,* the major fungal pathogen responsible for Fusarium head blight (FHB) in cereal crops world-wide. To investigate adaptive evolution of FHB pathogens, we performed population-level analyses to characterize genomic structure, signatures of natural selection and differences in gene content among isolates. Genome-wide patterns of SNP diversity revealed that most isolates with the novel NX-2 (3α-acetoxy, 7α,15-81 dihydroxy-12,13-epoxytrichothec-9-ene) toxin type represent a genetic population (termed NA3) that is distinct from the native (NA1, largely 15-acetyl-deoxynivalenol toxin type) and invasive (NA2, largely 3-acetyl-deoxynivalenol toxin type) populations inhabiting North America, although genetic exchange among populations was documented. The three populations differed in gene content, with 134 genes showing population-specific patterns of conservation. In addition, each population had unique genetic signatures of adaptive selection that were largely focused in hypervariable regions of chromosomes. Sixteen candidate loci, varying in size from 10-40 kb, showed genetic signals of adaptive divergence, in that alleles were highly differentiated among populations but showed reduced diversity within populations. The strongest signals of selective sweeps were observed at the trichothecene biosynthetic gene cluster. However, functional annotation of population-differentiating genomic regions revealed numerous genes involved in host invasion, toxin production and secondary metabolism, and implicated plant hosts, microbial competitors, and temperature and light as major drivers of adaptive divergence. Collectively, our results show that North American populations of *F. graminearum* harbor unique sets of adaptations that contribute to differences in how these pathogens exploit the agricultural landscape.

238W Functional genomics of lipid accumulation in *Rhodosporidium toruloides.* *Samuel Coradetti*[1,2], Dominic Pinel[2], Gina Geislman[2], Adam Arkin[2], Rachel Brem[1,3], Jeffrey Skerker[2] 1) Buck Institute for Research on Aging, Novato, CA; 2) Department of Bioengineering, University of California, Berkeley, CA; 3) Plant and Microbial Biology, University of California, Berkeley, CA.

Wild isolates of the basidiomycete yeast *Rhodosporidium toruloides* can accumulate more than 70% of their biomass as nuetral lipid in the form of triacylglycerol and produce relatively high concentrations of carotenoids. *R. toruloides* can also utilize a wide spectrum of plant cell wall sugars and lignin derivatives, and has a natural tolerance for common inhibitors in biomass hydrolysates. These properties make *R. toruloides* an attractive host for sustainable production of hydrocarbon chemicals and fuels from low-value feedstocks. While the *R. toruloides* research community is growing rapidly, relatively sparse biochemical data limits current engineering efforts. To rapidly interrogate gene function on a genomic scale, we applied high efficiency *Agrobacterium tumefaciens* mediated transformation to build a 300,000+ member strain library of barcoded random insertions. By tracking the relative abundance of barcoded strains in competition experiments and physical enrichments, we can simultaneously assign phenotypes for deletions of thousands of genes in a given condition. We have used this technique to identify a large cohort of genes (both novel and homologous to well-characterized genes in other systems) with roles in various aspects lipid metabolism including central carbon metabolism, nucleation and maturation of lipid bodies, protein trafficking, and nutrient sensing.

239T Genomic analysis of phenotype reversion in *cuf1-* strains of *Cryptococcus neoformans var neoformans*. Shannon Ellis[2], Morgan Coslett[3], Jocelyn Teagarden[1], Tanner Bross[1], *Jeramia Ory*[1] 1) Department of Basic Sciences, Saint Louis College of Pharmacy, Saint Louis, MO ; 2) Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Department of Biology, King's College, Wilkes-Barre, PA.

Cryptococcus neoformans is a ubiquitous soil fungus that is a major cause of meningitis in immunocompromised patients worldwide. One virulence factor responsible for the pathogenesis of *C. neoformans* is the fungus' ability to produce melanin using a wide variety of environmental substrates. The gene responsible for melanin synthesis, *CNLAC*1, has been identified and shown by others to encode a copper dependent laccase. In addition, proper copper import and processing has been shown by multiple studies to be necessary for pathogenesis. *CUF1* codes for the major copper sensing transcription factor, and controls expression of numerous genes related to copper metabolism. To further explore the role of copper metabolism in cell fitness and pathogenesis, we constructed a *CUF1* deletion strain of *C. neoformans var neoformans* (*cuf1*-). Studies by our lab and others show that the *cuf1*- strain grows quite poorly on Yeast Peptone Dextrose (YPD) media without supplemented copper. To characterize the growth phenotype and identify possible gene regulatory networks, we measured microarray whole genome expression of both wild type (JEC21) and *cuf1*- strains in various copper supplemented conditions. Expression patterns are similar to those found for *C. neoformans var grubii*, but analysis is hindered by metabolic stress found in the *var neoformans cuf1*- strain that is not present in the *var grubii cuf1*- strain. In low copper environments the *var neoformans cuf1*- strain is unable to express copper import genes as expected, but also shows lower levels (compared to wild type) of citric acid cycle and glycogen storage enzymes and higher levels of saccharide processing and breakdown enzymes. The metabolic stress caused by insufficient copper import also appears to impart significant selective pressure. We find the *var neoformans cuf1*- strain repeatedly and stably reverts to fitness levels close to wild type when serially passaged on YPD without supplemented copper. We are currently collecting whole genome short read data on independent revertants, and are analyzing them for mutations or duplication events that would explain the mechanism of reversion.

240F Adaptations of *Exophiala dermatitidis* in stressful environments. *Jyothi Kumar*, Anthony Mosher, Steven D Harris Department of Plant Pathology, Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE.

Fungi are known to inhabit extreme environments that are constantly exposed to high salt, high temperature, cold and desiccation stress. A multitude of factors enable colonization of these environments, including melanization and UV resistance. *Exophiala dermatitidis* is a model black yeast extremophile that causes cutaneous and subcutaneous infections in humans. Studies indicate that it can produce carotenoids, melanins, and oxidative enzymes in response to stress and as a means to survive extreme environments. Our objectives are to investigate the mechanisms that underlie stress resistance in *E. dermatitidis.* In this study, we performed a transcriptomic analysis of *E. dermatitidis* using RNA sequencing (RNA-seq) to investigate the role of the conserved GTPases Cdc42 and Rac1 in stress tolerance. Differential expression analyses revealed important genes / processes involved in light responses, carotenoid synthesis, oxidative stress, heat shock proteins and MAPK signaling pathways. Transcription factors like RLM1, SMP1, PHO2 involved in the regulation of melanin synthesis, the HOG1 pathway and phosphate synthesis were also modulated. *E. dermatitidis* requires photoinduction for carotenogenesis and has an intriguing set of light sensing genes. We studied the role of light in regulating pigmentation by phenotypic and expression profiling of genes involved. Our results show that light and maltose repress the morphological transition from yeast to filamentous form and CDC42/Rac1 module is a positive regulator for the switch from yeast to hyphal form.

241W Improved genome sequence of Diplodia seriata, a tool for comparative genomics of Diplodia species. *G. Robert*[1], J. Vallet[2], E. Abou-Mansour[3], J. Xu[4], P. Rey[5], C. Bertsch[6], C. Rego[7], P. Larignon[8], F. Fontaine[2], MH. Lebrun[1] 1) BIOGER, INRA, Thiverval-Grignon, FRUMR 1290 BIOGER, INRA, AgroParisTech, Campus AgroParistech, Ave Bretignières, 78850, Thiverval-Grignon, France; 2) Université de Reims Champagne-Ardenne, URVVC EA 4707, Laboratoire Stress, Défenses et Reproduction des Plantes, BP 1039, 51687 Reims Cedex 2, France. ; 3) Plant Biology Department, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland; 4) BGI-Shenzhen, 518083 Shenzhen, China; 5) UMR1065 SAVE, Santé et Agroécologie du Vignoble, INRA, Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France; 6) Université Haute Alsace, Laboratoire Vigne Biotechnologie et Environnement EA 3991, 33, rue de Herrlisheim, 68008 Colmar Cedex, France; 7) Institut Supérieur d'Agronomie, Tapada da Ajuda, 1349-017 Lisbonne, Portugal; 8) Institut Français de la Vigne et du Vin Pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France.

The Ascomycotina *Diplodia seriata* is one of the causal agent of grapevine trunk diseases. Here, we present an improved genome sequence of *D. seriata* isolate F98.1, isolated in 1998 at Perpignan (France) from the trunk of a diseased grapevine. *D. seriata* is one of the most common *Botryosphaeriaceae* species associated with grapevine trunk diseases. This species is also frequently isolated from woody tissues of trees such as *Acer* sp., *Prunus* sp. or *Quercus* sp.. Sequencing was performed using Illumina HiSeq 2500 at a coverage of 270 X. Assembly with SOAPdenovo 1.05 led to 512 contigs and 112 scaffolds (37.27 Mb, GC%: 56.8). This high quality genome (Scaffold *N*50, 2.9 Mb; Min scaffold length, 1.007 bp; gaps, 250 kb) has 13 scaffolds with a size higher than 1 Mb (90% of total sequence), likely corresponding to chromosomes. Using GLEAN, 8.087 CDS were identified, 93 % being supported by RNAseq (mycelium on PDB or MM for 4 days). Recently, the genome sequence of *D. seriata* isolate DS831 from an infected grapevine (USA, 2011) was released. The genome size of DS831 (37.13 Mb) is almost similar to F98.1. However, its assembly is 5 times more fragmented (1391 contigs, 695 scaffolds) and it carries 9398 CDS. Bidirectional best blast hit (BDBH) analysis revealed that 82% of F98.1 and DS831 CDS are similar, 1507 genes are specific to F98.1 strain, and 2763 are specific to DS831 strain. Using Blast, we showed that 2686 (97.2%) of DS831 apparently specific genes were present in F98.1 genome sequence, and reversely 1440 (95.6%) of F98.1 apparently specific genes are present in DS831 genome sequence. Therefore, the difference in CDS numbers between DS831 and F98.1 is likely a consequence of using different annotation software (Glean vs Augustus). Fusion of two annotation leads to a total of 10,500 genes. The genomes of the two species *Diplodia sapinea* and *Diplodia scrobiculata* closely related to *D. seriata* have been sequenced. These two genome have a size of, respectively, 36,97 and 35,85 Mb, and 13020 and 13624 genes respectively. These differences could reflect recent massive gene losses in *D. seriata*.

242T Unravelling potato wart disease; genome assembly and annotation of an obligate parasitic chytrid fungus. *B.T.L.H. van de Vossenberg*[1,2], H. Nguyen[3], M. van Gent Pelzer[1], S. Warris[1], L.V. Bakker[1], H.C. van de Geest[1], D.S. Smith[4], D. Joly[5], C.A. Levesque[3], J.H Vossen[1], R.G.F. Visser[1], T.A.J. van der Lee[1] 1) Wageningen University, Wageningen, Gelderland, NL; 2) National Reference Centre, Dutch National Plant Protection Organization, Wageningen, Gelderland, NL; 3) Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ca; 4) Pathogen Identification Research, Canadian Food Inspection Agency, Ottawa, Canada; 5) Université de Moncton, Moncton, New Brunswick, CA.

The obligate fungal parasite *Synchytrium endobioticum* (Chytridiomycota) is the causal agent of potato wart disease, a severe disease of

cultivated potato that leads up to 100% yield loss. Over the last decades a multitude of pathotypes emerged that break resistance. Once introduced into the soil, the soil itself cannot be used for further crop production due to robust resting spores that can remain viable and infectious for over four decades. Worldwide quarantine measures are in place to restrict the spread of *S. endobioticum.* In addition, the pathogen is included on the HHS and USDA Select Agent list.

To better understand the obligate parasitic lifestyle of the fungus and its interaction with potato, we sequenced and assembled the genomes of two different strains: MB42 from the Netherlands and LEV6574 from Canada. As this pathogen cannot be cultured, generating DNA for this fungus free from its host and other contaminants proved to be impossible. A comparative read-mapping approach (referred to as ZOO selection) with 21 HiSeq or Miseq datasets generated from 17 *S. endobioticum* strains, RNAseq of both reference strains, and HiSeq data from healthy potato allowed the identification of contaminant scaffolds. The effect of ZOO selection was visualised using the Blobology tool. The resulting genome size ranged from 21.6 to 23.5 Mb and show only low levels of DNA polymorphisms between the two strains. RNAseq-based structural annotation using the BRAKER pipeline resulted in 8400-9000 predicted gene models for the respective strains. The annotation completeness of the gene dense and intron rich genomes was assessed using the BUSCO v2 benchmark, resulting in 91.4% and 92.1% annotation completeness for the respective strains. InterProScan was used to functionally annotate the predicted genes and to assign them to KEGG pathways.

Orthologous gene families shared between S. endobioticum and culturable saprobic or pathogenic chytrid species were predicted using Orthofinder and allowed the identification of genes present in all culturable chytrids, but absent in both obligate parasitic S. endobioticum genomes. In addition, orthologues gene families with transposons were found to be highly expanded in both S. endobioticum genomes compared to the other chytrid genomes. We hypothesise that these transposons may have had a significant impact on the genome evolution and perhaps its reduced genome size.

243F Genetic diversity of 100+ *Aspergillus* species - the aspMine analysis resource. *T. C. Vesth*[1], J. L. Nybo[1], S. THEOBALD[1], R. P. DE VRIES[4], I. V. GRIGORIEV[3], S. E. BAKER[2], M. R. ANDERSEN[1] 1) Department of Bioingineering, Technical University of Denmark, Lyngby, Denmark; 2) Joint Bioenergy Institute, Berkeley, CA, USA, Berkeley, CA, USA; 3) Joint Genome Institute, Walnut Creek, CA, USA, Walnut Creek, CA, USA; 4) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, Utrecht, The Netherlands.

The filamentous fungal species of the Aspergillus genus are of broad interest to the scientific community including applied, medical and basic research. These fungi are prolific producers of native and heterologous proteins, organic acids, and secondary metabolites (including bioactives and toxins such as ochratoxin A). Because of these abilities, they represent a substantial economic interests in pharmaceutical, biotechnology, and bioenergy applications. In a project collaboration with the US Joint Genome Institute and JBEI we are de novo sequencing 300 different species of Aspergillus and establishing an online analysis platform for the scientific community, aspMine. The goal of this project is to develop a targeted tool to expand and improve our knowledge and expertise about this versatile group of fungi. At time of writing, 200 genomes are in various stages of sequencing and a bioinformatic pipeline has been established to analyze and store the data. This project covers a wide range of biologically interesting ideas surrounding the concept of speciation, such as genetic diversity, primary and secondary metabolism and proteome diversity. Complementary to the tools offered by FungiDB and JGI, the aspMine analysis resource offers tools for tracking genes and functions across species, allowing for investigation of shared genes and clusters across the genus as well as species- and clade-specific genes. The online platform also offers comparative analysis of secondary metabolism gene clusters with focus on synteny and functional conservation across species. The aspMine is implemented as a number of web applications created in R shiny, a graphical interface for analysis. The different tools are collected on a webpage which also includes method descriptions and relevant literature. The webpage is available from the beginning of 2016 and will be continually expanded. It is our goal to provide a comprehensive analysis platform for the community for comparative analysis of Aspergillus species.

244W Insight into *Cryptococcus neoformans* lineages with different clinical patterns using 800 whole genome sequences. *P.M. Ashton*[1], P.H. Trieu[1], L.T. Thanh[1], D.V. Anh[1], T.T. Chau[1], S. Baker[1,2], G.E. Thwaites[1], F.M. Kibengo[4], N.T.K. Cuc[5], W. Chierakul[6], A.K. Chan[7], M. Mayxay[8], J. Beardsley[1,2], S. Harris[3], J.N. Day[1,2] 1) Oxford University Clinical Research Unit, Ho Chi Minh city, VN; 2) Centre for Tropical Medicine, University of Oxford, UK; 3) Wellcome Trust Sanger Centre, Hinxton, UK; 4) MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda; 5) Hospital for Tropical Disease, Ho Chi Minh City, Vietnam; 6) Mahidol Oxford Tropical Medicine Research Unit, Bankgkok, Thailand; 7) Dignitas International, Zomba, Malawi; 8) Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Laos.

The vast majority of cryptococcal meningitis in immunodepressed patients is caused by C. *neoformans* var. *grubii.* In Viet Nam, we see disease in apparently immunocompetent patients due to a specific cluster of C. *neoformans* var. *grubii* (VNIγ, lineage 5). This causes 84 % of infections in HIV-negative patients, but only 35% in HIV-positive patients. These data suggest that VNIγ is not the dominant genotype in the environment, but is associated with HIV-negative patients. We hypothesize that the VNIγ cluster has increased ability to infect immuno-competent people compared with other VNI strains. Here, we report initial results from the use of whole genome sequencing of 800 isolates of *C. neoformans var grubii* collected as part of clinical trials to investigate this hypothesis.

Gene multiplication has been shown to be a source of genetic diversity in *C. neoformans* which can result in significant phenotypic shifts (Chow et al., 2012). Here, we examined our dataset for other gene multiplication events. There were a total of 1267 putative multiplication events affecting 501 genes identified in this dataset. Most genes are only multiplied in one strain (73%). Furthermore, 93% of the singletons are accounted for by just 5 strains, with one large 465 kb duplication accounting for 46% of duplications present in only one strain. However, some genes are duplicated in a larger number of strains, suggesting that these genetic events have been selected for. There was an average of 17 duplication events per genome, with a maximum of 370 and a minimum of 0.

Our phylogenetic analysis shows that *C. neoformans* var grubii is divided into at least 5 lineages, separated by considerable genetic distance. Each lineage caused infections in both South East Asia and East Africa, but their prevalence differs markedly. The fact that all

lineages were present in both SE Asia and East Africa, but the prevalences varied markedly suggests that the different lineages have characteristic environmental or host adaptations that result in this difference.

In conclusion, whole genome sequencing of 800 *C. neoformans* isolates has generated a rich resource for the investigation of this organism and will allow us to further investigate the clinical differences between lineages of this species.

245T Whole genome sequence of the causal agent of Asian pear sab disease, *Venturia nashicola* for comparative genomics analyses toward identifying host-specificity determinants in pear scab fungi. *Shakira Johnson*[1,2], Joanna Bowen[3], Helge Dzierzon[3], Dan Jones[3], Carl Mesarich[5], Vincent Bus[4], Hideo Ishii[6], Kim Plummer[1,2] 1) Animal, Plant and Soil Sciences, La Trobe University, Bundoora, VICTORIA, AU; 2) Plant Biosecurity Cooperative Research Centre, Canberra, Australia; 3) Plant and Food Research, Auckland, New Zealand; 4) Plant and Food Research, Hawke's Bay, New Zealand; 5) Massey University, Palmerston North, New Zealand; 6) Kibi Internation University,Takahashi, Okayama, Japan.

Pear scab is a major disease of pears worldwide; it is caused by two distinct fungal species, *Venturia pirina* (causes European pear scab) and *V. nashicola* (causes Asian pear scab, a quarantine threat outside Asia). The two species display host specificity: *V. pirina* exclusively infects European pears (*Pyrus communis);* while *V. nashicola* exclusively infects Asian pears (*P. bretschneideri, P. ussuriensis* and *P. pyrifolia*). The distinction between the host specificity of *V. pirina* and *V. nashicola* is likely to be determined by differences in their effector gene arsenal. It is hypothesized that these effectors are important factors in determining the non-host resistance relationships (i.e. in the incompatible interactions). The *Pyrus*-*Venturia* patho-system provides a unique opportunity to dissect the underlying genetics of non-host interactions and to understand host pathogen coevolution of this potentially more durable form of resistance. Genome sequencing technologies have greatly increased the power to perform comparative analyses that assist in the understanding of the molecular mechanisms involved in pathogenesis. Here we report the first draft genome sequence of *V. nashicola,* which will be used to perform comparative genomics with a WGS of *V. pirina* to identify unique and essential effectors that govern host-specificity of these pear-infecting fungi. In addition to these sequences, we have re-sequenced a number of *V. pirina* and *V. nashicola* isolates to reveal effector candidates under either diversifying or purifying selection pressure, with the aim to use these effector candidates to screen experimental *Pyrus* germplasm for durable resistance.

246F Understanding genome evolution and adaptation in the Dothideomycetes. *S. Haridas*[1], Ryan Albert[2], Asaf Salamov[1], Pedro Crous[3], Joseph Spatafora[4], Igor Grigoriev[1,2] 1) DOE Joint Genome Institute, Walnut Creek, CA. USA; 2) Plant and Microbial Dept., University of California Berkeley, Berkeley, CA. USA; 3) CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; 4) Oregon State University, Corvallis, OR. USA.

Dothideomycetes is the largest and most diverse class of ascomycete fungi with 23 orders 110 families, 1300 genera and over 19,000 known species. We have sequenced over 50 Dothideomycetes genomes across the phylogenetic tree. This, along with the previously published 40 Dothideomycetes genomes provides an opportunity and challenge for large scale genomic studies across 300 million years of evolutionary history. We were able to clarify the phylogenetic positions of several species whose origins were unclear in previous morphological and sequence comparison studies. We also show some of the challenges in analyzing such a diverse dataset and present some recent breakthroughs in understanding genome evolution and adaptation.

247W Wild-isolates of nematode-trapping fungi in Taiwan. *S. Lin*, Y. Hsueh Institute of Molecular Biology, Academia Sinica, Taipei, TW.

Nematodes- trapping fungi use different mechanisms to trap and digest nematodes. However, little is known about how they evolve to identify their prey in different environments. To understand the distribution and ecological niche of nematode-trapping fungi, we collected ~60 soil samples from 12 locations in Taiwan and isolated 6 different *Arthrobotrys* species including *A. oligospora*. Among these species, *A. oligospora* appeared to be the most ubiquitously distributed one. Characterization of the phenotypes of *A. oligospora* wild-isolates revealed that these wild isolates exhibited significant difference in certain phenotypic traits such as growth at high temperature and nematode-trapping ability. We plan to use GWAS to understand the potential links between the genotypes and phenotypes in these nematode-trapping fungi.

248T A new genetic code in yeasts. *Robert Riley*[1], Sajeet Haridas[1], Kenneth H. Wolfe[2], Mariana R. Lopes[3], Chris Todd Hittinger[3], Asaf Salamov[1], Christopher H. Calvey[4], Aisling Y. Coughlan[2], Cletus P. Kurtzman[5], Meredith Blackwell[6], Igor V. Grigoriev[1], Thomas W. Jeffries[3,4] 1) US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Walnut Creek, CA; 2) University College Dublin, Dublin, Ireland, ; 3) University of Wisconsin-Madison, Madison, WI, ; 4) Xylome Corporation, Madison, WI, ; 5) US Department of Agriculture ARS NCAUR, Peoria, IL, ; 6) Louisiana State University, Baton Rouge, LA.

The ascomycete yeasts, which include the model organism *Saccharomyces cerevisae*, are a diverse group of fungi spanning a billion years of evolution, and with enormous biotechnological potential. Unique among eukaryotes, some yeasts are known to have a genetic code change, in which CUG codons are translated to serine rather than the usual leucine. Such genetic code changes are interesting due to their extreme rarity, but may also complicate efforts to exploit the yeasts' biotechnological potential through exogenous expression of genes (which might have genetic codes incompatible with hosts). We sequenced the genomes of 16 ascomycete yeasts with the goal of discovering biotechnologically useful genes. From the genome sequences, we predicted that a xylose-fermenting yeast, *Pachysolen tannophilus*, translates CUG codons neither to canonical leucine nor previously known serine, but to alanine. Mass-spectrometry proteome analysis and genetic transformation experiments confirmed this prediction. The existence of multiple genetic codes in the ascomycete yeasts has implications for their exploitation in biotechnology and synthetic biology.

249F Resolving the Mortierellaceae phylogeny through Multi-Locus Sequence Typing (MLST) and phylogenomics. *Natalie Vande Pol*[1], Jason Stajich[2], Kerry O'Donnell[3], Alessandro Desiro[1], Gregory Bonito[1] 1) Michigan State University, East Lansing, MI; 2) University of California at Riverside, Riverside, CA; 3) Mycotoxin Prevention & Applied Microbiology Research Unit, NCAUR-ARS-USDA, Peoria, IL.

The Mortierellaceae (Mortierellomycotina) are a diverse family of fungi that are of evolutionary and ecological relevance. They are the closest lineage to the arbuscular mycorrhizae (Glomeromycotina) and include some of the first species to evolve fruiting body production. The Mortierellaceae are estimated to contain at least 100 species classified within six polyphyletic genera that cannot be resolved with ribosomal markers. With advances in DNA sequencing technology, it is now feasible to generate sequence data from many loci in parallel (MLST), or to perform low-coverage genome (LCG) sequencing to identify phylogenetically informative loci. In collaboration with the ZyGo Life consortium and the Joint Genome Institute, we sequenced 68 LCGs representing 50 unique species of Mortierellaceae. From these, we identified 400 informative loci and used RaxML to build a concatenated tree. The resulting phylogeny has very strong bootstrap support and a very different structure from existing ribosomal trees. In parallel, we analyzed three *de novo* sequenced *Mortierella* genomes and extracted 13 informative loci. For each locus, we designed PCR primers for multiplexed PCR amplification across 333 Mortierellaceae isolates, which included the 68 LCG isolates. We will discuss the strengths and limitations of these two approaches.

250W Comparative genomics and transcriptomics of *Russulaceae*. Brian Looney[1,2], Dan Jacobson[1], Kerrie Barry[3], Alan Kuo[3], Igor Grigoriev[3], Francis Martin[4], *Jessy Labbé*[1,2] 1) Bioscience Division Oak Ridge National Laboratory, Oak Ridge TN 37830; 2) University of Tennessee, Knoxville, TN 37996; 3) US DOE Joint Genome Institute, Walnut Creek, CA 94598; 4) INRA UMR1136, 54280 Champenoux, France.

Russulaceae is a diverse fungal family including the genera *Russula*, *Lactarius*, *Lactifluus*, and *Multifurca*, and is one of the most widespread and species rich ECM lineages. In a recent collaborative effort, the Joint Genome Institute has sequenced genomes and transcriptomes of representative groups across *Russulaceae*, including a saprotrophic outgroup. We present here an overview of the first insight into the dense genome sampling within the family to capture specific genomic features and investigate i) to what extent genes involved in plant biopolymer degradation have been maintained within a single, diverse, ECM lineage and ii) to examine functional diversity within this ecologically important clade, specifically focused on their ability to scavenge nitrogen sources from recalcitrant organic matter. Indeed, preliminary evidence suggests that members of this family, though being mutualists, have retained a restricted set of genes coding for lignin peroxidases and copper oxidoreductases which may be responsible for the degradation of lignin derivatives accumulating in soil organic matter.

251T Genome sequencing reveals a heterothallic mating system in the *Eucalyptus* pathogen *Chrysoporthe austroafricana*. A.M. Kanzi[1], B.D. Wingfield[1], E.T. Steenkamp[2], *N.A. van der Merwe*[1] 1) Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, Gauteng, ZA; 2) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, Gauteng, ZA.

Chrysoporthe austroafricana is a fungal pathogen that affects commercial forestry establishments of *Eucalyptus* species and native species of *Myrtales* in Southern Africa by causing deadly cankers. The mating system of *C. austroafricana* has not been characterized, but sexual structures have been observed on native *Syzygium* trees. Homothallism was proposed as the mating system in this species, but this hypothesis could not be fully tested due to the lack of mating-type information. Thus, genome sequence data of two *C. austroafricana* isolates and two close relatives, *C. cubensis* and *C. deuterocubensis*, which are also known to cause disease on *Myrtales*, were analyzed. A putative *MAT1-1* idiomorph was found in one of the *C. austroafricana* isolates and a putative *MAT1-2* idiomorph, containing a truncated *MAT1-1-1* gene, was observed in the other *C. austroafricana* isolate. The presence of a single idiomorph in each isolate suggests that this fungus is heterothallic. The ratio of *MAT1-2* to *MAT1-1* isolates in a South African population of *C. austroafricana* was 2:1. In contrast, the ratios of *MAT1-2* to *MAT1-1* in populations from neighbouring Southern African countries ranged from 1:1 to 1:6. The non-proportional ratios in some populations suggest that these populations may be the result of recent introductions. The sibling species *C. cubensis* and *C. deuterocubensis* are apparently homothallic, since the *MAT1* locus of these isolates included genes associated with both *MAT1-1* and *MAT1-2* idiomorphs.

252F Comparative genomics to assess myco-parasitism and pathogenicity in understudied Oomycetes. Ramesh R. Vetukuri[1], Kurt H. Lamour[2], *Laura J. Grenville-Briggs*[1] 1) Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden; 2) Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee, USA.

The oomycetes are a lineage of filamentous Eukaryotes that resemble fungi, but are most closely related to the heterokont (brown) algae. Many oomycetes are plant pathogens including the new threat, *Phytophthora colocasiae* causing taro blight, however, some Pythium species are parasitic to fungi and other oomycetes (*Pythium oligandrum* and *Pythium periplocum*) whilst others such as *Lagenidium giganteum* are parasites of insects including mosquitos that vector Zika virus and Dengu fever. We have carried out *de novo* genome sequencing and genome-wide transcriptomics (RNA-seq) of four understudied oomycetes, *P. oligandrum*, *P. periplocum*, *L. giganteum* and *P. colocasiae* using the SciLifeLab Illumina Hiseq platform using both paired and mate pair sequencing. We are currently investigating the genetic and molecular determinants of host specificity in all the four sequenced oomycetes and mycoparasitism in the Pythium species. The sizes of the these four genomes range from 36 Mb to 100 Mb. RxLR class effectors were found in *P. colocasiae* but not in the three Pythium species. Crinkler (CRN), and elicitin proteins were detected in all the four oomycetes sequenced. Novel effector families are present in the insect pathogen *L. giganteum*. RNA-seq data from *P. oligandrum* myco-parasitising the potato late blight oomycete *Phytophthora infestans* shows specific up-regulation of 1940 *P. oligandrum* genes 12 hrs after infection and 300 genes at 24hrs. Some of these genes are involved in secondary metabolism and terpenoid biosynthesis, transport and cell wall degradation. Novel transcripts from *P. infestans* reveal how this oomycete defends itself against mycoparasitic attack and may provide vital clues to control potato late blight in a more durable way in the future. Comparative analyses of the hyperaggressive mycoparasite *P. oligandrum* versus the weaker mycoparasite *P. periplocum* are providing vital clues to the success of *P. oligandrum.* Identification of common and specific pathogenicity determinants and defence pathways opens the way for more sustainable oomycete control measures and better use of oomycetes as biological control agents.

253W Pervasive Glyphosate Resistance in *S. cerevisiae* Lays Outside the Chorismate Pathway. *J. Gallagher*, X. Rong-Mullins, C. Blackwood, K. McNeal, Z. Lonergan, J. Creamer, A. Biega, A. Ravishankar Dept Biology, West Virginia Univ, Morgantown, WV.

S. cerevisiae are associated with different crops and environments and are consequently subject to a wide range of selective pressures whether intentional or by happenstance. First marketed as RoundUp™, glyphosate is the most widely used herbicide. Glyphosate inhibits

the chorismate pathway which is present in many organisms but not in mammals. Agriculture-associated isolates of *S. cerevisiae* isolated during the last forty yeast had high glyphosate resistance. Despite sequence similarity between the target protein, the plant EPSPS and the yeast orthologous protein Aro1, and the prediction that yeast would be resistant to glyphosate, there was a wide dose range of yeast growth inhibition to a commercial preparation of glyphosate. Growth variation seen in the subset of yeast tested was not due to polymorphisms within Aro1, instead, it was caused by genetic variation in a multiple-drug transporter and amino acid permease. Recently collected yeast from conventional and organic farms, urban and rural areas had glyphosate resistant yeast. However, in remote areas or areas only recently exposed, the number of glyphosate resistant yeast was significantly lower. Therefore, close association with challenging environments is changing the wild *S. cerevisiae* populations either through selection by directed application of glyphosate or other unforeseen selective pressures.

254T Comparison of orthologous gene clusters and carbohydrate-active enzymes of *Ophiosphaerella* spp. the causal agents of spring dead spot of bermudagrass. *N. Graf Grachet*[1], B. Couger[2], C. D. Garzon[1], S. Marek[1], N. Walker[1] 1) Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK; 2) High Performance Computing Center, Oklahoma State University, Stillwater, OK.

In the United States, turfgrasses and their maintenance are estimated to be a $40 billion industry. Bermudagrass (*Cynodon dactylon* and *C. dactylon* x *C. transvaalensis*) is a warm-season grass commonly cultivated as turfgrass in the southern US. Spring dead spot is considered the most destructive disease of bermudagrass in this region. Control of this disease requires high rates of fungicides. Spring dead spot is caused by three fungi: *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari*. The fungi infect and colonize below ground organs of bermudagrass. Although in the same genus, the species vary in asci and melanin formation when cultured. In addition, the species differ in virulence, origin, and host range. The objective of this study was to examine genomic information of each species. Whole-genome of 11 isolates and total RNA of 6 isolates of *Ophiosphaerella* spp. were sequenced using Illumina HiSeq and PacBio SMRT technology. Average genome size was 47 Mb in *O. narmari*, 65 Mb in *O. herpotricha*, and 70 Mb in *O. korrae*. Number of protein coding genes varied from 12 to 14 thousand across all species. The difference in genome size was due to expansion of intergenic bases. Ortholog gene clustering in OrthoMCL revealed that approximately 85% of the protein coding genes were shared between the three *Ophiosphaerella* spp. The same analysis showed that *O. korrae* had more unique genes (638) than *O. narmari* (147) and *O. herpotricha* (77). Preliminary results of SignalP predicted an average of 1,000 signal peptides among all three species. *Ophiosphaerella* spp. shared similar abundances of the CAZymes. There was an expansion of auxiliary activities families 3, 4, and 11, and of carbohydrate esterases family 1. The majority of *Ophiosphaerella* spp. enzymes were homologs of plant-acting enzymes. It was hypothesized that these groups act on plant substrates such as lignin to facilitate root colonization.

255F Genome sequence and its use in developmental, genetic and population studies of Shiitake mushroom *Lentinula edodes*. *H.S. Kwan*[1,2], Yang Xiao[1,3], Lei Li[1,2], Chun Hang Au[1,2], Man Chun Wong[1,2], Qianli Huang[1,2], Xuanjin Cheng[1,2], Man Kit Cheung[1,2], Jinhui Chang[1,2], Wenyan Nong[1,2] 1) School of Life Sciences, Chinese Univ of Hong Kong, Hong Kong, SAR; 2) Food Research Centre, Chinese Univ of Hong Kong, Hong Kong SAR; 3) Institute of Applied Mycology, Huazhong Agricultural University, 430070, Hubei Province, P. R. China.

The Shiitake mushroom *Lentinula edodes* is one of the most popular edible mushrooms in the world. We sequenced the genome of the *L. edodes* monokaryon L54A. Comparative analyses on genome sequences of basidiomycetes and ascomycetes revealed genes expanded in genomes of mushroom-forming fungi. Four functional categories, Signal transduction mechanisms [T], Posttranslational modification, protein turnover, chaperones [O], Transcription [K] and Carbohydrate transport and metabolism [G], dominate in the expanded families. We examined kinome, ubiquitome, transcription factories and CAZymes. AGC kinase subfamily, F-box and paracaspase domain-containing E3 like proteins are significantly expanded in mushroom-forming genomes. We also performed RNA-Seq of multiple stages and identified genes differentially expressed. Transcriptome age index (TAI) profile and transcriptome divergence index (TDI) profile showed a molecular hourglass pattern over the developmental stages. Young fruiting body stage was the “waist” of the hourglass that expressed the evolutionarily oldest transcriptome. The genome sequence is used to generate a high-density genetic map for Expression Qualitative Trait (eQTL) studies. In addition, the genome sequence also served as the reference sequence for analysis of wild and cultivated populations in China. Our works have generated rich resources for genomics, transcriptomics, and population studies of mushrooms.

256W Genome sequence of the pathogenic white-rot fungus *Phellinus noxius* OVT-YTM/97 reveals insights on pathogenesis. *K.S.W. Tsang*[1], Y.C. Lam[2], W.Y. Nong[1], C.K. Wong[1], H.S. Kwan[1] 1) The Chinese University of Hong Kong, Hong Kong SAR; 2) Muni Arborist Limited, Hong Kong SAR.

Phellinus noxius is a pathogenic white-rot fungus of the phylum Basidiomycota that causes brown root rot disease, common to pan-tropical and subtropical regions with a host range of over 200 plant species. The fungal pathogen causes irrevocable damage in plant hosts and if left untreated, can lead to swift deterioration of the host health within a year. In Hong Kong, occurrence of *P. noxius* infected trees has increased dramatically in the past several years and many of which are old and valuable with cultural or historical significance. Currently there is no cure for this disease, despite the development of several biocontrol methods and fungicides.

We have assembled the first genome sequence of *P. noxius* OVT-YTM/97, isolated from a root sample obtained from an infected *Ficus microcarpa* tree in Hong Kong. This strain was found to have a particularly aggressive pathogenicity and exhibited resistance towards biocontrol agents. The genome was sequenced on Illumina Mi-Seq and PacBio RSII platforms, followed by *de novo* assembly using the Celera assembler v8.3 and annotated.

A total of 9,957 protein-coding genes were predicted using GeneMark-ES and carbohydrate-active enzyme (CAZyme). Analyses using dbCAN v5 revealed 478 CAZymes and a strong resemblance to that of necrotrophic plant pathogens, with large proportions of glycoside hydrolases and carbohydrate esterase families 1 and 10, which are required for the complete breakdown of plant cell walls for infection. In addition, analyses using the Database of Fungal Virulence factors (DFVF) indicated an abundance of β (1-3)-glucan synthases and class I and II chitin synthases, which control cell wall synthesis and septa production, providing structural stability for the hyphae. The presence of these carbohydrate-active enzymes and virulence factors could explain the high virulence of the pathogen and its ability to cause catastrophic fatalities in infected trees. The results generated from this study allow us to have a deeper understanding of the mechanisms of

action and the hypervirulence of this pathogen. It also provides a basis for the development of preventation and treatment to protect trees from this pathogen.

257T Comparative genomics integrated with association analysis identifies candidate effector genes corresponding to *Lr20* in phenotype-paired *Puccinia triticina* isolates from Australia. *J.Q. Wu*[1], S. Sakthikumar[2], C.A. Cuomo[2], R.F. Park[1] 1) Plant Breeding Institute, Faculty of Agriculture and Environment, The University of Sydney, Narellan, NSW, Australia; 2) Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, MA 0214, USA.

Leaf rust is one of the most common and damaging diseases of wheat, and is caused by an obligate biotrophic basidiomycete, *P. triticina* (*Pt*). In the present study, 20 *Pt* isolates from Australia, comprising 10 phenotype-matched pairs with contrasting pathogenicity for *Lr20*, were analysed using whole genome sequencing. Compared to the reference genome of the American *Pt* isolate 1-1 BBBD Race 1, an average of 404,690 single nucleotide polymorphisms (SNPs) per isolate was found and the proportion of heterozygous SNPs was around 87% in the majority of the samples, demonstrating a high level of polymorphism and a high rate of heterozygosity. From the genome-wide SNPs, a phylogenetic tree was inferred, which consisted of a large clade of 15 isolates representing diverse presumed clonal lineages including 14 closely related isolates and the more diverged isolate 670028 and a small clade of five isolates characterised by lower heterozygosity level. Principle component analysis detected 3 distinct clusters, corresponding exactly to the two major subsets of the small clade and the large clade comprising all 15 isolates without further separation of isolate 670028. While genome-wide association analysis identified 302 genes harbouring at least one SNP associated with *Lr20* virulence ($p<0.05$), a Wilcoxon rank sum test revealed that 36 and 68 genes had significant ($p<0.05$) and marginally significant ($p<0.1$) differences in the counts of non-synonymous mutations between *Lr20* avirulent and virulent groups, respectively. Twenty of these genes were predicted to have a signal peptide without a transmembrane segment, which were identified as candidate effector genes corresponding to *Lr20*. Future studies are thus warranted to investigate the biological functions of the candidate effectors as well as the gene regulation mechanisms at epigenetic and post-transcription levels. Our study is the first to integrate phenotype-genotype association with effector prediction in *Pt* genomes, an approach that may circumvent some of the technical difficulties in working with obligate rust fungi and accelerate avirulence gene identification.

258F Blue light perception in the maize foliar pathogen, *Exserohilum turcicum*. *J. Liversage*[1,2], S.H. Stoychev[3], B.G. Crampton[1,2] 1) Department of Plant and Soil Science, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; 2) Genomics Research Institute, University of Pretoria, South Africa; 3) Council for Scientific and Industrial Research, Biosciences, Pretoria, South Africa.

Northern corn leaf blight (NCLB) is a devastating maize foliar disease in South Africa, with the causative agent being *Exserohilum turcicum*. To understand how the fungus infects maize and how this links to its photobiology is unexplored. Here we firstly set out to identify all the *E. turcicum* circadian clock proteins, which include the White collar-1 (blue light photoreceptor), White collar-2 (forms part of light responsive transcription factor) and Vivid (photoadaptation). Lastly, we aimed to identify and characterize an alternative blue light photoreceptor, CRY-DASH, the function of which remains unexplained in the circadian clock and pathogenicity. Local databases were set up of each of the genes from other fungi, after which a BLAST search against the sequenced genome of *Setoshearia turcica* ET28A v1.0. was performed. Retrieved gene sequences were analyzed for correct domain architecture and there after qPCR primer sets were designed. *E. turcicum* cultures were grown in constant darkness and flash frozen following light pulses of 15, 30 and 60 min as well as a 24 hr pulse. Following, RNA extraction and cDNA synthesis the light response profile of the clock genes were generated. The function of CRY-DASH was determined through producing a knock-out via Agrobacterium-mediated transformation and the generation of a Green Fluorescent CRY-DASH fusion protein. All the classical clock genes are present in the genome sequence of *E. turcicum*. The traditional light expression pattern of these genes differ from that in literature. Cry-DASH is a promising alternative blue light photoreceptor in fungi.

259W Developing durable disease control strategies for rice blast disease in Sub-Saharan Africa by genetic and genomic analysis of *Magnaporthe oryzae* populations. *V.M. Were*[1], D. Mwongera[1], S. Mutiga[2,4], F. Rotich[4], M.J. Kershaw[1], D.M. Soanes[1], G.R. Littlejohn[1], X. Yan[1], B. Zhou[3], J.W. Harvey[2], I. Ouédraogo[6], G-L. Wang[5], T. Mitchell[5], J. Correll[4], N.J. Talbot[1] 1) Biosciences, University of Exeter, UK; 2) BecA-ILRI, Nairobi, Kenya; 3) International Rice Research Institute, Los Baños, The Philippines; 4) Department of Plant Pathology, University of Arkansas, USA; 5) Department of Plant Pathology, Ohio State University, Columbus, Ohio; 6) Institute of Environment and Agricultural Research, Ouagadougou, Burkina Faso.

The rice blast fungus, *Magnaporthe oryzae*, causes the most serious disease of cultivated rice. We are characterising effector proteins from *M. oryzae* and using this knowledge to develop new strategies for durable rice blast disease control in Sub-Saharan Africa (SSA). We have collected more than 500 rice blast isolates from 9 countries and carried out extensive pathotype analysis on monogenic rice lines carrying 24 rice blast resistance genes *Pia, Pib, Pik, Pik-h, Pi-km, Pik-p, Pik-s, Pish, Pit, Pita, Pita-2, Piz, Piz-5, Piz-t, Pi1, Pii, Pi3, Pi5 (t), Pi7 (t), Pi9, Pi11 (t), Pi12 (t), Pi19 (t),* and *Pi20*. We have used this analysis to identify gene combinations that might lead to durable resistance. We have found that *Pi-9, Pita-2, Pi-12, Piz* and *Pi-z5* offer the greatest possibility for exclusion of the prevailing rice blast population. We have begun to introgress these genes and other potential sources of resistance into high yielding locally adapted rice cultivars. To understand the relationship between the effector repertoire of *M. oryzae* and their potential recognition as avirulence determinants in blast populations in SSA, we have sequenced the genomes of 21 *M. oryzae* isolates. We have combined this comparative genome analysis with RNA-seq analysis of rice blast infections to identify a set of putative avirulence gene candidates. We have characterised allelic variation at these loci and then carried out targeted gene deletion and complementation analysis to determine their functions. Progress towards the characterisation of these effectors and their use to guide future rice breeding programmes in Africa will be reported.

260T MagNet: the integrated gene network of the rice blast fungus *Magnaporthe oryzae*. *H. Kim*[1], K. Cheong[1], K.T. Kim[2], J. Jeon[1], G. Choi[1], Y.H. Lee[1,2,3] 1) Interdisciplinary Program in Agricultural Genomics, Seoul National University, Seoul 08826, Korea; 2) Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea; 3) Center for Fungal Genetic Resources, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea.

Magnaporthe oryzae, the rice blast fungus, plays a role as a model organism in the area of molecular plant-microbe interaction research.

Studies on pathogenic mechanism of this fungus revealed many genes in signaling pathways are essential for appressorium formation and penetration. As multi-omics data are being available, genomic-level researches have been conducted to uncover the underlying biological process during the pathogenesis of *M. oryzae*. Identifying genome-wide protein-protein interaction (PPI) network is one of omics-level approaches which can help to understand signaling and regulatory pathways. However, existing biological network resources of *M. oryzae* are not sufficient to decipher molecular mechanisms of pathogenesis. In this study, PPI network analysis platform of *M. oryzae*, MagNet, was constructed with three methods: homology-based 'Interolog' search, co-expression network construction, and domain-domain interaction based prediction. Interologs within *M. oryzae* proteins which have orthologues in model species were predicted with six PPI repositories. Co-expression networks were built with RNA sequencing data from the infection stages and those from the vegetative stage. In addition, we gathered the information of domain-domain interactions (DDIs) from two integrated DDI databases, and predicted interactions between domain-annotated proteins. With three approaches all together, the pan-network with 6,600,976 interactions was generated including highly confident 215,731 interactions found in more than three sub-networks. Experimental data on *M. oryzae* PPIs support that our highly confident PPI network can predict PPIs with higher sensitivity (89.65%) and specificity (78.57%) compared to the previously constructed databases. MagNet would provide integrated biological network data which can help to understand the molecular mechanisms of the rice blast fungus and beyond.

261F Global proteome analysis of *Magnaporthe oryzae* during nitrogen starvation. *Yeonyee Oh*[1,2,5], Suzanne Robertson[3,4,5], Jennifer Parker[3,4,5], David Muddiman[3,4,5], Ralph Dean[1,2,5] 1) Center for Integrated Fungal Research; 2) Department of Entomology and Plant Pathology; 3) W. M. Keck FT-ICR Mass Spectrometry Laboratory; 4) Department of Chemistry; 5) North Carolina State University, Raleigh, NC.

Fungi are constantly exposed to nitrogen limiting environments and the efficient regulation of nitrogen metabolism is essential for fungal survival, growth, development and pathogenicity. To understand how the rice blast pathogen, *Magnaporthe oryzae* deals with nitrogen shortage, we performed global proteome analyses. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we identified a total of 5,498 *M. oryzae* proteins during nitrogen starved or non-starved conditions. Comparative analysis showed 363 proteins were significantly induced or uniquely expressed under the nitrogen starved condition and 266 proteins under non-starved condition. A functional analysis of differentially expressed proteins suggested major biological pathway changes during nitrogen starvation including nitrogen repression, melanin biosynthesis, protein degradation and protein translation. Nitrogen starvation induced accumulation of various extracellular proteins including a number of small extracellular proteins suggesting a link between nitrogen starvation and pathogenicity in *M. oryzae*. The results from this study provide a better understanding of fungal response to nitrogen availability.

262W *Genome and Transcriptome Analysis of Phellinus linteus for a Biosynthetic Pathway for Hispidin Compounds*. *D. Pathiraja*, H. Park, B. Min, K. Jang, J. Byun, I. Choi Korea University, Seoul, KR.

Phellinus linteus is a medicinal mushroom used as traditional medicine in East Asia. *Phellinus linteus* has been studied for various favorable pharmacological effects such as anti-inflammatory, anti-allergic, anticancer, immunomodulatory and anti-oxidant effects. These effects were found to be associated with bioactive secondary metabolites of *Phellinus linteus*. We carried out genome and transcriptome sequencing to identify and characterize those metabolic pathways. Here, we report a comparative genomics of *Phellinus linteus* strains known as 'Changsu' and 'Goryo' in South Korea. Genome sequencing of two medicinal mushrooms was performed in the Illumina MiSeq platform. The size of *P. linteus* Changsu was estimated as 40.6 Mb, whereas *P. linteus* Goryo was estimated as 31.9 Mb. Unique genetic patterns of these two genomes were surveyed by comparing to well-studied mushroom genomes: *Ganoderma lucidum*, *Agaricus bisporus*, and *Laccaria bicolor*. We have identified several important secondary metabolic pathways in these genomes. Among them, the gene cluster responsible for the biosynthesis of Hispidin, a well-known anti-oxidant, was predicted in both Changsu and Goryo strains.

263T Genomics of mycorrhizal fungi. *S. Unruh*[1], F. Martin[2], L. Zettler[3], S. Perotto[4], C. Hudson[5], G. Conant[1], J.C. Pires[1] 1) Biological Sciences, University of Missouri, Columbia, MO; 2) Institut National de la Recherche Agronomique (INRA), Nancy, France; 3) Department of Biology, Illinois College, Jacksonville, IL; 4) University of Turin, Italy; 5) Systems Biology Research Group, Sandia National Laboratories, Livermore, CA.

Orchids have a unique relationship with a certain subset of fungi in that the seeds of these plants rely on nutrients from fungi to germinate and develop. The fungi involved are not all in other more typical mycorrhizal interactions, so the criteria for what makes a fungus an orchid mycorrhizal fungus is a mystery. With a Community Sequencing Project, my team is attempting to better understand the genomes of various orchid mycorrhizal fungi, their relationships to each other, and what genes are expressed solely in the symbiosis. To do this, we are sequencing whole genomes of 15 fungi, shallowly sequencing up to 400 fungal isolates to perform phylogenetic analysis, and performing RNAseq on orchid protocorms infected with mycorrhizal fungi.
The whole genome sequencing is underway, the shallow sequencing is also in progress and new barcodes are being developed that are more useful in this group than ITS. The RNAseq experiment will start shortly after the fungal genetics conference.

264F Understanding the evolution and functioning of symbiosis : *Pisolithus sp*. as model. *A. Kohler*[1], M. de Freitas Pereira[1], E. Morin[1], V. Singan[2], A. Kuo[2], J.M. Plett[3], M.D. Costa[4], I. Grigoriev[2], F. Martin[1] 1) UMR IAM, LABEX ARBRE, INRA Centre de Nancy, Champenoux, Lorraine, FR; 2) US Department of Energy, Joint Genome Institute (JGI), Walnut Creek, California, USA; 3) Hawkesbury Institute for the Environment, University of Western Sydney, Richmond NSW, Australia ; 4) Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

All ectomycorrhizal fungi sequenced so far exhibit a reduced set of genes encoding plant cell wall–degrading enzymes (PCWDE) compared to their ancestral wood decayers and the analysis of mycorrhizal transcriptomes revealed the involvement of both conserved and clade-specific genes. Induced are genes coding for the same functions but without orthology, like (often clade-specific) small-secreted proteins, transporters, redox metabolism or carbohydrate active enzymes, suggesting a convergent evolution. These findings are also true for two *Pisolithus* genomes, *Pisolithus tinctorius* and *P. microcarpus*, sequenced in the frame of the JGI Mycorrhizal Genomics Initiative (MGI). Both species are frequent fungi in forest ecosystems but colonize different host trees, pine and eucalypt, respectively. Comparison of the *Pisolithus* mycorrhizal transcriptomes revealed a set of small-secreted proteins induced in interaction with their hosts that are conserved

between the two genomes but expanded in gene copy number compared to other genomes.
Further, a *Pisolithus microcarpus* monocaryotic strain collection isolated from the same fruiting body showed striking differences in the ability to form mycorrhiza. Genome resequencing and gene expression analyses during mycorrhiza formation allowed further insights into genetic differences within this *Pisolithus* progeny.

265W Extensive genome diversity among isolates of a model arbuscular mycorrhizal fungus. *E.Chun-Hung. Chen*[1], S. Ndikumana[1], E. Morin[2], F. Martin[2], J. Noel[1], D. Beaudet[1], J. Giorgi[1], N. Corradi[1] 1) Biology, University of Ottawa, Ottawa, Ontario, CA; 2) UMR 1136, INRA-Nancy Université, Interactions Arbres/Microorganismes, Champenoux, FR.

Arbuscular mycorrhizal fungi (AMF) are best known for their beneficial effects on the health of many land plants and their surrounding ecosystem through establishment of the mycorrhizal symbiosis. It has been proposed that that intra-specific diversity is an essential component of a successful symbiosis with the plant host. Recent evidence of a mating-driven processes in a model AMF has revealed some of the mechanisms involved in creating intra-specific diversity in this group, but how the genome content and structure varies within one species is still poorly understood. To address this question, we compared the genome sequences of five isolates of previously proposed to be part of the *Rhizophagus irregularis* clade. Our analyses show that inter-isolate genome diversity is present at all levels, and includes large amounts of isolate-specific genes, sequence and gene copy number polymorphisms and inter-isolate rearrangements indicative of inter-isolate recombination. Plasticity affects all biological functions, leads to re-interpretations of population-based, and underscores potential problems with the species concept in these widespread plant symbionts.

266T PhytoPath: harnessing community expertise to fight plant disease. *Helder Pedro*[1], Uma Maheswari[1], Nishadi De Silva[1], Paul Kersey[1], Kim Hammond-Kosack[2], Alayne Cuzick[2], Martin Urban[2] 1) EMBL-EBI, Hinxton, Cambridge, GBEMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK; 2) Rothamsted Research 2016 Harpenden, Hertfordshire, AL5 2JQ.

Plant pathogens continue to threaten food security and have a significant economic impact. For instance, it is estimated that late blight of potato and tomato (caused by *Phytophthora infestans*) incur annual global losses in excess of US$6.7 billion. With burgeoning populations, maintaining (and increasing) the yields of crop plants through pesticide research and plant breeding has never before being more important. For this, an understanding of gene function and host-pathogen interactions is critical, and accurate annotation of genomic sequence is important to both enable and disseminate this knowledge.
PhytoPath (www.phytopathdb.org), established in 2010, is a bioinformatics resource for genome scale data for important fungal and protist plant pathogens. It integrates resources from the Ensembl Genomes and PHI-base databases to organise genome sequence data, genetic variation, comparative analyses and phenotypic data to facilitate research on plant pathogenesis. Currently PhytoPath houses over 250 genomes from 115 plant pathogen species, from which over 1800 genes have been associated with disease phenotypes. We run automated pipelines at regular intervals to acquire and analyse new fungal and protist genomes deposited in the public data archives, and provide visualisations using the Ensembl software suite and numerous routes for programmatic data access.
Furthermore, we facilitate the refinement of genome annotations by capturing manual community curation through WebApollo, an online gene editing tool. In 2015, we trained close to 50 species experts from the *Botrytis cinerea* community who systematically reviewed and revised the entire gene set for this organism. Following a thorough QC process, these updates have been integrated into subsequent releases of PhytoPath and Ensembl Genomes and made available to the wider research community. Following this successful initiative, we are currently in the process of engaging with the communities for *Zymoseptoria tritici, Blumeria graminis* and *Fusarium culmorum* and expect to commence curation of these species in the near future.

267F *HAP3 of Phomopsis longicolla influences growth, development, and pathogenesis* . *M. Zaccaron*, W Hawkins, B Dhillon, Y Ramegowda, J Ridenour, B Bluhm Plant Pathology, University of Arkansas, Fayetteville, AR.

Phomopsis longicola (Hobbs), the most important soybean seed pathogen, is the causal agent of *Phomopsis* seed decay. Currently, little is known about the molecular basis of pathogenesis in *P. longicolla*, in part because crucial tools for molecular genetics, such as targeted gene deletion, have not been demonstrated in this organism. In other filamentous fungi, the heterotrimeric CCAAT-binding complex is involved in diverse aspects of growth and development, including secondary metabolism, morphogenesis, and pathogenesis. In this study, a putative component of the CCAAT-binding complex (HAP3) was identified in *P. longicolla* and characterized through functional genomics. With agrobacterium-mediated transformation, *HAP3* gene was successfully deleted via homologous recombination, and the mutant was genetically complemented via reintroduction of the wild-type gene. Deletion of *HAP3* substantially impaired growth and development as well as the ability of *P. longicolla* to colonize soybean seeds and stems. A RNAseq experiment reviewed massive reprograming in a ?*hap3* strain (H101) transcriptome while colonizing soybean seeds. A total of 2080 upregulated and 1732 downregulated genes were found in H101, accounting, respectively, for 12 and 14% of *P. longicolla* predicted protein coding genes (adjusted $P < 0.05$). This study reviews the important role of the *HAP3* component of the CCAAT-binding complex in growth, development and pathogenesis of *P. longicolla*. More importantly, our work illustrates the feasibility of targeted gene deletion in *P. longicolla*. Phenotypic similarities between *HAP3* deletion mutants of *P. longicolla* and other plant pathogenic fungi potentially indicate broad involvement of the CCAAT-binding complex in plant pathogenesis.

268W DNA methylation and chromatin architecture contribute to pathogenic fungal genome organization and adaptation. *David E. Cook*[1,2], Michael F. Seidl[1], Martin Kramer[1], Bart PHJ Thomma[1] 1) Laboratory of Plant Pathology, Wageningen University, Wageningen, Netherlands; 2) Department of Plant Pathology, Kansas State University, Manhattan, KS USA.

Fungal pathogens have evolved diverse strategies to overcome host immunity. During fungal-plant interactions, invading fungi utilize secreted proteins, termed effectors, to avoid or subvert the plant immune response through varied biochemical mechanisms. Effector genes are not randomly distributed across a genome, but often reside in polymorphic regions of the genome, clustering with repetitive DNA. Despite the ubiquity and importance of fungal effectors, our mechanistic understanding of their transcriptional regulation and genome organization remains inadequate. As such, we are addressing two key questions 1) How are *in planta* effectors transcriptionally regulated? 2) How does repetitive DNA contribute to the evolution of highly variable genomic regions that contribute to fungal virulence? Using a variety of genetic and computational approaches, we are characterizing how DNA modifications and chromatin structure (the organization of

DNA in a cell) contribute to the evolution of virulence using the soil-borne fungal pathogen *Verticillium dahliae.* The genome of *V. dahliae* is predicted to express numerous homologs of known DNA and chromatin modifying proteins, including three putative DNA methyltransferases. We have identified that a single DNA methyltransferase controls a significant portion of the observed DNA methylation at repetitive DNA. Interestingly, repetitive DNA arising from recent segmental genome duplications are devoid of DNA methylation and are more transcriptionally active relative to repetitive DNA at other loci. Additionally, we are assaying the genome for open chromatin to develop a comprehensive view of how gene regulation and chromatin architecture impacts the evolution of fungal virulence.

269T A virulence interactome of the wheat pathogen Fusarium graminearum. *A. Mirmiran*[1,2], C. Tsai[1], C. Mogg[1,2], G. Subramaniam[1,2], D. Desveaux[1,3] 1) University of Toronto, Toronto, ON, Canada; 2) Agriculture and Agri-Food Canada, Ottawa, ON, Canada; 3) Centre for the Analysis of Genome Evolution and Function, Toronto, ON, Canada.

Fusarium Head Blight (FHB) is a disease affecting wheat (*Triticum aestivum*) and other cereal crops worldwide resulting in the loss of over $3 billion in the US alone since the 1990s. This disease caused by the fungal pathogen *Fusarium graminearum* results in the loss of yield, quality, and food safety in wheat due to its production of trichothecene mycotoxin. The trichothecene, deoxynivalenol (DON), is responsible for both the spread of the disease within the host as well as the inhibition of protein synthesis when consumed resulting in symptoms such as anorexia, vomiting, and immune response alteration. As the primary agent responsible for FHB in wheat, *F. graminearum* forms its toxin while under specific environmental conditions such as low pH, light, and nutrient-limited environments. It has been established that the trichothecene biosynthetic pathway is expressed through the TRI gene cluster Expression of this pathway is regulated by two particular members of the TRI family: TRI6 and TRI10. Studies have shown that disruption of either regulator results in complete suppression of DON production rendering the fungus non-pathogenic. Our interests lie in further exploring the regulation of DON synthesis to uncover novel regulatory pathways that contribute to mycotoxin production and *F. graminearum* virulence. To do so, we have created a protein-protein interaction map of genes that are co-regulated during DON production. We will discuss the hypotheses we have generated from our preliminary analysis of this network and our efforts to functionally test them.

270F Characterization of a 26S protease ortholog, *CpPts1*, from *Cryphonectria parasitica* in response to several stress. *Yo-Han Ko*[1], Jung-Mi Kim[2], Jyotiranjan Bal[1], Kum-Kang So[1], JeeSun Chun[1], Seung-Moon Park[3], Moon-Sik Yang[1], Dae-Hyuk Kim[1] 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Department of Bioactive Material Sciences, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Chonbuk 570-749, Korea; 3) Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan, Jeonbuk 570-752, Republic of Korea.

The chestnut blight fungus, *Cryphonectria parasitica*, and its hypovirus comprise an useful model system to study the mechanisms of hypoviral infection. Pathoproteomic analysis revealed a protein spot, showing upregulation in the presence of Cryphonectria hypovirus1 (CHV1) and tannic acid supplementation. The corresponding protein spot was identified as the 26S protease homologue of *Cryphonectria parasitica* by tandem mass analysis. Sequence analysis of cDNA revealed that, it comprised of four exons, and three introns. It also contained a canonical AAA (ATPase associated with various activities) domain, consisting of Walker A motif and Walker B motif, which are involved in binding and hydrolysis of ATP. The encoded protein had a predicted mass of 51.8 kDa and a pI of 5.05. Sequence similarity and phylogenetic analysis indicated that the cloned gene encodes a homolog of the 26S protease of *C. parasitica* and refers as *CpPts1*. Northern blot analysis confirmed that the transcript of the cloned gene was upregulated by CHV1. Functional analysis of the cloned gene was conducted using gene deletion analysis. We obtained a *CpPts1*-null mutant among 192 candidates. The *CpPts1*-null mutant showed similar phenotype as that of wild type strain on PDAmb media. However, when the *CpPts1*-null mutant was challenged by the CHV1 infection, dramatic changes in colonial growth and morphology were observed.

271W Comparative Phosphoproteomics of the Pga1-mediated heterotrimeric G protein signaling pathway in *Penicillium chrysogenum*. *U. Carrasco-Navarro*[1], R. O. García-Rico[2], R. Vera-Estrella[3], B. J. Barkla[4], F. Fierro[1] 1) Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, México; 2) Departamento de Microbiología, Universidad de Pamplona, Colombia; 3) Instituto de Biotecnología, Universidad Nacional Autónoma de México, México; 4) Southern Cross Plant Science, Southern Cross University, Australia.

Introduction. The *Penicillium chrysogenum* heterotrimeric Gα protein Pga1 regulates several cellular processes and the biosynthesis of penicillin (**1, 2**). We have recently performed a proteomic analysis of the Pga1-mediated signaling pathway, finding two phosphorylated proteins as effectors of the pathway (**3**). Protein phosphorylation plays a important role in signal transduction pathways. To advance in the characterization of the Pga1 signaling pathway we have performed a comparative phosphoproteomic analysis using mutant strains with different levels of Pga1 activity and varying cAMP intracelular concentrations, aiming to find changes in the phosphorylation patterns of proteins between strains in order to identify new effectors and gain insight in the functioning of this signaling pathway. **Methods**: Two different methodologies were used: 2D-DIGE with phosphoprotein-enriched extracts followed by identification of individual spots by LC-MS/MS, and a *Label-Free* phosphoproteomics approach with phosphopeptide-enriched extracts (using TiO_2 affinity chromatography) submitted to LC-MS/MS. Three strains were used: Wis54-1255, *Δpga1* and G42Rpyr-T, plus a fourth condition: *Δpga1*+cAMP (increased cAMP intracelular concentration). **Results**: The 2D-DIGE analysis showed changes in abundance of phosphorylated proteins when comparing the strains, but little information on differential phosphorylation. The *Label-Free* phosphoproteomic analysis showed changes in the phosphorylation patterns of proteins between strains, which allowed to identify new effectors of the Pga1 signaling pathway. One of these proteins was Pc16g10400 (protein with a Bromo Adjacent Homology domain and a PHD zinc finger domain), with two phosphorylation sites at S-738 and S-742, of which the S-742 site is present only in the *Δpga1* strain. **Conclusions**: New effectors of the Pga1 signaling pathway were identified by comparative phosphoproteomic analysis. **References**: **1**) Res Microbiol. 2007;158(5):437-46. **2**) Microbiology. 2008;154:3567-78. **3**) Microb Cell Fact. 2016 Oct 6;15(1):173.

272T Proteome adaptations during growth on beech wood of the early sapwood decay fungi *Trametes versicolor* and *Schizophyllum commune* . Amjad Zia, Andrzej Majcherczyk, *Ursula Kües* Molecular Wood Biotechnology, University of Göttingen, Göttingen, DE.

Trametes versicolor with 14,296 predicted genes in its genome is an aggressive white rot wood degrader. It reduced beech wood weight within 90 days by 51% in dark, by 43% in light and by 47% in a 12 h dark/12 h light scheme. Light conditions had effects on the fungal morphology. The surface mycelium in light and in light/dark was massive and primordial fruiting body structures were regularly formed. In dark, surface mycelium was sparse without any aggregated structures. Therefore, distinct extra- and intracellular proteomes are expected to be produced under different illumination schemes. In first attempts to unravel the secretome of *T. versicolor* by LC-MS, extracellular proteins were isolated by different protocols from wood samples incubated in dark. Results distinguished in the number of isolated secreted proteins (between 181 and 224) and contaminations by intracellular proteins (between 77 and 414). In total, 142 secreted proteins were common in all extractions. Among the extracellular proteins identified so far from *T. versicolor* are ligninolytic enzymes (laccase, peroxidase; 6%), enzymes acting in carbohydrate metabolism (e.g. carbohydrate esterase, glycoside hydrolase, glucose dehydrogenase; 44%), cell wall/membrane biogenesis proteins (lysophospholipase, acetylcholinesterase; 4%), protein degrading enzymes (e.g. serine protease, peptide hydrolase, aspartyl peptidase, carboxypeptidase; 14%), phosphatases (acid phosphatase, phosphoglycerate mutase; 3%), and a few enzymes of other functions (6%). 23% of the identified secreted proteins have no known function. *T. versicolor* is in nature often found on wood together with *S. commune*. This fungus is positioned in between white and brown rot and is depicted as weak wood degrader (weight losses of beech wood within 90 days: 4% in dark, 3% in light and in a 12 h dark/12 h light scheme). Surface mycelium of *S. commune* on wood under all conditions was thin and there were no fruiting bodies. The fungus (13,210 predicted genes) is equipped with restricted groups of lignocellulolytic genes. Further experiments will target on production of proteins from early to late wood decay of both species alone and in dual culture in order to study the possible types of interactions that occur between the fungi.
Our work is supported by the Ministry of Lower Saxony by the MaFoHolz PhD program and by DFG funding for a TripleTOF 5600 mass-spectrometer (DFG-GZ: INST 186/1085-1 FUGG).

273F Using meta-transcriptomics to dissect the *Ocimum basilicum* and *Peronospora belbahrii* interaction. *G. DeIulio*[1], R Pyne[2], J Simon[2], L Ma[1] 1) UMass Amherst, Amherst, MA; 2) Rutgers University, New Brunswick, NJ.

The production of sweet basil (*Ocimum basilicum*) is threatened by the Oomycete downy mildew pathogen *Peronospora belbahrii*. However, economically viable cultivars lack usable resistance traits while some economically inviable cultivars display resistance. Through a comprehensive genetic breeding program, a resistant cultivar MRI showing resistance to *P. belbahrii* was selected. A previous genetic study suggested the presence of two potential resistance genes in MRI. This research dissects the *Ocimum basilicum* and *P. blbahrii* interaction to identify these potential resistant genes. We will compare the meta-transcriptomics of *P. belbahrii* infecting the resistant cultivar MRI with *P. belbahrii* infecting a closely related but susceptible sweet basil cultivar SB22. We have sequenced total mRNA of *P. belbahrii* infected tissue from cultivars MRI and SB22 at four times over the course of 72 hours post inoculation. Using meta-transcriptomics we will identify candidate basil resistance genes *P. blbahrii* resistant cultivar MRI for further downstream characterization.

274W Evolution of Plant Penetration by Fungal Pathogens. *Cristina Miguel Rojas*[1], Zheng Wang[2], Jeffrey P. Townsend[2], Frances Trail[1,3] 1) Department of Plant Biology, Michigan State University, 612 Wilson Rd, East Lansing, MI, 44824; 2) Department of Biostatistics, Yale School of Public Health, Yale University, 135 College Street, New Haven, CT 06520-8106; 3) Department of Plant, Soil and Microbial Sciences, Michigan State University, 612 Wilson Rd, East Lansing, MI, 44824.

Fungal spores are responsible for initiation and propagation of the majority of biotic plant diseases. Although spore germination is the first step in most fungal diseases, the genetics of spore germination has never been comparatively explored across multiple fungal lineages. Here we use comparative transcriptomics of spore germination among five fungal plant pathogens and one saprotroph representing two fungal classes, to determine how expression of orthologous genes has changed during evolution, and to predict genes whose knockouts will exhibit phenotypic differences in the spore germination and host penetration processes. We have chosen fungi which represent different approaches to plant penetration. *C. graminicola* and *M. oryzae* form melanized appressoria, while *F. graminearum* penetrates through natural openings associated with flowers, lacking melanization. Finally, *V. inaequalis* produces an intermediate form, with a melanized ring at the base of the appressorium. To provide a basis for comparison among species and to identify infection-specific expression patterns, we compare transcriptional profiles during germination on a single defined medium (saprotrophic conditions) as well as differences during germination on hosts (plant pathogens) and natural substrates (saprotroph). We will estimate ancestral gene expression for orthologous genes common among all species to identify genes that undergo transcriptional shifts during the spore germination process, as well as those that are unique to infective germination, and those that are unique to specific fungi. Lastly, functional assays of a subset of genes exhibiting species-specific and infection-type specific upregulation will be performed to assess the roles of these genes in conidial germination in these fungi. These experiments contribute to our understanding of how shifts in gene expression drive the evolution of conidial germination in a wide range of fungi.

275T Comparative genomics of *Ashbya gossypii* and *Saccharomyces cerevisiae*. *Fred S. Dietrich*, Jonathan Grego, Andrew Wilcox MGM, Duke Univ, Durham, NC.

The hyphal fungus *Ashbya gossypii* is a member of the Saccharomycetaceae. While it is closely related to *S. cerevisiae* it has an unduplicated genome that is overall about 3Mb smaller than *S. cerevisiae*. While *S. cerevisiae* lives in a wide range of environments, *A. gossypii* lives only in the gut of specific insects, the Milkweed bug and members of the genus *Dysdercus*. In comparison of the gene sets between these species *A. gossypii* share a core set of genes, but there are many additional genes found in *S. cerevisiae.* We are using this comparison to identify genes of novel function including a putative epoxide hydrolase of bacterial origin in some strains of *S. cerevisiae,* and a 5'-oxoprolinase gene of bacterial origin in other *S. cerevisiae* strains. We will be discussing what is the core set of genes and what are the variable genes in these organisms, and the mechanisms by which additional genes are acquired in these fungi. These comparisons take advantage of the genome sequences species related to *A. gossypii*, including *A. aceri, Holleya sinecauda*, *Eremothecium coryli*, and *Eremothecium cymbalariae,* as well as the tremendous diversity seen among the more than 100 *S. cerevisiae* genomes that have been sequenced.

276F Evolution of secondary metabolism and host association in insect pathogens. *Kathryn E. Bushley*[1], Stephen A. Rehner[2], Joseph W. Spatafora[3] 1) Plant and Microbial Biology, University of Minnesota, Saint Paul, MN; 2) Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, MD; 3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Fungal secondary metabolite genes including nonribosomal peptide synthetases, polyketide synthases, terpenes, and alkaloids are among the most rapidly evolving of fungal genes. They respond to selective pressures in the environment, often enabling fungi to adapt to specific environments or parasitize specific hosts. We investigate population genomic variation of secondary metabolite genes and clusters in two insect pathogenic fungi, the beetle pathogen *Tolypocladium inflatum* and the wide host-range insect pathogen and biocontrol agent *Beauveria bassiana*. Using Pac Bio single molecule real time sequencing, *we* improved the resolution of the sequenced reference strain *T. inflatum* NRRL 8044 and have assembled de novo genomes of additional strains isolated from distinct environments. For the reference strain and one additional strain, we have also used a Hi-C chromosome mapping approach to provide evidence for chromosomal level assemblies. The nearly complete chromosomal assemblies produced using these methods have allowed investigation of fine-scale evolutionary genetic mechanisms contributing to the rapid evolution of secondary metabolite genes and clusters and to evaluate the relative contributions of transposition, duplication/deletion, cluster rearrangement, and horizontal transfer in cluster evolution. Similarly, using Illumina technology, we have sequenced ten strains of *B. bassiana* and several outgroup *Beauveria* species *(B. brongniartii, B. asiatica, B. australis)* to analyze the evolution of secondary metabolite clusters and other genes involved in host-specific interactions (e.g. G-protein coupled receptors, SSPs). In addition to being an insect pathogen, *B. bassiana* has recently been shown to colonize a wide-range of plant species as an endophyte. Differential expression in plant and insect infection assays examines the role(s) of specific metabolites and other genes in recognition, colonization, and pathogenesis of plant versus insect hosts. The results of our analysis shed light on the role of metabolites in shaping the interaction of these fungi with distinct hosts and in turn on the effects of host-specialization versus generalism in driving the evolution of metabolite clusters.

277W Identifying more than 300 biosynthetic gene clusters with potential resistance genes in over 75 *Aspergillus* species using resistance gene-guided genome mining. *I. Kjaerboelling*[1], T.C. Vesth[1], J.C. Frisvad[1], J.L. Nybo[1], S. Theobald[1], I.V. Grigoriev[2], A. Salamov[2], T.O. Larsen[1], U.H. Mortensen[1], M.R. Andersen[1] 1) Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, DK; 2) Joint Genome Institute, Walnut Creek, CA, USA.

With the increasing number of genome sequences, an increasing number of orphan /cryptic secondary metabolite biosynthetic gene clusters (BCGs) has followed. These clusters potentially encode pathways for bioactives, however linking genes to compound and elucidating bioactivity requires extensive work. Thus, the question remains how to select the most interesting clusters in a rational manner and find their bioactivity. By using Resistance Gene-Guided Genome mining, it is possible to identify novel bioactive compounds and their clusters.

The hypothesis for this approach is that some BGCs include a gene which is a resistant form of the enzyme targeted by the compound produced by the cluster. In filamentous fungi, this mechanism has been seen for mycophenolic acid as well as Fellutamide B. By using this knowledge and a large number of fungal genomes, we have set up a pipeline, which extracts predicted clusters with potential resistance genes.

A total of over 300 clusters were found to exhibit this specific pattern by running our algorithm on more than 75 *Aspergillus* species and 5000 (SMURF) predicted gene clusters. With further filtering and analysis, we ended up with a manageable number of potential resistance genes and clusters. The filters used in this algorithm will return clusters where one gene in the cluster has a homolog conserved in 98% of the examined species. Although we use a conservative approach it ensures that the identified clusters are most likely of interest. Selected clusters and resistance genes are now being validated experimentally both to confirm the resistance mechanism and to identify the compounds produced.

278T Genus level analysis of secondary metabolism reveals the origin of hybrid NRPS-PKS gene clusters. *S. Theobald*[1], T.C. Vesth[1], J.L. Nybo[1], I. Kjærbølling[1], J.C. Frisvad[1], K.F. Nielsen[1], T.O. Larsen[1], I.V. Grigoriev[3], A. Salamov[3], U.H. Mortensen[1], S.E. Baker[2], M.R. Andersen[1] 1) Department of Biotechnology and Biomedicine, Technical University of Denmark, Lingby, DK; 2) Joint Bioenergy Institute, Berkeley, CA, USA; 3) Joint Genome Institute, Walnut Creek, CA, USA.

The World Health Organization is reporting a rising number of multiple drug resistant pathogens every year, increasing the need for new drug development. However, current methods for natural product discovery rely on time consuming experimental work, making them unable to keep up with this demand.

In the Aspergillus genus sequencing project, we are sequencing and analyzing over 300 species of Aspergilli, a group of filamentous fungi rich in natural compounds. Natural products are encoded by genes located in close proximity, called secondary metabolic gene clusters (SMGC), which makes them interesting targets for genomic analysis. Important classes of SMGC include non ribosomal peptide synthetases (NRPS), polyketide synthases (PKS) and hybrids thereof.

We use a modified version of the Secondary Metabolite Unique Regions Finder (SMURF) algorithm, combined with InterPro annotations, network analysis and approximate maximum likelihood (ML) trees of conserved domains, giving insights into the secondary metabolism gene diversity and evolution. In this study we describe the diversity of hybrid SMGC among Aspergilli, horizontal gene transfers within and outside Aspergilli and identify possible ancestors of hybrids.

ML analysis indicated PKS-NRPS hybrids being of bacterial origin and having an ancestor cluster in the Streptomyces and Achromobacter genus. NRPS-PKS hybrids showed a wide diversity with orthologs being found in different Ascomycete classes like Dothideomycetes, Eurotiomycetes, Sordariomycetes and others. Here, our genus level study enables us to determine which hybrids might have been horizontally transferred to the precursor of a whole section, driving speciation of this section by a selective advantage. Examining these events helps us to identify the origin of secondary metabolites in Aspergilli and also provides us with new variants of compounds for industrial applications.

279F Comparative genomic analysis of secondary metabolite biosynthetic gene clusters in 207 isolates of *Fusarium*. *H.-S. Kim*, R.H. Proctor, D.W. Brown Mycotoxin Prevention and Applied Microbiology, USDA, ARS, NCAUR, Peoria, IL.

Fusarium species are known for their ability to produce secondary metabolites (SMs), including plant hormones, pigments, mycotoxins, and other compounds with potential agricultural, pharmaceutical, and biotechnological impact. Understanding the distribution of SM biosynthetic gene clusters across the phylogenetic diversity of *Fusarium* should provide insight into how changes in selection for production of SMs affect genome dynamics in the fungus. Using genome mining tools, such as antiSMASH and OrthoFinder, we identified known, novel, and putative SM gene clusters in 207 genomes from 159 species representing 25 species complexes of *Fusarium*. We found multiple homologs of 45 gene clusters responsible for biosynthesis of known mycotoxins and other SMs (i.e., trichothecenes, fumonisins, zearalenone, and fusaric acid). A total of 9403 SM gene clusters were detected in the 207 genomes, including 2276 clusters with non-ribosomal peptide synthetase genes, 2267 with polyketide synthase genes, and 1743 with terpene synthase genes. Closely related species within a species complex have similar numbers of SM clusters. For example, members of the *F. fujikuroi* species complex had 43 – 61 SM clusters, whereas distantly related species from different species complexes had 26 – 68 SM clusters. The comparative genomic analysis indicated that the gibberellin (plant hormone), fumonisin and zearalenone (mycotoxins) gene clusters have limited distribution, whereas the carotenoid and fusarubin (pigments) clusters are present in all members of most species complexes; they are absent only in some basal lineages of *Fusarium.* These data indicate that SM gene clusters differ markedly in when they were acquired and how long they have persisted during the evolutionary diversification of *Fusarium.*

280W Genomic and bioinformatics analyses of biosynthesis and production enhancement of a novel antifungal antibiotics, FR901469, from a filamentous fungus. *Masayuki Machida*[1,4], Makoto Matsui[2,4], Tatsuya Yokoyama[2,4], Hiroya Itoh[2,4], Akira Ohyama[3,4], Takashi Shibata[2,4] 1) Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo, Japan; 2) Biotechnology labs, Astellas Pharma Inc., Japan; 3) in silico biology, inc., Yokohama, Japan; 4) Technology Research Association of Highly Efficient Gene Design (TRAHED), Tokyo, Japan.

A novel antifungal antibiotics, FR901469, is a non-ribosomal peptide (NRP) compound produced by a filamentous fungus, No. 11243. We have sequenced an entire genome of this strain, revealing genome size of 21.7 Mb with 9,920 predicted genes. Of the 4 putative non-ribosomal peptide synthetase (NRPS) genes existing on the genome, we have identified a 45 kbp NRPS gene for the core biosynthesis of FR901469 and associated nine genes as a gene cluster of 79 kbp in length. To date, fourteen mutants producing higher amount of FR901469 than the parental strain (up to 10 times) have been obtained by UV irradiation. Genome-wide mutation analysis revealed 14-183 mutations for each strain, among which those for genes categorized as “Replication, recombination and repair”, “Signal transduction mechanisms” and “Transcription” are highly enriched. Interestingly, the region consisting of approximately 1.6 Mbp in size that showed 50% higher depth of mapping of the short reads from NGS possessed the FR901469 biosynthesis gene cluster above. Transcription expression analysis by RNA-sequencing revealed strong relationship between the expression of NRPS and the FR901469 productivity. In accordance of this result, artificial overexpression of a C6 zinc cluster transcription factor found in the gene cluster of the parental strain showed 3- to 4-times higher production of FR901469. Besides the enhancement of transcription expression of the cluster member genes, we have found genes relating to amino acid biosynthesis were overexpressed commonly in the mutant strains as well.
During the progress above, we have developed a software tool to further analyze secondary metabolism (SM) gene clusters, In silico Molecular Cloning Genome Design Suite (IMCDS). Based on the unique feature of this tool, by which multiple clusters are aligned according to the sequence similarity of each cluster member gene, and in combination with MIDDAS-M and MIPS-CG, which we previously developed to predict SM gene cluster in a sequence motif-independent manner, further characterization of the FR901469 biosynthesis gene cluster will be discussed

281T Genetic drivers of diversity in secondary metabolic gene clusters in Aspergillus fumigatus populations. *Abigail Lind*[1], Jennifer Wisecaver[2], Fernando Rodrigues[3], Gustavo Goldman[4], Antonis Rokas[2] 1) Biomedical Informatics, Vanderbilt University, Nashville, TN; 2) Biological Sciences, Vanderbilt University, Nashville, TN; 3) Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal; 4) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil.

Filamentous fungi produce a diverse array of secondary metabolites that play ecological roles in defense, virulence, and inter- and intra-species communication. The biosynthetic genes required for the production of individual secondary metabolites are arranged in contiguous genomic clusters. These pathways are narrowly taxonomically distributed and highly diverse between species, and are among the most fast-evolving protein-coding elements in filamentous fungal genomes. To gain insight into the diversity of secondary metabolic gene clusters in fungal populations and its drivers, we examined the conservation and divergence of secondary metabolic gene clusters across the genomes of 40 representative isolates of the cosmopolitan opportunistic pathogen *Aspergillus fumigatus*. We found that a core set of secondary metabolic gene clusters were present in all isolates, but that other gene clusters were only present in subsets of isolates. We additionally identified multiple different types of gene cluster polymorphisms, including fusion of different gene clusters, alternative genomic locations for gene clusters, and clusters with multiple alternative idiomorphs (i.e., non-homologous alleles). In particular, two gene clusters flanked by transposable elements were consistently found in different genomic locations; one of these clusters showed a phylogenetic pattern consistent with horizontal gene transfer between fungi. Our results suggest that multiple genetic factors, including recombination, gene loss, and horizontal gene transfer, drive the diversification of secondary metabolism pathways.

282F A forward genetic screen coupled with a novel target enrichment sequencing approach reveals new insight into the regulation of cercosporin biosynthesis in *Cercospora zeae-maydis*. *B.H. Bluhm*, S. Sharma, J. Ridenour, B. Dhillon Plant Pathology, University of Arkansas, Fayetteville, AR.

Cercospora zeae-maydis causes gray leaf spot of maize (*Zea mays*), a disease that affects maize production throughout the world. During pathogenesis, *C. zeae-maydis* produces cercosporin, a non-host-specific phytotoxin that is an important virulence factor for many species of *Cercospora*. Although the gene cluster underlying cercosporin biosynthesis has been identified, the genetic regulation of cercosporin biosynthesis has not been elucidated. In this study, a forward genetic screen for aberrant cercosporin production was coupled with a novel, target enrichment sequencing approach to identify genes involved in the regulation of cercosporin biosynthesis. Over 3000

genetically tagged mutants of *C. zeae-maydis* were created and screened for altered levels of cercosporin production. A total of 75 mutants produced significantly higher or lower levels of cercosporin compared to the wild-type strain. To characterize the site(s) of insertion in each mutant, biotinylated oligonucleotides were designed to probe the borders of the mutagenesis cassette and selectively enrich cassette-genome break junctions. After target enrichment, bar-coded libraries were sequenced with the Ion Torrent PGM platform. This approach identified 49 mutants with single insertions of the mutagenesis cassette, and 26 mutants with multiple insertions. Novel genes potentially regulating cercosporin biosynthesis were identified, including genes involved in signal transduction, primary and secondary metabolism, growth and development, and stress responses. Additionally, one mutant contained an insertion in *CTB1*, a polyketide synthase gene previously demonstrated to be involved in cercosporin biosynthesis. RNAi lines created for seven of the single-copy mutants confirmed associations between genes disrupted by insertional mutagenesis and cercosporin-related phenotypes. This approach has shed new light on the genetic regulation of cercosporin biosynthesis, and could easily be adapted to dissect the genetic basis of secondary metabolism in a wide range of filamentous fungi.

283W Genome-wide survey of sexual stage-dependent non-coding RNAs in *Fusarium graminearum*. *W. Kim*, F. Trail Department of Plant Biology, Michigan State University, East Lansing, MI.

Although the global functional significance of non-coding RNAs (ncRNAs) remains controversial, there has been growing evidence that ncRNAs play critical roles in morphological transition and meiosis in yeasts. In filamentous fungi, however, little is known about the role of ncRNAs during the sexual development. Here we identified sexual stage-dependent ncRNAs in *F. graminearum*, a model for studying sexual development. To discover novel ncRNAs expressed during the sexual development of *F. graminearum*, transcripts were assembled from time-series transcriptome data across the sexual development, and compared to the reference annotation. Among the novel transcripts not annotated in the reference genome, transcripts harboring little coding potential and differentially expressed (DE) in at least one-time point during the developmental stages were classified as sexual stage-dependent ncRNAs (DE-ncRNAs). A total of 578 DE-ncRNAs were identified and the expression patterns of the DE-ncRNAs were largely developmental stage-dependent, while only 20% of the DE-ncRNAs (113 out of 578) were expressed during asexual spore germination. Interestingly, many of the DE-ncRNA expressions (243 out of 578) peaked at the stage of ascus formation during which meiosis occurs. Many of the DE-ncRNAs were antisense to protein-coding genes, forming 357 sense–antisense pairs. Functional enrichment analyses showed that the sense genes were significantly enriched for the Gene Ontology term 'DNA metabolism', raising a hypothesis that DE-ncRNAs play regulatory roles in DNA synthesis and degradation. In addition, DE-ncRNAs were searched against the RNAcentral database v5 (http://rnacentral.org) to find ncRNAs conserved in other organisms. Functional roles of selected DE-ncRNAs are currently under investigation. This study will provide a foundation for functional characterization of ncRNAs during the sexual development.

284T Comparative genomics and transcriptomics of sexual development in a nematode-associated strain of *Fusarium neocosmosporiellum*. *W. Kim*[1], B. Cavinder[1], R.H. Proctor[2], K O'Donnell[2], Z. Wang[3], J.P. Townsend[3], F. Trail[1] 1) Department of Plant Biology, Michigan State University, East Lansing, MI; 2) Mycotoxin Prevention and Applied Microbiology Research Unit, USDA, Peoria, IL; 3) Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT.

Fusarium neocosmosporiellum (formerly *Neocosmospora vasinfecta*) is a ubiquitous saprobic fungus that has been isolated from plants, fungi, nematodes, dung and soil. It is phylogenetically placed in a clade near a lineage of fusaria farmed by ambrosia beetles within the *F. solani* species complex. Here we obtained the genome sequence of *F. neocosmosporiellum* NRRL 22166 strain isolated from soybean cyst nematode to conduct a comparative study with *F. graminearum*, a model for studying sexual stage (perithecium) development. Completeness of the sequenced genome was quantified by the presence of core eukaryotic genes and shown to be comparable to that of the finished *F. graminearum* genome. The *F. neocosmosporiellum* mating type locus included both *MAT1-1* and *MAT1-2* idiomorphs, but the gene order differed from that in *F. graminearum*. We identified several secondary metabolite gene clusters that exhibited increased expression during perithecial development, including *pksN* gene responsible for red perithecial pigmentation. To dissect transcriptional changes during perithecial development, we performed a time-series transcriptomic analysis and identified genes that are differentially expressed (DE) between two successive developmental stages. Overall, many DE genes were upregulated in later stages. Functional enrichment analyses showed that upregulated DE genes were enriched for Gene Ontology (GO) terms 'protein phosphorylation' and 'lipid metabolism' at the beginning of perithecia formation, and enriched for GO terms related to meiosis during ascus formation. Unlike *F. graminearum*, perithecia of *F. neocosmosporiellum* exhibited a marked increase in size at the stage immediately preceding ascus formation. Concordantly, *F. neocosmosporiellum* genes upregulated during this increase were enriched for GO terms 'transcription' and 'carbohydrate transport'. This study provides a comprehensive understanding of transcriptional changes during sexual development in *F. neocosmosporiellum*, a potential biological control agent for soybean cyst nematode.

285F The Bck1 homolog gene, *CpBck1*, from the chestnut blight fungus *Cryphonectria parasitica* is related to sectorization and changes in DNA methylation. *Kum-Kang So*[1], Yo-Han Ko[1], Jyotiranjan Bal[1], Jeesun Chun[1], Junhyun Jeon[2], Jung-Mi Kim[3], Jaeyoung Choi[4], Yong-Hwan Lee[4], Jin Hoe Huh[5], Dae-Hyuk Kim[1] 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Department of Bioactive Material Sciences, Chonbuk National University, Jeonju, Chonbuk 561-756, South Korea; 2) Department of Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk, 38541, South Korea; 3) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Chonbuk 570-749, South Korea; 4) Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea; 5) Department of Plant Science, Seoul National University, Seoul 151-921, Korea.

Mutation in *CpBck1*, an ortholog of the cell wall integrity MAPKKK of *Saccharomyces cerevisiae*, of the chestnut blight fungus *Cryphonectria parasitica* resulted in a sporadic sectorization as the culture proceeded. The progenies from the sectored area maintained the characteristics of sector, a massive morphogenetic change including robust mycelial growth without differentiation. Epigenetic changes as the genetic reason for this sectorization were investigated. Quantification of DNA methylation and whole-genome bisulfite sequencing revealed the genome-wide DNA methylation of the wild-type at the each nucleotide level and changes in the DNA methylation of the sectored progeny. Compared to the wild type, the sectored progeny exhibited marked genome-wide DNA hypomethylation. *In trans* complementation of the sectored progeny of the *CpBck1*-null mutant using the wild-type *CpBck1* gene recovered the phenotype as well as methylation indicating that the phenotypic and epigenetic changes was due to the *CpBck*1 gene. In addition, functional analysis using

mutant phenotypes of corresponding DNMTases demonstrated that a mutant of *CpDmt1*, an ortholog of RID of *Neurospora crassa*, resulted in the sectored phenotype but the *CpDmt2* mutant suggesting the genetic basis of fungal sector appeared to be more complex. The current study revealed that a mutation in a signaling pathway component resulted in sectorization accompanied with changes in genome-wide DNA methylation, which suggests that this signal transduction pathway is important for epigenetic control of sectorization via regulation of genes involved in DNA methylation.

286W Functional characterization of *CpSlt2*, yeast *SLT2* homologue, from the chestnut blight fungus *Cryphonectria parasitica*. *Kum-Kang So*[1], Jung-Mi Kim[2], Kwang Yeop Jahng[3], Dae-Hyuk Kim[1] 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Department of Bioactive Material Sciences, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea; 2) Department of Bio-Environmental Chemistry, Institute of Life Science and Natural Resources, Wonkwang University, Iksan, Chonbuk 570-749, Korea; 3) Department of Life Sciences, Chonbuk National University, Jeonju-si, 561-756, Republic of Korea.

In fungi, mitogen-activated protein kinases (MAPKs) are involved in pathways that are required for numerous processes related to growth and differentiation. The sequential activation of the MAPK cascade eventually results in the activation of transcription factors and the expression of specific set of genes in response to environmental stimuli. Interestingly, the MAPKs play important roles in the pathogenicity of various pathogenic fungi. The *SLT2* mitogen-activated protein kinase has been well studied in *Saccharomyces cerevisiae* and is required for cell wall integrity. Functional characterization of the *SLT2* homologue in several pathogenic fungi has indicated that this MAPK is well conserved among fungal pathogens and plays important roles during plant infection. In this study, we cloned a yeast *SLT2* homologue, *CpSlt2*, from the chestnut blight fungus *Cryphonectria parasitica* and disrupted *CpSlt2* gene for examining gene functions. Analysis of *CpSlt2* mutant revealed defects in sporulation, vegetative growth, aerial mycelia, and pigmentation. The *CpSlt2*-null mutant showed increased sensitivity to the cell wall-disturbing agents and electron microscopy revealed the presence of intrahyphal hyphae. In addition, the sectorization was observed in *CpSlt2*-null mutant and sectored phenotypes were maintained in the progenies. The phenotypic changes of the *CpSlt2*-null mutant were recovered through *in trans* complementation of the *CpSlt2* gene, indicating the *CpSlt2* gene was responsible for these changes. These results indicate that putative biological functions of *CpSlt2* are related to cell wall integrity and sectorization.

287T Identification and characterization of necessary sites on Ran-binding protein 1, essential for survivability of *Cryphonectria parasitica*, through heterokaryon and gene disruption mutant analysis. So Yeun Choi[1], *Yo-Han Ko*[1], Kum-Kang So[1], Myeongjin Jo[1], Jeesun Chun[1], Jung-Mi Kim[2], Dae-Hyuk Kim[1] 1) Institute for Molecular Biology and Genetics, Department of Bioactive Material Sciences, Chonbuk National University, Jeonju , Chonbuk 54896, Korea; 2) Department of Bio-Environmental Chemistry, Institute of Life Science and Natural Resources, Wonkwang University, Iksan, Chonbuk 54538, Korea.

The chestnut blight fungus, *Cryphonectria parasitica*, and its hypovirus is known to be a useful model system to study the mechanisms of hypoviral infection. So we attempted a proteomic analysis of the virus-free EP155/2 strain and its isogenic virus-infected UEP1 strain in response to tannic acid(TA), which is abundant in the bark of the chestnut trees. The patho-proteomic analysis presented a differential-regulated protein spot, which showed a high similarity to the known fungal Ran-binding protein 1. The corresponding gene was determined by inspection of the draft genome sequence of *C. parasitica* (http://genome.jgi-psf.org/Crypa1/Crypa1.home.html). The cloned gene was predicted to encode a protein product containing a unique Ran-binding domain. Sequence similarity and phylogenetic analysis indicated that the cloned gene encodes a homolog of the Ran-binding protein 1 of *C. parasitica* and refers as *CpRbp1*. For function analysis, we constructed the *CpRbp1*-null mutant through homologous recombination and obtained two mutant candidates. PCR analysis and southern analysis revealed that both transformants were heterokaryons consisting of mixture of the wild-type and null-mutant nuclei. We designed several complementing vectors of *CpRBP1* based on the conserved Ran-binding protein 1 domains. The complementing experiments allowed function test of specific domain and amino acid residue of the *CpRbp1* gene, which helps us to understand the essential domains or residues within a lethal gene in a filamentous fungus

288F Characterization of a GSP1/Ran ortholog gene, *CpRan1*, from a Chestnut blight fungus, *Cryphonectria parasitica* through point-mutation based heterokaryon analysis. *Yo-Han Ko*[1], Jung-Mi Kim[2], Jyotiranjan Bal[1], Kum-Kang So[1], So Yeun Choi[1], Dae-Hyuk Kim[1] 1) Institute for Molecular Biology and Genetics, Center for Fungal Pathogenesis, Department of Bioactive Material Sciences, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea; 2) Department of Bio-Environmental Chemistry, Wonkwang University, Iksan, Chonbuk 570-749, Korea.

Pathoproteomic analysis revealed a protein spot down regulated by the presence of Cryphonectria hypovirus1 (CHV1) and tannic acid supplementation. The corresponding protein spot was identified as the GSP1/Ran homologue of *Saccharomyces cerevisiae* by tandem mass analysis. The Ran (Ras-related nuclear protein) also known as GTP-binding nuclear protein is a protein that is involved in transport into and out of the cell nucleus during interphase and also in mitosis. It is a member of the Ras superfamily. Through genomic sequence analysis, a near full-length cDNA clone was obtained. As a result, sequence comparison with the corresponding genomic sequences revealed that the cloned gene consisted of five exons, with four intervening sequences. The deduced protein product consisted of 216 amino acids, with an estimated molecular mass of 24.5 kDa and a pI of 6.11. Homology searches using the deduced amino acid sequence indicated that the protein product of the cloned *CpRan* gene is related to other fungal GSP1/Ran homologs from *Verruconis gallopava* (93%), *Fusarium avenaceum* (94%), *Colletotrichum gloeosporioides* (94%), *Trichoderma virens* (95%), *Aspergillus nidulans* (91%) and *Saccharomyces cerevisiae* (87%). Functional analysis of the cloned *CpRan1* was conducted using gene deletion analysis. We obtained the *?CpRan1* mutant from heterokaryotic transformants consisting of mixed nuclei of wild-type and *?CpRan1* genome. Single-spore analysis followed by genetic identification indicated that the cloned *CpRan1* gene is essential for growth. We design several point-mutation structures of the *CpRan1* gene to confirm that which is an essential part of the CpRan1 for succinylation, uniqutination, and phosphorylation.

289W Regulation of plant biomass degradation in Ascomycete fungi. *T. Benocci*[1], M V Aguilar Pontes[1], B Seiboth[2], R De Vries[1] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, Utrecht, Netherlands; 2) Vienna University of Technology - Institute of Chemical Engineering. Vienna, Austria.

Fungi play a major role in the global carbon cycle because of their ability to utilize plant biomass (polysaccharides, proteins, and lignin) as

a carbon source and convert it to small molecular compounds. Due the complexity and heterogenic composition of plant biomass, fungi need to produce a broad range of degrading enzymes, matching the composition of (part of) the prevalent substrate. This process is dependent on a network of regulators that not only control the extracellular enzymes that degrade the biomass, but also the metabolic pathways needed to metabolize the resulting monomers.
Genomic and phylogenetic analysis of the currently known transcriptional regulators involved in this process have revealed a high level of diversity among Ascomycete fungi, with respect to the presence and absence of orthologs of these regulators. Together with results from functional studies this revealed that the organization of plant biomass conversion varies from species to species and is likely linked to their natural environment. Highlights will be presented.

290T Complete genome sequences and comparative analyses of red and white *Antrodia cinnamomea* polyporus mushrooms reveal new insights for fungal life cycle and evolution. *Chia-Ling Chen*[1], Chien-Hao Huang [1,2], Chung-Yu Chen[5], Ko-Yun Lo[1], Wan-Chen Li[1,3], Yi Yang[1], Guo-An Chang[6], Wen-Der Yang[7], Zong-Ming Yeh[5], Ching-Hua Su[4], Ting-Fang Wang[1] 1) Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan; 2) Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan; 3) Institute of Life Sciences, National Defense Medical Center, Taipei 115, Taiwan; 4) Department of Microbiology and Immunology, Taipei Medical University, Taipei 110, Taiwan; 5) Shen Nong Fungal Biotechnology Co. Ltd., Taoyuan 330, Taiwan ; 6) KFK Biotech Co. Ltd., Kaohsiung 802, Taiwan; 7) HIMA Foundation, Taipei 111, Taiwan.
Antrodia cinnamomea (syn. *Taiwanofungus camphoratus*), an endemic mushroom-forming fungus, has long been used in traditional medicine in Taiwan. Most wild dikaryotic strains produce lustrous and orange-red fruiting bodies in 1-2 years or even longer. Up to now, only the genome draft sequence (32.15Mb, 360 scaffolds and 9254 genes) of an orange-red monokaryon has been reported. Deletion of *pks63787,* one of the polyketide synthesis genes, displayed a reduced red phenotype and deficient in the biosynthesis of several aromatic metabolites. There are also few wild dikaryotic strains can develop mushrooms faster (within 3-6 months), but the fruiting bodies are in milky white color. To reveal the phylogenetic relationship between orange-red and white *A. cinnamomea* strains, we isolated four sexually compatible monokaryons (W1, W2, V5, V7). W1 and W2 are derived from the arthorspores of a white dikaryotic strain (SN1), whereas V5 and V7 are from the arthospores of an orange-red dikaryotic strain (HC1). Next, we applied PacBio RSII and Illumina sequencing platforms to these four monokaryons for high-quality genome assembly. Our results reveal that these four monokaryons all have 14 chromosomes. Comparative analyses of gene organization and expression in the four monokaryons and the corresponding dikaryons will be presented.

291F The dynamic and varied genome of the oomycete pathogen *Phytophthora infestans* . *M.E.H. Matson*[1,2], J. Shrivastava[1,2], H.S. Judelson[1,2] 1) Genetics, Genomics, & Bioinformatics Graduate Program, University of California-Riverside, Riverside, CA; 2) Department of Plant Pathology & Microbiology, University of California-Riverside, Riverside, CA.
As the causal agent behind the late blight disease of tomato and potato, the oomycete *Phytophthora infestans* causes considerable crop losses around the world. Controlling epidemics via host-mediated resistance is hampered by the remarkable adaptability of the P*. infestans* genome, since new R gene introductions into commercial varieties are quickly overcome. To investigate mechanisms driving genome plasticity, we used whole genome Illumina sequences of progeny from two sexual crosses and >50 diverse global individuals to identify copy number variation (CNV), structural variation (SV), and loss of heterozygosity (LOH) events. We determined through examining SNP allele ratios that the four parental isolates behind our two sexual crosses were mostly 2N, but that about 10% of progeny were mostly 3N. Local CNVs larger than 5kb comprised around 4.5% of genomic regions which could be uniquely mapped, and affected as many as 400 individual genes depending on the strain. Structural variants in the form of inversions and deletions were also identified among nine examined diverse global isolates using Pindel and Breakdancer. Finally, we observed considerable tracks of LOH among our progeny using the YMAP toolset. To investigate the functional consequence of LOH, we sequenced four progeny which spontaneously produced fast-growing sectors in the presence of the fungicide metalaxyl. These exhibited numerous sequence differences which may reveal the basis of resistance to the fungicide.

292W Comparative Genomics and Transcriptomics of *Panellus stipticus* Strains for Elucidating Fungal Bioluminescence. *B. Min*[1], H. Park[1], J. Byun[1], D. Pathiraja[1], R. Ohm[2,3], R. Riley[2], I. Grigoriev[2], I. Choi[1] 1) Korea University, Seoul, KR; 2) Joint Genome Institute, Walnut Creek, CA, USA; 3) Utrecht University, 3508 TB Utrecht, Netherlands.
Bioluminescence occurs in various clades of organisms, such as marine vertebrates and invertebrates, microorganisms, and in some fungi. Fungal bioluminescence has been reported in mostly mushroom-forming fungi and a few ascomycete. Genome sequences of several bioluminescent fungi are available, including *Omphalotus*, *Armillaria*, and *Panellus*, but the molecular mechanism and genetic basis of bioluminescence has been hardly understood yet. We announced genome sequences of two *Panellus* strains representing luminescent *Panellus stipticus* LUM and non-luminescent *Panellus stipticus* KUC 8834 isolated in Korea. Two strains have similar genome sizes of 57 Mbp and 53 Mbp, respectively. It is assumed that at least two enzymes are related to fungal bioluminescence such as reductase and luciferase enzymes. We predicted candidate gene families involved in bioluminescence from comparative genome analysis of two strains. We focused on differentially expressed transcripts and orphan genes in the LUM genome. Transcriptome analysis was performed to retrieve expression patterns for targeting metabolic pathway for bioluminescence. By comparing two genetically similar but phenotypically different genomes, we suggest potential genetic basis of fungal bioluminescence.

293T Assembling the *Parastagonospora nodorum* genome to the bitter end: long reads and optical mapping for reference genome completion and genome structure insights. *S. Bertazzoni*[1], K.C. Tan[1], R.A. Syme[1], T.L. Friesen[2], B.A. McDonald[3], R.P. Oliver[1], J.K. Hane[1] 1) Centre of Crop Disease Management, Dept. of Environment and Agriculture, Curtin University, Bentley 6102, Western Australia; 2) United States Department of Agriculture-Agricultural Research Service, Northern Crop Science Laboratory, Cereal Crops Research Unit, Fargo, ND, 58102; 3) Institute of Integrative Biology, Plant Pathology Group, Swiss Federal Institute of Technology, ETH Zentrum, LFW, CH-8092 Zürich, Switzerland.
Parastagonospora nodorum causes septoria nodorum blotch (SNB), a prevalent disease inflicting damage to wheat crops worldwide. Necrotrophic fungal pathogens such as *P. nodorum* exploits an inverse gene-for-gene interaction where fungal effectors must be recognised by a specific host receptor for infection to occur. A quality annotated reference genome is critical to discover the pool of

necrotrophic effectors produced by the pathogen. Improved understanding of *P. nodorum*-wheat interactions is an effective way to address yield losses. The previous reference genome for *P.nodorum* was a fragmented assembly which prevented the efficient study of genes, their placement and genome evolution mechanisms. Resequencing of the SN15 strain with the Pacific Biosciences sequencing platform (PacBio) allowed assembly into 36 contigs. Contigs were placed onto a ~38.5 Mbp whole-genome restriction map (OpGen), resulting in 23 chromosomes comprising 37.89 Mbp of assembled sequence with an N50 of ~1.6 Mbp and L50 of 9.
Annotation of coding genes has been updated and manually curated, incorporating new RNAseq data from both *in vitro* and *in planta* experiments and applying new fungal-specific gene and effector prediction softwares. The resulting gene content was improved. Comparisons between SN15, other isolates of *P. nodorum* and the closely related *P. avenaria* show that the smallest (~441Kbp) chromosome exhibits presence-absence variation (PAV) suggesting conditionally dispensable behaviour. The size and PAV patterns are consistent with a previously reported wheat/barley-specific chromosome suggesting that chromosome 23 may potentially harbour host-specific pathogenicity genes or play a role in driving genome plasticity and gene adaptation. We believe this improved genome assembly and the related analyses will assist effector discovery and illuminate new evolutionary studies across the Dothideomycetes.

294F JGI fungal single cell genomics pipeline. *Doina Ciobanu*[1], Alicia Clum[1], Asaf Salamov[1], Wiliam Andreopoulos[1], Steven Ahrendt[1,2], Alisha Quandt[3], Igor Grigoriev[1,2], Timothy James[3], Jan-Fang Cheng[1] 1) Genomics, Joint Genome Institute, Walnut Creek, CA; 2) Plant and Microbial Biology, University of California, Berkeley, CA, 94720, USA; 3) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, 48109, USA.

We developed a single cell genomics pipeline for noncultured fungal species and used it for sequencing seven early diverging fungi: *Blyttiomyces helicus* [Chytridiomycota], *Caulochytrium protostelioides* [Chytridiomycota]*, Rozella allomycis* [Cryptomycota]*, Syncephalis pseudoplumigaleata* [Zoopagomycotina], *Thamnocephalis sphaerospora* [Zoopagomycotina], *Piptocephalis cylindrospora* [Zoopagomycotina], *Dimargaris cristalligena* [Kickxellomycotina], and one Dikarya: *Metschnikowia bicuspidata* [Ascomycota]. The established pipeline for fungal single-cell genome recovery consists of seven steps: environmental sample collection and analysis, single cell isolation, single cell lysis and whole genome amplification, single cell amplified genome (SCAG) quality screening, best SCAG genome deep sequencing and assembly and finally best SCAG or combined SCAG (C-SCAG) annotation and functional analysis. Each of these steps can have an impact on the quality of the resulting genome and annotation. We validated each step of the pipeline using a set of quality check criteria such as start genome amplification (SGA), fold genome amplification (FGA), random twenty-mer uniqueness (RTU), genome GC content, assembled genome size plus a set of metrics for genome assembly quality and evaluated each using criteria for its predictability power in relationship to genome completeness, which was estimated using CEGMA (CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. G. Parra, K.Bradnam, I.Korf. Bioinformatics, V23, Iss 9, pp1061-1067). We examined all the factors that have a critical impact for fungal single cell genome recovery, the most important of which is amplification bias, which can be characterized by the combination of SGA, FGA, RTU and genome GC content. We offer a suite of protocols for a large scale workflow that can be applied to a wider range of organisms than fungi.

295W SMRT sequencing reveals a transferrable pathogenicity chromosome in *Fusarium oxysporum* f.sp. *radicis-cucumerinum*. *Peter van Dam*[1], Like Fokkens[1], Michelle van der Gragt[1], Anneliek ter Horst[1], Balázs Brankovics[2], Petra M. Houterman[1], Martijn Rep[1] 1) Molecular Phytopathology, University of Amsterdam, Amsterdam, The Netherlands; 2) CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

The genome of *Fusarium oxysporum* (Fo) is typically divided into a set of eleven 'core' chromosomes, shared by most strains and responsible for housekeeping, and one or several 'accessory' chromosomes. In Fo f.sp. *lycopersici*, one of these chromosomes was shown to be horizontally transferrable and responsible for pathogenicity towards tomato. In *de novo* Illumina assemblies, these accessory chromosomes are dispersed over many contigs, making it impossible to identify how many accessory chromosomes are present in a strain. We sequenced a strain of Fo f.sp. *radicis-cucumerinum* (Forc) using PacBio SMRT sequencing. The majority of the core chromosomes was assembled into single contigs, and a chromosome that shows all the hallmarks of a pathogenicity chromosome comprised two contigs. Part of this chromosome is very rich in repeats and transposons and contains all identified candidate effector genes, including homologs of *SIX6, SIX9, SIX11* and *SIX13.*
Through individual knockout of several *SIX* homologs as well as Horizontal Chromosome Transfer (HCT) towards a non-pathogenic strain (Fo47), we were able to show that (a) *SIX6* is an important effector for this root rot pathogen and (b) that this chromosome is indeed a pathogenicity chromosome for cucurbit infection. Strains that received this chromosome into a Fo47 background are fully pathogenic, essentially changing Fo47 into a virulent root rot pathogen infecting multiple cucurbits. One strain obtained by means of HCT showed a duplication of the entire pathogenicity chromosome and is still pathogenic, while another shows partial duplications and is reduced in virulence towards cucumber and watermelon but not towards muskmelon.
These observations allow us to conclude that also non-wilt-inducing Fo pathogens like Forc rely on effector proteins for successful infection and that the Forc pathogenicity chromosome contains all the information necessary for causing root rot of cucurbits. Moreover, the fact that three out of nine HCT strains have undergone large-scale chromosome alterations shows the enormous plasticity of Fo genomes.

296T The genome of Paramicrosporidium saccamoebae highlights alternate adaptations to obligate intracellular parasitism in Cryptomycota and Microsporidia evolution. *A. Quandt*[1], D. Beaudet[2], D. Corsaro[3], J. Walochnik[4], R. Michel[5], N. Corradi[2], T. James[1] 1) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 2) Canadian Institute for Advanced Research, Department of Biology, University of Ottawa, ON, Canada; 3) CHLAREAS Chlamydia Research Association, Nancy, France; 4) Molecular Parasitology, Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria; 5) Laboratory of Medical Parasitology, Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany.

Phylogenomic analyses have suggested that a clade comprising eukaryotic parasites with the smallest known genomes, Microsporidia, and the phylum known primarily from environmental sequences, Rozellomycota, are at the base of the fungal phylogeny. However, the ecological and genetic similarities between these distant relatives remains unclear. To gain insights into the genome diversity and biology of rozellids and their close relatives, we compared genome data of Rozellomycota and Microsporidia with the newly acquired nuclear and mitochondrial genomes of *Paramicrosporidium saccamoebae*; an intranuclear parasite of amoebae. Our analyses demonstrate that

Microsporidia are nested within Rozellomycota, which forms a paraphyletic clade, and reveal both lineage-specific loss of genes and previously unknown biological similarities between *P. saccamoebae* and its rozellid relatives. We show that the loss of complex I of oxidative phosphorylation and mitochondrial reduction has occurred more than once in the evolution of Rozellomycota, and that aspects of primary metabolism such as purine biosynthesis and the ability to synthesize several amino acids have been lost in *P. saccamoebae* but offset by horizontally transferred permeases that may help in acquiring these from their hosts. The general pattern of genome size and content reduction during the evolution in Rozellomycota and Microsporidia shows consistent but independent losses of genes as the result of variation in parasitic strategies (host, subcellular localization) within the clade.

297F Comparative genomics of the *Aspergillus* section *Flavi*. *A. Sato*[1], K. Matsushima[1], K. Ito[1], T. Mituyama[2] 1) R&D, Kikkoman Corporation, Noda, JP; 2) Artificial Intelligence Research Centre, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan.

Aspergillus section *Flavi* includes industrial fungi; *A. sojae* (*As*), *A. oryzae* (*Ao*), as well as aflatoxigenic fungi; *A. parasiticus* (*Ap*) and *A. flavus* (*Af*). It is difficult to clarify the evolutionary relationships among these four species because these fungi exhibit considerable morphological and physiological variations which hinder ordinary phylogenetics analysis. In order to clarify the relationship of these fungi at single base resolution, we performed re-sequencing and re-assembling of *As* NBRC 4239 upon our previous sequencing and obtained high quality 39.4-Mb chromosomal sequences with a 29-kb mitochondrial genome. We conducted computational genome comparison for these fungi and found that *As* is more homologous to *Ap* than to *Ao* and *Af*. In order to identify major factors to explain differences of genomic sizes (As and Ap 39Mb, Ao 37.2Mb, Af 36.9Mb) among these species, we conducted multiple compuation analyses. We found that genomic duplication can not be one of the factors because we did not find blocks to infer that *As* and *Ap* gained extra DNA by genome duplication. We conducted computational gene prediction by using Scipio (http://www.webscipio.org/) for these genomes. Then we computed gene-wise similarities among the species. The averaged alignment score over all predicted genes between *As* and *Ap* is 0.93 while that between *As* and *Ao* is 0.85. These results suggest that these fungi can be classified into two groups. We compared *As* and *Ap* genomes in terms of genomic regions of putative gene clusters for secondary metabolites. We found that *As* is deficient in some backbone genes which are essential for the secondary metabolite synthesis. This result suggests that *As* has lost abilities to generate some mycotoxins in the course of evolution. We developed a database of *As* in order to share our genome analysis results with the research community. Our database provides *As* genome browsing feature using Zenbu browser software (http://fantom.gsc.riken.jp/zenbu/). We integrated our genome annotation results into a GenBank formatted file which is supplied to the Model SEED server (http://modelseed.org/) to generate a SBML formatted genome-scale model. They can be downloaded from our database server at https://genome.cbrc.jp/sojae/.

298W Genome comparisons reveal transposable elements played a role in the evolution of *Ceratocystis albifundus*. *Magriet A van der Nest*, Michael J Wingfield, Brenda D Wingfield Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Comparative genomics provides a powerful tool to investigate the processes that underlie the biology of fungi over evolutionary time. Genome-based studies have, for example, shown that transposable elements (TEs) play important roles in the evolution of pathogens and their adaptation to new environments. Here, we investigated the possible role that TEs might have had in the evolution and biology of the aggressive tree pathogen *Ceratocystis albifundus*. The publicly available genome of *C. albifundus* was complemented with sequenced genomes of four additional isolates from a wide geographic range and on different hosts. The genomes were compared in terms of size, sequence similarity, gene content, and the pathways and processes that they encode. Finally, we used the REPET pipeline to identify and annotate the TEs present in five *C. albifundus* genomes. The results showed that these genomes were similar in genome size, gene content and overall sequence similarity. These similarities also extended to the pathways and processes encoded by each genome. The most obvious differences among the five isolates were evident in the distribution and number of repeated sequences, TEs, and/or proteins involved in TE activity. The presence and activity of these elements are commonly responsible for the accelerated evolution in the genomes of fungal pathogens and they most likely contributed significantly to the biology and evolution of *C. albifundus*.

299T Characterization and manipulation of sesquiterpene biosynthetic pathways in *Hypholoma* species. *Suhad Al-Salihi*, Andy Bailey, Gary Foster Molecular Plant Pathology and Fungal Biology Group, School of Biological Sciences, University of Bristol 24 Tyndall Ave, Bristol BS8 1TQ, UK.

The increase in microbial resistance to antibiotics highlights the need to intensify efforts to discover novel biological active compounds which have the ability to kill or inhibit resistant pathogenic bacteria. Despite the prominence of Basidiomycetes as bioactive compounds producers, only a few species have been studied in detail as a source of novel antibiotics. Both *Hypholoma fasciculare* (Hfas) and *Hypholoma sublateritium* (Hsub), are a prolific producers of pharmaceutically important sesquiterpenoids and triterpenoids, and their antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces service* has been confirmed in this work.

Two “foreign” genes (hygromycin resistant and green fluorescent protein) have been expressed heterologously via a developed ATMT method, and the use of the Hsub *gpd* promoter as an efficient regulator element is described for the first time in this research.

Both the in-house generated genome of Hfas and the published genome of Hsub, were mined for terpenoid core genes using antiSMASH and local blast search. This has revealed 17 and 16 terpene synthases from Hfas and Hsub respectively. Phylogenetic comparison with characterized basidiomycete terpene cyclases has categorized these into three different groups based on predicted of cyclization patterns, confirming that these two mushrooms are capable of producing a wide range of terpenoids. Further syntenic comparison of the two genomes carried out and six twinned gene clusters were characterized in both *Hypholoma*.

Chemical investigation so far has confirmed the production of the sesquiterpene compound “Naematolin” from both species. The structure of this compound has been confirmed via NMR analysis, and a further work is on-going to identify the gene cluster responsible for the production of this antimicrobial compound.

300F *De Novo* genome sequencing and transcriptome analysis of two *Venturia inaequalis* strains. *Cristina Miguel Rojas*[1], Brad Cavinder[1], Janna Beckerman[2], George Sundin[3], Jeffrey P. Townsend[4,5], Frances Trail[1,3] 1) Plant Biology, Michigan State University, East Lansing, MI; 2) Department of Botany and Plant Pathology, Purdue University, IN; 3) Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; 4) Department of Biostatistics, Yale University, New Haven, CT; 5) Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT.

Venturia inaequalis is a Dothideomycete fungus that causes apple scab, one of the most devastating diseases of apple. Recently, *Venturia* has become a powerful model to study the pathogenesis of hemi-biotrophic fungal plant pathogens. We have performed *de novo* high-throughput whole-genome sequencing of two *Venturia inaequalis* strains featuring different virulence genes. To determine the genetic basis of the differences in virulence, we are comparing their genomes. To further identify infection-specific genes, we carried out a transcriptomic analysis of the spore germination process for one of the two strains during germination on a defined medium and during germination on apple leaves. The evolution of the transcriptome will additionally be analyzed in a set of species possessing divergent mechanisms for plant penetration.

301W Whole genome DNA-methylation (methylome) profiling during heterokaryosis in the Agaricomycetes. *R.A. Powers*, T.Y. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

DNA methylation of the 5' position of cytosines, a well-known form of 'epigenetic' modification, has been shown to be important in such diverse processes as the formation of human cancers, development in multicellular eukaryotes, and the silencing of transposons and repetitive elements in plants, animals, and fungi. Despite the importance and apparent conservation of DNA methylation across diverse clades of eukaryotes, we still lack a basic understanding of its roles in the mushroom-forming fungi of the Agaricomycetes. While it has been shown that DNA methylation in CpG (5' Cà G 3') contexts is important for the silencing of repetitive DNA elements in fungi, we lack a comprehensive understanding of DNA methylation at the whole genome level in the Basidiomycota. In particular, we know little of how whole genome DNA methylation ("methylome") patterning differs both during different life-stages within a species, as well as how methylomes differ between taxa. Using whole-genome bisulfite sequencing (WGBS), which involves the treatment of genomic DNA with sodium-bisulfite followed by whole genome next generation sequencing, we have generated genome-wide methylation profiles of two haploid homokaryotic isolates from five taxa across diverse clades of the Agaricomycetes: *Coprinopsis cinerea, Heterobasidion irregulare, Wolfiporia cocos, Coprinellus disseminatus* and *Cyathus stercoreus*. Here, we report that the amount of genomic methylation of cytosines in CpG contexts is species specific and ranges from of a low of ~2% in *Heterobasidion irregulare* to ~12.5% in *Cyathus stercoreus*. Further, we show that there is a high degree of variation in CpG methylation percentages between haploid strains of the same species for most of the species tested. Lastly, we examined the role of changes in methylome patterning during mating and heterokaryosis and show that DNA methylation patterns change during the transition for haploid to heterokaryon, and that these changes are also species specific.

302T Applications of the genome sequence of *Cercospora zeina*, causal agent of gray leaf spot disease of maize. *Dave Berger*[1], Nicholas Olivier[1], Velushka Swart[1], Yao-Cheng Lin[2], Bridget Crampton[1], Irene Barnes[3], Yves Van de Peer[2,4], Pedro Crous[5], Burton Bluhm[6] 1) Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; 2) VIB Department of Plant Systems Biology, Department of Plant Biotechnology and Bioinformatics, Ghent University, Belgium; 3) Department of Genetics, FABI, University of Pretoria, South Africa; 4) Genomics Research Institute, University of Pretoria, South Africa 5) CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; 5) Department of Plant Pathology, University of Arkansas, Fayetteville, USA; 6) CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Cercospora zeina is a Dothideomycete fungus with a lifestyle adapted to invasion and multiplication in maize leaves. Symptoms on susceptible maize leaves are characterized by grayish matchstick-like lesions parallel to leaf veins, hence the name gray leaf spot (GLS) disease. Although *C. zeae-maydis* has been better studied as agent of GLS in the USA, *C. zeina* is the causal agent of GLS in Africa. We sequenced the genome of an African isolate of *C. zeina*, and will illustrate three applications from these data. First, the sequence was mined for microsatellite markers, which were used for a population genetics study of the pathogen from commercial maize in South Africa. We observed a diverse panmictic population across the country indicating widespread dispersal. Second, the cercosporin biosynthetic pathway was annotated, revealing pseudogenization of an oxido-reductase gene, providing an explanation for lack of cercosporin production by this species. Third, we are developing a bioinformatics pipeline to identify single copy syntenic genes across sequenced Dothideomycetes that can be used to resolve unidentified species within the genus *Cercospora*.

303F Whole-genome haplotype sequencing of Puccinia coronata f. sp. avenae, causal agent of oat crown rust disease. *M. Miller*[1,2], V. Omidvar[1], Y. Zhang[1], B. Schwessinger[6], J. Rathjen[6], C. Hirsch[1], C. Raley[5], R. Park[3], P. Dodds[1,4], S. Kianian[1,2], M. Figueroa[1] 1) University of Minnesota, MN, USA; 2) USDA-ARS Cereal Disease Laboratory, MN, USA; 3) University of Sydney, NSW, Australia; 4) CSIRO Agriculture and Food, ACT, Australia; 5) Leidos Biomedical Research, MD, USA; 6) Australian National University, ACT, Australia.

Oat crown rust caused by *Puccinia coronata* f. sp. *avenae* (*Pca*) is one of the most destructive pathogens affecting grain production. *Pca* shows high genetic variability and resistance genes deployed in the field are often overcome in just a few years, which is of particular importance given that domestic oat production cannot keep pace with U.S. demand. The genomic resources necessary for a greater understanding of molecular mechanisms of virulence and more targeted resistance deployment strategies of *Pca* are underdeveloped. We generated *de novo* genome assemblies of two *Pca* isolates with contrasting virulence phenotypes (races LBBB and STTG). Genomic analyses of rust fungi are complicated by both the dikaryotic nature in the most readily accessible life stages and also the high percentage of repetitive elements present in the genome. To overcome these obstacles, we implemented emerging technologies to assemble haplotigs of urediniospores to produce a high quality reference genome. Long-read single-molecule PacBio and short-read Illumina sequences were analyzed in parallel to accurately phase the genome using FALCON and FALCON-Unzip genome assembly algorithms. The high quality genome references were annotated utilizing expression data from a variety of tissues and life stages, including haustoria isolated from infected tissues, and a catalog of predicted effectors was generated. Moreover, we compared sequence variation and repetitive element repertoires to assess the variability between nuclei and inter-isolate comparisons were used to characterize the genomic landscape of this fungal species. We are also embarking on a resequencing project of 60 isolates to describe the pan-genome of *Pca* and variability among

isolates. Ultimately, this project will provide genomic resources to study heterozygosity, conserved and variable genomic regions, and ultimately the evolution of pathogenicity mechanisms in *Pca*.

304W Penicillium genomics to solve postharvest apple decay. *Jiujiang Yu*[1], Guangxi Wu[2], Guohua Yin[3], Hui Peng[4], Verneta Guskins[1], Wayne, W. Jurick II[1], Joan, W. Bennett[3] 1) USDA/ARS, Beltsville Agricultural Research Center, Beltsville, MD; 2) Colorado State University, Fort Collins, CO; 3) Rutgers University, New Brunswick, NJ; 4) Pennsylvania State University, University Park, PA.

Post-harvest decay of apple and pears is caused by *Penicillium*s pecies, mainly *P. expansum.* It is commonly called the Blue mold. The highly virulent species *P. expansum* (R19) is the major fungal species that causes blue mold. On the contrary, *P. solitum* (RS1) is the weakly virulent species we identified. Genome-wide analysis of the genetic components and their regulatory mechanisms may provide insight into their virulence for devising pathogen-specific control strategies. For that reason, the two species were sequenced and analyzed. The genome similarities and differences were examined. A total of 10,560 and 10,672 protein coding sequences were identified and contained 41 and 43 unique genes in *P. expansum* (R19) and *P. solitum* (RS1), respectively. The genes responsible for infection in apple decay could be among those unique genes identified in the virulent strain *P. expansum* (R19). Further investigations to characterize their specific function of these genes are underway through gene knock-out experiments.

305T A haplotype phased reference genome and a transformation system for the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici*. *B. Schwessinger*[1], Y. Hu[1], W. Cuddy[2,3], R. Park[2], J. Rathjen[1] 1) Research School of Biology, The Australian National University, Acton ACT 2601, Australia; 2) Department of Plant and Food Sciences, Faculty of Agriculture and Environment, University of Sydney, Plant Breeding Institute, Private Bag 4011, Narellan, NSW, 2567, Australia; 3) Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Woodbridge Road, Menangle, NSW, 2568, Australia.

Stripe rust of wheat, caused by the obligate biotrophic fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major threat to wheat production world-wide with an estimated yearly loss of US$ 1 billion. *Pst* undergoes a complex multi-spore life-cycle and infects wheat during its asexual stage mostly via reinfection by dikaryotic urediniospores. The highly heterozygous genome together with its obligate biotrophic life-style has hindered progress in understanding the molecular and genetic mechanisms of *Pst* pathogenesis and evolution. Current genome assemblies are highly fragmented and unphased making downstream analysis at a whole genome level challenging. Here we present a 80% haplotype phase reference genome for one Australian *Pst* founder race based on single molecule long read sequencing technologies. We will explore differences between the two haplotypes in regards to nucleotide and structural variations, transposable element and gene content. In addition, we will present a new Agrobacterium based *in vitro* transformation protocol for *Pst*, which might be applicable to rusts in general.

306F Molecular Characterization of Horizontally Transferred Genes in a Zygomycete Fungus *Choanephora cucurbitarum*. *B. Min*, H. Park, J. Byun, H. Shin, I. Choi Korea University, Seoul, KR.

A zygomycete fungus, *Choanephora cucurbitarum* is a causal agent for blossom rot in cucurbits and other plants. We recently reported the genome sequence of *Choanephora cucurbitarum* KUS-F28377 isolated from a Korean squash. The genome was assembled to a size of 29.1 Mbp and 11,977 protein-coding genes. Comparing to ten Mucorales and two Glomerales genomes, we identified 1,326 orphan genes only found in the genome. One orphan gene family had 31 members presenting F-box domain. It is interesting that the genome contained 11 Streptomyces subtilisin inhibitor-like domains (SSIDs). SSID has been found only in bacterial genomes mainly in *Streptomyces* species. SSIDs are known to be related to defense against plant immunity. We cloned and characterized the molecular function of the SSID-containing genes in *E. coli*. Overall, these findings will help us to understand how zygomycetes are associated with plants.

307W *Trichoderma reesei* complete genome sequence reveals chromosome architecture, repeat-induced point mutation and partitioning of CAZyme gene clusters. *Wan-Chen Li*[1,2,3], Chien-Hao Huang[3,4], Chia-Ling Chen[3], Yu-Chien Chuang[3], Shu-Yun Tung[3], Ting-Fang Wang[1,3] 1) Taiwan International Graduate Program in Molecular and Cellular Biology, Academia Sinica. Taipei 115, Taiwan; 2) Institute of Life Sciences, National Defense Medical Center, Taipei 115, Taiwan; 3) Institute of Molecular Biology, Academia Sinica. Taipei 115, Taiwan; 4) Institute of Genome Sciences, National Yang-Mang University, Taipei 112, Taiwan.

Trichoderma reesei (Ascomycota, Pezizomycotina) QM6a is a model fungus for a broad spectrum of physiological phenomena, including plant cell wall degradation, industrial production of enzymes, light responses, conidiation, sexual development, polyketide biosynthesis and plant-fungal interactions. We integrated Pacific Biosciences and Illumina sequencing platforms for the highest-quality genome assembly yet achieved, revealing seven telomere-to-telomere chromosomes (34,922,528 bp) with 2009 newly-predicted genes and >1.5 Mb of new sequences. Most new sequences are located on AT-rich isochores, including 7 centromeres, 14 subtelomeres and 2349 interspersed AT-rich islands. The seven QM6a centromeres separately consist of 24 conserved repeats and 20 centromere-encoded genes. We demonstrate that sexual crossing readily induced cytosine-to-thymine point mutations on both tandem and unlinked duplicated sequences. Comparative bioinformatics analyses reveal that *Trichoderma reesei* has evolved a robust repeat-induced point mutation (RIP) system to accumulate AT-rich sequences, with longer AT-rich isochores having more RIP mutations. Our analyses, together with the complete genome sequence, provide a better blueprint for biotechnological and industrial applications.

308T Genomics of the first 100 Aspergilli. *A. Kuo*[1], R. Riley[1], S. Mondo[1], S. Haridas[1], A. Salamov[1], F. Korzeniewski[1], B. Simmons[2], S. Baker[2], M. Andersen[3], I. Grigoriev[1] 1) DOE Joint Genome Institute, Walnut Creek, CA; 2) DOE Joint BioEnergy Institute, Emeryville, CA; 3) Technical University of Denmark, Kongens Lyngby, Denmark.

Aspergillus is a ubiquitous and phenotypically diverse genus of filamentous Ascomycota, many of which play key roles as fermenters in food production, platforms for biotechnology and industrial production of enzymes and chemicals, plant and opportunistic animal pathogens, and agents of agricultural toxigenesis and biomass conversion for bioenergy. As part of a Joint BioEnergy Institute initiative to characterize the entire genus, the Joint Genome Institute will sequence, assemble, and annotate the genomes of each of the ~300 species of the genus *Aspergillus*. To accomplish this massive task in a timely manner without sacrificing quality, we streamlined and optimized our processes for *Aspergillus* genomes. Over the past year we have released on MycoCosm the genomes of 100 *Aspergillus* sp. which represent a broad spectrum of phylogenetic diversity and gene content, including significant variability of transporters, carbohydrate-active enzymes,

proteases, and secondary metabolism clusters. The high resolution of genomic differences between closely related species is being mapped to their distinctive phenotypes to improve gene annotation in the entire genus. The next 100 species are expected soon.

309F Comparative genomics of *Aspergillus flavus* S and L morphotypes provides insights into niche adaptation. *Mana Ohkura*, Peter Cotty, Marc Orbach School of Plant Sciences, University of Arizona, Tucson, AZ.

Aspergillus flavus is a widely distributed saprotrophic fungus and the most common causal agent of aflatoxin contamination on food crops. Despite the notoriety of *A. flavus* to produce aflatoxin, strains vary greatly in their levels of toxin production and atoxigenic strains are not uncommon in nature. Within *Aspergillus flavus,* there are two distinct morphologies, namely morphotypes: the S morphotype produces numerous small sclerotia and limited conidiospores and the L morphotype produces relatively fewer large sclerotia and abundant conidiospores. S strains are consistently toxigenic, whereas L strains vary greatly in toxin production ranging from atoxigenic to highly toxigenic. Morphological differences between the two morphotypes suggest the production of abundant sclerotia in S strains is advantageous for long-term survival in the soil, whereas the production of abundant conidia in the L strains is advantageous for aerial dispersal to the phyllosphere. The selection pressure to maintain aflatoxin production may be higher in the soil compared to the phyllosphere, leading to the retention of consistently toxigenic S strains in the soil. In contrast, the selection pressure may be lower in the phyllosphere, allowing the survival of atoxigenic or low toxin-producing L strains in that niche. To develop hypotheses on differential niche adaption, we compared the genomes of three S strains and three L strains to identify structural differences and genes unique to each morphotype. A 530 kb inversion was identified between the morphotypes that affects a secondary metabolite gene cluster and a cutinase gene on the margins. The L strain genomes contained 10 deletions, many of which involve secondary metabolite genes. Each morphotype had unique genes that play a role in carbon/nitrogen metabolism, secondary metabolism, and antimicrobial defense. These findings indicate that the genomes of the two morphotypes differ beyond developmental genes, and that they may have diverged as they adapted to their respective niches.

310W Are G protein-coupled receptor proteins involved in thigmoregulation of aflatoxin inhibition by *Aspergillus flavus*? *R.R. Sweany*, K.E. Damann, Jr. Plant Pathology and Crop Phys, Louisiana State University, Baton Rouge, LA.

Aspergillus flavus can contaminate corn, groundnuts and other oil seed crops with acutely toxic and carcinogenic aflatoxin. Atoxigenic biocontrol strains of *A. flavus* reduce aflatoxin of toxigenic strains in a thigmoregulated manner. G protein-coupled receptor (GPCR) mutants of *A. flavus* were obtained from Nancy Keller at the University of Wisconsin. Since GPCRs are membrane bound proteins involved in signaling, we determined whether loss of signal receptor proteins affected intraspecific aflatoxin inhibition. Aflatoxin production was quantified using HPLC of extracts from four-day old, glucose-salts medium cultures grown in 24-well plates. Several atoxigenic isolates, both commercially developed biocontrol strains and isolates from Louisiana, were screened against the wild-type CA14 and the CA14N1 *Nit* mutant (the genetic background for gene knock outs) for intraspecific aflatoxin inhibition. Only two strains from Louisiana completely inhibited aflatoxin production of CA14 and CA14N1. One inhibitory biocontrol strain was grown with the GPCR mutants to look for loss of biocontrol function. The biocontrol strain inhibited aflatoxin production in all mutants. Toxigenic strains can also have biocontrol ability and inhibit aflatoxin production, therefore the mutants were grown with four toxigenic strains representing small or large sclerotial strains of both mating types. Only two GPCR mutants did not inhibit aflatoxin production of the toxigenic strains. When non-inhibitory GPCR mutants were germinated 12 hours prior to inoculation (due to slow growth) with competing toxigenic strains, the ability to inhibit toxin production was restored. Giving the slow-growing GPCR mutants a head start presumably reduced aflatoxin production because competing germinated hyphae now touched within the 1st 12 hours. This reiterates earlier observations that touch must occur early during the germination of the toxigenic isolate, otherwise aflatoxin production will be unaltered by a competing strain. Single G protein-coupled receptor protein knockouts did not change aflatoxin inhibition, but further studies are needed during the 12-18 hour critical window to understand what signaling leads to successful intraspecific aflatoxin inhibition.

311T Centromere dynamics and the formation of dicenteric chromosomes in *Zymoseptoria*. *Klaas Schotanus*[1,2], Christoph J. Eschenbrenner[1,2], Lanelle R. Connolly[3], Mareike Moeller[1,2], Kristina M. Smith[3], Michael Freitag[3], Eva H. Stukenbrock[1,2] 1) Environmental Genomics, Christian-Albrechts University of Kiel, Kiel, Germany; 2) Max Planck Institute for Evolutionary Biology, Plön, Germany; 3) Department of Biochemistry and Biophysics, Oregon State University, Corvallis OR, USA.

Fungal plant pathogens in the genus *Zymoseptoria* have diverged only 12,000 years ago, and since then *Z. tritici* has specialized to infect wheat and become an important crop pathogen with worldwide distribution. In contrast, *Z. pseudotritici* and *Z. ardabiliae* occur on wild grasses at the center of origin in Iran.

All three species have two types of chromosomes, namely gene-rich core chromosomes enriched with euchromatic histone marks, and gene-poor accessory chromosomes enriched with heterochromatic histone marks. We previously showed that centromeres and telomeres of core and accessory chromosomes are indistinguishable in terms of sequence composition and structure, and demonstrated that centromeres of *Z. tritici* are small, are not enriched with DNA repeats and sometimes have actively transcribed genes abutting or within the short, 5-14 kb centromeric DNA sequences.

To address the evolution of centromeres we conducted a comparative analysis of centromeres in *Z. tritici*, *Z. pseudotritici* and *Z. ardabiliae*. The centromere specific histone H3 (CenH3) was tagged at the amino terminus with GFP in *Z. ardabiliae* and *Z. pseudotritici*, allowing us to identify centromeric sequences in the genome of the two species by ChIP-seq and mapping of Illumina reads to PacBio assemblies of *Z. ardabiliae* and *Z. pseudotritici*.

Overall, centromeres of *Z. pseudotritici* and *Z. ardabiliae* resembled those of *Z. tritici* as they are short (5-16 kb) and contain a low amount of repetitive DNA. However, compared to *Z. tritici*, five *Z. pseudotritici* centromeres were found in non-syntenic regions and lack homology between the species, a remarkable finding considering that we previously found that centromere position is conserved in syntenic regions of more divergent *Fusarium* species.

We also found two small dicentric chromosomes in *Z. pseudotritici*, though it remains to be seen whether both centromeres are functional. They are separated by 11 & 50 kb of interstitial regions that are enriched for transposable elements and the gene density left and right of the two centromeres differs. We hypothesize that the dicentric chromosomes could be the products of a fused chromosome with different gene densities. By comparative genomics, we identified homologous sequences of the dicentric chromosomes in 16 and 19 isolates

respectively, out of 27 total isolates sequenced, suggesting that the sequences of the dicentric chromosomes are conserved in the majority of the *Z. pseudotritici* isolates sequenced.

312F Advances in chemical genetic tools and impact on the research and development of novel crop protection products. Siân Deller, Helen Carter, Helen Clake, Eileen Scott, *Michael Csukai* Syngenta, Jealott's Hill, GB.

Chemical control agents are likely to remain a part of the mixture of control strategies open to farmers for the foreseeable future. In order to support the discovery and development of new active ingredients a wide range of chemical genetic tools are utilised and are under constant development. Historically many of these tools used the model organism *Saccharomyces cerevisiae,* but improvements in genomic information and tools make it possible to perform research directly in field-relevant plant pathogens. Some of the wide range of tools used in support of new fungicide discovery will be outlined, including:

- Forward genetic tools, which have long been the mainstay of mode of action diagnosis, provide an unbiased approach for the identification of the molecular target of a chemical inhibitor. If resistant mutants can be generated, the technique can now be applied to any pathogen, providing a reference genome is available and single nucleus cells/spores can be isolated.
- Libraries of engineered model organism strains. Chemistry with lower resistance risk is often associated with the inability to isolate resistant mutants in the plant pathogen of interest. In this case unbiased genetic tools are still of value and model organisms are employed. Haploinsufficiency or overexpression-induced resistance within a yeast library can provide information on proteins or protein families targeted by novel chemistry. Hypotheses can then be based on the mutations seen, and compared to information from additional phenotypes such as biochemical pathway inhibition and microscopic observations.

The improvements in transformation procedures, genetic engineering techniques and availability of multiple selectable markers make it possible to test hypotheses in fungal pathogens of economic importance such as *Zymoseptoria*. Additionally, genes from any species can be tested in a uniform host cell background, for instance, by heterologous expression. Furthermore, these tools can be used to assess the lifetime of a new fungicide product. Mutations induced in the lab or discovered in the field can be assessed for their importance in different genetic backgrounds and an assessment made of the impact on sensitivity and fitness. Advancing molecular biology tools and understanding of phytopathogens are helping to inform and support the development of ever safer and more effective chemical crop protection strategies. How these tools have been used to study Zymoseptoria will be shown.

313W New Zealand: The Home of Hobbits and Fungicide Resistant Yellow Rust? *N.M. Cook*[1], J. Maintz[2], S. Chng[3], D.G.O. Saunders[1,2] 1) Earlham Institute, Norwich, GB; 2) John Innes Centre, Norwich, GB; 3) Plant & Food Research, Christchurch, NZ.

Every year crops come under attack from cereal pathogens, which reduces their yield and the economic viability of growing crops. One major threat to wheat production worldwide is caused by yellow rust (*Puccinia striiformis* f. sp*. tritici*) which can lead to severe yield losses as 88% of wheat varieties are susceptible to yellow rust infection. This disease can be treated effectively by the use of fungicides but these are under threat by the potential for yellow rust to develop fungicide resistance. For instance, recently developed single site fungicides can easily be overcome by a single mutation within the fungicide target site. One such class of fungicides are the demethylation inhibitors (DMIs) which target the CYP51 gene involved in sterol biosynthesis.

In this study, we identified for the first time six isolates of yellow rust from New Zealand which contain a Y134F mutation within the CYP51 gene. This is homologous to the Y137F mutation previously characterised in many other fungal pathogens including *Zymoseptoria tritici* and is known to cause a reduction in sensitivity to DMIs. The yellow rust isolates containing this mutation were from two distinct genetic backgrounds suggesting the mutation arose twice independently in New Zealand. Although the Y134F mutation is heterozygous in these isolates, the mutated allele was shown to be preferentially expressed in all six isolates. Fungicide spray tests to determine if the Y134F mutation does indeed confer fungicide resistance for wheat yellow rust are currently underway.

314T Transcriptome analyses of differentially expressed genes in the *Lentinula edodes*. *H Song*[1], D Kim[2], J Kim[1] 1) Bio-Environmental Chemistry Dept, WKU, South Korea; 2) Molecular Biology Dept, JBNU, South Korea.

The edible mushroom *Lentinula edodes* has been utilized as a popular medicine, as well as food, because extracts from its mycelium and fruiting body have revealed several therapeutic properties. However, the deficit of *L. edodes* genomic information study has been barrier to the advancement of functional genomics research. High-throughput Illumina RNA-seq was used for deep gene analyses of the transcriptome of poly (A)+RNA from mycelium and fruiting body in *L. edodes*. About 141.77 million reads were obtained, trimmed, and de novo assembled into 32,001 transcripts. Based on sequence orientations determined by a BLASTX search against the NR fungi, Uniprotkb fungi, COG, GO, and KEGG databases, 27,569 (86.15%) contigs were assigned putative descriptions. When the expression profiles of mycelium and fruiting body were compared, 2,898 DEGs were detected, including 880 upregulated and 2,018 downregulated. Among the 27 COG categories, the cluster for general function prediction represents the largest group followed by 'Secondary metabolites biosynthesis, transport, and catabolism'. Results of GO analysis, DEGs were involved in diverse biological process. The most highly enriched GO category was cytoplasm, intracellular membrane-bounded organelle, and protein metabolic process. Analysis of 19 KEGG categories revealed that the DECs were significantly enriched in metabolic and signal transduction pathway. The pathways with most representation by the unique sequences were translation and carbohydrate metabolism.

315F Comparative genomics of lichen mycobionts. *A. Kuo*[1], D. Culley[2], O. Mueller[3], P. Dyer[4], J. Magnuson[2], F. Lutzoni[3], I. Grigoriev[1] 1) DOE Joint Genome Institute, Walnut Creek, CA; 2) Pacific Northwest National Laboratory, Richland, WA; 3) Duke University, Durham, NC; 4) University of Nottingham, Nottingham, UK.

Lichens are mutualistic symbioses usually between an alga (the photobiont) and a fungus (the mycobiont). The photobiont contributes fixed carbon to the partnership, while the mycobiont provides a physical structure that encloses the photobiont, roots the partners to a substrate, and acquires nutrients often of air-borne origin. Lichens are an ancient (possibly 600 Mya) and highly successful adaptation, covering 8% of terrestrial ecosystems, and comprising as much as 20% of fungal species diversity. The lichen lifestyle has evolved multiple times and occurs in at least 5 fungal classes. As part of an ongoing effort to represent the full phylogenetic and ecological diversity of fungi in the Joint Genome Institute MycoCosm and the 1000 Fungal Genomes Project, we have partnered with members of the lichen research community to sequence, assemble, and annotate several lichen mycobionts and photobionts. Initial comparison of 5 publicly

available mycobiont genomes from 3 distinct lichen-forming clades with their nearest non-lichenizing relatives has revealed varied combinations of secondary metabolite synthase, transporter, and protease gains and losses. The diversity of gains and losses suggest that the independent evolutionary transitions to the lichen lifestyle may have involved different gene-level adaptations. We expect to expand on these findings by examining additional functional characters, and by sequencing more genomes, as well as importing more genomes from our partners and from the public domain.

316W Comparative mitochondrial genomics of nine *Malassezia* species reveals diverse mitochondrial genome organization and evidence of homologous recombination between large inverted repeats. *C. D. Baudo*[1], E. Westhus[2], J. C. Kennell[1] 1) Department of Biology, Saint Louis University, Saint Louis, MO; 2) Center for Outcomes Research, Saint Louis University, Saint Louis, MO.

Malassezia yeast species occupy broad ecological niches from deep-sea vents to the skin of animals where they are associated with a number of chronic skin diseases. Mitochondrial (mt) haplotypes have been proposed to be associated with virulence in the closely related fungus *Cryptococcus gatti* highlighting the need to characterize mt genomes of other fungal pathogens. In this report, the mt genome of 19 isolates from nine *Malassezia* species were assembled, annotated, and compared. Four of the nine species, *M. sympodialis*, *M. restricta*, *M. globosa*, and *M. pachydermatis* share identical gene order and form a distinct branch on a maximum likelihood phylogenetic tree. A distinguishing feature detected in all but one species, *M. yamatoensis*, is the presence of a large inverted repeat (LIR) ranging from 3.5-8.7 kb within the mt genome. Closely related species have a higher percent nucleotide identity in the LIR than the intergenic regions, yet this relationship reverses as evolutionary distance increases between the species. Substitution rate (Ks) analysis of a protein coding gene (PCG) in the LIR, *atp9*, was found to have lower Ks than PCGs in the single copy region. These data suggest relaxed selection pressures exist on noncoding sequences in the LIR compared to the intergenic regions and PCGs are preserved through copy correction. The *M. sympodialis* ATCC 42132 mt genome was also assembled from single molecule, real-time (SMRT) reads and inspection of reads extending from the LIR into the inter-LIR region found the LIR region present in multiple orientations. The analysis indicates homologous recombination occurs between repeat regions that inverts the inter-LIR region producing variable genomic configurations. The comparative genomic analysis presented here provides valuable insight into the evolution of mt genomes and the role of homologous recombination in generating novel genomic arrangements.

Fungal Diversity

317T How accurate biological source information enables data verification and reuse. *B. Robbertse*[1], P. Strope[1], R. Gazis[2], P. Chaverri[3,4], C. Schoch[1] 1) National Center for Biotechnology Information, National Library of Medicine, NIH, Bethesda, MD; 2) University of Tennessee, Department of Entomology and Plant Pathology, Knoxville, TN ; 3) University of Maryland, Department of Plant Science and Landscape Architecture, College Park, MD; 4) Escuela de Biología, Universidad de Costa Rica, San Pedro, San José, Costa Rica.

The ITS (internal transcribed spacer) RefSeq project (NCBI BioProject accession PRJNA177353) aims to provide accurate organism name to sequence associations and additional information (public specimen sources) to confirm this association by independent evaluation. Since curators at NCBI have started collecting and annotating type material (identifiers that ties formal biological names to physical specimens), the most immediate impact has been improving the accurate identification of genome and barcode data. Unfortunately, the primary sequence records from type material frequently contain only the minimum information with no other metadata beyond strain or isolate identifiers. Although the RefSeq ITS project does add public collection identifiers and verify the specimen-sequence-name triangle, no additional source data is added on a regular basis. Gathering source information from protologues (species descriptions) scattered among several journals, most of which are not easily accessible, is a high curation burden. However, when researchers in the community get involved, it becomes a more feasible task. As an example, a thorough curation of one of the top ranking important fungal genera, namely Trichoderma was recently completed. The effort resulted in several verified data associations beyond name-sequence associations (eg: isolation source, location etc.). We present the outcome of this process and highlight the importance of source information.

318F Molecular analysis of the dust mycobiome from homes of patients with aspergillosis. *Ahlam Alanazi*[1], Paul Bowyer[2], Malcolm Richardson[2] 1) Faculty of Applied Medical Sciences, University of Hafr Al-Batin, Kingdom of Saudi Arabia; 2) Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, University of Manchester, UK.

Objective: Fungi are common members of the microbiota of the indoor environment and constitute a serious public health threat in homes and workplaces. A major component of the fungal mycobiome in these environments is *Aspergillus fumigatus* which is responsible for a spectrum of pulmonary diseases

The present study was designed to investigate the diversity of allergenic and pathogenic fungi in the settled dust of homes of patients with chronic pulmonary aspergillosis to assess levels of exposure and disease severity. Dust is a valuable matri to analyse since it reflects the history of the patients' home.

Methods: Dust samples were collected from the vacuum cleaners of control and patients' houses and analysed by conventional culture and molecular techniques. Sequencing of ribosomal DNA from environmental fungi offers a valuable tool for analyzing the mycobiome of indoor environments. DNA extracted from 21 dust samples as amplified and analysed using the ITS-1 forward primer and the ITS-4 reverse primer.

Results: Based on conventional culture methods a high diversity of environmental fungi was detected. It was possible to identify three different species of Aspergillus: *A. niger, A. fumigatus* and *A. flavus*. *Rhizomucor, Penicillium* and *Mucor*, were also detected. In addition to filamentous fungi, a variety of yeasts were present. *A. fumigatus* was present in the majority of houses. The ITS region of rDNA was amplified by PCR which confirmed the presence of fungi in our dust samples.

Conclusions: The analysis of settled dust by PCR provides a sensitive technique for the investigation of the diversity of the fungal mycobiome in indoor environments and will potentially identify houses where fungal exposure by patients with aspergillosis needs to be

controlled. Morphological characterizations and molecular techniques such as PCR were used to further confirm the identity of the mycobiome of dust. Current work includes microbiome analysis by next - gen sequencing.

319W Field dynamics and patho-genomics of Asian soybean rust pathogen *Phakopsora pachyrhizi*. *Y.K. Gupta*[1], J. Ku[1], D MacLean[1], H.M. Murithi[3,5], M.H.A.J. Joosten[5], G. Tabor[4], K.E. Broglie[4], S.H. Brommonschenkel[2], H.P van Esse[1] 1) The Sainsbury Laboratory, Norwich, Norfolk, GB; 2) Dep. de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Brazil; 3) International Institute of Tropical Agriculture (IITA), Dar es Salaam, Tanzania; 4) Agricultural Biotechnology, DuPont-Pioneer, Wilmington, Delaware, USA; 5) Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands.

Soybean (*Glycine max*) is an important legume crop and a rich source for plant proteins and vegetable oil. A major constraint for soybean production is Asian soybean rust (ASR) which causes yield losses of up to 80%. ASR is caused by the obligate biotrophic fungus *Phakopsora pachyrhizi* and can only be controlled by fungicide application. Extensive efforts have been made to find genetic resistance against *P. pachyrhizi* however, the pathogen has rapidly overcome major resistance genes *Rpp1-5.* Remarkably, *P. pachyrhizi* infects over 153 different legume species under suitable conditions, which suggests that *P. pachyrhizi* maintains diverse pathogenicity factors. The degree and distribution of genetic variation in the pathogen population is key for informed deployment of novel resistance genes but is largely unknown for *P. pachyrhizi.* To understand the field variation and population structure of *P. pachyrhizi*, infected field samples of soybean are collected from different locations across Brazil and east Africa. We have developed a next-generation sequencing approach to explore the population structure and genetic variation of *P. pachyrhizi* in these very different geographic locations. Sequencing of field samples will allow us to study sequence polymorphisms in *P. pachyrhizi*. We will aim to obtain a detailed understanding of the effector diversity in the field population of *P. pachyrhizi*. We will use this information for the sustainable deployment of genes that confer resistance to Asian Soybean Rust in transgenic soybean.

320T Genomic and phenotypic population heterogeneity of *Candida lusitaniae* in chronic lung infections. *E. Demers*[1], S. Masonjones[2], N. Grahl[1], A.R. Biermann[1], A.W. Crocker[1], J.E. Stajich[2], D.A. Hogan[1] 1) Geisel School of Medicine, Dartmouth College, Hanover, NH; 2) University of California, Riverside, CA.

Despite the fact that fungal isolates recovered from infections frequently test as sensitive to antifungal drugs *in vitro*, chronic fungal infections are notoriously hard to treat. The mechanisms behind this resistance are not well understood, though recent studies of bacterial infections and human tumors are starting to acknowledge that heterogeneity within these populations may lead to an alteration in our assessment of drug susceptibility from testing one isolate. We have collected numerous isolates from three subjects with cystic fibrosis who have chronic, high fungal burden infections; we propose these represent an important model for studying population heterogeneity in chronic fungal infections. We identified the fungus in all three subjects (A, B and C) as *Candida lusitaniae*, which is an uncommon pathogen but has been previously studied because of its ability to rapidly develop resistance to amphotericin B and more recently echinocandins. Using a mixture of Illumina sequencing technology and phenotypic assays we have characterized numerous isolates from three subjects for genome sequence heterogeneity, MIC for a variety of drugs, growth rate and metabolic activity. Interestingly, we observed a high level of heterogeneity in drug resistance in all three subjects for multiple classes of antifungals, despite the fact that none of the subjects has received antifungal therapies in the previous year. Additionally, whole genome sequencing analysis revealed a high level of heterogeneity within each subject's fungal population, with at least 100-1000 SNPs varying among isolates from an individual subject when compared to a reference genome. Comparison of phenotypic and genomic analysis among Subject A isolates revealed a correlation between one of the most variable loci, *MRR1*, which encodes a known regulator of antifungal resistance, and fluconazole (FLC) MIC, which in some isolates was quite high. In addition, both deletion of *MRR1* from the clinical isolate D2Y8 and analysis of mating progeny from mating with a highly FLC sensitive strain, indicated that $MRR1^{D2Y8}$ confers high FLC resistance. These studies may prompt a reexamination in the way in which we view and study chronic fungal infections, introducing the need to examine multiple isolates from individuals to accurately assess infecting populations and potentially altering treatment strategies to account for isolates with heterogeneous drug resistance harbored within the population.

321F Patterns of mycofloral diversity and composition in Korean traditional fermentation starter *nuruk* as influenced by fermentation temperature and external humidity. Jyotiranjan Bal[1], Suk-Hyun Yun[1], Soo-Hwan Yeo[2], Jung-Mi Kim[3], *Dae-Hyuk Kim*[1] 1) Department of Molecular Biology, Institute for Molecular Biology and Genetics, Chonbuk National University, Jeonju, Republic of Korea; 2) Fermented Food Science Division, Department of Agrofood Resource, NAAS, RDA, Wanju-gun, Republic of Korea; 3) Department of Bio-Environmental Chemistry, Institute of Life Science and Natural Resources, Wonkwang University, Iksan, Republic of Korea.

The traditional Korean fermentation starter *nuruk* is typically composed of moistened coarsely ground cereals, packed into a mold, and left to ferment. Korea's popular alcoholic beverages are brewed using *nuruk*. Emerging popularity of Korean alcoholic beverages demands for its quality enhancement. The *nuruk* mycobiome greatly influences both fermentation and palatability enhancement. In our previous experiments, we have assessed the mycofloral diversity across varying temperatures and internal moisture contents of *nuruk.* In continuation of our efforts to explore traditional *nuruk* mycobiome, we further evaluated mycofloral dynamics of two barley based-*nuruk* samples (E and F) consisting of barley and green gram, fermented under 30°C and 40°C, respectively, for a period of 30 days. The initial external humidity was maintained at 70% for three days following which it was reduced to 50%. The average observed fungal richness during the 30 day *nuruk* fermentation was higher in *nuruk* E, as compared to *nuruk* F. Variations in both abundance and diversity of mycoflora was observed with different fermentation temperatures as well as changes in external humidity. Day 6 of *nuruk* fermentation revealed greater abundance of *Saccharomycopsis fibuligera*. The genera *Saccharomycopsis* predominantly inhabited both *nuruks*. However, *Aspergillus* dominated *nuruk* E and *Rhizopus* dominated *nuruk* F. In *nuruk* E at 70% humidity, *Aspergillus* predominated whereas *Saccharomycopsis* at 50%. With increase in fermentation duration, *Aspergillus* abundance increased whereas that of *Saccharomycopsis* decreased. Most of the mycofloral communities present abundantly in the 0 day samples either perished or diminished with the progress in fermentation. These findings are a step forward in our aim of understanding *nuruk* microbiome and thereby its quality enhancement.

322W Fungal diversity of marine sediments from the Gulf of Mexico. *L. Vargas Gastelum*[1], A.L. Romero-Olivares[6], J. Catalán-Dibene[7], A. Rocha-Olivares[2], C. Brizuela[3], S. Herzka[4], A.E. Escalante[5], M. Riquelme[1] 1) Department of Microbiology, Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE, Mexico; 2) Department of Marine Ecology, CICESE, Ensenada, Mexico; 3) Department of Computer Sciences, CICESE, Ensenada, Mexico; 4) Department of Biological Oceanography, CICESE, Ensenada, Mexico; 5) Instituto de Ecología, Universidad Nacional Autónoma de México UNAM, Ciudad Universitaria, Mexico; 6) Departments of Ecology and Evolutionary Biology, University of California-Irvine, California, USA; 7) Physiology and Biophysics, University of California-Irvine, California, USA.

Fungi inhabiting marine sediments interact with other organisms and contribute to the biogeochemical cycling of elements. These important ecological processes are susceptible to anthropogenic disturbances such as oil spills. The impact of these disturbances is only measurable if the habitat was characterized prior to the disaster. Our aim was to establish the baseline of the benthic mycobiota of the Mexican Exclusive Economic Zone of the Gulf of Mexico in order to detect changes of these communities as a result of future potential disturbances. To achieve this, ten stations between parallels 19 to 25°N and 86 to 97°W were sampled. Total genomic DNA was recovered and used as template to amplify the ITS region by PCR followed by Illumina MiSeq sequencing. A total of 2,051,282 sequences were recovered and analyzed, resulting in 1,149 OTUs. The fungal community appears to be influenced by the geographic location (Zone 3, deepest stations; Zone 4, stations closer to the coast). Zones 3 and 4 shared only 68 OTUs. Zones 3 and 4 displayed 72 and 55 unique OTUs, respectively, indicative of specific functions correlated to the underlying characteristics of each zone. The remaining 954 OTUs were shared between two or more stations. The Ascomycota and the Basidiomycota represented the main components of the community. The most abundant orders were Sordariales and Malasseziales. A group of unidentified sequences, *Neurospora terricola, Malasseziales* sp. and *Capnobotryella* sp. were the most abundant phylotypes. A wide variety of the identified fungi have been described as facultative marine fungi (derived from terrestrial ecosystems). Ongoing annual samplings will allow us to expand our understanding of the deep-sea fungal community.

323T Diversity of Spalting Fungi in the Peruvian Amazon. *Sarath Vega Gutierrez*, Sara Robison Wood Science and Engineering, Oregon State University, Corvallis, OR.

Most of the research related to fungi of the Peruvian Amazon is focused on edible mushrooms and pathogens. Other important fungi, such as spalting fungi, are not broadly studied because most of them do not produce fruiting bodies, are difficult to locate, and do not present an immediate value to the forager. Finding spalting fungi involves a complete sampling of downed trees and branches in a given area through the opening of the xylem to identify potential pigmenting and zone line producing fungi. This methodoly was employed in the district of Las Piedras, Madre de Dios, Peru. Fungi suspected of causing internal pigment and zone lines were collected, cultured, isolated, and sequenced. The species found belonged to the orders of the Helotiales, Xylariales, Hypocreales, Russulales, Polyporales, Botryosphaeriales and two specimens of the class Leotiomycetes. Most of the fungi produced pigments or zone lines in wild conditions and all of them were part of the wood decomposing fungi. Findings from this research will benefit Amazonian communities as well as Peru as whole, through identification and location of value-added wood products that can widen the commercial wood market currently focused on dark, old growth trees. This also gives more inherent value to the forest, potentially saving the land from being converted to plantation or pasture.

324F Hidden diversity in the oomycete genus Olpidiopsis is a global threat to red algal cultivation. *Y. Badis*[1], M. Strittmatter[1], P. Murua[1], g.h Kim[2], c Gachon[1] 1) Microbial and Molecular Biology, Scottish Ass. for Marine Science, Oban, Argyllce; 2) Dept of Biology, Kongju National University, Kongju, Korea.

Seaweed cultivation is the **fastest-growing of all aquaculture sectors**, with an annual growth rate of 8 % and a value in excess of $5bn. Emerging diseases are a threat to sustainable development. The oomycete genus *Olpidiopsis* notably harbours pathogens of red algae, and include the most economically damaging disease of *Pyropia* (ex-*Porphyra*) farms in Asia. Using microscopy and molecular analysis, we identified in Scotland three new *Olpidiopsis* species: *Olpidiopsis palmariae*, *O. muelleri,* and *O. polysiphoniae* spp. nov. A Scottish variety of *Olpidiopsis porphyrae*, a devastating pathogen previously thought to be restricted to Japanese seaweed farms, is also described as *O. porphyrae* var. *scotiae*. . Importantly, two of the new species infected the red seaweed *Porphyra* and *Palmaria* sp, which are subject extensive farming trials in Europe and North America. To further assess the extent of undescribed *Olpidiopsis* diversity and the threat it might pose to aquaculture in different regions, a metagenomics survey of eukaryotes associated from *Porphyra umbilicalis* thalli collected across the East and West Northern Atlantic shores was conducted, and combined to publicly available environmental sequences. Screening efforts were extended to global metagenomics barcoding campaigns Over 700 new Olpidiopsis sequences were detected with a truly worldwide distribution and grouped in three major Olpidiopsis clades. Close relatives of the Korean *O. pyropiae* are also reported for the first time in Europe and the United States. In the light of our restricted sampling, our results highlight the diversity and abundance of *Olpidiopsis* associated to red macroalgae worldwide, most of which are undescribed. In the context of worsening impact of *Olpidiopsis* pathogens in Asia, where red algal cultivation has grown steadily over the last two decades, this worldwide distribution should be treated as a serious threat to the global seaweed industry, and to the conservation of wild red algal populations near farms. Our data calls for more efforts towards the documentation of these pathogens, and for adequate biosecurity measures to be developed.

325W Microbial communities associated with water-damaged New York City public housing residences. Iman Sylvain[1], *John Taylor*[1], Michal Spilak[2], Michael Waring[2], Rachel Adams1[1] 1) Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA; 2) Department of Civil, Architectural and Environmental Engineering, Drexel University, USA.

The use of high-throughput sequencing technologies has elucidated the high biodiversity of microorganism in the built environment. Fungi found indoors in healthy buildings appear to be a random subset of the diversity found outdoors, but our knowledge of fungi associated with unhealthy buildings indoors is still quite limited, and largely informed by culture-based studies. To date a number of studies have shown that as water availability indoors increases, fungal biomass and richness indoors also increases. Damp, moldy housing is associated with a number of adverse health outcomes, principally asthma and respiratory symptoms, as well as anxiety and depression. It is well-established that housing quality is a major social determinant of health, and differential access to adequate housing is believed to be the fundamental cause of ethnic/racial health disparity in America.

Since Hurricane Sandy hit the Eastern Seaboard in 2012, the presence of mold has been a major problem in New York City public housing. In this study we have used high-throughput sequencing technologies to compare the indoor microbioata of New York City public and private residences impacted by Hurricane Sandy. Using quantitative-PCR and Illuminia MiSeq we have assessed community composition, diversity, and biomass of fungi and bacteria found in the house dust of 60 residential units in Brooklyn and Manhattan, over two seasons. Our microbiological analysis was paired with records of dwelling characteristics, environmental parameters, and occupant behavior over the course of one-month.

By amplifying the ITS1 and 16S rRNA regions for fungi and bacteria in over 600 dust samples collected from vacuums, swabs, electrostatic wipes, and empty petri dishes, we see that there are distinct microbial communities indoors in public and private apartments in New York City. Results showing significant differences in fungal community composition in public and private housing were also observable at the neighborhood level, i.e. within Brooklyn and Manhattan. While public housing had greater observable water-damaged than private housing, the presence or absence of water-damage did not appear to be the major factor driving differences in microbial communities across units or boroughs. The long-standing distinction in building maintenance in public and private housing seems to pre-date the water-damage caused by the hurricane, and has impacted both the microbial and human occupants.

326T Evolution of host preference and sorus location in the plant parasitic genus *Microbotryum*. *Martin Kemler*[1], Dominik Begerow[1], Matthias Lutz[2] 1) Ruhr-Universität Bochum, Bochum, DE; 2) Eberhard Karls Universität Tübingen, Tübingen, DE.

Microbotryum is a basidiomyceteous genus known for being the causing agent of anther smut in Caryophyllaceae. Monophyletic genera within the Caryophyllaceae recurrently have been colonized once, with subsequent radiation and specialization to many different host species. The host range of *Microbotryum* however is considerably larger than Caryophyllaceae and includes host species in many other plant genera and families. Additionally, other locations of sorus formation than anthers have evolved. In this study we present an updated phylogenetic approach using a denser sampling of species from different host families. The questions of this study are: 1) What is the ancestral state of host genus parasitized by the genus *Microbotryum*? 2) What is the ancestral state of sorus location in the genus *Microbotryum*? 3) Are monophyletic clades of parasites found on monophyletic groups of hosts? Our results indicate and corroborate previous results that the ancestor of the genus evolved on Polygonaceae and that most likely it already formed its spores in inflorescences. As in the Caryophyllaceae, many monophyletic groups of hosts have been colonized once with subsequent radiation. These results will be discussed in the light of some life-history traits of hosts and parasites.

327F Biodiversity and phylogeny of *Marasmius* (Agaricales, Basidiomycota) from Madagascar. *J.E. Shay*[1], D.E. Desjardin [1], B.A. Perry[2], C.L. Grace[1], D.S. Newman[1] 1) Department of Biology, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, USA; 2) Department of Biological Sciences, California State University East Bay, 25800 Carlos Bee Blvd., Hayward, CA 94542 USA.

Prior to this monographic treatment, limited research on the genus *Marasmius* (Basidiomycota, Agaricales) had been conducted in Madagascar. Based on field work in January 2013 and January–February 2014, which generated 45 specimens of *Marasmius sensu stricto*, supplemented by herbarium exsiccatae and published literature, 35 species of *Marasmius* were documented from Madagascar. Of these, a few key species are selected to demonstrate the variation in micro- and macromophology of *Marasmius* from Madagascar. Line drawings of salient micromorphological features and color photographs of basidiomes are provided for selected species. Species delineations are based on comprehensive morphological descriptions and molecular sequence data from the internal transcribed spacer (ITS) region.

328W Diversity and Genome Analysis of Marine Fungi. *F. Kempken*, P. Phule, A. Kumar Botanisches Institut, Christian-Albrechts University, Kiel, DE.

The number of species and distribution of marine fungi is largely unknown as marine fungi have been much neglected for a long time, although respective studies were published decades ago (1). Current estimate for numbers of marine fungal species is over 10,000 (2), but may be much higher (3). Nevertheless, their role in marine ecology is elusive (4).

To investigate the number of cultivatable fungi from marine sediments we have analyzed 16 samples from the Baltic Sea seafloor with a depth of 150 to 350 m. We were able to obtain 60 fungal species including isolates of *Chaetomium, Emericellopsis, Microascus, Penicillium, Phoma, Preussia*. The genome of the Preussia isolate has recently been sequenced and is currently being annotated. In sediment from the Mid-Atlantic ridge we did identify yeast and filamentous fungi. Several other fungi were isolated from beaches of the Baltic Sea in Germany, and the US west coast. Among the latter, *Asteromyces cruciatus* was re-identified, which previously had been described as a marine fungus.

At current, we have sequenced and annotated four marine genomes, *Scopulariopsis brevicaulis* (5) (from a mediterreanean sponge), *Pestalotiopsis* sp., *Calcarisporium* sp., and a new *Fusarium* species from the Baltic Sea (unpublished). The enormous biodiversity of marine fungal isolates is mirrored by the molecular diversity of their secondary metabolites (6–8). Yet, these studies are mainly chemistry based, and marine fungi remain tremendously underexplored with regard to species, distribution and applications (9, 10). We report here 44 NRPSs, 62 PKSs and seven hybrid NRPS-PKS genes for *Pestalotiopsis* sp. and 52 NRPSs, 66 PKSs and seven hybrid NRPS-PKS genes for *Calcariosporium.* This number of secondary metabolites is enormous and emphasis the potential of marine fungi for the discovery of new metabolites.

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329T Evolution and diversity of *Monosporascus* in arid-land environments. *A.J. Robinson*, D.O. Natvig, M.I. Hutchinson Department of Biology, University of New Mexico, Albuquerque, NM.

The genus *Monosporascus* represents an enigmatic group of fungi important in agriculture and widely distributed in arid ecosystems. Only three species of *Monosporascus* are currently described, and two are devastating pathogens on the roots of cantaloupe and other cucurbits. Nearly all research on this genus is limited to agricultural settings although members of the genus are nearly ubiquitous in surveys of root endophytes in arid environments of the southwestern United States and Europe. We have focused on isolates obtained from the roots of grasses, shrubs and herbaceous plants from central New Mexico. Comparison of the ribosomal internal transcribed spacer (ITS) DNA region and other genes indicates substantial diversity among strains. The New Mexico isolates include close relatives of the three described species and strains that appear to represent previously unrecognized lineages. There currently is no evidence for pathogenicity among any of the New Mexico lineages. To resolve the evolutionary relationships within the genus and the placement of the genus within the Ascomycota, we have sequenced and assembled the genomes of *M. cannonballus*, *M. ibericus* and several isolates obtained in New Mexico. Laboratory experiments suggest important life-cycle differences between described species and isolates from New Mexico. In contrast with *M. cannonballus* (homothallic), sexual reproduction has not been observed among any of the New Mexico isolates. Preliminary characterization of the genomes of 10 strains indicates that the genus likely belongs to the Xylariales, not to the Sordariales where it has generally been placed. The genomes of the isolates are consistently substantially larger than what is typical of other members of the Sordariomycetes and also raise interesting questions about life cycle biology and sexual reproduction. To date, we have failed to identify in these genomes clear homologs of any of the three genes associated with mating type A (Mat 1-1) in other heterothallic and homothallic filamentous Ascomycota.

330F Study on diversity of root-associated fungi of an Orchid *Crepidium acuminatum* (D. Don) Szlach: A rare and medicinally important taxon. *J. Thakur*, P. Uniyal Department of Botany, University of Delhi, India, New Delhi, Delhi, IN.

Mycorrhizal association is known to be essential for seed germination and seedling establishment in majority of orchids. Orchids possess embryo which lacks endosperm, the food reservoir. Hence, orchid roots harbour a large diversity of fungal species to procure carbon and other mineral nutrients to complete their life cycle. *Crepidium acuminatum* (Orchidaceae) is a rare medicinal plant that grows under shady and moist forest floor where light remains for a very short period of time. Thus, it is speculated that light is one of the key factors that enforces this species to form symbiotic association with diverse group of fungi. Identification of fungi that form mycorrhizae with orchids is of crucial importance for orchid conservation. We used both morphological as well as molecular approaches to study this plant-fungal interaction. Scanning electron microscopy showed that fungi grow and proliferate in the middle layers of cortex. Also, spiral root hairs were found along with roots hairs, which is an unusual observation. We believe that spiral root hairs improve fluid absorption, entrance of colonisers and increase adherence to the substrate. Furthermore, root samples were collected from four states in India. Fungal genomic DNA was isolated and fungal ITS regions were PCR-amplified using primer combinations ITS1F/ITS4 and ITS1/ITS4tul. ITS sequences were analyzed with BLAST against the NCBI sequence database to know the closest sequence matches in the GenBank database. Samples were identified to be belonging to three main genera i.e., *Tulasnella*, *Aspergillus* and *Penicillium*. Results indicate that mycorrhizal association is necessary for the growth and development of plant. In addition, these symbiosis influence the distribution and rarity of this medicinally valuable taxon. Specific fungal partners may lead to enhanced seed germination rate and increased efficiency of nutrient exchange between both the partners. Hence, knowledge of mycorrhizal fungi is essential for future *in vitro* germination and seeding establishment programs, because they rely on fungi for germination. Identification of mycorrhizal fungi can be used for orchid propagation, conservation and reintroduction in natural habitats.

331W Dissecting intraspecies diversity in fungal wood decay. *S. Casado Lopez*[1], S Purvine[2], A Dohnalkova[2], G Orr[2], E Robinson[2], E Zink[2], S Baker[2], K Hildén[3], M Mäkelä[3], R de Vries[1] 1) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre, Utrecht, NL; 2) Environmental Molecular Sciences Laboratory, Department of Energy, USA; 3) Department of Food and Environmental Sciences, University of Helsinki, Finland.

Dichomitus squalens is a white rot fungus that colonizes mainly softwood and is commonly found in the northern parts of Europe, North America and Asia. In nature, compatible monokaryons of *D. squalens* cross sexually to form a dikaryon. At this point it is not clear whether monokaryons can efficiently colonize and degrade wood, or whether this only occurs after the dikaryon is formed.
Here we studied in detail the process of wood colonization, degradation and utilization by *D. squalens*, using a combination of transcriptomics, proteomics, metabolomics and high-resolution microscopy enabled by the combined facilities of the JGI and EMSL institutes.
In this study, we compared a set of related mono- and dikaryotic strains, originating from two isolates. All strains were grown on solid spruce sticks and sampling was performed after two and four weeks to evaluate the variation between the strains during the wood decay process. Monokaryons, compared to dikaryons, were notably slower in colonizing wood, which was reflected in the analysis of the various datasets obtained from the samples.

332T Heterokaryon incompatibility and phenotypic characterisation of *Aspergillus flavus* isolates in low and high risk zones in Kenya. *Alfred Mitema*[1,2], Sheila Okoth[2], Revel Lyer[2], Amelia Hilgart[2], Suhail Rafudeen[2] 1) Molecular and Cell Biology, University of Cape Town, Cape Town, Western Cape, ZA; 2) University of Nairobi, Kenya.

Species *Aspergillus flavus* (*A. flavus*) has three morphotypes: S-, L- and S_{BG}-type strains, with the first repeatedly being associated with acute aflatoxicosis. Aflatoxicosis in Kenya is a serious health problem with outbreak 317 cases and 215 deaths. In 2013, we choose to address this crisis by characterising the diversity of *A. flavus* isolates through comparison of vegetative compatibility groups (VCGs), phenotypic characteristics and mycotoxin profiles across various agricultural regions in Kenya where aflitoxicosis has occurred previously. Using diagonal transect random household sampling, Maize kernels were collected from Makueni, Homa Bay, Nandi, and Kisumu counties

between November and December 2013. Out of 37 isolates, the nitrate non-utilising auxotrophs (*nit* mutants) complementation test revealed 20 VCGs. KVCG14 and KVCG15 had highest distribution frequency (*n* = 13; 10.8%) while KVCG10 and KVCG20 (*n* = 1; 0.8%) the least. Analyses of VCG diversity using the Shannon Index (H) showed that Nandi (*H'*= 0.108) and Kisumu (*H'*= 0.324) counties recorded the lowest and highest VCG diversities respectively. Results also showed that, with a few exceptions, within-county isolates were self-compatible but they were incompatible across any two adjacent counties and across all the sampled counties. Heterokaryon incompatibility in Nandi (*n* = 6; 67%) and Makueni (*n* =3; 33%) were the greatest and least respectively. Mycotoxin detection by coconut cream agar under UV light (365 nm) revealed blue fluorescence {(57%, *n* =21) aflatoxin B} and green {(43%, *n* =16) aflatoxin G}. The study further revealed L-, S- type strains (57%; *n* =21 and 7%; *n* =2) respectively. The findings of the study could provide reliable information in determining biocontrol strategies to mitigate aflatoxin contamination of maize by *A. flavus* in the studied counties.

333F Mycobiome in Anorexia Nervosa. *K. Mueller*[1], E. Huh[1], E. Bulik-Sullivan[3], H. Zhang[1], Y. Wang[1], C. Bulik[3,4,5], I. Carroll[3], S. Lee[1,2] 1) Biology, The University of Texas at San Antonio, San Antonio, TX; 2) South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, TX; 3) Medicine, The University of North Carolina at Chapel Hill, NC; 4) Nutrition, The University of North Carolina at Chapel Hill, NC; 5) Psychiatry, The University of North Carolina at Chapel Hill, NC.

Anorexia nervosa(AN) is a mental illness characterized by a fear of gaining body weight, which results in severe weight loss and malnutrition. Persistent malnutrition, along with anxiety as a common comorbidity, contributes to AN having the highest mortality rate of mental illnesses. Typical treatment for AN involves re-nourishment of patients, which has proven to be insufficient in treating the disease, as relapse rates after treatment reach 50%. As such, finding new treatments for AN is of great importance.

Previous studies have shown an overall lowered bacterial diversity in AN patients, as well as a significant association between the bacterial diversity of AN patients and anxiety. However, these studies have overlooked the possible role of fungal diversity in the gut. Intestinal fungi have been implicated in diseases of the gastrointestinal tract, and previous studies have shown that disruption of normal gut bacteria may lead to enteric fungal dysbiosis. It then stands to reason that the fungal gut community, as well as an interplay between the fungal and bacterial communities, also plays a role in AN associated dysbiosis.

DNAs extracted from stool samples were used to analyze mycobiota in the guts of AN patients and healthy controls (HC). Utilizing fungal specific ITS1 primers and bacterial specific 16s rDNA primers, qPCR analysis demonstrated a significant increase in number of gut fungi relative to bacteria in the undernourished phase (T1) of AN patients as compared to the post-refeeding phase (T2) of the same patients. Furthermore, an increase in *C. albicans* was observed upon patients undergoing the refeeding treatment, providing a specific role of this fungus in lower weight phase of AN patients. Illumina sequencing of the ITS1 amplicons was performed to examine the dynamics of fungal species present in AN patients and HC. The obtained sequence data were filtered to identify taxa via IPITS pipeline. The OTU abundance from each sample was assessed with QIIME. Measurements of alpha diversity (T1 vs T2 vs HC) with the number of species and the Chao-1 index revealed less fungal species present in the gut of AN patients compared to that of HC. This result is similar to previous studies considering lower bacterial diversity in AN. Together, these findings suggest that fungal dysbiosis in the intestines may play a part in perpetuating microbiota in AN patients. Addressing this dysbiosis may then offer new methods for the treatment of AN.

334W *Nothophytophthora* prov. nom., a new sister genus of *Phytophthora*. T. Jung[1,2,3], B. Scanu[4], J. Bakonyi[5], D. Seress[5], A. Durán[6], E. Sanfuentes von Stowasser[7], L. Schena[8], S. Mosca[8], P. Q. Thu[9], C. N. Minh[9], S. Fajardo[7], M. González[7], A. Pérez-Sierra[10], H. Rees[10], C. Maia[1], *M. Horta Jung*[1,2] 1) Laboratory of Molecular Biotechnology and Phytopathology, Center for Mediterranean Bioresources and Food (MeditBio), University of Algarve, 8005-130 Faro, Portugal; 2) Phytophthora Research Centre, Mendel University, 613 00 Brno, Czech Republic; 3) Phytophthora Research and Consultancy, 83131 Nußdorf, Germany; 4) Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Università degli Studi di Sassari, 07100 Sassari, Italy; 5) Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Hungary; 6) Ontario Forest Research Institute, ?P6A 2E5 Sault Ste. Marie, Canada; 7) Laboratorio de Patología Forestal, Facultad Ciencias Forestales y Centro de Biotecnología, Universidad de Concepción, Concepción, Chile; 8) Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, 89124 Reggio Calabria, Italy; 9) Forest Protection Research Centre, Vietnamese Academy of Forest Sciences, Duc Thang Ward, Northern Tu Liem District, Hanoi, Vietnam; 10) Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom.

During various surveys of *Phytophthora* diversity in Europe, Chile and Vietnam slow growing oomycete isolates were obtained from soil samples and small streams in natural and planted forest stands. Phylogenetic analyses of ITS, β-tubulin, LSU and *cox1* sequences placed them into six new species belonging to a new genus, officially described as *Nothophytophthora* gen. nov., which clustered sister to *Phytophthora*. *Nothophytophthora* species share numerous morphological characters with *Phytophthora*: persistent (all *Nothophytophthora* spp.) and caducous (*N. caduca*, *N. chlamydospora*, *N. valdiviana*, *N. vietnamensis*) sporangia with variable shapes, internal differentiation of zoospores and internal, nested and extended (*N. chlamydospora*, *N. caduca*) and external (all *Nothophytophthora* spp.) sporangial proliferation; smooth-walled oogonia with amphigynous (*N. amphigynosa*) and paragynous (*N. amphigynosa*, *N. intricata*, *N. vietnamensis*) insertion of the antheridia; chlamydospores (*N. chlamydospora*) and hyphal swellings. Comparisons of morphological structures of both genera allow clues about the morphology and ecology of their common ancestor which are discussed. Including *Nothophytophthora* in coalescence analyses will give new insights into the evolutionary history of *Phytophthora*. Production of caducous sporangia by *N. caduca*, *N. valdiviana* and *N. chlamydospora* from Valdivian rainforests and *N. vietnamensis* from a wet mountain forest in Vietnam indicates a partially aerial lifestyle as adaptation to these humid habitats. Presence of tree dieback in all forests from which *Nothophytophthora* spp. were recovered and partial sporangial caducity of several *Nothophytophthora* species suggests they may be facultative pathogens. Pathogenicity tests are urgently required to clarify whether the individual *Nothophytophthora* species have a pathogenic or saprophytic lifestyle.

335T Diversity of *Phytophthora* species from natural and semi-natural ecosystems in Portugal, Chile and Vietnam. T. Jung[1,2,3], B. Scanu[4], J. Bakonyi[5], D. Seress[5], A. Durán[6], E. Sanfuentes von Stowasser[7], L. Schena[8], S. Mosca[8], P. Q. Thu[9], C. N. Minh[9], S. Fajardo[7], M. González[7], A. Pérez-Sierra[10], H. Rees[10], C. Maia[1], B. Mora Sala[11], G. Carella[12], S. Moricca[12], A. Cravador[1], *M. Horta Jung*[1,2] 1) Laboratory of Molecular Biotechnology and Phytopathology, Center for Mediterranean Bioresources and Food (MeditBio), University of Algarve, 8005-130 Faro, Portugal; 2) Phytophthora Research Centre, Mendel University, 613 00 Brno, Czech Republic; 3) Phytophthora Research and Consultancy, 83131 Nußdorf; 4) Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), Università

degli Studi di Sassari, 07100 Sassari, Italy; 5) Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Hungary; 6) Ontario Forest Research Institute, ?P6A 2E5 Sault Ste. Marie, Canada; 7) Laboratorio de Patología Forestal, Facultad Ciencias Forestales y Centro de Biotecnología, Universidad de Concepción, Concepción, Chile; 8) Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, 89124 Reggio Calabria, Italy; 9) Forest Protection Research Centre, Vietnamese Academy of Forest Sciences, Duc Thang Ward, Northern Tu Liem District, Hanoi, Vietnam; 10) Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom; 11) Instituto Agroforestal Mediterráneo, Universitat Politècnica de Valencia, Edificio 3H, Camino de Vera s/n, 46022 Valencia, Spain; 12) Department of Agri-Food Production and Environmental Sciences, Plant Pathology and Entomology Division, University of Florence, Piazzale delle Cascine, 28, 50144 Florence, Italy.

Surveys of *Phytophthora* diversity were performed using baiting assays and direct plating of necrotic plant tissues. Isolates were identified using both classical identification and sequence analysis of ITS and *cox1*.

In Portugal, 68 forest stands, 12 forest nurseries, 38 river systems and 4 lagoon ecosystems were surveyed. The isolates obtained belonged to 29 known species, 3 informally designated taxa, and1 previously unknown taxon of *Phytophthora*, nine new *Phytophthora* hybrid taxa from Clades 6 and 9, *Nothophytophthora homothallica* nom. prov., 1 known and 10 new *Halophytophthora* species, 7 known species and one new taxon of *Phytopythium*, and multiple *Pythium* species were isolated.

In Chile, the survey was performed in 13 natural forest stands and 20 forest streams located in two protected areas near Valdivia and in a temperate mountain forest in the Concepción area, and in each one planted stand of the introduced tree species *Castanea sativa* and *Fagus sylvatica*. Eight described species (including *P. kernoviae*) and 2 previously unknown taxa of *Phytophthora* were isolated. In addition, a diverse array of Clade 6 hybrids, and *Nothophytophthora caduca* nom. prov., *Nothophytophthora chlamydospora* nom. prov. and *Nothophytophthora valdiviana* nom. prov., were obtained.

In Vietnam the survey was performed in 23 natural forest stands and 10 forest streams and rivers in temperate montane and tropical lowland regions, and in 14 rubber plantations. Sixteen described species (including *P. ramorum*), 3 designated taxa and 23 previously unknown taxa of *Phytophthora*, amongst them 9 Clade 9 hybrid taxa, were isolated. In addition, *Nothophytophthora vietnamensis* nom. prov. and a diverse array of known and new taxa of *Phytopythium*, *Pythium* and *Elongisporangium* were recovered.

The implications of these findings for plant biosecurity and the development of a deeper understanding of the evolution and adaptability of the genus *Phytophthora* will be discussed

336F Fungi challenge global food security. *Sarah Gurr*, Dan Bebber, Helen Fones Biosciences, Exeter University, Exeter, GB.

Over the past centuries, crop diseases have led to the starvation of the people, the ruination of economies and the downfall of governments. Of the various challenges, the threat to plants of fungal infection outstrips that posed by bacterial and viral diseases combined. Indeed, fungal diseases have been increasing in severity and scale since the mid. 20th Century and now pose a serious threat to global food security and ecosystem health.

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are on the move poleward in a warming world.

This presentation will highlight some current notable and persistent fungal diseases. It will highlight the evolutionary drivers underpinning emergence of new diseases and allude to the accelerators of spread. I will set these points in the context of recent disease modelling, which shows the global distributions of crop pathogens and their predicted movement and will discuss the concept of crop disease saturation.

I shall conclude with some thoughts on future threats and challenges, on fungal disease mitigation and of ways of enhancing global food security.

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337W Role of plant-endophyte interactions in shrub encroachment. *M.I. Hutchinson*, A.J. Robinson, D.O. Natvig Department of Biology, University of New Mexico, Albuquerque, NM.

Desertification is a significant global phenomenon, and in many dryland areas shrub encroachment is considered a primary factor. Shrub encroachment, the conversion of grasslands into shrublands, is a well-documented process but its causes are debated. Here, we

investigate how interactions between plants and fungal root endophytes may explain the expansion of shrubs into arid grasslands by examining the fungal colonization of roots from two Chihuahuan desert plants, the C3 shrub *Larrea tridentata* (creosote) and the C4 grass *Bouteloua eriopoda* (black grama). Our sampling spanned two years (2012 and 2015) and a narrow geographical gradient at the Sevilleta National Wildlife Refuge, NM, where there is a distinct ecotone between shrubland and grassland. For each sampling period we collected roots from 10 creosote plants, 5 black grama plants within the creosote stand, and 5 black grama plants from the grassland. To assess fungal colonization, we sequenced the fungal ribosomal internal transcribed spacer (ITS) from both cultured endophyte isolates and from the total DNA of harvested roots via high-throughput sequencing. ITS sequences showed consistency across years but significant differences among the fungal communities of the three sample types (although grama samples were more similar to each other than to creosote). A majority of cultures from creosote roots and a portion of cultures from the transition-zone black grama roots were from the genus *Monosporascus*. No cultures from grassland black grama roots were represented by *Monosporascus*, although sequences from members of the genus were observed among 454 sequences. While previous studies demonstrated an aridland fungal endophyte community shared across diverse plant species at the Sevilleta, our results demonstrate that endophyte communities are also shaped by host species, supporting the possibility that endophytes influence encroachment.

338T Intestinal mycobiome variation across geography and phylogeny in the snail genus *Conus*. *A. Quandt*, A. Glasco, T. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

This project seeks to use the extensive collections of a hyper-diverse, predatory genus of marine snails, *Conus*, at the University of Michigan Museum of Zoology to fill a gap in our understanding of the fungi that associate with mollusks and how the mycobiome (fungal community) varies across geographic space, phylogenetic diversity, and ecological niches of the hosts. Several species of *Conus* (including *C. ebraeus*), have near global distributions and are particularly well represented in the collections, yet we have no knowledge of whether the mycobiome of such animals will be driven by their geographic location or their phylogenetic relationships. To determine mycobiome compositions, we dissected the intestines and digestive gland tissues from more than 30 *Conus* spp. from over 20 different countries, extracted DNA, and used fungal-specific amplicon-based high throughput sequencing of the ribosomal DNA region. First, we identify which fungi are commonly and uniquely associated with the digestive tracts of *Conus* spp., all of which are dominated by *Malassezia* spp. We also explore whether *Conus* phylogeny or geography causes greater variation in fungal community structure, and discuss techniques for targeted sequencing of preserved museum specimens for studies.

339F Plant-Microbe Interfaces: Characterizing the diversity and function of the ectomycorrhizome of *Populus trichocarpa*. *J.Alejandro. Rojas*[1], Brian Looney[2,3], Sunny Liao[1], Jay Chen[2], Jessy Labbé[2], Rytas Vilgalys[1] 1) Biology, Duke university, Durham, NC; 2) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; 3) University of Tennessee, Knoxville, TN.

The *Populus* root microbiome harbors a diverse community of ectomycorrhizal fungi (EMF) that significantly increase nutrient uptake and acquisition by the plant host while also providing protection against antagonistic parasites. Over 30 genera of EMF are known to associate with *Populus*, including many groups of mushroom-producing families including Boletaceae, Russulaceae, Cortinariaceae, Tricholomataceae, and Amanitaceae. A major aim of the PMI project is the collection, isolation, and characterization the diversity and function of major EMF fungal associates of *P. trichocarpa* across its range in the Pacific Northwest. In Autumn 2015 and 2016 (Oct/Nov 2015 and 2016) we surveyed macrofungal diversity under native *P. trichocarpa* forests from five core watersheds on the Squamish (BC), Snohomish (WA), Puyallup (WA), Columbia (OR and WA), and Willamette (OR) rivers. This resulted in over 150 collections of EMF fruit body collections and sampling of bulk soil used in bioassay studies. All sporocarp collections were plated on modified Melin-Norkrans medium, photographed, spore printed, and dried for identification and accession into fungal herbaria. The first fungal collections have been identified while the second samples from the second campaign are being identified by a consortium of taxonomic experts using the ITS barcode marker as well as morphological features.

340W Transcriptome analysis of deep-sea fungi from the Gulf of Mexico. *P. Velez*, R. Riquelme Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California Norte, MX.

The Gulf of Mexico is a biodiverse ecosystem that provides a wide array of valuable resources. Particularly, petroleum exploitation in this region sustains a multi-millionaire industry. However, these operations have resulted in major environmental disasters of unknown ecological consequences. Understanding the effect that oil spills have on marine ecosystems is limited to the extent of our knowledge on the autochthonous biota. Currently a great amount of information is available on bacteria inhabiting deep-sea sediments, however the occurrence of fungi in this environment has been poorly studied. Fungi are involved in key ecological deep-sea processes, yet these microorganisms have been rarely cultured and preserved from deep-sea samples. Therefore, the aim of this work is to analyze the microfungal diversity isolated from deep-sea sediment samples from the Gulf of Mexico, and to evaluate their metabolic response when exposed to hydrocarbons. We chose a culture-based approach in order to obtain fungal isolates, which will provide the opportunity for *a posteriori* studies and the possibility of *ex situ* preservation of fungal genetic resources. Fungal taxa were identified based on the evaluation of sequence data from the nuclear ribosomal internal transcribed spacer including the 5.8 rDNA region. Our results agree with previous work on deep-sea fungi reporting low levels of cultivable diversity, with Ascomycota as the dominant phylum. We did not observe significant differences among several culture media tested, indicating that perhaps these taxa tolerate a wide range of nutrient conditions. Ongoing work focuses on the transcriptome analysis on selected deep-sea fungal isolates, in order to provide the first insight of the genetic expression in response to hexadecane and 1-hexadecene. This is the first analysis exploring cultivable fungal diversity from deep-sea sediments in the Gulf of Mexico, and their response to hydrocarbons input, shedding light on a better understanding of the deep-sea ecosystem dynamics and bioremediation using deep-sea native taxa.

341T The exciting content in your cup of tea. *I. Skaar*[1], Y Zhang[2], M Sulyok[4], X Liu[5], M Rao[6], J Taylor[3] 1) Mycology, Norwegian Veterinary Institute, Oslo, NO; 2) School of Life Sciences, Shanxi University, Taiyuan, Shanxi, China; 3) Department of Plant and Microbial Biology, University of California, Berkeley, California, USA; 4) Center for Analytical Chemistry, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Tulln, Austria; 5) State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China and; 6) Bureau of Culture and Sports, Puer, Yunnan, China.

Tea is one of the most popular and widely consumed beverages in the world. Tea has important physiological effects on consumers due to the presence of compounds such as polyphenols, amino acids, vitamins, carbohydrates, caffeine, and purine alkaloids, all of which can have health benefits On the other hand, tea may also offer a complex microbiological diversity which can both contribute to the terroir of the tea, but also lead to presence of adverse secondary metabolites. Some teas are produced by microbiological fermentation. An example of such teas is the Pu-erh tea, produced in Yunnan, China, by fermentation of fresh *Camellia sinensis* leaves. The production and quality of Pu-erh tea is consequently closely related to microbial activity, making it important to understand the Pu-erh microbiome.
The fungal diversity in teas and the corresponding fungal metabolites as revealed both by culture dependent and culture-independent approaches will be discussed.

We will present a study of fungal and bacterial communities leaves and two types of Pu-erh teas by high-throughput, rDNA-amplicon sequencing and by quantitative liquid chromatography-tandem mass spectrometry of their metabolites.

We identified 390 fungal and 629 bacterial OTUs from leaves and both Pu-erhs. Major findings were: 1) fungal diversity drops and bacterial diversity rises due to raw or ripened fermentation, 2) fungal and bacterial community composition changes significantly between fresh leaves and both raw and ripened Pu-erh, 3) aging causes significant changes in the microbial community of raw, but not ripened, Pu-erh, and, 4) ripened and well-aged raw, but not young, Pu-erh have similar microbial communities. Twenty-five toxic metabolites, mainly of fungal origin, were detected, with patulin and asperglaucide dominating. We show that principles of community ecology can be applied to microbially fermented foods, that the acceleration of Pu-erh fermentation by the ripened process approximates the slower, traditional process, and that discarding the first preparation of Pu-erh tea and saving the wetted tea to brew a second cup for consumption has a sound mycotoxicological basis.

342F A phylogenetic approach based on PCR target enrichment and high-throughput sequencing for fungi: The *Ilyonectria* complex. *J.Alejandro. Rojas*, Khalid Hameed, Rytas Vilgalys Biology, Duke university, Durham, NC.

The use of high throughput sequencing (HTS) has facilitated the study of the systematics and phylogenetics of organisms. Nonetheless, non-model organisms and complex systems in fungi require large datasets to study the phylogenetic relationships among their members, which has been traditionally approach by sanger sequencing multiple genes to resolve their taxonomy. The use of HTS could provide large amounts data for multiple genes suitable for phylogenetics analyses. The present study used ten unlinked genes, eight nuclear genes (LSU, SSU, ITS, RPB1, RPB2, TUB, ACT and EF1-a) and two mitochondrial genes (ATP6 and mitSSU) that can be amplified from a single individual, using a barcoding approach per individual. We describe a novel approach using PacBio sequencing to obtain ~1kb sequences, using a multilocus sequence typing (MLST) approach and the downstream analysis to genotype 100 individuals in single sequencing run, providing enough loci to characterize a species complex. This approach was applied to *Ilyonectria* complex, an endophyte present in cottonwood (*Populus trichocarpa* and *Populus deltoides*), but also associated with black-foot disease on grapevines. The genus *Ilyonectria* was recently separated from other *Cylindrocarpon*-like anamorphs, and 17 species have been described up to now, most of them associated with disease. The HTS-MLST approach was used on endophytic and pathogenic isolates of *Ilyonectria* to resolve the species complex associated with *Populus.* The methodology could be applied to study the phylogenetics and systematics of other fungal groups.

343W Species and trichothecene genotype of Fusarium head blight Pathogens in Nebraska. *E. Valverde-Bogantes*[1], S. Wegulo[2], H. Hallen-Adams[1] 1) Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE; 2) Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE.

Fusarium head blight (FHB) is a devastating and economically important disease that affects wheat, barley, oats and other small grain cereals. FHB is particularly problematic because it poses a threat to both food security and food safety, due to yield reductions and mycotoxin contamination. FHB is caused by many *Fusarium* species, but *F. graminearum* is the most prevalent causal agent in North America. The most common mycotoxins produced are trichothecenes, mainly deoxynivalenol (DON), acetylated derivatives and nivalenol (NIV), which are harmful both to humans and animals. Early studies reported the 15-ADON genotype as the dominant genotype in North America. However, genetically divergent populations with the 3-ADON and NIV genotypes have been reported, making the introduction of these genotypes into Nebraska feasible and highlighting the need for increased vigilance. This research aims to identify the species and trichothecene genotype of FHB pathogens in Nebraska, in order to identify the major toxigenic risks in the state. A total of 33 affected wheat samples were collected from Nebraska fields – 23 from 2015 and 10 from 2016. DNA was extracted directly from plant material, as well as from single-spore isolates, in order to compare results and reduce testing time. A PCR reaction was performed using two alternative sets of primers for *F. graminearum* – galactose oxidase (GO) gene and UBC85 primers. Additionally, a multiplex PCR using two alternative sets of primers based on the *Tri3* and *Tri12* genes was used to determine trichothecene genotype. Preliminary results show that *F. graminearum* of the 15-ADON genotype is still the most prevalent FHB pathogen in Nebraska. Thus far, similar results have been obtained when using plant-extracted DNA and single spore isolate DNA, except for two single-spore isolates that produced unusual results with the trichothecene genotype primers. Since isolates with the 3-ADON and NIV genotype have been increasing in range due to selective advantages, constant surveillance is important for the deployment of proper management strategies and to ensure detection methods are appropriate.

344T Variations of mutation in *AVR1* and *AVR2* genes in the field isolates of tomato wilt fungus, *Fusarium oxysporum* f. sp. *lycopersici*. *T. Arie*[1,2,3,4,5], K. Akai[1,3], S. Tamura[3], S. Kikuchi[4], Y. Ayukawa[5], Y. Akagi[6], K. Komatsu[2,3,5], M. Kodama[6] 1) Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT); 2) Institute of Global Innovation Reserch, Tokyo University of Agriculture and Technology (TUAT); 3) Laboratory of Plant Pathology, Department of Agriculture, Tokyo University of Agriculture and Technology (TUAT); 4) Leading Graduate School, Tokyo University of Agriculture and Technology (TUAT); 5) United Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT); 6) United Graduate School of Agriculture, Tottori University.

Three races (race 1, 2 and 3), determined by the host-specificity to cultivars, have been reported in the tomato wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). Molecular mechanisms of divergence of races in *Fol* have been explained by holding and mutation of the avirulence genes *AVR1* and *AVR2*; race 1, *AVR1 AVR2 AVR3*; race 2, *avr1 AVR2 AVR3*; race 3, *avr1 avr2 AVR3*. We analyzed the patterns of mutation of avirulence genes in the field isolates of *Fol*. Five patterns of mutation were recognized in *AVR1*; *AVR1*-null, *AVR1*

is truncated at nts. 380 or 685 with a transposon *hormin* (*avr1*^{th380} or *avr1*^{th685}), or expression of *AVR1* is hindered by the insertion of a transposon *fot-5* at 380 bp. upstream of *AVR1* (*avr1*$^{tf\text{-}380}$), or by truncation at 212 bp. upstream of *AVR1* (*avr1*$^{t\text{-}212}$). Six patterns of mutations were recognized in *AVR2*; nucleotide substitutions at nts. 121 (*avr2*^{G121A}), 134(*avr2*^{G134A}), 137(*avr2*^{G137C}), 122(*avr2*^{T122A}), and 146(*avr2*^{C146T}), or *AVR2*-null. In total 4 biotypes of race 2 and 7 biotypes of race 3 were identified in the collection by holding and mutation pattern of *AVR1* and *AVR2*. Three biotypes of race 2 and five biotypes of race 3 were isolated after 2000 in Japan. We discuss the evolutional path of development of races in *Fol*.

345F Vertical transmission of cryptic alleles in *Aspergillus flavus*. *R.A. Olarte*, K.E. Bushley Plant and Microbial Biology, UMN, St. Paul, MN.

Aspergillus flavus commonly infects agricultural staple crops, but is also a common species in natural systems. It and other *Aspergillus* species produce aflatoxins and cyclopiazonic acid (CPA), both of which are mycotoxins and secondary metabolites. *A. flavus* is the most potent producer of aflatoxins, and along with *A. parasiticus* are considered to be the *Aspergillus* species of agronomic importance. Differing lineages of *A. flavus* may be prone to chromosomal instability in the aflatoxin gene cluster, evidenced by variation among strains within a lineage in their ability to produce aflatoxin or CPA. For example, *A. flavus* lineage 1B is prone to large-scale gene loss events within the aflatoxin cluster, which results in a non-functional aflatoxin gene cluster. However, the rates of chromosomal instability across the diverse lineages of *A. flavus* and their consequences for variation in aflatoxin production remain unknown. Through PCR, progeny isolates were found to possess genetic elements (*i.e.*, cryptic alleles) that were not detected in either parental strain. Cryptic alleles are believed to be extra-chromosomal elements that may result from genomic instability because inheritance of these alleles occurs in a non-Mendelian fashion. We sequenced a set of isolates, including the two parental strains as well as several progeny isolates, with Illumina HiSeq 4000 with conditions that allowed us to recover a minimum genomic coverage of at least 100x. Deep sequencing will confirm whether or not cryptic alleles found in the progeny are also present in the chromosomal genomes of the parental strains. *De novo* genome assemblies will be produced for isolates harboring cryptic alleles to be able to locate the positions of the identified cryptic alleles in the genomic landscapes of these isolates. Future research will determine whether these cryptic alleles are associated with fitness advantages and/or consequences.

346W The genome of an uncultured nematode-destroying fungus and its role in resolving the zygomycete tree of life. *K.R. Amses*, T.Y. James Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Phylogenomic analyses of the artificial Zygomycota *s.l.* show support for its reorganization into two segregate phyla, each containing three subphyla. Of these six subphyla of zygomycetes, many are represented by only one or a few draft genomes, leading to some ambiguity in analyses. The sampling of more taxa from these poorly represented groups will bolster the strength of phylogenomic hypotheses formed at these deep nodes, strengthening the lines delineating the proposed taxonomic groupings. The Zoopagomycotina, a subphylum comprised of obligate parasites of other zygomycetes and small soil animals, is represented by a single draft genome in the most recent analyses. Establishment of axenic cultures has proven a central obstacle to the genome-enabled study of these cryptic soil fungi as acquisition of sufficient material for traditional whole genome sequencing is difficult. Single cell genomics approaches offer a solution to these complications, allowing for the sequencing and assembly of whole genome data sets from miniscule amounts of input material summing to only one or a few cells. Leveraging these techniques in order to address the underrepresentation of Zoopagomycotina in phylogenomic analyses, we have sequenced the genome of *Stylopage hadra*, a cryptic but ubiquitous fungus that captures and consumes nematodes in natural and agricultural soils. Our single cell genomics approach generated four sequencing libraries with an average cumulative scaffold size of 22.81 Mbp and genome completeness scores of 56.65%, suggesting a *S. hadra* genome size of approximately 40 Mbp. Preliminary phylogenetic analyses based on concatenated RPB1 and RPB2 sequences support *S. hadra*'s placement in the Zoopagomycotina. Sequencing libraries contain a putative endosymbiont of *S. hadra* belonging to the beta-proteobacterian genus *Glomeribacter* with cumulative scaffold sizes indicating a genome size of approximately 1.80 Mbp. Known from the cytoplasm of arbuscular mycorrhizal fungi and species of *Mortierella*, species of *Glomeribacter* comprise a group of opportunistic soil-borne endosymbionts that tend to reduce the growth rates of their associated fungi, indicating a weakly parasitic interaction.

347T Genomics and sampling of the Ustilaginomycotina subphylum. *S.J. Mondo*[1], T. Kijpornyongpan[2], M.C. Aime[2], I.V. Grigoriev[1] 1) Fungal Genomics, Joint Genome Institute, Walnut Creek, CA; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN.

Despite their importance as plant and animal pathogens, as a whole Ustilaginomycotina remains one of the most poorly understood lineages of Basidiomycota. As genomes are becoming available for more orders, comparative genomics is becoming an attractive tool for further characterization of this subphylum. At the JGI, we now have annotated genomes available for 16 Ustilaginomycotina, representing 9 different orders. Most of these genomes are small (ranging 8 to 27 Mb) and very compact. However, this sampling still represents a small subset of the total diversity present within Ustilaginomycotina. Here we discuss the sequencing progress and preliminary results comparing these genomes to one another and those from other fungal groups. Through collaborations with the Ustilaginomycotina community, our aim is to provide a comprehensive set of genomes to facilitate deeper exploration of this subphylum.

348F *First report of Rhizoctonia solani AG-4 on tomato in Pothwar region of Pakistan*. *A.S. Gondal*[1], A. Rauf[2], G. Irshad[3], F. Naz[4] 1) Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi , Rawalpindi, Punjab, PK; 2) Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi , Rawalpindi, Punjab, PK; 3) Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi , Rawalpindi, Punjab, PK; 4) Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi , Rawalpindi, Punjab, PK.

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* (Frank) Donk) is the most important soil-borne fungal pathogen cause causes significant establishment with typical symptoms of collar rot, seedling death, stunted growth and root rot in tomato. Fungus isolated from diseased plants was identified as *Rhizoctonia solani* by comparing its morphological characters. Isolates were further confirmed as AG-4 by hyphal fusion test with tester isolates. Present study is the first report of *R. solani* AG-4 effecting tomato in Pothwar region, Pakistan.

349W Whole genome sequence of *Endogone lactiflua* reveals signatures associated with ectomycorrhizal lifestyle. *Y. Chang*[1], A. Desirò[2], G. Bonito[2], J. Spatafora[1] 1) Botany and Plant Pathology, Oregon State University, Corvallis, OR; 2) Plant Soil and Microbial Sciences, Michigan State University, MI.

Endogone (Endogonales, Mucoromycotina) is a species-poor but globally distributed genus and is known primarily from its truffle-like sporocarps. Ecologically *Endogone* includes saprobic to ectomycorrhizal (ECM) species and represents an independent origin of ECM outside Dikaryon. Together with its relatives in the early-diverging fungal lineage of Mucoromycotina, *Endogone* plays an important role in our understanding of the evolution of various plant-fungus associations and the molecular basis for these associations, especially for ECM.

In this study we did shotgun sequencing of the ECM species *E. lactiflua* from sporocarp tissue. We binned the sequence data using both Blast-based and nucleotide-composition-based methods before final assembly and annotation. We identified a large number of Mycoplasma-related endobacteria (MRE) sequences during the binning process. This is consistent with the observation that many *Endogone* species harbor MREs. The genome size of *E. lactiflua* is much larger than the average fungal genome, with an estimation of ~ 180 MB. Its large size is primarily due to the presence of various repetitive elements. Compared to its saprobic relatives, *E. lactiflua* has low diversity and low copy numbers of genes coding Carbohydrate-Active Enzymes (CAZymes) in its genome. This is consistent with the hypothesis that ECM lifestyle requires a small number of plant-degrading enzymes to avoid damaging host plant cells and eliciting a host plant defense response.

350T Appalachia Fungal Diversity, their Importance in Industry Representation, and the Phenotypic Variation of Newly Isolated Strains. *Matthew Winans* Biology, West Virginia University, 53 campus drive, Morgantown, WV, 26505.

Saccharomyces cerevisiae is one of the most powerful tools for the genomic fungal scientific community. The genomic sequences of many *Saccharomyces* clade members show nucleotide divergence similar to the divergence between humans and birds. Lab strains are typically used for genomic analysis for their flexibility in the laboratory setting, which in many cases reduces genetic and phenotypic diversity. Typically, lab strains are used to account for diversity in biofilms and flocculation abilities which is important the medical research setting, accounting for $148B per year and in addition in the brewing industry which accounts for $220B per year in the US alone. Isolating new strains of *Saccharomyces* clade members makes it possible to gain a larger picture of the genetic variation present in the wild and determine how they are evolutionarily adapted to their environments. Assays to determine chemical tolerances of strains in response to glyphosate, copper, and copper nanoparticles were performed. Additionally, in this study, over 300 environmental samples were collected, isolated, and analyzed through optimization of isolation techniques, from trees, flowers, fruit, soil and mammalians in Appalachia, Mexico, and Chile at varying temperatures. These findings have shown great ubiquity not only in the *Saccharomyces* clade, but of yeasts in general and their unique phenotypes.

351F Using intra-species fungal diversity to get different epigenetic imprints on fungal exometabolite production. *J. Frisvad* CMB, Dept Systems Biol - DTU, Kongens Lyngby, DK.

Fungi that are primarily Competition-selected (C-selected), such as species of *Aspergillus*, *Penicillium Paecilomyces* and *Talaromyces*, have been reported to produce many different secondary metabolites (exometabolites). Some of these exometabolites are produced on commonly used agar or broth media, while others need to be epigenetically induced by exometabolites from other species. Alternatively histone de-methylation or deacetylation inhibitors may be added to a medium in order to increase the number and kinds of exometabolites produced by the fungus. Often the use of an additional medium will increase the number of members of one biosynthetic family of exometabolites. On the commonly used media Czapek yeast autolysate (CYA) agar and yeast extract sucrose (YES) agar *Aspergillus taichungensis* did not produce any prenylated indol alkaloids, while on rice the same isolate produced 21 different taichunamides. However exometabolites coded by apparently silent gene clusters can also be discovered by examining isolates of the same species from different geographic regions. *Penicillium antarcticum* is a marine-derived fungal species producing a series of bioactive exometabolites. 47 strains from all over the world were analyzed using HPLC-DAD after growth on CYA and YES agar. Of these 45 produced asperentins, 40 patulin, 31 antarones, 28 fischerin, 25 atlantinone A, 23 chrysogines, 22 phthalides, 12 penitrems, 12 deacetoxyfructigenine, 5 a terrestric acid-like metabolite, 4 atrovenetins, 4 austalides, 3 cytochalasins, 1 an orthosporin-like metabolite and 1 patulodins. Furthermore potentially new extrolites (chromophore families not observed in any other *Penicillium* species) were produced by 26, 11, 5, 4, 3, 2, and 6 singleton isolates respectively. Examining several isolates within a species from different niches and geographic regions is an alternative way of discovering potentially bioactive exometabolites that could otherwise only be discovered by genome sequencing and using exometabolite gene cluster search algorithms. These observations indicate that Aspergilli and Penicillia can be epigenetically imprinted to express their exometabolites in certain instances.

352W Genetic diversity of *Ustilago esculenta*, a smut fungus associated with water bamboo in East Asia. *W.C. Shen* Plant Pathology & Microbiology, National Taiwan Univ, Taipei, TW.

Ustilago esculenta Henning is a smut fungus associated with a wild rice species, *Zizania latifolia* Turcz. With the infection of this smut fungus, swollen gall is developed at the basal part of the plant and becomes a favorable vegetable, so called water bamboo or makomotake, in East Asia. Sexual differentiation is required for the establishment of fungus-plant association and development of edible galls is resulted from phytohormone imbalance. In this study, we aim to characterize the mating type loci and mating system of *U. esculenta* and determine its genetic diversity. *U. esculenta* isolates were first collected from teliospores and gall tissues of field materials in Taiwan and Japan extensively. To identify the mating type loci and idiomorphic polymorphism, we conducted NGS genomic and PCR screening approaches. Conservations of pheromone, pheromone receptor and homeodomain transcriptional factors were confirmed and three idiomorphs are identified. Interestingly, transposon was found to insert at the mating type locus of *U. esculenta.* Genetic evidence indicate that *U. esculenta* contained a bipolar heterothallic mating system involved extremely large mating type loci. To understand its genetic diversity, we have targeted transposon and simple sequence repeats in *U. esculenta* genome and tried to develop molecular markers for fingerprinting study. These information will be utilized to breed for new varieties for commercial production.

353T Microbial communities associated with external fur surfaces of bats and the discovery of novel isolates with antifungal activity against *Pseudogymnoascus destructans*. *Paris Hamm*[1], Shiloh Lueschow[2], Ara Winter[3], Nicole Caimi[3], Diana Northup[3], Ernest Valdez[4], Debbie Buecher[5], Christopher Dunlap[2], David Labeda[6], Andrea Porras-Alfaro[1] 1) Department of Biological Sciences, Western Illinois University, Macomb, IL; 2) Crop Bioprotection Research Unit, US Department of Agriculture, Peoria, IL; 3) Department of Biology, University of New Mexico, Albuquerque, NM; 4) United States Geological Survey, Fort Collins Science Center, Albuquerque, NM; 5) Buecher Biological Consulting, Tucson, AZ; 6) Mycotoxin Prevention and Applied Microbiology Research Unit, US Department of Agriculture, Peoria, IL.

White-nose Syndrome (WNS), a bat fungal disease caused by the psychrophilic fungus *Pseudogymnoascus destructans*, has been estimated to have caused the death of more than six million bats in the eastern U.S. and Canada. Fungal and bacterial surveys were conducted to explore bats' natural microbial communities as a possible defense against this pathogen. Our WNS research efforts have mainly focused on the study of microbial communities associated with bats using next generation sequencing, metagenomic analysis, and the evaluation of cultured Actinobacteria to determine antifungal activity against *P. destructans.* Metagenomic analysis showed that fungi on bat fur surfaces belong to Chytridiomycota, Ascomycota, Basidiomycota, Glomeromycota, and Neocallimastigomycota. Distinct fungal and bacteria communities were detected on WNS (+) bats from Illinois and Indiana and WNS (-) bats from New Mexico and Arizona using 454 pyrosequencing. Further, we evaluated the antifungal potential of naturally occurring Actinobacteria isolated from WNS (-) bats. Bacteria colonizing bat fur and membranes were isolated from 12 healthy bat species providing approximately 2,700 isolates. We have screened over 1,300 bacterial isolates using a bi-layer method, of which 36 isolates show antifungal activity against *P. destructans*. Of the antifungal producing Actinobacteria, 32 (89%) were from the genus *Streptomyces*, known for their antibiotic production. Fifteen of the isolates with antifungal activity against *P. destructans* were identified as novel *Streptomyces* species after morphological and multi-gene phylogenetic analysis. This study includes one of the largest surveys of microbial communities associated with healthy bats. Our results show that bats in western North America possess novel bacterial microbiota with the potential to inhibit *P. destructans*.

354F Elucidating fungal endosymbiont interaction mechanisms between *Mortierella elongata* and *Mycoavidus cysteinexigens*. *Jessie Uehling*[1], Fred Dietrich[1], Pawel Misztal[2], Gregory Bonito[3], Tim Tschaplinski[4], Allen Goldstein[2], Chris Schadt[4], Jessy Labbe[4], Rytas Vilgalys[1] 1) Genetics & Genomics, Duke University, Durham NC 27708, USA; 2) Universtiy of California at Berkeley, Berkeley, CA 94270 USA; 3) Michigan State University, East Lansing, MI 48823 USA; 4) Biosciences Division, Oak Ridge National Laboratory, PO Box 2008 MS 6445, Oak Ridge, TN 37831-6445, USA.

Recently efforts to catalog beneficial microbes associated with *Populus* have yielded cultures of diverse fungi including several *Mortierella elongata* (*Mortierellomycotina*) isolates. Some of these fungi including *M. elongata* strain AG77 harbor bacterial endosymbionts belonging to the Burkholderiales. We have sequenced the genome of *M. elongata* AG77 and its bacterial endosymbiont Mycoavidus cysteinexigens (AG77), and used comparative phylogenomics to infer this symbiosis is ancient and grounded in shared metabolites. We have used and developed methods to assemble single genomes from meta-genomic data and inferred several gene losses that make culturing endosymbiont *M. cysteinexigens* (AG77) challenging. To circumvent this research obstacle, we used an antibiotic treatment to create fungal strains with and without their long-term endosymbionts for comparative multi-omic studies. The ability to contrast cleared *M. elongata* strains and their wild type, endosymbiont-housing relatives in the same genetic background allows us to ask the following questions: *1. How does the presence or absence of long-term, co-evolved endosymbionts impact health and physiology of host fungi? 2. Which currently available methods can be used and modified to study interaction dynamics for systems involving multiple intractable microbes?* Our research goals through this work were to develop -omics and imaging pipelines that allow the study of bacterial fungal interaction mechanisms. To this end we have used genome sequencing, transcriptomics, metabolomics, and volatomics for studying fungal endosymbiont interaction dynamics. The results of these experiments will be further discussed.

355W Diversity of fungal communities and co-occurrence with bacterial communities at the MSU-KBS Long Term Ecological Research wheat/corn/soybean crop rotation. Kristi Gdanetz MacCready, *Frances Trail* Michigan State University, East Lansing, MI.

The recent launch of the Phytobiomes Alliance calls for a holistic approach to studying plant health, one aspect of this is the plant microbiome. We need a thorough understanding of the populations of microbes inhabiting different plant niches before we can successfully manipulate microbial communities to benefit our crop health. Michigan State University's Kellogg Biological Station Long Term Ecological Research site harbors six replicate plots of four management treatments: conventional management, no-till, reduced inputs, and organic. These plots are on a three-year wheat/corn/soybean rotation cycle, providing an ideal site to conduct long-term characterization of these economically important row crops. We compared the diversity of the microbial communities, using fungal ITS2 and bacterial 16S amplicon sequencing, across the four management styles and three different plant organs for one cycle of the crop rotation. We assessed how below-ground and above-ground microbial communities differed, identified the unique community members for each crop, as well as the differences between the four management styles. Microbe communities, across plant organs and within a crop, are similar at the early vegetative stages; at later growth stages, the diversity increases. Compared with fungal communities, greater variation was observed in the structure of bacterial communities between organs and crops. Host plant species and plant organs had a stronger influence on community structure compared to plot management. Increased microbe diversity with time might be explained by a pattern of succession, or response to changes in plant metabolites and biochemistry across growth stages. Manipulating microbial communities to increase diversity, during critical growth stages when pathogens infect, may be beneficial to crop health.

356T Genotypic analysis of *Rhodotorula* strains isolated from environmental and clinical samples. Alex Metzger[1], Natalie Akers[2], Abigail Jacobs[1], Lauren McKean[2], *Amy Reese*[1] 1) Basic Sciences, St. Louis College of Pharmacy, St. Louis, MO; 2) Biological Sciences, Cedar Crest College, Allentown, PA.

Rhodotorula species are pink to coral encapsulated basidiomycete yeasts that have been isolated from various environmental locations. They are also increasingly linked with disease in immunocompromised patients and are particularly associated with central venous catheter infections and meningitis. Little is known about the biology of these fungi, including their natural reservoirs and the role of their capsule. Clinical isolates are known to be naturally resistant to fluconazole, voriconazole, and caspofungin, leaving few antifungals available for treatment. We have collected environmental *Rhodotorula* species from trees, domesticated dog coats, and avian excreta. In

addition, we have obtained agricultural strains from a plant pathology lab and clinical isolates from a medical laboratory, resulting in a set of about 50 strains in total. To characterize and compare our strains we have grown them on various cell wall integrity-challenging media and at various temperatures, assessed urease production, performed capsule size analysis, and detected their ability to bind of our anti-capsular antibody. In order to correlate our phenotypic and biochemical data with genotypic information, the internal transcribed spacer (ITS) regions of eight environmental and eight clinical strains were amplified with ITS1 and ITS2 primers from genomic DNA and the amplicons sequenced directly with the same primers. These results verified that all strains were *Rhodotorula,* but the data were not ideal for species-level identification. We have extracted genomic DNA from all samples to amplify longer ITS regions with ITS1 and ITS4 primers to then subclone into plasmids for improved sequencing reads for detailed species-level identification. There is limited genetic data available for these fungi in fungal databanks and the methods used to determine the species of clinical isolates is often vague or missing in the literature. Phenotypic and genotypic analyses for *Rhodotorula* species would allow a better understanding of these fungi and could prove instructive as more infections arise in our immunocompromised patient population from this emerging pathogen.

Gene Regulation

357F A novel phosphoregulatory switch controls the activity and function of the major catalytic subunit of protein kinase A in *Aspergillus fumigatus*. *E.K. Shwab*[1], P.R. Juvvadi[1], G. Waitt[2], E.J. Soderblom[2], M.A. Moseley[2], N.L. Nicely[3], Y. Asfaw[4], W.J. Steinbach[1,5] 1) Department of Pediatrics, Division of Pediatric Infectious Diseases, Duke University Medical Center, Durham NC, USA ; 2) Duke Proteomics and Metabolomics Core Facility, Center for Genomic and Computational Biology, Duke University, Durham NC, USA ; 3) Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC, USA; 4) Department of Laboratory Animal Resources, Duke University Medical Center, Durham NC, USA; 5) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham NC, USA.

Invasive aspergillosis, caused by the filamentous fungal pathogen *Aspergillus fumigatus,* is a major cause of death among immunocompromised patients. The cyclic-AMP/protein kinase A (PKA) signaling pathway is essential for hyphal growth and virulence of *A. fumigatus,* but the mechanisms of PKA regulation remain largely unknown. Here we have discovered a novel mechanism for the regulation of PKA activity in *A. fumigatus* via phosphorylation of key residues within the major catalytic subunit, PkaC1. Phosphopeptide enrichment and tandem mass spectrometry revealed the phosphorylation of PkaC1 at four sites (S175, T331, T333 and T337) with implications for important and diverse roles in the regulation of *A. fumigatus* PKA. While the phosphorylation at one of the residues (T333) is conserved in other species, the identification of three other residues represents previously unknown PKA phosphoregulation in *A. fumigatus*. Site-directed mutagenesis of the phosphorylated residues to mimic or prevent phosphorylation revealed dramatic effects on kinase activity, growth, conidiation, cell wall stress response, and virulence in both invertebrate and murine infection models. Three-dimensional structural modeling of *A. fumigatus* PkaC1 substantiated the positive or negative regulatory roles for specific residues. Suppression of PKA activity also led to downregulation of protein expression in an apparent novel negative feedback mechanism. Based on these findings, we propose a model in which PkaC1 phosphorylation both positively and negatively modulates its activity. This work paves the way for the future discovery of fungal-specific aspects of this key signaling network.

358W Recognition for Calcineurin in *Aspergillus fumigatus* by CRISPR-Cas9 mutagenesis. *C. Zhang*, X. Meng, L. Lu College of Life Sciences, Nanjing Normal Univeristy, Nanjing, jiangsu, CN.

Calcineurin (CaN) as a conserved protein phosphatase activated through the binding of Ca^{2+}-calmodulin, is a heterodimer composed of a regulatory (CnaB) and a catalytic (CnaA) subunit. In all eukaryotes, the calcineurin pathway is an important signaling cascade. In fungal pathogens, calcineurin is required for hyphal growth; stress responses and virulence and calcineurin inhibitors are suggested to be effective antifungals against the major invasive fungal infection. However, in mammals, calcineurin inhibitors are widely used immunosuppressive drugs, which limit therapeutic effectiveness as antifungals. Through gene deletion combined with complementation test, previous studies reported that calcineurin A embeds fungal-specific residues which are required for phosphorylation and virulence, indicating this specific region could be a effective antifungal target in filamentous fungal pathogen *Aspergillus fumigatus*. However, the précised *in situ* molecular characterization of calcineurin remains unknown. In this study, to carry out precise genome editing, we set up a highly efficient CRISPR mutagenesis system for exploring the function of calcineurin. Different from data obtained in model organism *A. nidulans*, we found that the *cnaA* null mutation can be significantly suppressed by adding calcium chelator EGTA or by deletion of the subunit of high affinity calcium channel *cchA* while this suppression only occurred in the presence of a calcineurin binding protein calcipressin CbpA. These results indicate that the hyphal growth defects caused by loss of calcineurin are primarily due to the influx of extracellular calcium *via* the high affinity calcium channel in *A. fumigatus*. Furthermore, through site-direct point mutation approaches, we found that previous identified the fungal-specific Serine Proline Rich Region (SPRR) located between two conserved the CnaB-binding helix and the CaM-binding domain are not required for hyphal growth since the in situ 4SA mutant (in situ point mutation 4SA containing S406A, S408A, S410A and S413A in Serine Proline Rich Region) has almost a normal colony phenotype, which is inconsistent to previous reported. In contrast, in situ point mutation W374G at the CnaB-binding helix domain caused severe hyphal growth defects, suggesting this domain is required for hyphal growth. Further verification for detail is ongoing.

359T The Negative Cofactor 2 complex mediates azole resistance through transcriptional regulation of an efflux transporter and ergosterol biosynthesis in *Aspergillus fumigatus*. Takanori Furukawa[1], Fabio Gsaller[1], Josie Parker[2], Steve Kelly[2], Scott Moye-Rowley[3], Paul Bowyer[2], *Mike Bromley*[1] 1) Manchester Fungal Infection Group, University of Manchester, Manchester, UK; 2) Institute of Life Science, Swansea University Medical School , UK; 3) Department of Molecular Physiology and Biophysics, University of Iowa.

A. fumigatus is the most important airborne mould pathogen and allergen worldwide. Estimates suggest that over 3 million people have invasive or chronic infections that lead to in excess of 600,000 deaths every year. Very few drugs are available to treat the various forms of aspergillosis and we rely predominantly on the azole class of agents (Itraconazole, Voriconazole, Posoconazole and the recently licensed Isavuconazole). Resistance to the azoles is emerging. For individuals that are infected with a resistant isolate the mortality rate exceeds 88%. Therapy failure is in part attributed to delays in administering alternative therapies so methods to rapidly detect resistance is critical. While resistance in around 50% of clinical isolates has been linked to modification of the gene encoding the target of the azoles,

cyp51A, our understanding of what leads to resistance in the remaining strains is lacking. To examine the mechanistic basis of non-target driven azole resistance we have carried out a systematic evaluation of the role of transcription factors in drug tolerance.
We have screened a library of 401 transcription factor *A. fumigatus* knockout strains and identified a cohort of factors governing azole resistance and sensitivity. Here we report on two CBF/NF-y family transcription regulators, AFUB_029870 (NctA) and AFUB_045980 (NctB) where loss of function leads to azole resistance. The orthologues of these regulators in yeast, known respectively as BUR6 and NCB2, are subunits of a heterotrimeric transcriptional regulator called Negative Cofactor 2 (NC2). In keeping with the hypothesis that NctA and NctB are part of the same transcriptional regulatory complex, we show the transcriptional regulons governed by these two factors are essentially identical. Furthermore, reciprocal co-immunoprecipitation studies using S-tagged versions of the proteins indicate that they interact directly with each other.
With a view to understanding which genes are directly regulated by NctA, we performed genome-wide protein-DNA interaction analysis using ChIP-seq. Over 1500 NctA interacting loci were identified, including 13 genes which encode members of the ergosterol biosynthetic pathway, and the efflux transporter cdr1B. Eight of these sterol biosynthetic genes along with cdr1B are transcriptionally upregulated in our NctA null leading to elevated levels of ergosterol and Cdr1B protein. Evaluation of the clinical significance of these findings is under investigation.

360F The opposing roles of SrbA and the HapX/CCAAT binding complex in the regulation of sterol biosynthesis and azole tolerance in *Aspergillus fumigatus*. *F. Gsaller*[1], P. Hortschansky[2], T. Furukawa[1], P.D. Carr[1], B. Rash[1], J. Capilla[3], C. Müller[4], F. Bracher[4], H. Haas[5], A.A Brakhage[2,6], M.J. Bromley[1] 1) Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, GB; 2) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 3) Microbiology Unit, Medical School, Universitat Rovira i Virgili, Spain; 4) Department of Pharmacy, Center for Drug Research, Ludwig-Maximilians University of Munich, Germany; 5) Division of Molecular Biology, Biocentre, Medical University of Innsbruck, Austria; 6) Institute for Microbiology, Friedrich Schiller University Jena, Jena, Germany.
Azole drugs selectively target fungal sterol biosynthesis and are critical to our antifungal therapeutic arsenal. Resistance to this class of agents in the major human mould pathogen *Aspergillus fumigatus* is reaching levels that suggest that they could be lost for clinical use. One family of pan-azole resistant isolates, characterized by the presence of a tandem repeat of at least 34 bases (TR34) within the promoter of sterol C14-demethylase encoding gene *cyp51A* dominates and is found globally. We demonstrate the transcriptional mechanisms underpinning TR34 mediated resistance showing the repeat is bound by both the sterol regulatory element binding protein SrbA, and the CCAAT binding complex (CBC). We show that the CBC acts complementary to SrbA as a negative regulator of ergosterol biosynthesis and TR34 driven overexpression of *cyp51A* results from effective duplication of SrbA but not CBC binding sites leading us to suggest possible routes to tackle the problem of TR34 mediated resistance.

361W Ascospore-specific gene expression analysis in *Aspergillus nidulans*. Mi-Kyoung Lee[1], Jong-Hwa Kim[2], Jae-Hyuk Yu[1], *Kap-Hoon Han*[2] 1) Department of Bacteriology, University of Wisconsin-Madison, WI, USA; 2) Department of Pharmaceutical Engineering, Woosuk University, Wanju, Jeonbuk, South Korea.
Developmental process and spore formation in a model filamentous fungus *Aspergillus nidulans* is environmentally and genetically regulated. Asexual spores or conidia differentiation is controlled by various orchestrated developmental pathways, including the *brlA* gene-mediated conidiophore and conidia morphogenesis. However, a few genes have been elucidated for playing an important role in sexual development and ascospore formation. The *nsdD*, *nsdC* and *veA* genes are well-known key regulators of sexual and asexual developmental processes. However, unlike conidia, physiological and genetic studies of ascospores are remained to be characterized. To know more about the ascospores biology, we performed RNA-seq analysis from from *A. nidulans* conidia and ascospores RNA samples. Comparative analysis of transcription profiles of conidia and ascospores revealed many genes that are expressed differentially in both spores. Detailed investigation of the differentially expressed genes is in progress.

362T Genetic interactions among G-protein coupled receptors GprH, I, and –M affect nutrient sensing, sexual development and secondary metabolite production in *Aspergillus nidulans*. *Laura Mellado*[1], Jessica M. Lohmar[2], Ana M. Calvo[2], Gustavo Henrique Goldman[1] 1) University of São Paulo, Ribeirão Preto, Brazil; 2) Department of Biological Sciences, Northern Illinois University, Dekalb, USA.
All living cells have the capacity to sense and respond to external signals, fundamental for cellular functions and survival. In eukaryotic signal transduction, the heterotrimeric G protein system plays a crucial role. In fungi, heterotrimeric G proteins (GPCRs) mediate responses to cell growth and division, mating, virulence and pathogenicity and the production of secondary metabolites. In spite of its biological importance, only a few have been functionally characterized. Our previous study in *Aspergillus nidulans* revealed the increased expression of a putative uncharacterized GPCR, *gprH*, during carbon starvation. We have demonstrated that GprH is a putative glucose and tryptophan receptor and also a sexual development repressor. We identified two other putative GPCRs, *gprI* and *gprM,* as additional elements of the carbon starvation response. Microarray hybridization analysis and qRT PCR experiments showed a *gprH*-dependent expression of *gprI* and *gprM* under carbon starvation. The presence or absence of each protein has an impact on spore germination and/ or hyphal proliferation, making cells able to respond to the presence or absence of glucose in the culture media especially post-carbon starvation. GprH and GprI also negatively regulate sexual development. *ΔgprH* and *ΔgprI* mutants showed an increase in cleistothecia production, and subsequently, a reduction in conidiospores formation under light conditions. This phenotype is still conserved in darkness for the *ΔgprH* mutant but not for the *ΔgprI*. These GPCRs might play an important role in secondary metabolite production. Our results show that the deletion of these genes affect the production of the sterigmatocystin toxin, and it is dependent on the VeA transcription factor and the exposure to light. Double mutant analysis showed genetic interaction among these genes affecting their individual phenotypes. In summary, we propose that GprH, GprI and GprM are nutrient receptors and they are involved in the germination, hyphal proliferation, sexual development and secondary metabolite production in *A. nidulans.* Intriguingly, there is crosstalking among glucose starvation, sexual development and light influencing the GPCRs signaling that seems to be mediated by VeA.
Financial support: FAPESP and CNPq, Brazil

363F Global Analysis of CreA Regulatory Network in *Aspergillus nidulans* . *Yingying CHEN*[1], Liguo DONG[1], Md Ashiqul Alam[2], Fang Wang[1], Joan Kelly[2], Koon Ho Wong[1] 1) Faculty of Health Science, University of Macau, Macau, CN; 2) Department of Genetics and Evolution, School of Biological Science, The University of Adelaide.

Carbon metabolism is central to all living organisms governing many physiological processes. In *Aspergillus nidulans*, carbon metabolism is tightly controlled at the transcription level by a process known as carbon catabolite repression (CCR). CCR is mediated by a global transcriptional repressor called CreA. Despite decades of research on CreA, the full spectrum of CreA targets and how it globally regulates and coordinates expression of genes remain unclear. In this research, we applied two powerful approaches RNA-seq and ChIP-seq for a genome-wide study of CreA under repressing and derepressing conditions. We discovered that CreA binds to several thousands promoters in the genome. The bindings are found mainly at the nucleosome-depleted promoter regions and are enriched with GC-rich motifs along with many other motifs. The set of CreA direct targets include genes involved in biosynthetic process, transmembrane transport, response to stimulus, regulation of transcription, ion homeostasis and sexual reproduction. Some of these roles have been confirmed by functional tests. Interestingly, our analysis reveals that the CreA binding intensity, the number of CreA binding sites and additional motifs are associated with different sets of CreA target genes of different physiological functions. Moreover, our genome-wide ChIPseq data also revealed that CreA constitutively binds to the target promoters at similar levels under both repression and de-repression conditions, even for genes whose expression is markedly increased (i.e. de-repressed) under the de-repression condition. This observation strongly indicates that CreA binding alone is not sufficient for CreA regulation, and in turn implies that post-translational modification(s) play(s) crucial role in CreA regulation. Transcription profiling analysis by RNAseq of wildtype and *creA*? mutant strains reveals that CreA not only acts as a transcription repressor, but also has a positive role for many target genes. Surprisingly, we found that expression of more than half of CreA bound genes are not significantly affected in the *creA*? strain. This suggests that either CreA pauses at many promoters pending modification for function or the activators responsible for activating those genes are not available for function under our experimental conditions. Taken together, our results reveal a comprehensive global CreA regulatory network at a whole-genome level and illuminate novel CreA regulating patterns and functions.

364W Phosphoproteomic and transcriptomic analysis of PKA activity in the filamentous fungus *Aspergillus nidulans*. *L.F.C. Ribeiro*[1], C. Chelius[1], K. Boppidi[1], S. Linconl[2], R. Srivastava[2], J. Kumar[3], S.D. Harris[3], M.R. Marten[1] 1) Chemical, Biochemical and Environmental Engineering Department, University of Maryland, Baltimore County (UMBC), Baltimore, MD, United States; 2) Chemical & Biomolecular Engineering Department, University of Connecticut, Storrs, CT, United States; 3) Center for Plant Science Innovation, University of Nebraska - Lincoln, Lincoln, NE, United States.

The ability to sense and respond to changing nutrient conditions is an essential function for all cells. After a fungus senses a change in its external nutrient environment, several internal processes occur in a coordinated fashion, including transcription of relevant transport and metabolic genes, ribosome biogenesis, up or down-regulation of translation machinery, and expression of proteins which direct morphogenesis. A major pathway involved with nutrient sensing and regulation of cell metabolism is activated by cAMP-dependent protein kinase A (PKA). While some PKA substrates have been characterized, these are not sufficient to explain the significant range of PKA activity in the cell. To better understand PKA systemic action in *Aspergillus nidulans*, we carried out phosphoproteomic and transcriptomic analysis of two *A. nidulans* strains: PKA+ (wild type) and PKA- (D*pkaA*). The data have allowed us to identify a number of proteins affected by PKA, many of which have not been previously associated with PKA. This approach also allowed us to identify specific phosphorylation sites which appear to mediate the impact of PKA. For example, CreA, a transcription factor responsible for carbon catabolite repression, was observed to be phosphorylated only in the wild type. Since CreA is responsible for repressing the transcription of cellulolytic enzymes, cellulolytic activity was quantified for both strains cultivated in rich medium with glucose, and the D*pkaA* strain yielded significantly higher (2.5x) cellulase activity. These results suggest PKA phosphorylates CreA (directly or indirectly) at an identified phosphorylation site. Additional data imply the lack of this phosphorylation site inhibits the repressor activity of CreA.

365T CRISPR/dCas9-mediated transcriptional activation in *Aspergillus nidulans*. *Shuhui Guo*, Chirag Jayantibhai Parsania, Liguo Dong, Chris Koon Ho Wong Faculty of Health Sciences, University of Macau, Macau SAR, China.

The well-known and powerful CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat and associated protein Cas9) system has been extensively applied for genome engineering in many organisms. The system can be modified for transcriptional activation or suppression of gene-of-interest by linking a mutated Cas9 that lost the nuclease function (called dCas9) with a transcriptional activation element (e.g. VP16, VP64, VPR and SAM) or a repression element (e.g. KRAB, SID), respectively. In this study, we aim to establish and optimize a CRISPR/dCas9 system for transcriptional activation in *A. nidulans*. We have generated a strain that expresses the dCas9-VP64 fusion protein and a gRNA expression plasmid carrying an internal fragment of the wA gene for targeting to the wA locus. An efficient strategy for introducing gRNA sequences to the expression plasmid has also been optimized. Similar to the CRISPR/Cas9 system, targeting of dCas9 to specific genomic regions is also mediated by sequence specific gRNAs to PAM sites. However, the location of PAM sites is critical and can greatly influence the degree of transcriptional activation and repression by the CRISPR/dCas9 system. Since many PAM sites can be found across a given promoter region, it is not immediately obvious which one would be ideal for activation/repression. Based on the well-established characteristics (e.g. nucleosome density, chromatin modifications, DNA bendability, sequence conservation, etc.) underlying transcription factors function, we have designed an approach to predict PAM sites suitable for the purpose. As a proof-of-principle, we have tested the CRISPR/dCas9 activation system on genes located within active (e.g. house-keeping genes) or silent heterochromatin (e.g. genes within secondary metabolite clusters) regions in *A. nidulans*. The success and the level of transcriptional activation were determined using RT-PCR. A “PAM site for activation” prediction program will be developed for public use. We believe that the CRISPR/dCas9 activation system is not only useful for artificial transcriptional activation, but also offer a novel method for activating cryptic secondary metabolite gene clusters in filamentous fungi.

366F L-rhamnose induction and metabolism in *Aspergillus niger*. *C. Khosravi*, Maria Victoria Aguilar-Pontes, Eline Majoor, Ronald De Vries CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

Aspergillus species are potent producers of enzymes involved in plant polysaccharide degradation. In nature, *Aspergillus* degrades the polysaccharides to monomeric sugars that can serve as a carbon source. Therefore, *Aspergillus* uses a variety of catabolic pathways to

efficiently convert all the monomeric components of plant biomass.
L-rhamnose catabolism in fungi has been described for the yeasts *Pichia sptipitis* and *Debaryomyces hansenii* (Watanabe *et al.*, 2008). In *Aspergillus niger* this pathway is poorly studied, and none of the putative L-rhamnose pathway genes or enzymes have been characterized. This project aims to confirm the function of the putative L-rhamnose pathway genes and to identify which metabolite in the L-rhamnose pathway is the inducer of the L-rhamnose responsive regulator (RhaR) (Gruben et al., 2014). After identification of the candidates, deletion mutants for these genes were obtained. Growth profile result showed no or only minor growth for all of the metabolic mutants. To study the effect of deletion of these genes in more detail, transcriptomics analysis of the reference strain and the KO strains Δ*lraA*, Δ*lraB*, Δ*lraC* and Δ*rhaR* have been done. The results indicate that L-rhamnose, L-rhamnono-Y-lactone and L-rhamnonate are not the inducers of RhaR. Further experiments will be required in order to determine if the inducer is located at the end of the metabolic pathway.

367W Subcellular localization and stability of deubiquitinase CreB involved in carbon catabolite repression in *Aspergillus oryzae*. *S. Ichinose*, M. Tanaka, T. Shintani, K. Gomi Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan.
Aspergillus oryzae produces large amounts of amylolytic enzymes in the presence of maltooligosaccharides, In the presence of glucose, however, their production is repressed by carbon catabolite repression (CCR) system. In filamentous fungi, it has been proposed that CCR is regulated by Cys_2His_2 type transcriptional factor CreA, and *creA* gene deletion results in high production level of α-amylase in *A. oryzae* (1). In addition, it is known that the ubiquitin processing protease CreB is also involved in CCR regulation. We generated a *creB* deletion mutant in *A. oryzae*, and revealed that CCR was relieved by deleting the *creB* gene. However, it is not clear how CreB is involved in CCR regulation. In this study, to elucidate the mechanism of CCR regulation by CreB, we generated GFP- or 3FLAG-fused CreB and observed the subcellular localization and stability of CreB. CreB-GFP was localized in the cytoplasm in both glucose and maltose media. In contrast, the amount of CreB-3FLAG in maltose medium was larger than that in glucose medium. Concomitantly, the *creB* gene expression level in maltose medium was much higher than that in glucose medium. These results suggested that CreB is regulated at transcriptional level and the *creB* gene is induced under CCR relieved condition. In *Aspergillus nidulans*, it was reported that CreB interacts with WD40 repeat protein CreC. Thus, we generated a *creC* deletion mutant in *A. oryzae*, and observed that CCR was also relieved by deleting the *creC* gene in *A. oryzae*. Interestingly, *creC* gene deletion resulted in a significant decrease in the amount of CreB-3FLAG and increase in the *creB* gene expression level compared with the wild-type, while the subcellular localization of CreB was not substantially altered. These results suggested that stability and transcriptional level of CreB was regulated by CreC
(1) Ichinose et al., *Appl. Microbiol. Biotechnol.,* **98**, 335–343 (2014)

368T Development of genome editing method by direct introduction of Cas9-protein in *Asepergillus oryzae*. K. Shimamoto[1,2], R. Saitou[1], Y. Wada[3], O. Mizutani[1], *K. Oda*[1], M. Okuda[1,2], K. Iwashita[1,2] 1) Brewing mycology, National Research Institute of brewing, Higashi-hiroshima, Hirhoshima, JP; 2) Hiroshima Univ; 3) FASMAC Co.Ltd.
Aspergillus oryzae is the one of attractive industrial strains used for traditional fermentation industries and recent enzyme production and pharmaceutical industries. Molecular breeding of *A. oryzae* is still difficult and troublesome, because of its genetical difficulty such as multi-nuclear and deficient of cross. Recently, genome editing with CRISPR/Cas9 is focused on for editing target genes. This system was generally introducing *cas9* gene with plasmid vector and expressed in mycelium, and as a result the transformants becomes gene modified organisms. Considering the utilization in industry, we developed the genome editing method by directly Cas9 introducing into the experimental strain of RIB40 and applied to various industrial strains.
We chose 5-FOA selectable *pyrG* in *A. oryzae* for genome editing target. Cas9 and *pyrG* single guide RNA (sgRNA) are mixed and formed ribonucleoproteins (RNPs), then introduced into *A. oryzae* by conventional protoplast-PEG method. 289 candidates were selected on 5-FOA containing plates. We confirmed the sequence of *pyrG* locus in 24 candidates and found that about half of the strains occurred 1bp deletion at target locus, indicating that genome editing was succeeded by this method. We further examined the amount of Cas9 use, and found that more than 10 mg is enough for *A. oryzae* genome editing by this method.
To confirm the practicality of the method, we carried out *pyrG* genome editing against some *A. oryzae* industrial strains, such as OIS01 (current Japanese Sake brewing), RIB128 (ancient Sake brewing), RIB163 (Japanese Sake brewing) and RIB915 (Soy sauce making) strains. We acquired genome editing candidates in the all industrial strains, indicating that this Cas9 direct introducing genome editing method is useful for molecular breeding of *A. oryzae* industrial strains.

369F Functional and subcellular localization analyses of transcription factors, AtrR and SrbA, involved in azole drug resistance in *Aspergillus oryzae*. K. Sugiyama[1], A. Ohba-Tanaka[1], D. Hagiwara[2], S. Kawamoto[2], M. Tanaka[1], T. Shintani[1], *K. Gomi*[1] 1) Graduate School of Agricultural Science, Tohoku University, Sendai, JP; 2) Medical Mycology Research Center, Chiba University, Chiba, JP.
We previously demonstrated that a novel $Zn(II)_2Cys_6$ transcriptional factor, AoAtrR, regulates gene expression of the ABC transporters that would function as drug efflux pumps and contributes to the azole drug resistance in *Aspergillus oryzae, and* a disruption mutant of the *AoatrR* ortholog (*AfatrR*) in *Aspergillus fumigatus* was similarly hypersensitive to azole drugs. In addition, RNA-seq analysis in *A. fumigatus* showed that AtrR regulated several ergosterol biosynthetic genes including *erg11*. Surprisingly, most of these ergosterol biosynthetic genes regulated by AtrR were nearly identical to those regulated by the basic helix-loop-helix transcription factor, SrbA (1). Therefore, we are interested in and investigate the difference in function between AtrR and SrbA in *Aspergillus oryzae*.
The expression of ergosterol biosynthetic genes such as *erg11*, *erg24*, and *erg25*, as well as ABC transporter genes, was significantly down-regulated in the *AoatrR* disruptant. Similarly, *AosrbA* disruption resulted in remarkable down-regulation of ergosterol biosythetic genes same as in *A. fumigatus*, but not ABC transporter genes. In contrast, *AoatrR* overexpression did not resulted in up-regulation of ergosterol biosythetic genes. These results suggested that AtrR and SrbA coordinately regulate ergosterol biosynthetic genes in aspergilli. However, *AoatrR* or *AosrbA* disruption had apparently no effect on another gene expression level, suggesting their expression would be regulated independently of each other. On the other hand, the *AoatrR* disruptant was more hypersensitive to azole drugs compared to the *AosrbA* disruptant and sensitive comparable to the *AoatrR*/*AosrbA* double disruptant, suggesting that hypersensitivity of the *atrR* disruptant to azole drugs is attributed not only to lowered ergosterol levels owing to down-regulation of ergosterol biosynthetic genes, but also to reduced efflux transport of the drugs owing to down-regulation of ABC transporter genes. Subcellular localization analysis of AoAtrR and AoSrbA showed that AoAtrR was constitutively localized in the nucleus and AoSrbA was likely localized in the nuclear envelope and/or

endoplasmic reticulum.
(1) Hagiwara et al., *PLoS Pathogens*, in press.

370W Relationship between stability and subcellular localization of carbon catabolite repression regulator, CreA, in *Aspergillus oryzae*. *M. Tanaka*, T. Shintani, K. Gomi Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, JP.

Aspergillus oryzae can produce considerable amounts of amylolytic enzymes in the presence of maltose, but expression of amylolytic enzyme genes is repressed by carbon catabolite repression (CCR) in the presence of glucose. CCR in filamentous fungi is regulated by the C_2H_2-type transcription regulator, CreA. When the 3FLAG-fused CreA was expressed under the control of the *creA* own promoter in *A. oryzae*, abundance of 3FLAG-CreA protein was dramatically reduced after addition of maltose or xylose, whereas this reduction was not observed after glucose addition. To examine the stability of CreA protein, 3FLAG-CreA was expressed under the control of the thiamine-repressible *thiA* promoter, and half -life of 3FLAG-CreA protein after addition of various sugars was measured.
After addition of maltose, xylose, and fructose, half-life of 3FLAG-CreA protein was calculated as approximately 11 minute. On the other hand, half-life of 3FLAG-CreA protein after glucose addition was approximately 25 minute. These results indicated that 3FLAG-CreA was stabilized by glucose addition. To investigate the relationship between stability and subcellular localization of CreA, we generated the mutated CreA harboring mutation in nuclear export signal (NES). Fluorescence microscope analysis of GFP-fused CreA showed that this mutated CreA remained into the nucleus after maltose addition, although intact CreA was exported to the cytosol. After maltose addition, half-life of 3FLAG-CreA harboring mutation in NES was calculated as approximately 33 minute. These results suggested that CreA was rapidly degraded in the cytosol under the CCR non-inducing condition.
This study was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry, and Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

371T Intron within 5′ untranslated region enhances transcription of the enolase-encoding gene (*enoA*) in *Aspergillus oryzae*. *Taishi Inoue*, Hiroki Toji, Mitsuru Takama, Mizuki Tanaka, Takahiro Shintani, Katsuya Gomi Tohoku University, Sendai, Miyagi, Japan.

Back ground: The glycolytic pathway is the primary metabolic process which is essential to metabolize a wide range of carbon sources in fungi. Hence, it is important for fungal survival to optimize the level of glycolytic genes expression in response to various environments. Interestingly, in *Aspergillus oryzae*, several genes which are involved in both glycolysis and gluconeogenesis, have alternative transcription start sites (TSSs). In addition, we demonstrated that selection of TSSs was dependent on two types of carbon sources; one is metabolized via glycolysis such as glucose and fructose, and another via gluconeogenesis such as acetate and ethanol. Particularly, stringent selection of alternative TSS was observed in the enolase-encoding gene (*enoA*). The similar transcriptional control of enolase gene was suggested in *Aspergillus nidulans* (Hynes et al., 2007). These findings seem to be important because it provides novel insight into environmental adaptation in the stage of transcriptional regulation of glycolytic genes in *Aspergillus* spp. However, the molecular details of alternative TSS selection remain to be elucidated. Remarkably, in enoA, there is an intron containing a downstream TSS (dTSS) within 5′ untranslated region (5′ UTR) when it is transcribed from an upstream TSS (uTSS). The length of the intron is 440 bp and quite long in fungi. In this study, we investigated the importance of this 5′ UTR intron in *enoA* expression.
Results: To this end, we analyzed the enoA promoter plus 5′ UTR whose intron was deleted (P*enoA*-Δi) or mutated at a splice site (P*enoA*-issm) using GUS reporter system. Under culture condition with acetate where transcription from uTSS is induced while transcription from dTSS is suppressed, both the GUS activity and the mRNA level were significantly decreased in P*enoA*-Δi. On the other hand, in P*enoA*-issm, the mRNA level was unaffected but the GUS activity was almost lost, presumably caused by emergence of uORF within intron unspliced. These results indicated that the deletion of dTSS within intron does not contribute to the reduction of gene expression in P*enoA*-Δi. Additionally, when the *enoA* gene was expressed by P*enoA*-Δi under condition that a resident *enoA* expression is suppressed, a resulting strain showed a significant reduction of the mRNA transcribed from uTSS and a poor growth under acetate culture condition. These results suggested that 5′ UTR intron enhances the *enoA* transcription from uTSS in *A. oryzae*.

372F Light-induced gene expression in *Botrytis cinerea* involves GATA-transcription factors and the stress-activated MAP kinase module. *Julia Schumacher*[1], Kim Cohrs[1], Adeline Simon[2], Jaafar Kilani[2], Muriel Viaud[2], Sabine Fillinger[2] 1) IBBP, WWU, Muenster, Germany; 2) BIOGER, INRA, Grignon, France.

Botrytis cinerea is a plant pathogen that exhibits prominent light responses including the formation of the reproduction structures (photomorphogenesis), secondary metabolites/ pigments, and antioxidant enzymes. A complex regulatory network of photoreceptors, transcription factors (TFs) and chromatin modifiers is supposed to initiate, transmit, and fine-tune the responses to different wavelengths of light on the transcriptional level that finally leads to the observable phenotypes. As the formation of the reproduction structures is strictly regulated by light in this fungus - conidia are formed in the light, sclerotia in the dark - the output can be easily monitored. The GATA-type TFs BcWCL1 (as part of the White Collar complex (WCC)) and BcLTF1 are important regulators as their deletions result in light-independent conidiation (“always conidia”) due to the deregulation of BcLTF2 (Schumacher et al. 2014; Canessa et al. 2013; Cohrs et al. 2016). Study of light-induced gene (LIG) expression in both deletion mutants highlighted the role of the TFs in activating EARLY and in repressing LATE LIG expression, including *bcltf2* encoding the master regulator of conidiation and further LTFs that may function downstream or in parallel with BcLTF2 in regulating the conidiation process. As the group of LIGs also contained genes that are induced by various stresses in a BcSAK1-dependent manner, the phosphorylation status of the stress-activated MAPK BcSAK1 was studied and shown to increase after exposure to light. Deletion of *bcsak1* impairs LIG expression suggesting that BcSAK1 functions as a co-activator of the WCC in inducing EARLY genes, and as a component releasing the LATE promoters (e.g. *bcltf2)* from repression by BcLTF1 and the WCC resulting in the expression of the conidiation genes. This preliminary model is in agreement with the observations that the deletion of BcSAK1 results in a “never conidia” phenotype (Segmüller et al. 2007), and that the exposure of the wild type to heat and osmotic stress bypasses the requirement of light for conidiation.

373W Investigation of six conserved transcriptional factors in *Candida tropicalis* in regulation of hyphae formation and biofilm development. *Y. CHEN*, C. Lin Biochemical Science and Technology, National Taiwan University, Taipei, TW.

Candida species are opportunistic fungi, which can cause superficial and systemic infections in immunocompromised individuals. It has been known that regulatory network composed of Bcr1, Brg1, Efg1, Ndt80, Rob1 and Tec1 is involved in biofilm development in *C. albicans*. In this study, we compared the function of the six orthologous genes in *C. tropicalis* to those of *C. albicans* in biofilm and hyphae formation. Results showed that deletion of *BRG1*, *EFG1* or *TEC1* gene resulted in biofilm development deficiency and reduced its hyphae formation significantly in both *C. tropicalis* and *C. albicans*. Although mutant strains of *bcr1Δ, ndt80Δ,* and *rob1Δ* of *C. tropicalis* also caused a significant reduction in the biofilm mass, the hyphal formation displayed distinct outcomes compared to the mutants strains of orthologous genes in *C. albicans*. We therefore used cross-species gene transfer strategy to investigate their roles in biofilms and hyphae between two *Candida* species. Transformation of *C. tropicalis BCR1, NDT80,* and *ROB1* into each corresponding mutant of *C. albicans*, respectively, showed that *CtROB1* is unable to recover biofilm formation and filamentation. Reintroduction of the functional *CtBCR1* into *Cabcr1Δ* did not affect hyphae formation, but restored the developmental process of biofilms. Under serum condition, 64.3% and 41.7% of the wild-type strains of *C. albicans* and *C. tropicalis* cells formed filaments, respectively. Interestingly, mutant strains of *Candt80Δ* and *Ctndt80Δ* caused biofilm defects but had 24.5% and 63.1% of filamented cells in *Candt80Δ* and *Ctndt80Δ*, respectively, suggesting that Ndt80 in *C. tropicalis* might be a negative regulator in filamenetation. Thus, the results imply that the Ndt80 has the conserved function in biofilm formation, but the entire hyphal regulatory circuit might be different between two *Candida* species. Indeed, our results further demonstrated that expression of *CaNDT80* into *Ctndt80Δ* could repress the filamentation ability of the *Ctndt80* mutants. Taken together, our study demonstrates that each single transcriptional gene holds the conserved function in biofilm and in filamentation, but have evolved a sophisticated regulatory network by a group of factors for the control of hyphae formation.

374T *ZCF32*, a Zn(II)2Cys6 transcription factor, suppresses the virulence of *Candida albicans*. *P. Kakade*[1], V. Nagaraja[1,2], K. Sanyal[2] 1) Indian Institute of Science, Bangalore-560012, Karnataka, India; 2) Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore-560064, Karnataka, India.

Candida albicans, an opportunistic human pathogen, carries an expanded family of Zn(II)2Cys6 zinc binuclear cluster transcription factors. Zcf32, a Zn(II)2Cys6 transcription factor, is the member of this expanded family of proteins. In the present study, we intend to gain insights into the role(s) played by this transcription regulator in the virulence of *C. albicans*. Global transcriptome analysis carried out for the *zcf32/zcf32* null mutant and wild-type revealed that biofilm development is the most altered pathway followed by iron homeostasis and hyphal development pathways. To delineate the functional correlation between *ZCF32* and the different traits regulated by it, we determined the set of genes directly regulated by Zcf32. Our data suggest that Zcf32 regulates biofilm formation by repressing the expression of adhesins, chitinases and a significant number of other GPI-anchored proteins. We have established that there is lesser recruitment of Zcf32 on the promoters of biofilm genes in biofilm condition compared to the planktonic mode of growth. This shows that the transcription factor *ZCF32* negatively regulates biofilm development in *C. albicans*. Iron homeostasis maintenance is considered as an important fitness attribute in *Candida* pathogenesis. While growth studies showed that *zcf32/zcf32* null mutant grows as good as wild-type in low as well as high iron condition, the molecular data hints at the role of Zcf32 in high iron homeostasis maintenance. Lastly, the virulence regulation of the pathogen by this transcription factor was studied in mice model of candidiasis. Notably, *zcf32/zcf32* null mutant displayed hypervirulence in comparison to the wild-type. Thus, *C. albicans,* a gut commensal as well as a pathogen, has evolved with a negative regulator of virulence which might have helped the organism to maintain the commensal state.

375F Functional Analysis of the Cph1-regulated Genes of *Candida albicans* White Cells during the Response to Pheromone. *F. Deng*, C. Lin Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei, TW.

Candida albicans is the most important fungal pathogen in human, particular in some immunocompromised patients. The propensity for causing *C. albicans* infection has been closely linked with its ability to form biofilms. Central to understanding the *C. albicans* behavior is the white-opaque phenotypic switch, in which cells can undergo an epigenetic transition between the white state and the opaque state. The phenotypic switch regulates multiple properties including biofilm formation, virulence, and sexual mating. In particular, it is now apparent that sexual pheromones secreted from opaque cells can initiate biofilm formation in white cells. Our studies have identified the transcriptional regulator of pheromone-induced cell adhesion as Cph1 (Ste12). However, the mechanism of how white cells in response to pheromone remains obscure. Based on the array profiling, we have identified 10 downstream genes regulated by Cph1 in the *C. albicans* white state, but not in the opaque state. We therefore hypothesized that these genes might play important roles in pheromone-induced white response specifically. Deletion of each gene revealed that most of mutant strains affected the pheromone-stimulated cell adhesion in white cells. In particular, *orf19.1539Δ*, *orf19.1725Δ* and *orf19.3643Δ* showed a significant reduction in cell adhesion during the response to pheromone. Due to adherent ability is an important first step in establishing biofilm formation. *orf19.1539Δ*, *orf19.1725Δ* and *orf19.3643Δ* were further investigated to understand if each of them is also involved in the formation of conventional biofilms. Results showed that mutant strains of *orf19.1725Δ* lacked its ability to form biofilms and repressed hyphal formation. Introduction of a functional copy of *ORF19.1725* returned its function of biofilm development and hyphal formation. Together, our data has discovered some genes that might be important for the white cell response, but not for the opaque cell response. It might provide a clue that why white and opaque cells display distinct behaviors during the response to pheromone.

376W DNA Methylation Remodeling in *Candida albicans* Plays a Key Role in Epigenetic Regulation of Phenotype- Dependent Pheromone Responses. *S. Hoo*[1], Y. Huang[2], A. Archana[2], P. Chen[2], C. Lin[1] 1) Biochemical Science and Technology, National Taiwan University, Taipei, TW; 2) Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan.

Candida albicans is the most frequently isolated fungal pathogen in immunocompromised patients with hospital-acquired infections, as the phenotypic plasticity enables easy adaption of *C. albicans* to different niches in immunodeficient patients. Among the phenotypic plasticity, there is a heritable and reversible phenotypic switching between white and opaque cells, called white-opaque switching. Herein, mating competent opaque cells secrete sexual pheromone to promote opaque cell mating projection, the process is called opaque cell pheromone response. Besides, the pheromone can also stimulate cell adhesion and sexual biofilm formation of mating incompetent white cells through white cell pheromone response. Interestingly, white and opaque cells are sharing the same pheromone signaling pathway and major transcription factor Cph1 despite having different cell specific response and gene expression during pheromone stimulation.

According to the previous research, DNA methylation efficiently regulates expression of cell specific gene between different cell types of *C. albicans*. In our study, the results of AzaC (5-azacytidine, DNA methylation inhibitor) treatment show that DNA demethylation inhibits white cell pheromone response, which is associated with downregulated white cell pheromone response genes (*EAP1* and *PBR1*). By contrast, opaque cell pheromone response is raised by DNA demethylation and opaque cell pheromone response genes (*FUS1* and *FIG1*) are also upregulated. Also, whole genomic bisulfite sequencing and RNA sequencing data further suggest that DNA methylation remodeling (include critical DNA demethylation and *de novo* methylation process) regulates different gene expressions and causes distinct cell behaviors in white and opaque cells during pheromone stimulation. Therefore, we suppose that DNA methylation remodeling in *C. albicans* may play a key role in regulating phenotype-dependent pheromone responses.

377T The role of *C. neoformans* chitin deacetylases in chitosan mediated virulence. *R. Upadhya*[1], W.C Lam[1], L.G Baker[1], M.J Donlin[2], C.A Specht[3], J.K Lodge[1] 1) Molecular Microbiology, Washington University School of Medicine, Saint louis, MO; 2) Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine; St. Louis, MO; 3) Department of Medicine, University of Massachusetts Medical School, Worcester, MA.

Chitosan is one of the major virulence factors of C. *neoformans*. Mutant strains deficient in chitosan are avirulent in mice and are rapidly cleared from the host. Enzymatic conversion of chitin to chitosan is catalysed by chitin deacetylases. We have discovered that deletion of three deacetylase genes: *CDA1, CDA2* and *CDA3* are required for generating a chitosan deficient *C. neoformans* strain. This *cda1?2?3?* strain is avirulent and when cleared induces protective immunity to a lethal infection by its virulent, progenitor strain KN99. To further understand the role of individual chitin deacetylases in fungal pathogenesis we generated mutant strains deleted of either a single CDA gene or combinations of two CDA genes. Individual CDA deletion strains did not show any defect in the amount of chitosan under standard growth conditions, in vitro. However, when tested using inhalation model of cryptococcosis, we found that only *cda1*? was avirulent and both *cda2?* and *cda3?* exhibited wild-type virulence. These results indicate that *C. neoformans CDA1* is the major chitin deacetylase that is critical for establishing infection and eventual mortality to infected animals. The importance of *CDA1* is further supported by its specific upregulation during growth in the infected lung while *CDA2* and *CDA3* exhibit downregulation. In addition, c*da1*? cells in the infected lung produced less chitosan than KN99. Interestingly, the infected host lung was not able to clear *cda1?* cells. Of the three double deletion combinations only *cda1?2?* was also cleared. These data support a minor role of *CDA2* in the persistence of fungus inside the host. In conclusion, in *C. neoformans* the chitosan produced by *CDA1* is critical for fungal virulence.

378F *Botrytis cinerea*, more than just a pathogen: a new platform to dissect integration of environmental signals and circadian mechanisms. *Paulo Canessa*[1,3], Montserrat Hevia[2,3], Hanna Müller-Esparza[2,3], Luis Larrondo[2,3] 1) Centro de Biotecnologia Vegetal, Universidad Andres Bello, Santiago, RM, CL; 2) Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, RM, CL; 3) Millennium Nucleus for Fungal Integrative and Synthetic Biology (FISB), Santiago, RM, CL.

BcWCL1 and BcFRQ1 are two major components of the recently characterized *Botrytis cinerea* circadian clock. These molecular machineries have emerged at least three times during evolution and are cell-contained time-devices that allow temporal compartmentalization of several functions, both at the cellular level (i.e. gene expression) and at the systemic level (i.e. general physiology, metabolisms and defense mechanisms). As previously demonstrated, BcWCL1 serves light-dependent developmental-associated functions, while BcFRQ1 is the central circadian clock pacemaker that impacts time-dependent virulence in *B. cinerea*.
An exhaustive bioinformatic examination of several fungal genomes has allowed the identification of the clock negative element (FRQ) outside a couple of previously identified fungal clades. In the case of BcFRQ1, close examination of the protein sequence reveals conservation of several domains and regions of low structural complexity, thus supporting its function as the clock pacemaker. Nevertheless, both phenotypical and molecular data, derived by comparison of genome-wide expression data of *bcwcl1* and *bcfrq1* null mutants, indicate new “supra-circadian” functions for this clock protein. Thus, in addition to its role in orchestrating daily changes in the *B. cinerea* virulence potential, BcFRQ1 appears to be at the crossroads between nitrogen metabolism and asexual/sexual developmental programs. FUNDING: MN-FISB NC120043, FONDECYT Inicio 11140678 and FONDECYT 1131030.

379W Interconnected network of circadian rhythms and glycogen metabolism in *Neurospora crassa*. M. Baek[1], S. Virgilio[2], A. Dovzhenok[3], T. Lamb[4], O. Ibarra[4], S. Lim[3], D. Bell-Pedersen[4], M.C. Bertolini[2], *C.I. Hong*[1] 1) Department of Molecular & Cellular Physiology, University of Cincinnati, Cincinnati, OH, USA; 2) Departamento de Bioquímica e Tecnologia Química, Instituto de Química, Universidade Estadual Paulista, UNESP, 14.800-060, Araraquara, São Paulo, Brazil; 3) Department of Mathematical Sciences, University of Cincinnati, Cincinnati, OH 45221-0025, USA; 4) Department of Biology, Texas A&M University, College Station, TX 77843, USA.

Circadian rhythms provide temporal information to other cellular processes including metabolism, which align organisms’ physiological processes with the external environment. In this report, we utilized mathematical modeling and experimental validations to investigate molecular mechanisms that link circadian rhythms and glycogen metabolism in *Neurospora crassa*. We discovered complex regulation of the expression of key enzymes that regulate glycogen metabolism, glycogen synthase (*gsn*) and glycogen phosphorylase (*gpn*), by three different transcription factors (CSP-1, VOS-1, and WCC). This combinatorial TF regulation of *gsn* and *gpn* results in robust circadian oscillations of glycogen abundance, which is abolished in circadian arrhythmic mutants (frq^{ko} and $wc\text{-}1^{ko}$). Our findings elucidate key players and mechanisms that regulate rhythmic abundance of glycogen, which is critical for glucose homeostasis.

380T Analysis of circadian regulated transcripts under cellulose growth conditions in *Neurospora crassa*. *Adrienne Mehalow*[1], Alexander Crowell[1], Kerrie Barry[2], Igor Grigoriev[2], Jennifer Loros[1], Jay Dunlap[1] 1) Molecular and Systems Biology, Dartmouth Medical School, Hanover, NH; 2) US Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598.

Circadian clocks are highly conserved in eukaryotic organisms and confer the ability to anticipate regular changes in the environment resulting from the 24 hour day on earth. *Neurospora crassa* has been utilized to investigate the molecular clockworks for more than 30 years, due to readily observable circadian phenotypes, availability of mutants, and ease of manipulation in the lab. The *Neurospora* clock consists of a core clock oscillator with interconnected transcription and translation feedback loops. This oscillator produces a self-sustaining rhythm, is entrainable to environmental cues, and regulates the behavior of downstream clock-controlled genes (*ccg*s). *Neurospora* is capable of metabolizing a wide variety of carbon sources, although is most commonly cultured on glucose. RNA-seq

completed by this lab (Hurley *et al*., PNAS 111: 16995-17002, 2014) and others has identified 10-40% of the *Neurospora* genome that is circadianly regulated under glucose metabolizing conditions. When cultured on pure cellulose, over 100 genes are upregulated, cellulases are produced in large quantities, and additional biochemical pathways such as the unfolded protein response (UPR) are activated (Tian *et al.,* PNAS 106: 22157-22162, 2009). While amongst the upregulated genes are many that have been identified as circadianly regulated under glucose metabolizing conditions, confirmation of their circadian behavior during cellulose metabolism remains incomplete.
The goal of this work is to transcriptionally profile *Neurospora* cultures grown on 2% microcrystalline cellulose (Avicel) over a 48 hour high resolution time course to identify circadian sense and anti-sense transcripts. Samples collected at two hour intervals and assessed for circadian behavior of core clock genes and *ccg*s by real-time PCR provide the RNA for subsequent high-throughput sequencing; analysis of primary data with JTK cycle detects rhythmic changes in abundance of both sense and anti-sense transcripts.
These data will provide the first global assessment of circadian transcripts under cellulose metabolism conditions, and of circadian regulation of anti-sense transcripts from *Neurospora*. Understanding the differential regulation of core clock components and downstream *ccg*s under cellulose metabolism will be valuable for optimization of biofuel production.

381F Cation-Stress-Responsive Transcription Factors SltA and CrzA Regulate Morphogenetic Processes and Pathogenicity of Colletotrichum gloeosporioides. A. K. Dubey[1], N. Luria[1], S. Barad[1,2], D. Kumar[1], E. A. Espeso[3], *D. Prusky*[1] 1) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, Rishon LeZion, IL; 2) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 3) Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas (C.I.B.), Madrid, Spain.

Growth of *Colletotrichum gloeosporioides* in the presence of cation salts NaCl and KCl inhibited fungal growth and anthracnose symptom of colonization. Previous reports indicate that adaptation of *Aspergillus nidulans* to salt- and osmotic-stress conditions revealed the role of zinc-finger transcription factors SltA and CrzA in cation homeostasis. Homologs of *A. nidulans* SltA and CrzA were identified in *C. gloeosporioides*. The *C. gloeosporioides* CrzA homolog is a 682-amino acid protein, which contains a C_2H_2 zinc finger DNA-binding domain that is highly conserved among CrzA proteins from yeast and filamentous fungi. The *C. gloeosporioides* SltA homolog encodes a 775-amino acid protein with strong similarity to *A. nidulans* SltA and *Trichoderma reesei* ACE1, and highest conservation in the three zinc-finger regions with almost no changes compared to ACE1 sequences. Knockout of *C. gloeosporioides crzA* (?*crzA*) resulted in a phenotype with inhibited growth, sporulation, germination and appressorium formation, indicating the importance of this calcium-activated transcription factor in regulating these morphogenetic processes. In contrast, knockout of *C. gloeosporioides sltA* (?*sltA*) mainly inhibited appressorium formation. Both mutants had reduced pathogenicity on mango and avocado fruit. Inhibition of the different morphogenetic stages in the ?*crzA* mutant was accompanied by drastic inhibition of chitin synthase A and B and glucan synthase, which was partially restored with Ca^{2+} supplementation. Inhibition of appressorium formation in ?*sltA* mutants was accompanied by downregulation of the MAP kinase *pmk1* and carnitine acetyl transferase (*cat1*), genes involved in appressorium formation and colonization, which was restored by Ca^{2+} supplementation. Furthermore, exposure of *C. gloeosporioides* ?*crzA* or ?*sltA* mutants to cations such as Na^+, K^+ and Li^+ at concentrations that the wild type *C. gloeosporioides* is not affected, had further adverse morphogenetic effects on *C. gloeosporioides* which were partially or fully restored by Ca^{2+}. Overall results suggest that both genes modulating alkali cation homeostasis, have significant morphogenetic effects that reduce *C. gloeosporioides* colonization.

382W You don't know what you've got until it's gone: novel RNAi components in *Cryptococcus neoformans*. *Shelby Priest*, Blake Billmyre, Joseph Heitman Duke University Medical Center, Durham, NC.

Whole genome sequencing from *Cryptococcus deuterogattii*, a species responsible for an ongoing outbreak in immunocompetent individuals, revealed loss or significant truncation of fourteen conserved genes relative to the *Cryptococcus* pathogenic species complex. The fourteen genes include three canonical RNAi components and previous studies have shown that six more of these genes are involved in mitotic-induced silencing (MIS), sex-induced silencing (SIS), or both. We investigated the role of two additional genes identified from this comparative genomics approach, *MEH1*, a microsomal epoxide hydrolase, and *CDP1*, a chromodomain protein. We initially hypothesized that *CDP1* would likely play a role in at least one of the silencing pathways, while *MEH1* would not. However, assaying for MIS and SIS through fluctuation assays and genetic crosses, respectively, in genetic deletion mutants of *MEH1* and *CDP1* revealed roles for both genes in MIS and SIS. These unexpected phenotypes drove us to investigate the genomic landscape of *MEH1*. Interestingly, there is significant overlap between the 3' UTRs of *MEH1* and its neighboring upstream gene, *RNH70*, an RNA exonuclease. Unlike the previously observed silencing phenotypes, independent *MEH1* deletion mutants produced a modest increase in MIS and a large increase in SIS during bilateral crosses. Conversely, deletion of *RNH70* resulted in complete abolishment of MIS and decreased SIS during unilateral crosses. Bilateral crosses between *rnh70*Δ mutants generated primarily bald basidia and sparse basidiospores, suggestive of a meiotic defect. These new findings represent a case of a neighboring gene effect, where off-target effects of a gene deletion may be responsible for the observed phenotypes, and provide an opportunity to investigate how 3' UTRs of convergently oriented genes contribute to genetic regulation.

383T Plant homeodomain (PHD) finger proteins regulate cryptococcal yeast- hypha transition. *Yumeng Fan*, Yunfang Meng, Xiaorong Lin Biology, Texas A&M University, College Station, TX.

Cryptococcus neoformans, an opportunistic human fungal pathogen, is responsible for a million infections and half a million deaths each year globally. This fungus can undergo morphological transition from the yeast to the hypha form. Like other dimorphic fungal pathogens, this morphological transition of *Cryptococcus* is tightly linked to its virulence. Previously, our lab identified a transcription factor Znf2 as the master regulator that bridges cryptococcal morphology and its virulence potential. However, the regulatory circuit controlling this potent anti-virulence factor is still unclear.

Morphotype transition indicates remodeling of gene expression. Epigenetic factors as well as genetic factors are shown to be critical in regulating morphogenesis in other fungal species. The post-translational histone modifications can impact gene expression by altering chromatin structure or recruiting histone modifiers. However, nothing is known about epigenetic regulation in cryptococcal yeast-hypha transition. The plant homeodomain (PHD) finger proteins are known as epigenetic effectors in plants and mammals by reading and effecting

histone modifications. We deleted and characterized 15 PHD finger genes and found that five PHD finger containing proteins affect switching to the filamentous form dramatically. The *rum1*Δ and the *znf1*Δ mutants both showed enhanced initiation of filamentation but failed to maintain filamentous growth. The *bye1*Δ and the *phd11*Δ mutants exhibited enhanced filamentation while the *set302*Δ mutant displayed reduced filamentation. Ectopic overexpression of these five PHD genes in the corresponding null mutants partially or completely restored the defect in filamentation. Thus, these PHD finger proteins are activators or repressors of filamentation. We further demonstrated that Phd11 and Set302 regulate yeast-hypha transition through the known master morphogenetic regulator Znf2. Collectively, these findings indicate the existence of novel pathways to control cryptococcal morphogenesis. Currently, I am focusing on dissecting the detailed mechanism of epigenetic regulation of filamentation. Exploring this layer regulation will not only help understand *Cryptococcus* development, but also identify targets for therapeutic interventions and against this fatal pathogen.

384F Pas3 regulates cryptococcal morphogenesis through Bre1/Npl3-Mediated alternative splicing. *Y. Zhao*, X. Lin Biology, Texas A&M University, College Station, TX.

Per-ARNT-Sim (PAS) proteins function as signal receptors, transducers, or regulators. In the human fungal pathogen *Cryptococcus neoformans*, two PAS proteins, Bwc1 and Bwc2 (white collar 1 and 2), are known to regulate mating, virulence, and tolerance of ultraviolet radiation. To comprehensively understand the regulatory role of PAS proteins in regulating morphogenesis and pathogenicity, all 9 PAS domain coding genes were analyzed in *C. neoformans*. One of the PAS domain proteins, Pas3, was found to be critical in cryptococcal filamentation and virulence. Deletion of *PAS3* in either the serotype D reference strain XL280 or the serotype A reference strain H99 severely impaired filamentation during either unisexual (a alone) or bisexual (**a**-a) reproduction. Interestingly, Pas3 was localized to the nucleus despite absence of NLS, implicating regulatory roles in addition to transducing signals. Through co-immunoprecipitation (Co-IP) coupled with mass spectrometry, we identified Bre1 and Npl3 as two potential interacting protein-partners of Pas3. Bre1, as an E3 ligase, mono-ubiquitinates histone H2B subunit, and may regulate cryptococcal morphogenesis through changing the chromatin structure at filamentation-associated genetic loci, resulting in altered gene transcription. In addition to chromatin modification effects, Bre1and Npl3 may function together in regulating alternative splicing of selective pre-mRNAs. Deletion of *BRE1* and *NPL3* individually partially recapitulated the filamentation defect of *pas3*D, indicating that Bre1 and Npl3 may function both in parallel and overlapped manners downstream of Pas3 in regulating morphogenesis. Fluorescently labeled Bre1 gave fluorescent signals all over the nucleus and also one bright non-nucleolus dot, indicating its specific role in regulating alternative splicing in addition to ubiquitination of H2B. Our forward genetic screening for non-filament mutants identified the splicing factor Cus1 as an important regulator of filamentation. This corroborates the hypothesis that alternative splicing regulates cryptococcal morphogenesis. Currently we are analyzing the alternative splicing profile in *cus1*D relative to wild type via RNA-seq. Identification of the genetic connection among Pas3, Bre1 and Npl3, and dissection of mode of action by alternative splicing in regulating fungal morphogenesis in *C. neoformans* will expand the knowledge about signal transduction and post-transcriptional regulatory network in fungal development.

385W *Aspergillus flavus aswA* regulates sclerotial development and biosynthesis of sclerotium-associated secondary metabolites. *P. Chang*[1], L. Scharfenstein[1], R. Li[2], J. Diana Di Mavungu[3] 1) Southern Regional Research Center, New Orleans, ARS, USDA, Louisiana, USA; 2) Animal Genomics and Improvement Laboratory, ARS, USDA, Maryland, USA; 3) Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium.

Proteins with a $Zn(II)_2Cys_6$ DNA-binding domain are pathway-specific regulators. They bind to promoter regions of controlled genes and up-regulate gene expression via a transcriptional activation domain. *Aspergillus flavus aswA* (AFLA_085170) is a gene encoding such a protein. Deletion of *aswA* yielded strains that made a truncated gene transcript and produced a greatly increased number of sclerotia. These sclerotia were odd-shaped and non-pigmented (white), different from mature *A. flavus* sclerotia, which are oval and pigmented (dark brown to black). Transcriptomic analysis of the Δ*aswA* strain grown on potato dextrose agar plates and Wickerham agar plates showed that expression of clustering genes involved in the biosynthesis of three sclerotium-associated secondary metabolites was down-regulated. These included gene clusters of asparasone, aflatrem, and aflavarin. In contrast, those of aflatoxin, cyclopiazonic acid and kojic acid were not affected. Metabolite analysis of non-pigmented sclerotia confirmed that they contained aflatoxin and cyclopiazonic acid but not other aforementioned metabolites plus three asparasone analogs and dihydroxyaflavinine. Impairment in *aswA* gene function can stall normal sclerotial development, which in turn prevents biosynthesis and accumulation of sclerotium-specific metabolites occurring at the maturation stage.

386T Interspersed homology patterns influence the detection of unpaired DNA during meiosis in *Neurospora crassa*. N. Rhoades[1], P. Manitchotpisit[1], A. Boyd[1], T. Malone[1], T. Reed[1], E. Gladyshev[2], *T. Hammond*[1] 1) School of Biological Sciences, Illinois State University, Science Laboratory Building, Normal, Illinois, 61790, USA; 2) Department of Molecular and Cellular Biology, Harvard University, 52 Oxford Street, Cambridge, Massachusetts 02138, USA.

Neurospora crassa possess a phenomenon known as meiotic silencing by unpaired DNA (MSUD). MSUD has the remarkable ability to identify gene-sized fragments of DNA that are not at the same location on each member of a pair of homologous chromosomes. It is as if MSUD is searching for genes that lack pairing partners. If a gene is found to lack a pairing partner, it is silenced for the duration of meiosis. Thus far, it has been shown that the identification of pairing partners depends on the level of sequence identity between the partners and on the proximity of the partners to each other on the homologous chromosomes. Here, we report that interspersed homology patterns are also critical for the pairing-partner detection process. Our results are similar to those reported recently for the homology search process of repeat induced point mutation (RIP). RIP scans the *N. crassa* genome for repeated gene-sized fragments of DNA. Our findings raise the intriguing possibility that the homology search processes of MSUD and RIP are mechanistically and evolutionarily related.

387F Regulation of fungal effector gene expression through chromatin de-condensation. *M. Kramer*[1], D.E. Cook[1,2], M.F. Seidl[1], B.P.H.J. Thomma[1] 1) Laboratory of Phytopathology, Wageningen University & Research, Wageningen, NL; 2) Department of Plant Pathology, Kansas State University, Manhattan, KS.

Fungal plant pathogens require tight control over the expression of their effector genes which encode secreted proteins that facilitate host invasion. Failure to express such genes at the appropriate time or location during host invasion may lead to interception by the plant host,

and thus failure of the infection. In many fungal plant pathogens, effector genes are not randomly distributed over the genome, but localized in distinct genomic regions that are enriched in transposable elements (TEs). TEs can transpose over the genome and thereby affect gene expression or functionality, which may negatively affect the organism. To control TE activity, TE-containing genomic regions are usually structured as heterochromatin, a highly condensed genomic structure that is not accessible to the transcription machinery. Consequently, TEs are generally silenced. Due to the close proximity of many effectors to TEs, they may be held in a co-silenced state. Consequently, upon encountering a host-plant, pathogens will require de-condensation of heterochromatin to appropriately express effector genes. To investigate whether the genomic regions containing effector genes are actively de-condensed during host colonization, we are using chromatin immunoprecipitation (ChIP) to selectively isolate heterochromatic DNA of the broad host-range fungus *Verticillium dahliae* grown *in vitro* and *in planta*. Additionally, we map the genome-wide positioning of nucleosomes to investigate differences between effector genes in TE-rich regions and genes that reside within core regions of the genome. This research will lead to a better understanding of the regulation of effector genes and reveal the importance of chromatin dynamics in this process.

388W Validation and characterization of development-related microRNA-like RNAs in the mushroom-forming fungus *Coprinopsis cinerea* **Yuet Ting Lau[1], Xuanjin Cheng[1] & Hoi Shan Kwan[1*]. School of Life Sciences, The Chinese University of Hong Kong[1], Shatin, New Territories, Hong Kong.*Correspondence: hoishankwan@cuhk.edu.hk.** *Y.T Lau*, H.S Kwan School of Life science, The Chinese University of Hong Kong, Hong Kong, HK.

Introduction. *Coprinosis cinerea* is a model organism for the study of homobasidiomycete fungi due to its short life cycle and easy cultivation in the laboratory. The function and regulation mechanism of microRNA (miRNA) of animals and plants have been extensively studied. Although emerging studies have suggested that microRNA-like RNAs (milRNAs) are present in fungi and their biogenesis pathway might not be identical as conventional miRNA in plants and animals, milRNAs are still elusive for. The lack of studies on the role of milRNAs in fungi hinders the improvements of mushroom strains.

Aim. We aim to understand the development of mushroom-forming fungi at the molecular level, including RNA-mediated and protein-mediated regulation.

Methods. The presence of milRNAs has been validated experimentally through Northern blotting and Stem-loop Reverse Transcription Real-time PCR. Computational methods have been applied subsequently to characterize the roles of milRNAs in the development of mushrooms, especially during the transition from mycelium to primordium stage.

Results. We have identified, from *C.cinerea* genome, 16 putative milRNAs candidates with sizes around 21nt and a group of Dicer homologs (CC1G_00230, CC1G_03181, CC1G_13988) that are specific to mushroom-forming fungi. The PZA domain is present in the Dicer-like (DCLs) proteins of mushroom, while it is absent in all other fungal DCLs. Besides, for these 16 putative milRNAs candidates, one of them showed higher expression in mycelium stage and 15 showed higher expression in primordium stage.

Discussion. The mushroom-specific PAZ domains were grouped closely with the animal PAZs and plant PAZs and the Dicer_dimer domain sequences alone could distinguish fungal DCLs from their homologs in animals and plants. The DCLs domains of mushroom suggest Dicer genes duplicated and diversified independently in early evolution of all three multicellular kingdoms-animals, plants and fungi. A better understanding of the biogenesis pathways and regulatory mechanisms milRNAs in *C.cinerea* could improve the development of homobasidiomycete fungi, such as edible ad medicinal mushrooms, contributing to evolutionary studies of miRNA among kingdom in the near future.

389T Transcriptomic analysis of the development of basidiocarp in *Ustilago maydis*. *C. Leon-Ramirez*, J. Cabrera-Ponce, D. Martínez-Soto, J. Sánchez-Arreguín, J. Ruiz-Herrera, J. Cabrera-Ponce Genetics engeniering, CINVESTAV - CIE6010281U2, Irapuato, Guanajuato, MX.

Ustilago maydis is a Basidiomycota pathogenic fungus of corn and teozintle. The fungus has a saprophytic life cycle, and other virulent infective stage which carries out within its host. This phase ends when teliospores germinate with formation of a phragmobasidium that gives rise to four meiotic products. Although *U. maydis* has been classified as a non-forming basidiocarp species, Cabrera-Ponce et al 2012, showed that when *U. maydis* is grown in a dual culture with an embryogenic maize callus under controlled growth conditions, it has the ability to form basidiocarps. The developed structures showed the presence of a hymenium composed of skeletal hyphae, generative hyphae, clamp connections, hyphal pore, and more impressive, holobasidia (not phragmobasidia), to give rise to basidiospores and thus complete this sexual cycle.

One of the tools mostly used to identify the genes involved in the regulation of of development are microarray analyses. Using several online programs such as MIPs, FunCat. JGI, Smart; Pfam, NCBI, we used this technique to analyze the transcriptomic changes occurring when the fungus is transformed from the yeast stage to young basidiocarp, and its its further establishment as a mature basidiocarp. 2002 genes were found to be regulated in the first step, and 1064 in the second step. Classification of the genes using FunCat software gave ten differents categories. The most highly represented categories were Unclassified (31%), and Metabolism and energy with about 25% of the total regulated genes. Search of *U. maydis* homologous regulated genes to those previously described as important in the formation of fruit bodies in other fungal models gave positive results for a number of them. Some genes important for the process encoded a MAPkk, transcription factors *Ace2, Foxo3a, Mig3, Pro1,* and *TEC1*; and copper, ABC and MFS transporters, some cytochromes, and hydrophobins. These results indicate the complexity of the developmental process, that show important similarities to fruiting body formation by other fungi.

390F The histone methyltransferases FfSet2 and FfAsh1 deposit H3K36 methylation at specific loci, having a major impact on vegetative growth, sporulation, secondary metabolism and virulence in *Fusarium fujikuroi*. *S. Janevska*[1], L. Baumann[1], J. Ulrich[2], J. Kaemper[2], B. Tudzynski[1] 1) Institute of Plant Biology and Biotechnology, University of Muenster, Muenster, DE; 2) Institute for Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, DE.

Fusarium fujikuroi is well-studied due to its ability to produce highly bioactive plant hormones, gibberellic acids, that cause the *bakanae* disease of rice plants. In addition, *F. fujikuroi* possesses a great potential to synthesize a vast range of other secondary metabolites (SMs). The manipulation of chromatin-mediated regulation represents a powerful tool for the activation of “cryptic” SM gene clusters without yet assigned product. In the present work, we focused on the histone 3 lysine 36 methylation (H3K36me) mark which is generally associated

with transcriptional activation. In budding yeast, one single methyltransferase ScSet2 confers H3K36me, while there are several enzymes dedicated to this methylation in higher eukaryotes. In filamentous fungi, only the Set2-homolog has been described as H3K36-specific methyltransferase so far.
We show that the *F. fujikuroi* Set2-homolog FfSet2 is responsible for conferring the bulk amount of H3K36me2/me3, however a significant activity remains in Δ*ffset2* mutants. Bioinformatic analysis identified a second homolog of the Set2-family in *F. fujikuroi*, FfAsh1, which is conserved among filamentous fungi. Deletion of *FfASH1* resulted in only a slight decrease of global H3K36me3. But ChIP-Seq analysis revealed that both methyltransferases deposit H3K36me3 at different and very specific loci. Δ*ffset2* and Δ*ffash1* mutants are viable, however strongly attenuated in their vegetative growth and conidiation. Microarray analysis underlined a large impact on the expression of both known as well as unknown SM gene clusters. Production of gibberellic acids is nearly fully abolished *in vitro*, going in line with a decreased pathogenicity of the deletion mutants in rice infection assays.
Furthermore, to shed more light onto the role of H3K36me in the regulatory network of gene expression, we also analyze the H3K36-specific demethylase, which counteracts the activity of FfSet2 and FfAsh1.

391W New regulatory tricks for an old toxin cluster. *Daren W. Brown*[1], Alessandra Villani[2], Antonella Susca[2], Antonio Moretti[2], Robert H. Proctor[1], Susan P. McCormick[1] 1) Mycotoxin Prevention and Applied Microbiology Research Unit, USDA/ARS, Peoria, IL; 2) Institute of Sciences of Food Production, CNR, via Amendola 122/0, 70126 Bari, Italy.

Trichothecenes are among the mycotoxins of greatest concern to food and feed safety and are produced by at least two lineages of *Fusarium*: the *F. sambucinum* (FSAMSC) and *F. incarnatum-equiseti* (FIESC) species complexes. Trichothecene biosynthesis begins with the formation of a cyclic sesquiterpene followed by up to eight oxygenation and four acylation reactions. Most trichothecene biosynthetic genes (*TRI*) are co-regulated and located in a cluster. All *Fusarium TRI* cluster homologs characterized to date include two regulatory genes that encode a C_2H_2 zinc finger (*TRI6*) and a fungal transcription factor domain (*TRI10*). Functional analysis of FSAMSC members indicate that *TRI6* regulates expression of structural genes in the *TRI* cluster, while *TRI10* regulates *TRI6* expression. Here, we characterized a novel gene, *TRI21*, located in the *TRI* cluster of FIESC but not FSAMSC, which encodes a Zn_2Cys_6 transcription factor. Gene deletion and precursor feeding analyses with FIESC isolates indicate that *TRI21* is required for expression of two *TRI* cluster genes (*TRI11* and *TRI13*) responsible for two later oxygenation reactions, but not for expression of other cluster genes. In contrast, *TRI6* is required for expression of genes required for earlier and some later steps in trichothecene biosynthesis but not for *TRI11* and *TRI13* expression. These results indicate fundamental differences in the regulation of the *TRI* cluster in FIESC and FSAMSC. Furthermore, the presence of a pseudogenized *TRI21* in some members of FSAMSC indicates that the evolution of *TRI* cluster regulation in FSAMSC has included loss of *TRI21* and expansion of the role of *TRI6* in trichothecene biosynthesis.

392T Transcriptional profiling and phenotypic analysis to identify genes involved in stress response and conidiogenesis in *Fusarium graminearum*. C. Blaschke, Z. Bilton, K. Lambert, J. Hamilton, R. Manspeaker, *J. Flaherty* Coker College, Hartsville, SC.

Fusarium graminearum is an important plant pathogen that causes major yield-limiting diseases like head blight of wheat and ear rot of maize. *F. graminearum* persists in the soil in the absence of a host and reproduces asexually through the production of conidia (asexual spores) to initiate repeating cycles of infection. Our project involves undergraduate students on both gene discovery in *F. graminearum* using bioinformatics approaches and phenotyping analysis of mutant strains. Candidate genes putatively involved in stress responses/conidiogenesis have been identified through two gene expression projects. RNAseq data obtained from *F. graminearum* PH-1 and two mutant strains, all grown in conditions either conducive or non-conducive for conidiation, was processed using cluster analysis (based on unbiased measures of similarity rather than sorting objects into predefined categories). Attributes for clustering genes from each strain were locus and change in expression. Analysis of this data provides a robust way of examining gene expression patterns to identify candidate genes and gene clusters for future investigation. Towards characterizing specific candidate genes, a series of mutant strains were confirmed as targeted disruption mutants and four of these, found to be closely linked *F. graminearum* genes FGSG_12704, _12705, _12744, and _05476, were interrogated for phenotypes related to growth and development. The characterization of candidate genes identified in this study will help to fill gaps in our understanding of fungal development and facilitate building models depicting developmental processes required for the survival and dissemination of *F. graminearum*.

393F *FgSRPK1*, a SR protein kinase is involved in vegetative growth, sexual reproduction and virulence in *Fusarium graminearum*. *G. Wang*[1], Z. Gong[1], P. Sun[1], J. Xu[2], Z. Wang[1] 1) Fujian-Taiwan Joint Center for Ecological Control of Crop Pests, Fujian Agriculture and Forestry University, Fuzhou, China; 2) College of Plant Protection, Northwest Agriculture and Forestry University, Yangling, Shaanxi, China.

The wheat scab fungus *Fusarium graminearum* has 116 protein kinases genes,that are involved in various aspects of growth, development, stress responses, sexual reproduction and pathogenicity. *FgSRPK1* is homologous to mammalian SR protein kinase *SRPK1*, which is involved in mRNA alternative splicing. In a previous study, the *FgSRPK1* kinase gene was firstly identified as a fungal virulence factor. In this study, we further characterized the roles of *FgSRPK1* gene. Here we show that the deletion of *FgSRPK1* significantly reduced the growth and DON production, and completely blocked sexual reproduction and plant infection. In *FgSRPK1*-GFP transformants, GFP signals were mainly localized to the cytoplasm in conidia and hyphae, but we also observed that GFP signals were localized in nucleus in only a small proportion of hyphae. Interestingly, in both conidia and hyphae FgSrpk1 were specifically localized to the center of septal pores , and the same result was observed in rice blast fungus, suggesting that the *FgSRPK1* may have specific functions in filamentous fungi. The *FgSRPK1* gene contains two conserved kinase domains (K1 and K2) that are separated by a spacer domain. We found that expressing of *FgSRPK1* lacking the spacer domain caused nuclear localization of FgSrpk1 in conidia and hypha and significantly restored the defects of *Fgsrpk1* mutant in growth, sexual reproduction and plant infection indicating that the spacer domain may function as an anchor fastening FgSrpk1 in cytoplasm, and FgSrpk1 has constitutive kinase activity and performs its specific functions by changing subcellular localization. Furthermore, we found that the K2 domain contributes the septal pores localization, because exclusively expressing K2-GFP led to GFP localization in septal pores. Furthermore, we detected that the FgSrpk1 lacking spacer domain can interact with SR proteins FgNpl3, FgHrb1 and FgSrp1 respectively by BiFC assays. Recently, we are trying to identify the *SRPK1*-interacting proteins in cytoplasm, nucleus and septal pore by protein affinity purification.

394W Characterization of the ADAT genes in *Fusarium graminearum*. *Z. Bian*[1], H. Liu[2], Y. Li[1], L. Chen[2], J. Xu[1,2] 1) Botany and Plant Pathology, Purdue University, west lafayette, IN; 2) College of Life Sciences, Northwest A&F University, yanglin, shanxi.

RNA editing is an event that recodes hereditary information by changing the nucleotide sequence of mRNA molecules. In animals, Adenosine Deaminase Acting on RNA (ADAR) enzymes catalyze the A to I mRNA editing. The wheat scab fungus *Fusarium graminearum* lacks ADAR orthologs but has genome-wide RNA editing specifically during sexual reproduction. The preference of edited As in the loop indicates that fungal A-to-I editing is similar to editing of tRNA in anticodon loop catalyzed by Adenosine Deaminase Acting on RNA (ADAT) enzymes. Three predicted ADAT genes in *F. graminearum*, FGRRES_16992, FGRRES_11590, and FGRRES_01444 are orthologous to yeast *TAD1*, *TAD2*, and *TAD3*, respectively. Deletion of *TAD1* had no detected phenotypes but *TAD2* and *TAD3* appeared to be essential genes. Both *TAD2* and *TAD3* were increased during sexual reproduction in *F. graminearum*. Overexpression of *TAD3* had no effects on vegetative growth but resulted in defects in ascospore morphogenesis and releasing. A number of point mutations that affected ascosporogenesis and release but not vegetative growth or perithecium formation were identified in *TAD3*. Further characterization of these point mutations in *TAD3* on A-to-I RNA editing is under the way. Several proteins interacting with Tad3 specifically during sexual reproduction were identified by the affinity approach. Their functions in RNA editing and sexual reproduction also will be presented.

395T Epigenetics of a cereal killer: fungal pathogen *Fusarium graminearum*. *Christopher T Bonner*[1,2], Owen Rowland[1], Rajagopal Subramaniam[2] 1) Biology, Carleton University, Ottawa, Ontario, CA; 2) Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Ca.

Fusarium graminearum is a fungal pathogen of cereal crops and causes fusarium head blight disease in wheat. Identifying how *F. graminearum* regulates gene expression is of the utmost importance in understanding pathogenicity. Recent literature has indicated that epigenetics, such as histone modification, plays a key role in regulating genes responsible for producing secondary metabolites. This study focuses on the role that DNA epigenetics, specifically DNA methylation, has on regulating gene expression under changing environmental conditions. Expression of key secondary metabolism genes, such as the deoxynivalenol toxin pathway regulator gene, TRI6, changes rapidly with changing environmental conditions. We hypothesize that changes in DNA cytosine methylation patterns may help regulate environmentally responsive genes, including many secondary metabolite related genes. DNA methylation was assessed through whole genome bisulfite sequencing (WGBS). Methylation sites on genes of interest were validated by methylation specific PCR. This study has identified DNA methylation in *F. graminearum*. Furthermore, methylation patterns change rapidly in response to environmental conditions such as under nutrient rich vegetative growth or nutrient poor, stress growth. Preliminary data indicates that targeted gene deletions of DNA methyltransferase enzymes result in altered morphology, growth, sporulation and pathogenicity on susceptible wheat. Future efforts involve characterizing DNA methyltransferase mutants, and their role in the infection process. The purpose of this study of DNA methylation is to help identify mechanisms of rapid gene regulation in response to changing environmental conditions in the fungal pathogen *F. graminearum*, as well as in other filamentous ascomycete fungi.

396F Functional analyses of the RNAi pathway required for sexual development in the cereal pathogen *Fusarium graminearum*. *Da-Woon Kim*, Hee-Kyoung Kim, Sung-Hwan Yun Dept Med Biotech, Soonchunhyang Univ, Asan, Chungnam, KR.

Fusarium graminearum, the causal agent of Fusarium head blight in cereal crops, produces sexual progeny (ascospore) as an important overwintering and dissemination strategy for completing the disease cycle. This homothallic ascomycetous species carries two opposite mating-type (*MAT*) loci in a single nucleus to control sexual development. Recently, we have identified putative target genes or regulatory pathways controlled by the MAT proteins, among which a RNA interference (RNAi) pathway has been suggested to control a late stage of sexual development. Using several molecular strategies, we have determined the functions of several genes involved in the putative RNAi pathway such as *FgSAD-1*, *FgSAD-3*, *FgDCL-1*, *FgDCL-2*, *FgRecQ-2*, *FgQIP*, *FgSMS-2*, *FgQDE-3*, *FgQDE-2* and *FgQDE-1*. In particular, we have focused on the role of *FgSMS-2* encoding an Argonaute-like protein, which is a part of the RNA-induced silencing complex for specific cleavage of target mRNAs, in *F. graminearum*. Both gene deletion- and gene overexpression-strains of *FgSMS-2* were defective in ascospore/asci maturation. A GFP-tagging analysis showed that FgSMS-2 was specifically localized on perinuclear regions inside the immature asci. A BiFC analysis revealed that FgSMS-2 was able to bind to a Dicer-like protein, FgDCL-1 in cytoplasmic region during the early stage of sexual development. However, three isoforms of the AGO2 protein from *Drosophila melanogaster* were not able to complement the defects caused by *FgSMS-2* in *F. graminearum*, indicating an evolutionary divergence of the AGO-2 like proteins between these two eukaryotes. In addition, we identified a putative target gene (*TAG1*) of FgSMS-2, which showed an unknown protein similarity. The overexpression of *TAG1* led at least 12 h-delay of ascospore formation in *F. graminearum*. Overall, it is likely that the a RNAi pathway plays an important role during the sexual development in *F. graminearum*, particularly the Argonaute-like protein, FgSMS-2 protein, controls a set of mRNAs that might be unnecessary during the meiotic event in *F. gramineaerum*.

397W The cyclase-associated protein FgCap1 has both PKA-dependent and -independent functions during DON production and plant infection in *Fusarium graminearum*. *Cong Jiang*[1], Tao Yin[1], Qiang Zhang[1], Jianhua Wang[2], Huiquan Liu[1], Chenfang Wang[1], Jin-rong Xu[1,2] 1) Northwest A&F University, Yangling, CN; 2) Purdue University, West lafayette, US.

Fusarium graminearum is a causal agent of wheat scab and a producer of trichothecene mycotoxin deoxynivalenol (DON). The expression of trichothecene biosynthesis (*TRI*) genes and DON production are regulated by the cAMP-PKA pathway and two pathway-specific transcription factors (*TRI6* and *TRI10*). Interestingly, mutants deleted of *TRI6* had reduced expression of several components of cAMP signaling, including the *FgCAP1* adenylate binding protein gene that has not been functionally characterized in *F. graminearum*. In this study, we showed that FgCap1 interacted with Fac1 adenylate cyclase and deletion of *FgCAP1* reduced the intracellular cAMP level and PKA activities. The *Fgcap1* deletion mutant was defective in vegetative growth, conidiogenesis, and plant infection. It also was significantly reduced in DON production and *TRI* gene expression, which could be suppressed by exogenous cAMP, indicating that a PKA-dependent regulation of DON biosynthesis by FgCap1. The wild type but not *tri6* mutant had increased levels of intracellular cAMP and *FgCAP1* expression under DON producing conditions. Furthermore, the promoter of *FgCAP1* has one putative Tri6-binding site that was important for its function during DON biosynthesis but dispensable for hyphal growth, conidiogenesis, and pathogenesis. In addition, FgCap1 has an actin-like localization to the cortical patches at the apical region of hyphal tips. Phosphorylation of FgCap1 at S353 was

identified by phosphoproteomics analysis. The S353A mutation in *FgCAP1* had no effects on its functions during vegetative growth, conidiation, and DON production. However, expression of the *FgCAP1*^{S353A} allele failed to complement the defects of *Fgcap1* mutant in plant infection, indicating the importance of phosphorylation of FgCap1 at S353 during pathogenesis. Taken together, our results suggested that *FgCAP1* is involved in the regulation of DON production via cAMP signaling and subjected to a feedback regulation by *TRI6*, but phosphorylation of FgCap1 at S353 is likely unrelated to the cAMP-PKA pathway because S353A mutation only affected plant infection.

398T Nucleosome dynamics in the plant pathogen *Fusarium graminearum*. E. ZEHRAOUI[1], M. MONTIBUS[1,2], *N. PONTS*[1] 1) UR 1264 - MycSA, INRA, Villenave d'Ornon Cedex, FR; 2) Laboratoire de Biologie FCBA, Bordeaux, France.

Nucleosome dynamics are the first level of regulation of all eukaryotic molecular processes that use genomic DNA as a template, including gene expression. Abundant litterature on various organisms , notably in yeasts, reports that the position of nucleosomes and their relative stabilities are major parameters influencing gene expression. Changes in nucleosome positionning during cell differentiation and growth are commonly observed in eukaryotes in general. In the yeast *Candida albicans*, such mechanisms were shown to be involved in morphological plasticity that plays a role in virulence.

Here, we report the investigation of nucleosome dynamics during the developement *in vitro* of the mycotoxin-producing phytopathogen *Fusarium graminearum*. Nucleosome landscapes were investigated using MNase-Assisted Isolation of Nucleosomal Elements coupled to deep sequencing, or MAINE-seq. The general nucleosomal organisation extensively described in various organisms appears conserved in *F. graminearum*, with most nucleosomes arrayed and well-positionned relative to start and stop codons of genes. In the details, nucleosome dynamics were observed between three and four days of culture, *i.e.,* when growth speeds up and toxin production becomes detectable in our conditions of culture. The observed events regard mostly differences in nucleosome stability, sometimes referred to as occupancy. Additional transcriptomics data provide leading information regarding the significance of these observations.

399F Identification of novel genes involved in the photoregulation of carotenoid biosynthesis in *Fusarium oxysporum*. *O. Parra-Rivero*, J. Avalos , M.C. Limon Department of Genetics, Faculty of Biology, University of Seville, Seville, ES.

The genus *Fusarium* comprises hundreds of saprophytic and pathogenic fungal species widely distributed in nature. In addition to their roles as pathogens, these fungi stand out for their complex secondary metabolism, which includes the production of carotenoids, terpenoid pigments providing the characteristic orange colour to their colonies when grown in the light. As a regulatory model of secondary metabolism in filamentous fungi, our group investigates the molecular mechanisms underlying the control of carotenoids production by *Fusarium*. The stimulation of carotenoid biosynthesis by light is mainly achieved through the induced transcription of three structural genes, *carRA*, *carB,* and *carT*, presumably mediated by the White collar protein WcoA/Wc1. Besides the up-regulation by light, the pathway is down regulated by the RING-finger protein CarS, as indicates the enhanced expression of the structural genes and the high carotenoid accumulation in the *carS* mutants. The *carS* gene is preceded by a 4-kb sequence with no predicted ORFs, where we have identified by RNAseq analysis a novel 1.2-kb transcript. Its sequence lacks significant ORFs and it was disregarded in the annotation of the *F. oxysporum* genome, suggesting that the transcript is a long non-coding RNA (lncRNA). Deletion of this gene, whose orientation was determined by northern and PCR assays, results in an albino phenotype and in a major loss of photoinduction of the structural *car* genes. Due to its presumed role in photoregulation, we named this putative lncRNA *carP*. Interestingly, the *carP* mutant exhibits higher levels of *carS* mRNA, suggesting a connection between *carP* and photoregulation through *carS* expression. At the 5' end of the 4-Kb *carS* upstream region, and divergently transcribed from *carP*, there is a gene coding for a protein of unpredicted function that we called *carF*. Deletion of *carF* causes a decrease in carotenoid production in the light. However, the photoinduction of the structural *car* genes in the *carF* mutant was at least as efficient as in the wild type, suggesting a post-transcriptional regulatory function. In conclusion, this communication suggests the occurrence in *Fusarium* of a *car* regulatory cluster, consisting of gene *carS* and at least two novel regulatory genes participating in the control by light of carotenoid biosynthesis in *Fusarium* through novel regulatory mechanisms, involving the mediation of at least a long non-coding RNA.

400W A Mitogen-Activated Protein Kinase Gene (*VmPMK1*) is Involved in Virulence and Regulates Pectinase Activity in *Valsa mali*. *Y. Wu*, L. Xu, L. Huang College Of Plant Protection, NORTHWEST A&F UNIVERSITY, Yangling, shaanxi, CN.

Mitogen-activated protein kinases (MAPKs) play a critical role in signaling pathways and are involved in the transduction of a variety of extracellular signals and regulation of different developmental processes such as appressorium-formation, conidiation, infectious growth, and regulation of hydrolytic enzymes in many fungal phytopathogens. In the present study, a FUS3/KSS1 MAPK superfamily gene, *VmPmk1*, has been identified and characterized in Valsa mali. *VmPmk1* null mutants show a reduced growth phenotype (-33.2 %) on PDA. When inoculated onto apple leaves and twigs, ΔVmPmk1 mutants showed a decrease in lesion size by about 80 %. Furthermore, transcript levels of pectinase genes were decreased in mutants during infection. VmPmk1 deletion mutants also showed a significant reduction of biomass and enzyme activity on pectin medium. Meanwhile, conidiation have not been found in PDA or 30 % ABA medium, and sensitivity to H_2O_2 were promoted significantly in mutants. In conclusion, VmPmk1 could affect virulence and regulated the pectinase and essential for conidiation and oxidative stresses in *V. mali*.

401T Terbinafine affects transcription pattern of an ERAD encoding gene in *Trichophyton rubrum*. *T.A. Bitencourt*, P.R. Sanches, V.M. Oliveira, A. Rossi, N. M. Martinez-Rossi University of São Paulo, Ribeirão Preto Medical School, Department of Genetics, Ribeirão Preto,SP, Brazil.

The genome of *Trichophyton rubrum*, the main causative agent of dermatophytosis worldwide, was recently sequenced, enlarging knowledge about its genetic structure. Pre-mRNA splicing events occur as an adaptive genetic response to a myriad of cellular stressors. After translation, the newly synthesized proteins are loaded into the endoplasmic reticulum (ER) lumen, where they undergo folding and posttranslational modifications. ER stress is the consequence of the imbalance between protein synthesis and folding, accumulating misfolded proteins, or even triggered by disturbs of the cell wall integrity. Under this stress, the cells switch on the unfolded protein response (UPR), which regulates the ER-folding and ER-associated degradation (ERAD) machinery. The homeostasis of these processes is required to cytoprotection and apoptosis prevention. In this context, we aimed to assess the transcription pattern of an ERAD encoding gene in *T. rubrum* (TERG_04442), and also to examine the retention of intron-3 during exposure of *T. rubrum* to the sub-lethal dose of

terbinafine. To assess gene modulation and intron retention we employed RT-PCR and qRT-PCR techniques by exposing *T. rubrum* mycelia to terbinafine for 12h (MIC70). Our results show that terbinafine exposure leads to a decrease in transcript levels of ERAD encoding gene, and an up-regulation of intron-3 retention. Due to intron-3 retention, the Der1-like domain of the protein is interrupted, probably leading to conformational changes, and possibly interfering with the affinity for the substrates. These data may suggest that an adaptive strategy assures cytoprotection toward an antifungal compound that acts on the ergosterol pathway, which in turn can cause cell wall damage, implying in a fine regulation of mRNA processing and mRNA decay.
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402F Drug resistance via RNAi-dependent epimutations in *Mucor circinelloides*. *Z. Chang*[1], B. Billmyre[1], S.C. Lee[2], J. Heitman[1] 1) Duke University, Durham, NC; 2) University of Texas at San Antonio, San Antonio, TX.

The opportunistic fungal infection mucormycosis is notable for high morbidity and mortality as well as increasing incidence. Treatment is complicated by the fact that *Mucor circinelloides*, a major cause of mucormycosis, demonstrates high intrinsic resistance to the majority of antifungal agents. However, the mechanisms driving this extensive resistance remain poorly understood. Previous work demonstrated that *Mucor* is capable of developing transient resistance to the antifungal FK506 through a novel, RNA interference-dependent mechanism known as epimutation. Epimutants silence the drug target gene and can be selected by exposure to FK506; the target gene is re-expressed in these strains following passage in the absence of FK506 selective pressure. This silencing process involves the generation of small RNAs (sRNA) against the target gene via core RNAi pathway proteins including RNA-dependent RNA polymerases, Dicer, and Argonaute. To further investigate the role of epimutation in *Mucor*'s intrinsic antifungal resistance, we studied the development of resistance to a second drug, 5-fluoroorotic acid (5-FOA). We have identified epimutants that exhibit resistance to 5-FOA without mutations in either of the target genes, *pyrF* or *pyrG*. We conducted sRNA hybridization analysis to document the presence of sRNA against *pyrF* or *pyrG* in these epimutants, and demonstrated this sRNA is lost after reversion to drug sensitivity. Analysis of sRNA libraries generated from *pyrF* and *pyrG* epimutants demonstrated expression of sRNA against the *pyrF* and *pyrG* loci, respectively. From this work, we conclude that epimutation is a general mechanism through which *Mucor* can develop resistance to multiple antifungal agents. Elucidation of the role of RNAi in epimutation will afford us a fuller understanding of mucormycosis and also fungal pathogenesis and drug resistance more generally.

403W Comparative analysis of LysM genes in the dermatophyte *Trichophyton rubrum*. *L. Lopes*[1], E. A. S. Lang[1], N. T. A. Peres[2], A. Rossi[1], N. M. Martinez-Rossi[1] 1) Department of Genetics, School of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; 2) Department of Morphology, University of Sergipe , Aracaju, Sergipe, Brazil.

Trichophyton rubrum is a dermatophyte that shows a high incidence of cutaneous infections in humans, are persistent and difficult to treat. Therefore, studies are crucial to elucidate the mechanisms involved in pathogenicity and the disease process. The genome of dermatophytes presents a high number of genes encoding proteins with the LysM domains that bind chitin and related carbohydrates. However, it is unclear whether these proteins are related to chitin ligation in the fungal cell wall, and if this interaction could be involved in the relationship between dermatophytes and the host. Our aim is to characterize these LysM genes in *T. rubrum* using gene expression studies and in silico analysis. Our analysis revealed that LysM proteins showed signal peptide and hydrophobic regions, suggesting a possible anchorage in the cytoplasmic membrane. Furthermore, there was no accumulation of LysM transcripts in the conidia and, during growth for 48, 72 and 96 hours, some genes were expressed only in later times of cultivation, in different keratinous substrates, suggesting that they have function preferentially at alkaline pH. These results provide new information and contribute to the better understanding of the functions of LysM genes in dermatophytes.
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404T New insights into the transcriptional regulation of carotenoid genes in *Fusarium fujikuroi*. *Steffen Nordzieke*, Macarena Ruger-Herreros , M. Carmen Limón, Javier Avalos Department of Genetics, University of Seville, Sevilla, ES.

Carotenoids include over 600 different terpenoid pigments, produced by bacteria, plants, and fungi. Their diverse individual abilities include coloration, protection against light induced or oxidative damage, and their use as a source for other physiologically active compounds. All structural genes of the carotenoid pathway (*car* genes) in *Fusarium fujikuroi* have been identified, while their regulation remains to be elucidated. The *car* genes are organized both in the form of a cluster and autonomously, and their light induced expression leads, among others, to the production of neurosporaxanthin.
CarS, a major regulator of the cluster in this fungus, down-regulates transcription of all involved car genes despite its inability to bind directly to the corresponding promoters. Thus, we postulate the participation of other intermediating proteins as part of the regulation process.
By analyzing mutant phenotypes in combination with promotor-binding studies, Y2H, and co- immunoprecipitation experiments, we aim to describe a network of *car*-interacting proteins and transcription factors: The analysis of the proteins binding the promoters of the *car* genes show that transcriptional regulation of the carotenogenic pathway in *F. fujikuroi* is likely to include transcription factors of the high-mobility group. Interestingly, the identification of different sets of putative binding proteins suggest that different mechanisms govern the parallel biosynthetic pathways leading to the production of Neurosporaxanthin and retinal. Other results suggest that the impact of CarS on the transcription of the *car* genes is highly regulated through alternative splicing, an infrequent event in filamentous fungi, known to influence only 1.3 to 4.3% of transcripts. Using a combined RNAseq / RT PCR approach, we were able to identify four different splicing variants of CarS in *F. fujikuroi*, leading to proteins with alternative C-terminal parts. However, the functional significance of this alternative splicing process remains to be investigated.

405F Identifying carbohydrate sensing pathways in *Neurospora crassa*. *L.B. Huberman*, S.T. Coradetti, N.L. Glass Plant and Microbial Biology, UC Berkeley, Berkeley, CA.

Identifying and utilizing the nutrients available in the most efficient manner is a challenge common to all organisms. The model filamentous fungus, *Neurospora crassa*, is capable of utilizing a variety of carbohydrates: from simple sugars to the complex sugar chains found in plant cell walls. Expression of the genes necessary for degrading these complex carbohydrates is energetically expensive, so tight regulation of these genes is important. Several transcription factors necessary for cellulose utilization in *N. crassa* have been identified, but the method by which these transcription factors are activated is not clear. We identified a novel repressor of the cellulolytic response, which

in combination with other, more general nutrient sensing pathways appears to play an important role in regulating cellulase expression in *N. crassa*.

406W Regulation of Sulfur Metabolism in *Neurospora crassa*. *J.V. Paietta* Biochemistry and Molecular Biology, Wright State University, Dayton, OH.

The sulfur regulatory system of *Neurospora crassa* is composed of a set of structural genes involved in various aspects of sulfur metabolism that are controlled by the CYS3 transcriptional activator and SCON (sulfur controller) negative regulators. We are analyzing the entire set of structural genes involved in sulfur metabolism for control by the CYS3/SCON regulators. While previous work has focused on transsulfuration, we examine here in detail two steps connected to this important metabolic process. The *met-5*$^+$ and *met-8*$^+$ genes were cloned and characterized to this regard. *met-5*$^+$ encodes homoserine O-acetyltransferase which produces O-acetylhomoserine from homoscysteine and acetylCoA. O-acetylhomoserine can be subsequently converted into cystathionine (which is central to transsulfuration). *met-8*$^+$ encodes methionine synthase which generates methionine from 5-methyl tetrahydrofolate and homocysteine (a product of transsulfuration). Using Northern blot analysis, transcripts were detectable under high sulfur growth conditions for *met-5*$^+$ and for *met-8*$^+$; however, under low sulfur growth conditions the transcript levels were substantially up-regulated. Further experiments using a *cys-3* deletion mutant showed a loss of the up-regulation observed under the low sulfur (or derepressing) conditions for both *met-5*$^+$ and *met-8*$^+$, while maintaining basal transcript levels. Additional experiments detected the presence of CYS3 activator binding sites on the promoters of the *met-5*$^+$ and *met-8*$^+$ genes. The data further adds to our understanding of the regulatory dynamics involved in sulfur metabolism.

407T Functions for fission yeast splicing factors SpSlu7 and SpPrp18 in pre-mRNA splicing, alternative splice-site choice and stress-regulated splicing. G. Melangath[1], T. Sen[1], P. Bawa[2], R. Kumar[1], S. Banerjee[1], N. Vijaykrishna[1], S. Srinivasan[2], *U. Vijayraghavan*[1] 1) Microbiology/Cell Biology Labs, Indian Inst Sciences, Bangalore, Karnataka 560012, IN; 2) Institute of Bioinformatics and Applied Biotechnology, Bangalore Karnataka 560100, IN.

The fission yeast genome with numerous short introns, degenerate splice site elements and factors for alternative splicing is an apt model for studies on splicing by intron-definition as relevant to most fungi. Here, we report *Schizosaccharomyces pombe* SpSlu7 and SpPrp18 have essential, wide-spread but intron-specific splicing functions. Our analyses using strains with conditional expression of *SpSlu7* or *SpPrp18* and in mis-sense mutants reveal their genome-wide splicing functions. Statistical analysis of these global data ascribe multiple intronic features such as branch nucleotide-to-3' ss distance and A/U content at the 5' end co-relate with an intron's dependence on SpSlu7. However, features like polypyrimidine tracts between 5'ss and branch nucleotide and presence of non-consensus nucleotides in the 5'ss co-relate with a role for SpPrp18. Primer extension analyses, on *tfIId*+ intron 1 mini-transcript show the absence of either factor causes arrest with high levels of pre-mRNA and little or no lariat intron-exon 2 splicing intermediate. These data and the genetic interactions of *spprp18* and *spslu7* with mutants in other early-acting factors show novel early splicing functions for *SpSlu7* and *SpPrp18* that differ from the role of their budding yeast orthologs in the later step of second step 3'ss cleavage.
Use of alternative 5' or 3'ss in introns of a transcript can create spliced isoforms that vary in their relative abundance. Our studies on *ats1*+ isoforms in wild type cells and in mutants show SpSlu7 can influence the exon 3 skipped mRNA isoform levels. Whereas SpPrp18 affects the abundance of the intron 2 retained isoform. Environmental stresses too alter the ratio of *ats1*+ transcript isoforms where all but the fully spliced mRNA are RNA species destined for degradation. Overall, our data imply substrate-splicing factor relationships in the multi-intron transcripts of fission yeast differ from that in budding yeast and our data show alternative spliced isoforms allow for an additional level gene regulation in fission yeast.

408F Bacteria induce pigment formation in the basidiomycete *Serpula lacrymans*. *J. Tauber*[1], V. Schroeckh[2], D. Hoffmeister[1], E. Shelest[2], A. Brakhage[2] 1) Friedrich-Schiller-University, Jena, Germany; 2) Leibniz Institute for Natural Product Research and Infection Biology– Hans Knöll Institute, Jena, Germany.

The basidiomycete *Serpula lacrymans* produces chromophoric natural products derived from the quinone precursor atromentin such as the pulvinic acid-type variegatic acid. These compounds have been shown to function in redox cycling and were induced from nutritional cues [1,2,3]. Atromentin is a widespread pigment. Hence, we addressed the question if there are other environmental cues, such as microbes, that could also cause induction of these pigments and how such induction may be regulated for Basidiomycota. *Serpula lacrymans* was co-incubated with one of three diverse terrestrial bacteria, *Bacillus subtilis*, *Pseudomonas putida*, or *Streptomyces iranensis* [4]. Analysis of pigment induction was accomplished two-fold. First, gene expression was quantified by qRT-PCR from the clustered genes encoding a quinone synthetase, an aminotransferase, and an alcohol dehydrogenase. Secondly, pigment secretion was measured chromatographically by HPLC. We showed that the atromentin gene cluster of *S. lacrymans* was up-regulated and that secreted pigments accumulated (variegatic, xerocomic, and atromentic acid) during co-incubation with each bacterium indicating induction by a diverse set of microbes. Analysis of the promoter region of each clustered gene across 12 different basidiomycetes containing putatively orthologous atromentin gene clusters indicated a common motif, and thus conserved regulation. We concluded that because a diverse set of bacteria caused pigment induction (in addition to nutritional cues), and given the widespread, conserved presence of functional atromentin-derived pigments and its regulation, these pigments are an essential aspect for basidiomycetes in nutrition uptake, protection, or both.

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409W Codon Usage is an Important Determinant of Gene Expression Levels Largely Through its Effects on Transcription. Zhipeng Zhou[1], Yunkun Dang[1], Mian Zhou[1,3], Lin Liu[2], Chien-Hung Yu[1], Jingjing Fu[1], She Chen[2], *Yi Liu*[1] 1) The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390, USA; 2) National Institute of Biological Sciences, 7 Life

Science Park Rd., Changping District, Beijing 102206, China ; 3) State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, 200237.

Codon usage biases are found in all eukaryotic and prokaryotic genomes and preferred codons are more frequently used in highly expressed genes. The effects of codon usage on gene expression were previously thought to be mainly mediated by its impacts on translation. Here we show that codon usage strongly correlates with both protein and mRNA levels genome-wide in the filamentous fungus *Neurospora*. Gene codon optimization also results in strong up-regulation of protein and RNA levels, suggesting that codon usage is an important determinant of gene expression. Surprisingly, we found that the impact of codon usage on gene expression results mainly from effects on transcription and is largely independent of mRNA translation and mRNA stability. Furthermore, we show that histone H3 lysine 9 trimethylation is one of the mechanisms responsible for the codon usage-mediated transcriptional silencing of some genes with non-optimal codons. Together, these results uncovered an unexpected important role of codon usage in open reading frame sequences in determining transcription levels and suggest that codon biases are adaptation of protein coding sequences to both transcription and translation machineries. Therefore, synonymous codons not only specify protein sequences and translation dynamics but also help determine gene expression levels.

410T The transcriptional activators RhaR, AraR and GaaR co-regulate pectin degradation in *Aspergillus niger*. *J. E. Kowalczyk*, R. J. M. Lubbers, E. Battaglia, R. P. de Vires Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, the Netherlands.

Aspergillus niger produces an arsenal of extracellular enzymes that allow step-by-step degradation of organic matter found in its environment. Pectin is a heteropolymer abundantly present in the primary cell wall of plants. The complex structure of pectin requires multiple enzymes to act sequentially. Production of pectinolytic enzymes in *A. niger* is highly regulated, which allow flexible and efficient capture of nutrients. So far, three transcriptional activators have been linked to regulation of pectin degradation in *A. niger.* The L-rhamnose-responsive regulator RhaR controls the production of enzymes that degrade rhamnogalacturonan-I (RG-I) [1]. The L-arabinose-responsive regulator AraR controls the production of enzymes that decompose the arabinan and arabinogalactan side chains of RG-I [2]. The D-galacturonic acid-responsive regulator GaaR controls the production of enzymes that act on the polygalacturonic acid backbone of pectin [3]. This project aims to understand how RhaR, AraR and GaaR co-regulate pectin degradation. For that reason, we constructed single, double and triple disruptant strains of those regulators and analyzed their growth phenotype and pectinolytic gene expression in *A. iger* grown on different pectins.

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411F The effects of modification of the cellulase regulator *ace3*. *Mari Valkonen*, Marika Vitikainen, Markku Saloheimo, Tiina Pakula Industrial Biotechnology, VTT Technical Research Centre of Finland Ltd., Espoo, Finland.

The *ace3* gene was originally identified from transcriptome data of *Trichoderma reesei* cultivated in the presence different carbon sources and it was found to be co-induced with cellulase/hemicellulase genes. Deletion of the gene was detrimental to the production of cellulase activity, reduced hemicellulase gene expression as well as expression of the activator *xyr1.* Overexpression of *ace3*, which is located next to a co-regulated β-glucosidase gene (*cel1b)* and a candidate sugar transporter gene in its original locus, resulted in a significantly increased production of cellulase and xylanase activity as compared to the parental strain. Therefore, *ace3* can be considered to code for a novel master regulator of cellulase expression and a modulator of xylan degrading enzyme expression. Increased cellulase production was obtained by overexpression of the *ace3* according to the predicted gene model in the QM6A.

However, the gene model predicted later for Rut-C30 is different. The Rut-C30 gene model contains an additional short exon separated by a very long intron (>400bases) in the 5´end of the gene, so that the DNA binding domain is split by the intron. In the QM6A model the DNA binding domain is partial. RNA seq data supports the idea that both short and long form may exist, the gene region for the N-terminal exon gives weaker signals.

We have tested the effects of the overexpression of the different predicted *ace3* forms in *T. reesei* and were able to conclude that the QM6A annotated gene model improves cellulase activity production the most. The gene model with longer 5' end of *ace3* gene has little effect to cellulase activity in the strains studied. Expression profiling experiments show that deletion of *ace3* causes large amount of differences in the data. In the data, both negative and positive effects of *ace3* on gene expression were seen.

412W RNA-Editing sites controlling CAZyme production in fungus *Coprinopsis cinerea*. *J. Chang*, H. S. Kwan School of life sciences, The Chinese University of Hong Kong, Sha Tin, NT, HK.

Our goal is to elucidate the regulatory process of the enzymatic degradation of different lignocellulosic substrates by the model mushroom Coprinopsis cinerea, and identify RNA editing sites involved in lignin degradation regulation on different substrates or inducers in Coprinopsis cinerea by in silico analyses.

C. cinerea is a model organism in studies of homobasidiomycetes biology for its short life cycle under laboratory conditions. This fungus has been a target to study carbohydrate active enzymes (CAZymes) that take part in lignocellulose degradation, an important step in biomass to biofuel process. We know little about the regulation of CAZymes production and signal transduction pathways in fungi growing on different lignocellulosic substrates. Supported by next generation sequencing, there are many studies on the transcriptome studies of mushroom forming fungi. Our laboratory has studied the biology of fungi for more than 15 years. We have recently obtained RNA-seq gene expression profiles of 46 single spore isolates of C. cinerea and conducted eQTL analysis. Unlike general belief that gene expression varies with different genotypes of the transcription factors, we could not observe SNPs in transcription factors that associate with CAZymes expression . On the other hand, we found that gene expression is associated with the RNA editing sites on several transcription factors. Based on this

observation, we developed an expression Quantitative Trait RNA editing sites (eQTR) strategy to identify regulators harboring RNA editing sites. eQTR represents a recoding base in the RNA different from its template DNA. Such variation between individuals could affect mRNA abundance, a quantitative gene expression trait. The basic model included the gene expression level as a response variable, with the RNA editing type as the predictor. We have used the *C. cinerea* genome sequences and RNA sequences to call RNA editing sites. Together with the gene expression profile from the same set of samples, some transcription factors were identified that link and may regulate CAZyme expression.

To our knowledge, this would be the first functional study on RNA editing sites for roles in CAZymes production in mushroom forming fungi. Identification and functional characterization of regulators in CAZymes production will contribute to our understanding of the fungal lignocellulose degradation with potentials to benefit enzyme production industry.

413T LaeA regulation of a MAK-1 sensor complex is required for cellular communication in *Aspergillus flavus*. *x. zhao*[1,2], J. Sparker[3], J. Bok[1], Z. He[2], N. Keller[1,3] 1) medical microbiology and immunology, University of Wisconsin-Madison, Madison, WI; 2) MOE Key Laboratory of Aquatic Product Safety, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China; 3) Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706-1598, USA.

Aspergillus flavus is a saprophytic soil fungus that poses a serious threat worldwide as it contaminates many food and feed crops with the carcinogenic mycotoxin called aflatoxin. *Aspergillus flavus* survives as sclerotia in soil which enables survival in harsh environmental conditions. Sclerotial formation by *A. flavus* depends on successful cell communication and hyphal fusion events. Loss of LaeA, a member of the conserved developmental Velvet Complex, abolishes sclerotial formation in this species whereas overexpression (OE) of *laeA* results in enhanced sclerotia production. Here we demonstrate that sclerotial loss and inability to form heterokaryons in *A. flavus* ? *laeA* is mediated by a MAK-1 sensor complex encoded by homologs of the *Neurospora crassa ham* (hyphal anastomosis mutants) genes termed *hamF-I* in *A. flavus*. LaeA positively regulates *ham* gene expression with near loss in ?*laeA* and higher expression in an OE::*laeA* strain. Phenocopying ?*laeA,* deletion of *hamF*, *G*, *H*, or *I* results in both heterokaryon and sclerotial loss. This is accompanied by a significant decrease in aflatoxin synthesis in the *ham* mutants, presumably due to sclerotial loss and decreased heterokaryon formation.

414F Light controls dimorphic transitions in the human fungal pathogen *Histoplasma capsulatum*. *S. Beyhan*[1,2], M. Voorhies[2], A. Sil[2] 1) J. Craig Venter Institute, La Jolla, CA; 2) University of California, San Francisco, San Francisco, CA.

Histoplasma capsulatum (*Hc*), a dimorphic fungal pathogen, is the most common cause of fungal respiratory infections in immunocompetent host. In the soil, *Hc* grows in a filamentous form. Once inhaled, the filaments and the associated spores convert to a yeast form that expresses virulence genes and causes disease. Host temperature is the key signal that triggers a developmental switch in *Hc*. In the laboratory, cells grow in the filamentous form at room temperature (RT), whereas growth at 37ºC is sufficient to trigger growth in the yeast form. Through previous studies, we identified four key regulators, Ryp1,2,3,4, that are required for yeast-phase growth at 37ºC. Ryp1 and Ryp4 are transcriptional regulators that are conserved in the fungal kingdom. Ryp2 and Ryp3 are both members of the Velvet protein family, which regulates development and secondary metabolism in many fungi. Interestingly, in *A. nidulans*, Velvet family proteins can form a complex with various photosensors to regulate sexual and asexual development in response to light. In *Hc*, the role of light in regulating cell morphology and virulence has not been fully explored. Furthermore, it is unknown whether Velvet family proteins are involved in light response in *Hc.* In this study, our goal is to investigate the role of light in the regulation of phenotypic traits in *Hc*. In our preliminary studies, we found that conidiation was dramatically affected by light. Additionally, we found that the yeast-to-filament transition of *Hc* is light-regulated. Wild-type yeast cells (grown at 37°C) were shifted to RT under light and dark conditions. Morphological changes were monitored by microscopy and transcriptional changes were monitored by RNAseq. As a result, we found that cells that were grown in the dark persisted in the yeast form for a longer period of time before they ultimately switched to filaments. These morphological changes were accompanied by changes in transcript levels of *RYP* genes. We found that *RYP2* and *RYP4* transcript levels were lower in light compared to dark conditions at RT, suggesting that accumulation of these transcripts is light-responsive. Overexpression of *RYP2* or *RYP4* in light conditions was sufficient to prevent the yeast-to-filament transition at RT. Taken together, our results suggest that dark promotes (or light inhibits) yeast-phase growth via regulation of Ryp transcript accumulation, and that light is an important environmental signal for the physiology of *Hc.*

415W Light conservatively regulates the ergosterol biosynthetic pathway in the mold pathogens *Aspergillus* and *Fusarium.* *Kevin Fuller*[1], Mike Zegans[2], Robert Cramer[3], Jennifer Loros[4], Jay Dunlap[1] 1) Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Department of Ophthalmology, Dartmouth-Hitchcock Medical Center, Lebanon, NH ; 3) Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH; 4) Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

We have shown that light serves as an important environmental cue that regulates growth, development, and stress resistance in the mold pathogen *Aspergillus fumigatus* (Fuller et al., 2013 & 2016). Whole-genome microarray analysis revealed that several genes in the ergosterol biosynthetic pathway are down-regulated by light in this fungus, including those encoding the sterol 14-demethylase (Erg11/Cyp51b), the hydroxymethylglutaryl CoA synthase (Erg13), and the C4-methyl sterol oxidase (Erg25). qRT-PCR analysis confirmed the light-repression of these genes in the two commonly studied *A. fumigatus* strains, Af293 and CEA10. Furthermore, the influence of light on their expression was lost upon deletion of the *lreA* (blue light receptor) and *fphA* (red light receptor), indicating that these discrete photosystems cooperatively regulate sterol metabolism. Because Erg11 is the target of the azole class of antifungals, we next hypothesized that the light environment could augment the sensitivity of *A. fumigatus* to these drugs. Indeed, E-test and microbroth dilution assays demonstrated that the fungus was more sensitive to voriconazole when cultured in the light.

We reasoned that the influence of light on fungal sensitivity to sterol-targeting drugs may be particularly relevant in the context of fungal eye infections (keratitis). Accordingly, we sought to determine whether the light-repression of ergosterol genes observed in *A. fumigatus* was conserved in species commonly associated with fungal keratitis (namely *Fusarium* species). We identified orthologs of *erg11*, *erg13*, and *erg25* for both *F. oxysporum* and *F. solani* and found that, in both species, all three genes were down-regulated upon light exposure when

assessd by qRT-PCR. Taken together, these data suggest that light may play a conserved role in regulating sterol metabolism in fungi and that this could be exploited to a therapeutic end in certain clinical contexts.

416T The expression of the rice blast effector *PWL2* is induced during appressorium-mediated and hyphal penetration into live plant cells. *J. Zhu*, D.W. Kim, C.H. Khang Department of Plant Biology, University of Georgia, Athens, GA.

Rice blast, caused by the filamentous fungus *Magnaporthe oryzae*, is one of the world's most devastating agricultural diseases. During plant infection, the fungus produces various secreted proteins, called effectors, to modify plant cell structure and function to facilitate colonization. Given the significant roles played by effectors, there is surprisingly little information about how the expression of effector genes is regulated. To investigate effector gene expression at the cellular level, we generated transgenic *M. oryzae* strains expressing a fluorescent reporter gene under control of the endogenous promoter of the *M. oryzae* effector gene *PWL2*. Using this method, we revealed that the expression of *PWL2* was strongly induced in the appressorium upon plant penetration and subsequently decreased during the proliferation of invasive hyphae (IH) in the first invaded rice cell. Interestingly, *PWL2* expression was induced again when IH moved into adjacent rice cells. This up-down-up expression pattern was consistent with our qRT-PCR data. We further determined that strong induction of *PWL2* expression is not host specific, but requires hyphal penetration into live plant cells. A promoter deletion study determined that a 388-bp sequence upstream of the *PWL2* coding sequence was sufficient for high-level promoter activity *in planta*. Taken together, we propose that *M. oryzae* recognizes an unknown live plant-derived cue(s) during plant penetration to induce the effector gene expression.

417F Dissecting the molecular basis of temperature response in H. capsulatum. *D. Assa*, M. Voorhies, A. Sil Sil Lab, Microbiology and Immunology Dept., Univ. of California, San Francisco, San Francisco, CA.

Histoplasma capsulatum is the causative agent of histoplasmosis, the most common endemic mycosis in the United States. Thermally dimorphic fungi use temperature as a critical signal to sense whether they are growing in the soil or in a mammalian host: environmental temperatures trigger a growth form that is specialized for soil colonization whereas mammalian body temperature triggers a growth form that is specialized for growth in the host. These organisms utilize unknown signal transduction pathways to dramatically alter cell shape and gene expression programs in response to temperature. I hypothesize that temperature change triggers a conformational change in an unknown sensor molecule(s), resulting in activation of a thermosensory pathway that allows the fungus to adapt to growth in the environment or in the host. This conformational change could affect the activity or stability of the sensor molecule. I propose a multipronged approach to explore aspects of thermodetection in *H. capsulatum*, by (1) inspecting a potential thermosensory mechanism in the transcript of *RYP1*, a master regulator of morphology, (2) conducting a forward genetic screen to identify previously unknown regulators that participate in the immediate response to temperature change, and (3) taking an unbiased, whole genome approach to identify RNA molecules that alter their conformation *in vivo* in response to temperature and partake in temperature response.

418W Investigating how a thermally dimorphic fungal pathogen, *Histoplasma capsulatum,* senses and responds to its host environment. *Lauren Rodriguez*, Sarah Gilmore, Sinem Beyhan, Anthony Myint, Mark Voorhies, Anita Sil Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, Ca.

Thermally dimorphic fungal pathogens utilize temperature as a signal to distinguish between growth in the environment and growth within a mammalian host. Growth at 37ºC triggers alterations in morphology and gene expression. We study *Histoplasma capsulatum,* which exists in a multicellular hyphal phase in the soil that transitions to a unicellular yeast phase after inhalation by a mammalian host. Here we describe the identification of insertion mutants that are locked in the yeast phase independent of temperature. These mutants are either defective in sensing temperature or transducing the temperature signal. One insertion mutation disrupts the regulation of the *MSB2* gene, whose ortholog encodes a putative osmosensor in *Saccharomyces cerevisiae*. Gene expression profiling of the *MSB2* mutant revealed that, unlike wild-type cells, the transcription factor Efg1 fails to be induced at room temperature in the yeast-locked mutant. Since Efg1 promotes filamentation in *C. albicans*, we explored the role of Efg1 in filamentation in *H. capsulatum*. We determined that overexpression of Efg1 is sufficient to promote filamentation even at 37ºC. Taken together, these data suggest that Msb2 may be required for Efg1 induction to promote filamentation in response to temperature. We are currently characterizing the role of *MSB2* in temperature response pathways in *H. capsulatum*.

419T Evidence for post-transcriptional control of alternative oxidase expression in Neurospora crassa. *Natasa Bosnjak*, Iman Asaria, Frank Nargang Biological Sciences, University of Alberta, Edmonton, Alberta, CA.

In the event of a disruption of the standard mitochondrial electron transport chain, the nuclear-encoded *aod-1* gene is induced in *Neurospora crassa*. *Aod-1* encodes an alternative oxidase (AOX) that provides a branch point off the standard chain. When present, AOX transfers electrons from coenzyme Q directly to oxygen. In normal (non-inducing) conditions, AOX is transcribed at very low levels and no protein is detectable. We previously identified a strain of *N. crassa* that carries a mutation in the tyrosinase gene (T), that produces relatively high amounts of *aod-1* transcript in non-inducing conditions. However, we are unable to detect AOX protein in the strain. Growth of the strain in inducing conditions results in an increase in transcription and production of the protein. Two questions arise from these findings. First, why are there *aod-1* transcripts present in this strain non-inducing conditions and second, why is there no AOX protein present in these cells? Genetic analysis has shown that the tyrosinase mutation is not responsible for the increased levels of *aod-1* transcript. In addition, we have shown that there are no significant alterations in the *aod-1* promoter region of the strain nor in the transcription factors controlling *aod-1* expression. Thus, we suggest that an unknown factor(s) results in the transcription of the *aod-1* gene in this strain under non-inducing conditions. Similarly, an unknown factor(s) prevents the transcript from being translated under non-inducing conditions.

420F Circadian clock control of translation in *Neurospora crassa*. *K.D. Castillo*, S.Z. Caster, D Bell-Pedersen Biology, Texas A&M University, College Station, TX.

The circadian clock controls daily rhythms in a wide variety of physiological and behavioral processes in diverse organisms, allowing anticipation of daily environmental cycles and maintenance of internal temporal order. Rhythms in mRNA accumulation are observed for up to 40% of the eukaryotic genome. Interestingly, about 50% of the oscillating proteins in the mouse liver are produced from mRNAs that are not clock-controlled, suggesting a prominent role for clock regulation of post-transcriptional processes. While the basic mechanisms of

circadian transcriptional control are known, the extent of clock control of mRNA translation is understudied. The filamentous fungus *Neurospora crassa* is a simple model organism for determining how the clock controls gene expression. In *N. crassa*, we discovered that the phosphorylation of the highly conserved translation elongation factor 2 (eEF-2), by the serine/threonine kinase RCK-2, is rhythmic in wild-type cells, but not in Δ*rck-2* or clock mutant Δ*frq* cells. These data suggested that clock control of eEF-2 activity plays a key role in rhythmic translation of all, or specific, mRNAs. RNA-seq and ribosome profiling experiments from cultures harvested at different times of the day revealed that clock regulation of eEF-2 activity influences temporal translation of specific mRNAs, including glutathione-S-transferase 3 (*gst-3*). Experiments are currently underway to determine the mechanism of this specificity.

421W Changes in the proteome of *Neurospora crassa* in Response to Quinic Acid. Kayla Brown[1,2], Katie Allen[1,2], Dana Tirabassi[1,2], Gary Walker[1,2], *David Asch*[1,2] 1) Biological Sciences, Youngstown State University, Youngstown ; 2) Center for Chemical Biology.

The filamentous fungus , *Neurospora crassa*, serves as an ideal model for eukaryotic organisms. Like many fungi, *N. crassa* is able to utilize many different carbon sources for energy. This requires the presence genes that encode for metabolic pathways which are not always needed. One such pathway is the pathway to metabolize quinic acid. The genes coding for this pathway, the quinic acid (*qa*) gene cluster, have been studied for many years as a model of eukaryotic gene regulation. Recently it has been noted that the expression of many other genes outside the *qa* gene cluster is affected by the presence of quinic acid. In this study we have compared the proteomes of *N. crassa* grown on sucrose with that of *N. crassa* grown on quinic acid. We have identified several proteins that are present during growth on quinic acid but not present during growth on sucrose. We are presently confirming these observations using qRT-PCR.

422T DNA sequence homology triggers cytosine-to-thymine mutation by a heterochromatin-related pathway. *Eugene Gladyshev*, Nancy Kleckner Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

In many eukaryotes, chromosomal regions with highly repetitive DNA are normally associated with cytosine methylation (5mC) and histone H3 lysine-9 di-/trimethylation (H3K9me2/3) in the context of heterochromatin. In the fungus *Neurospora crassa*, where the pathway of heterochromatin assembly has been characterized in great detail, H3K9me3 and 5mC are catalyzed, respectively, by a conserved SUV39 lysine methylase DIM-5 and a DNMT1-like cytosine methylase DIM-2. Here we show that DIM-2 can mediate cytosine-to-thymine (C-to-T) mutation of repetitive DNA during a process known as "RIP" (Repeat-Induced Point mutation). We further show that DIM-2-mediated mutation requires DIM-5 as well as a number of additional heterochromatin factors including HP1. The pathway can act effectively on tandem repeat arrays as well as on individual pairs of closely positioned or widely separated sequence copies. These results, when combined with our model for repeat recognition during RIP, define a new process by which heterochromatin can be nucleated specifically on repetitive DNA. Existing molecular mechanisms rely on cis-acting proteins or non-coding RNAs. We propose, instead, that formation of constitutive heterochromatin can be initiated by direct interactions between homologous double-stranded DNA segments. Given that the number of potential homologous interactions will scale with the number of repeat copies, this process could be particularly effective and important in heterochromatic silencing of large repetitive regions such as those associated with (peri)centromeric and (sub)telomeric regions. Also, irrespective of the repeat-recognition mechanism, our results implicate the DIM-5/DIM-2 pathway in the accelerated evolution of pathogenic genes that are typically found near repetitive elements in the genomes of many filamentous fungi.

423F Circadian clock regulation of translation initiation through eIF2α phosphorylationShanta Karki and Deborah Bell-Pedersen.Center for Biological Clocks Research, Department of Biology, Texas A&M University, College Station, TX 77840. *Shanta Karki* Dept of Biology, Texas A&M University, College Station, TX.

Roughly half of eukaryotic mRNAs accumulate with a circadian rhythm, demonstrating the profound impact of the clock on gene expression. In addition, mounting evidence supports a role for the circadian clock in controlling mRNA translation, extending the influence of the clock on gene expression beyond its role in rhythmic transcription control. However, the mechanisms and extent of translational regulation by the clock are largely unknown. Previous studies in our lab revealed that the clock, through rhythmic activation of the stress-associated p38 MAPK pathway, regulates the activity of translation elongation factor eEF-2 in *Neurospora crassa*. This discovery prompted us to also examine if the clock controls translation initiation, where we first focused on determining if the activity of eIF2α, a conserved component of the translation initiation machinery, is clock-controlled. In fungi, phosphorylation of eIF2α is accomplished by GCN2 kinase, which is activated in response to nutrient starvation. While phosphorylation of eIF2α blocks translation initiation of most mRNAs, some mRNAs are actively translated, including mRNAs encoding proteins involved in adaptation to stress. We discovered that phosphorylation of eIF2α is clock-controlled, peaking during the subjective day, and that the Neurospora eIF2α kinase CPC-3, a homolog of GCN2, is necessary for phosphorylation of eIF2α. In addition, we showed that the levels of *cpc-3* mRNA and CPC-3 protein accumulate rhythmically. Consistent with these data, we found using *in vitro* translation assays that mRNA translation is reduced using cell-free translation extracts made from WT cells harvested at the peak of phospho-eIF2α levels, as compared the trough. As predicted, this translation rhythm was abolished in clock mutant and Δ*cpc-3* cells. Experiments are in progress to determine the impact of rhythmic phospho-eIF2α levels on mRNA translation and rhythmic protein accumulation using ribosome profiling, coupled with RNA-seq, in wild type versus *Δcpc*-3 cells.

424W Transgenic Editing of *Neurospora Crassa* as a Tool for Studying Oscillatory Reproductive Cycle Synchronization. *Brooke Hull*[1], Michael Judge[1], Zhaojie Deng[2], Carmen Rodriguez[1], James Griffith[1], Jonathan Arnold[1], Leidong Mao[2] 1) Genetics, University of Georgia, Athens, GA; 2) Regenerative Bioscience Center, University of Georgia, Athens, GA.

Circadian rhythms are a well-characterized emergent property in *Neurospora crassa* with known responses to external factors such as temperature and light, but the effect of intercellular communication on this system is not understood. Our group has recently demonstrated synchronicity of cells over time in interactive cell populations, but not in those which are physically barred from cell-to-cell interaction. We therefore hypothesize cells can communicate circadian time to each other, resulting in synchronization of cell clock phase. To directly measure the synchronicity of cells over time we have engineered a second construct with yellow fluorescence. Venus, a yellow fluorescent protein was introduced into the genome in codon optimized form under the control of the promoter of the *clock controlled* gene 2 using CRISPR Cas9 guided homologous recombination. When used in tandem with an existing red fluorescence construct, this new strain allows visualization and direct study of circadian synchronicity in *N. crassa* on both a single cell and on a population scale.

425T The RUV-1/2 proteins have a functional role in heat shock response in *Neurospora crassa*. Pablo Acera Mateos, Jonatas Erick Campanella, Fernanda Freitas, *Maria Célia Bertolini* Departamento de Bioquímica e Tecnologia Química, Instituto de Química, UNESP, Araraquara, BR.

In a previous work, using a combination of pulldown assay and mass spectrometry, we identified the *Neurospora crassa* RUV-1 protein that binds to STRE (STress Response Element), a motif present in promoters of stress-responsive genes. This protein, together with its paralogue RUV-2, belongs to the AAA^+ ATPase protein family, and is annotated in the *N. crassa* database as an ATP-dependent DNA helicase. The proteins have been identified as components of several macromolecular complexes being implicated in many cellular processes. Both paralogous proteins are described in different organisms (*Homo sapiens* and *Saccharomyces cerevisiae*) as partner proteins that interact with each other in different complexes, such as histone acetyltransferase Tip60 complex, the chromatin remodelling complexes Ino80 and SWR-C, and the telomerase complex, among others. In this work, we demonstrate by EMSA that recombinant RUV-1 binds specifically to the STRE motif, confirming our previous results. Gene expression assays showed that the *ruv-1* transcript and the RUV-1 protein are up-regulated in the fungus submitted to heat stress. The *ruv-2* transcript is not regulated under heat stress; however, *ruv-2* mRNA undergoes an alternative splicing (intron retention) under heat stress (45ºC), in which a 75 bp intron is not removed leading to the synthesis of a truncated protein having the 97 N-terminal amino acid residues. Cellular localization analyses showed that both proteins translocate to nucleus under heat stress. Experiments to investigate if both proteins interact each other are currently underway. Financial Support: CNPq and FAPESP

426F Effects of *mus-52* gene disruption and extracellular phosphate availability in the regulation of an ATP Synthase in *Neurospora crassa*. Maíra Martins, *Eriston Gomes*, Pablo Sanches, Wellington Pedersoli, Nilce Martinez-Rossi, Antonio Rossi Ribeirão Preto Medical School, Genetics Department, University of São Paulo, SP – Brazil.

The completion of *N. crassa* genome sequence and the availability of a set of mutant strains individually knocked-out in particular genes, such as the *mutagen sensitive-52* (*mus-52*), provide an efficient tool for gene targeting. *N. crassa mus-52* (NCU00077) is homologous to human *KU80*, which is involved in telomere maintenance; apoptosis process; DNA replication; gene transcription regulation; and especially in non-homologous end-joining of double-stranded DNA breaks. In the absence of mus-52 the homologous recombination is considerably increased, a molecular event and its consequences that are yet not well understood. This work aimed to estimate the effects of inorganic phosphate (Pi) availability and the *mus-52* deletion in gene transcription regulation, using two *N. crassa* strains (74A [wild-type] - FGSC#2489 and *Δmus-52* - FGSC#9568). All strains were maintained on Vogel's minimal medium, pH 5.8, at 30°C. Conidia from each strain were germinated for 5 h at 30°C in an orbital shaker (200 rpm), in low-Pi (10μM) and high-Pi (10mM) media. *N. crassa* hypothetical protein (NCU06977), which showed over than 70% of identity with a *Colletotrichum graminicola* ATP synthase F0 subunit, was used as gene reference. Thus, when the wild-type strain was subjected from low-Pi to high-Pi, the relative expression was up-regulated; interestingly, when the mutant *Δmus-52* was submitted to the same condition, the expression was down-regulated. When compared the expression fold-change between the wild-type and *Δmus-52* in low-Pi, the mutant strain showed an up-regulation, but in high-Pi condition, the expression was down-regulated. These results indicate that the *N. crassa* wild-type strain modulates the ATP synthase transcription according to with Pi availability. But the absence of *mus-52* indeed affects the regulation of this gene, being oppositely modulated in both strains and all conditions tested probably by the directly binding of Mus-52 or the reducing access of the enhancers and transcription factors to the ATP synthase promoter region.
Financial Support: FAPESP, CNPq, CAPES, and FAEPA.

427W Epigenetic suppression of transposons co-opted as global regulator of virulence gene expression in a fungal phytopathogen. *Adam P. Taranto*, Megan C. McDonald, Peter S. Solomon Plant Sciences Division, Research School of Biology, The Australian National University, Canberra, Australia.

Many fungal plant-pathogens display a rapid transcriptomic switch in gene expression upon infection of a susceptible host. We have explored the role of epigenetic suppression in regulation of this shift towards virulence in the wheat-pathogen *Parastagonospora nodorum*. We have characterised the methylome of *P. nodorum* via whole genome bisulfite sequencing and assessed the contribution of DNA-methylation to gene expression by RNA-seq. Genomic regions subject to epigenetic control were enriched in transposable elements, effector-like genes, and putatively horizontally acquired genes. Key necrotrophic effectors, *ToxA*, *Tox1* and *Tox3* occur in close proximity to transposons. Molecular disruption of epigenetic silencing mechanisms active in these regions (H3K9me3, H3K27me3, and 5mC DNA methylation) allowed for partial recapitulation of the early-infection transcriptomic profile of *P. nodorum*. Similar epigenetic reprogramming of pathogen gene expression was achieved through application of chemical inhibitors of epigenetic pathways.

428T The photoreceptor WcoB interacts with carotenogenic proteins in the cytoplasm of *Phycomyces blakesleeanus.* *A. Miralles Durán*, MA Sánchez-Romero, LM Corrochano Genetics, University of Seville, Seville, Sevilla, ES.

Phycomyces blakesleeanus is sensitive to environmental signals such as light, wind, gravity and pressure. Light modifies the direction of growth of the fruiting body, sporangiophore, (phototropism), stimulates the production of beta-carotene in the mycelium and regulates the development of the sporangiophores. Blue light is sensed through the Mad complex, a transcription factor complex composed of MadA and MadB. MadA and MadB are homologs of WC-1 and WC-2 from *Neurospora crassa*. The *Phycomyces* genome has three genes homologous to *wc-1: madA, wcoA* and *wcoB*; and four genes homologous to *wc-2: madB, wctB, wctC* and *wctD*. WcoB contains a LOV domain for chromophore binding, but lacks the Zn finger domain. We have characterized the localization of WcoB in the mycelium of Phycomyces using an antibody raised against a peptide of WcoB. The gene *wcoB* is induced by light in vegetative mycelia producing an increase in the amount of WcoB. In order to identify the cellular localization of WcoB we performed cellular fractionations using cultures grown in the dark or exposed to 30 min of light. We detected WcoB in the cytoplasmic fraction of cellular extracts, while the nuclear fraction was devoid of WcoB. Immunofluorescence assays with spores or germinating mycelia showed that WcoB was detected as localized patches in the cell membrane. Our results suggest that WcoB does not act as a transcription factor and is located in the cell membrane. In order to identify proteins that interact with WcoB we performed immunoprecipitation assays. Candidate proteins were excised from the electrophoresis gel and sequenced. Several proteins were immunoprecipitated with WcoB: HMG-CoA, CarRA, CarB and CarS. To

corroborate the location of WcoB and other WC proteins *in vivo*, we have used *Aspergillus* as a model due to the absence of an efficient transformation system for *Phycomyces*. In addition we have confirmed the interactions between WcoB and *Phycomyces* enzymes using the Bimolecular Fluorescence Complementation System (BiFC) in *Aspergillus.* Our results suggest that WcoB is a photoreceptor for the regulation of the enzymes that participate in the biosynthesis of beta-carotene and pheromones.

429F Comparative proteomics and phosphoproteomics to decipher signal transduction in *Botrytis cinerea*. *J. Kilani*[1,3], M. Davanture[2], M. Zivy[2], S. Fillinger[1] 1) UMR BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; 2) PAPPSO, UMR GQE, INRA, CNRS, Univ Paris-Sud, AgroParisTech, Université Paris-Saclay, Gif-sur-Yvette, France; 3) Univ. Paris-Sud, Université Paris-Saclay, France.

Adaptation to changing environmental conditions takes place rapidly in all living organisms: cells must respond to a wide variety of signals. Through successive protein phosphorylation and de-phosphorylation steps, signal transduction cascades mediate between the perception of the extracellular conditions and the adaptation of intracellular processes.

Botrytis cinerea is a necrotrophic, polyphageous plant pathogen, that causes gray mold disease and can infect over 1000 plant species including several agronomically important crops (grapevine, strawberry, tomatoes …). Fungicides remain the most effective means to combat this disease. However *B. cinerea* rapidly adapts to fungicides. Presently, the phenylpyrrole fludioxonil is one of the most efficient fungicides against *B. cinerea*. Therefore deciphering the response to fludioxonil in *B. cinerea* is crucial.

In *Botrytis cinerea*, the fungicide fludioxonil activates the Sak1 (Hog1-like) and Bmp3 (Slt2-like) MAPKs, which are respectively involved in osmoregulation, cell wall integrity, development and pathogenicity. In order to trace the transduction of fludioxonil to the MAPK pathways, we have undertaken a comparative proteomics and phosphoproteomics study of the *B. cinerea* osmosensing pathway challenged by the fungicide fludioxonil. We compared mutants of the sensor histidine-kinase Bos1 and of the MAPK Sak1 to the parental wild-type. Strains were exposed (or not) to fludioxonil for 15 min during exponential *in vitro* growth. Shotgun proteomics revealed considerable differences in protein content among the strains, but no treatment effect. These results indicate a strong transcriptional and/or translational regulation under Bos1 and Sak1 control, respectively, under standard conditions. One of the most relevant result is the abundance of 263 proteins controlled by Bos1 and Sak1 independently. Proteins of the oxidative stress response are under negative control of Bos1, while some proteases are positively regulated. In addition, proteins involved in translation are specifically regulated by Bos1. It appears that Bos1 is important in different biological processes. Sak1, on its turn, controls the abundance of proteins involved in oxidative stress response, early secretome, protein folding, primary metabolism and secondary metabolism.

Quantitative phosphoproteomics is ongoing and it will permit to verify a clear response to the fludioxonil treatment as well as (de)phosphorylation events controlled independently by Bos1 or Sak1, or by both kinases.

430W Identifying targets of Autophagic mRNA decay in *S. cerevisiae*. *J.F. Garcia*[1], R Parker[2,3] 1) Molecular Biology, Colorado College, Colorado Springs, CO; 2) Dept. of Biochemistry, CU Boulder, Boulder, CO; 3) HHMI.

Autophagy is conically thought of as mechanism by which to degrade proteins. However, the autophagy of cytoplasmic RNA granules and rRNAs, and the existence of a ribonuclease in the lysosome or yeast vacuole, suggests that autophagy may also act as a novel mRNA decay pathway. To test this, we performed RNA-seq on total RNA isolated from *S. cerevisiae* that harbor deletions of vacuolar ribonuclease, Rny1, or, a key autophagy factor, atg15, to identify potential mRNA targets of autophagic mRNA decay. Our analysis of mRNAs isolated from exponentially growing rny1? cells revealed that mRNA levels remained unchanged by the removal of Rny1. When mRNA was isolated from rny1? cells grown past the post-diauxic shift, a subset of mRNAs increased in abundance when Rny1 was not present. This suggested that Rny1 acts to modulate levels of mRNAs in a temporal manner. Approximately 189 mRNAs with increased levels in rny1? and atg15? post-diauxic cells were identified as potential targets for autophagic mRNA decay. Interestingly 40% of mRNAs within this subset are mRNAs that encode for proteins associated with mitochondria function. Further computational analysis showed that this subset of potential mRNA targets tended to be preferentially translated on the mitochondria surface, or localized to mitochondria. Lastly, rny1? displays slight respiration defects. Though further analysis into the mechanism by which Rny1 and autophagy selectively regulate mRNAs that encode for mitochondrial proteins needs to be done, our current findings suggests that mRNAs associated with the mitochondria are preferentially degraded by Rny1 during the post-diauxic phase through a novel autophagic mRNA decay mechanism.

431T Evidence for a demethylase-independent role of KdmB, a chromatin regulator of secondary metabolism in *Aspergillus nidulans*. *S. Bachleitner*[1], L. Studt[1], A. Gacek-Matthews[1], M. Sulyok[2], J. Strauss[1] 1) Division of Microbial Genetics and Pathogen Interactions, Department of Applied Genetics and Cell Biology, BOKU-University of Natural Resources and Life Science Vienna, Campus Tulln Austria; 2) Center for Analytical Chemistry, Department of Agrobiotechnology, BOKU-University of Natural Resources and Life Science Vienna, Campus Tulln, Austria.

Fungi produce a plethora of secondary metabolites (SMs), low-molecular-weight compounds involved in cellular protection, defence and signalling. The genes involved in the biosynthesis of these substances are usually physically linked in gene clusters and tightly regulated to prevent an unnecessary use of resources. Efficient transcription of the clusters involves posttranslational modifications (PTMs) of histones which influence the status of chromatin in and around these gene clusters. The exact molecular mechanisms by which histone PTMs influence silencing and activation of SM genes, however, remains largely unknown. Previously, we showed that KdmB, a member of the KDM5-family of jumonji histone demethylases is a regulator of genome-wide H3 lysine 4 trimethylation (H3K4me3) and functions in both gene repression and activation. Specifically, normal induction of several SM-associated genes depend on KdmB. To determine whether the demethylase domain of KdmB is required for SM gene activation we expressed a KdmB mutant carrying point mutations in the highly conserved catalytic JmjC domain and subsequently analysed SM-gene expression and chromatin marks in selected gene clusters and their associated SM metabolite levels. H3K4me3 marks did not differ much between wild type and the JmjC mutant at the selected SM gene loci in agreement with the generally low levels of this PTM found previously by ChIP-seq even in active SM genes[1]. Interestingly, the integrity of the JmjC-demethylase domain is required for the activation of most studied SM gene clusters suggesting a structural role of this domain for the putative activation regions containing PHD-type and other Zn-fingers. However, KdmB-mediated SM gene repression was also found to be independent of the JmjC domain suggesting that KdmB also acts as a recruitment platform for other repressors.

[1]Gacek-Matthews et al., 2016 PLoS Genet. 12:e1006222

432F A CRE1-regulated cluster is responsible for light dependent production of Dihydrotrichotetronine in *Trichoderma reesei*. *Alberto Alonso Monroy*[1], Eva Stappler[1], Andre Schuster[2], Stefan Böhmdorfer[3], Rainer Schuhmacher[4], Michael Sulyok[4], Monika Schmoll[1] 1) AIT - Austrian Institute of Technology GmbH, Health & Environment, Bioresources, Konrad-Lorenz-Straße 24, 3430 Tulln, AUSTRIA; 2) TU Wien, Institute of Chemical Engineering, Research Area Molecular Biotechnology, Gumpendorfer Straße 1a, 1060 Vienna; 3) University of Natural Resources and Life Sciences Vienna, Department of Chemistry, Division of Chemistry of Renewable Resources, Konrad-Lorenz-Straße 24, 3430 Tulln, AUSTRIA; 4) University of Natural Resources and Life Sciences Vienna, Department for Agrobiotechnology (IFA-Tulln), Center for Analytical Chemistry, Konrad-Lorenz-Straße 20, 3430 Tulln, AUSTRIA.

Changing light conditions, caused by the rotation of earth resulting in day and night or growth on the surface or within a substrate, result in considerably altered physiological processes in fungi. For the biotechnological workhorse *Trichoderma reesei* (syn. *Hypocrea jecorina*), regulation of glycoside hydrolase gene expression, especially cellulase expression was shown to be a target of light dependent gene regulation. Investigation of genes regulated in response to light in *T. reesei* and their distribution within the genome revealed several genomic clusters. One of them comprises genes involved in secondary metabolism and is regulated by the carbon catabolite repressor CRE1 in a light dependent manner as well as by photoreceptors. High performance thin layer chromatography (HPTLC) revealed clear differences in secondary metabolite production in light and darkness as well as changing patterns in mutants of this cluster.
Two polyketide synthases as well as one monooxygenase were found to be involved in production of Dihydrotrichotetronine and Trichodimerol in a light dependent manner. Accordingly, these genes show a positive feedback cycle in darkness, but negative feedback in light, indicating a cellular sensing mechanism for the products of these enzymes. The transcription factor residing within the cluster regulates the cluster genes in a light dependent manner, but transcriptome analysis shows that also other secondary metabolism related clusters are targeted. Additionally, an interrelationship of this cluster with regulation of cellulase gene expression was detected that suggests sophisticated distribution of resources either to degradation of substrate (feed) or to antagonism of competitors (fight), which is influenced by light.

433W ChIP-seq analysis of the velvet protein PcVelA from *Penicillium chrysogenum*: Methyltransferase PcLlmA is a downstream regulator of fungal development. *U. Kück*[1], K. Becker[1], S. Ziemons[1], K. Lentz[1], M. Freitag[2] 1) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-University Bochum, Bochum, DE; 2) Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, USA.

A velvet multisubunit complex was previously described for *Penicillium chrysogenum*, the major industrial producer of the beta-lactam antibiotic penicillin. A core component of this complex is velvet A (PcVelA), a global regulator, which is highly conserved within filamentous euascomycetes. In *P. chrysogenum*, PcVelA regulates secondary metabolite production, hyphal morphology, conidiation, and pellet formation. Here we present a detailed chromatin immunoprecipitation next-generation sequencing (ChIP-seq) analysis of PcVelA. We provide experimental evidence that PcVelA acts as a direct transcriptional regulator at the DNA level in addition to functioning as a regulator at the protein level in *P. chrysogenum*, which was previously described. We identified many target genes that are related to processes known to be dependent on PcVelA, e.g., secondary metabolism as well as asexual and sexual development. We also identified seven PcVelA target genes that encode putative methyltransferases. Yeast two-hybrid and bimolecular fluorescence complementation analyses showed that one of the putative methyltransferases, PcLlmA, directly interacts with PcVelA. Furthermore, functional characterization of PcLlmA demonstrated that this protein is involved in the regulation of conidiosporogenesis, pellet formation, and hyphal morphology, all traits with major applied relevance.

Becker K, Ziemons S, Lentz K, Freitag M, Kück U (2016) Genome-wide ChIP-seq analysis of the velvet protein PcVelA identifies methyltransferase PcLlmA as a novel downstream regulator of fungal development. mSphere 1(4):e00149-16

434T Genome-wide ADAR-independent A-to-I RNA editing is generally adaptive for sexual reproduction in *Neurospora crassa*. *Huquan Liu*[1], Yang Li[2], Daipeng Chen[1,2], Qinhu Wang[1], Cong Jiang[1], Jin-Rong Xu[2] 1) Plant Pathology, Northwest A&F University, Yangling, Shaanxi, P. R. China; 2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, United States of America.

ADAR enzymes mediated A-to-I editing is the most prevalent RNA modification in animals. Recently, A-to-I editing was reported to occur specifically during sexual reproduction in *Fusarium graminearum*. Here, we identified over 41000 A-to-I sites that specifically occurred during sexual stages in the model filamentous fungus *Neurospora crassa* by strand-specific RNA-seq with hyphae, wild-type perithecia harvested 3, 4, 5, and 6 days-post-fertilization (dpf), and 5-dpf perithecia of the *stc1* and *sad-1* mutants that are defective in ascus or ascospore development. RNA editing events were found in premeiotic stage but increased significantly during ascus development and ascospore formation. Many phase-specific A-to-I sites and sites with different editing levels across sexual stages were also identified, suggesting that these may affect stage-specific functions. Over 81% of the A-to-I sites in CDS regions are nonsynonymous recoding events, which is significantly higher than synonymous one (18.7%). In addition, the editing level of recoding sites is also significantly higher than that of synonymous sites, with the tendency of the higher the editing level, the higher percentage of recoding events. These results indicate that recoding events are generally beneficial, and editing sites with high editing levels may be more beneficial than those with low editing levels. RNA-seq analysis also showed that A-to-I editing occurred in sexual stage of *N. tetrasperma*. Approximately 52% of the *N. tetrasperma* editing sites are conserved in *N. crassa*, of which 454 were also conserved in *F. graminearum*. The conserved editing sites tend to have higher editing levels and frequency of recoding events, suggesting that the conserved recoding events are likely functionally important and have adaptive advantages during evolution. Consistent with this observation, recoding editing was found to be enriched in genes important for sexual reproduction and meiotic cell cycle. Furthermore, most of the genes encoding enzymes or proteins important for RNA silencing, DNA methylation, and histone modifications are subjected to extensive recoding editing, suggesting that the RNA editing may have important regulations to other known epigenetic phenomena in *N. crassa*. Overall, *N. crassa* has great genetic resources to further characterize the molecular mechanism and function of RNA editing in filamentous fungi. Several genes with conserved editing sites were selected for functionally characterization.

435F ChIP-seq analyses of the α-domain transcription factor MAT1-1-1 in *Penicillium chrysogenum* point towards a dose-dependent regulation of target genes. *T.A. Dahlmann*, K. Becker, S. Krevet, U. Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44801 Bochum, Germany.

The filamentous ascomycete *Penicillium chrysogenum* is the main industrial producer of the β-lactam antibiotic penicillin and is therefore of great economical and medical importance. Like many related fungi, it has been considered as asexual for more than a century. However, it was recently demonstrated that *P. chrysogenum* is able to complete a heterothallic sexual life cycle [1]. In *Penicilli*, sex is determined by the mating-type ideomorphs *MAT1-1* and *MAT1-2*, each characterized by presence of a single gene, called *MAT1-1-1* and *MAT1-2-1* [2]. Deletion and overexpression of these genes resulted in various phenotypic changes and functional characterization of both proteins revealed functions beyond sexual development. Most interestingly, deletion and overexpression of *MAT1-1-1* resulted in similar phenotypes. To identify target genes of transcription factor MAT1-1-1 (MATα1) and to enlighten the genetic reasons for the observed phenotypes, ChIP-seq experiments of a GFP-tagged MAT1-1-1 protein were performed. Up to now, these studies were only performed under overexpression conditions using a strong constitutive promoter [3]. We have now extended our analysis using the native MAT1-1-1 promoter. Under high overexpression conditions 238 MAT1-1-1 target sites were identified [3]. 139 of these were now validated and 98 additional targets were found exclusively under control of the native promoter. These results point towards a dose-dependent regulation of MAT1-1-1 target genes by this important regulator representing a master switch for development and metabolism.

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436W The root endophyte *Piriformospora indica* modulates growth and stress response in *Glycine max*. *R. Bajaj*, K.E. Bushley Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN.

Piriformospora indica is an endophytic symbiotic fungus, which was isolated from rhizosphere of the xerophytic woody shrubs from the Thar deserts of Rajasthan, India. This fungus colonizes plant roots endophytically to promote plant growth while also providing additional benefits of biotic (e.g. pathogens and herbivores) and abiotic (e.g. drought, salinity, heat) stress tolerance to a wide range of host plants. This study investigated the effects of *P. indica* on soybean (*Glycine max*) in a biological growth chamber study that included a uninoculated Control (C), 2% (w/w) *P. indica* (Pi), and 2% (w/w) *P. indica* + Nutrient Hoaglands solution (Pi + N). Plants were harvested 60 days after germination and root colonization was determined using lactophenol cotton blue. Transcriptome analysis was performed on roots of two biological replicates for each treatment plus *P. indica* grown in liquid PDA. Differential gene expression analyses were performed using Tophat and Cuffdiff. GO ontology (GO) and metabolic pathway enrichment analyses were performed using Soybase tools and SoyCyc, respectively. Results confirmed 41.47% and 28.89%, colonization in Pi and Pi + N treatments, respectively. Colonization with *P. indica* also significantly impacted plant growth and nutrient status, increasing shoot dry weight while decreasing root dry weight and increasing macronutrients (P, N, K) in shoots. Differential expression analyses showed that biosynthetic pathways and GO categories pertaining to lignin biosynthesis and regulation of iron transport were highly enriched in the C vs. Pi comparison. The C vs. Pi + N comparison also yielded significant upregulation of genes and pathways pertaining to cell wall biogenesis/lignin biosynthesis, iron/transition metal homeostasis, as well as response to drought and salinity stress. Higher colonization rates in the C vs. *Pi* treatment were associated with downregulation and over-representation of various plant defense categories, namely response to chitin, respiratory burst, and salicylic acid and jasmonic acid signaling/biosynthesis as well as genes involved in response to heat and high-light intensity. Overall, these results suggest that, in the presence of the root endophyte *P. indica*, the soybean plant induces a positive growth and abiotic stress response while down-regulation major plant defense pathways, potentially indicative of a measured accommodation to the endophytic symbiont.

437T Antisense transcription licenses nascent transcripts to mediate transcriptional gene silencing. Yunkun Dang[1], Jiasen Cheng[2], Xianyun Sun[3], Zhipeng Zhou[1], *Yi Liu*[1] 1) UT Southwestern Medical Center, Dallas, TX. USA; 2) Huazhong Agricultural University, Wuhan, Hubei, China; 3) Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

In eukaryotes, antisense transcription can regulate sense transcription by induction of epigenetic modifications. We previously showed that antisense transcription triggers Dicer-independent small interfering RNA (disiRNA) production and disiRNA loci DNA methylation (DLDM) in *Neurospora crassa*. Here we show that the conserved exonuclease ERI-1 is a critical component in this process. Antisense transcription and ERI-1 binding to target RNAs are necessary and sufficient to trigger DLDM. Convergent transcription causes stalling of RNA polymerase II during transcription, which permits ERI-1 to bind nascent RNAs in the nucleus and to recruit a histone methyltransferase complex that catalyzes chromatin modifications. Furthermore, we show that in the cytoplasm ERI-1 targets hundreds of transcripts from loci without antisense transcription to regulate RNA stability. Together, our results demonstrate a critical role for transcription kinetics in long non-coding RNAs-mediated epigenetic modifications and identify ERI-1 as an important regulator of co-transcriptional gene silencing and post-transcriptional RNA metabolism.

438F The SAGA complex mediates the transcriptional up-regulation of antiviral RNA silencing in the chestnut blight fungus. Ida Bagus Andika, Atif Jamal, Hideki Kondo, *Nobuhiro Suzuki* Institute of Plant Science and Resources, Okayama University, Kurashiki, JP.

RNA silencing, which is conserved across eukaryotic organisms, functions as an innate antiviral defense with the key components including Dicer with RNase III-like dsRNA cleavage activities and Argonaute with RNase H-like activities. Although RNA silencing-related genes, if not all, are known to be transcriptionally up-regulated upon virus infection in some host organisms, little is known about its induction mechanism. In this regard, a phytopathogenic ascomycete, *Cryphonectria parasitica* (chestnut blight fungus) and its viruses provide a particularly advantageous system to study RNA silencing activation, because its infection by certain RNA viruses highly induces the transcription of dicer-like 2 (*dcl2*) and argonaute-like 2 (*agl2*), two major antiviral silencing players. In this study, we developed a screening protocol to identify host factors governing activation of antiviral RNA silencing in *C. parasitica*. The protocol entails multiple

sequential transformations of the host fungus with a cDNA copy of a mutant of the prototype hypovirus lacking the RNA silencing suppressor (CHV1-Dp69), a reporter construct consisting of an enhanced green fluorescent protein gene under the control of the *dcl2* promoter, and a random mutagenic construct with a drug resistance gene. Screening for GFP-negative colonies allowed for identification of *sgf73*, a component of the SAGA **(***S*pt–*A*da–*G*cn5 *a*cetyltransferase) complex, known to be a transcriptional co-activator of a subset of genes. Knockout of other known SAGA components showed that the histone acetyltransferase module regulates transcriptional induction of *dcl2* and *agl2*, while the histone deubiquitinase is involved in regulation of only *agl2*. Interestingly, full-scale induction of *agl2* and *dcl2* by CHV1-Dp69 required both DCL2 and AGL2, whereas that by another RNA virus, mycoreovirus 1 (MyRV1), required only DCL2, supporting the previously proposed positive feedback by DCL2 and AGL2 to this RNA silencing activation. Taken together, these results showed that SAGA mediates the regulation of virus-induced activation of RNA silencing in *C. parasitica*.

439W Nuclear accumulation of the *Aspergillus nidulans* GATA transcription factor AreA is independent of DNA binding. *C. Hunter*[1], M. Hynes[2], J. Fraser[2], D. Clarke[2], M. Davis[2], R. Todd[1] 1) Department of Plant Pathology, Kansas State University, Manhattan, KS; 2) Department of Genetics, The University of Melbourne, Melbourne, AU.

The primary regulator of nitrogen metabolic genes in fungi is the GATA transcription factor AreA. Subcellular localization of AreA in *A. nidulans* is dependent on nutrient availability. When glucose and nitrogen sources are available, AreA is found throughout the cytoplasm and the nucleus. When the cell becomes nitrogen starved, AreA accumulates in the nucleus due to blocked AreA nuclear export. We have recently found redundancy in the nuclear localization signals (NLSs) of AreA. Five conserved canonical NLSs and one conserved noncanonical arginine-based bipartite NLS within the zinc-finger DNA binding domain all function together to mediate nuclear import. Only mutation of the noncanonical bipartite NLS within the DNA binding domain had any significant effect on AreA function, completely abolishing transcriptional activity. In order to determine whether AreA DNA binding affects the intracellular localization of AreA we have HA-epitope-tagged and analyzed the two classical DNA binding mutants; an altered DNA binding specificity mutant, AreA102, and a non-binding mutant, AreA217. The AreA102^{HA} mutant protein showed a similar pattern of subcellular localization to wildtype AreA except when transferred to uric acid (a nitrogen source the *areA102* mutant cannot utilize). On uric acid AreA102^{HA} accumulated in the nucleus as observed during nitrogen starvation. The AreA217^{HA} non-binding mutant protein accumulated in the nuclei of nitrogen-starved cells, demonstrating that DNA binding is not required for AreA nuclear accumulation. AreA217^{HA} does not show nuclear accumulation when ammonium is present. In contrast to wildtype, AreA217^{HA} accumulated in the nucleus when alternative nitrogen sources were available. These findings suggest that while nuclear accumulation of AreA is independent of DNA binding, nuclear export is dependent on AreA DNA binding function. We show using nitrogen metabolism mutants that this is likely due to the requirement of AreA for metabolic signaling of nitrogen availability.

440T Elucidation of the role of the truncated version of Cre1 (Cre1-96) in cellulase production in *Trichoderma reesei*. *Alice Rassinger*, Thiago Mello-de-Sousa, Robert Mach, Astrid Mach-Aigner Institute of Chemical Engineering, Vienna, AT.

Rut-C30 is a cellulase-hyperproducing *Trichoderma reesei* (*T. reesei*) strain and, consequently, became the ancestor of most industry strains used in the production of plant cell wall-degrading enzymes, in particular cellulases. Due to three rounds of undirected mutagenesis its genetic background differs from the wild-type QM6a in many ways, of which two are the lack of a 83 kb large sequence in scaffold 15 and the partial lack of the gene encoding the Carbon catabolite repressor 1 (Cre1). Nonetheless, it remained still unclear, whether the truncated protein Cre1-96 has remained with any functional feature of a transcription factor and if its role remained repressing or changed to an activator. The truncated protein Cre1-96 has been left with a zinc finger domain and a nuclear localization signal *in silico*. On the one hand, the DNA binding was investigated within the Cre1-target genes (*xyr1*, *cbh1* or *xyn1*). On the other hand, trans-regulatory effects were further elucidated such as differences in cellulase activity and chances in the DNA accessibility in cellulase encoding promoters (e.g. *pcbh1* and *pcbh2)*. To this end, it should further the understanding of the function of a putative new transcription factor in *T.reesei*. The gained knowledge could be used for further strain improvement.

441F RNA-editing on transcription factors in model mushroom *Coprinopsis cinerea*. *Y. Xie*, J. Chang, H.-S. Kwan School of Life Sciences, the Chinese University of Hong Kong, Shatin, NT, HK.

Introduction. RNA-editing is an unusual form of post-transcriptional event which can recodes the hereditary information by changing the nucleotide sequence of RNA molecules. It diversifies the transcriptome and proteome of the organism by several means. To date, research on RNA-editing in fungi is rare. *Coprinopsis cinerea*, a typical mushroom, is one of the model organisms to study homobasidiomycetous fungi. It has a short life-cycle and is easy to be cultivated in laboratory. The complete genome sequence of *C. cinerea* has been released.
Aims. To identify RNA-editing sites and editing pattern in *C. cinerea*. Also, to determine the influence of RNA-editing on transcription factors (TFs) and the expression of downstream genes.
Methods. We first sequenced and analyzed the total RNA collected from mycelium of medium cultured C. cinerea to predict RNA-editing candidates. The RNA was then reverse transcribed with specific primers which target on the predicted sites. cDNA prepared were sequenced separately using Sanger method. To confirm the RNA-editing event, we aligned the sequencing read to the reference genome. Expression level is measured by real-time PCR.
Results. *In silico* analysis of RNA-seq data revealed that several TFs, involving in *CAZymes* expression, kinases expression and other cell activities, are likely to have RNA-editing. We have identified one TF that one of the base of its mRNA is edited from A to I. This editing event changes the expression level of downstream genes. We also looked into the mechanism of the editing event. However, no ADAR gene was found. The genome of *C. cinerea* has 3 predicted ADAT genes but the function has not been confirmed.
Discussion. In this study, we uncovered one A-to-I RNA-editing site on the transcription factor of *C. cinerea*, which is the first report of RNA-editing in this model mushroom. Here, we have not analyzed the editing pattern of the whole genome or different developmental stages. RNA-editing can help to improve the diversity of gene expression products in an organism, which means that it may help the creature to survive under stress environments or provide extra diversities in protein coding genes. Stage-specific RNA-editing events and stress-stimulated RNA-editing events worth further studies.

442W Crosstalk of LaeA and CreA in controlling virulence and secondary metabolism in *Penicillium expansum* . *J. Tannous*[1], X. Luo[2], D. Kumar[3], S. Barad[3,4], Y. Chen[5], A. Dubey[3], N.G. Matana[3,4], S. Tian [5], B. Li [5], D. Prusky[3], N. Keller[1,2,6] 1) Department of Medical Microbiology and Immunology, University of Wisconsin – Madison, Madison, WI 53706-1598, USA; 2) Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI 53706-1598, USA; 3) Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel; 4) Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; 5) Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China; 6) Department of Bacteriology, University of Wisconsin -Madison, Madison, WI 53706-1598, USA.

The plant pathogenic fungus *Penicillium expansum* is a major concern of the global food industry due to its wide occurrence and ability to produce various mycotoxins, of which the most significant is patulin. Although the patulin gene cluster has been recently identified and characterized, little is known on how it is regulated. Here we demonstrate that LaeA, a global regulator of mycotoxins in other fungi, is also required for the production of not only patulin but also other putative toxic secondary metabolites (SMs) produced by *P. expansum* as well as virulence on apple. We find that an increase of the sucrose molarity in the culture medium from 15 to 175 mM positively impacts *creA* expression, but conversely, decreases the expression of *laeA* and the accumulation of patulin by 5 and 175 fold, respectively. The increase in sucrose also negatively influences the expression of most of the SM synthase genes in *P. expansum* Pe-D1 strain. Independently, LaeA was deemed as a positive regulator of at least 6 SM gene clusters. Here we address the hypothesis that CreA regulation of *laeA* mediates many of the ΔlaeA phenotypes including regulation of SM clusters and virulence on apple.

443T AP-1-type transcription factor PcYap1 is involved in regulation of penicillin biosynthesis in *Penicillium chrysogenum*. *W. Perez Perez*[1], U. Carrasco Navarro[1], J. Barrios Gozalez[1], M. Gutierrez Ruiz[2], F. Fierro Fierro[1] 1) Departamento de Biotecnologia, Universidad Autonoma Metropolitana - Iztapalapa, Mexico city, Mexico city, MX; 2) Departamento de Ciencias de la Salud, Universidad Autonoma Metropolitana - Iztapalapa, Mexico city, Mexico city, MX.

AP-1 transcription factors are found in eukaryotes from yeasts to mammals, they contain a bZIP domain, regulate transcription of genes in response to a variety of stimuli and their highest affinity binding sequence is TGACTCA. In yeasts, a family of eight transcription factors, named Yap, was described which showed structural similarities to AP-1 factors and whose preferred DNA binding sequence is TTACTAA. *Yap1* is involved in the oxidative stress response, and orthologs of this gene has been described in filamentous fungi participating in the same process. In *Penicillium chrysogenum*, a transcriptional activator of the penicillin biosynthetic gen *pcbAB* was detected by electrphoretic mobility shift assay (EMSA) which bound the sequence TTAGTAA in the promoter of the gene, but its identity remained unclear. in this work, we cloned the *Yap1* ortholog of *P. chrysogenum* (*PcYap1*), expressed and purified the His-tagged Pcyap1 protein from *Pichia pastoris* and tested its ability to bind the *pcbAB* promoter region containing the sequence TTAGTAA. We also attenuated the expression of PcYap1 by RNAi and studied its effect on different processes.
Results: The PcYap1 transcription factor, purified by Nickel-affinity chromatography, bound and caused a mobility shift to a 28 bp DNA probe from the *pcbAB* gene promoter containing the TTAGTAA sequence. No binding was observed when a probe containing the mutated sequence CTAGTCA was used. Then we analyzed the role of PcYap1 in the cell by attenuating its expression through the RNAi pathway. For this purpose, a 458 bp DNA fragment from the exon 2 of *PcYap1* was introduced in the vector pGpdPki-RNAi to generate a dsRNA in *P. chrysogenum* transformants. The transformants showed reduced conidiation, lower tolerance to H_2O_2 and decrease in penicillin production.
Conclusion: We have identified the transcription factor PcYap1 of *P. chrysogenum* as the transcriptional activator binding to the TTAGTAA sequence in the *pcbAB* promoter. Our results suggest that PcYap1 mediates regulation of *pcbAB* transcription by oxidative stress.

444F Deciphering the regulatory network between SREBP pathway and secretory pathway in *Neurospora crassa*. *L. Qin*, M Reilley, V Wu, N.L. Glass Energy Biosciences Institute, The University of California, Berkeley California, USA 94720.

Sterol regulatory element binding proteins (SREBPs) are first found in mammalian cells and their major function is regulating cholesterol uptake and synthesis. Recent studies demonstrate the SREBP pathway is conserved in fungi. In addition to regulate the sterol homeostasis, fungal SREBPs have been reported to involved in hypoxia adaptation and virulence. Furthermore, our previous study demonstrates that disruption of SREBP pathway in *Neurospora crassa* and *Trichoderma reesei* results in hyper production of cellulases. However, how fungal SREBPs mediate the protein secretion is unknown and has never been investigated so far. To address this question, in this study, we identified the homolog components of SREBP pathway in *N. crassa* and utilized the next generation RNA-Seq technology to compare the transcriptional profiling of WT, Δsre-1, Mclr-2 and MΔsre-1 strains under sucrose in which the expression of cellulases genes are repressed in WT and Δsre-1 but induced in Mclr-2 and MΔsre-1. Our results demonstrated that the function of SREBP in ergosterol biosynthesis and hypoxia response was conserved in *N. crassa*. In addition, our data revealed that SREBP pathway was highly activated in cellulase induction condition and the activation of SREBP limited the expression of genes encoding cellobiose dehydrogenase and most of the copper-dependent polysaccharide monooxygenase. Moreover, a set of genes which potentially involved in protein modification, sorting, and trafficking were repressed by sre-1. Furthermore, disruption of SREBP pathway can restored the growth defect of ?hac-1 mutant under cellulose condition. The analyses presented here demonstrated new insights into how SREBPs regulated protein secretion and provided new clue for genetic engineering of filamentous fungal organisms to improve their production of extracellular proteins.

445W Introns regulate gene expression in *Cryptococcus neoformans*. *G. Janbon* Institut Pasteur, Paris, FR.

Cryptococcus neoformans is a basidiomycetous opportunistic pathogen leaving in the environment responsible for more than 500 000 deaths every year. Our recent work suggests that a fascinating, complex pattern of RNA molecules composes its transcriptome and this fungus is emerging for different aspects as an ideal model to study RNA metabolism in eukaryotes. It is also tempting to hypothesize that this complex RNA metabolism provides a mechanism for this yeast to respond to different environmental cues and to be an efficient pathogen. The most prominent features of this transcriptome are introns. Our recent re-annotation of the *C. neoformans* genome revealed that nearly all the expressed genes contain introns. These introns can be present within the coding sequence but also within UTR regions. We previously demonstrated that these introns are essential for gene expression. Here, we identified a large number of regulated

alternative splicing events. However, alternative splicing in this yeast seems to be more a means to regulate gene expression than to diversify the proteome.

446T Analysis of Clp1-dependent UPR-modulation in *Ustilago maydis*. *N. Pinter*, K. Heimel Department of Molecular Microbiology and Genetics, Georg-August-University, Göttingen, Lower Saxony, DE.

The unfolded protein response (UPR) is conserved eukaryotic signaling pathway to counteract endoplasmatic reticulum (ER) stress through extensive restructuring of the secretory pathway. UPR activation is mediated by unconventional splicing of *cib1* mRNA, encoding the central UPR regulator in the corn smut fungus *Ustilago maydis*. During the life cycle of *U. maydis* the UPR is inactive during saprophytic growth but specifically activated after successful penetration of the host. We identified Cib1 (Clp1 interacting bZIP1) to physically interact with Clp1, an important developmental regulator of *U. maydis* and the decisive factor for the induction of fungal proliferation after successful host penetration. Both genes are crucial for pathogenic development and deletion strains are blocked in development after penetration of the leaf surface. Cib1-Clp1 complex formation results in stabilization of both Clp1 and Cib1 and alters the transcriptional program of the UPR, rendering cells hyper-resistant towards ER-stress. Since a constitutive active UPR is deleterious for cell growth, we hypothesize that Clp1-mediated modulation of the UPR prevents deleterious UPR hyperactivation to facilitate long-term UPR activation during biotrophic growth *in planta*. Transcriptional profiling of Clp1-mediated UPR modulation and subsequent gene deletion analysis identified a previously uncharacterized UPR target gene, being specifically required for biotrophic growth of *U. maydis*. While growth under axenic conditions and ER-stress resistance is not affected, deletion strains are fully avirulent in infection assays. Our results suggest that the induction of proliferation *in planta* and modulation of the UPR pathway are connected processes to re-shape the secretory pathway and align fungal development with the cellular physiology.

447F Transcriptional Regulation in haploid cells of *Ustilago maydis* may tie together the High Affinity Ammonium Transporter, Ump2, and the mating program. *R. Margaret Wallen*, Kirsten Richardson, Madison Furnish, Allison Dentinger, Michael H. Perlin Biology, University of Louisville, Louisville, KY.

Ustilago maydis is a biotrophic pathogen of maize. In order to cause disease in the host, the fungus must undergo a dimorphic switch from a yeast-like, budding state to a filamentous state that allows for plant penetration and propagation of the infection. Different environmental cues can induce filamentous growth and some, specifically low nitrogen availability, are essential for mating to occur in nature. In haploid wild type strains of *U. maydis*, low nitrogen availability also results in a non-infectious filamentous growth response. Mating is governed by the coordinated expression of genes from the mating loci in *U. maydis*, the *a* locus and the *b* locus. The *b* locus in particular must play an additional role in haploid cells, as deletion of the locus prevents filamentation due to nitrogen limitation. Previous work found that the *U. maydis* genome encodes two ammonium transports (AMTs), Ump1 and Ump2. While deletion of *ump1* does not yield an observable phenotype, deletion of *ump2* in haploids results in the loss of filamentous growth as a response to low nitrogen, similar to what is observed with deletion of the *b* locus. Overexpression of the high affinity ammonium transporter, Ump2, results in filamentation under non-inducing conditions and can rescue the filamentation defect of the *b* locus deletion mutant. In light of this discovery, we hypothesized that Ump2, like its homolog in *Saccharomyces cerevisiae*, acts as a transceptor, both transporting ammonium across the membrane and signaling low ammonium availability; furthermore, we here present evidence that functional Ump2 is required for transcriptional regulation related to mating, and that there is interaction at the transcriptional level between *ump2* and the *b* locus.

448W The master is switched off, long live the new master: phase specific hierarchical transcriptional control in *Ustilago maydis*. *J. Ulrich*[1], J. Fortenbacher[1], K. Heimel[1,2], F. Finkernagel[3], J. Kämper[1] 1) Karlsruhe Institut of Technology, Institute for Applied Biosciences, Karlsruhe, GER; 2) Georg-August-University-Göttingen, Institute for Microbiology and Genetics, Göttingen, GER; 3) Philipps-University Marburg, Institute for Molecular Biology and Tumor Research, Marburg, GER.

In *Ustilago maydis*, the change from yeast-like saprophytic growth to the formation of pathogenic filamentous hyphae is controlled by a closely interconnected and cross-controlled network of transcription factors. The network integrates pheromone signaling during the mating-associated cell fusion events as well as the subsequent onset of the biotrophic development by formation of the bE/bW heterodimeric transcription factor complex. bE/bW activation triggers the expression of Rbf1, the master regulator for most bE/bW-regulated genes. bE/bW expression alone is sufficient to trigger pathogenic development, ectopic Rbf1 expression at least initiates pathogenic development, stressing the central role of both factors. During plant penetration, function of both proteins is inhibited by the interaction with Clp1; at later stages, expression of the “master regulator” Rbf1 is not detectable.

Via a combination of microarray-data and ChipSeq analysis, we show the complex combinatorial control of bE/bW, Rbf1 and Clp1 of genes during the switch to pathogenic development on various promoters. Both promoter-binding as well as expression data show that at later stages the two transcription factors Biz1 and Hdp2, both essential for plant infection, overtake the central function of Rbf1. Apparently, *U. maydis* uses distinct sets of regulators to integrate and/or differntiate the different signals before and after infecting its plant host.

449T Utilizing next generation sequencing to revitalize a forward genetic screen for mutants deficient in the production of sterigmatocystin in Aspergillus nidulans. *B. T. Pfannenstiel*[1], K. J. Affeldt[2], J. Bok[2], R. A. E. Butchko[3], T. Choera[2], G. J. Fischer[1], B. P. Knox[2], F. Y. Lim[2], X. Luo[4], A. A. Soukup[1], J. E. Spraker[4], K. Throckmorton[1], P. Wiemann[2], N. P. Keller[2,5], J. M. Palmer[6] 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI, 53706, USA; 2) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, 53706, USA; 3) Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA; 4) Department of Plant Pathology, University of Wisconsin-Madison, Wisconsin 53706; 5) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA; 6) Center for Forest Mycology Research, Northern Research Station, U.S. Forest Service, Madison, WI 53706, USA.

The study of aflatoxin regulation in *Aspergillus* spp. has warranted the attention of many researchers due to its carcinogenic properties and frequency as a food and feed contaminant. Significant progress on the regulation of the AF cluster has been conducted in the model organism *Aspergillus nidulans* by assessing regulation of the sterigmatocystin (ST) gene cluster as ST is the penultimate precursor of AF. A screen using a chemical mutagen identified 23 loci involved in regulating ST production. Only six of these loci were characterized from this screen using classical mapping (*mcsA)* and complementation with a cosmid library (*laeA*). Recently the remaining mutants were

backcrossed and sequenced using an Ion Torrent PGM™. Each mutant contained one or more SNPs in predicted genes. Deletion of these genes resulted in identification of mutant alleles responsible for the loss of ST production in 12 out of the 17 remaining mutants. Three of the causative mutations were in uncharacterized genes unknown to be involved with ST production, of which two appear to regulate the ST cluster via transcriptional regulation of its cluster specific transcription factor, *aflR*. Based on protein domains and homologs, the remaining uncharacterized gene has a predicted role in the plasma membrane fusion. Nine mutations were in genes already known to affect ST synthesis (*laeA*, *mcsA*, *fluG*, *stcA*).

450F Local rates of nucleosome turnover are influenced by transcription and chromatin modifications in *Neurospora crassa*. *M. Kamei*, A. Courtney, Z. Lewis Department of Microbiology, University of Georgia, Athens, GA.

Nucleosome turnover occurs when old nucleosomes are removed from the chromatin fiber and replaced with new nucleosomes. Turnover happens regularly during nuclear processes such as transcription, DNA replication, and DNA repair. Several studies have shown that different genomic regions exhibit different rates of nucleosome turnover, yet the molecular basis of differential histone turnover is poorly understood. We developed a transgenic strain of *N. crassa* that enables us to measure rates of new histone incorporation across the genome. Specifically, we replaced the *N. crassa csr-1* locus with a single, epitope tagged copy of histone H3 (H3-FLAG) under control of the *tcu-1* gene promoter. Expression of the H3-FLAG transgene is tightly regulated by the presence or absence of copper in the growth medium. We induced H3-FLAG for 0, 2, 4, or 8 hours and performed ChIP-seq (Chromatin-immunoprecipitation followed by Illumina sequencing) using anti-FLAG antibodies to enrich for sites that preferentially incorporate newly synthesized H3-FLAG. Induction of the transgene led to rapid incorporation of H3-FLAG in the promoters and +1 nucleosomes of highly expressed genes, indicating high rates of nucleosome turnover at these sites. As expected, the rate of nucleosome turnover in heterochromatin was low. Heterochromatin formation depends on DIM-5, an H3K9 methyltransferase. We found that *dim-5* strains exhibit increased chromatin accessibility in silent heterochromatin domains and discrete heterochromatic loci displayed increased rates of nucleosome turnover relative to wildtype. Currently, we are analyzing nucleosome turnover rates in additional mutants that are defective for specific chromatin modifying activities. We will summarize our progress in identifying chromatin determinants of nucleosome turnover.

451W Pervasive adenine N6-methylation of active genes in fungi. *S.J. Mondo*[1], R.O. Dannebaum[1], R.C. Kuo[1], A. Gryganskyi[2], J. Magnuson[3], T.Y. James[4], M.A. O'Malley[5], J.E. Stajich[6], J.W. Spatafora[7], A. Visel[1], I.V. Grigoriev[1] 1) Fungal Genomics, Joint Genome Institute, Walnut Creek, CA; 2) L. F. Lambert Spawn Co, Coatesville, PA; 3) Pacific Northwest National Laboratory, Richland, WA; 4) Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI; 5) Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA; 6) Department of Plant Pathology and Microbiology, University of California, Riverside, California; 7) Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Epigenetics plays a critical role in regulation and expression of genetic information. Many important epigenetic marks are encoded as DNA-based modifications, for example 5-methylcytosine (5mC) or N6-methyldeoxyadenine (6mA). 6mA is a non-canonical eukaryotic DNA base modification reported genome-wide in only four species: an alga, *Chlamydomonas reinhardtii* and three animals, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Mus musculus*. However, the genome-wide abundance of 6mA in these organisms ranges from low (0.4% of all A's in *C. reinhardtii*) to extremely low (0.001%-0.07% in *D. melanogaster*). Despite low levels, 6mA is critical for proper development, as it plays an important role in regulation of gene expression. Regrettably, exploration of 6mA thus far has been limited to single model eukaryotes and therefore its relevance in many major branches of Eukaryota is still unknown. Here we conduct the first kingdom-wide exploration of both 6mA and 5mC, where we interrogate the Fungi, an ancient and extremely diverse eukaryotic lineage. We found heavy utilization of 6mA in early-diverging fungi where up to 2.8% of all adenines were methylated, vastly exceeding the levels observed in other eukaryotes and more derived fungi, whereas 5mC was primarily in the CpG context and restricted to repeats. Interesting, with one exception 6mA and 5mC were mutually exclusive (i.e. lineages with high 6mA had no 5mC and vice versa). 6mA occurred symmetrically at ApT dinucleotides, where it concentrated in dense 6mA 'islands' at gene promoters. Islands were positioned nearby promoter thymine-blocks and ≈95% of methylated genes were expressed. Our results demonstrate the importance of 6mA as a broadly conserved epigenomic mark in eukaryotes as well as identify key components involved in positioning of genomic 6mA. Furthermore, our results implicate 6mA as an epigenomic mark transmissible across nuclear division and suggest its involvement in nucleosome organization. We anticipate that our study will provide a foundation for deeper exploration of 6mA not only in fungi, but across all Eukaryota.

452T Genome-wide analysis of the GATA transcription factor AreA in *Aspergillus nidulans*. *A. Li*[1], Z. Miao[1], D.J. Downes[2], G.Y. Busot[2], R.B. Todd[2], K. Wong[1] 1) Faculty of Health Sciences, University of Macau, Macau, CN; 2) Department of Plant Pathology, Kansas State University, KS.

In *Aspergillus nidulans,* nitrogen utilization is precisely regulated at the gene expression level. Expression of genes encoding enzymes to utilize alternative nitrogen sources or to scavenge nitrogen compounds during nitrogen starvation is dependent on the GATA transcription factor AreA. In general for typical nitrogen metabolic genes, AreA-dependent activation is minimal during nitrogen sufficiency, increased when nitrogen is limiting, and strongest during nitrogen starvation. Although many AreA target genes are known, the genome-wide targets of AreA and AreA DNA binding properties remain unknown. To address this, we profiled AreA DNA binding under nitrogen sufficiency, nitrogen limitation and nitrogen starvation via Chromatin Immuno-precipitation followed by Sequencing (ChIP-seq) and identified more than 1,600 promoters bound by AreA. Interestingly, AreA binds to more than 400 promoters under nitrogen sufficiency, when its protein level in the nucleus is low. Expression analysis shows that less than 5% of these targets are affected by *areA* deletion. In contrast, under nitrogen starvation expression of more than 40% of AreA bound genes is significantly altered by *areA* deletion. These results indicate that AreA binds to many target promoters poised to activate upon appropriate signaling. Comparisons of targets among the different nitrogen conditions revealed many (~200) common binding sites as well as nitrogen source-specific AreA binding events, indicating that AreA targets different promoters according to nitrogen sources and availability. De novo motif discovery analysis showed that besides "GATA", several other motifs are also enriched among AreA bound regions, consistent with cooperation of AreA with pathway-specific transcription factors. In addition to nitrogen metabolism, Gene Ontology analysis of AreA target genes suggests roles in development and stress response. Moreover, our data also provides compelling evidence for positive feedback control of AreA on the co-repressor NmrA that

negatively regulates its function. Therefore, this work not only reveals the genome-wide functions of AreA, but also uncovers a new regulatory loop for nitrogen metabolism repression in *A. nidulans*.

453F Evidence for inducer function of 2-keto-3-deoxy-L-galactonate in D-galacturonic acid induced gene expression in *Aspergillus niger*. *E. Alazi*[1], C. Khosravi[2], T.G. Homan[1], M. Arentshorst[1], M. Di Falco[3], M. Peng[2], M.V. Aguilar Pontes[2], A. Tsang[3], J. Visser[1], R.P. de Vries[2], A.F.J. Ram[1] 1) Molecular Microbiology and Biotechnology, Institute of Biology Leiden, Leiden University, The Netherlands; 2) Fungal Physiology, CBS-KNAW Fungal Biodiversity Centre & Fungal Molecular Physiology, Utrecht University, The Netherlands; 3) Centre for Structural and Functional Genomics, Concordia University, Canada.

Pectin is an important carbon source for the saprotrophic fungus *Aspergillus niger*, which is an efficient producer of pectin-degrading enzymes. D-galacturonic acid (GA) is the main product of pectin degradation. In *A. niger*, GA is transported into the cell by the sugar transporter GatA and subsequently catabolized into pyruvate and glycerol through a pathway consisting of four enzymes: GaaA, D-galacturonate reductase; GaaB, L-galactonate dehydratase; GaaC, 2-keto-3-deoxy-L-galactonate aldolase; and GaaD, L-glyceraldehyde reductase. It has been shown that GA or a derivative of GA is required for the induction of the genes needed for pectin degradation, GA transport, and GA catabolism. In order to identify the GA derivative that acts as an inducer, we constructed GA catabolic pathway deletion mutants (*?gaaA*, *?gaaB*, *?gaaC* and *?gaaD*) anticipating that the mutants would accumulate the substrate of the deleted enzyme when grown on GA. The growth of both *?gaaB* and *?gaaC* was abolished on GA pointing out that there are no redundant enzymes replacing GaaB and GaaC. *?gaaB* and *?gaaC*, pregrown in fructose medium and then transferred to GA medium, accumulated pathway intermediates L-galactonate and 2-keto-3-deoxy-L-galactonate, respectively. Northern analysis showed that the expression of the GA induced genes is drastically reduced in *?gaaB* and highly increased in *?gaaC* compared to the wild type strain. Genome wide gene expression analysis via RNA-seq indicated that the accumulation of 2-keto-3-deoxy-L-galactonate in *?gaaC* results in the induction of the GA induced genes. Identification of the inducer of the pectinase genes, together with the recent identification of the transcriptional activator-repressor module controlling pectinase expression would facilitate the industrial use of *A. niger* in pectinase production.

454W Multidrug resistance caused by a gain-of-function mutation in a novel fungal specific transcription factor in *Sclerotinia homoeocarpa*. *H. Sang*[1], J. Hulvey[2], R. Green[1], T. Chang[3], G. Jung[1] 1) Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA; 2) Biology Department, University of Massachusetts, Amherst, MA; 3) School of Ecology and Environmental System, Kyungpook National University, South Korea.

The ongoing emergence of multidrug resistance (MDR) in human and plant pathogenic fungi threatens global human and plant health. To combat multidrug resistant fungal pathogens, understanding the MDR mechanisms is a crucial step, but is limited in the filamentous plant pathogenic fungi. In this study, we used genomics and molecular genetic tools to uncover the key factors for MDR mechanisms in the filamentous ascomycete fungus *Sclerotinia homoeocarpa* (the causal agent of dollar spot on turfgrass). We showed evidence that overexpression of cytochrome P450s (CYP450s) in drug metabolism and ATP-binding cassette (ABC) transporters in drug secretion are involved in MDR. Moreover, a gain-of-function mutation in a fungal specific transcription factor detected from the field MDR isolates is responsible for constitutive and induced overexpression of the CYP450s and ABC transporters, resulting in resistance to structurally different chemicals. Our establishment of a molecular mechanism for the MDR pathway in this fungal pathosystem has implications for controlling plant pathogenic fungi and possibly human pathogens.

Pathogenic and Mutalistic Interactions

455T Hidden in Plain Sight: Heterogeneity in Asexual Spores of *Aspergillus fumigatus*. *S.E. Kang*, M. Momany Department of Plant Biology, University of Georgia, Athens, GA.

Aspergillus fumigatus spores are found ubiquitously in the environment. The asexual clonal spores (conidia) produced in the environment are inhaled where they can germinate and cause high mortality rates in immunocompromised patients. Germination of conidia is the most crucial step in the development of aspergillosis. Given that conidia are produced in a variety of environments, we tested whether sporulation conditions impact the ability of conidia to break dormancy and grow. Spores from a single colony were bulked on complete media and cultured with environmentally- and medically-relevant stress conditions. Spores from each condition were collected and transferred to all other conditions. Germination and growth kinetics were analyzed for 25,000 conidia from each condition swap experiment using flow cytometry. We found that clonal populations of conidia are heterogeneous for germination and growth phenotypes and the stress increases this phenotypic heterogeneity.

456F *Aspergillus fumigatus* carbon catabolite repression is essential for virulence in established infection microenvironments. *S. Beattie*[1], K. Mark[2], L. Reis[3], S. Dhingra[1], C. Black[6], C. Cheng[2,4,5], G. Goldman[3], R. Cramer[1] 1) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH USA; 2) Department of Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH USA; 3) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil; 4) Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Lebanon, NH USA; 5) Institute for Quantitative Biomedical Sciences, Geisel School of Medicine at Dartmouth, Lebanon, NH USA; 6) Department of Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH USA.

Purpose: The filamentous fungus *Aspergillus fumigatus* is responsible for a disproportionate number of cases of invasive mycoses, relative to other filamentous fungi found in the environment in similar or even higher quantities. Therefore, unique aspects of *A. fumigatus* biology must account for the ability to grow within the host and cause disease. Here, we hypothesize that carbon availability during an infection is dynamic, and the ability to optimize carbon utilization within the host is critical for optimal virulence throughout the fungus-host interaction.

Methods: To test this hypothesis, we characterized the carbon catabolite repression (CCR) system of *A. fumigatus* through generation of a *creA* genetic null mutant. Murine models of invasive aspergillosis were used to assess the contribution of CCR to pathogenesis. Transcriptomics, metabolomics, and *in vitro* growth and stress susceptibility assays were further utilized to determine the role of CCR in *A.*

fumigatus fitness.

Results: In contrast to what has been observed in yeasts, we identified a novel role for CreA and CCR in the virulence of *A. fumigatus*. While CCR was not required for the establishment of infection, loss of CreA dramatically reduced virulence after infection establishment. Therefore, we hypothesized the virulence defect to be a result of altered *in vivo* host carbon metabolism and observed a critical role for CreA in controlling redox homeostasis in the face of a dynamic nutrient environment.

Conclusions: Collectively, our results support the conclusion that transcriptional regulation of fungal bioenergetics to optimize *in vivo* utilization of carbon and nitrogen sources is critical for human fungal virulence.

457W The *A. fumigatus* Fumiquinazoline C is potentially cytotoxic to macrophages and soil amoeba *Dictyostelium discoideum*. Marina Campos Rocha[1], Taicia Pacheco Fill[2], Juliana Issa Hori[3], Lilian Pereira Silva[4], João Henrique Tadini Marilhano Fabri [1], Anderson Ferrira da Cunha[1], Gustavo Henrique Goldman[4], *Iran Malavazi*[1] 1) Departamento de Genética e Evolução, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, Brazil. ; 2) Instituto de Química, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. ; 3) Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil. ; 4) Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil.

Fungi remarkably produce a variety of secondary metabolites as a consequence of different environmental stimuli. These compounds can ultimately provide fitness attributes to the producing organism. Recently, we characterized two components of the *A. fumigatus* cell wall integrity pathway (CWI), *pkcA* and *rlmA* and observed that in addition to the cell wall related-phenotypes, the perturbation of the signaling circuit coordinated by the PkcA-MpkA-RlmA module impacts on the production of fumiquinazolines (Fq). FqC is the major Fq produced by *A. fumigatus* which accumulation was associated with conidia formation. Here we show that *pkcA*^{G579R} and Δ*rlmA* mutant strains produce lower FqC (24.7% and 27.9%, respectively) and that FqC concentrations were 10.5- fold lower in the Δ*mpkA* strain. This decrease is accompanied by global down-regulation in mRNA expression of the Fq cluster genes during the asexual development. Aiming to understand if other cell stresses could influence the production of FqC, we performed a screening using different null mutants and found that the deletion of the transcription factor SebA, (primarily involved in heat shock and oxidative stress) overproduced FqC (about 4.5-fold increase) indicating that *sebA* is a negative regulator of FqC production. *A. fumigatus* is sensitive to FqC and this tolerance is decreased in the CWI pathway mutants and increased in the Δ*sebA* strain. In addition, FqC can induce pore formation on the membrane of macrophages and highly stimulates the secretion the pro-inflammatory cytokine TNF-α by this cell type. We also used the soil amoeba Dictyostelium *discoideum* to study the phagocytic interaction of this organism with conidia from the Δ*sebA* strain. Interestingly, conidia of the Δ*sebA* were significantly less phagocytized by *D. discoideum* and the opposite occurred when conidia from the CWI pathway mutants were tested. Our results suggest that Fq production is regulated at different levels in *A. fumigatus* and that FqC can serve as a defense compound against other microorganisms or soil predators. Although we could not detect FqC in the lung of infected mice, this molecule is potentially cytotoxic to fungi and mammalian cells.

458T Culture independent detection of *Aspergillus fumigatus* Cyp51A in human lung. *Aiah Khateb*[1,2], Michael Bromley[2], Paul Bowyer[2] 1) Faculaty of Applied Sciences, Medical Laboratory Technology, University of Taibah, Medina, Kingdom of Saudi Arabia ; 2) Fungal Infection Group, Centre for Respiratory Medicine and Allergy, Faculty of Biology, Medicine and Health,Institute of Inflammation and Repair, Manchester Academic Health Science Centre, The University of Manchester and University Hospital of South Man.

Background *Aspergillus fumigatus* is the main causative agent of aspergillosis. Traditional culture methods recover a small fraction of all the lung resident species and therefore resistant isolates are usually missed. Long-term triazole oral therapy, particularly itraconazole and voriconazole has been used in treatment of chronic pulmonary Aspergillosis or allergic aspergillosis. Azole resistance is associated with poorer disease outcome in allergic and chronic disease and has increased in recent years. This study aims to detect resistant mutations in *cyp51A* in the context of uncultured *Aspergillus* strains present in clinical samples.

Methods DNA was extracted directly from 38 BAL samples (8 healthy controls, 9 ABPA, 8 SAFS, 7 SA and 3 MA). The *cyp51A* gene was directly amplified from extracted DNA and amplicons sequenced using illumina Miseq. Sequence analysis was done using the following software pipline; trimmomatic 0.36>bowtie2>samtools1.2>bcftools htslib 1.3.1.>VarScan.

Results 61 SNPS were identified, 29 resulted in amino acid change. Three SNPs matched cyp51A mutations that have previously been shown to confer azole resistance; M220I/M220T and F46L. 9 further mutations occurred only in azole treated patients; N218T, F219Y, N218Y, M220T, M220I, I217S, A62C, I135T, T112I and 17 were common to sequences obtained from patients with no azole treatment history.

Conclusions This study has identified new *cyp51A* mutations in the healthy, chronically colonized patients. It also broadened our understanding of *cyp51A* resistance mechanisms in the context of uncultured *Aspergillus* strains present in clinical samples.

459F Experimental evolution of *A. fumigatus* in hypoxia results in increased virulence. *C.H. Kowalski*[1], A.K. Caffrey[2], S.R. Beattie[1], J.J. Obar[1], J.E. Stajich[3], R.A. Cramer[1] 1) Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA; 2) Department of Immunology & Infectious Diseases, Montana State University, Bozeman, Montana, USA; 3) Department of Plant Pathology & Microbiology and Center for Plant Cell Biology, University of California-Riverside, Riverside, California, USA.

Purpose: The ability of microbes to thrive in hypoxic environments is important for virulence. Hypoxia fitness of *Aspergillus fumigatus* strains strongly correlates with virulence in murine models of invasive aspergillosis (IPA). Despite this observation, the molecular mechanisms of hypoxia adaption and its mechanistic link to virulence remain elusive. Here we aim to utilize an experimental evolution approach to elucidate underlying mechanisms of hypoxia and host fitness.

Methods: Conidial populations of a low virulent, hypoxia fitness deficient strain of *A. fumigatus* were passaged in hypoxia for 80 days on glucose minimal medium. Single spore clones from the population were isolated and phenotypic, virulence, genomic and transcriptomic sequencing, and metabolic analyses were carried out in comparison to ancestral strains.

Results: After 80 days in hypoxia, an experimentally evolved strain, EVOL20, was isolated that displays increased fitness in hypoxia highlighted by metabolic derepression, altered cell wall composition, and acidification of the culture media. Interestingly, EVOL20 is more

virulent in murine models of IPA and has altered immunopathogenesis in immune competent mice. Transcriptomic analysis reveals large scale expression changes in EVOL20 compared to the ancestor in hypoxia; with expression changes greater than 4-fold in 172 genes (55 increased, 112 decreased). Genome sequence analysis of EVOL20 identified 106 synonymous SNPs and only 3 nonsynonymous SNPs in 3 uncharacterized genes. Molecular genetic characterization of these genes in the context of hypoxia fitness and fungal virulence is underway.

Conclusions: Experimental evolution in hypoxia of a low virulent *A. fumigatus* strain with reduced hypoxia fitness dramatically improved *A. fumigatus* virulence compared to the ancestor strain. We conclude that hypoxia selection pressure may promote evolution of *A. fumigatus* virulence mechanisms.

460W Full length and five short segments of TRI6 RNA interference constructs that reduce deoxynivalenol production in Fusarium graminearum reveal consistent patterns of siRNA and different silencing efficiencies. *Thomas Baldwin*, Phil Bregitzer National Small Grains Germplasm Research Facility, USDA-ARS, Aberdeen, Idaho, United States of America.

Deoxynivalenol (DON) contamination of small grains caused by Fusarium head blight is a problem of economic and health importance that may be addressed by RNAi interference (RNAi)-based host-induced gene silencing (HIGS). RNAi in higher eukaryotes, including fungi, involves processing double stranded RNA (dsRNA) into small interfering RNA (siRNA) that silence genes based on homology. Six transgenic *F. graminearum* (strain PH1) mutants containing the RNAi vector pTRM-TRI6 were studied, each containing a full length inverted repeat of *TRI6* inserted in a different genomic position. *TRI6* is a transcription factor that positively regulates DON production. The mutant lines showed drastic reductions of DON production in infected plant tissues and in TBI media, and reduced virulence on wheat. The sRNA populations of three mutant lines had abundant siRNA species, not present in PH1, that mapped to *TRI6*, and ~22 nt siRNA was the most abundant size class. There was a discontinuous and repeatable pattern among the mutants of *TRI6*-specific siRNA. The 5'-most base of 83% of siRNAs was uracil, indicative of dsRNA processing by a fungal dicer. Subsequent experiments, were conducted with additional mutants in which one of five shorter (200-250 nt) *TRI6*-specific inverted repeats (each targeting a different region of *TRI6*). The pattern of siRNA processing for each individual inverted repeat was similar to the pattern of the full-length construct for the corresponding section of *TRI6*. All mutants with short constructs showed reduced expression of a *TRI6*-regulated gene, *TRI5,* on TBI media, but not as much as observed for the full length construct. Dicer patterns for dsRNA processing have implications for design of efficient RNAi silencing vectors. Understanding the siRNA profiles that result from RNAi constructs is critical to optimizing RNAi applications that are designed to reduce pathogenicity and mycotoxin production in the field.

461T The fungal nitrooxidative stress response suppresses rice innate immunity during blast disease. Margarita Marroquin-Guzman, *Richard Wilson* Plant Pathology, University of Nebraska at Lincoln, Lincoln, NE.

Understanding how microbes manipulate plant innate immunity and colonize host cells is a major goal of plant pathology and crop health improvement. Here, we report that the *Magnaporthe oryzae* nitrooxidative stress response quells host defenses and facilitates fungal growth and development in rice cells. Nitronate monooxygense (Nmo) catalyzes the oxidative denitrification of nitroalkanes to their corresponding carbonyl compounds and nitrite. The *M. oryzae* genome carries five *NMO* genes. *NMO2* expression was subject to nitrogen metabolite repression by Nut1, and carbon catabolite repression by Tps1. Deleting *NMO2* revealed, counter intuitively, that the resulting Δ*nmo2* mutant strain was nitrate (NO_3^-) and nitrite (NO_2^-) non-utilizing. We determined this was because Δ*nmo2* strains, relative to wild type (WT), could not mitigate nitrooxidative cellular damage caused by reactive nitrogen species by-products of NO_3^- and NO_2^- metabolism. Δ*nmo2* mutant strains were also sensitive to the NO generator nitroprusside and reactive oxygen species (ROS). On plants, Δ*nmo2* mutant strains formed functional appressoria that penetrated rice cuticles, but growth of invasive hyphae (IH) in underlying epidermal cells was restricted and accompanied by granular depositions in rice cells, host ROS accumulation and the induced expression of rice pathogenesis-related (PR) genes *PBZ1* and *PR-1*. Δ*nmo2* strains expressing Bas4 fused to GFP and the cytoplasmic effector Pwl2 fused to mCherry:NLS showed that both effectors were expressed in rice cells. This signified that the elevated host defense responses elicited by Δ*nmo2* mutant strains did not kill the fungus. However, whereas Pwl2:mCherry:NLS expressed in WT IH localized to a single, punctate blast interfacial complex (BIC) (as reported previously), Pwl2:mCherry:NLS in Δ*nmo2* was localized to multiple foci throughout IH. Inhibiting the host ROS burst by treatment with diphenyleneiodonium (DPI), or quenching host ROS with sodium ascorbate, avoided triggering the rice immune response in cells infected with Δ*nmo2* mutant strains. This allowed Δ*nmo2* mutant strains to grow and develop normal IH with single BICs per host cell. Thus, *NMO2* is required for maintaining redox balance in host cells in order to avoid triggering plant defenses that restrict the growth and development of *M. oryzae* in rice. This work provides new knowledge on molecular plant-microbe interactions and, more broadly, identifies a new requisite for NO_3^- metabolism in fungi.

462F A novel *Botrytis cinerea* MFS transporter provides tolerance to glucosinolate breakdown products. D. Vela-Corcia, A. Dafa-Berger, O. Barda, *M. Levy* Hebrew Univ Jerusalem, Plant Pathology & Microbiology, Rehovot, Israel.

Glucosinolates are secondary metabolites that accumulate mainly in cruciferous plants. Glucosinolates and their hydrolytic products have antifungal properties and play a role in plants' resistance against pathogens. *Botrytis cinerea*, a necrotrophic pathogen, has variable sensitivity to glucosinolates. Here we studied the mechanisms of tolerance to glucosinolates in the fungus *B. cinerea*. Exposure of *B. cinerea* to glucosinolate breakdown products induced expression of *Botrytis cinerea* major facilitator superfamily transporter suggested to be involved in Isothiocyanates detoxification (*BcmfsI*). *B. cinerea* inoculated on wild-type *Arabidopsis thaliana* or on *IQD1*^{OE} plants, transgenic for high glucosinolate level, activated *BcmfsI* expression to higher levels than *B. cinerea* on glucosinolate-deficient *A. thaliana* mutants *cyp79B2/79B3*. *B. cinerea* strains lacking functional MFSI (Δ*BcmfsI*) were more sensitive to glucosinolate breakdown products *in vitro* and *in planta*. Δ*BcmfsI* strains were less virulent on wild-type *Arabidopsis* plants, but not on glucosinolate-deficient mutants *cyp79B2/B3.* Furthermore *Saccharomyces cerevisiae* expressing the *BcmfsI* gene was more resistance to the fungitoxic glucosinolate breakdown products. We demonstrate here that the *B. cinerea* MFSI transporter is a virulence factor that increases tolerance of the pathogen to glucosinolates. We also demonstrate that *BcmfsI* can confer tolerance to hydrolytic products of glucosinolate in the yeast *S. cerevisiae*.

463W Antifungal activity of the human gut metabolome. *C.E. Garcia-Rangel*[1], F Tebbji[1], MC Daigneault[2], NN Liu[3], JR Ko¨hler[3], E Allen-Vercoe[2], A Sellam[1] 1) Infectious Diseases Research Centre-CRI, CHU de Québec Research Center, Quebec City, Quebec, Canada; 2) Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada; 3) Boston Children's Hospital/Harvard Medical School, Boston, MA, USA.

The human gut contains a variety of commensal microbes which are composed of diverse organisms that belong to all three domains of life with Eukaria primarily represented by fungi. The commensal/opportunistic yeast *Candida albicans* has been reported as the most common fungus in the gut of healthy humans. Recent study has shown that commensal microbiota play a critical role in the protection of the gut against colonization by other bacterial pathogens and pathobionts. However, so far, whether *C. albicans* overgrowth or pathogenicity are controlled by other fecal microbiota is not known. In this study, we showed that the secreted human gut metabolome (HGM) exerts an antifungal activity against different intestinal-resident yeasts including *C. albicans*, *C. tropicalis* and *C. parapsilosis.* The HGM inhibited the growth of both sensitive and drug-resistant strains of *C. albicans.* To uncover the mechanism of action of the HGM associated with its antifungal property, a genome-wide genetic screen was undertaken and identified key components of the conserved TOR (Target Of Rapamycin) pathway as required for HGM tolerance. The HGM reduced the phosphorylation state of the known TOR effector, RPS6 confirming thus that the HGM inhibit *C. albicans* growth through TOR pathway. Given the central role of TOR pathway in modulating cell growth in response to nutrients cues, these data support a model where gut microbial cohabitants control *C. albicans* nutritional competitiveness through the modulation of TOR pathway activity. Furthermore, hyphal growth, a critical virulence trait of *C. albicans*, as well as the inducibility of hyphae-specific genes were significantly reduced. In accordance with the inhibitory effect on hyphae formation, we also showed that the HGM reduced significantly the damage inflicted to the human colon epithelial cells by *C. albicans*. To identify microbial specie(s) that produce the antifungal molecule(s), individual isolates form continuous-culture chemostat of fecal extracts of two healthy human donors were screened. Our data revealed that common butyrate-producing bacteria from the two donors exhibited an apparent antifungal activity. Together, these emphasize that the novel cross-kingdom interaction mediated by butyrate-producing bacteria contribute to the control of both the growth and virulence traits of *C. albicans* and provide a new paradigm where the commensal growth of this major human pathogen in the gut is dictated by the surrounding microbiota.

464T Phagosomal neutralization by the fungal pathogen *Candida albicans* induces macrophage pyroptosis. S. Vylkova[1,2], *Michael Lorenz*[3] 1) ZIK Septomics, Friedrich-Schiller University, Jena, Thuringia, DE; 2) Hans-Knöll Institute, Jena, Thuringia, DE; 3) Univeristy of Texas Health Science Center at Houston, Houston, TX, USA.

The interaction of *Candida albicans* with the innate immune system is the key determinant of the pathogen/commensal balance and has selected for adaptations that facilitate the utilization of nutrients commonly found within the host, including proteins and amino acids; many of the catabolic pathways needed to assimilate these compounds are required for persistence in the host. We have shown that *C. albicans* co-opts amino acid catabolism to generate and excrete ammonia, which raises extracellular pH, both *in vitro* and *in vivo* and induces hyphal morphogenesis. Mutants defective in the uptake or utilization of amino acids, such as those lacking *STP2,* a transcription factor that regulates the expression of amino acid permeases, are impaired in multiple aspects of fungal-macrophage interactions resulting from an inability to neutralize the phagosome. Here we identified a novel role in amino acid utilization for Ahr1p, a transcription factor previously implicated in regulation of adherence and hyphal morphogenesis. Mutants lacking *AHR1* were defective in growth, alkalinization and ammonia release on amino acid-rich media, similar to *stp2*? and *ahr1Δstp2Δ* cells, and occupied more acidic phagosomes. Notably, *ahr1*? and *stp2*? strains did not induce pyroptosis as measured by Caspase-1-dependent IL-1β release, though this phenotype could be suppressed by pharmacological neutralization of the phagosome. Altogether, we show that *C. albicans*-driven neutralization of the phagosome promotes hyphal morphogenesis, sufficient for induction of Caspase-1 mediated macrophage lysis.

465F Mapping host-pathogen transcriptional dynamics using sorted cell populations of *Candida albicans*-infected macrophages. *J.F. Munoz*[1], T. Delorey[2], C.B. Ford[1], D.A. Thompson[1], R.P. Rao[2], C.A. Cuomo[1] 1) Broad Institute of MIT and Harvard, Cambridge, MA; 2) Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA.

Encounters between immune cells and *Candida albicans*, a member of the healthy human microbiome and a major pathogen in immunocompromised individuals, determine the course of infection. Phagocytes such as macrophages are key players during *C. albicans* infection, and their interactions have diverse outcomes based on microscopic observation; however, little is known about the molecular contribution of these heterogeneous subpopulations to the infection process. To characterize the variability that may be important for infection outcome, we developed a system to sort distinct subpopulations during *C. albicans*-macrophage interactions using fluorescent markers, and simultaneously profile the transcriptional responses of the host and pathogen using RNA-sequencing. Here we show we can successfully isolate macrophages and yeast cells interacting extracellularly and macrophages with phagocytized live or dead fungus. Using this system, we trace the phenotypic and expression variability for distinct infection fates. We have identified specific sets of *C. albicans* genes in sorted subpopulations that show a coordinated response needed for the commensal to pathogenic transition, and the adaptation and survival of the fungus in the nutrient-limited and stressful environment within the macrophages. Simultaneously, we examined the transcriptional profile of macrophage subpopulations, and characterize the coordinate host immune response, including several pathways and regulatory genes needed for the migration, pathogen recognition, activation of engulfment, cellular communication, and phagocytosis. This system offers finer scale analysis compared to bulk population studies and enables examination of different host-pathogen phenotypes and their distinct expression variability to better understand the evolution of drug resistance, host adaptation and immunomodulatory effects.

466W Functional genomic analysis reveals fungal modulators of host immune responses. *Teresa O'Meara*, Kwamaa Duah, Leah Cowen Molecular Genetics, University of Toronto, Toronto, Ontario, CA.

Candida albicans is a leading human fungal pathogen that causes life-threatening systemic infections. The first line of defense against this pathogen is the innate immune response. Macrophages readily engulf *C. albicans*, and the engulfed *C. albicans* cells subsequently undergo a morphogenetic switch from yeast to a filamentous growth. This change in morphology is temporally coupled to the induction of macrophage pyroptosis, an inflammatory host cell death program mediated via activation of NLRP3 and caspase-1. Recently, we discovered that it is the remodeling of the fungal cell wall in response to the macrophage environment that exposes the trigger of host cell

death, not the filament itself. Further, we found that heat-killed previously phagocytized *C. albicans* cells could drive macrophage lysis, unlike their counterparts that had not been phagocytized prior to killing, suggesting that this fungal cell wall remodeling is sufficient to drive macrophage lysis, and that the remodeling occurs specifically in response to the macrophage phagosome. However, the specific trigger and mechanisms involved remain enigmatic.

To address this question, we developed a high-content imaging platform to monitor interactions between *C. albicans* and macrophages, and have leveraged this platform to identify *C. albicans* mutants that are defective in pyroptosis. Using murine macrophages expressing an ASC-fluorescent protein (FP) reporter, we quantified ASC-FP paranuclear specks, which are indicative of NLRP3 inflammasome activation. Our primary screen allows us to simultaneously quantify not only pyroptosis, but also *C. albicans* filamentation within macrophages. Genes required for filamentation in the macrophage cannot be predicted *a priori* given that distinct genes regulate filamentation in response to distinct cues. Our initial analysis of 96 filamentation-competent conditional expression strains that enable transcriptional repression of cell wall genes identified 34 genes that are important for activation of pyroptosis. This included genes that encode glucan biogenesis enzymes, mannosyltransferases, and GPI-anchored proteins, and their potential regulators. Together, we have identified novel circuitry regulating fungal induction of host immune cell death.

467T The active site of trehalose-6-phosphate synthase in *Candida albicans* impacts growth at elevated temperatures, hyphal growth in serum, and biofilm formation. *J.L. Tenor*[1], Yi Maio[2,3], D.L. Toffaletti[1], S.A. Maskarinec[1], R.G. Brennan[2], J.R. Perfect[1] 1) Division of Infectious Diseases, Department of Medicine, Duke University School of Medicine, Durham, NC; 2) Department of Biochemistry, Duke University School of Medicine, Durham, NC; 3) Present address: Department of Molecular and Cellular Physiology, Stanford University, Palo Alto, CA.

The trehalose pathway is commonly found in fungi with putative roles in glycolytic regulation, signaling, protection against stressors, and energy by serving as a carbon source. The enzyme, trehalose-6-phosphate synthase (Tps1), is responsible for the first committed step in the trehalose biosynthetic pathway. In *C. albicans*, TPS1 affects hyphae formation in serum, growth at elevated temperatures, and survival within macrophages. We have recently solved the crystal structure of CaTps1. Structural analysis of CaTps1 has identified key amino acid residues predicted to impact its activity. Site-directed mutagenesis of 4 residues of the active site were created (Y89F, K285A, D379A, D387A) and resulted in loss or reduced ability to grow at elevated temperatures, defective hyphal growth in serum at 37°C, and abnormal biofilm formation. Mutagenesis of 2 residues predicted to effect dimerization, E341 (E341R) and E364 (E364R), exhibited wild-type phenotypes. The absence of the trehalose pathway in humans and the importance of this pathway for *C. albicans* and other fungal pathogens to cause disease provides us with an attractive target for antifungal drug development.

468F *Debaryomyces hansenii* may antagonize *Candida* yeasts in the human gut. *H.E. Hallen-Adams*, N. Banjara Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE.

Over a century of studies, more than 250 species of fungi have been reported from the human gut. Fungi detected in the healthy human gut vary widely between individuals, and within the same individuals at different time points. However, much of this variation is illusory, as many of the fungi detected in fecal samples are environmental or foodborne and incapable of growth under the conditions in the human GI tract. A much smaller pool of organisms is commonly detected in the human gut, and these are the taxa to consider as true members of the gut ecological community. In addition to colonizing fungi, some species may be unable to persist long term but nevertheless be commonly detected and play a role in gut ecology due to dietary or environmental exposure. *Saccharomyces cerevisiae*, reported in 20 of 37 studies published between 1917 and 2016, is the classic example. The foodborne yeast *Debaryomyces hansenii* was reported in 7 of 37 studies, and in 18 of 85 fecal samples profiled individually, where its presence was negatively correlated with that of *Candida albicans, C. tropicalis* and other *Candida* species. More than half of the *D. hansenii* strains we have isolated from cheese show killer activity against *C. albicans* and *C. tropicalis in vitro*. *D. hansenii* neither grows nor produces killer toxin at 37 C and crude extracts of the toxin lose their killer activity after a few hours at elevated temperature, presumably due to proteolysis. Killer toxin in cheese naturally colonized with *D. hansenii* retains its anti-*Candida* activity for several days, suggesting a mechanism by which *D. hansenii* could affect gut fungal dynamics.

469W Purification and characterization of a symbiosis-induced endocellulase from the ectomycorrhizal symbiont *Laccaria bicolor*. *F Zhang*[1], C Champion[2], M Haon[2], G Anasontzis[2], M Kemppainen[3], A Pardo[3], Y Daguerre[1], A Deveau[1], C Veneault-Fourrey[1], A Kohler[1], MN Rosso[2], B Henrissat[4], JG Berrin[2], F Martin[1] 1) UMR 1136 INRA-Université de Lorraine 'Interactions Arbres/Microorganismes', Laboratoire d'Excellence ARBRE, Centre INRA-Lorraine, 54280, Champenoux, France; 2) UMR 1163 INRA-Biodiversité et Biotechnologie Fongiques, Polytech Marseille, Faculté des Sciences de Luminy, 13 009, Marseille, France; 3) Laboratorio de Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Provincia de Buenos Aires, Argentina; 4) CNRS, UMR 7257 & Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Marseille, France.

In forest soils, ectomycorrhizal fungi establish a mutualistic symbiosis with tree roots. The mutualistic fungi trade host photoassimilates against soil nitrogen and phosphorus. Differentiation of symbiotic roots induces extensive cell wall architectural modifications in the host apoplastic space. The origin of enzymes involved in these cell wall modifications has been the subject of debate for several decades. The ectomycorrhizal basidiomycete *Laccaria bicolor* has a restricted set of carbohydrate-active enzymes (CAZymes) degrading plant cell wall polysaccharides. However, several of those genes are upregulated upon symbiosis. We speculate that several of the symbiosis-induced CAZymes are involved in the remodeling of the host apoplastic space. Here, we characterize the sole GH5 endoglucanase with a cellulose-binding motif (CBM1) domain (LbGH5) identified in the genome of *L. bicolor*. We showed that the *LbGH5* gene is induced five-fold in ectomycorrhizal roots using qPCR and RNA-Seq. RNAi mutants with a decreased expression of *LbGH5* have a lower ability to form ectomycorrhizal roots. Yeast secretion trap (YST) functional screen confirmed that LbGH5 is a secreted protein. We then produced and purified the recombinant protein LbGH5 with and without its CBM1 domain in *Pichia pastoris*. The recombinant LbGH5 displayed highest actitivities towards carboxymethyl cellulose (CMC) and cellulose extracted from aspen roots. In contrast, LbGH5 displayed no activities toward *L.bicolor* cell walls or aspen hemicellulose. *In situ* localization of LbGH5 in ectomycorrhizal roots by indirect immunofluorescence confocal microscopy demonstrated that the enzyme accumulates in hyphal cell walls forming the mantle and Hartig net. These data suggest

that cell wall modifications within ectomycorrhizal roots arise from cell wall–modifying enzymes of fungal origin.
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470T Remodeling of the cell wall of endophytic hyphae of *Epichloë festucae* in the symbiotic interaction with *Lolium perenne.* *N. Noorifar*[1], M.S. Savoian[1], T. Weikert[2], B. Moerschbacher[2], B. Scott[1] 1) Institute of Fundamental Science, Massey University, Palmerston North, Manawatu, NZ; 2) Institute for Biology and Biotechnology of Plants Westfälische Wilhelms-Universität, Münster, Germany.

Epichloë festucae is a filamentous fungus, which forms symbiotic associations with aerial tissues of *Lolium* and *Festuca* grass species. Scanning confocal microscopy (SCM)-based analysis of leaf tissue infiltrated with the chitin-specific molecular probe, WGA-Alexa-488, showed that just septa of endophytic hyphae bound this probe while the entire cell wall was labelled in epiphyllous hyphae. These results suggest that hyphal cell wall chitin is either masked or remodeled in endophytic hyphae. The aims of this project are (i) to test whether *E. festucae* LysM-containing proteins have a role in masking chitin and thereby preventing PAMP-triggered immunity as has been shown for the *Cladosporium fulvum*-tomato pathogen interaction and (ii) to analyze the composition of the cell wall of endophytic and epiphytic hyphae.
Analysis of the *E. festucae* genome identified seven genes encoding LysM domains. The expression of two of these genes, *lymA* and *lymB*, were down-regulated in the transcriptome of three different *E. festucae* symbiosis mutants suggesting they are functionally important in symbiosis. Interestingly, both are divergently transcribed from chitinase-encoding genes that are also down regulated in the symbiosis mutants. To functionally analyse the roles of these genes in symbiosis we have generated D*lymA*, D*lymB,* D*chiA*, D*chiB* single deletion and D*lymA/B* double deletion mutants. Plants singly infected with D*lymA*, D*lymB* or D*chiA* mutants have the same plant-interaction phenotypes as wild type as shown by both SCM and transmission electron microscopy analyses. Phenotypic analyses of the D*chiB* and D*lymA*/*B* mutants are still in progress. To check fungal cell wall structure we used cell wall component-specific eGFP-based biosensors to understand the differences in structure of *E. festucae* endophytic and epiphytic hyphae. Preliminary results suggest that cell wall chitin is converted to chitosan in endophytic hyphae. This structural change is consistent with a lack of a defence response when *E. festucae* forms a mutualistic symbiotic association with *L. perenne*.

471F Investigating the circadian clock of *Verticillium dahliae* and its influence on pathogenicity. *E. Cascant-Lopez*[1,2], M. K. Sobczyk[1], L. J. Johnson [2], R. J. Harrison[1] 1) NIAB/EMR, West Malling, Kent, U.K; 2) University of Reading, Reading, Berkshire, U.K.

Many organisms have evolved to adapt themselves to the rhythmicity of their environment. A broad knowledge of the molecular mechanism of circadian clocks has been gained using the fungal model organism *Neurospora crassa*. Nevertheless, little is known about the existence of circadian clocks in other fungi. As a Sordariomycete, *N. crassa* is related to many important plant pathogens including the vascular wilt *Verticillium dahliae.* We aimed to investigate the presence of a circadian clock in *V. dahliae*, and to study whether the clock influences growth and pathogenicity.

Having identified homologues of the *N. crassa* clock oscillator proteins (FRQ, WC-1, WC-2, FRH, FWD-1 and VVD) in *V. dahliae,* we found high conservation in clock protein domains, which suggested the existence of a functional circadian clock. To characterize the clock system in *V. dahliae*, we studied if the daily formation of conidia and miscrosclerotia (infective propagules) were regulated in a circadian manner. No evidence for an entrainable, free-running rhythm was observed. This suggests that the morphological phenotype is only a response to cycles in light and temperature. Deleting core clock genes such as *frq* does not have any obvious effect on fungal morphology. Furthermore, temporal gene expression profiling with qRT-PCR in constant darkness showed a lack of rhythmic *frq* expression. However, deletion of the transcription factor/photoreceptor WC-1 prevents ring production in light:dark cycles. Thus, WC-1 is involved in the production of the daily developmental rhythm by mediating transcriptional responses to white light.

To understand the possible loss of clock functionality in *V. dahliae*, we performed an orthology analysis with 29 Sordariomycetes species. We found that among *Verticillium* spp. only *Verticillium alfalfae* had lost an oscillator protein homolog: WC-2. We then investigated the presence of a *N. crassa* Clock Box in the *frq* promoter of *V. dahliae*. The C-Box (5'CGATCCGCT3') is essential in regulating rhythmic expression of *frq* in *N. crassa.* Of all the species tested, only *Magnoporthe poae* presented an identical motif in the *frq* promoter. However, in *Verticillium spp.* a similar motif (5'CGATCCCCT 3') was found, and is present in other related species in the Sordariales. In conclusion*, V. dahliae* presents all the necessary genetic loci for a functional clock, but there is no evidence of rhythmicity in either morphological traits or in gene expression.

472W Phosphate status-dependent control of interactions with pathogenic and endophytic fungi in *Arabidopsis thaliana*. *K. Hiruma*[1,2], M. Tanaka[1], L. Tae-Hong[1], N. Kitagawa[1], P. Schulze-Lefert[3], Y. Saijo[1,2] 1) Biological Sciences, NAIST, Ikoma, Nara, JP; 2) JST, PRESTO, JP; 3) Max Planck Institute for Plant Breeding Reserach, Cologne, Germany.

Plants often rely on root-associated beneficial fungi for uptake of inorganic phosphate (Pi) from soils, while restricting pathogen invasion. It remains elusive how plants modulate root immunity in response to Pi availability, to accommodate beneficial endophytes while retaining effective pathogen resistance. We report alterations in Arabidopsis defense responses against the beneficial root endophyte *Colletotricum tofieldiae* (*Ct*), which promote plant growth under low Pi conditions and the pathogenic relative of *Ct*, *C.incanum* (*Ci*). Genetic studies combined with RNA-seq analysis reveal that components of an intact plant phosphate starvation response are not only required for *Ct*-mediated plant growth promotion but proper defense responses against *Ct*. Furthermore, damage associated molecular pattern (DAMP) sensing and signaling via PEPRs is required for *Ci* restriction in low Pi conditions. We will present how these two separate pathways regulate the closely related pathogenic and endophytic fungi in low Pi conditions.

473T Comparative genomics reveals structural variations in the genome of the plant pathogenic fungus *Colletotrichum higginsianum*. *A. Tsushima*[1,2], P. Gan[1], N. Kumakura[1], M. Narusaka[3], Y. Takano[4], Y. Narusaka[3], K. Shirasu[1,2] 1) Center for Sustainable Resource Science, RIKEN, Yokohama, Japan; 2) Graduate School of Science, The University of Tokyo, Bunkyo-ku, Japan; 3) Research Institute for Biological Sciences Okayama, Okayama, Japan; 4) Graduate School of Agriculture, Kyoto University, Kyoto, Japan.

The members of the genus *Colletotrichum* cause anthracnose disease on a broad range of crops and have a devastating economic impact. Further, the interaction between *Colletotrichum higginsianum* and its host, the model plant *Arabidopsis thaliana*, has been a useful pathosystem to study fungal hemibiotrophic infection of plants. Therefore, the interaction between *C. higginsianum* and *A. thaliana* has been the focus of many studies on *Colletotrichum* to date. Genomic analyses of other fungal plant pathogens have revealed that fast-evolving genomic compartments are often enriched in genes encoding effector proteins, which are diverse, small, and secreted proteins involved in pathogenicity. In order to investigate genomic variations in *C. higginsianum*, we sequenced the genome of strain MAFF305635 from Japan with PacBio RSII, yielding an assembly of 49.8 Mb consisting of 28 contigs. Then, it was compared to the genome of strain IMI349063 from Trinidad and Tobago (Zampounis *et al.*, 2016). Despite the geographic isolation, the two strains were found to be closely related, sharing 88.2 % of sequence (≥ 99 % identity, ≥ 15 kb). However, this analysis also revealed the presence of extensive genomic rearrangements between the two strains. Among these rearrangements, 6 inter-chromosomal translocations and 4 intra-chromosomal inversions were identified. The whole-genome comparison also revealed the presence of strain-specific regions in the genome of this species. Interestingly, it was found that large-scale genomic rearrangements and strain-specific regions tend to associate with transposable elements. These results suggest that transposable elements may increase the genomic flexibility of this pathogen by contributing to homology-based recombination through their repetitive sequences and alternative translocation. The new insights from our research are expected to accelerate effector candidate gene mining of *C. higginsianum* by considering structural variations in its genome.

474F *Cryptococcus neoformans* suppresses immune cell infiltration into the brain via a secreted immunomodulatory polysaccharide. *S.T. Denham*, S. Verma, J.M. Daugherty, R. Reynolds, T.E. Lane, J.C.S. Brown Pathology, University of Utah, Salt Lake City, UT.

The basidiomycetes yeast *Cryptococcus neoformans* is the most prevalent cause of fungal meningitis, responsible for ~1 million diagnosed cases and ~600,000 deaths annually. *C. neoformans* infection begins in the lungs after the inhalation of infectious particles. In immunocompromised individuals, where symptomatic disease is most prevalent, *C. neoformans* systemically disseminates from the lungs. Disseminating *C. neoformans* exhibits a particular tropism for the brain, leading to cryptococcal meningitis (CM). CM patients often suffer high titers of infecting fungal cells within the brain, but exhibit limited neuroinflammation and relatively mild initial symptoms. Consequently, many patients do not seek care until fungal burden is damagingly high and therefore difficult to treat. *C. neoformans* conditionally produces a protective polysaccharide capsule, consisting primarily of the polysaccharide glucuronoxylomannan (GXM). GXM is both attached to the cell surface and secreted into the extracellular space as exo-GXM. GXM has numerous immunosuppressive properties, and its levels in the cerebrospinal fluid (CSF) of human patients negatively correlates with CSF white blood cell count. We thus hypothesized that exo-GXM suppresses inflammation and immune infiltration into the brain during infection. Moreover, this pauci-inflammation of CM is likely not only due to the immunocompromised state of patients, as we and others have found that *C. neoformans* suppresses the immune response of immunocompetent mice.

After infecting C57BL/6J mice intranasally with wild-type *C. neoformans*, fungal cells eventually disseminate to the brain. Upon analysis of infected brains, we discovered very little immune infiltration (neutrophils, macrophages, CD4+, and CD8+ cells), despite high fungal burden and histopathology. We also microscopically observed significant levels of exo-GXM in the brain. When mice were infected intracranially with an acapsular strain (*cap60Δ*), numbers of brain infiltrating immune cells significantly increased over wild-type. Likewise, administration of purified GXM to *cap60Δ* infected mice suppressed immune infiltration and increased fungal burden. We have also identified mutants deficient in exo-GXM production and the regulation of the switch between cell surface retention and exo-GXM secretion. Our results support a model in which *C. neoformans* actively secretes exo-GXM to modulate the host's immune response and promote fungal survival in the brain.

475W Evaluation of Synergistic Molecules Discovered from High-throughput Screen. *M. Wambaugh*, J. Brown Department of Pathology, University of Utah, Salt Lake City, UT.

Antimicrobial resistance is a major problem in the modern world. For systemic fungal infections, antifungal drug resistance only adds to pre-existing challenges: few antifungal targets, severe side effects, and treatments that can last for a year or longer. One strategy to combat resistant fungal infections is treatment with synergistic drug combinations – when two drugs combined have far greater activity than predicted by the sum of each individual activity. These combinations have even been shown to inhibit growth of organisms that are resistant to one of the drugs in the pair. However, systematic identification of synergistic pairs is challenging, as they are relatively rare (4-10% of combinations) and high-throughput screening has been impractical due to scale. We developed a method to identify synergistic drug interactions that is scalable and applicable to a wide range of pathogenic microbes. Using a large-scale chemical genetics dataset from the fungus *Cryptococcus neoformans*, we calculate chemical-genetic signatures – the set of genes whose knockouts are strongly sensitive or resistant to a small molecule – for the antifungal drug fluconazole and its known synergistic partners. We then identify genes whose knockouts respond similarly to all known synergistic partners. We use these "synergy prediction mutants" to screen a collection of FDA approved drugs for synergistic interactions with fluconazole. Our screen is five times faster than testing each combination individually and correctly identifies important interactions 57% of the time. We will present newly identified synergistic pairs identified by our screen, which includes interactions between fluconazole and antidepressants, anticholinergics, and antihistamines. Since *in vitro* synergy does not always translate to efficacy in patients, we also test these combinations in a zebrafish brain model of *Cryptococcus neoformans* infection to further evaluate efficacy. Finally, we investigate the molecular mechanisms underlying these interactions with the ultimate goal of rationally designing synergistic combinations to improve treatment of systemic fungal infections.

476T Re-wiring of a conserved stress response pathway connects development, secretion and effector gene expression in *Ustilago maydis*. *Kai Heimel*[1], Lara Schmitz[1], Florian Finkernagel[2], Gunther Doehlemann[3], Martin Hampel[1] 1) Department of Molecular Microbiology and Genetics, Georg-August-University Goettingen, Germany; 2) Institute of Molecular Biology and Tumor Research, Philipps-University Marburg, Germany; 3) Department of Terrestrial Microbiology, University of Cologne, Germany.

The unfolded protein response (UPR) represents a conserved cellular response to ensure homeostasis of the endoplasmic reticulum (ER). Under ER stress conditions, such as increased demands for protein secretion the UPR is activated and promotes ER expansion and an upregulation of the ER protein folding machinery. In pathogenic fungi a functional UPR is ubiquitously required for virulence, although

the underlying molecular details are not completely resolved, yet. In the corn smut fungus *Ustilago maydis,* the UPR pathway is required for various virulence related processes, including the adaptation to the host environment, execution of developmental transitions and efficient delivery of effector proteins. A direct connection to the signalling pathways controlling development is established through protein-protein interaction between the central UPR regulator Cib1 and Clp1, an essential regulator of fungal development. This interaction triggers fungal proliferation *in planta* and re-shapes UPR signalling for extended activation during pathogenic growth. Moreover, we observed suppressive effects of an active UPR on the MAPK mediated pheromone pathway, preventing developmental transitions at the early stages of the *U. maydis* life cycle. Consistenly, UPR-activation prevents conjugation tube-formation and filamentous growth. By contrast, pathogenic devlopment after plant infection is strictly dependent on an active UPR pathway. Moreover, a functional UPR is critical for secretion of effectors, and directly contributes to transcriptional regulation of genes encoding secreted effectors. Abolishment of the regulatory connection between the UPR and the Pit2 effector did not affect gene expression of *pit2 in planta*, but resulted in significantly reduced virulence. We therefore hypothesize that the UPR is critical for effector secretion and UPR-dependent transcriptional control of specific effector genes allows for a rapid adaptation of gene expression in response to changing intracellular or environmental conditions.

477F Similar outcomes but different ways to get there – Diversity of infection development and expression profiles in *Zymoseptoria tritici* isolates. *Janine Haueisen*[1,2], Christoph Eschenbrenner[1,2], Holger Adamiak[3], Jonathan Grandaubert[1,2,4], Eva H. Stukenbrock[1,2] 1) Christian-Albrechts-University of Kiel, Germany; 2) Max Planck Institute for Evolutionary Biology, Ploen, Germany; 3) independent, Kiel, Germany; 4) Institut Curie, Paris, France.

Zymoseptoria tritici is a wheat pathogen with a complex, poorly understood hemibiotrophic lifestyle. Although *Z. tritici* is specialized to infect domesticated wheat in putatively uniform agro-ecosystems we observe a high degree of intra-specific diversity. Our aim is to understand the relevance of this diversity during host-pathogen interaction. We conducted infection experiments with *Z. tritici* field isolates from the Netherlands, Denmark and Iran that show similar quantitative virulence phenotypes on the susceptible cultivar Obelisk. Confocal laser scanning microscopy of infected leaves allowed us to characterize four *Z. tritici* "core" infection stages. However, microscopic analysis revealed that during the core infection stages spatial and temporal infection development clearly differs between isolates. In particular, the extent of biotrophic fungal growth in the plant mesophyll varies, ranging from single hyphae limited to the tissue around stomata to dense hyphal networks in the inter-cellular space. We combined the comparative microscopy with stage-specific RNA-seq to capture dynamics in fungal gene expression as well as isolate-specific transcription profiles. Differential gene expression (DGE) analyses showed that transcription in *Z. tritici* isolates is highly dynamic during infection. Expression profiles representing the biotrophic stage are clearly distant from profiles of the necrotrophic stage and up to 7 % of all transcribed genes were identified to be differentially expressed between all four core stages. Moreover, we found these genes to be enriched in genes encoding small secreted proteins indicating that fine-tuned expression of effector candidates is important for infection progress. DGE analyses also revealed that many *Z. tritici* genes have very different expression profiles in the individual isolates. Comparing the stage-specific transcriptomes between the three isolates, we found that up to 20 % of all genes are differentially expressed. In summary, our analyses reveal a core *Z. tritici* infection program but also identify highly diverse infection development, expression profiles and molecular host-pathogen interaction. We conclude that the high levels of intra-species diversity enable rapid adaptations to changes in host and environment and thereby significantly contribute to the success of *Z. tritici* as one of the most damaging pathogens of wheat.

478W Fungal-bacterial interactions drive community assembly in cheese rind microbiomes. *Benjamin Wolfe*, Yuanchen Zhang, Casey Cosetta, Erik Kastman Biology, Tufts University, Medford, MA.

Despite a growing appreciation of the widespread co-occurrence of bacteria and fungi in many microbiomes, mechanisms that drive the ecology of bacterial species are largely studied while excluding neighboring microbial eukaryotes. My lab is using tractable microbiomes based on fermented foods to identify the processes and molecular mechanisms by which diverse fungi can shape the ecology of bacterial species and communities. We first use in situ metagenomics and culture-based approaches to identify putative bacterial-fungal interactions in naturally forming communities. We then combine in vitro community reconstructions, comparative genomics, transcriptomics, and metabolomics to identify the molecular mechanisms driving fungal-mediated bacterial competition and cooperation. Results from a variety of cheese rind biofilm models illustrate the diverse ways that fungi drive bacterial community development. In biofilms dominated by closely-related *Staphylococcus* species, the fungus *Scopulariopsis* allows *Staphylococcus* species that are weak competitors and colonizers to outcompete neighbors by altering iron and amino acid availability. In biofilms composed of both non-motile Firmicutes and Actinobacteria as well as motile Proteobacteria, the biophysical networks formed by *Mucor* can facilitate the dispersal of motile community members, leading to communities dominated by Proteobacteria. Most recently, we have found that volatile organic compounds produced by fungi can impact the growth of individual bacterial species and alter the development of multispecies bacterial communities. Collectively, our results demonstrate how fungi can mediate bacterial competition and community development in multispecies microbiomes through a variety of contact-dependent and contact-independent mechanisms. Ongoing work is exploring how manipulation of the bacterial-fungal interactions we have identified can be used to better manage the quality and safety of food microbiomes.

479T Functional ecology of mycobiome shifts associated with plant senescence - linking environmental and experimental re-synthesis metatranscriptomics. *Ko-Hsuan Chen*[1], Hui-Ling Liao[2], Hailee B. Korotkin[3], P. Brandon Matheny[3], Francois Lutzoni[1] 1) Department of Biology, Duke University, Durham, NC, USA; 2) Soil and Water Sciences Department, University of Florida, Gainesville, FL, USA; 3) Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA.

The same fungi are often found in healthy and dead tissues of the same plant species. Because plant tissues at different stages of senescence are usually separated by time and space, tracking functional transitions of their mycobiomes is often confounded with extraneous factors. The perennial moss *Dicranum scoparium* has a growth form with a continuous senescence gradient, making it an ideal system to study fungal trophic transitions. Our goal is to understand the function of fungi associated with healthy photosynthetic tissues to advanced states of decomposition. A metatranscriptomic approach was first applied to obtain the metabolic (rRNA) and functional (mRNA) activities of fungi associated with top (photosynthetic), middle (senescent), and bottom (decomposing) layers for the naturally occurring *D. scoparium*. Our study of nutrient-related gene expression in each layer, revealed that the highly expressed genes in the top layer are mostly

related to carbon exchange, whereas in the middle and bottom layers we also found highly expressed genes that are involved in nitrogen and phosphate transport. In parallel to our metatranscriptomic study of environmental samples of *D. scoparium*, we isolated fungal strains from the three layers of this moss and grew *D. scoparium in vitro* from spores. By comparing and integrating the fungal rRNA sequences generated by metatranscriptome and culture-based approaches, we assigned fungi into three types of association categories: 1) High activity in photosynthetic tissues, 2) High activity in decomposing tissues and 3) Low activity throughout the gametophyte but abundant when using culture-based detection. Based on these categories, seven fungal strains representing distinct fungal lineages (four Ascomycota, two Basidiomycota, one Mortierellomycotina) were selected to establish fungus-plant re-synthesis pairs in the laboratory with both living and dead gametophytes. For every fungus-plant pairs, growth rates were monitored for two months after fungal colonies reached the moss. Metatranscriptomes of these fungus-plant pairs were obtained at the end of this period.

480F Biochemical, structural, and functional characterization of the Avr4 core effector family in fungi. Li-Hung Chen[1], Amanda Kohler[1], Anthony Salvucci[1], Nicholas Hurlburt[2], Benjamin Schwessinger[1], Andrew Fisher[2,3], *Ioannis Stergiopoulos*[1] 1) Plant Pathology, UC Davis, Davis, CA; 2) Chemistry, UC Davis, Davis, CA; 3) Molecular and Cellular Biology, UC Davis, Davis, CA.

Effectors are secreted microbial proteins that facilitate infections. Research in our lab and elsewhere has revealed the presence of abundant homologous core effectors across different fungal species. One such core effector with homologs in many fungal species of Dothideomycete is Avr4, a secreted chitin-binding effector with a conserved CBM14 (carbohydrate-binding module family 14) domain in its structure that was first characterized in the tomato pathogen *Cladosporium fulvum*. Despite their low sequence homology, Avr4 effectors have a similar biological function in protecting fungal cell wall chitin against chitinases of plant and microbial origin. This suggests that next to deregulation of host immunity during infections some effectors can facilitate general fungal fitness and survival. Remarkably, the majority of Avr4 orthologs are still perceived by Cf-4, a trans-membrane receptor-like protein (RLP) from tomato that recognizes Avr4 and mediates resistance. To identify how Cf-4 can perceive so diverse in sequence Avr4 effectors, we have solved the 1.7 Å X-ray crystal structure of *Pf*Avr4 from the tomato pathogen *Pseudocercospora fuligena* and used site-directed mutagenesis to identify the molecular determinants necessary for ligand-binding and recognition in *Pf*Avr4. Our analysis indicated that, in contrast to expectations that the indispensability of the chitin-binding domain (ChtBD) would make it a prime target for recognition by Cf-4, individual chitin-binding residues do not have a direct effect on the *Pf*Avr4/Cf-4 interaction. This further indicates that the *Pf*Avr4 ligand-binding function (virulence) is structurally distinct or does not fully overlap with the property of recognition (avirulence). We have also discovered that several Dothideomycetes carry a paralog of Avr4 that we refer to as Avr4-2. Like Avr4, Avr4-2 proteins are small cysteine-rich secreted proteins with a CBM14. The intrinsic function of Avr4-2 is not yet known but preliminary data from biochemical and functional analyses suggest that it has a role during plant infections that is diversified from that of Avr4. Current efforts are concentrated on determining the substrate specificity and biological function of Avr4-2, and obtaining a co-crystal of Avr4 with its chitin substrate in order to definine with higher accuracy the molecular basis for the broad-recognition of core effectors by cognate immune receptors.

481W Identification and monitoring of effector proteins in the spinach downy mildew pathogen *Peronospora farinosa*. *Joël Klein*[1], Marcel van Verk[1,2], Guido van den Ackerveken[1] 1) Plant-Microbe Interactions, Department of Biology, Utrecht University, Utrecht, NL; 2) Bioinformatics, Department of Biology, Utrecht University, Utrecht, NL.

Peronospora farinosa f. sp. *spinaciae* (*Pfs*) is an obligate biotrophic oomycete pathogen of spinach, on which it causes downy mildew disease. *Pfs* employs secreted effector proteins to modulate plant innate immunity and enable infection. Resistance genes in the spinach host provide protection against *Pfs*, by recognizing the pathogen, through its effectors. Although newly bred resistant cultivars are initially protected, new *Pfs* races rapidly break the employed resistance genes by adaptation of their recognized effector proteins. A reference genome of *P. farinosa* race 1 (*Pfs1*) of 32 Mbp was generated by sequencing using PacBio, and Illumina technology, followed by a hybrid assembly. Sequencing of *Pfs1* mRNA of 7 infection stages before and during infection contributed significantly to generate accurate gene models. The corresponding protein models have been used to identify more than 60 *Pfs1* effectors, by selecting proteins with signal peptides followed by conserved translocation motifs. To study adaptation of effectors we sequenced 14 other *Pfs* races using Illumina. The genomes of these races were *de novo* assembled, and the effector repertoires determined. Polymorphisms in effector sequences of these races are under study to provide insight into effector evolution. Furthermore, we assembled the mitochondrial genomes of *Pfs1* and the 14 other races to infer their phylogenetic relatedness. Combined with the data on effector evolution this will give us insight into mechanisms by which this downy mildew breaks resistance in spinach cultivars.

482T Roles of specific *Fusarium graminearum* secreted proteins during Fusarium head blight disease. *A.K. Machado*[1], W-S Lee[1], M. Urban[1], F. Olmez[2], K. Hammond-Kosack[1] 1) Plant Biology Crop Science, Rothamsted Research, Harpenden, United Kingdom; 2) Central Research Institute for Field Crops, Ankara, Turkey.

Fusarium graminearum (Fg) is a major fungal pathogen of wheat crops globally, causing Fusarium head blight (FHB) disease. Like many other plant pathogens, *Fg* is predicted to produce *in planta* secretred proteins that modulate plant metabolism to suppress or re-programme plant defences. Understanding the molecular functions of *Fg* effectors will help to elucidate the processes underlying wheat spike colonisation and fungal pathogenicity.

With the aim of identifying *Fg* effector proteins that can suppress host plant defences we selected using various criteria a set of small secreted proteins (SSP) to express *in planta* using the *Barley stripe mosaic virus* over-expression system (BSMV-VOX). We then tested whether expression of any of these SSPs enhanced *Fg* fungal infection of susceptible wheat spikes.

Amongst the set of *Fg* SSP tested, three (FgSSP5, FgSSP6 and FgSSP7) appear to enhance FHB disease when overexpressed in wheat ears prior to infecting with *Fg.*

FgSSP6 and FgSSP7 belong to the cerato-platanin protein (CPP) family. In several other plant pathogenic fungi, CPPs have been implicated in a number of virulence and plant protection mechanisms, including induction of host plant cell death or expansin-like activity. *FgSSP5* encodes a protein that possesses the pfam domain RALF (Rapid alkalinization factor; PF05498.6). RALF domain-containing proteins are predominately found in plants and play a role in plant development regulating tissue expansion and/or negatively regulating pollen tube elongation. BLAST analyses identified RALF domain containing proteins in a restrict range of different pathogen species. Based on those results and biochemical tests, our hypothesis is that pre-elevated cerato-platanins (FgSSP6 and FgSSP7) levels in the

apoplast/surrounding the hyphae could induce an intense defence response culminating in cell death to benefit the necrotrophic phase of *Fg* by increasing nutrient availability. FgSSP5 may be a specific virulence factor that manipulates a key plant process, by alkalinizing the plant environment during infection, and using same plant receptor used to recognise plant proteins. Once the mechanisms are further understood, these genes/proteins could potentially be novel intervention targets either for conventional chemistries and/ or for methods such as host-induced gene silencing to achieve FHB disease and/or mycotoxin control. The characterisation of single and double gene deletion *Fg* mutants is in progress.
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483F A *SIX1* homolog in *Fusarium oxysporum* f.sp *cubense* is required for virulence towards banana. *Sri Widinugraheni*[1,5], J.N Sanchez[2], H.C van der Does[1], P. van Dam[1], F. Garcia Bastidas[3], N. Ordonez[3], S. Subandiyah[4], H.C. Kistler[2], G.H.J Kema[3], M. Rep[1] 1) Molecular Plant Pathology, University of Amsterdam, Amsterdam 904, 1098 XH, NL; 2) ARS USDA, 1551 Lindig Street, St. Paul, MN 551086052, USA; 3) Biointeraction Unit, PRI-WUR, Radix 107 Droevendaalsesteeg 1, Wageningen, NL; 4) Dept. Plant Protection, Gadjah Mada University, Yogyakarta, Indonesia; 5) Fac.of Agriculture, Nusa Cendana University, Kupang, East Nusa Tenggara, Indonesia.

Fusarium oxysporum f.sp *cubense* (Foc) causes Panama disease in banana. Foc strains are divided into races according to their host specificity. Race 1 infects Gros Michel and Silk varieties, Race 2 infects Bluggoe, a cooking banana variety, and Sub-Tropical Race 4 (ST4) and Tropical Race (TR4) can infect a vast majority of banana varieties, including Cavendish, the variety that is resistant to Race 1 and Race 2.

The *SIX1* effector gene is known to contribute to virulence in *Fusarium oxysporum* f.sp *lycopersici* (Fol) and also has an avirulence function by activating the *I-3* resistance gene of tomato. In Foc, effectors and avirulence factors are still unknown. We found that *SIX1* has three homologs in the Foc Tropical Race 4 (TR4) genome described as *SIX1a/b/c*, of which similarity to Fol SIX1 is 86%, 84% and 82% respectively on the genomic level. At least, *SIX1b* has the highest expression *in planta* 48 hours after inoculation [1]

To find out which virulence factors are associated with the disease caused by Foc, we chose to first investigate *SIX1a* function by knocking out and complementing the gene. We chose this gene because *SIX1a* is present in all races of Foc. It is also the most similar to Fol *SIX1*, and may therefore be able to activate the *I-3* gene like Fol *SIX1.*

A *SIX1a* deletion mutant of was generated in the Foc TR4 II5 strain. The *SIX1a* gene from II5 strain was then ectopically reinserted in the FocΔ*SIX1a* strain. Disease assays in banana showed that the FocΔ*SIX1a* results in a lower average disease index in comparison to the wild type, and that the virulence of the complementation strains are restored to wild type levels. We conclude that *SIX1a* is required for full virulence of Foc TR4 towards Cavendish banana. We are also investigating whether any of the Foc*SIX1* homologs can induce *I-3* mediated resistance in tomato to reveal a potential avirulence function.

[1] Guo,L., L. Han, L. Yang., *et al*. (2014). Genome and Transcriptome Analysis of the Fungal Pathogen Fusarium oxysporum f.sp cubense Causing Banana Vascular Disease. Plos One. Vol 9 April (4).

484W Biotic and abiotic factors influence the expression of effectors in Leptosphaeria maculans during axenic growth. Michel Meyer[1], Salim Bourras[2], Julie Gervais[1], *Isabelle Fudal*[1], Marie-Hélène Balesdent[1], Thierry Rouxel[1] 1) BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; 2) Institute of Plant Biology, University of Zürich, Switzerland.

Plant pathogens secrete effector proteins into host tissues to promote infection through the manipulation of host processes. Sequencing and analyses of the genomes of fungal phytopathogens have shown that they contains tens to hundreds of genes predicted to encode putative effectors. Moreover, global analyses of gene expression revealed that several waves of concerted expression of effector genes take place during host invasion. In sharp contrast with the situation described inplanta, the expression of the effectors is difficult to detect and quantify in axenic cultures because their genes are expressed at a very low level. In the present study, we investigate biotic and abiotic factors that may relieve suppression of expression of effectors during axenic growth. Biotic factors (such as carbon source, nitrate source, antibiotics) as well as abiotic factors (pH, temperature) can influence their expression. Of major interest, incubation of the fungal mycelium with 1ug/ml of an antibiotic of the aminoglycoside family allowed an increase of effector gene expression 20-fold to 60-fold compared to regular axenic growth. An RNAseq analysis aiming at identifying the set of effectors up- and down-regulated in a culture medium supplemented or not with the antibiotic has been performed and results obtained will be presented. This simple system could be a good starting point to characterize the plant signals that trigger fungal effector gene expression.

485T Towards understanding the mechanism of cytoplasmic effector translocation during biotrophic development of *Magnaporthe oryzae*. *E. Oliveira Garcia*, B. Valent Plant Pathology, Kansas State University, Manhattan, KS , KS.

Rice blast caused by *Magnaporthe oryzae* (synonym of *Pyricularia oryzae*), a hemibiotroph and facultative pathogen, is the most destructive disease of rice worldwide. During the infection process, *M. oryzae* secretes various effectors, which are hypothesized to be involved in effective host infection. Effectors are classified by their destinations in the interaction court, with apoplastic effectors residing in the extracellular plant compartment and cytoplasmic effectors translocating into the cytoplasm of living plant cells. Notably, cytoplasmic effectors of *M. oryzae* are associated with a specialized interfacial structure, the biotrophic interfacial complex (BIC). To date, little is known about the mechanisms of effector uptake into plant cells during fungal infection. Here we show evidence for translocation of the cytoplasmic effectors Bas1, Pwl1 and Pwl2 in vesicles from BICs to rice cytoplasm during biotrophic development. Using fluorescent protein tagging, we found that cytoplasmic effectors Bas1, Pwl1 and Pwl2 are sorted into different vesicles in BICs formed on primary hyphae (PH), revealing new levels of functional complexity for this biotrophic structure. In contrast, most of the vesicles from BICs on mature bulbous hyphae showed colocalization of the cytoplasmic effectors. Whereas BICs on primary hyphae deliver effectors in micro-vesicles, BICs on mature bulbous hypha deliver effectors in macro-vesicles, at times reaching diameter sizes over 3 µm. Furthermore, we demonstrate that endocytosis inhibitors Cantharidin, Triclosan and Wortmannin induce abnormally-shaped and swollen BICs as well as the accumulation of cytoplasmic effectors in the BICs. Moreover, Cantharidin, Triclosan and Wortamannin treatment induced the accumulation of the

cytoplasmic effectors under penetration pores, suggesting that effector uptake begins even before host penetration. Based on these results, it appears that cytoplasmic effector translocation is mediated by vesicle formation and may be characteristic of appressoria as well as biotrophic invasive hyphae. Our results also suggest a potential role of *M. oryzae* effectors for manipulation of the host cell endocytosis process.

486F A highly polymorphic avirulence gene in *Zymoseptoria tritici* induces resistance in wheat. Lukas Meile, Clemence Plissonneau, Fanny Hartmann, Parvathy Krishnan, Daniel Croll, Bruce McDonald, *Andrea Sánchez-Vallet* Institut fur Integrative Biologie, ETH, Zurich, CH.

Zymoseptoria tritici is a globally distributed pathogen that colonizes wheat plants, causing devastating damages. The fungus has a peculiar infection cycle including a long asymptomatic phase followed by a necrotrophic phase that coincides with a high increase in fungal biomass. Several resistance proteins from specific host cultivars control the development of particular avirulent isolates. These resistance proteins are thought to recognize specific avirulence factors. Until now, only one avirulence gene has successfully been cloned. By means of genetic mapping, we have identified a new avirulence gene. The gene is upregulated upon infection and encodes a cysteine-rich small secreted protein, which indicates that it has a role during infection. Disruption of this gene in the avirulent isolate led to an increase in virulence. Complementation experiments showed that polymorphism in the coding sequence is responsible for the difference in virulence between the two isolates. The avirulence gene is present in all of 131 investigated isolates from across the world and exhibits high sequence polymorphism. The genomic region surrounding the gene has low conserved synteny and only in the virulent isolate two insertions rich in transposable elements are present. Remarkably, in vitro expression of a selection marker under a constitutive promoter is substantially lower when inserted at the position of the avirulence gene compared to a random position in the genome. These data indicate that this region is silenced when the pathogen is not in contact with the plant and that the transposable elements might regulate the expression of the gene. Highly controlled gene expression regulation, high sequence polymorphism and localization in a highly dynamic genomic environment highlight a major role of this avirulence gene in plant colonization.

487W Characterization of *Magnaporthe oryzae* effectors and their interacting partners in rice. *X. Yan*, D.M. Soanes, G.R. Littlejohn, M. Martin Urdiroz, L.S. Ryder, M. Oses-Ruiz, B. Tang, M.J. Kershaw, N.J. Talbot School of Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, GB.

The first line of defense against fungal pathogens by plants is the detection of pathogen-associated molecular patterns by pattern recognition receptors to trigger PAMP-triggered immunity (PTI). Pathogens that overcome PTI and successfully invade host cells do so by delivering effectors that interfere with PTI, leading to effector-triggered susceptibility (ETS). We have identified a family of effector-encoding genes in the rice blast fungus *Magnaporthe oryzae*. We first using RNA-seq to identify genes that were differentially expressed during plant infection and which were temporally co-regulated during the early stages of tissue colonisation by the fungus. The putative effector genes were then characterized by targeted gene deletion and their protein products localized using live-cell imaging. We found a group of apoplastic effectors that outlined invasive hyphae while cytoplasmic effectors accumulated at the biotrophic interfacial complex (BIC). We then identified potential effector targets in rice using yeast two hybrid analysis and co-immunoprecipitation in infected plant tissue. Putative protein-protein interactions were independently confirmed using Bimolecular Fluorescence Complementation (BiFC) assays. In this way, we have characterized a set of 32 *MEP (Magnaporthe effector protein)* genes. We will report progress on their characterization and, specifically, the role of *MEP1* and *MEP3* in fungal virulence and host immune suppression, respectively.

488T The *Sporisorium reilianum* effector Sad1 targets the maize RGLG2-like protein to suppress apical dominance in maize ears. *N. Agrawal*, F. Drechsler, M. Romich, J. Schirawski RWTH Aachen University, Applied Microbiology, Aachen, North Rhine Westphalia, DE.

When the biotrophic smut fungus *Sporisorium reilianum* infects maize, it suppresses apical dominance of maize ears. The secreted fungal effector protein Sad1 is responsible for this phenotype but its mechanism of action is unknown. When expressed in *Arabidopsis thaliana*, GFP-Sad1 localizes to the cytoplasm and the nucleus and leads to increased inflorescence branching. From a yeast two-hybrid screen, the maize E3 ubiquitin ligase RGLG2 was identified as one of the strongest interaction partners of Sad1. We show by BiFC that Sad1 interacts with RGLG2 at the plasma membrane in *Nicotiana benthamiana*. RGLG2 has a proposed myristoylation site at its N-terminus. Accordingly, interaction of RGLG2 with Sad1 in yeast two-hybrid experiments was only seen, when the C-terminal end of RGLG2 was used. RGLG2-GFP localized to the plasma membrane when expressed in *Nicotiana benthamiana*, but not when the N-terminal Glycine was mutated to Alanine. In *A. thaliana*, RGLG2 is known to move from the plasma membrane to the nucleus upon stress, and there to function in dampening the stress response. We hypothesize that interaction of Sad1 with RGLG2 interferes with RGLG2 function or transport into the nucleus, which would lead to a prolonged stress response. A prolonged stress response has been suggested before to cause suppression of apical dominance. Hence, Sad1 might lead to suppression of apical dominance by inhibiting the stress response suppression function of RGLG2.

489F Functional characterization of CRN-like effector proteins in the arbuscular mycorrhiza symbiosis. *S. Walter*[1], R. Betz[1], N. Corradi[2], N. Requena[1] 1) Karlsruhe Institute of Technology, Karlsruhe, DE; 2) University of Ottawa, Canada.

The arbuscular mycorrhiza symbiosis (AMS) between fungi of the phylum Glomeromycota and more than 80% of all living land plants is characterized by the formation of tree-like fungal structures, arbuscules, within cortical root cells, that function as the interface for the bidirectional nutrient exchange. Prior to this, a highly complex communication is necessary in order to establish the interaction. In plant-pathosystems, effector proteins are secreted during colonization that lead to modifications in the plant cell program, essentially to facilitate the infection process. Evidence is now accumulating that mutualistic fungi also use effector proteins when colonizing their host plants. Recent success in sequencing the genome of *Rhizophagus irregularis* revealed a new effector protein family, which resembles the modular structure of a protein family called Crinkler (CRN). CRNs are present in all pathogenic oomycetes and in some chytridiomycetes. Many contain a signal peptide for secretion and share a highly conserved N-terminal region, with a characteristic LFLAK domain for translocation, and a divergent C-terminal region to execute effector activity. Expression analyses of *R. irregularis* CRN-like genes showed two of them being highly expressed in mycorrhizal roots, suggesting their involvement in the symbiosis. To functionally characterize them, expression of

RiCRN1 and *RiCRN2* in *planta* was carried out. Unlike many oomycete CRNs neither RiCRN1 nor RiCRN2 induced cell death in *Nicotiana benthamiana* but both led to a strong phenotype in transgenic *Arabidopsis thaliana* that were smaller and displayed anthocyanin accumulation. Fusions with GFP and transient expression in *N. benthamiana* leaves showed nucleo-cytoplasmic localization for RiCRN1 and RiCRN2. Most interestingly preliminary experiments to downregulate these CRN-like proteins during symbiosis in *M. truncatula* using host induced gene silencing showed a severe impairment of the symbiosis. We therefore postulate that these CRN-like proteins work to promote symbiosis. Experiments are in progress to identify the *in planta* targets responsible for such a positive role.

490W Determining the mechanism of action of the Histoplasma capsulatum virulence factor Cbp1. *D.R. Azimova*, B.C. English, A. Sil Microbiology and Immunology, UCSF, San Francisco, CA.

Intracellular pathogens have developed diverse strategies to evade detection by the immune system and spread effectively through the host. *Histoplasma capsulatum* (*Hc*), an intracellular pathogen of macrophages, is a thermally dimorphic fungus endemic to the Ohio and Mississippi river valleys. The yeast form invades alveolar macrophages and resides in a modified phagolysosome that escapes acidification. In culture, one of most abundant yeast phase-specific proteins that *Hc* secretes is Calcium Binding Protein 1 (Cbp1), a critical virulence factor required for apoptosis of macrophages during intracellular infection. Cbp1 is a 78 amino acid protein with no known calcium-binding domains and no known molecular function. Previous work from our lab showed that Cbp1 is required for transcriptional induction of pro-apoptotic factors tribbles homolog 3 (TRB3) and C-EBP homologous protein (CHOP) during *Hc* infection, as well as activation of apoptotic executioner caspases 3 and 7 and lysis of host cells. We show here that Cbp1 localizes to the cytosol during infection of primary murine macrophages using fractionation. This key breakthrough shows, for the first time, that a fungal effector of an intracellular pathogen of humans enters the cytosol during infection. We are currently utilizing a library of Cbp1 mutants to determine the amino acid residues in Cbp1 that are necessary for cytosolic localization. Additionally, to characterize the molecular mechanism of Cbp1 action, we are performing affinity purification of a Strep-tagged allele followed by mass spectrometry (AP-MS). These data will shed light on host-binding partners of Cbp1 that allow it to modulate macrophage biology.

491T *Verticillium nonalfalfae* effectors: from –omics approaches to individual function. *S. Berne*, H. Volk, K. Marton, M. Flajšman, V. Progar, B. Javornik Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

Plant pathogens express an assortment of effector molecules to modulate plant biological processes and aid infection of specific plant species. Studying effector biology is therefore central to understanding plant-pathogen interactions, including molecular mechanisms of pathogenicity and plant defence responses. For almost a decade, we have been investigating Verticillium wilt of hops, predominantly caused by hemibiotrophic sordariomycete *Verticillium nonalfalfae*, using genome sequencing, RNA-Seq and proteomics approaches. Here, we present identification of a repertoire of *V. nonalfalfae* effectors and their subsequent characterization. The *V. nonalfalfae* secretome, comprising 962 putatively secreted proteins, was predicted with an in-house generated pipeline. After a filtering step, 330 candidate secreted effector proteins (CSEPs) with effector-specific domains or without known Pfam domains were obtained and ranked according to various selection criteria (e.g. expression *in planta*). For the 48 best ranked CSEPs, spatio-temporal expression profiling was performed with RT-qPCR in root and shoot samples from infected and control plants, for both susceptible and resistant hop cultivars. Pathogenicity assays in susceptible hop using ATMT-generated effector knock-out mutants revealed 4 CSEPs with reduced virulence and 1 with increased virulence. Two effector proteins *Vna*SSP4.2 and *Vna*CBP8.213 were selected for functional characterization in biochemical assays. *Vna*SSP4.2, a *Verticillium*-specific small basic protein, was purified from *E. coli* BL21(DE3)pLysS. It bound to multilamellar lipid vesicles in sedimentation assays and formed SDS- and DTT-resistant dimers in POPC:PG, POPC:PE, POPC:SM and POPC:PS vesicles. *Vna*CBP8.213, a carbohydrate binding protein with six hevein domains, was purified from inclusion bodies in *E. coli* SHuffle cells and will be tested for chitin binding, induction of ROS and HR in host and model plants. Unravelling an effector's mode of action may reveal mechanisms essential for *V. nonalfalfae* virulence and provide candidate host proteins that could be exploited in engineering resistance.

492F A *Phytophthora capsici* virulent RXLR effector targets plant PP2a isoforms that confer *Phytophthora* blight resistance. *X. Chen*[1,2], G. Sheng[1], Y. Li[1], Y. Xing[1], Y. Zhai[2], W. Ma[2] 1) College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, Jiangsu Province, China; 2) Department of Plant Pathology and Microbiology, University of California-Riverside, Riverside, California, USA.

Plant pathogens deliver an array of effectors to alter host physiology and defense responses. To understand the molecular basis underlying these, it is important to identify and characterize the target proteins of effectors in plants. Here we show that the RXLR effector PcAvh1 of *Phytophthora capsici* is highly conserved across *Phytophthora* genus. The encoding gene *PcAvh1* is upregulated during the plant infection stages versus barely expressed during the developmental stages. The effector is important for the pathogen virulence on pepper and *Nicotiana benthamiana* via activity in the host nucleus and cytoplasm and of RNA silencing suppression. PcAvh1 interacts with two host protein phosphatase 2a (PP2a) structural isoforms. Silencing of the *PP2a* isoforms facilitates pathogen infection. Furthermore, silencing of the isoforms together severely compromises the growth of plants. These results demonstrate that host PP2a activity is required for the plant resistance against the pathogen. Taken together, we conclude that PcAvh1 is an important virulence determinant that targets host PP2a isoforms to attenuate plant resistance.

493W Presence of race-specific resistance against oat crown rust in *Brachypodium* species. *M. Figueroa*[1], V. Omidvar[1], S.E. Rottschaefer[1], F. Li[1], D. Singh[2], R.F. Park[2], M. Ayliffe[3], M. Moscou[4], P.N. Dodds[1,3], S.F. Kianian[1,5] 1) Plant Pathology, University of Minnesota, St.Paul, MN; 2) University of Sydney, Cobbitty, NSW, Australia; 3) CSIRO Agriculture and Food, ACT, Australia; 4) The Sainsbury Laboratory, Norwich NR4 7UH, UK; 5) USDA-ARS, Cereal Disease Laboratory, St. Paul; MN, USA.

Puccinia coronata f. sp. *avenae* (Pca), the causal agent of crown rust, represents a serious worldwide agronomic problem in the production of oat (*Avena sativa*). Genetic resistance to control oat crown rust is rather limited; therefore the identification of new resources to reduce yield losses associated with Pca outbreaks is a priority. *Brachypodium* species such as *B. distachyon* and *B. hybridum* show non-host resistance (NHR) against Pca. A collection of *Brachypodium* accessions of these two species was examined to determine the genetic variation for resistance against various North American and Australian Pca pathotypes. Using phenotypic and fungal colonization assays we identified several *Brachypodium* accessions that are ideal for dissecting the genetic architecture controlling NHR against Pca. Our analyses indicate the presence of race specific resistance in both species, highlighting the contribution of effector-triggered immunity to NHR that is likely due to the short evolutionary divergence between oat and *Brachypodium*. Pca isolates with broad virulence in oat showed higher

colonization levels in *Brachypodium* accessions than a Pca isolate with restricted virulence in oat. Transcriptional profiling of several defense-related genes in two *B. distachyon* accessions in response to three Pca isolates during early stages of infection was also conducted. Overall, genes involved in salicylic acid and ethylene signaling pathways were upregulated during the first 48 h after infection. Interestingly, a hexose transporter gene, *BdSTP13*, was also upregulated, which is deemed to be a putative susceptibility factor to wheat rusts. The amplitude of transcriptional upregulation of these genes correlated with rust colonization levels. A study of the genetic basis of resistance to Pca has been initiated in order to discover the factors governing recognition of Pca in *B. distachyon* and to provide foundational knowledge to direct genetic engineering programs in oat improvement.

494T The discovery of the virulence gene *ToxA* in the wheat and barley pathogen *Bipolaris sorokiniana*. *Megan C. McDonald*[1], Dag Ahren[2], Steven Simpfendorfer[3], Andrew Milgate[4], Peter S. Solomon[1] 1) Plant Science, The Australian National University, Canberra, ACT, Australia; 2) Department of Biology, Lund University, Lund, Sweden; 3) NSW Department of Primary Industries Tamworth Agricultural Institute, Tamworth, NSW, Australia; 4) NSW Department of Primary Industries Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia.

Bipolaris sorokiniana is the causal agent of multiple diseases on wheat and barley and is the primary constraint to cereal production throughout South Asia. Despite its significance, the molecular basis of disease is poorly understood. To address this, the genomes of three Australian isolates of *B. sorokiniana* were sequenced and screened for known pathogenicity genes.

Sequence analysis revealed that the isolate BRIP10943 harboured the *ToxA* gene which was previously associated with disease in the wheat pathogens *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*. The proteinaceous necrotrophic effector, ToxA, was first identified in culture filtrates of the fungal wheat pathogen *Pyrenophora tritici-repentis* where the culture filtrate alone caused necrotic lesions on susceptible wheat cultivars, in a genotype-specific manner. Later, a near identical 11kb region containing *ToxA* was found in the genome sequence of the wheat pathogen *Parastagonospora nodorum.* Susceptibility of wheat to ToxA was eventually linked to the presence of a host immune-like gene, *Tsn1.*

Analysis of the regions flanking *ToxA* within *B. sorokiniana* revealed that it was embedded within a 12kb genomic element nearly identical to the corresponding regions in *P. nodorum* and *P. tritici-repentis*. A screen of 35 Australian *B. sorokiniana* isolates confirmed that *ToxA* was present in 12 isolates. Sequencing of the *ToxA* genes within these isolates revealed two sequence haplotypes, which differ by a single non-synonymous nucleotide substitution. Pathogenicity assays show a *B. sorokiniana* isolate harbouring *ToxA* is more aggressive on wheat lines that contain the sensitivity gene when compared to a non-*ToxA* isolate. Based on available data, we speculate on the evolutionary origins of this gene in these three fungal wheat pathogens. We propose that *ToxA* and its surrounding 12kb is a highly promiscuous genomic element, that has the ability to cross species barriers to facilitate disease on wheat. Acquisition of this element can dramatically increase the fitness of pathogenic strains, which in an agricultural setting can have devastating economic and social impacts.

495F Structural and functional characterization of *Leptosphaeria maculans* effectors: the example of AvrLm4-7. *Yohann Petit-Houdenot*[1], Françoise Blaise[1], Clémence Plissonneau[1], Thierry Rouxel[1], Marie-Hélène Balesdent[1], Karine Blondeau[2], Noureddine Lazar[2], Inès Gallay[2], Théo Le Moigne[2], Herman van Tilbeurgh[2], Isabelle Fudal[1] 1) INRA/AgroParisTech UMR1290 BIOGER, French National Institute for Agronomical Research, Thiverval-Grignon, FR; 2) UMR I2BC Université Paris-Sud / CNRS / CEA, Orsay, France.

During plant infection, pathogens secrete an arsenal of effectors, key elements of pathogenesis which modulate innate immunity of the plant and facilitate infection. Fungal effector genes typically encode small proteins, predicted to be secreted, with no homology in databases, and absence of known motif. As such their function or role in pathogenesis is mostly unknown. The phytopathogenic ascomycete *Leptosphaeria maculans* is the causal agent of stem canker of oilseed rape. More than 650 putative effector-encoding genes have been identified in its genome, 7 of them corresponding to avirulence proteins. We develop a project aiming at elucidating the involvement of *L. maculans* effectors in pathogenicity through the structural and functional characterization of a few major effector proteins and the determination of their interactants.

Our strategy is illustrated here with AvrLm4-7, a 143 amino-acid long secreted protein important for fungal fitness and recognized by two oilseed rape resistance proteins, Rlm4 and Rlm7. One single amino-acid change is sufficient to lose recognition by *Rlm4* while maintaining recognition by *Rlm7*. 3D-structure of an isoform of AvrLm4-7 only recognized by Rlm7 was previously determined, allowing us to define regions implicated in recognition by Rlm7 and translocation into plant cell. We recently determined the 3D-structure of another isoform of AvrLm4-7 recognized both by Rlm4 and Rlm7, showing that the amino-acid change allowing to escape *Rlm4*-recognition was located on an external loop and did not change the overall structure of the protein. AvrLm4-7 was also recently shown to suppress recognition of another *L. maculans* avirulence gene, *AvrLm3*, by its cognate resistance gene *Rlm3*, leading us to hypothesize a suppression of Effector-Triggered Immunity (ETI) by AvrLm4-7. In order to test that hypothesis, we transiently expressed AvrLm4-7 and several cell-death inducers in *Nicotiana benthamiana* epidermal cells: AvrLm4-7 was able to suppress cell death induced by BAX and AvrPto. We also generated transgenic lines of *Arabidopsis thaliana* constitutively expressing AvrLm4-7 and are currently characterizing the lines for their susceptibility to pathogens with contrasted lifestyles and for their ability to suppress recognition of *Pseudomonas syringae* avirulence proteins. A better understanding of the role of an effector implicated in the masking of another effector will allow us to develop alternative strategies to genetically control stem canker disease.

496W The mode of action and origin of RNase-like effectors in powdery mildew fungi. *Pietro Spanu*[1], Linhan Li[1], Michal Przydacz[1], Summer Zhang[1], Helen Pennington[2], Laura Davis[3], Ian Dry[3] 1) Department of Life Sciences, Imperial College London, London, United Kingdom; 2) The Sainsbury Laboratory, Norwich, United Kingdom; 3) CSIRO Plant Industry, Australia,.

The fungi causing powdery mildews on cereals and other plants possess extraordinarily large repertoires of proteins with typical features of pathogenicity effectors. The most prominent of these are a large superfamily of proteins that resemble canonical RNases: the **R**N**A**se-**L**ike **P**roteins associated with **H**austoria: the "RALPH" effectors. Here, we provide evidence that RALPH effectors interfere with host immune responses that target ribosomal RNA. RALPH effectors are highly expressed in the plant host and are subject to high rates of selective pressure to diversify; this suggests that plants adopt mechanisms for recognising RALPH effectors which, in turn, drives effector

diversification. In the last year, several seminal publications have demonstrated that, as predicted, cereal immune receptors encoded by resistance (R) genes recognise RALPH effectors. RALPH effectors are present in all powdery mildew fungi analysed so far. We report here that RALPH effectors appear to have evolved from an ancestral *ur*-RALPH. The *ur*-RALPH effector was likely to have been crucial to the early evolution of powdery mildew fungi in the Cretaceous at least 70 MYr ago.

497T The role of *Phytophthora infestans* effector *Avr1-like* in modulating plant defense. *R.R. Vetukuri*[1], P. B. Kalyandurg[2], P Boevink[3], S. C. Whisson[3], E. I. Savenkov[2], L Grenville-Briggs[1] 1) Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, SE; 2) Department of Plant Biology, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden; 3) Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK.

Phytophthora infestans secretes many RXLR effectors potentially involved in infection of host plants. *Avr1-like* is one such RXLR effector that acts as a virulence factor, promoting colonization. Avr1-like is closely related to Avr1 but does not trigger R1-mediated resistance. Unlike Avr1, Avr1-like is present in all the modern European isolates tested in our study. High numbers of transcripts encoding *Avr1-like* were present throughout infection stages, suggesting that this gene is specifically induced in contact with host tissue to modulate plant defences, as seen with other known avirulence effectors such as *Avr3a* and *Avr2*. In this study we demonstrate that *Avr1-like* acts as a suppressor of RNA silencing. Using mutagenesis analysis, we found that a motif in Avr1-like that plays a major role in its suppression activity. Subcellular localization of AVR1 in *Nicotiana benthamiana* by transient expression revealed that this RXLR effector was localized to both the nucleus and the cytoplasm.

498F *In planta* localisation of *Leptosphaeria maculans* effectors and identification of their plant targets. *C. Marais*[1], Y. Petit[1], F. Blaise[1], B. Ollivier[1], J. Gervais[1], T. Le Moigne[2], T. Rouxel[1], M.H. Balesdent[1], I. Gallay[2], K. Blondeau[2], N. Lazar[2], H. van TilBeurgh[2], I. Fudal[1] 1) BIOGER, INRA, Thiverval-Grignon, FR; 2) UMR I2BC, Université Paris-Sud, CNRS, CEA, Orsay, FR.

Fungal effector genes are very diverse and typically encode small proteins, predicted to be secreted, with no or low homology in databases, and absence of known motif. As such their function or role in pathogenesis is mostly unknown. On these bases, the StructuraLEP project aims at elucidating the involvement of *L. maculans* effectors in pathogenicity through the structural and functional characterization of a few major effector proteins and the determination of their interactants. We are investigating six *L. maculans* effectors chosen for their biological significance (involvement in fungal fitness, cognate R gene identified) or because they may represent novel modes of interaction with their plant target (two AVR genes have to be recognized by a specific R gene). We present here the strategies developed within the project to answer two questions: (i) “Where do *L. maculans* effectors act during plant infection?” and (ii) “Which proteins interact with *L. maculans* effectors?”. In order to localise *L. maculans* effectors into plant cells and to identify their plant targets, we will transiently express effectors with a fluorescent tag into tobacco leaf epidermal cells and observe effector localisation by confocal microscopy. Tobacco leaves expressing effectors will be used to perform pull-down assays and tobacco proteins interacting with effectors will be identified through mass spectrometry. A more exploratory but biologically relevant strategy will also be tested. *L. maculans* transformants stably expressing effectors with HA-tag will be used to infect oilseed rape leaves. Localisation of effectors in oilseed rape leaves will be performed by immunocytolocalisation using antibodies against the HA-tag. The infected oilseed rape leaves will also be used to perform pull-down assays in order to identify plant proteins targeted by *L. maculans* effectors.

499W Characterization of effector candidates from the soybean rust fungus that suppress plant immunity. *M. Qi*[1], T Link[2], R Voegele[2], T Baum[1], S Whitham[1] 1) Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA; 2) Institut für Phytomedizin, Universität Hohenheim, Otto-Sander-Straße 5 70599 Stuttgart, Germany.

Soybean rust (SBR) is an important disease caused by *Phakopsora pachyrhizi* (*Pp*). Like other rust fungi, *Pp* forms specialized cells called haustoria, which are key hubs of molecular exchange with host cells. Haustoria express effector proteins that are transferred to plant cells where they promote fungal growth. We have recently sequenced the *Pp* haustorial transcriptome and predicted candidate effector proteins. To gain insights into functions of *Pp* effector candidates (*Pp*ECs), we have cloned 82 *Pp*EC coding sequences and delivered the proteins into host and non-host plants using a bacterial type III secretion system-based delivery vector. Several *Pp*ECs suppressed immune responses associated with effector- and pattern-triggered immunity, and we are in the process of characterizing these further. One interesting example is *Pp*EC23, a small, cysteine-rich effector candidate that attenuated the hypersensitive response caused by *Pseudomonas syringae* pv *tomato* DC3000 on soybean and tobacco, and it also suppressed basal defense on tobacco and Arabidopsis. *Pp*EC23 interacts with a transcription factor that our data suggests negatively regulates soybean defenses. We are now using host-induced gene silencing and transcriptomics to determine the effects of *Pp*EC23 and the interacting soybean transcription factor in immunity or virulence. The results are expected to provide mechanistic insights into the molecular basis of SBR and may be relevant to other rust diseases.

500T The poplar rust fungus effector biology: challenges of functional characterization of effectors in a non-model pathosystem. *C. Lorrain*[1], Benjamin Petre[1], Diane Saunders[2], Jan Sklenar[3], Joe Win[3], Sophien Kamoun[3], Christine Delaruelle[1], Jérémy Petroswky[1], Pascal Frey[1], Arnaud Hecker[1], Sébastien Duplessis[1] 1) UMR 1136 IAM, Centre INRA Nancy-Lorraine, Champenoux, Lorraine, FR; 2) Earlham Institute, Norwich Research Park, Norwich, United Kingdom; 3) The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.

Rust fungi are devastating biotrophic pathogens manipulating host processes by delivering effector proteins into the plant cells. The poplar leaf rust fungus *Melampsora larici-populina* genome analysis revealed a large set of secreted proteins that some have been considered as candidate effectors. The understanding how these effector proteins function in the host cells has been the key question of effector biology for the last decade. Many efforts have been made in the field plant-microbe molecular interactions to unravel their role in the colonization of plant tissues. The poplar-poplar rust pathosystem although considered, as a genomic model in the study of tree-microbe interactions is actually non-model pathosystem when it comes to functional characterization of effectors. Absence of easy-going transformation systems for poplar and rust fungi is a major drawback. However, we combined several tools and approaches to help at elucidate the roles of *M. larici-populina* effector proteins such as heterologous *in planta* expression, recombinant protein production or structural approach. All these diverse approaches have led to partially unravelling the role of numerous *M. larici-populina* effector

candidates. Summary report of the ongoing research aimed at elucidating candidate effectors functions will be presented, rising questions about the methodological limits of the study of effector biology in non-model pathosystems.

501F An insight into necrotrophic effector gene regulation and host-specific virulence of two major Pleosporales fungal pathogens of wheat. Kasia Rybak, Huyen Phan, Pao Theen See, Robert Syme, Caroline Moffat, Richard Oliver, *Kar-Chun Tan* Centre for Crop Disease Management, Department of Environment and Agriculture, Curtin University, Bentley, WA 6102, Australia.

The fungus *Parastagonospora nodorum* is the causal agent of septoria nodorum blotch (SNB) of wheat (*Triticum aestivum*). The pathosystem is mediated by multiple fungal necrotrophic effector-dominant host sensitivity gene interactions. The three best characterised effector-sensitivity gene systems are SnToxA-*Tsn1*, SnTox1-*Snn1* and SnTox3-*Snn3*. These necrotrophic effector genes are highly expressed during early infection. However, it is not known how these necrotrophic effector genes are regulated in *P. nodorum* until recently. We identified and characterised a gene, referred to as *PnPf2*, which encodes a putative zinc finger transcription factor. *PnPf2* is expressed maximally during early infection like *SnToxA*, *SnTox1* and *SnTox3*. *PnPf2* deletion resulted in down-regulation of *SnToxA* and *SnTox3* expression. Virulence on *Tsn1* and *Snn3* wheat was highly reduced. *SnTox1* expression and virulence on *Snn1* wheat remained unaffected in the deletion background. We also determined that SnTox1-*Snn1* is epistatic to the SnTox3-*Snn3* interaction during SNB. The mechanism of epistasis can be explained - in part – by the suppression of *SnTox3* expression on the presence of SnTox1. However, *SnTox1* deletion in the *pnpf2* background did not restore *SnTox3* expression. This indicates that *PnPf2* is dominant over SnTox1-mediated epistasis of *SnTox3*. We have also identified and deleted an orthologous *PtrPf2* from the tan spot fungus *Pyrenophora tritici-repentis* which possesses a near-identical ToxA that was acquired from *P. nodorum* via horizontal gene transfer. *PtrPf2* deletion also resulted in the down-regulation of *PtrToxA* expression and a near-complete loss of virulence on wheat. We have demonstrated, for the first time, evidence of a functionally conserved signalling component that plays a role in the regulation of common/horizontally transferred effector found in two major pathogens of wheat.

502W Functional analysis of *Epichloë festucae* small secreted proteins in the symbiotic interaction with *Lolium perenne*. *B. Hassing*[1], C. H. Mesarich[2], C. J. Eaton[1], B. Scott[1] 1) Institute of Fundamental Science, Massey University, Palmerston North, Manawatu, NZ; 2) Institute of Agriculture and Environment, Massey University, Palmerston North, Manawatu, NZ.

Epichloë endophytes form stable and mainly mutually beneficial associations with a wide range of cool-season grasses. In a transcriptome analysis, 14 genes encoding small secreted proteins (SspB–SspO) were identified as differentially expressed in four different symbiosis-deficient strains of *E. festucae* during *in planta* colonization of *Lolium perenne*[1]. Many Ssps, also called effectors, have been shown to interfere with the host defence response in pathogenic and symbiotic interactions. All 14 putative effectors were analysed with bioinformatic tools, and from these, four, specifically SspL, SspM, SspN and SspO, were chosen for further functional analysis. Their secretion was verified using a secretion signal-trap assay and Western analysis. The deletion of *sspL*, *sspM*, *sspN* and *sspO* did not result in an obvious whole-plant interaction phenotype distinguishing WT- and deletion mutant-infected plants, except for a slight increase in anthocyanin levels in the *sspO* deletion strain infected plants. Furthermore, the analysis of infected plant tissue with confocal microscopy and TEM did not identify any obvious differences in cellular phenotype. Currently the effect of Ssp overexpression on whole-plant interaction and cellular phenotypes is being explored. In addition, Ssp localization studies are being conducted to gain further information about the function of these proteins *in planta*. In the future the effect of the Ssp on *L. perenne* gene expression will be analysed and *in planta* interaction partners will be identified.

[1]Eaton C. J., Dupont P. Y., Solomon P., Clayton W., Scott B., Cox M. P. (2015). A Core Gene Set Describes the Molecular Basis of Mutualism and Antagonism in *Epichloë spp.*. Mol Plant Microbe Interact. 28:69-85.

503T An antimicrobial and phytotoxic ribonuclease secreted by the wheat pathogen *Zymoseptoria tritici*. *G.J. Kettles*, C. Bayon, C.A. Sparks, K. Kanyuka, J.J. Rudd Plant Biology & Crop Science, Rothamsted Research, Harpenden, Hertfordshire, UK.

The dothidiomycete fungus *Zymoseptoria tritici* (*Z. tritici*) is amongst the most prevalent and damaging pathogens of wheat throughout the world. During early (symptomless) colonisation of wheat leaves, there is minimal activation of the host immune system. Later, there is a switch to necrotrophy, accompanied by host defence gene induction and extensive cell death in infected tissues. We recently identified a secreted ribonuclease (Zt6) as a candidate effector protein based on transcriptomic analysis of *Z. tritici* infection of susceptible wheat. Zt6 displays an unusual double-peak expression pattern during wheat infection, with maximal expression both shortly after spore germination on the leaf surface (1 dpi) and after the transition to necrotrophy (14 dpi). Expression at transition (9 dpi) was comparatively low, in contrast to the majority of putative secreted protein effectors identified to-date.

Agrobacterium-mediated transient expression of Zt6 in the non-host model plant *Nicotiana benthamiana* revealed this effector protein to be a highly potent inducer of cell death. Both full-length protein and its mature form (lacking signal peptide) were equally potent in cell death induction. Particle co-bombardment of GFP- and Zt6-expressing constructs into wheat leaves resulted in no GFP expression, indicating Zt6 phytotoxicity against the natural host. Further co-bombardment experiments utilising a 22-amino acid N-terminal loop deletion (?1-22) and catalytic mutation (H52A) revealed both regions of Zt6 to be essential for full functionality and suggested a role for the N-terminal loop in Zt6 translocation into the host cytoplasm. Interestingly, recombinant Zt6 protein showed a ribotoxin-like activity by cleaving native rRNA semi-specifically, with production of distinct cleavage fragments. Finally, microbial toxicity assays revealed Zt6 to be highly toxic to both bacteria and yeast but not to more complex filamentous fungi, including *Z. tritici* itself. Together, our data reveal a new multi-functional cytoplasmic ribotoxin-like effector that is toxic to both host and non-host plants, and also to microbial competitors.

504F Functional characterization of a protein complex formed by four *Ustilago maydis* effectors essential for virulence. *N. Ludwig*[1], L. Liang[1], K. Schipper[1,2], S. Reißmann[1], D. Aßmann[1], T. Glatter[1], J. Altmüller[3], R. Kahmann[1] 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Heinrich Heine University Düsseldorf, Düsseldorf, Germany; 3) Cologne Center for Genomics (CCG), Cologne University, Cologne, Germany.

The fungus *U. maydis* causes smut disease in maize. *U.maydis* is a biotrophic pathogen requiring living plant tissue for colonization. For a successful infection *U.maydis* needs to suppress plant defense responses and manipulate host physiology for its own benefit. To

accomplish this, *U.maydis* secretes a cocktail of about 300 effector proteins. The majority of these proteins lack a functional annotation and their function remains to be uncovered. Our current work focuses on effectors expressed early during infection. Systematic deletion of the most highly expressed effector genes in this class resulted in the discovery of three mutants unable to cause disease. Their mutant phenotype resembled previously identified *stp1* (stop after penetration) mutants, and the newly identified genes were designated *stp2*, *stp3* and *stp4*. A similar phenotype was also observed for mutants lacking the essential effector *pep1* (Döhlemann *et al*.,2009). *stp1-4* deletion strains were able to form appressoria that penetrate maize epidermal cells, but their growth was arrested in epidermal tissue. Their growth arrest was accompanied with the elicitation of plant defense responses and plant cell death. Co-IP with individually tagged effectors followed by mass-spectroscopic analysis revealed that Stp1, Stp3, Stp4 and Pep1 form a complex, while Stp2 is not part of the complex. We will discuss our current efforts to localize the complex and to functionally characterize its components as well as Stp2. Furthermore, we are testing possible theories for the existence of the complex, such as a function in translocation or the shielding of Avr proteins.

505W Identification of effector candidates for avirulence genes in the wheat stripe rust fungus (*Puccinia striiformis* f. sp. *tritici*) by secretome analysis. C. Xia[1], M. Wang[1], O. Cornejo[2], D. Jiwan[1], D. See[1], *X Chen*[1,3] 1) Department of Plant Pathology, Washington State University, Pullman, WA; 2) School of Biological Sciences, Washington State University, Pullman, WA; 3) USDA, ARS, Pullman, WA.

Puccinia striiformis f. sp. *tritici* (*Pst*) causes stripe rust (yellow rust, *Yr*), one of the most important diseases of wheat. Planting resistant cultivars is effective to control this disease, but resistance can be overcome by new virulence. Races of *Pst* have been characterized by testing isolates on a set of wheat lines with different *Yr* genes, but no avirulence genes have been genetically identified. To identify avirulence genes, we selected 7 *Pst* isolates for re-sequencing and characterized them, together with 7 previously sequenced isolates, for their avirulent/virulent profiles on the 18 *Yr* single-gene differential lines. The 14 isolates had a 1:1 ratio for avirulence and virulence to 12 of the *Yr* genes. From the *Pst* sequences, we characterized 2,146 secreted protein (SP) genes and identified 48,059 high quality variants including 41,764 SNPs and 6,295 Indels in the SP genes. Secretome comparison with other rust fungi identified 923 *Pst*-specific SPs. Effector candidates were identified by characterizing *Pst* SPs based on cysteine-rich, EffectorP predicted, haustorial differentially expressed, *Pst*-specific, and polymorphic among the isolates. Of the 2,091 SPs that fit at least one criterion, 32 fitting all criteria could be considered as effector candidates. We also identified effector candidates significantly associated to avirulence genes corresponding to five *Yr* genes by correlating their polymorphisms to avirulence/virulence profiles. One effector candidate for *AvYr76* was present in all avirulent isolates and absent in all virulent isolates, suggesting that deletion of the gene is a mechanism responsible for changing from avirulence to virulence.

506T *Leptosphaeria maculans* effectors involved in the oilseed rape systemic colonization. *J. Gervais*[1], C. Plissonneau[1], J. Linglin[1], M. Meyer[1], K. Labadie[2], C. Cruaud[2], B. Ollivier[1], I. Fudal[1], T. Rouxel[1], M-H. Balesdent[1] 1) INRA, UMR 1290 BIOGER INRA-AgroParisTech, 78850 Thiverval-Grignon, France; 2) CEA-Institut de Génomique, GENOSCOPE, Centre National de Séquençage, Evry Cedex, France.

The stem canker disease, caused by *Leptosphaeria maculans*, is one of the most devastating diseases of oilseed rape (canola). It colonizes the plant in two stages: a short and early colonisation stage corresponding to cotyledon or leaf colonisation, and a late colonisation stage during which the fungus colonises systemically and symptomlessly the plant during several months before stem canker appears. To date, determinants of the late colonisation stage remain poorly understood.

By a transcriptomic approach, we previously identified two waves of effector candidate expression during the early and late colonisation stages (Gervais et al, 2016). The late effector candidates are located in gene-rich genomic regions, whereas the early effector genes are located in gene-poor regions of the genome.

Among the late effector candidates identified, we selected 6 genes for further characterization. We created mutants silenced for these effector candidates. For one of these genes, its expression level correlated negatively with the size of the necrosis observed in the stem.

The identification of new effector genes would contribute to the identification of new resistance genes specific to these effectors. To easily identify matching resistance genes in oilseed rape, we created transgenic isolates expressing these 6 late effectors at the early steps of infection to provide medium-throughput strategies to screen more efficiently different cultivars. Preliminary results indicate that some cultivars with adult resistance were more resistant to these transgenic isolates in cotyledon assays. With this approach, we also identified a cultivar carrying a specific resistance to one these 6 effector candidates.

Reference

Gervais, J., Plissonneau, C., Linglin, J., Meyer, M., Labadie, K., Cruaud, C., Fudal, I., Rouxel, T. and Balesdent, M.H. (2016) Different waves of effector genes with contrasted genomic location are expressed by *Leptosphaeria maculans* during cotyledon and stem colonization of oilseed rape. *Mol. Plant Pathol.*

507F Delivery of Phytophthora sojae effector Avr1b in planta requires PI3P-binding, but does not require N-terminal cleavage. *Brett M. Tyler*[1], Qunqing Wang[1], Biao Gu[2], Felipe Arredondo[1], Eli Perez[1], Shiv D. Kale[3] 1) Center for Genome Research and Biocomputing, Oregon State University, ALS3021, Campus Way, OR; 2) College of Plant Protection, College of Plant Protection, Northwest A&F University, Yangling, 712100, China ; 3) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA24061, USA.

A major class of effectors produced by oomycetes contains RxLR motifs that mediate entry of these effectors into plant cells. We previously showed that these effectors can bind to specific lipids including phosphatidylinositol-3-phosphate (PI3P). PI3P-binding requires the RxLR motif, plus in some cases, C-terminal regions of the protein. In order to validate that PI3P-binding mediates host cell entry in planta, we have shown that heterologous PI3P-binding proteins such as yeast VAM7p can functionally replace the RxLR domain of *Phytophthora sojae* effector Avr1b, and can deliver this effector into soybean cells during a natural *P. sojae* infection. The Avr1b and various derivative mutant proteins can be specifically detected in culture supernatants after de-glycosylation, indicating that Avr1b is post-translationally modified. Some RxLR effectors such as Avr1b also undergo N-terminal cleavage following secretion, but others such as Avr4/6 do not. Cleavage of Avr1b occurs upstream of the RxLR motif, and the RxLR motif is not required for cleavage. Mutants of Avr1b

that are not cleaved are delivered normally. We conclude that N-terminal cleavage is not required for delivery *in planta*. We are now using CRISPR-mediated gene replacement to refine these experiments.

508W Effectors Required for *Epichloë*-Wheat Interactions. Benjamin Moody[1], Christine Voisey[1], Rosie Bradshaw[2], Paul Maclean[1], Carl Mesarich[2], Martijn Rep[3], *Linda Johnson*[1] 1) Forage Science, AgResearch Limited, Palmerston North, Manawatu, NZ; 2) Massey University, Palmerston North, New Zealand; 3) Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, Amsterdam, The Netherlands.

The fungal endophyte *Epichloë bromicola* forms mutualistic symbioses with grasses of the *Elymus dahuricus* complex. Artificial inoculation studies have shown that *E. bromicola* strains AR3060 and AR3018 are also able to colonise the commercial spring wheat cultivar Monad. Unlike the compatible and symptomless associations they form with their native hosts, colonisation of wheat by AR3060 causes host dwarfing but the endophyte is still able to undergo its life-cycle and be vertically transmitted via seed. AR3018 infection is less compatible and causes severe stunting in wheat, followed by plant death and/or the formation of endophyte-free tillers. Transcriptome analysis of compatible versus incompatible interactions between AR3060 and AR3018 with their native and non-native hosts, show that many genes predicted to encode secreted effector-like proteins are highly up-regulated during interactions with host plants. One of most abundant transcripts *in planta* encodes a LysM-domain containing protein that has high homology to *Cladosporium fulvum* effector Ecp6 based on its secondary and tertiary structure. Our results indicate that *Epichloë* endophytes may manipulate grass physiology via the production of effector proteins to produce an environment favourable for fungal infection. Through bioinformatics approaches, over 30 *E. bromicola* candidate effectors with potential to regulate compatibility between *Epichloë* fungi and their hosts have been identified. Transient expression of effectors in non-host plants, combined with targeted gene deletion studies, are being conducted to explore their roles in regulating defence responses and symbiotic interactions between *Epichloë* and its novel wheat host. Long term, this study aims to facilitate fully compatible interactions between *E. bromicola* and wheat to enable these fungi to confer biotic and abiotic stress protection to commercial wheat.

509T Identification of candidate genes encoding the avirulence effector AvrRvi8 from the plant pathogenic fungus *Venturia inaequalis*. Brogan McGreal[1], Jared Bennett[1], Saskia Bosman[1], Linda Collard[1,3], Cecilia Deng[1], Kim Plummer[2], Erik Rikkerink[1], Matthew Templeton[1,3], *Joanna Bowen*[1] 1) The New Zealand Institute for Plant & Food Research, New Zealand; 2) La Trobe University, Australia; 3) The University of Auckland, New Zealand.

The fungus *Venturia inaequalis* causes scab disease of apple. The interaction between the host, *Malus*, and the pathogen follows the 'gene-for-gene' model, with 17 avirulence (*Avr*) and cognate resistance (*R*) gene pairings currently being recognised. Two *R* genes have been cloned from *Malus* (*Riv6* and *Rvi15*), but no avirulence effector genes. Resistance responses in apple governed by 'gene-for-gene' interactions are phenotypically diverse and not restricted to a rapid hypersensitive response. It is hypothesised that the Rvi8 R protein recognises an effector (AvrRvi8), and initiates a stellate necrosis resistance response approximately 10-14 days post inoculation (dpi). Although this response restricts sporulation, rendering the host field-resistant, extensive sub-cuticular fungal growth, typical of a susceptible interaction, is also observed. A bulked segregrant/NGS approach was adopted to identify candidate *AvrRvi8* genes. Progeny (80) from an *in vitro* cross between isolates B04 (*AvrRvi8*) and 1639 (*avrRvi8)* have previously been phenotyped with respect to *AvrRvi8*[1]. DNA from two pools (*AvrRvi8* and *avrRvi8*), each comprising 12 progeny, were sequenced using Illumina technology, and 213 candidates for *AvrRvi8*, with SNPs in the orthologues between the pools, identified. Of these, only one candidate was situated between two novel markers for *AvrRvi8* on a single scaffold in the B04 genome. The nucleotide sequence of this candidate gene was identical in the *AvrRvi8* progeny and a further isolate phenotypically classified as *AvrRvi8*, but there were two predicted amino acid substitutions in the sequence from all *avrRvi8* progeny. The candidate gene is predicted to encode a small (200 amino acids with seven cysteines), putatively secreted protein and had a 4 and 6 log2-fold increase in expression *in planta* compared with *in vitro* at 2 and 7 dpi, respectively, as measured by RNA-seq. The candidate gene belongs to a small gene family and is currently being functionally analysed by a complementation strategy.

[1]Broggini et al. (2011) *Fungal Genet Biol.* 48:166-76

510F A game of omes: hunting for effectors in fungal plant pathogens using comparative genomics. *James Hane*[1,2], Alison Testa[1], Robert Syme[1], Stefania Bertazonni[1], Chala Turo[1], Mahsa Mousaviderazmahalleh[1,3], Haifei Hu[1], Paula Moolhuijzen[1], Richard Oliver[1] 1) Centre for Crop & Disease Management, Curtin University, Perth, Australia; 2) Curtin Institute for Computation, Curtini niversity, Perth, Australia; 3) The University of Western Australia, Perth, Australia.

The outcome of host-pathogen interactions in plant diseases caused by Fungi is largely determined by the presence or absence of "effector" molecules produced by a pathogen, and corresponding resistance or sensitivity loci in its host. Thus determining the effector component of a pathogen's genome is a crucial step in molecular plant pathology: e.g. in facilitating subsequent isolation of effectors to be used to screen host accessions for resistance/susceptibility loci. However the major fungal taxa abundant in plant pathogens (Pezizomycotina and Basidiomycota) exhibit taxon-specific types of genome mutations and/or a higher observed rate of common types of mutations. Thus, unlike for other microbial pathogens, proteinaceous fungal effectors have proven to be especially difficult to predict, due to an overall lack of sequence conservation. The difficulty in producing a reliable and concise effector candidate list and a limited capacity to experimentally test candidates, has created a strong impetus to develop improved bioinformatic methods for the molecular plant pathology community.

While the plasticity of fungal genomes often obscures clear effector candidates from view, in some cases bioinformatics can turn the tables by leveraging knowledge of these mechanisms and the patterns they leave behind to reveal effectors. We have studied the dynamics of mutation between the genomes of multiple plant-pathogenic fungal species and present methods by which two mechanisms (Repeat-induced point mutation (RIP) and lateral-gene transfer) can be exploited for effector prediction. We have also developed additional methods to support various points in the effector prediction process. These methods and/or the outcomes of prediction across multiple species can be accessed at http://effectordb.com. Additionally our efforts to understand the common and distinguishing properties of fungal effectors have allowed us to make new observations of their defining features, develop new predictive methods and explore parallels with

cytotoxic peptides from a wide range of non-fungal species.

511W The two-speed genome of *Verticillium dahliae* mediates emergence of potent virulence factors. *B.P.H.J. Thomma*, L. Faino, J. Li, X. Shi-Kunne, J. Depotter, M. Kramer, G.C. van den Berg, D.E. Cook, H. Rovenich, M.F. Seidl Laboratory of Phytopathology, Wageningen University, Wageningen.

Genomic plasticity enables adaptation to changing environments, which is especially relevant for pathogens that engage in “arms races” with their hosts. In many pathogens, virulence genes reside in highly variable, transposon-rich, physically distinct genomic compartments. However, understanding of the evolution of such compartments, and the role of transposons therein, remains limited. We show that transposons are the major driving force for adaptive genome evolution in the fungal plant pathogen *Verticillium dahliae*, and that highly variable lineage-specific (LS) regions evolved by genomic rearrangements that are mediated by erroneous double-strand repair, often utilizing transposons. Remarkably, LS regions are enriched in active transposons, which may contribute to local genome plasticity. Thus, we provide evidence for genome shaping by transposons, both in an active and passive manner, which impacts the evolution of *V. dahliae* virulence. Based on this knowledge, we are now able to identify crucial virulence factors of *V. dahliae*, which also allows investigating causal relationships between particular effectors and pathotypes.

512T Investigating pathogenicity and genetic diversity within the clinically relevant Fusarium solani species complex. *L. Wendell*, J. Coleman Entomology and Plant Pathology, Auburn University, Auburn, AL.

Members of the genus *Fusarium* are capable of causing severe disseminated infections (fusariosis), which can be difficult to treat and are becoming more common. Most frequently associated with these infections are a number of species within clade 3 of the *Fusarium solani* species complex (FSSC), which are found regularly in the environment. As the FSSC represents >60 phylogenetically distinct species, we were interested in investigating their genetic diversity and variance in pathogenicity. A total of 33 FSSC isolates from clinical sources were compared using multi-locus sequence typing (MLST) and phylogenetic analyses. In accordance with previous studies, all of the isolates collected from patients belonged to clade 3 of the FSSC, and the species most often isolated belonged to *F. keratoplasticum* (FSSC 2), *F. falciforme* (FSSC 3+4), and the currently unnamed species FSSC 5. Karyotypes were analyzed by pulsed-field gel electrophoresis (PFGE) under conditions to evaluate small chromosomes (between 1 and 3 Mb). Every isolate resolved by PFGE was found to have small chromosomes, concentrated between 2.7 Mb and approximately 0.5 Mb. Karyotype diversity was significant between phylogenetic species, and not a single duplicate chromosomal profile was found within any species. All isolates were fitness tested for growth at elevated temperatures, and subsequently screened for virulence in the heterologous host *Galleria mellonella* and a murine model. Those with the greatest ability to grow at 37°C belonged to FSSC groups 2, 3+4, 5, and 20. When tested in animal models, the most virulent isolates were members of FSSC 2, 3+4, and 5. This research demonstrates that phylogenetically closely related species representing a fraction of the FSSC are responsible for a majority of the clinical FSSC infections, and these isolates are both thermotolerant and more virulent than other species within the FSSC.

513F Functional and molecular characterization of genes involved in antagonisms between two maize endophytes, *Fusarium verticillioides* and *Sarocladium zeae*. *M. Gao*[1], X. Gu[2], A. Glenn[3], S. Gold[3] 1) Department of Plant Pathology, The University of Georgia, Athens, GA; 2) Institute of Bioinformatics, The University of Georgia, Athens, GA; 3) Toxicology and Mycotoxin Research Unit, USDA-ARS, Athens, GA.

Fusarium verticillioides (*Fv*) is a prevalent seed-borne maize endophyte capable of causing severe kernel rot and fumonisin mycotoxin contamination. Within maize kernels, *Fv* is primarily confined to the pedicel, while another seed-borne fungal endophyte, *Sarocladium zeae* (*Sz*), is observed in embryos. *In vitro* assay has indicated that *Sz* was able to inhibit the growth of *Fv*. Two lactam-containing antibiotics by *Sz*, named pyrrocidine A (PA) and B (PB), are associated with antagonism of *Fv* by *Sz*. In order to explore the mechanism of antagonism, RNA-seq experiments were conducted by challenging the Fv liquid culture with PA and PB at subinhibitory concentrations to induce transcriptomic changes. A total of 27 genes with dramatic induction in both PA and PB treatments, including 17 genes encoding proteins with a beta-lactamase domain, were selected as targets to generate deletion mutants with our OSCAR protocol. Phenotypic and functional analyses of the mutants are being conducted to help us better understand the molecular mechanisms underlying the metabolic antagonism between these fungal endophytes. We will present current results on the bioinformatic analyses of RNA-seq experiments and functional characterization of the related mutants.

514W StuA is a key regulator of fumonisin production and virulence in *Fusarium verticillioides*. Manisha Rath[1], Nicole Crenshaw[2], *Scott GOLD*[2] 1) Department of Plant Pathology, University of Georgia, Athens, GA; 2) Toxicology & Mycotoxin Research, USDA-ARS, Athens, GA.

Fusarium verticillioides is one of the most important pathogens of maize, producing fumonisin mycotoxins during infection. Ingestion of fumonisin-contaminated corn causes fatal toxicity in livestock and is associated with neural tube birth defects and growth stunting in children. It is also a potential human carcinogen. It is critical to understand the underlying molecular mechanisms associated with fumonisin production in order to generate novel control approaches. StuA, an APSES class transcription factor, is a global regulator with StuA homologs regulating crucial developmental processes in various fungal species such as sporulation, virulence and toxin synthesis among others. Our objective was to investigate StuA’s role in virulence and fumonisin production in *F. verticillioides* via functional and transcriptomic analysis of ?*stuA* mutants. The mutants generated via *Agrobacterium*-mediated transformation of *F. verticillioides* with an OSCAR deletion construct, have stunted aerial hyphae and reduced macro- and micro-conidiation. Three of the ?*stuA* mutants were selected for transcriptome analysis and cultured in GYAM medium to induce toxin production. A total of 437 genes were differentially regulated across the 3 mutants compared to wild type with ≥ 3-fold change. Out of these, 328 were downregulated and 109 upregulated. Five genes in the FUM (fumonisin) cluster and 8 genes in the FUS (Fusarin C) cluster were dramatically downregulated (≥ 4 fold for FUM and ≥ 8 fold for FUS). Analysis of fumonisin levels in GYAM shows reduced fumonisin production by ?*stuA* mutants (≥ 100-fold). Additionally, maize seedlings inoculated with ?*stuA* mutants showed greater shoot height and weight as compared to seedlings inoculated with wild type, indicating reduced virulence of the mutants and thus an important role in virulence for the *stuA* gene.

515T Suppression of genes of *Fusarium verticilloides* using Virus induced Gene Silencing. *J.E. Jurgenson* Biology, University of Northern Iowa, Cedar Falls, IA.

Virus induced Gene Silencing (VIGS) is a technique that can be used to silence genes in plants. plants. Several reports have shown that this approach can also silence genes in organisms (such as pathogens) associated with the plant. Experiments are being conducted that will determine if this technique can be used to silence the genes of the corn pathogen *Fusarium verticilliodes* in the same manner that was used to silence stripe rust genes in wheat.(2011,Yin etal.) These experiments involve the introduction of double stranded RNA with sequence homology to selected fungal genes into host corn plants via a virus vector. The ability of subsequent expression of these genes are being evaluated using standard Quantitative PCR techniques. We are using two vectors: one developed from Brome mosaic virus and one from Foxtail mosaic virus.

The objectives of this project are to: 1) Determine the efficacy of *in-planta* expressed RNAs in silencing *Fusarium verticillioides* genes by examining constructs containing sequences derived from genes involved in infection of host corn plants. 2) Use VIGS constructs to conduct high throughput suppression assays of *F. verticillioides* genes to determine if transcripts are essential essential for fungal pathogenicity or reproduction. *F. verticillioides* genes tested will include those that have homology to other fungal pathogens of corn and to genes of other *Fusarium* species known to be essential for host plant infection. These gene homologs are documented by experimental results published in the literature. 3) Make corn plants that stably express interfering RNAi for genes that show promise for controlling *Fusarium* in VIGS assays.

Chuntao Yin, James E. Jurgenson, and Scot H. Hulbert. (2011) Development of a Host Induced RNAi System in the Wheat Stripe Rust Fungus Puccinia striiformis f. sp. Tritici. MPMI Vol. 24, No. 5, 2011, pp. 554–561

516F A nonribosomal peptide facilitates cell-to-cell invasion of *Fusarium graminearum* in wheat. Leijie Jia, Tinglu Yuan, Dong Zhang, *Weihua Tang* Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, CN.

Fusarium graminearum is an important pathogen of wheat. Deletion of the putative secondary metabolite biosynthetic gene cluster FG3_54 compromised its ability to infect wheat coleoptiles and spikes. Wild-type hyphae invade internal tissues through cell-to-cell penetration, while hyphae of mutants lacking FG3_54 or its member gene FGM4 failed to progress beyond the first layer of wheat cells where papillae-like cell wall depositions were observed. Filtration extracts of FG3_54-expressing *F. graminearum* cultures, when applied to inoculation sites together with FG3_54 deletion strains, facilitated hyphal penetration beyond the first layer of cells. We identified a tripeptide as one of the responsible metabolites. The application of chemically-synthesized tripeptide, restored cell-to-cell invasion in FG3_54-deleted *F. graminearum*, and even enabled colonization of wheat coleoptiles by a banana pathogenic *Fusarium oxysporum* strain. Thus this tripeptide represents an effector that facilitates hyphal cell-to-cell penetration in wheat.

517W Fusarium G-protein coupled receptors: Modulators of pathogenesis and targets for disease control. *N.A. Brown*, T. Dilks, K.E. Hammond-Kosack Plant Biology and Crop Science, Rothamsted Research, Harpenden, Hertfordshire, GB.

Fusarium graminearum is a globally important cereal pathogen and mycotoxin producer. When *F. graminearum* lands on a plant, the fungal germling must decide if it is suitable, where to infect, and when to deploy different virulence strategies. But how *F. graminearum* senses the “touch and taste” of its environment through external receptors is largely unknown.

Bioimaging, bioinformatics [1-2] and now transcriptomics has shown how *F. graminearum* hyphae spatiotemporally regulate a complex array of characterised and putative virulence mechanisms during wheat infection. This includes the dramatic up-regulation of genes involved in the biosynthesis of the trichothecene mycotoxin, deoxynivalenol, plus other secondary metabolite clusters, during symptomless infection. Distinct groups of putative secreted effector proteins were up-regulated either during symptomless or symptomatic infection, while hydrolytic CAZyme induction followed a two-step mechanism, resulting in elevated expression during the development of disease symptoms. Collectively, this implies that *F. graminearum* hyphae must sense alterations in the microenvironment within the host and coordinate virulence accordingly.

G-protein coupled receptors (GPCRs) are the largest class of extracellular receptors in eukaryotes and *F. graminearum* possesses more than other model fungi. Interestingly, a group of non-classical GPCRs were highly expressed during the establishment of symptomless infection. Now a collection of *F. graminearum* mutants lacking individual GPCRs genes has been assessed, revealing their involvement in the establishment of wheat infection.

1. Brown N.A. *et al* (2010) The infection biology of *Fusarium graminearum*: Defining the pathways of spikelet to spikelet colonisation in wheat ears. *Fungal Biology*. 114(7):555-71.
2. Brown N.A. *et al* (2012) The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: A refined comparative analysis. *Plos One*. 2012:7(4).

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518T Novel *Fusarium graminearum* cyclic peptides possess phytotoxic activity. *Linda Harris*[1], Adilah Bahadoor[2], Elizabeth Brauer[1], Whynn Bosnich[1], Danielle Schneiderman[1], Yves Aubin[3,4], Jeremy Melanson[2], Izhar Khan[1], Steve Gleddie[1], Barbara Blackwell[1] 1) Ottawa Research & Development Centre, Agriculture & Agrifood Canada, Ottawa, ON, CA; 2) Measurement Science and Standards, National Research Council Canada, Ottawa, ON, Canada; 3) Centre for Biologics Evaluation, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON, Canada; 4) Department of Chemistry, Carleton University, Ottawa, ON, Canada.

Fusarium Head Blight, a devastating disease of cereal crops in temperate regions globally, is mainly caused by the broad host pathogen *Fusarium graminearum*. A significant number of predicted *F. graminearum* secondary metabolism genes are expressed during host infection but are not yet associated with a metabolite. In an effort to identify and link secondary metabolites to their respective biosynthetic genes, we have isolated two novel cyclic peptides, named gramilin A and B, produced by *F. graminearum* isolates cultured in liquid media.

A combination of LC-HRMS, 1D and 2D-NMR experiments were performed to elucidate their structure. Gene disruption has revealed the nonribosomal peptide synthetase gene responsible for the biosynthesis of these compounds. Gramilin A/B are phytotoxic towards both monocot and dicot plants. Infiltration of either maize or Arabidopsis leaves with gramilin causes cell death within three to four hours. Gramilin A/B do not appear to possess antimicrobial activity against Gram negative or Gram positive human-associated pathogenic bacterial species tested. We are continuing to characterize the mechanism of toxicity.

519F Transcriptomics and experimental proof that compound appressoria are arsenals of *Fusarium graminearum*. *M. Mentges*[1], A. Glasenapp[1], M. J. Boenisch[2], A.-L. Martínez Rocha[1], M. Münsterkötter[3], U. Güldener[3], B. Henrissat[4], M.-H. Lebrun[5], J. Bormann[1], W. Schaefer[1] 1) Molecular Phytopathology, Biocenter Klein Flottbek, University of Hamburg, Germany; 2) Cereal Disease Lab, USDA-ARS, St. Paul, United States of America; 3) Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Germany; 4) CNRS & Aix-Marseille Université, Marseille, France ; 5) INRA BIOGER-CPP, Versailles, France.

Fusarium graminearum is one of the most destructive plant pathogens. Its infection of cereals causes significant losses due to yield reduction and contamination with mycotoxins, e.g. deoxynivalenol (DON). *F. graminearum* infects the floral leaf by defined structures: runner hyphae are formed for colonization of the floral surface and compound appressoria, e.g. infection cushions, are developed to facilitate multiple penetrations of the plant cell wall.

In this study, we created und compared RNAseq data sets of three different fungal cell types, i.e. infection cushions and runner hyphae (isolated via laser capture microdissection from the surface of paleae), and in culture grown mycelium. Validation of the data by qRT-PCR showed similar gene regulation patterns.

The analysis showed that 3916 genes (about one-third of all genes) are specifically regulated during the infection of the plant compared to mycelium. In total 653 genes are exclusively transcribed: 235 in mycelium, 77 in runner hyphae and 341 in infection cushions.

In particular, we show that 1. in runner hyphae, the highly expressed fungal pigment aurofusarin acts as an antibiotic agent against bacteria and fungi, 2. infection cushions are arsenals of virulence factors, harboring plant cell wall degrading enzymes, specific metabolites like DON, and putative effector proteins, 3. the putative effector protein FgEF1 is localized in the plant cell after colonization by *F. graminearum*.

520W Ubiquitin-dependent protein degradation during infection structure development by the plant pathogenic fungus *Fusarium graminearum* . *G. Baermann*, W. Schaefer Molecular Phytopathology, Biocenter Klein Flottbek, University of Hamburg, Germany.

The fungal plant pathogen *Fusarium graminearum* is the most important causal agent of Fusarium head blight of small grain cereals and cob rot of maize worldwide. During the initial infection *Fusarium graminearum* forms two morphologically distinct structures, non-invasive runner hyphae and invasive compound appressoria, especially infection cushions.

Transcriptome analyses of runner hyphae and infection cushions showed a high number of genes coding for enzymes involved in the regulation of these structures. In total we identified 158 different fungal genes that are expressed during development on wheat flower leaves and are most likely involved in ubiquitin-dependent protein degradation. Based on sequence homology, there are 19 ubiquitin carboxyl-terminal hydrolases type 1/2, 3 ubiquitin/SUMO-activating enzymes (E1), 23 ubiquitin-conjugating enzymes (E2), 10 ubiquitin-conjugating enzymes with zinc finger motives of the RING-type, 2 Small ubiquitin-related modifier (SUMO) and 53 F-box proteins among these genes.

The project focuses on the role of selected genes during the ubiquitination process in the fungal pathogen, especially during development of fungal infection structures. In a first attempt we deleted five genes which encode for F-box proteins and ubiquitin-conjugating enzymes. Three mutants showed different deficiencies in growth, conidiation, cell wall integrity, ROS stress and virulence.

A detailed analysis of the involvement of protein degradation in the life cycle of *Fusarium graminearum* will be presented.

521T *Fusarium oxysporum* and its biocontrol. *Maria-Ermioni Constantin*, Francisco de Lamo, Frank Takken, Martijn Rep Molecular Plant Pathology, University of Amsterdam, Amsterdam, North Holland, NL.

Fusarium oxysporum (*Fo*) is known as a fungal pathogen capable of causing vascular wilt disease in over 100 different hosts. Although most studies focus on its ability to cause disease, *Fusarium* is also capable of colonizing plants without triggering disease symptoms, as an endophyte. These non-pathogenic strains of *Fo* have been shown to confer protection against Fusarium wilt on tomato caused by *Fo* f.sp. *lycopersici* (*Fol*). We have received from collaborators about 80 *Fo* strains, some of which could have biocontrol potential against wilt disease. These strains were obtained from different locations in Australia, USA and Spain. From the collection, 44 isolates have been selected for genome sequencing. In addition, we developed a high-throughput screening set-up that allows us to test these strains for their ability to confer protection against tomato wilt disease. Preliminary results show variation in the efficiency of protection against wilt disease by non-pathogenic strains. We are currently trying to understand the mechanism behind this protection.

522F Accessory chromosomes and pathogenicity in *Fusarium oxysporum*. *JIMING LI*, Ido Vlaardingerbroek, Shermineh Shahi, Martijn Rep University of Amsterdam, Amsterdam, NL.

Fusarium oxysporum is a species complex, harbouring pathogenic and non-pathogenic strains. Pathogenic strains cause disease in more than 120 different plants, but each pathogenic strain generally causes disease in one or a few related plant species. Comparative genome studies of *Fusarium graminearum*, *F. verticillioides*, *F. solani* and *F. oxysporum* f.sp. *lycopersici* (*Fol*) has shown that *Fol* contains core and accessory chromosomes, and one of the accessory chromosomes contains virulence genes that promote disease in its host tomato. This 'pathogenicity chromosome' can be transferred to a non-pathogenic strain, resulting in acquired pathogenicity. Conversely, loss of the Fol pathogenicity chromosome leads to loss of virulence. How does the pathogenicity chromosome transfer happen? Fusion of genetically different strains results in formation of heterokaryons. However, heterokaryons are not stable because they contain different vegetative incompatibility loci, which leads to heterokaryon incompatibility reactions. We have observed that conidial pairing of incompatible Fol and Fo (non-pathogenic) strains under carbon starvation can result in the formation of viable cells. After formation of heterokaryons, the chromosomes from the Fol parental strain are lost, but sometimes one or two chromosomes remain in the progeny, including the

pathogenicity chromosome. We now wish to determine the minimal chromosome regions or genes on the Fol pathogenicity chromosome that can cause disease on tomato plants, and/or can be transferred horizontally. We will also investigate the underlying mechanisms of selective chromosome loss in heterokaryons.

523W *FTF2*, the core genome gene of the *Fusarium oxysporum FTF* family, is involved in conidiation and recognition by the host plant. Virginia Casado-del Castillo, Jonathan Niño-Sánchez, Francisco J. Hernández-Aparicio, Liszeth Báez-Ojeda, Ernesto P. Benito, *José María Díaz-Mínguez* CIALE-UNIVERSIDAD DE SALAMANCA, SALAMANCA, SPAIN.

The *FTF* gene family of *Fusarium oxysporum* encodes $Zn(II)_2Cys_6$ binuclear cluster DNA-binding transcription factors. We have previously shown that *FTF2* is a single copy gene well conserved in filamentous ascomycetes that is located in the *F. oxysporum* core genome; on the contrary, the *FTF1* paralogues are variable in number, exclusive to *F. oxysporum* and are located in the adaptative genome.
Null mutants of *FTF2* in the HV strain FOP-SP1 of *F. oxysporum* f. sp. *phaseoli* (SP1D*FTF2*) show reduced virulence when inoculated in common bean plants. The colonization pattern is similar to that displayed by weakly virulent strains (which do not contain *FTF1* paralogues) and the silenced *FTF* mutants, showing a reduction in the number of infected xylem vessels and an increase in parenchymal colonization. However SP1D*FTF2* mutants do not show a significative reduction of fungal biomass in colonized plants as it is the case with the *FTF* silenced HV strains. Further functional analysis demonstrated that *FTF2*$^-$ mutants produce high amounts of macroconida in solid media, but not in liquid media, and induce upregulation of the common bean *PR1* gene which is involved in the plant defensive response mediated by salicylic acid.
To shed light on the specific FTF2-responsive genes, comparative trancriptomic analysis were carried out in a genetic background free of *FTF1* paralogues (FOP-SP4/SP4DFTF2). Several genes showing strong differential expression were confirmed by RT-qPCR analysis. Interestingly, the expression of two genes encoding hydrophobin type II proteins was confirmed to be absent in the *FTF2*$^-$ mutant, and one of them was specifically upregulated during root colonization. These findings indicate that FTF2 is involved in spore production and spore/hypha masking to prevent recognition by the host plant.
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524T Deletion of the AP1 transcription factor in the anthropophilic dermatophyte *Trichophyton rubrum* reveals that it is not required for oxidative stress and antifungal drug resistance. *N. T. A. Peres*[1,2], E. A. S. Lang[2], V. M. Oliveira[2], T. A. Bitencourt[2], A. Rossi[2], N. M. Martinez-Rossi[2] 1) Morphology, Federal University of Sergipe,BR; 2) Genetics, Ribeirão Preto Medical School, University of São Paulo, BR.

Trichophyton rubrum is the major causative agent of tineas, i.e., skin and nails infections, worldwide. Pathogenesis of tinea is a dynamic and complex process, in which the host inflammatory response and the release of fungal proteolytic enzymes are the major responsible factors for tissue damage during the infection, leading to erythematous and itchy lesions. Functional studies have been helping to understand the mechanisms that allow fungal adaptation to the host environment and stressful conditions. Genetic manipulations are valuable tools to understand the regulation and functionality of genes giving insights into the pathogenesis of fungal infections. However, genetic manipulations of the anthropophilic dermatophyte *T. rubrum* is very laborious given the very low frequency of transformation and homologous recombination. In this work, we describe the obtainment of a null mutant for the AP-1 transcription factor of *T. rubrum*, and its functional characterization in response to several environmental conditions. The knockout strain was obtained by replacing the *ap-1* gene by the *hph* gene that confers resistance to hygromicin B. The gene target cassette was constructed by fusion PCR, and inserted into *T. rubrum* by protoplast transformation. By increasing the size of the flanking homologous regions in the cassette, it was possible to isolate a null mutant strain for the *ap-1* gene. Gene expression analysis revealed that *ap-1* is upregulated during human nail and skin ex vivo infection, growth in keratin, and in response to the exposure to amphotericin B, nystatin, and terbinafine. Functional analyses of the mutant strain revealed that inactivation of the *ap-1* gene did not alter fungal growth or sensitivity to oxidative stress and the antifungal drugs analyzed. However, the null mutant showed a higher growth in nail fragments, hyperpigmentation, and increased conidia sensitivity to calcofluor white, compared to the wild type. These results suggest that in *T. rubrum*, the AP-1 transcription factor is not required for oxidative stress adaptation and antifungal drug resistance, but it is required for fungal growth control during nail infection.

525F A difference in hydrophobin gene expression profiles among *Verticillium* isolates corresponds with the gene's highly specific role in resting structure development. *A. Klimes*, S. Bibi, T. Q. Nguyen Western New England University, Springfield, MA.

Verticillium dahliae causes a vascular wilt disease in a wide range of economically significant crops. The soil-borne pathogen produces melanized resting structures known as microsclerotia that stymie eradication efforts in the field. *Vdh1* is a predicted hydrophobin gene that was among the first *V. dahliae* genes found to be involved in microsclerotia production; in the Race 1 tomato isolate Dvd-T5, disruption of *Vdh1* inhibits microsclerotial development and results in accelerated disease development. We identified a gene that shares 100% nucleotide identity with *Vdh1* in the genome sequence of the Race 2 *V. dahliae* lettuce isolate VdLs.17. Expression of this gene, designated *Vdh1-L*, is down regulated during microsclerotial development. In marked contrast, *Vdh1* was previously shown to be specifically expressed in developing microsclerotia. In *V. alfalfae* (isolate VaMs.102), which produces relatively simple and non-persistent resting structures known as dark resting mycelium, the *Vhd1* homologue *Vdh1-A* shows the expected induction of expression during resting structure development, indicating that repression of *Vdh1-L* during microsclerotia development results from isolate-specific gene regulation patterns. *Vdh1-L*'s role in development appears to be isolate-specific as well, because disruption of *Vdh1-L* did not inhibit microsclerotia production; conversely, under specific nutrient availability conditions, *Vdh1-L* disruption mutants appear to produce more microsclerotia. Our results suggest that developmentally important genes may have highly specific regulation patterns that could be relevant to differences in host-specificity among various *V. dahliae* isolates. Comparison of expression patterns of hypothetical genes during resting structure development in VdLs.17 and VaMs.102 yielded results that support this suggestion: of 9 hypothetical genes whose expression profiles we examined, at least one showed a marked difference in expression between the two species. Comparing genome-scale expression profiles may therefor help us pinpoint important developmental differences between *V. dahliae* isolates with different host-specificities, even in instances of high genome sequence identity.

526W Functional characterization of Phospholipase D likes in *Phytophthora infestans*. *Harold Meijer*[1,2], Charikleia Schoina[2], Shutong Wang[2,3], Klaas Bouwmeester[2], Chen-lei Hua[2], Francine Govers[2] 1) Wageningen Plant Research, Wageningen University and Research, Wageningen, NL; 2) Laboratory of Phytopathology, Wageningen University and Research, Wageningen, NL; 3) College of Plant Protection, Agricultural University of Hebei, CH.

Invasion of plant host tissue by oomycete pathogens, like *Phytophthora infestans,* is dependent on modifications of the host plasma membrane to facilitate two-way transfer of proteins and other compounds. This modulation of the host plasma membrane, e.g. to establish extrahaustorial membranes, might be accompanied by extracellular (phospho)lipase activities. Phospholipases have been implicated as virulence factors in pathogens and since the *P. infestans* genome harbors a range of small phospholipase Ds (PLD-likes) with a signal peptide, these oomycetes could use similar weaponry during the infection process. We show that three small PLD-likes, belonging to three PLD classes, are expressed during various oomycete life-stages, including infection. *In planta* expression of small PLD-likes promotes local cell-death which highly depends on silencing suppression, calcium presence and intact catalytic motifs. We further demonstrate that *in planta* expression of the wild type small PLD-likes facilitates *P. infestans* infection and supports sporangia formation. These results suggest that the small PLD-likes are catalytically active and function by executing membrane modifications to support *Phytophthora* growth and development *in planta*.

527T CgXbp1 modulates temporal transcriptional dynamics in human fungal pathogen *Candida glabrata* during interaction with host macrophages. *M. N. Rai*, C. Parsania, R. Rai, K. H. Wong Faculty of Health Sciences, University of Macau, Macau, MO.

Candida glabrata, an opportunistic human fungal pathogen in immunocompromised patients, remodels its carbon metabolism to survive in nutrient-deprived intracellular macrophage microenvironment. Here, we have identified a transcription factor, CgXbp1, required for remodeling carbon metabolic pathways in *C. glabrata* upon exposure to host macrophages. Employing the next generation sequencing approaches, we showed that *Cgxbp1Δ* mutant exhibited constitutively active fatty acid catabolic and proteolytic pathways even in the presence of sufficient carbohydrate in the growth medium, suggesting that carbon metabolic processes are deregulated upon *CgXBP1* deletion. Next, we demonstrated that CgXbp1 is essential for arresting the cells in G1 phase of cell cycle during carbon starvation. Global gene expression analysis on *Cgxbp1Δ* mutant revealed that genes associated with GO terms – mitochondrion organization, mitochondrial transport and mitochondrial translation were highly enriched among down-regulated genes, indicating that CgXbp1 may have pivotal roles in cellular energy homeostasis maintenance. Comparison of the transcriptional profiles of macrophage-engulfed wild-type and *Cgxbp1Δ* mutant cells revealed that unlike wild-type cells, *Cgxbp1Δ* mutant was unable to repress genes associated with GO terms - Translation, Ribosome biogenesis and RNA metabolic processes during early stage of macrophage infection. Overall, our gene expression data indicated that CgXbp1 not only is central in carbon metabolic remodeling, but also affects global transcriptional response of *C. glabrata* cells in host macrophages.

528F Transcriptome changes in a seed-transmitted endophyte during host floral development are related to stress and reactive oxygen responses. Padmaja Nagabhyru[1], Randy D. Dinkins[2], *Christopher L. Schardl*[1] 1) Plant Pathology, University of Kentucky, Lexington, Kentucky, KY; 2) Forage-Animal Production Research Unit, USDA-ARS, Lexington, Kentucky, KY.

Epichloë coenophiala is the common systemic symbiont (endophyte) of tall fescue (*Lolium arundinaceum*), the most widely planted cool-season forage grass in the U.S., and the fungal endophyte strictly and efficiently transmits vertically by colonizing floral primordia, ovaries and embryos. We investigated differences in endophyte and host transcriptomes associated with the plant reproductive tissues, as well as vegetative pseudostems and floral bracts. Remarkably, ovaries of *Epichloë*-symbiotic plants showed almost no significant differences in gene expression compared to those of asymbiotic plants. In contrast, 277 fungal genes showed significantly higher expression in ovaries than pseudostems, and many of these were indicative of responses to stress and reactive oxygen species (ROS). Examples are genes for heat-shock proteins, trehalose biosynthesis enzymes, catalases, superoxide dismutases, and bax inhibitor-1 protein. Gene expression profiles of floral primordia and bracts indicated that this endophyte response began early in host floral development. Out of 150 fungal genes with significantly higher expression in pseudostems compared to ovaries, many were alkaloid biosynthesis genes, or sugar and amino acid transporter genes. The major changes in the fungal transcriptome during plant reproductive development indicate a shift away from specialized metabolism and towards enhanced stress tolerance, and suggest that response to ROS is important for vertical transmission of the endophyte.

529W RNA-sequencing of susceptible and resistant spinach downy mildew interactions. *Steven J. Klosterman*[1], Roberto Ornelas[2], Kevin Stoffel[3], Amanda Hulse-Kemp[3], Sebastian Reyes-Chin-Wo[3], Lida Derevnina[3], Steven T. Koike[4], Richard Michelmore[3], Allen Van Deynze[3] 1) USDA-ARS, Salinas, CA; 2) California State University, Monterey Bay, Seaside, CA; 3) University of California, Davis, Davis, CA; 4) University of California, Cooperative Extension, Salinas, CA.

Peronospora effusa is an oomycete plant pathogen that causes downy mildew, a widespread and destructive disease on spinach. Symptomatic spinach leaves infected with *P. effusa* display yellow lesions in an otherwise dark green background, severely limiting their fresh market value. In this study, RNA sequencing was performed to detect gene expression in the pathogen and host in *P. effusa*-spinach interactions. Susceptible and resistant spinach cultivars, Viroflay and Solomon, respectively, were inoculated with *P. effusa*, while control plants of both cultivars were uninoculated. As both host and pathogen genome resources are available, gene expression levels of *P. effusa* and the two inoculated cultivars were evaluated by RNA-sequencing. We detected over 700 different transcripts of the pathogen as expressed in the inoculated Viroflay leaves, including two genes encoding effector homologs. The results of this work provide initial insights into genetic responses of *P. effusa* during infection, and also the resistant and susceptible host responses.

530T Identification and Prevalence of Proteins Predicted to be Intrinsically Disordered in the Secretome of the Fungal Plant Pathogen *Venturia inaequalis*. *L. Collard*[1,2], C. Deng[1], M. Templeton[1,2], E. Rikkerink[1], K. Plummer[3], J. Bowen[1] 1) The New Zealand Institute For Plant & Food Research, Auckland, NZ; 2) The University of Auckland, Auckland, New Zealand; 3) La Trobe University, Melbourne, Australia.

Apple scab disease is caused by the Ascomycete fungus, *Venturia inaequalis*, an apoplastic dwelling and host-specific pathogen. This pathosystem is consistent with the gene-for-gene model of resistance interactions and 17 avirulence (*Avr* gene) and resistance (*R* gene)

interactions have been identified thus far. As yet, no effectors involved in the suppression or evasion of host defence have been characterised in this organism.
Several hundred candidate effector genes were identified in draft whole genome sequences (WGS) of a set of five isolates of *V. inaequalis* with differential virulence phenotypes against hosts containing specific resistances (MNH120, B04, 1639, Race 5 on *Malus* × *domestica* cultivars and 1389 on *Eryobotria japonica*). Criteria for effector prediction included: low molecular weight (less than 500aa), secretory signals, differentially upregulated expression during infection compared with *in vitro* expression, cysteine enrichment and lack of sequence similarity to known proteins. This large candidate gene set was further bioinformatically interrogated to prioritise a subset for functional characterisation. Disulphide bridges are important structural elements thought to confer stability of proteins in the protease rich apoplast and various configurations of cysteine bridges were predicted in candidates using sequence based prediction tools. Highly stable configurations including inhibitory cysteine knots (ICKs) were detected in some candidates. In addition to these elements, regions of intrinsic structural disorder were frequently detected in the candidates. Lack of secondary structure could enable manipulation of protein-protein interactions, diversity of function of a protein, sensitivity to host cues or evasion of recognition by host resistance proteins. The sensitivity of many effector prediction tools may be enhanced by inclusion of IDP consideration. Candidate atg7917, in the *V. inaequalis* isolate MHN120, contains a relatively large disordered segment with an ICK at the C terminal and has been prioritised for functional analysis using knock out and protein expression strategies.

531F Characterization of the interaction between bacteria and *Magnaporthe oryzae*. *X. Zhang*, JR Xu Botany and plant pathology, Purdue University, west lafayette, IN.

Like many other foliar pathogens, the rice blast fungus *Magnaporthe oryzae* form appressoria for plant penetration. Interestingly, bacteria attached to appressoria are often observed in in vitro assays for appressorium formation. In this study, *Pseudomonas fluorescens* strain TR3 was isolated from rice leaves and used for studying for its interaction with *M. oryzae*. TR3 could attached to conidia, germ tubes, and appressoria and caused fungal cell death. In co-cultivation assays, TR3 was inhibitory to colony growth in a contact-based manner, excluding the possibility of small antifungal metabolites. Attachment of TR3 to fungal spores was inhibitory to germination and plant infection. Antifungal activities were specifically detected in culture filtrates of TR3 grown in M9 medium together with *M. oryzae,* suggesting the involvement of the type 3 secretion system (T3SS). Mutants detected of the *hrcC* gene encoding the outer membrane pore forming protein of T3SS failed to form flagella and had reduced inhibitory activities against *M. oryzae*. In contrast, deletion of the *pilC* gene that is essential for T4SS had no impact on its antifungal potential although affected attachment to fungal hyphae or conidia. The phosphorylation level of Pmk1 MAP kinase was significantly increased after incubated with TR3 for 2 h, which was not observed in the *sho1* deletion mutant. Taken together, these results indicate that the interaction of *P. fluorescens* with *M. oryzae* is inhibitory to fungal growth, development, and pathogenesis and likely involves T3SS effectors affecting intracellular signaling pathways.

532W Application of Host-Induced Gene Silencing (HIGS) for The Control of Rice Blast. *M. Wang*, R. Dean Department of Entomology and Plant Pathology, NCSU, Raleigh, NC.

Rice blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae,* is the most destructive disease of rice world-wide. To overcome readily broken-down disease resistance, HIGS (host induced gene silencing) is being developed as a strategy to create a new type of disease resistant plants. Eight genes (*CRZ1, Pmc1, MagB, Lhs1, CYP51A, CYP51B, Exo70, Sec5*) that play important roles for the pathogenicity and development of *M. oryzae* were chosen to make HIGS vectors. The vectors were transformed into rice calli through the *Agrobacterium*-mediated method, generating RNA silencing signals in transgenic rice that will silence the target genes of invasive fungus. T0 and T1 generations of transgenic rice plants were generated and the insertion of HIGS fragment confirmed by genomic DNA PCR and Southern blotting. Generation of siRNAs was confirmed by Northern blotting. Silenced and control transgenic rice plants have been challenged with *M. oryzae* and the results will be presented. In addition, treating *M. oryzae* with naked diced siRNA derived from the target genes silenced their expression in the fungus and caused functional collapse. These findings suggest RNA silencing signals can be transferred from host to invasive fungus and HIGS has potential to generate resistant rice against *M. oryzae*.

533T Functional characterization of SUMOylation-associated genes in the rice blast fungus, *Magnaporthe oryzae*. *You-Jin Lim*[1], Yong-Hwan Lee[1,2] 1) Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea; 2) Center for Fungal Genetic Resources, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea.

Among the post-translational modifications, SUMOylation is widely conserved process of attaching SUMO (small ubiquitin related modifier) to a substrate protein in eukaryotic organisms. This process controls many important biological mechanisms including transcriptional regulation, protein localization, cell cycling, DNA repairing, and pathogenesis. In *Saccharomyces cerevisiae*, SUMOylation is required for DNA repair, growth, sporulation, and stress resistance. However, SUMOylation is still uncharacterized in plant pathogenic fungi including *Magnaporthe oryzae*. The SUMO-activating enzymes (E1), SUMO-conjugating enzymes (E2), SUMO ligases (E3), and SUMO protease are the four main enzymes in the SUMOylation. In this study, to elucidate the roles of SUMOylation in fungal development and pathogenicity, four SUMOylation-associated genes encoding two E1 (*MoAOS1* and *MoUBA2*), one E2 (*MoUBC9*), and one SUMO protein gene (*MoSMT3*) were functionally characterized by gene deletion strategy. All deletion mutants of four genes, Δ*Moaos1*, Δ*Mouba2*, Δ*Moubc9* and Δ*Mosmt3*, showed significant defects on mycelial growth, conidiation, conidial morphology, conidial germination, appressorium formation, and pathogenicity. These data suggest that SUMOylation is essential for fungal development and pathogenicity in *M. oryzae*. This study would enhance to comprehensively understand the role of the SUMOylation in the rice blast fungus and beyond.

534F Genome-wide profiling of small RNA transcriptome at single nucleotide level in the rice blast fungus. *G. Choi*[1], J. Jeon[1], Y.H. Lee[1,2] 1) Interdiscplinary Program in Agricultural Genomics, Seoul National University, Seoul 08826, Korea; 2) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea.

RNA silencing is the universal strategy to regulate diverse biological pathways. Since RNA-induced silencing complex inhibits complementary mRNA activity, sequence changes by single nucleotide polymorphism (SNP) or RNA modification may affect the target process. In fungi, post-transcriptional gene silencing is known to be involved in genome stability and viral defense. Functional small RNAs

were also predicted in several fungal species including plant pathogens. However, the role of the fungal small RNAs was not well understood in the pathogen-host interactions. As a first step to decipher the roles of small RNAs in the rice blast fungus, *Magnaporthe oryzae*, small RNA transcriptome data from in planta and under stress conditions were collected and analyzed. Small RNA reads were matched to *M. oryzae* genome and read counts were normalized using upper-quartile scaling. Sequences were classified as specific and conserved based on the number of libraries where they were expressed. Higher abundance and lower diversity were detected in conserved sequences compared to specific sequences. Contrary to conserved sequences, specific sequences were mapped more on the genic region than the repeat region across libraries. Small RNA reads matched to the genome with one mismatch were also analyzed compared to perfectly mapped reads. Mismatches were enriched at the 3' end position of these reads, which may imply post-transcriptional modification of small RNAs. Some imperfectly matched small RNAs had the higher copy numbers than perfectly matched small RNAs on the corresponding chromosomal position. These data suggest that those sequences often discarded in the small RNA analysis pipeline could be functional as isomers. This study would provide new insights on understanding the roles of fungal small RNAs in the rice blast fungus and beyond.

535W Structure of a fungal heme-iron acquisition pathway. Ziva Weissman[1], Shir Yaish[1], Lena Nasser[1], Udita Roy[1], Cristina Avila[1], Guy Horev[2], Hay Dvir[3], *Daniel Kornitzer*[1] 1) Faculty of Medicine, Technion - I.I.T., Haifa, Israel; 2) Bioinformatics Knowledge Unit, Technion - I.I.T., Haifa, Israel; 3) Technion Center for Structural Biology, Technion - I.I.T., Haifa, Israel.

Iron acquisition poses a general challenge for all organisms due to the low solubility of elemental iron, and a particular challenge for pathogenic microorganisms due to the iron withholding mechanisms deployed by the host. Many microbial pathogens have therefore evolved mechanisms for extracting iron from hemoglobin, the largest iron store in the host. In fungi such as *Candida albicans,* the heme-iron utilization pathways includes an extracellular cascade of small secreted and GPI-anchored proteins containing a CFEM domain, defined by a sequence signature consisting of 8 cysteines. These proteins all are able to capture heme from hemoglobin and to exchange heme among themselves, consistent with a pathway in which heme is extracted from hemoglobin outside the cell and is then transferred from one CFEM protein to the next across the cell wall, until it reaches the plasma membrane. We solved the structure of the hemophore Csa2, and found that its CFEM domain adopts a novel protein fold, held together by 4 disulfide bonds generated by the 8 conserved cysteines. Heme is bound via a flexible N-terminal extension and a unique redox-sensitive heme-iron coordination mechanism, consistent with rapid ligand binding and release. At the plasma membrane, the heme is endocytosed by a mechanism involving the ESCRT pathway and a β-arrestin. To identify the predicted transmembrane heme receptor that connects the extracellular CFEM network with the endocytic pathway, we turned to phylogenetic profiling: taking advantage of the hundreds of fungal genomes available, we screened for genes jointly present across fungi with the CFEM heme transfer cascade genes. This enabled us to identify a new class of plasma membrane proteins that is essential for heme uptake. The hemoglobin-iron utilization pathway that emerges from these studies, which is distinct from bacterial systems, indicates that fungi have evolved a unique solution to the heme-iron acquisition problem. Phylogenetic profiling is shown here to represent a powerful method for unraveling cellular pathways in organisms that are recalcitrant to conventional genetic screening.

536T Genome wide expression profiling of the response of *Neurospora crassa* to the antifungal plant defensin MtDef4. *Z.Allen. Snodgrass*[1], K. El Mounadi[1], D. Shah[2] 1) Biology, Kutztown University of Pennsylvania, Kutztown, PA; 2) Donald Danforth Plant Science Center.

Defensins are small, cysteine-rich antimicrobial proteins present in all plants. MtDef4 from *Medicago truncatula* is an evolutionarily conserved plant defensin that exhibits strong antifungal activity towards an array of fungal pathogens. Overexpression of this defensin in transgenic plants significantly increases the resistance of these plants to fungal pathogens. In order to understand the mechanisms by which MtDef4 mediates its antifungal activity, we have investigated the changes in the gene expression profile of *Neurospora crassa* in presence of the defensin. A total of 254 genes were differentially expressed in response to MtDef4 and encoded proteins involved in various metabolic and cellular processes. The majority of genes highly expressed in presence of the defensin control cellular stress responses. Other up-regulated genes are involved in DNA and protein synthesis, cell cycle control, signal transduction, regulation of gene expression and lipid metabolism. Deletion mutants of several up-regulated genes were further characterized to determine the contributions of these genes to MtDef4's mode of antifungal action.

537F Molecular and cellular responses of the ascomycete fungi *Neurospora crassa* and *Fusarium graminearum* to the antifungal plant defensin MtDef4. *K. El Mounadi*[1], K. Islam[2], D. Shah[2] 1) Biology, Kutztown University of Pennsylvania, Kutztown, PA; 2) Donald Danforth Plant Science Center.

Defensins play an important role in plant defense against fungal pathogens. The plant defensin, MtDef4, inhibits the growth of the ascomycete fungi, *Neurospora crassa* and *Fusarium graminearum,* at micromolar concentrations. Previous studies have suggested that MtDef4 is transported into the cytoplasm of these fungi and exerts its antifungal activity on various intracellular targets. Here, we show that *N. crassa* and *F. graminearum* respond very differently to MtDef4 challenge. MtDef4 permeabilizes the plasma membrane of *F. graminearum*, but not of *N. crassa*. We find that it is targeted to different subcellular compartments in each fungus. Internalization of MtDef4 in *N. crassa* is energy-dependent and involves endocytosis. By contrast, MtDef4 appears to translocate into *F. graminearum* autonomously using partially energy-dependent pathway. MtDef4 has been previously shown to bind to the phospholipid phosphatidic acid (PA). We provide evidence that the plasma membrane localized phospholipase D (PLD1), involved in the biosynthesis of PA, is needed for entry of this defensin in *N. crassa*, but not in *F. graminearum*. To our knowledge, this is the first example of a plant defensin which inhibits the growth of two ascomycete fungi via different mechanisms.

538W Perturbation of *Tim54* expression affects Hyphal Interference and increases life-span in *Podospora anserina*. Alex Mercier, Colin Clairet, David Morais, Philippe Silar, *Sylvain Brun* LIED-UMR 8236, Univ Paris-Diderot, Paris, FR.

Fungi are very successful microorganisms capable of colonizing virtually any ecological niche where they must constantly cope with competitors such as other fungi, bacteria, nematodes, *etc.* We have shown that the ascomycetes *Podopora anserina* exhibits Hyphal Interference (HI), an antagonistic response triggered by direct contact of competing fungi hyphae (Ikediugwu and Webster, 1970). Indeed, when challenged by *Penicillium chrysogenum*, *P. anserina* produces hydrogen peroxide at the confrontation and kills the hyphae of *P.*

chrysogenum (Silar, 2005). This analogy with the oxidative burst taking place during innate immune response in higher eukaryotes was further strengthened when we demonstrated that as in animals and in plants, HI requires the NADPH oxidase (Nox) PaNox1 in *P. anserina* (Silar, 2005).
Here, we report the characterization of the *PDC*^{2218} mutant affected in HI. When challenged with *P. chrysogenum*, the *PDC*^{2218} mutant produces a massive oxidative burst at the confrontation compared to the wild type but this increased production of hydrogen peroxide is not correlated to a higher mortality of the cells of *P. chrysogenum*. Hence, the oxidative burst and cell death of the challenger are uncoupled in *PDC*^{2218}. We first proceeded to the positional cloning of the mutation and identified a deletion spanning 1560 bp in the mutant genome. Even though the deletion disrupts the *PaNip30* putative CDS as well as the 3' UTR (UnTranslated Region) of the neighboring *PaCdk5* putative gene, we eventually show that neither *PaCdk5* nor *PaNip30* play a role in HI. Instead, the gene responsible for the phenotype of the *PDC*^{2218} mutant is located more than 1000 bp away from the deletion. This gene is *PaTim54* encoding the homologue of the budding yeast mitochondrial internal membrane import machinery component Tim54p. We show that *PaTim54* is essential in *P. anserina* and that the phenotypes displayed by the hypomorphic *PDC*^{2218} mutant (renamed *PaTim54*^{2218}) is the consequence of a drastic reduction in the expression of *PaTim54*. Finally, the pleiotropic *PaTim54*^{2218} mutant displays increased life-span, a phenotype in accordance with its observed altered mitochondrial physiology.
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539T Characterization of necrosis-inducing NLP proteins in *Leptosphaeria maculans* and *L. biglobosa*. *Georgia Mitrousia*[1], Pryank Patel[1], Henrik Stotz[1], Henk-Jan Schoonbeek[2], Christopher Ridout[2] 1) School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK; 2) Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK.

Proteins of the superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) are produced by various phytopathogenic microorganisms. These secreted proteins have been studied in bacteria, fungi and oomycetes. They are involved in cell death but also in activation of host innate immune responses (Qutob et al. 2006). Protein prediction following the *Leptosphaeria maculans* genome sequencing has identified two similar-to-Nep1 proteins (Lm_NLP1 and Lm_NLP2). Recent work showed that Lm_NLP1 is required for the complete virulence of *L. maculans* (Haddadi et al. 2015). Here, the Lb_NLP gene was identified in the *L. biglobosa brassicae* genome, based on the conserved GHRHDWE heptapeptide motif that is characteristic for the NLP family. The three NLPs from the *Leptosphaeria* spp. were found to belong in the Type I of NLPs, based on their amino acid sequence (Gijzen and Nürnberger 2006) in a conserved region. We observed that a peptide motif derived from a fragment of type I NLPs from bacteria, oomycetes and fungi that is not required for necrosis can induce PAMP triggered immunity in *Arabidopsis*. Further, a 24 amino acid peptide from Lm_NLP1 is recognised by some *Arabidopsis* and *Brassica* lines as a PAMP and induces a transient ROS-burst. Recombinant proteins of the three identified *Leptosphaeria* NLPs with or without the signal peptide were used to examine their cytotoxic activity. This will enable us to determine how the different proteins exert their biological activities. Analysis of RNAseq data of *B. napus* seedlings following *L. maculans* inoculation revealed the up-regulation of four NEP-1 interacting genes. The role of these genes following infiltration of the recombinant proteins is discussed.

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540F *Brachypodium distachyon* - A new model host for *Claviceps purpurea*. *S. Kind*, S. Schurack, J. Hinsch, P. Tudzynski University of Muenster, Muenster, DE.

The ergot fungus *Claviceps purpurea* is a biotrophic phytopathogen with a broad host range including important crop species. The infection is highly specific to unfertilized ovaries and causes intoxication of the harvest with ergot alkaloids, thus, it is unsuitable for food and feed (Hinsch and Tudzynski, 2015). To study the infection process rye is currently used as standard host but its cultivation conditions are complex, its large genome hinders genetic modifications and generally there is only little knowledge about typical pathogen responses. In contrast, the grass species *Brachypodium distachyon* combines several qualities of a suitable model host and allows new perspectives for molecular analysis of the infection process (Fitzgerald et al 2015). Its susceptibility to *C. purpurea*- infection was tested by the appearance of macroscopic infection symptoms, microscopic observation and altered expression profiles of typical disease responsive genes. Our results show that *B. distachyon* and *C. purpurea* can establish a compatible interaction featuring micro- and macroscopic infection symptoms. However, the appearance of macroscopic symptoms is reduced compared to rye which hinders statistically significant evaluation. We explored different methods to quantify the infection process such as determination of the ratio from fungal to plant biomass and plant gene expression. We observed that plant responses matches results from known compatible interactions. Taken together, those tools enable us to study the interaction between *C. purpurea* and Brachypodium. We want to use this system to test the general relevance of virulence factors from the *Claviceps*-rye system and compare the plant response to *C. purpurea* to other pathogens with regard for fungal derived phytohormones, especially cytokinins.
Hinsch and Tudzynski in Patterson and Lima (eds) Mol. Biol. of [..] Mycotoxigenic and Mycotic Fungi (2015): 229
Fitzgerald et al. "Brachypodium as an emerging model for cereal–pathogen interactions." Annals of botany (2015): 717

541W Identification of host targets for effectors from the anther smut fungus, *Microbotryum lychnidis-dioicae*. *V.S kuppireddy*, M.C Tsai, C Cahill, B Carman, M.H Perlin Department of Biology, University of Louisville, Louisville, KY.

The anther smut disease caused by *Microbotryum lychnidis-dioicae* and its close relatives may be considered a sexually transmitted disease, spreading from host-to-host via pollinators that would normally transmit the means of host reproduction. In the male plant, the fungus replaces the pollen with its own spores, whereas in female plants the ovaries abort and develop spore bearing pseudoanthers. Fungal pathogens have evolved highly advanced mechanisms to engage their hosts in intimate contact and sabotage host immune

responses; commonly, this is done by secreting effector proteins into host cells to target regulators of defense or plant development. Many of these small secreted proteins, specifically produced during plant infection, manipulate host cells to facilitate parasitic colonization by biotrophic fungi, e.g., by suppressing plant innate immunity or enhancing nutrient availability. However, the roles of these predicted effectors and how they perform their functions remain unresolved. We have characterized the roles of several effectors by identifying their host targets using the yeast 2-hybrid technique to identify protein-protein interactions. Some interesting potential plant proteins that will interact with these fungal effectors were identified, including at least one predicted to be involved in pollen tube elongation. Further experiments will reveal the significance of these fungal effectors in pathogenicity and also their possible location inside the host plant once it is infected. This overall study will provide more insights into the mode of pathogenicity of this fungus and explore not just how a biotroph behaves inside a host but also reveal aspects of the symbiotic behavior between the host and the fungus.

542T Secreted Nep1-like proteins of oomycetes, fungi, and bacteria trigger immunity in *Arabidopsis*; genetic dissection of NLP-induced plant defense. Tom Raaymakers, Ruben Hijne, Tijmen Van Butselaar, *Guido Van den Ackerveken* Plant-Microbe Interactions, Utrecht University, Utrecht, NL.

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are secreted by a wide range of plant-associated microorganisms, in particular by many phytopathogenic oomycetes and fungi. Both cytotoxic and non-cytotoxic NLPs act as a microbe-associated molecular pattern (MAMP) in *Arabidopsis thaliana*. Specifically, a 24 amino acid fragment, nlp24, derived from a conserved region of oomycete, fungal, and bacterial type 1 NLPs triggers defense. The widespread distribution of NLPs makes this family the first proteinaceous MAMP identified in three different kingdoms of life. A receptor complex consisting of RLP23–SOBIR1–BAK1 mediates NLP-triggered immunity (NTI) in *Arabidopsis*. Here, we present our data on the genetic dissection of NTI, using a transgenic *Arabidopsis* line expressing an estradiol-inducible version of *HaNLP3* of the Arabidopsis downy mildew pathogen *Hyaloperonospora arabidopsidis*. When treated with estradiol plants become severely stunted and plant immunity is strongly activated. M2 seeds of an EMS-mutagenized population were screened for loss of NLP-triggered growth inhibition. We selected ~40 mutants showing normal growth after estradiol treatment and insensitivity to the nlp24 peptide. The obtained *decreased NTI* (*dni*) mutants are analysed for defects in responsiveness to other MAMPs and their susceptibility to pathogen infection. We will report on our progress on *dni* mutant analysis and cloning of the corresponding *DNI* genes.

543F Taking a host genetics approach to understand how the intracellular fungal pathogen *Histoplasma capsulatum* survives within and lyses host macrophages. *A.L. Cohen*[1], N.V. Prooyen[1], M.C. Bassik[2], A. Sil[1] 1) Microbiology and Immunology, UCSF, San Francisco, CA; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA.

Histoplasma capsulatum (*Hc*) is a pathogenic soil fungus that is inhaled by mammalian hosts, including humans. Subsequently, *Hc* is phagocytosed by macrophages, where it utilizes largely unknown strategies to survive and replicate within the macrophage phagosome. Robust proliferation of *Hc* within the phagosome is followed by host-cell death and the release of live fungal cells. Our goal is to understand how *Hc* manipulates host cell processes, including phagosome maturation, to facilitate intracellular replication within macrophages and successful infection. Our lab and others have shown that, unlike most microbes ingested by macrophages, *Hc* resides within a neutral phagosome, and *Hc*-containing phagosomes do not acquire late lysosomal markers such as cathepsins. However, little is known about the molecular players underlying host manipulation by this fungus. We are taking a two-pronged approach to elucidate host pathways that are targeted by *Hc* during intracellular growth and lysis. First, we are conducting a pooled CRISPR-Cas9 based gene disruption screen in human macrophage-like cells to identify host genes required for death following *Hc* infection. We will then study how the corresponding gene products and pathways are directly or indirectly manipulated by *Hc* to promote intracellular infection. Second, we have discovered that a host lysosomal trafficking regulator, LYST, is required for *Hc* to lyse host macrophages during infection. We are investigating the role of LYST in allowing *Hc* to promote host cell lysis. These studies will hopefully uncover molecular mechanisms of intracellular fungal pathogenesis as well as novel insights into host cell biology.

544W MicroRNA expression during Metarhizium anisopliae-host pathogenic interaction. M.A.K. Alenezi[1], M. Hitchings[2], *D.C. Eastwood*[1] 1) Department of Biosciences, Swansea University, Swansea. GB; 2) School of Medicine, Swansea University, Swansea. GB.

Entomopathogenic fungi, such as *Metarhizium* spp., target a wide range of insect species and have been employed as biological control agents reducing the need of chemical pesticides. Infection of an insect host involves a developmental progression from spore attachment and germination, host cuticle degradation and penetration, systemic proliferation as blastospores, and late stage mycelial growth and sporulation. Pathogenic determinants and gene expression during the infection process have been studied, while mechanisms regulating morphogenetic change are still to be fully elucidated. This study aimed to determine whether microRNAs have a functional role during the infection time course of *Galleria mellonella* (wax moth) larvae with *Metarhizium anisopliae*. Methodology for miRNA extraction and was developed and, following Illumina MiSeq sequencing, *Metarhizium*-specific miRNAs differentially expressed at different stages of infection that align to coding and non-coding regions of the *M. robertsii* genome sequence were identified.

545T Alteration of polyamine metabolism negatively affects *Aspergillus flavus* development and pathogenesis during its interaction with corn kernels. *R. Majumdar*[1], J. Cary[1], S. Minocha[2], M. Lebar[1], B. Mack[1], C. Sickler[1], C. Carter-Wientjes[1], K. Rajasekaran[1], D. Bhatnagar[1] 1) Food and Feed Safety Research Unit, USDA-ARS, New Orleans, LA; 2) Dept. of Biological Sciences, University of New Hampshire, Durham, NH.

Polyamines (PAs) are low molecular weight polycations that regulate DNA replication, transcription and translation in living cells. They have been shown to positively influence growth, development, and pathogenicity in both plant and human pathogens. Among the three predominant polyamines (putrescine, Put; spermidine, Spd; spermine, Spm), Spd is absolutely required for cell division. Spermidine (triamine) is synthesized from Put (diamine) by spermidine synthase. Spermidine serves as the donor of a 4-aminobutyl group to the Lys50 residue of eukaryotic elongation factor 5A (eIF5A) to produce the rare amino acid, hypusine. Hypusination-mediated activation of eIF5A is critical for initiation of translation. *A. flavus* is an opportunistic fungus that infects corn and other oilseed crops and produces the toxic and carcinogenic mycotoxins, aflatoxins, upon infection. To explore the role of spermidine synthase (s*pds)* in *A. flavus* pathogenesis, we disrupted the single copy of the *spds* gene and studied the ability of *spds* mutants to grow on artificial media as well as infected corn

kernels. Inactivation of s*pds* significantly reduced mycelial growth and sporulation on artificial media and an exogenous supply of Spd (auxotroph) was required to support fungal growth and development. Infection of corn kernels using *spds* mutant, shows significantly less fungal growth and sporulation (vs. controls). These results suggest that impairment of Spd biosynthesis negatively affects *A. flavus* pathogenesis and pre-treatment (prior to seed inoculation) of a *spds* mutant with Spd and possible Spd acquisition from the host, are not sufficient to restore wild-type levels of pathogenesis during seed infection. On the other hand, dual disruption of the ornithine decarboxylase (*odc)* gene (involved in Put biosynthesis) and a putative PA uptake transporter had little effect on fungal growth and sporulation. The significance of these observations will be discussed in context to the expression of *A. flavus* genes associated with polyamine metabolism (biosynthesis, uptake, and catabolism) and aflatoxin biosynthesis, and aflatoxin contents in the *spds* mutant vs. wild-type *A. flavus* infected corn kernels.

546F Ramularia collo-cygni and barley host-pathogen interactions. *E. Sjökvist*[1,5], R. Lemcke[2], J.-B. Lopez[3], M. Kamble[3], S. Visentin[3,4], M. Blaxter[5], A. Newton[4], E. James[4], S. Dam[3], R. Hjortshøj[6], N. Havis[1], M. Foged Lyngkjær[2], S. Radutoiu[3] 1) SRUC, Crop and Soil, Edinburgh, UK; 2) Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; 3) Department of Molecular Biology and Genetics, Aarhus University, Denmark; 4) The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK; 5) Institute of Evolutionary, Biology School of Biological Sciences, University of Edinburgh, UK; 6) Sejet Planteforædling, Horsens, DK.

Ramularia collo-cygni is a fungal pathogen of barley, and the causal agent of Ramularia leaf spot (RLS), which is now a major economic disease in many countries. R. collo-cygni has a global distribution and is spread both via the barley seed and through airborne conidiospores. The fungus has a long asymptomatic growth stage in the host plant and only causing necrotic spots after the plant experiences a stress e.g. waterlogging or flowering. To uncover the underlying genetics of the R. collo-cygni host/pathogen interaction and the transition from an endophytic to necrotrophic lifestyle we have combined RNA-sequencing, genome sequencing, proteomics, effector prediction and microscopy work. We will present the up-to-date results from this on-going
Danish government funded collaborative project.

547W VdNUC-2 regulates *Verticillium dahliae* infection through a novel Signaling pathway. *S. Deng*, C.Y. Wang, S.Q. Luo, X. Zhang, Q. Wang, L. Lin Jiangsu Academy of Agricultural Sciences, Nanjing, China.

Purpose: In soil-borne fungal vascular wilt pathogen *Verticillium dahliae*, we characterized a pathogenicity-related gene *VdNUC-2*, named after its homolog *NUC-2* in *Neurospora crassa*. *NUC-2* and its homologs (such as *Pho81* in *Saccharomyces cerevisiae*) belong to cyclin-dependent kinase inhibitor, and are known for their essential roles in the regulation of the phosphate (Pi) responsive signaling and metabolism (PHO) pathways, which keep the homeostasis of Pi in fungal cells. Except the PHO pathway, our recent results indicated that *VdNUC-2* participates a pathogenic regulation pathway which is indispensable for the penetration of host physical barriers.
Methods and materials: The wild-type strain V07DF2 was isolated from infected cotton in natural field. The original *VdNUC-2* mutant, named 6C4, was obtained by T-DNA random insertion. Other two *VdNUC-2* targeted deletion mutants, two ectopic complementation strains and three *VdNUC-1* targeted deletion mutants were introduced. VdNUC-1 is a crucial transcription factor located downstream of VdNUC-2, and it is positively regulated by VdNUC-2 in the PHO pathway. We observed cultural phenotypes of these strains under normal or low Pi culture condition, and accessed their virulence by employing the root irrigation method (intact root inoculation) or the root-dip method (wounded root inoculation). For further investigating the pathway(s) VdNUC-2 involved in, we performed yeast two-hybrid analysis and RNA-seq (total RNA isolated from inoculated plant roots).
Results and conclusion: ? All the strains grew normally on Czapek-Dox media with normal Pi content. However, compared with V07DF2 and the complementation strains, *VdNUC-2* and *VdNUC-1* mutants display growth defects under low Pi circumstances. ? The virulence of *VdNUC-2* mutants were significantly compromised on the cotton seedlings with intact roots, and that was unable to be restored by exogenous application of extra Pi. However, on the seedlings with wounded roots, the *VdNUC-2* mutants exhibit strong virulence and kill the hosts. Remarkably, the *VdNUC-1* mutants always displayed strong virulence as well as the wild-type strain regardless of inoculation method. These results proved that VdNUC-2 participates in at least two pathways, one is the PHO pathway, the other is pathogenic regulation pathway. ? Six proteins interacted with VdNUC-2 in yeast cells, and more than 800 genes were found with differential expression pattern in 6C4. Some of the differential expression genes play roles in cell wall hydrolysis, that might explain why the *VdNUC-2* mutants fail to penetrate the host physical barrier. For example, e*ndoglucanase EG1 gene* (VDAG_07825), encoding a Glycosyl hydrolase, has been proved to be a pathogenicity-related gene, and its log2-Ratio (6C4/V07DF2) are -1.85 (2 dpi) and -2.24 (5 dpi).

548T Identification of an NB-LRR-encoding gene conferring sensitivity to victorin in *Phaseolus vulgaris.* *Jenny Lorang*, Thomas Wolpert, Christina Hagerty Oregon State University, Corvallis, OR.

Breeding disease resistant plants is an essential, environmentally-favorable strategy for preventing crop loss due to disease. However, selection for resistance to one disease can inadvertently select for susceptibility to another. The species *Cochliobolus victoria* was described in 1946 as the casual agent of Victoria blight of oats but the pathogen was also associated with several weedy species, soybean, and barley. By the late 1940s Victoria blight of oat had reached epidemic proportions because susceptible oat genotypes had been widely planted as a source of resistance to the crown rust fungus *Puccinia coronata*. A dominant gene conditioning susceptibility to Victoria blight, *Vb,* was found to be genetically inseparable from the *P. coronata* resistance gene, *Pc2*. The *Vb* gene also was found to condition sensitivity to the *C. victoriae* toxin victorin. Recent studies of Arabidopsis, sorghum, barley and wheat suggest that necrotrophic pathogens are able to coopt resistance responses to cause disease. In this work we investigated the genetic basis of victorin sensitivity in *Phaseolus vulgaris*. We evaluated victorin sensitivity in Stampede x Red Hawk recombinant inbred lines (RILs) and identified a victorin sensitivity-associated QTL that contains Nucleotide Binding-Leucine Rich Repeat (NB-LRR) genes, a class of disease resistance (R) genes. One of these R genes conferred victorin-induced cell death when expressed in N. benthamiana and Arabidopsis. This gene is likely maintained in *P. vulgaris* for resistance functions, but could also be a source of necrotrophic disease susceptibility.

549F The RNAi system in the Brassicaceae smut fungus *Thecaphora thlaspeos*. *K. Boesch*[1,2], L. Frantzeskakis[1], K. Courville[1], M. Feldbrügge[1,2,3], V. Göhre[1,2,3] 1) Heinrich-Heine-University, Düsseldorf, NRW, DE; 2) Bioeconomy Science Center (BioSC), Jülich, NRW, DE; 3) Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, NRW, DE.

Smut fungi infect important crop plants, e.g. maize or potato. To develop novel plant protection strategies, the infection process has to be characterized molecularly. Genetic manipulation of the crop host plants is still difficult because of their complex genomes. For this reason, we want to characterize the interaction of the smut fungus *Thecaphora thlaspeos* with the model plant *Arabidopsis thaliana*.
T. thlaspeos infects different Brassicaceae throughout Europe, among them the perennial plant *Arabis alpina*. Its lifecycle differs in two important steps from crop smut fungi such as *Ustilago maydis*: First, teliospores only germinate in the presence of a plant-derived signal. Second, there is no dimorphism. Filaments emerge from teliospores that are directly infectious. Importantly, these filaments can be propagated as haploid cultures enabling genome sequencing and genetic manipulation.
Interestingly the genome encodes components of the RNAi machinery, which are absent in *U. maydis* but present in the smut fungus *Sporisorium reilianum*. Cross-kingdom RNAi is used by *Botrytis cinerea* to modulate *Arabidopsis thaliana*'s immune system. Based on reduced virulence of the DICER deletion mutant, it is hypothesized that sRNAs are generated in the fungus, transported into the host plant's cell and there silences expression of specific genes involved in immunity. With our new model system we will study how *T. thlaspeos* uses RNAi and ultimately investigate whether also a smut fungus utilizes cross-kingdom RNAi during infection.

550W Elucidating how *Histoplasma capsulatum*, a fungal intracellular pathogen, interferes with host cell biology. *Rosa Rodriguez*, Nancy Van Prooyen, Dervla Isaac, Anita Sil Dept. of Microbiology and Immunology, UCSF, San Francisco, CA.

Histoplasma capsulatum (Hc), a human intracellular fungal pathogen, subverts the normal biology of eukaryotic cells. *Hc* is a ubiquitous soil fungus that is aerosolized and inhaled into the mammalian lung when the soil is disrupted. Once in the lungs, *Hc* is phagocytosed by macrophages where it replicates within the phagosome by blocking acidification and maturation of this organelle through largely unknown mechanisms. The ability to colonize and lyse macrophages is thought to be a key step in successful infection. Our lab identified *Hc* insertion mutants that were defective in their ability to lyse bone-marrow-derived macrophages (BMDMs) during infection. One of these mutants harbors an insertion in the *SNX8* (sorting nexin 8) gene, so-named for its ortholog in mammalian cells. Sorting nexins (SNXs) are a family of proteins that are classified by the presence of phox-homology (PX) domains, which confer phosphoinositide (PtdInsP) lipid binding specificity. This lipid specificity recruits SNXs to PtdInsP rich early endosomes and SNXs are involved in a variety of cellular processes including membrane trafficking and remodeling. Our preliminary data indicate that the *Hc snx8* mutant is defective in manipulation of BMDMs. In culture, the *snx8* mutant has no growth defect; however, in BMDMs, it has a slight growth delay and fails to elicit host-cell lysis. Additionally, we used transmission electron microscopy (TEM) to compare the appearance of the phagosome during BMDM infection with either wild-type or *snx8* mutant *Hc*. We found that by 12 hours post infection (hpi), *snx8* mutants reside in a more spacious phagosome than wild-type *Hc*, indicating that the *snx8*-containing phagosome is fundamentally different from wild-type. We have also determined that the *snx8* mutant is avirulent in the mouse model of *Hc* infection, indicating that the inability of the *snx8* mutant to manipulate the host is detrimental to pathogenesis. We are using the *snx8* mutant as a tool to elucidate how wild-type *Hc* manipulates phagosome biology.

551T A first glimpse into the genetic architecture of pathogenicity of Zymoseptoria tritici on bread wheat. *T.C. MARCEL*[1], A. DUCASSE[1], J. COMPAIN[2], N. LAPALU[1,2], Z. ZHONG[3], F. HARTMANN[3], J. CONFAIS[1], H. GOYEAU[1], J. PALMA-GUERRERO[3], G.H.J. KEMA[4], J. AMSELEM[2], D. CROLL[3] 1) BIOGER, INRA, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France; 2) URGI, INRA, Universite´ Paris-Saclay, Versailles, France; 3) Plant Pathology Group, ETH Zürich, Zürich, Switzerland; 4) Plant Research International B.V., Wageningen, The Netherlands.

Zymoseptoria tritici is a fungal pathogen of wheat responsible for the septoria leaf blotch disease. This disease is a major concern in bread and durum wheats growing areas worldwide. It can be controlled by fungicide treatments and the use of resistant wheat cultivars. To date, 21 resistance genes and 89 QTLs for resistance to septoria leaf blotch have been mapped in bread wheat. Despite this large availability of resistant sources, it remains a challenge to improve the resistance levels of wheat elite cultivars because of the mostly quantitative nature of wheat-septoria interactions and of the very high level of genetic diversity within pathogen populations. Our objective is to understand the genetic architecture of pathogenicity in *Z. tritici* by identifying its determinants and revealing the specificity of their interaction with resistance determinants in wheat. We have undertaken a genome wide association mapping approach (GWAS) to identify pathogenicity genes in *Z. tritici*. A collection of more than 2000 isolates has been established in France. The genetic diversity has been characterized in this collection, and 109 isolates selected for resequencing and pathogenicity assays. In so doing, we obtained the virulence spectra of the 109 French isolates, revealing the efficiency of known resistance *STB* genes in France, and allowing to detect known and unknown *STB* genes present in French elite cultivars. Furthermore, combining the precision of our pathology assays, the selection of wheat genotypes well characterized for their resistance determinants, and the precision of GWAS in our *Z. tritici* population, we have identified several candidate avirulence genes interacting with known resistance genes in bread wheat. Thus far, the small secreted protein *AvrStb6* is the first avirulence gene to be identified and functionally validated in this important wheat pathogen.

552F A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. Ziming Zhong[1], Thierry Marcel[2], Clémence Plissonneau[1], Bruce McDonald[1], Daniel Croll[1], *Javier Palma-Guerrero*[1] 1) Plant Pathology Group, ETH Zürich, Switzerland; 2) UMR BIOGER, INRA, , France.

Zymoseptoria tritici is the causal agent of Septoria tritici blotch (STB), a major pathogen of wheat globally and the most damaging pathogen of wheat in Europe. The *Z. tritici* infection cycle includes an extended asymptomatic phase characterized by slow apoplastic growth followed by a switch to necrotrophy that coincides with the onset of plant cell death. Gene-for-gene (GFG) interactions between this apoplastic pathogen and resistant wheat cultivars have been postulated for many years, but until now none of the genes involved in this interaction were identified. We identified *AvrStb6* by combining QTL mapping based on a cross between two Swiss strains with a genome-wide association study (GWAS) using a natural population of ~100 strains coming from France. We functionally validated *AvrStb6* using ectopic transformations. *AvrStb6* encodes a small, cysteine-rich, secreted protein, with features of fungal effector proteins, which produces an avirulence phenotype on wheat cultivars carrying the *Stb6* resistance gene. *AvrStb6* shows peak of expression during the transition to the necrotrophic phase suggesting that the protein may act as a necrotrophic effector in susceptible cultivars. We found 16 non-synonymous SNPs among the tested strains, indicating that *AvrStb6* is evolving very rapidly. By analyzing the finished genomes of both parental strains used to establish the cross, we found that *AvrStb6* is located in a highly polymorphic subtelomeric region and is surrounded by transposable elements. This genomic environment may facilitate the rapid evolution of *AvrStb6* and enable *Z. tritici* to rapidly adapt to

overcome *Stb6* resistance. *AvrStb6* is the first avirulence gene to be functionally validated in *Z. tritici*, contributing to our understanding of avirulence in apoplastic pathogens and the mechanisms of GFG interactions between *Z. tritici* and wheat.

553W Construction and efficacy of Δ*cps1*, a live attenuated vaccine for coccidioidomycosis. *M. A. Mandel*[1,4], L. F. Shubitz[1], L. Lewis[1], H. T. Trinh[1], A. S. Buntzman[2], J. A. Frelinger[3], J. N. Galgiani[2], M. J. Orbach[1,4] 1) The Valley Fever Center for Excellence, Tucson, AZ; 2) Department of Medicine, University of Arizona, Tucson, AZ; 3) Department of Immunobiology, University of Arizona, Tucson, AZ; 4) The School of Plant Sciences, University of Arizona, Tucson, AZ.

Coccidioidomycosis (Valley Fever, VF) is a fungal disease of mammals endemic to the SW US and parts of Mexico, Central and South America, caused by *Coccidioides immitis* and *C. posadasii*. While most people experience mild flu-like symptoms, this pathogen can cause meningitis and is responsible for 160 deaths and $170 million in hospital costs in CA and AZ annually. Infection results in lifelong immunity, suggesting a vaccine against VF is attainable. We have developed a live attenuated vaccine by deletion of *CPS1*, the ortholog of a *Cochliobolus heterostrophus* virulence factor. Our *Δcps1* strain is non-pathogenic in susceptible mice, and provides dramatic protection against WT infection. Current analyses indicate that Cps1 is a member of the AMP-dependent synthetase/ligase family of proteins related to Dip2 in animals. The role of Cps1 in the parasitic phase is unknown, but evidence shows that the *Δcps1* strain initiates spherule formation but fails to propagate beyond a first generation in the host. We present data showing that vaccination results in long-term survival in mice given lethal doses of *Coccidioides spp.*, and that protection lasts at least 6 months following a single dose of live spores. The *Δcps1* strain forms defective thin-walled spherules that fail to endosporulate and that lyse prematurely. *In silico* analysis suggests Cps1 is a transmembrane protein and transcriptional analysis supports a role as a transcriptional regulator. The original *Δcps1* strain has the complete ORF replaced by the hygromycin phosphotransferase marker. The presence of this marker is a safety and regulatory concern for a live vaccine. To create a new *Δcps1* strain lacking an antibiotic resistance marker, we used Biolog nutritional and genomic data to determine that *Coccidioides spp.* are unable to use sucrose as a carbon source. We demonstrate that the *A. niger sucA* gene can be introduced into *Coccidioides,* allowing selection on sucrose and are using this gain-of-function strategy for the new vaccine strain design.

554T A basidiomyceteous fungi-specific *PsCaMKL1* encoding a CaMK-like protein kinase is required for the virulence and oxidative tolerance of *Puccinia striiformis* f. sp. *tritici*. M Jiao, ZS Kang, *J Guo* State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China.

Calcium/calmodulin-dependent kinases (CaMKs) are Ser/Thr protein kinases (PKs) that respond to changes in cytosolic free Ca^{2+} and play diverse roles in eukaryotes. In fungi, CAMKs are generally classified into four families CAMK1, CAMKL, RAD53 and CAMK-Unique. Among these, CAMKL is the largest family. In some fungal plant pathogens few CaMKLs have been shown to be responsible for pathogenesis, but little is known about their roles in rust fungi. In this study, we functionally characterized a novel PK gene, *PsCaMKL1*, from *Puccinia striiformis* f. sp. *tritici* (*Pst*). PsCaMKL1 belongs to a group of PKs that are evolutionarily specific to basidiomyceteous fungi. It shows a highly conserved intra-species polymorphism and cytoplasmic localization in wheat protoplasts. *PsCaMKL1* is highly induced at early infection stages. The CaMK inhibitor KN93 blocked *Pst* urediospore germination and suppressed transcription of *PsCaMKL1* during *Pst* germination. Overexpression of *PsCaMKL1* in fission yeast increased resistance to oxidative stress. Knock down of *PsCaMKL1* using host-induced gene silencing (HIGS) reduced the virulence of *Pst* accompanied by enhanced ROS accumulation and a hypersensitive response. These results suggest that *PsCaMKL1* is a novel and important factor that exerts it virulence function by regulating ROS production in wheat.

555F Identification of novel classes of effector proteins of multicellular fungi against nematodes by challenge of vegetative mycelia with fungivorous nematodes in microfluidics devices. *Annageldi Tayyrov*[1], Stefanie S. Schmieder[1], Aleksandr Goryachkin[1], Claire E. Stanley[2], Andrew J. DeMello[2], Markus Aebi[1], Markus Künzler[1] 1) Institute of Microbiology, Department of Biology, ETH Zürich, Vladimir-Prelog-Weg 4, CH-8093 Zürich, Switzerland; 2) Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Vladimir-Prelog-Weg 1, CH-8093 Zürich, Switzerland.

Multicellular fungi defend themselves against antagonists that share a same growth environment with them. For this purpose, the non-motile fungi have developed several defense lines. In addition to physical defense lines, such as the buildup of melanized outer layers or chitinous cell walls, fungi mainly rely on chemical defense mediated by effector molecules (toxins). Characterized classes of fungal defense effectors include secondary metabolites, peptides and proteins. The best known examples of these classes are penicillin, alpha-amanitin and fungal pore-forming toxins/protease inhibitors/lectins, respectively. Most likely, this list of fungal defense effectors is not complete and, thus, novel approaches for the identification of such molecules are needed in order to explore the fungal defensome.
It has been shown that genes coding for fungal defense effectors can be identified on the basis of differential gene expression upon challenge with antagonists. Hence, in this study we conduct a genome-wide analysis of transcription (RNA sequencing) of the model mushroom *Coprinopsis cinerea* challenged by the fungivorous nematode *Aphelenchus avenae* at different time points in a tailor-made microfluidics device. Based on the expression profile of genes coding for already characterized anti-nematode effector proteins of *C. cinerea*, we set up a list of putative novel classes of anti-nematode effector proteins of this fungus. The list contained two types of cytoplasmic proteins with putative lipase domains that showed nematotoxicity when heterologously expressed in *Escherichia coli* and tested for toxicity towards the bacterivorous model nematode *Caenorhabditis elegans*. Site-specific mutagenesis of predicted catalytic residues reduced the toxicity significantly. Our results suggest that cytoplasmic lipases may constitute a novel class of fungal effector proteins against nematodes.

556W Ancient horizontal transfer of a toxin biosynthesis cluster reveals novel mechanisms in the cercosporin biosynthesis pathway. *Melvin Bolton*[1], Ronnie de Jonge[2], Malaika Ebert[3], Bart Thomma[3] 1) USDA - ARS, Fargo, ND; 2) Department of Plant Microbe Interactions, Utrecht University, Utrecht, The Netherlands; 3) Department of Phytopathology, Wageningen University, Wageningen, The Netherlands.

Cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola* is considered to be the most destructive foliar disease of sugarbeet worldwide. The ability of the fungus to cause disease relies primarily on the production of a toxin called cercosporin during infection. Cercosporin is a photo-activated secondary metabolite toxic to a wide array of organisms including bacteria, mice, and plants but not to

cercosporin-producing *Cercospora* species. The cercosporin biosynthesis pathway has been well-characterized and thought to consist of eight cercosporin toxin biosynthesis (*CTB*) genes. We used phylogenomics to show that the *CTB* cluster is not limited to *Cercospora* species and has experienced an unprecedented number of duplications, losses, and horizontal transfers across a spectrum of plant pathogenic fungi during evolution. Since comparative genomic analysis revealed extensive gene collinearity adjacent to the established *CTB* cluster in all *CTB*-harboring species, we confirmed that *CTB* is larger than previously recognized and includes at least five additional genes, three of which are required for cercosporin biosynthesis. Additionally, the expanded cluster encodes the major facilitator superfamily transporter CFP, which contributes to cercosporin auto-resistance in *C. beticola*. Together, our results give new insight on the intricate evolution of the *CTB* cluster.

557T Functional characterization of TvCyt2, a p450 monooxygenase relevant during plant-fungus interaction and mycoparasitism by Trichoderma virens. *Vianey Olmedo-Monfil*, Daniela Porras-Troncoso, Claudia Ramírez-Valdespino, Alma Corrales-Escobosa , Kazimierz Wrobel DCNyE Universidad de Guanajuato. Guanajuato, Gto., MX.

Functional characterization of TvCyt2, a p450 monooxygenase relevant during plant-fungus interaction and mycoparasitism by *Trichoderma virens*

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In nature plants interact with harmful and beneficial organisms, in both cases the plant defense pathways can be activated through Microbe-Associated Molecular Patterns. In these interactions are also involved effector molecules, that can alterate structure and function of the host and are determinants in the type of association that is going to be established. The genus *Trichoderma* includes species of fungi widely known for its mycoparasitic activity. Furthermore, during its interaction with plants, activate defense mechanisms and promote plant growth and development, providing plant protection against infection by pathogens and abiotic stress. There is little information regarding *Trichoderm*a effector molecules and their possible role in establishing the biological interactions. With the interest of identifying these molecules, we select 15 genes encoding possible effector type proteins, from a predicted secretoma of *Trichoderma*. Validation by qRT-PCR showed that three of these genes increased their expression level during interaction with the plant, while one of them, named *Tvcyt2*, showed a significant decrease on its expression. *Tvcyt2* encodes an enzyme classified as a monooxigenase P450. This kind of enzymes are involved in metabolic pathways related to detoxification processes and secondary metabolites synthesis. In order to determine the possible role of this gene in *Trichoderma*, we analyzed the metabolic profile obtained from overexpressing (OE*TvCyt2*) and null mutant (Δ*tvcyt2*) strains, finding secondary metabolites present only in the OE*TvCyt2* strain. The strains showing this modified metabolic profile had a better antagonistic activity against *Rhizoctonia solani* and activated with greater intensity defense pathways in *Arabidopsis*. Additionally, these strains promoted an increase in biomass and fitness in tomato plants. Our results indicate that TvCyt2 participates in the synthesis of secondary metabolite, which have a relevant role during plant-fungus interaction and mycoparasitism by *Trichoderma virens.*

558F The role of BoiA, a Boi1-like protein, in fungal-plant symbiosis. *E. L. Bradley*[1,2], PY. Dupont[1,2], R. Bradshaw[1,2], A. Ram[1], B. Scott[1,2], C. J. Eaton[1,2] 1) Institute of Fundamental Sciences, Massey University, Palmerston North, NZ; 2) Bio-Protection Research Centre, Massey University NZ.

Reactive oxygen species (ROS) produced by the fungal NADPH oxidase (Nox) complex play an important role in regulating both mutualistic and antagonistic fungal-plant interactions. In the mutualistic symbiotic interaction between the fungal endophyte *Epichloë festucae* and its grass host *Lolium perenne*, loss of ROS signalling through disruption of *noxA* leads to a dramatic change in the host-interaction phenotype (Tanaka et al. 2006). Hyphae are defective in cell-cell fusion and undergo proliferative, instead of restrictive, growth in the leaves of the host. Plants infected with Δ*noxA* are severely stunted and prematurely senesce. The fungal NoxA complex consists of membrane bound NoxA and NoxD components and cytosolic subunits NoxR, RacA, BemA and Cdc24, which are recruited to the membrane in response to some as yet unidentified signal. Activation of the mammalian Nox2 (gp91phox) complex involves changes in the cell membrane phosophoinositides, which leads to recruitment of the PX-domain cytosolic components, p47phox and p40phox, together with p67phox and Rac1. Although BemA contains a putative phosphoinositide-binding PX domain, this has been shown to be dispensable for membrane localisation in *Neurospora crassa* (Schürg et al. 2012). Instead, we hypothesize that recruitment of BemA occurs via interaction with the phosphoinositide-binding Boi1-like protein, which is known to interact with Bem1 in yeast. *E. festucae* contains two Boi1-like proteins, designated BoiA and BoiB. Phylogenetic analysis suggests *E. festucae* genes encoding these proteins arose from a common ancestor shared with *Saccharomyces cerevisiae BOI1* and *BOI2*. Transcriptome studies showed that *boiA* is highly expressed *in planta* compared to in culture, and up-regulated in mutants that disrupt the mutualistic interaction, suggesting an important role for *boiA* in symbiotic maintenance (Eaton et al. 2015). Phenotype analysis of Δ*boiA* deletion mutants revealed a loss of hyphal cell-cell fusion in culture compared to wild-type. Plants infected with Δ*boiA* were hypertillered and had a significant reduction in tiller length compared to the wild-type strain. Hyphae of Δ*boiA*, unlike wild-type, formed convoluted structures within the leaves, colonised vascular bundles and were frequently broken. These results show that BoiA is required for establishment of a mutualistic symbiotic interaction between *E. festucae* and *L. perenne*.

559W Mollicutes-related endobacteria in the Mortierellomycotina. *A. Desirò*[1], Z. Hao[1], GMN. Benucci[1], N. Vande Pol[1], Y. Chang[2], K. Barry[3], I. Grigoriev[3], JW. Spatafora[2], G. Bonito[1] 1) Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI; 2) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; 3) Department of Energy, Joint Genome Institute, Walnut Creek, CA.

Bacterial interactions with animals and plants have been examined for over a century; in contrast, the study of bacterial-fungal interactions has received less attention until recently. Bacteria and fungi interact at different levels and bacterial endosymbionts, which dwell inside fungal cells, provide the most intimate example. The most notable bacterial endosymbionts are associated with fungi belonging to the phylum Mucoromycota. The coenocytic nature of these fungi offers a niche for two types of endobacteria: *Burkholderia*- and Mollicutes/*Mycoplasma*-related endobacteria (MRE). MRE have been identified within the mycelium and spore of two out of the three Mucoromycota subphyla, the Glomeromycotina and Mucoromycotina; differently, MRE have never been reported in the Mortierellomycotina. We screened over 350 Mortierellomycotina strains for the presence of MRE and detected novel endobacteria phylotypes in nine of them.

MiSeq 16S rDNA amplicon data demonstrated that a single MRE population thrives within each strain. We were successful in "curing" the fungal culture of its endosymbionts and designed experiments to assess impacts of these bacteria on their fungal host. We report on the morphology and phenotypic changes in fungal host biomass and growth when inhabited by MRE. Genome analysis and metatranscriptome data from MRE and their fungal host are expected to provide novel insights on the ecological and evolutionary role of these enigmatic microbes.

560T Extremotolerant fungi: survivability via symbiosis. *E. Carr*[1], S. Harris[2] 1) School of Biological Sciences, University of Nebraska Lincoln, Lincoln, NE; 2) Dept. of Plant pathology, Plant Science Innovation, University of Nebraska Lincoln, Lincoln, NE.

The black yeasts are a group of fungi from the subdivision Pezizomycotina that are known for their extremotolerance. Some of the conditions they can withstand are heat, freezing, desiccation, osmolarity, UV, heavy metals, and in some cases radiation. Unique features of these organisms make them intriguing to study alone, but may also provide insight into the phylogenetically related lichen-forming fungi. Since lichens and these polyextremotolerant fungi share similar resistance features, the black yeasts may enable study of the lichen lifestyle without the challenges of directly working with lichens. In addition to sharing similar traits, black yeasts are also almost always found in the same location as algae, even in desert and polar ecosystems. This raises the intriguing possibility that these organisms interact in a lichen-like manner. We propose that black yeasts and algae indeed interact in a mutualistic fashion that resembles lichens, and that observing these interactions will reveal clues about the nature of the fungal-algal communication that underlies lichen formation. To test our hypothesis we have isolated black yeasts and algae from biological soil crusts and rock surfaces, identified isolates using their ITS sequences, subjected isolates to multiple stressors and nutritional conditions to investigate their phenotypic diversity, and co-cultured the fungi and algae together in a pair-wise manner. Isolates that were taken from a biological soil crust in one location resulted in 24 black yeasts and 43 algal isolates. These have been extensively phenotyped for carbon utilization, nitrogen utilization, temperature resistance, UV resistance, and metal resistance allowing us to observe a moderate range of diversity. Current efforts are underway to integrate the phylogeny and phenotype data, as well as co-culturing the black yeasts with the algal isolates to observe symbiotic tendencies and any phenotypes associated with symbiosis.

561F SymB and SymC, two membrane associated proteins, are required for *Epichloë festucae* hyphal cell-cell fusion and maintenance of a mutualistic interaction with *Lolium perenne.* *K.A. Green*[1,2], Y Becker[1,3], A Tanaka[4], D Takemoto[4], H.L. Fitzsimons[1], S Seiler[5], H Lalucque[6], P Silar[6], B Scott[1,2] 1) Institute of Fundamental Science, Massey University, Palmerston North, Manawatu, New Zealand; 2) Bioprotection Research Centre, Massey University, Palmerston North, New Zealand; 3) Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany; 4) Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; 5) Freiburg Institute for Advanced Studies, Albert-Ludwigs Universität Freiburg, Freiburg, Germany; 6) Univ Paris Diderot, Sorbonne Paris Cité, Laboratoire des Energies de Demain, Paris, France.

Epichlo? festucae is a filamentous fungus that forms a mutually beneficial symbiotic association with *Lolium perenne*. The NADPH oxidase complex components *noxA, noxR* and *racA*, the transcription factor *proA*, and the cell wall integrity (CWI) MAP kinases, *mkkA* and *mpkA*, are required for mutualistic *E. festucae-L. perenne* associations and cell-cell fusion. Homologues of these genes in *Neurospora crassa, Sordaria macrospora* and *Podospora anserina* are required for cell-cell fusion and sexual fruiting body maturation. We examined whether *symB* and *symC*, the *E. festucae* homologs of *Podospora anserina* self-signaling genes *IDC2* and *IDC3*, are required for *E. festucae* hyphal fusion and host symbiosis. Deletion mutants of these genes were defective in hyphal cell fusion, formed intra-hyphal hyphae, and had enhanced conidiation. SymB-GFP and SymC-mRFP1 localize to plasma membrane, septa and points of hyphal cell fusion. Plants infected with Δ*symB* and Δ*symC* strains were severely stunted. Hyphae of the mutants colonized vascular bundles, were more abundant than wild type in the intercellular spaces and formed intra-hyphal hyphae. Although these phenotypes are identical to those previously observed for cell wall integrity MAP kinase mutants no difference was observed in the basal level of MpkA phosphorylation or its cellular localization in the mutant backgrounds. Both genes contain binding sites for the transcription factor ProA. Collectively these results show that SymB and SymC are key components of a conserved signaling network for *E. festucae* to maintain a mutualistic symbiotic interaction within *L. perenne*.

562W A novel bacterial endosymbiont related to *Mycoavidus cysteinexigens* inhabits the mycelium of *Mortierella minutissima*. *Gregory Bonito*[1], Alessandro Desiro[1], Jessie Uehling[2], Natalie Vande Pol[1], Kurt LaButti[3], Stephen Mondo[3], Kerrie Barry[3], Igor Grigoriev[3] 1) Michigan State University, East Lansing, USA; 2) Duke University, Durham NC, USA; 3) Joint Genome Institute, Walnut Creek CA, USA.

Mortierella are an early diverging genera of soil and plant associated fungi that are known to harbor bacterial endosymbionts. The genome of one of these symbiotic microbes, a *Burkholderia*-related bacterium named *Mycoavidus cysteinexigens*, was recently sequenced from two different *Mortierella elongata* strains isolated in Japan and North America. Here we report on another *Mycoavidus* species that we detected within *Mortierella minutissima.* We sequenced both the fungal host *M. minutissima* and endosymbiont genome with PacBIO. The 2.9 MB genome of this bacterium includes 2682 protein coding sequences and is the largest yet for a bacterium belonging to the fungal-associated Glomeribacter-lineage. The genome of this bacterium appears to have type II and type IV secretion systems and many phage capsule and tail protein coding genes. Phylogenetic analyses place this bacterium as sister species to *Mycoavidus cysteinexigens,* within the /glomerobacter lineage. Fungal isolates "cured" of their endobacteria show a difference in fitness when grown under various culture conditions. Transmission studies will be carried out to determine whether bacteria can be acquired horizontally between *Mortierella* species.

563T The role of SreA-mediated iron regulation in maintaining endophyte-grass symbioses. Natasha Forester[1], Paul Maclean[1], Geoffrey Lane[1], Iain Lamont[2], *Linda Johnson*[1] 1) Forage Improvement, AgResearch Limited, Palmerston North, Manawatu, NZ; 2) University of Otago, Dunedin, New Zealand.

In ascomycetes and basidiomycetes, an iron-responsive GATA-type transcriptional repressor is involved in regulating iron homeostasis. Functional characterisation of an orthologue, SreA, from *Epichloë festucae*, a fungal endosymbiont of cool-season grasses, indicates that regulation of iron homeostasis processes is critical for symbiotic maintenance. The deletion of sreA (*ΔsreA*) led to the gradual loss of the endophyte from host plants (perennial ryegrass) and confocal microscopy studies identified anomalies in hyphal growth *in planta*, including

misshapen hyphae particularly at branching junctions. SreA also negatively regulates siderophore biosynthesis and high affinity iron uptake systems by *E. festucae* as shown for other fungi. Preliminary studies indicate that the endophyte tightly controls iron acquisition from the host apoplast via SreA, as *ΔsreA* mutants are able to induce premature chlorosis in their host plants during low iron availability.
To understand the influence of iron availability on gene expression and to identify genes that are targets of SreA, we compared the transcriptomes of plants infected with *ΔsreA* versus WT (wild-type), and WT-infected plants grown hydroponically under high versus low iron supply. A disproportionally large number of fungal genes were up-regulated in *ΔsreA* versus WT, indicating that these genes are likely direct targets of SreA. Two such highly differentially expressed genes were clustered and putatively encode small secreted proteins (SSPs). Conserved GATA motifs present in promoter regions, combined with up-regulation of SSP gene expression under low-iron availability, suggest a novel role for these SSPs in iron metabolism. SreA-responsive genes included genes previously identified as SreA-regulated as well as a diverse group of genes not obviously associated with iron metabolic processes. Putative siderophore biosynthetic genes not previously identified from *E. festucae* were also found, including gene links to the ergosterol biosynthetic pathway. Changes in endophyte secondary metabolite gene expression (for lolitrem and ergot alkaloid biosynthetic genes) with respect to either iron availability or SreA presence/absence were also observed, highlighting the importance and interconnection of iron availability with fungal growth and secondary metabolite production.

564F The *Ustilago maydis* effector Rsp3 shields hyphae and blocks the anti-fungal activity of a secreted maize protein. *Lay-Sun Ma*[1], Lei Wang[1,2], Christine Trippel[1], Artemio Mendoza-Mendoza[3], Steffen Ullmann[4], Marino Moretti[1], Stefan Wawra[5], Stefanie Reissmann[1], Karin Münch[1], Alexander Carsten[1], Bernd Zechmann[6], Regine Kahmann[1] 1) Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Marburg, Germany ; 2) Max Planck Institute for Heart and Lung Research, Department of Pharmacology, Bad Nauheim, Germany; 3) Bio-Protection Research Centre, Lincoln University, New Zealand; 4) Heinrich-Heine-Universität Düsseldorf, Molecular Microbiology, Düsseldorf, Germany; 5) University of Cologne, Botanical Institute, Cologne, Germany; 6) Center for Microscopy and Imaging, Baylor University, Texas, USA.

The biotrophic fungus *Ustilago maydis* causes smut disease in maize. Hallmarks of the disease are plant tumors and anthocyanin biosynthesis. Here we functionally characterize the secreted effector Rsp3 which contains several repetitive as well as a Cys-rich domain. *rsp3* alleles obtained from field isolates have full biological activity despite of length polymorphisms due to reduced or expanded numbers of certain repeats. *rsp3* is highly expressed during the biotrophic stage and is required for virulence and anthocyanin accumulation. Rsp3-HA can be easily detected in culture supernatants when expressed from a constitutive promoter, but displays highly anomalous migration behavior on SDS-PAGE. During biotrophic growth, Rsp3 decorates the surface of biotrophic hyphae. Using immunoprecipitation and mass spectrometry, we show that Rsp3 interacts with secreted maize DUF26 domain-family proteins. One of these (designated AFP1) was expressed and purified from *Nicotiana benthamiana*. AFP1 was able to bind mannose and showed antifungal activity against the *U. maydis rsp3* deletion mutant but not against a strain over-expressing *rsp3*. This suggests that the Rsp3 effector blocks the antifungal activity of the DUF26-containing maize protein, presumably by inhibiting its binding to the fungal cell wall.

565W The *Ustilago maydis* effector Ten1 interacts with a maize protein phosphatase 2C. *P. Erchinger*[1], J. Wu[1,2], R. Kahmann[1] 1) Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; 2) Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Science, PR of China.

The fungus *Ustilago maydis* is a pathogen that establishes a biotrophic interaction with *Zea mays*. The interaction with the plant host is largely governed by more than 300 novel, secreted effector proteins, many of which are encoded in gene clusters. The deletion of cluster 10A consisting of 10 effector-encoding genes results in strongly reduced virulence after seedling infection. By generating subdeletions and by complementing the full deletion of cluster 10A with individual genes we could show that *um03744* (termed *ten1*) has a major contribution to virulence. After overexpression in *U. maydis* hyphae, secreted Ten1 protein could be detected in axenic culture supernatant. Through a yeast 2-hybrid screen ZmPP26, a maize protein phosphatase 2C was identified as interaction partner of Ten1. Interaction was further supported by Co-IP experiments after co-expression of Ten1 and ZmPP26 in *Nicotiana benthamiana*. Moreover, ZmPP26 was detected by mass spectrometry after IP of 3xHA-Ten1 from infected maize tissue 52 hours post inoculation. Heterologously expressed ZmPP26 showed strong type 2C-specific phosphatase activity *in vitro*. Current work focuses on the recombinant production of Ten1 to see whether and how the interaction of both proteins alters phosphatase activity of ZmPP26. Furthermore, we are about to identify the interacting domain of Ten1 to be able to test a non-interacting allele in plant infections.

566T Structure-function analysis of Cmu1, a secreted chorismate mutase in *Ustilago maydis*. *X. Han*[1], J. Schuhmacher[2], F. Hartwig[1], A. Djamei[3], T. Glatter[1], R. Kahmann[1], G. Bange[2] 1) MPI for Terrestrial Microbiology, Marburg, DE; 2) LOEWE Center for Synthetic Microbiology (Synmikro), Marburg, DE; 3) Gregor Mendel Institute of Molecular Plant Biology, Vienna, AT.

The basidiomycete *Ustilago maydis* is the causal agent for corn smut disease. To successfully colonize the host plant, *U. maydis* secretes a repertoire of around 300 effector proteins to manipulate host processes and suppress plant defense responses. These effector proteins function either in the intercellular space (apoplast) or translocate to plant cells to exert their functions. Previously we have shown that a secreted chorismate mutase Cmu1 is a translocated effector. Cmu1 enters plant cells by a yet unknown mechanism and interferes with salicylic acid biosynthesis to promote fungal infection. To gain more insight into the biological function of Cmu1, we have recently solved its crystal structure by X-ray crystallography using *E. coli* expressed protein. The structure revealed several unique features which might contribute to the function of Cmu1, including a surface-exposed acidic patch, a conserved disulfide bridge and a long loop region situated adjacent to the catalytic site. We have followed up on the relevance of these features for uptake by plant cells, protein stability and allosteric regulation/catalytic activity of Cmu1, respectively. In addition, we have shown that a secreted chorismate mutase from *Sclerotinia sclerotiorum* possessing a chloroplast transit peptide (M. Dickman, personal communication) could complement the virulence phenotype of the *cmu1* mutant. Furthermore, with the help of co-immunoprecipitation followed by mass spectrometry we have identified a secreted maize protein which might be a target of Cmu1 in the apoplast.

567F Systematic identification of glycoproteins involved in the infection process of *Ustilago maydis.* Ismael Moreno-Sanchez, Ramon Barrales, *Jose Ibeas* Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-CSIC-Junta de Andalucía, Seville,

Spain.

Ustilago maydis has raised as an excellent model for the study of plant-pathogen interactions, and its relation with maize plant is one of the systems in which studies can be tackled from both plant and pathogen perspective (Dean et al., 2012; Djamei & Kahmann, 2012). Protein glycosylation are critical processes in host-pathogen relations. In fact, mutations in essential genes for O-glycosilation (*pmt4*) and N-glycosilation (*gls1* and *gas2*) compromise *U. maydis* virulence on maize, affecting different steps in the infection process (Fernandez-Alvarez et al., 2009; Fernandez-Alvarez et al., 2013). We have now performed a proteome analysis in order to identify cytoplasmatic, secreted and cell wall glycoproteins, produced only when the virulence program is activated by over-expressing the transcription factor Biz1. Up today we have identified by mass spectrometry and MASCOT analysis 48 glycoproteins (33 cytoplasmatic, 11 secreted and 4 from the cell wall), all dependent of the virulence program activation. A systematic characterization of the role of all this glycoproteins in the infection process is currently in progress.

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568W The comparison of β-1,3-glucanase activity between mycovirus-cured *Trichoderma atroviride* NFCF028 and mycovirus-infected *T. atroviride* NFCF028. Han-Eul Yang, Song Hee Lee, Suk-Hyun Yun, *Jeesun Chun*, Dae-Hyuk Kim Department of Molecular Biology, Department of Bioactive Material Sciences, Institute for Molecular Biology and Genetics, Chonbuk National University, Jeonju, Chonbuk 561-756, Korea.

Molecular characterization of the most common dsRNA element from a green mold disease-causing *Trichoderma atroviride* indicated that it comprised 8,566 bp and encoded two large open reading frames (ORF1 and 2). The two ORFs were found to overlap by 46 bp with a typical (-1) slippery heptanucelotide sequence. The deduced protein sequences of ORF1 and ORF2 showed significant similarities to those of known mycoviral structural proteins and RNA-dependent RNA polymerases, respectively. Phylogenetic analysis indicated that this dsRNA is a member of a distinct species related to a group of unclassified mycoviruses; therefore, it was named Trichoderma atroviride mycovirus 1 (TaMV1). In order to analyze the effect of TaMV1 infection, β-1,3-glucanase, which is a cell wall-degrading enzyme for controlling plant pathogens and has been associated with the green mold disease by *Trichoderma* spp., was examined. The β-1,3-glucanase activity of TaMV1-cured isolate was higher than that of TaMV1-infected parental *T. atroviride* isolate. In addition, fungal metabolite from the TaMV1-cured isolate was more toxic to other fungal growth, which suggested that the TaMV1 infection resulted in hypovirulence of *T. atroviride*.

569T Identifying virulence factors in the broad host range necrotrophic pathogen *Sclerotinia sclerotiorum.* Kassaye Belay[2], Christopher Misar[1], Mitchell DuFour[2], Berlin Nelson[2], Robert Brueggeman[2], *William Underwood*[1] 1) Sunflower and Plant Biology Research Unit, USDA-ARS, Fargo, ND; 2) Department of Plant Pathology, North Dakota State University, Fargo, ND.

The necrotrophic fungal plant pathogen *Sclerotinia sclerotiorum* exhibits an exceptionally broad host range. This fungus causes significant damage on many agriculturally important broadleaf crops including canola, soybean, common bean, and sunflower. The pathogenicity determinants that allow *S. sclerotiorum* to successfully infect such a wide range of plant species are not well understood and to date, only a few potential effectors or virulence factors have been identified for this pathogen. We have established a diverse collection of 260 *S. sclerotiorum* field isolates collected from numerous host plants primarily in the north central United States. We are currently evaluating the virulence of this isolate collection on multiple plant species and utilizing genotyping-by-sequencing to generate high-density genotypic information for these isolates. Our results thus far indicate a broad range of virulence phenotypes on a single USDA sunflower inbred line and preliminary results indicate that at least some isolates exhibit unique virulence profiles on specific sunflower inbred lines. We are in the process of using this information to identify genetic factors contributing to virulence of this destructive broad host range pathogen.

570F Tame a virus: a novel defense gene controls virus spread in the cereal pathogen *Fusarium graminearum*. *J. Bormann*[1,2], C. Heinze[1], M. Mentges[1], A. Brockmann[1], A. Alder[1], C. Blum[1], J. Hartung[1], D. Adpressa[3], B. Plitzko[3], B. Josephson[2], S. Loesgen[3], M. Freitag[2], W. Schäfer[1] 1) Molecular Phytopathology, University Hamburg, Hamburg, DE; 2) Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA; 3) Dept. Chemistry, Oregon State University, Corvallis, OR, USA.

The kingdom of the fungi is unique in that defense reactions against pathogens have not been reported yet. Mycoviruses are found in all phyla of the true fungi. Infections are often fatal as they perturb sexual and asexual sporulation, growth, and, if applicable, virulence of the fungal host, a phenomenon known as “hypovirulence”. The molecular basis of this effect remains unknown. Here we describe how the plant pathogen *Fusarium graminearum* defends itself against a viral infection in a gene-for-gene manner. We identified the mRNA-binding protein “fungal defense 1” (*fd1*), as the central facilitator of fungal response to viral infection. The transcription level of *fd1* is markedly reduced in the presence of virus and in strains expressing a viral coat protein acting as a virus-associated molecular pattern. In order to confine virus spread, the fungus triggers *fd1* downregulation, thereby constraining its own vigor and hampering anastomosis formation, a prerequisite for virus transmission within a fungal colony. Deletion of *fd1* causes virus-like symptoms in the absence of virus and abolishes anastomosis formation while constitutive overexpression of *fd1* overrules the cytopathic effects of a virus infection leading to symptomless accumulation of virus particles. Our results extend the recent concepts of virus-host interactions revealing a potentially conserved host defense mechanism reminiscent of immune responses in higher organisms. Metabolomic studies and RNA-seq experiments support the hypothesis of causal connections between *fd1* and mycovirus infection and led to the identification of virus-induced tolerance-associated genes (VITA-genes). Functional characterization of these genes is ongoing and will provide valuable insights into the underlying signaling network for fungal defense. This advance in understanding fungal infection and defense may aid biological pest control approaches using whole mycoviruses or specific viral proteins to prevent future fungal epidemics.

571W Transposon mutagenesis as a tool to identify genes affecting the pathogenicity of *Talaromyces marneffei*. *D. Kaymakci*, A. Andrianopoulos School of BioSciences, University of Melbourne, Melbourne, Victoria, AU.

Advances in genome sequencing has expanded our knowledge of genomic architecture and led to novel gene discovery; however there is a notable lack of tools required for correlating genetic structure with biological function. In an era of escalating global health threatened by pathogenic fungi, correlation of genes to function are essential for identifying unique and essential factors that determine microbial virulence. Studies on disease-causing fungi have revealed that pathogenic potential depends on a complex interplay between the host and pathogen, which is governed by independent *in vivo* cellular pathways. This study utilises a high throughput mutagenesis system coupled with a candidate gene approach to identify genes essential for infection, survival and proliferation of the opportunistic pathogen *Talaromyces marneffei* during human macrophage infection.

572T Predator Yeasts: genomics and molecular biology of necrotrophic killer yeasts. *Jürgen Wendland*[1,2], Klara Junker[2] 1) Bioengineering Sciences, Vrije Universiteit Brussel, Brussel, BE; 2) Carlsberg Research Laboratory, Copenhagen, Denmark.

Yeast of the genus *Saccharomycopsis* can be isolated from diverse habitats around the globe. They exhibit a unique predacious behaviour, which allows them to feed on and kill suitable fungal prey cells. Under nutrient limiting conditions, particularly upon lack of organic sulphur supply, predator yeasts form penetration pegs with which they will penetrate and, consequently, kill their prey. The host range is wide and includes yeasts, such as *Saccharomyces cerevisiae* and *Candida albicans* but also filamentous fungi, e.g. *Ashbya gossypii*. We aim at understanding the biology of predation and characterize molecular pathways and genes required for successful killing of prey cells. To this end we have generated draft genomes of five predator yeasts: *Saccharomycopsis fodiens, S. fermentans, S. crataegensis, S. schoenii* and *Saccharomycopsis* spec. The genome sizes range from 12 Mb to 15 Mb. The genome data, which also include several contigs with telomeric repeats, suggest that loss of genes required for sulphate assimilation is causing methionine auxotrophy within *Saccharomycopsis* species. Interestingly, genomic signatures suggest that *Saccharomycopsis* species are part of the CTG clade, which reassigned the CTG codon from leucine to serine, e.g. also in *C. albicans*. This has guided our molecular approach towards tool development for studying predator yeasts. We have developed synthetic markers, e.g. *SAK1* providing resistance against the antibiotic G418. Selecting for resistance of *S. schoenii* agains 5-Fluoro-Orotic Acid generated a *ura5* mutant. Genome profiling indicated the presence of transposons and of gene families encoding proteins that may play a major role for predacious behaviour. This includes genes encoding proteins for cell-cell adhesion, so called flocculins; genes for cell wall degrading enzymes, e.g. chitinases; and proteases. These and other morphogenesis genes required for penetration peg formation offer excellent target genes to analyze predatatory behavior in *Saccharomycopsis*.

573F Transcriptional response of the opportunistic human pathogen *Mucor circinelloides* to macrophage interaction and phagocytosis. *C. Pérez-Arques*, M.I. Navarro-Mendoza, P. Martínez-García, F.E. Nicolás, V. Garre Department of Genetics and Microbiology, University of Murcia, Murcia, Spain.

Mucormycosis is an emerging fungal infection caused by Mucoralean opportunistic human pathogens. Despite the existence of a modern arsenal of antifungal drugs, mortality rates for this infectious disease remain devastatingly high since Mucorales present an unusual drug resistance. Consequently, a current demand of novel therapeutic targets is triggering the exploration of the genetic basis involved in mucormycosis.

This work aims to identify new virulence factors involved in mucormycosis, using *Mucor circinelloides* as a model due to the great assortment of molecular tools available to manipulate it. Transcriptomic analyses have been performed to compare gene expression in virulent and avirulent strains interacting *in vitro* with mouse macrophages. This comparison revealed a large number of *Mucor* genes (about 20% of the genome) differentially expressed in the interaction with macrophages, which can be clustered in groups according to their expression patterns. Among these differentially expressed genes, a representative sample has been validated by Northern blot, confirming that these expression patterns are triggered in response to macrophage interaction. Interestingly, a small cluster of approximately one hundred genes is differentially expressed in both virulent and avirulent strains in response to the interaction with macrophages, and could contain indispensable genes for fungal defense against phagocytosis. Another remarkable group includes more than five hundred genes that are specifically regulated in the virulent strains in response to the interaction with macrophages, which could represent virulence factors. A deeper analysis of this group of genes revealed an enrichment in functions associated with signal transduction mechanisms, cytoskeleton, cell wall and membrane biogenesis, which is in accordance with an important role in virulence. Thus, to identify essential components in this response, null mutants for ten putative upstream regulators of these signal transduction pathways have been generated and their virulence analyzed in animal host-pathogen interaction models.

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574W Effect of cytokinin level manipulation on virulence in the biotrophic fungus *Claviceps purpurea*. *S. Kind*[1], J. Hinsch[1], P. Galuszka[2], P. Tudzynski[1] 1) University of Muenster, Muenster, DE; 2) Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ.

The ergot fungus *Claviceps purpurea* is a biotrophic phytopathogen with a broad host range including economically important crops causing harvest intoxication upon infection. Its infection process is restricted to unfertilized ovaries without causing macroscopic defense symptoms. Thus, sophisticated host manipulation strategies are implicated. The plant hormones cytokinins (CKs) are known to regulate diverse plant cell processes and several plant pathogens alter CK levels during infection. For the first time, we could show that *C. purpurea* synthesizes CKs via two mechanisms and that fungus-derived CKs influence the host-pathogen interaction (Hinsch *et al.*, 2015 and 2016). For a better understanding of the CK biosynthesis and the CK contribution to the plant-fungus interaction we applied different approaches to generate strains with altered CK type composition. The first approach is based on the deletion of the two “LOG” encoding genes which are supposed to be essential for the release of active CKs. We generated both, single and double deletion strains and they were found to be able to produce all types of CKs. Apparently, LOG gene products are dispensable for the formation of CKs. The CK biosynthesis pathway

remains unaffected in the second approach as it is based on heterologous overexpression of CK degrading enzymes from maize (Ckx1) and *Rhodococcus fascians* (FasE). Preliminary results indicate that strains overexpressing *Zmckx1* have strongly increased Ckx-activity and drastically reduced CK levels. Evidently, overexpression of Zmckx1 resulted in the formation of functional protein. To our knowledge this is the first time a plant gene was successfully expressed in *C. purpurea*. Additionally, the strains showed reduced virulence dependent on the degree of the overexpression. Although characterization of the strains is not completed yet, so far this approach confirms previous findings that fungal-derived CKs are essential for a compatible plant-fungus interaction.

Hinsch *et al.* (2015) Environ Microbiol: 2935-2951
Hinsch *et al.* (2016) New Phytol: 980-992

575T A *Setosphaeria turcica* secondary metabolite effector prompts a resistance response on *Ht1* maize. *B. Gillian Turgeon*[1], Dongliang Wu[1], Santiago Mideros[1], Zeran Lin[1], Rebecca Nelson[1,2] 1) Section of Plant Pathology & Plant-Microbe Biology, SIPS, Cornell University, Ithaca, NY; 2) Section of Plant Breeding, SIPS, Cornell University, Ithaca, NY.

Setosphaeria turcica is the causal agent of Northern Leaf Blight of maize, one of the most significant maize foliar diseases. *S. turcica* is a hemibiotrophic pathogen and several races have been described based on the symptoms they cause on a panel of maize lines carrying the 'major resistance' genes, *Ht1, Ht2, Ht3,* and *HtN*. By definition, maize carrying *e.g., Ht1* is susceptible to *S. turcica* race 1. Our recent evidence suggests that a gene encoding a hybrid polyketide synthase:nonribosomal peptide synthetase (PKS:NRPS) enzyme for biosynthesis of a secondary metabolite in *S. turcica* race 23N sequenced strain 28A is critically important for the specific resistance interaction of this race on *Ht1* maize. Thus, mechanism of action is similar to the (hemi)biotrophic microbial effector protein-plant resistance protein model, but different in that a secondary metabolite, not a protein, acts as a fungal effector. Genotyping by sequencing and analysis of >200 progeny of a *S. turcica* race 23N by race 1 cross and concomitant phenotyping on *Ht1* maize identified a SNP, in the same *PKS:NPS* gene, that segregates perfectly with susceptible/resistant phenotypes. Co-inoculations with wild-type (resistance reaction) and mutant (susceptible reaction) strains in 1:1, 1:3, and 1:5 ratios complement the mutant phenotype. The *S. turcica PKS:NPS* gene is highly expressed *in planta* in the resistant interaction. On the maize side, qRT-PCR of maize inoculated with *S. turcica* wild type race 23N and the mutant demonstrate that both salicylic acid (SA) and jasmonic acid (JA) signaling are involved. Thus, a fungal small molecule associates in a specific manner with a plant resistance protein to prompt resistance.

576F A widely conserved type 2 Glycosyl Transferase is essential for fungal pathogenesis of plants through enabling hyphal growth across solid surfaces. Robert King[1], Martin Urban[1], Rebecca Lauder[2], Sreedhar Kilaru[3], Gero Steinberg[3], Kim Hammond-Kosack[1], *Jason Rudd*[1] 1) Wheat Pathogenomics Team, Rothamsted Research, Harpenden, GB; 2) BioImaging unit, Rothamsted Research, Harpenden, GB; 3) BioSciences Dept, Exeter University, Exeter, GB.

Fungal spores of plant pathogens must differentiate hyphal filaments in order to grow across and through plant surfaces to initiate infection. Failure to undergo this transition would in almost all cases result in a loss of virulence. From a T-DNA mutagenesis screen performed on the wheat leaf pathogen *Zymoseptoria tritici*, we recovered a mutant which was unable to correctly differentiate and extend hyphae on low nutrient agar, despite retaining full ability to (1) undergo 'yeast-like' blastic conidiogenesis on solid rich nutrient agar, (2) grow hyphae in liquid culture, and (3) generate aerial mycelium. The mutant also failed to form hyphae on wheat leaf surfaces resulting in a complete loss of virulence. The affected gene was identified and functionally validated through complementation and independent targeted deletion. It encoded a putative type 2 Glycosyl Transferase (*ZtGT2*) widely conserved throughout the fungal kingdom. The orthologous gene from the taxonomically un-related wheat ear-infecting fungus *Fusarium graminearum* was also functionally characterised. *ZtGT2* transcripts accumulated in conditions which stimulate hyphal growth of the wild-type strain and the protein was present only in the cell wall fraction. Global RNAseq gene expression analysis comparing Zt?GT2 versus the wild-type strain growing in liquid culture, and during early leaf infection, highlighted a rapid growth arrest of Zt?GT2 on the leaf surface, but a comparable early transcriptional adaptation, suggesting leaf surface sensing was largely unaffected. Comparison of fungal transcriptomes in liquid culture revealed an unexpected up-regulation of numerous candidate and functionally validated secreted effector proteins in Zt?GT2 including the chitin binding LysM domain effector *Zt3LysM*. These data highlight the essential role of a widely conserved GT2 in regulating both common and variable aspects required for fungal pathogenesis of plants, and suggest it may represent a viable target for future widespread control of plant diseases.

577W RNAi-based functional genomics identifies new virulence determinants in mucormycosis. *M.I. Navarro-Mendoza*[1], T.A. Trieu[1,2], C. Pérez-Arques[1], M. Sanchis[3], J. Capilla[3], P. Navarro-Rodríguez[3], L. López-Fernández[3], S. Torres-Martínez[1], V. Garre[1], R. Ruiz-Vázquez[1], F.E. Nicolás[1] 1) Department of Genetics and Microbiology, University of Murcia, Murcia, Spain; 2) Current address: Department of Genetics, Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam; 3) Microbiology Unit. University Rovira i Virgili. IISPV. Tarragona, Spain.

Mucorales are an emerging group of human pathogens that are responsible for the lethal disease mucormycosis. Unfortunately, functional studies on the genetic factors behind the virulence of these organisms are hampered by their limited genetic tractability, since they are reluctant to classical genetic tools like transposable elements or gene mapping. In this work, we describe an RNAi-based functional genomic platform that allows the identification of new virulence factors through a forward genetic approach firstly described in Mucorales. This platform contains a whole-genome collection of *Mucor circinelloides* silenced transformants that presented a broad assortment of phenotypes related to the main physiological processes in fungi, including virulence, hyphae morphology, mycelial and yeast growth, carotenogenesis and asexual sporulation. Selection of transformants with reduced virulence allowed the identification of *mcplD*, which encodes a Phospholipase D, and *mcmyo5*, encoding a probably essential cargo transporter of the Myosin V family, as required for a fully virulent phenotype of *M. circinelloides*. Knock-out mutants for those genes showed reduced virulence in both *Galleria mellonella* and *Mus musculus* models, probably due to a delayed germination and polarized growth within macrophages. This study provides a robust approach to study virulence in Mucorales and as a proof of concept identified new virulence determinants in *M. circinelloides* that could represent promising targets for future antifungal therapies.

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578T Unconventional recombination in the mating type locus of heterothallic apple canker pathogen *Valsa mali*. *Z. Yin*, X. Ke, Z. Li, X. Gao, L. Huang State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, China.

Sexual reproduction in filamentous ascomycetes is controlled by mating type (*MAT*) locus, including two idiomorphs *MAT1-1* and *MAT1-2*. Understanding the *MAT* locus can provide clues for unveiling the sexual development and virulence factors for fungal pathogens. The genus *Valsa* (Sordariomycetes, Diaporthales) contains many tree pathogens responsible for destructive canker diseases. Sexual stage of these ascomycetes is occasionally observed in nature and no *MAT* locus has been reported up till now. Thus, we identified the *MAT* locus of apple canker pathogen *V. mali*, which causes extensive damage and even death to trees. *V. mali* is heterothallic that each isolate carries either *MAT1-1* or *MAT1-2* idiomorph. However, the *MAT* structure is distinct from that of many other heterothallic fungi in Sordariomycetes. Two flanking genes *COX13* and *APN2* were cooperated into the *MAT* locus, possibly by intra-chromosomal rearrangement. After the acquisition of foreign genes, unequal recombination occurred between *MAT1-1/2* idiomorphs, resulting in a reverse insertion in *MAT1-2* idiomorph. Evolutionary analysis showed that these three complete *MAT1-1-2*, *COX13* and *APN2* genes in this region diverged independently due to different selection pressure. Additionally, the unbalanced distribution (*MAT1-1/2*, 3:1) of mating-type idiomorphs was revealed by PCR detection of 24 isolates from different geographic regions. The unconventional remodeling in *MAT* structure and unbalanced *MAT* ratio probably limits the regular sexual reproduction. These results provide insight into the evolution of the mating systems in Sordariomycetes.

579F Developing bi-parental fungal population and mapping of the genetic locus conditioning Ptr ToxC production in *Pyrenophora tritici-repentis*. *G. K. Kariyawasam*, Z. Liu Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA.

Pyrenophora tritici-repentis (Ptr) is a necrotrophic plant pathogen that causes tan spot disease on both bread and durum wheat worldwide. The fungal pathogen is known to produce at least three necrotrophic effectors including Ptr ToxA, Ptr ToxB, and Ptr ToxC as important virulence factors. Both Ptr ToxA and Ptr ToxB are a protein and the fungal genes encoding them have been cloned. However, Ptr ToxC is a non-ionic, polar, and low molecular mass molecule and the gene(s) conditioning its production remain unknown. Because Ptr is a homothallic fungus, it precludes the development of segregating fungal population for genetic analysis. In this work, we created heterothallic fungal strains by deleting one of the *MAT1* genes in isolate 86-124 (race 2, non Ptr ToxC-producer) and AR crossB10 (new race, Ptr ToxC-producer), and developed a bi-parental population by crossing them. The developed population consisted of 112 progenies and phenotyping of all progenies on Ptr ToxC differential lines suggested a single major gene conferring Ptr ToxC production in this population. The whole population is being genotyped using SSR and SNP markers. The obtained genotypic and phenotypic data will be used to map the genetic locus conditioning Ptr ToxC production. This work will help us to gain a better understanding of the disease system and aid in the breeding efforts to increase tan spot resistance.

580W Fatal attraction: nematophagous fungus *A. oligospora* mimics olfactory cues of sex and food to lure nematodes. *Y. Hsueh*[1], M. Gronquist[3], E. Schwarz[4], R. Nath[2], F. Schroeder[5], P. W. Sternberg[2] 1) IMB, Academia Sinica, Taipei, TW; 2) Howard Hughes Medical Institute and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA; 3) Department of Chemistry, State University of New York at Fredonia, Fredonia, NY 14063, USA; 4) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA; 5) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA.

To study the molecular basis for predator-prey coevolution, we investigated how *Caenorhabditis elegans* responds to the predatory fungus *Arthrobotrys oligospora*. *C. elegans* and other nematodes were attracted to volatile compounds produced by *A. oligospora*. Gas-chromatographic mass-spectral analyses of *A. oligospora*-derived volatile metabolites identified several odors mimicking food cues attractive to nematodes. One compound, methyl 3-methyl-2-butenoate (MMB) additionally triggered strong sex- and stage-specific attraction in several *Caenorhabditis* species; furthermore, when MMB is present, it interferes with nematode mating, suggesting that MMB might mimic sex pheromone in *Caenorhabditis* species. Forward genetic screening suggests that multiple receptors are involved in sensing MMB. Response to fungal odors involves the olfactory neuron AWCs. Single-cell RNA-seq revealed the GPCRs expressed in AWC. We propose that *A. oligospora* likely evolved the means to use olfactory mimicry to attract its nematode prey through the olfactory neurons in *C. elegans* and related species.

581T A Gpr1 receptor interactor protein from the Sur7-family is involved in cell wall stability and stress response in the mycoparasite *Trichoderma atroviride*. *Lea Atanasova*[1], Theresa Radebner[2], Sabine Gruber[1], Alexander Lichius[1], Gerhard Schütz[3], David Kreil[4], Susanne Zeilinger[1] 1) Institute of Microbiology, University of Innsbruck, Innsbruck, Austria; 2) Institute of Chemical Engineering, TU Wien, Vienna, Austria; 3) Institute of Applied Physics, TU Wien, Vienna, Austria; 4) Chair of Bioinformatics, University of Natural Resources and Life Sciences, Vienna, Austria.

The ability of fungi to feed on other fungi (necrotrophic hyperparasitism or mycoparasitism) - a trait prominent within the genus *Trichoderma* - represents a promising alternative to hazardous chemical fungicides for plant diseases control. We previously showed that the 7-transmembrane receptor Gpr1 is involved in prey sensing and regulation of mycoparasitism-related processes in *Trichoderma atroviride*. Based on transcriptome profiling and membrane-based yeast two-hybrid screening we identified several candidates among which a protein of the fungal-specific Sur7 superfamily was recognized to be transcriptionally regulated by Gpr1 as well as being a putative interactor of this receptor. Additionally, EGFP labeled Gpr1 and Sur7 proteins showed similar, partly overlapping localization patterns in *T. atroviride* hyphae. Sur7 family proteins are typical residents of membrane microdomains that exhibit a composition, structure, and biological function distinct from the surrounding membrane. In yeasts such Sur7 containing membrane compartment of Can1(MCC)/eisosome domains were recently discovered and represent a novel type of membrane domain that is important for plasma membrane organization, sphingolipid homeostasis, and cell wall morphogenesis. However, for filamentous fungi the function of Sur7 proteins is less known. We show that in the mycoparasite *T. atroviride* the deletion of the *sur7-15* gene causes significantly reduced antagonistic response against living prey fungi, whereas its overexpression triggers full overgrowth and killing of the prey. Chitinase and chitin synthase-encoding genes

were significantly upregulated in Δ*sur7* implying substantial cell-wall stress response. In accordance to that, degenerated resistance to cell-wall intercalation and activation of a specific self-response chitinase and actin in mutant's self-confrontation underlined the important role of *sur7-15* in the maintenance of the *T. atroviride* cell-wall integrity. Moreover, a regulatory impact of *sur7* on genes from the putative MCC/eisosome domain and its involvement in the *T. atroviride* cell membrane stability and osmotic tolerance was also indicated. Morphologically the *sur7* mutants showed a distinct phenotype with slower germination and enhanced hyphal and germ-tube branching.

582F Ménage à trois: fluorescent rhizosphere pseudomonads pursue combined strategies to control polarity and growth of pathogenic *Verticillium* fungi and their plant hosts. K. Nesemann[1], S. A. Braus-Stromeyer[1], *R. Harting*[1], H. Kusch[1], A. Hoefer[1], C. E. Stanley[2], M. Stöckli[3], M. Landesfeind[1], A. Kaever[1], A. Ambrosio[1], C. Timpner[1], K. Hoff[4], T. Pena-Centeno[4], I. Bulla[4], M. Starke[4], A. J. deMello[2], M. Künzler[3], M. Aebi[3], G. H. Braus[1] 1) Institute of Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-Universität Göttingen, DE; 2) Institute of Chemical and Bioengineering, ETH Zürich, CH; 3) Institute of Microbiology, ETH Zürich, CH; 4) Institute of Mathematics and Computer Science, Ernst-Moritz-Arndt-Universität Greifswald, DE.

The genus Verticillium includes several root-inhabiting fungi, which can colonize plants as endophytes or phytopathogens. The impact of genes from rhizosphere Pseudomonas species for secreted metabolites including phenazines or the genes controlled by the bacterial GacA/GacS two component regulatory system on fungal growth was investigated. *Pseudomonas fluorescens* from the rhizosphere of the rapeseed *Brassica napus* cannot synthesize phenazine or 2,4-diacetylphloroglucinol mycotoxins, but caused similar growth inhibitions towards the rapeseed pathogen *Verticillium longisporum* or *V. dahliae* infecting tomato as a different host. The fungal secondary metabolism control genes *Lae1* or *Csn5* are dispensable for the fungal-bacteria interaction. Bacterial co-cultivations with different strains supports media-dependent bacterial strategies to inhibit fungal growth during agar or liquid co-cultivation. Phenazines caused Verticillium growth inhibition on high glucose, but not on pectin/amino acid agar. Co-cultivation with *P. protegens* in liquid pectin/amino acid medium alters the relative fungal transcripts ratio including 16% up-regulated genes for mitochondrial activity, detoxification or melanine biosynthesis and about half decreased gene fractions for protein synthesis or pectin degradation. Bacterial-fungal interactions at the single cell level were probed in microfluid devices in liquid channels with pectin/amino acid medium. These confrontations with physical constraints revealed that rhizosphere Pseudomonads can inhibit 80% of fungal hyphal growth independently of phenazine and 2,4-diacetylphloroglucinol by complex combinations of metabolites. 90% of *V. longisporum* hyphal tips exhibited strong polarity defects with a 'curly' phenotype. Co-infection experiments of host plants with fungi and bacteria revealed that only the *gacA*-controlled Pseudomonas regulatory network protects plants against Verticillium induced pathogenicity.

Population and Evolutionary Genetics

583W Discovery of a novel azole-resistance mutation in *Aspergillus fumigatus* and the possible role of sexual reproduction in its evolution. *J. Zhang*[1], Bas Zwaan[1], E Snelders[1], S Schoustra[1], K Dijk[2], J Meis[3,4], F Hagen[3,4], E Kuijper[5], G Kampinga[6], J Zoll[4,7], W Melchers[4,7], P Verweij[4,7], A Debets[1] 1) Laboratory of Genetics, Wageningen University, Wageningen, NL; 2) Department of Medical Microbiology, Vu University Medical Centre, Amsterdam, The Netherlands; 3) Department of Medical Microbiology and Infectious Diseases, CWZ Hospital, Nijmegen, The Netherlands; 4) Expert Centre in Mycology Radboudumc/CWZ (EMRC); 5) Department of Medical Microbiology, Leiden University Medical Centre, Leiden, The Netherlands; 6) Department of Medical Microbiology, University Medical Centre Groningen, Groningen, The Netherlands; 7) Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands.

We investigated two compost-heaps, one containing azoles and one without azoles, for the presence of azole-resistant and azole-sensitive *A. fumigatus* isolates. The azole-free compost yielded 98% (49/50) sensitive and 2% (1/50) azole resistant isolates, whereas the azole-containing compost yielded 8% (4/45) sensitive and 92% (41/45) resistant isolates. From the latter, 84% (37/45) were of the highly resistant TR_{46} Y121F/ T289A genotype and 8% (4/45) had a novel pan–triazole-resistance harbouring a triple tandem repeat: TR_{46}^{3}/Y121F/ M172I/T289A/G448S. The stark contrast between the two composts indicates that azole–containing compost is a hot spot for the development and maintenance of azole resistance. Subsequent screening of the clinical *A. fumigatus* collection from the Dutch national surveillance programme indicated that this resistance mechanism was already present in 2012, and is now found in all participating medical centres. We were able to recover this novel TR_{46}^{3} mutation among the sexual progeny in the lab, from a cross between two TR_{46} isolates of opposite mating type that were from the same compost. This strongly indicates a role of sex in the emergence of this novel azole–resistance mechanism in *A. fumigatus*. Furthermore, we provide further evidence indicating the potential occurrence of sexual reproduction in compost, but this still needs further confirmation. Our findings alarm the fast development of the azole resistance issue in *A. fumigatus* and further indicate the role of sexual reproduction of *A. fumigatus* in the development of azole resistance.

Key words: *Aspergillus fumigatus*; novel new mutation; compost; azole resistance; hot spot; sexual reproduction; ascospores; conidiospores

584T The role of lifecycle in azole-resistance development in *Aspergillus fumigatus*. *E. Snelders*[1], J. Zhang[1], A.J.M. Debets[1], S.E. Schoustra[1], W.J.G. Melchers[2], B.J. Zwaan[1], P.E. Verweij[2] 1) Laboratory of Genetics, Wageningen University, Wageningen, NL; 2) Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, NL.

Aspergillus fumigatus is a ubiquitous fungus that plays an important role in carbon and nitrogen recycling in nature by degrading organic biomass. It is also a fungus that can cause several diseases in humans ranging from allergic conditions to acute invasive aspergillosis. To survive and thrive, *A. fumigatus* needs to rapidly adapt to these environments that often entail various challenges. Genetic adaptations by mutation and recombination can be defined as the acquisition of heritable modifications in an organism through natural selection, which enable it to survive and reproduce in the prevailing or new environment. One example of this adaptive process is the development of azole resistance. Triazoles have become the cornerstone of medical treatment of aspergillus-related diseases. Although triazole resistance can develop during patient therapy, another route of resistance selection in *A. fumigatus* can be through exposure to azole fungicides in the environment. Using the case of triazole resistance development, we show that the process of reproduction, sexual, parasexual, or asexual,

is crucial for the adaptive potential of *A. fumigatus*. In this study we describe the different life cycles in *A. fumigatus* and their characteristics and probability of occurring in the human host or in the environment.

585F Pivotal role for the alternatively spliced, spliceosomal twin intron in one-bp intron drift. *E. Fekete*[1], M. Flipphi[1], N. Ág[1], N. Kavalecz[1], G.C. Cerqueira[2], C. Scazzocchio[3], L. Karaffa[1] 1) Department of Biochemical Engineering, University of Debrecen, Debrecen, HU; 2) Broad Institute of MIT & Harvard, Cambridge MA, USA; 3) Dept. of Microbiology, Imperial College London, UK.

In the primary transcript of nuclear genes, coding sequences – exons – usually alternate with non-coding sequences – introns. The latter are removed and former are joined by means of splicing to create the mRNA ORF that translates into the functional peptide product. In the study of the control of fungal gene expression, the ubiquitous splicing process is largely ignored. In the evolution of genic intron-exon structure, introns are gained at new positions or lost from extant positions or they move to a new position. The latter "intron drift" or "intron slide" leads to discordant introns in ortholog genes.

Previously we described stwintrons (spliceosomal twin introns) in filamentous ascomycota (*). These are complex intervening sequences in which a canonical "internal" intron interrupts one of the three conserved domains of a canonical "external" intron and consequently, they can only be removed with two subsequent splicing reactions. A stwintron in which the donor of the external intron is interrupted between the first and second bp – [D1,2] – can also be removed alternatively if the acceptor of the other external intron is interrupted between the penultimate and ultimate bp – [A2,3] – where the resultant mRNA is identical for both splicing routes.

Here we present a new stwintron, the first uncovered in *Aspergillus nidulans*. Ortholog genes occur in 4 Pezizomycotina classes. Most encompass either a [D1,2] or an alternatively spliced [D1,2] /[A2,3] stwintron at the same position. However, 9 species harbor there a discordant, canonical intron that conforms a one-bp intron drift: 7 have a phase-2 intron and appear to miss the [D1,2] internal intron while two carry a phase-0 intron and they appear to lack the [A2,3] internal intron instead. This is one of the few instances of intron drift where its mechanism could be elucidated.

(*) Ág et al. (2015) Fungal Genet Biol 85:7 & Flipphi et al. (2013) Fungal Genet Biol 57:48.

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586W Sexual fertility in Aspergillus flavus: understanding genetic exchange and phenotypic inheritance through analysis of F1 progeny. *R.M. Gell*, I. Carbone Center for Integrated Fungal Research, Program of Genetics, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA.

The carcinogenic mycotoxin aflatoxin is a constant threat and economic burden to corn and oil seed crops grown within the United States and globally. Aflatoxin is produced by species in *Aspergillus* section *Flavi*, primarily *Aspergillus flavus*. Within the US and Africa, aflatoxin contamination is controlled through the high-density application of non-aflatoxin producing *A. flavus* strains. Though previously thought of as only asexual, *A. flavus* has recently been found to undergo sexual reproduction both in laboratory crosses and in the field. During the mating process, the sclerotium, a survival structure, of one strain acts as the female parent providing both the mitochondria and a matrix for the ascocarps and progeny to grow, while a spore or propagule from a second compatible strain fertilizes as the male. The fertility of mating pairs is highly variable and strongly influenced by the directionality of the cross but little is known about the genetic basis of fertility and how it is regulated. We are examining crosses that exhibit high fertility in one direction, but low fertility when male and female parents are reversed. Genome wide data using double digest Restriction Associated DNA sequencing was obtained for the progeny of these biased crosses. These data are being used to explore genome-wide recombination and as markers for mapping genomic regions that influence phenotypes, such as bias in fertility. By understanding this variation, we create opportunities to utilize strain fertility in the selection of biological control agents and increase our understanding of genetic exchange within populations of *A. flavus*.

587T The adaptive potential of *Candida albicans* in response to *in vivo* stress. G. Cromie[1], E. Jeffery[1], S. Filler[2], J. Berman[3], A. Dudley[1], *A. Forche*[4] 1) PNRI, Seattle, WA USA; 2) UCLA, Los Angeles, CA USA; 3) Tel-Aviv University, Tel-Aviv, Israel; 4) Bowdoin College, Brunswick, ME USA.

To understand the adaptive potential of *C. albicans* to its host, we characterized the genomes of ~1000 isolates recovered after single passage through mice from an oropharyngeal Candidiasis model (OPC) and a systemic model of Candidiasis (BSI). We used flow cytometry to determine ploidy and ddRADseq to assess whole genome karyotypes. Our analysis revealed high levels of aneuploidy and loss of heterozygosity (LOH) among isolates from both models. There was no significant difference in the overall frequencies of aneuploidy and LOH. However, chromosome (Chr) 6 trisomy was significantly higher in the OPC population. Intriguingly, Chr6 is particularly enriched for genes from multiple virulence gene families. Segmental aneuploidies and LOH occurred at similar frequencies in both model. Interestingly, all but one aneuploidy breakpoint (at the rDNA locus) were unique to each model. The majority of aneuploidy and LOH breakpoints were found near repetitive DNA and tRNAs. In addition, many LOH breakpoints were located near proposed origins of replication. This finding suggests that *in vivo* environments may trigger replication stress-induced genome instability, which could be due partially to the resolution of arrested replication forks. In contrast to similar overall frequencies of genome changes in the two models, genetic diversity within each mouse host was significantly higher for the OPC model, suggesting that the oral cavity represents a much more dynamic and diverse niche.

588F Transitions between tetrapolar and bipolar fungal mating type driven by chromosomal translocations involving intercentromeric recombination. *Sheng Sun*[1], Vikas Yadav[2], R. Blake Billmyre[1], Christina A. Cuomo[3], Minou Nowrousian[4], Jean-Luc Souciet[5], Teun Boekhout[6], Betina Porcel[7], Patrick Wincker[7], Joshua A. Granek[1], Liuyang Wang[1], Kaustuv Sanyal[2], Joseph Heitman[1] 1) Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC; 2) Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 3) Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA; 4) Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum, Germany; 5) Université de Strasbourg, CNRS UMR7156, Strasbourg, 67000, France; 6) CBS-KNAW Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands; Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands; 7) Commissariat à l'Energie Atomique (CEA), Institut de Génomique (IG), Genoscope, Evry, France, Université d'Evry, UMR 8030, Evry, France, Centre National de

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Species within the human pathogenic *Cryptococcus* species complex are major threats to public health, causing more than one million infections globally each year. *Cryptococcus amylolentus* is the most closely known related species of the pathogenic *Cryptococcus* species complex, and it is non-pathogenic. Additionally, while pathogenic *Cryptococcus* species have bipolar mating systems with a single large *MAT* locus that represents a derived state in Basidiomycetes, *C. amylolentus* has a tetrapolar mating system with two *MAT* loci (*P/R* and *HD*) located on different chromosomes. Thus, studying *C. amylolentus* could shed light on the origin and evolution of pathogenesis, as well as the transition from tetrapolar to bipolar mating systems in the pathogenic *Cryptococcus* species. In this study, we sequenced, assembled, and annotated the genomes of two *C. amylolentus* isolates, CBS6039 and CBS6273, which are interfertile. Genome comparison between the two *C. amylolentus* isolates identified the boundaries and the complete gene contents of the *P/R* and *HD* loci. Also, bioinformatics and ChIP-seq analyses showed that *C. amylolentus* has regional centromeres that are enriched with species-specific transposable and repetitive elements, similar to the centromeric structures in the pathogenic *Cryptococcus* species. Additionally, we found that while neither of the *P/R* and *HD* loci in *C. amylolentus* is physically linked to its centromere, both *MAT* loci showed centromere linkage in meiosis, suggesting the presence of recombination repressors and/or epistatic gene interactions in the inter *MAT*-CEN regions. Furthermore, genomic comparison between *C. amylolentus* and pathogenic *Cryptococcus* species provided evidence that chromosomal rearrangements mediated by intercentromeric recombination have occurred after the two lineages split from their common ancestor. We propose a model in which the evolution of the bipolar mating system was initiated by an ectopic recombination event mediated by repetitive elements located within the centromeric regions and shared between chromosomes. This translocation brought the *P/R* and *HD* loci onto the same chromosome, and was followed by chromosomal rearrangements that resulted in the two *MAT* loci becoming physically linked and eventually fused to form the single contiguous *MAT* locus that is now extant in the pathogenic *Cryptococcus* species.

589W Naturally occurring mismatch repair mutants mediate rapid phenotypic change and drug resistance in the Pacific Northwest *Cryptococcus gattii* outbreak. *R. Blake Billmyre*, Shelly Clancey, Shelby Priest, Joseph Heitman Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Pathogenic microbes confront a constant evolutionary conflict between the pressure to maintain genome stability and the need to adapt in response to mounting external stresses. Over the past fifteen years, an ongoing outbreak of the human fungal pathogen *Cryptococcus deuterogattii* has occurred in the Pacific Northwest of the United States and Canada. Whole genome resequencing of outbreak strains identified one lineage of the outbreak that harbors a nonsense mutation in *MSH2* and exhibits a hypermutator phenotype. Genetic analysis of progeny as well as independent deletions of *MSH2* demonstrate linkage of the hypermutator phenotype to *MSH2* mutation. This defect in mismatch repair destabilizes homopolymer runs throughout the *Cryptococcus* genome, resulting in inactivation of genes with coding homopolymer runs. As a result, resistance to the clinically-used immunosuppressive drugs FK506 and rapamycin is rapidly generated, as well as resistance to 5-fluorocytosine (5-FC), a frontline drug often used to treat Cryptococcal infections and for which spontaneous drug resistance occurs rapidly, necessitating use only in combination with amphotericin B. One environmental isolate with an *MSH2* mutation even exhibited an unselected FK506/rapamycin drug resistance phenotype attributable to a homopolymer run shift. Competition experiments demonstrate this hypermutator state is deleterious in rich growth conditions, but allows more rapid adaptation to stressful conditions. Ongoing work has identified strains with elevated mutation rate present throughout the population of the sister species of *Cryptococcus neoformans* and representing multiple independent origins of hypermutation. Hypermutator states may represent a general mechanism by which fungi adapt to changing environmental conditions or drug challenges.

590T A genetic comparison of *Exserohilum turcicum* isolates from maize and sorghum based on microsatellite haplotypes. *A. Nieuwoudt*[1], MP. Human[1], M. Craven[2], BG Crampton[1] 1) Plant and Soil Sciences, FABI, University of Pretoria, Pretoria, Gauteng, ZA; 2) Agricultural Research Council-Grain Crops, Potchefstroom, 2520, South Africa.

Exserohilum turcicum is the causal agent of northern leaf blight (NLB), a devastating foliar disease of both maize and sorghum in South Africa. Although *E. turcicum* is considered to cause disease in both crops, host specificity has been observed between isolates obtained from either maize or sorghum. The genetic differences underlying host specialization must still be determined. Population studies of *E. turcicum* isolated from maize has been conducted in Europe, America, Kenya, Northern China and South Africa, but studies comparing sorghum and maize isolates only refer to a few sorghum isolates. Therefore, a population genetics study was performed on *E. turcicum* isolates from both maize and sorghum to compare haplotypes of isolates from the different crops in KwaZulu-Natal (2014) and Mpumalanga (2015) in South Africa. Twelve microsatellite markers were used to screen 61 maize isolates and 64 sorghum isolates. No shared haplotypes were detected between the maize and sorghum isolates, but two shared haplotypes were identified between the maize isolates from the two locations. Principal coordinate analysis between maize and sorghum isolates showed no distinct separation between the maize and sorghum populations, since there is overlap in the central axis between the isolates from the different crops. Analysis of molecular variance indicated that isolates from the same host in the two locations are more similar than isolates from different hosts in the same location. Mating type distribution and indices of association gave no indication that random mating is taking place within the populations. However, haplotype diversities were too high to be associated with strictly clonal populations suggesting that a mixed reproductive life cycle is present. There is high genetic diversity in *E. turcicum* isolates from both maize and sorghum, evidence that *E. turcicum* follows a mixed reproductive strategy and there are indications for some differentiation between isolates based on the plant host from which isolates were collected.

591F The impact of heavy metal contamination on particular ectomycorrhizal fungi and associated plant hosts. *Jessica Velez*[1], Christopher Schadt[2] 1) University of Tennessee Knoxville, Knoxville, TN; 2) Oak Ridge National Laboratory.

Agriculture has become a multifaceted industry as the production of biofuels and the need for both greater productivity and sustainability grows in importance. However, there is a finite amount of land available, and a portion of this land is unusable due to the presence of major pollutants, such as heavy metal contaminants. As the demand for agricultural land rises, the viability of crops within soils that were previously considered unusable has become more appealing. There is a possibility that with modifications to the rhizosphere, a plant species may be able to survive in conditions that would otherwise be toxic to the plant, increasing overall land availability for use in agriculture. This would allow the planting of biofuel crops within fields that may not be optimized for the growth of crops intended for

consumption due to soil contamination, thereby avoiding the "food-for-fuel" tradeoff that has driven agronomic policy concerns in the corn-based ethanol industry. This research will aim to lead to a greater understanding of the exchange that occurs within a plant-fungal system and determine if there is an increase in the overall hardiness of the plant when the fungal species are present and how this varies with the genetic makeup of the symbionts. Looking to the future, this research will impact the direction of biofuel crop production as knowledge of the plant-fungal relationship increases, as well as potentially open new land for agricultural development.

592W Unusual evolutionary mechanisms to escape Effector-Triggered-Immunity in the fungal phytopathogen *Leptosphaeria maculans*. Clémence Plissonneau, *Thierry Rouxel*, Marie-Hélène Balesdent Plant Pathology, INRA-Bioger, Thiverval-Grignon, FR.

Leptosphaeria maculans is the fungus responsible for the stem canker disease of oilseed rape (*Brassica napus*). *AvrLm3* and *AvrLm7*, two avirulence effector genes of *L. maculans*, are involved in an unusual relationship: the presence of *AvrLm7* suppresses the *Rlm3*-mediated resistance of *AvrLm3*. Following the large-scale cropping of *Rlm7* cultivars in Europe, the on-going breakdown of the *Rlm7* resistance and the concomitant inactivation of *AvrLm7* was accompanied by the resurgence of isolates expressing the *AvrLm3* phenotype. Here, we evaluated adaptation mechanisms to escape *Rlm3*-mediated ETI by assessing *AvrLm3* polymorphism in a collection of 236 *L. maculans* isolates. In isolates virulent towards both *Rlm3* and *Rlm7* (a3a7), the loss of the *Rlm3*-mediated resistance response was due to two distinct mechanisms. First, when *AvrLm7* was inactivated (deletion or inactivating mutations), amino acid substitutions in AvrLm3 generated virulent isoforms of the protein. Secondly, point mutations in *AvrLm7* could maintain the masking of the AvrLm3 phenotype while also allowing escape from the Rlm7 recognition. This virulence mechanism was found in 56.4% of the a3a7 isolates, and these contained an avirulent allele of *AvrLm3*. Signatures of positive selection were observed in AvrLm3 and, in spite of the telomeric location of *AvrLm3*, no a3a7 isolates exhibited deletion events or inactivating mutations in *AvrLm3* as commonly observed for other avirulence genes of *L. maculans*. The complex evolutionary mechanisms enabling *L. maculans* to escape *Rlm3*-mediated resistance while preserving *AvrLm3* sequence integrity, along with observed reduced aggressiveness of isolates silenced for *AvrLm3,* serves to emphasize importance of this effector in pathogenicity towards *B. napus*.

593T Divergent evolution of two populations within a lineage of the hybrid fungal oilseed rape pathogen *Verticillium longisporum*. *Jasper R.L. Depotter*[1,2], Michael F. Seidl[1], Grardy C.M. van den Berg[1], Bart P.H.J. Thomma[1], Thomas A. Wood[2] 1) Laboratory of Phytopathology, Wageningen University and Research; 2) Department of Crops and Agronomy, National Institute of Agricultural Botany.

Population genetic structures represent evolutionary trajectories of organisms while they adapt to differential environmental conditions. Here, pathogen populations are additionally shaped by their host, as co-evolution between pathogen and host can lead to corresponding genetic co-structuring. Verticillium stem striping is a relatively new disease of oilseed rape that was mainly observed in continental Europe, but has recently expanded to other countries including the UK. Verticillium stem striping is caused by the hybrid fungal species *Verticillium longisporum* that originates from at least three separate hybridization events. Strains from the hybridization event between *Verticillium* species A1 and D1 are predominantly responsible for Verticillium stem striping. In this study, the population structure of *V. longisporum* lineage A1/D1 was investigated. Multi-locus genotype analysis revealed a hitherto un-described dichotomy that correlates with the geographic origin of the isolates. The genetic clusters are provisionally called "A1/D1 West" and "A1/D1 East" according to their relative location in Europe. Whereas A1/D1 East is the dominant population in Germany and Sweden, where Verticillium stem striping already occurs since the 1960s, the distribution of A1/D1 West reaches further than Europe, with isolates found in the USA and Japan. Genome comparison between representatives of the A1/D1 West and East clusters confirmed their mutual origin, excluding putative distinctiveness through separate hybridizations. An A1/D1 West population caused the sudden rise of Verticillium stem striping in the UK. Remarkably, the genetic diversity of the UK isolates was higher than that of the whole A1/D1 East cluster. Conceivably, the lower genetic variation within A1/D1 East indicates a founder effect, where A1/D1 West is the original population and A1/D1 East a founder population. A1/D1 East may have been able to establish by its initial capacity to cause Verticillium stem striping disease on oilseed rape, whereas A1/D1 West, until recently, occurred only on alternative hosts.

594F Exploring hybridization in fungi: a lesson from the forest pathogens *Heterobasidion* spp. *M.M. Garbelotto*[1], F. Sillo[2], L. Giordano[2,3], P. Gonthier[2] 1) ESPM, University of California Berkeley, 54 Mulford Hall, 94720 Berkeley (CA), USA; 2) DISAFA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy; 3) AGROINNOVA, University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy.

Hybridization is recognized as one of the major force to rapid adaptive evolution in fungi. However, the evolutionary potentials of hybrids are still poorly understood. The American forest pathogens *Heterobasidion irregulare* is currently hybridizing with the Eurasian sister species *H. annosum* in Italy. By using this model system, the aims of this work were I) clarifying the role of the mitochondrial genome in heterokaryotic hybrids, II) identifying introgressed genes from the invasive *H. irregulare* into the native *H. annosum*, III) estimating the pathogenic and saprobic fitness of hybrids. To reach these aims, artificial heterokaryotic hybrids were generated *in vitro* and their pathogenic and saprobic capabilities were assessed through pine seedling inoculations and growth experiments under controlled conditions. A target transcriptomic analysis through RT-qPCR was also performed. In addition, whole genomes of 9 natural homokaryotic hybrids collected in the sympatric area in Italy were sequenced and compared with that of pure genotypes of the two species.
In the saprobic assay, the heterokaryotic hybrids carrying only one mitochondrial genome perfectly mirror the respective parental donor of mitochondria, suggesting a role for the mitochondrion in saprobicity. RT-qPCR results support this hypothesis, since during saprobic processes heterokaryotic hybrids regulate their genes similarly to the respective parental donor of mitochondrial genome. The comparative genomic analysis in natural hybrids allowed to identify and quantify introgressed genomic regions in each genotype. Recurrent introgressed genes from the *H. irregulare* to *H. annosum* seem to be associated to saprobic processes and to secretory Golgi-related pathways. This work provides insights on the genomics of hybridization, towards a broader understanding of the evolutionary trajectories of hybrid fungal pathogens.

595W Evidence of birth and death evolution in the α-pheromone precursor gene of *Fusarium circinatum*. *O.O. Adenigba*[1], B.D. Wingfield[2], T.A. Duong[2], N.A. van der Merwe[2], M.J. Wingfield[1], E.T. Steenkamp[1] 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa.

Ascomycetes encode two classes of pheromones (α and a), which are expressed in a mating-type specific manner to initiate the process of sexual reproduction. Pheromone genes encode precursor proteins that undergo proteolytic processing to release tandem repeats of the respective pheromone peptides. We hypothesized that the evolution of these genes and their repeats are driven by birth-and-death and concerted evolution, as has been shown for multi-gene families. To test this hypothesis and to determine the mode of selection experienced by these genes, we utilized the α-pheromone precursor gene in the pine pitch canker pathogen, *Fusarium circinatum*. We amplified, sequenced and analysed the α-pheromone precursor gene in 69 isolates of *F. circinatum*. The aligned sequences of the repeats were used to construct a neighbour joining tree and to infer dN/dS ratios to investigate modes of selection. Among the 69 isolates, we identified 13 haplotypes, each with a unique combination of variants of the α-peptide repeat at the DNA level. The total number of tandem α-peptide repeats per haplotype ranged from 10 to 14, possibly due to the birth and loss of repeats. Phylogenetic relationships further indicated that repeats grouped according to repeat variant and not according to haplotype. All dN/dS ratios were also less than 1, suggesting that this gene and its repeats are subjected to negative selection. Collectively, our findings showed evidence of birth and death evolution and strong purifying selection acting on the α-pheromone precursor gene of *F. circinatum*. These findings could imply a need for increased dosage of a specific pheromone during sexual reproduction, while the occurrence of variant repeats suggested that new α-peptides with new or similar functions might evolve.

596T Fitness landscape driven by the interaction between gene mutation and aneuploidy. *H-J. Tsai*, A. Nelliat, R. Li Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD.

Aneuploidy, an abnormal genome state with gain or loss of chromosomes, has detrimental effects to cellular physiology due to dramatic changes in gene copy number. This unbalanced state of genome content leads to genome instability and developmental defects. Interestingly, aneuploidy also confers phenotypic variation in fungal populations to gain selective advantages for stress adaptation. For example, gain of chromosome 5 in pathogenic yeast *Candida ablicans* is highly associated with fluconazole resistance. Moreover, aneuploidy is widespread and well tolerated in wild yeasts to drive rapid phenotypic evolution as an adaptive strategy. The paradoxical nature between the beneficial and detrimental roles of aneuploidy in organismal fitness remain unclear. Here, we investigate how aneuploidy rewires genetic network to change population fitness by performing a genetic screen in a heterogeneous aneuploid population in *Saccharomyces cerevisiae*. We took advantage of triploid meiosis to generate a karyotypically heterogeneous aneuploid population and unbiasedly tested the growth impacts of aneuploidy. In parallel, we applied this strategy to aneuploid populations carrying a single gene mutation in each population. Thus, we were able to observe the fitness impact from the interaction between gene mutation and aneuploidy. Intriguingly, despite cell growth being compromised due to the negative effects of gene mutation or aneuploidy, majority of aneuploid populations carrying a gene mutation exhibited comparable fitness to euploids. To further examine this growth phenotype, we utilized a high-throughput, quantitative microscopy assay to measure the fitness distribution of microcolonies and revealed high cell-to-cell heterogeneity in aneuploid populations. Under the absence of stress in the environment, a wide range of growth capabilities existed in aneuploid populations, and few of them showed advantageous growth to euploid cells. The results suggest that aneuploidy may confer phenotypic variation due to the preservation of inner heterogeneity and could show high adaptability when stress presents.
In summary, we have mimicked the naturally occurring karyotypic complexity in a diverse aneuploid population to elucidate fungal adaptive evolution. Most importantly, our genetic screen and quantitative fitness analysis of the heterogeneous aneuploid populations could reveal detailed molecular trajectory in aneuploidy during evolution.

597F Multilevel selection in *Neurospora tetrasperma*. *C. Meunier*, S. Hosseini, Z. Maryush, H. Johannesson Systematic Biology, Evolution Biology Centre, Uppsala, SE.

Heterokaryosis, the coexistence of genetically divergent nuclei in the same mycelium, is a common trait in fungi. In the filamentous ascomycete *Neurospora tetrasperma*, a shift in the mating system has led to predominant heterokaryosis during the life cycle: both mating-type nuclei (*mat A* and *mat a*) are packaged together in conidia and ascospores- however, monokaryotic, haploid individuals can still be found. Such a shift can be considered as an evolutionary transition to higher complexity. During this transition, a potential threat to heterokaryons is discordant selection, when selection operating at level of haploid nuclei acts counter to selection operating at the level of the heterokaryon. Furthermore, a crucial step in the transition is the export of fitness heritability from monokaryon-level to heterokaryon-level, potentially leading to division of labor among nuclear types. In our study, we investigated pros and cons of heterokaryosis in *Neurospora tetrasperma*, studying nuclear ratios and contrasting fitness traits between the heterokaryotic mycelium and its two genetically divergent, totipotent and free ranging nuclear types. We showed that the ratio of the two nuclear types varies within a mycelium and verified that the ratio is homogeneous, but that it can deviate from a diploid-like 1:1-ratio. When measuring fitness traits of the mycelium and its component nuclear genotypes, we found that nuclear types have different fitness optima during the life cycle and that the phenotype of the heterokaryotic mycelium is reflecting the nuclear ratio in an additive manner. Thus, our data indicate subfunctionalization of the nuclear types for mycelial function, a form of division of labor. Our results also hint towards variation in nuclear ratio resulting in phenotypic flexibility of the mycelium. However, the occurrence of seemingly selfish nuclei, enjoying better replication and transmission than sister nuclei, yet being detrimental to the heterokaryotic organism, points towards ongoing discordant selection.

598W Can meiotic drive drive speciation? *A.A. Vogan*, Jesper Svedberg, Sandra L. Ament-Velásquez, Hanna Johannesson Systematic Biology, Uppsala University, Uppsala, SE.

Selfish elements can bias their own transmission from one generation to the next in a process referred to as meiotic drive. The driving element will be passed to the majority of offspring, thereby increasing in frequency over generations. The drive can occur regardless of its effect on fitness and may be a detriment to the organism as a whole. This intragenomic conflict could lead to strong selection against individuals carrying the element and promote reproductive isolation between so called killer and sensitive strains.

In ascomycetous fungi, meiotic drive manifests itself as a spore killing phenomenon. Briefly, ascospores carrying a killer element will induce the death of ascospores in the same ascus that do not carry the element. The killing is assumed to have a large cost to fitness, as a cross between killer and sensitive strains would result in the death of roughly half of all sexual progeny. We have examined 4 different spore killers in both *Neurospora* and *Podospora*. All of these examples of drive show patterns that suggest they may promote reproductive isolation between killer and sensitive strains.

In *N. sitophila*, we have indications that two separate defense mechanisms have evolved to protect against drive. Both Tahitian and European populations are polymorphic for spore killing. In Tahiti, a resistance locus has evolved that allows a number of sensitive spores to escape killing. In Europe, no such resistance has been observed. However, sensitive strains appear to be a distinct lineage from killer strains. This suggests that reproductive isolation may be a viable alternative to resistance as a way to protect against spore killing.

In *N. intermedia*, there are also indications of reproductive isolation between killer and sensitive strains. Crosses between certain populations produce little to no viable progeny when one parent carries a killer element, but produce moderate levels of viable progeny when both parents are sensitive. This pattern is also observed when crosses are conducted between *N. intermedia* and its sister species, *N. metzenbergii.*

The final example is from a population of *P. anserina* in the Netherlands, where the investigated spore killer element appears to be nearly fixed. A small number of individuals can be found that do not have this killer element and again, these isolates appear to be a distinct lineage from the other Dutch strains.

We are currently performing experiments to confirm the link between meiotic drive and speciation.

599T Impact of promoter evolution on gene expression levels in Zymoseptoria tritici. *C.J. Eschenbrenner*[1,2], A. Kumar[1], J. Haueisen[1,2], E.H. Stukenbrock[1,2] 1) Botanical Institute, University of Kiel, Kiel, Germany; 2) Max Planck Institute of Evolutionary Biology, Plön, Germany.

In a previous study, we compared transcriptome data from three isolates of the fungal wheat pathogen *Zymoseptoria tritici* during wheat infection. We used three isolates that cause the same level of disease symptoms on the susceptible wheat cultivar Obelisk. While the three isolates are confronted with the same host immune system and show the same ability to grow and reproduce in Obelisk wheat, we found that up to 20% of all shared genes are differentially expressed between isolates during host colonization. We concluded that isolates of *Z. tritici* use different transcriptional programs to successfully invade the same host. In this study, we address the underlying genetic and genomic basis of transcriptional variation in *Z. tritici* and focus on evolution in promoter sequences. For this purpose, we created *de novo* assemblies for 26 genomes of *Z. tritici* from several locations worldwide (Australia, Denmark, France, Germany and Iran). In addition, we used the Dutch isolate IPO323 as reference for all analyses. Genome assemblies of the 26 isolates were used to generate a multiple genome alignment with the *tba* program package. The alignment was further processed and filtered using the program *maffilter*. After filtering we retained a genome alignment of 26 Mb comprising only alignment blocks with sequence data for all 26 genomes. The filtered alignment was then used to identify 741,269 single nucleotide polymorphisms (SNPs). From the multiple genome alignments we extracted 500 bp sequences upstream of each aligned coding sequences and used the SNP data to compute a range of parameters for each promoter including SNP densities, Tajima's D, and the nucleotide diversity computed as Tajima's Pi. Genome-wide correlation analyses show a strong negative correlation between gene expression and genetic variation in promoters. Thus as expected, highly expressed genes have more conserved promoter sequences. We also find that genetic variation in promoters varies greatly according to the chromosomal context. Promoters in gene dense regions are more conserved than promoters in gene sparse regions in agreement with a stronger effect on background selection in gene rich regions.

600F Genome-wide genetic dissection of trait variation between species. Carly Weiss[1], Rylee Hackley[1,2], Jeremy Roop[1], Jeffrey Skerker[3], *Rachel Brem*[1,2] 1) Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA; 2) Buck Institute for Research on Aging, Novato, CA; 3) Energy Biosciences Institute, UC Berkeley, Berkeley, CA.

Geneticists since Mendel have sought to explain how and why traits vary among wild individuals. The industry standard toward this end is to test for DNA sequence variants that correlate with phenotype across a population, but such methods cannot be applied across reproductive barriers. To fill this analysis gap, we developed a high-throughput, interspecific version of the reciprocal hemizygote test. In a viable hybrid formed from the mating of two species, we introduce disrupting mutations at each allele of each gene in turn via transposon mutagenesis, and we quantify phenotypes of the resulting hemizygote mutants in a pooled format via Tn-seq. As a testbed for our approach, we focused on thermotolerance in yeasts. At 39°C, *Saccharomyces cerevisiae* grows faster and accumulates more biomass than its sister species *S. paradoxus*, to a degree far exceeding the variation among isolates of each species. Culture and sequencing of *S. cerevisiae* x *S. paradoxus* hemizygotes revealed a battery of loci at which the species' alleles conferred differences in thermotolerance. Few of these genes had known roles in resistance to heat shock or other stresses. At most mapped loci, the *S. cerevisiae* allele was associated with improved thermotolerance relative to the *S. paradoxus* allele. Independent transgenesis experiments verified the benefit at high temperature of *S. cerevisiae* versions of the housekeeping genes *AFG2*, *CEP3*, and *NIP100*. Together, these results elucidate the complex genetics of *S. cerevisiae* thermotolerance, and they pioneer a genetic mapping method that will be applicable across the tree of life.

601W An experimental approach to studying the role of Horizontal Gene Transfer in shaping fungal secondary metabolism. *P.E. de Reus*, K.F.N. Nielsen, R.J.N. Frandsen Biotechnology and Biomedicine, Technical University Denmark, Copenhagen, DK.

The *in-silico* discovery rate of putative secondary metabolite gene clusters in filamentous fungi is on a surge. With it come increasingly many reports of Horizontal Gene Transfer (HGT) of these gene clusters. Such events are inferred from discrepancies in the synteny, nucleotide composition or taxonomical distribution of the gene clusters in question. Clustered genes have been reported to undergo HGT more often than unclustered genes, for which various hypotheses can be offered. This 'genetic sharing economy' is very interesting from an evolutionary perspective, and yet *in-silico* studies leave fundamental questions about the functional implications of HGT unanswered.

Our study takes an experimental approach to studying the role of HGT in shaping fungal secondary metabolism, focusing on the immediate impact of a whole-cluster HGT event on the recipient. Four naphto-y-pyrone gene clusters - two confirmed and two putative - were selected from four different native fungal hosts (*Fusarium graminearum*, *Fusarium fujikuroi*, *Trichophyton rubrum*, and *Aspergillus eucalypticola*).

Each cluster was transferred to the same locus in host organism *Aspergillus nidulans* using a one-step PCR-based *in-vivo* recombination method. Alongside the clusters, a second library was created in which the cluster pathway-specific transcription factors were overexpressed in a second locus. In a third library, the transcription factors were overexpressed in strains lacking the cluster.

Transcriptional activity of the clusters was investigated using RT-qPCR to address splicing and trans-regulatory interactions as two of the potential barriers to the functional expression of HGT gene clusters in the new host. Metabolic profiling of the libraries was done by HPLC-UV/Vis-High Resolution MS to screen for production of pathway products and intermediates, as well as other novel metabolites arising from cross-chemistry or cross-regulation.

This case study, on a small scale, demonstrates the added value of *in-vivo* experimental work to complement *in-silico* findings and hypotheses, offering additional insights into the role of HGT in fungal secondary metabolism, as well as providing an additional tool for cluster validation.

602T Mating type loci suggest ancestral heterothallism and convergent evolution towards homothallism in *Ophiostomatales* lineages. *T.A. Duong*[1], M.J. Wingfield[1], Z.W. de Beer[2], B.D. Wingfield[1] 1) Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, ZA; 2) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, ZA.

The order *Ophiostomatales* (Ascomycota: Sordariomycetes) is a large group of fungi with diverse biological and ecological adaptations including the ability to cause disease in animals and plants. The order includes ten genera including more than 300 species. In many of these species, especially those residing in *Leptographium, Raffaelea* and *Sporothrix*, sexual structures are unknown and they have been presumed to be asexual. However, several recent studies have shown that many are actually heterothallic fungi most likely with cryptic sexual cycles. The mating type loci have also been fully characterized for a number of species and these all have a heterothallic mating system. The aim of this study was to characterize the mating type locus in selected homothallic species in the *Ophiostomatales*, and to compare this with the locus in heterothallic species. The overall aim was to gain insight into the evolution of the mating type locus in the Ophiostomatales. The genomes of several homothallic species were sequenced and their mating type loci characterized. Comparisons of the mating type loci in homothallic and heterothallic species belonging to five different genera in the *Ophiostomatales*, we were able to show that heterothallism is the ancestral state for the order. Furthermore, that convergent evolution has given rise to a small number of homothallic species residing in distantly related lineages. This is the first study to fully characterize the mating type loci of homothallic members of the *Ophiostomatales* and it has improved our understanding of the evolution of the mating systems in this interesting and important group of fungi.

603F Evolutionary genomics of Spore Killing in *Podospora anserina*. *S.L. Ament-Velasquez*[1], E. Bastiaans[1,2], A. Vogan[1], J. Svedberg[1], A.J.M. Debets[2], S.J. Saupe[3], H. Johannesson[1] 1) Systematic Biology, Department of Organismal Biology, Uppsala University, Norbyvagen 18D, 752 36 Uppsala, Sweden; 2) Laboratory of Genetics, Wageningen University, Droevendaalsesteeg, Wageningen, The Netherlands; 3) Institut de Biochimie et de Genetique Cellulaire, UMR 5095 CNRS, Universite de Bordeaux 2, France.

Mendel's law of equal segregation states that during meiosis each allele has the same probability of being transmitted to the offspring. However, some selfish genetic elements are known to "cheat" and promote their own transmission at the expense of the other allele. These *meiotic drivers* introduce intragenomic conflict within the host organism, which has potential implications for the evolution of genome architecture, population structure, and speciation. In ascomycete fungi, meiotic drivers are known as "spore killers" since half of the progeny (the ones with the killer locus) induce the death of the other half during ascus development. The pseudohomothallic fungus *Podospora anserina* exhibits a particularly complex case of spore killing, where up to six different coexisting spore killers (Psk's) have been described in a natural population sampled in the Netherlands. When confronted in matings, these Dutch Psk's can either kill or not kill other Psk in a hierarchical pattern. Although a novel type of gene (*spok*) conferring spore-killing abilities has been described in French strains, nothing is known on the genetic basis of Dutch Psk's or their effects on the organism fitness. In this context, we used Illumina Hi-Seq (paired-end) technology to sequence the entire Dutch collection (123 strains) plus two French strains. We further selected nine representatives of all Psk and sensitive strains and obtained chromosome-size genome assemblies using PacBio sequencing technology. We discovered that different *spok* paralogs segregate in a ~150kb translocation block located in either arm of chromosome 5 or in chromosome 3 of *P. anserina*, depending on the killer phenotype. The block has been subjected to internal rearrangements and deletions of *spok* genes. Moreover, although the gene *spok2* (placed outside of the block) is nearly fixed in the population, a few samples were found to be truly naive, having no *spok* genes at all. Preliminary population genomic analyses suggest that these naive strains are differentiated from the rest of the Dutch strains. To reconcile our findings with the observed killing hierarchy, we propose the existence of an unidentified killer locus unrelated to the *spok* genes. Future research will focus on functional verification of the *spok* genes for spore killing, fitness effects of carrying the *spok* block and its implications on the evolutionary dynamics of spore killing in *P. anserina*.

604W *Amanita*BASE: population genomics to elucidate the intersection of mating systems, genome organization, and invasion. *M. Holly Elmore*[1], Jaqueline Hess[2], Anne Pringle[3] 1) Organismic & Evolutionary Biology, Harvard University GSAS, Cambridge, MA; 2) University of Vienna, Vienna, AT; 3) Botany and Bacteriology, University of Wisconsin-Madison, Madison, WI.

We have sequenced and are assembling and annotating 99 *Amanita* genomes to enable *Amanita*BASE, a massive comparative genomics effort in this emerging system. In addition to previously sequenced genomes from *A. brunescens, A. muscaria var. guessowii, A. polypyramis, A. thiersii, and A. inopinata, Amanita*BASE will boast 70 specimens of *A. phalloides* from two invasive populations in California, 11 *A. phalloides* specimens from their native range in Portugal, 3 specimens of *A. thiersii*, a saprotrophic *Amanita*, and 11 *Amanita* specimens from across Europe provided by Kew Gardens. The 70 specimens from invasive *A. phalloides* populations in California were collected at three different timepoints over 10 years, which will give us insight into population dynamics. Each sequenced mushroom was meticulously mapped and photgraphed before being collected and detailed ecological notes were takne for each collection site. *Amanita* have a tetrapolar mating system, where compatible mates must have different alleles at both the homeodomain (HD) locus and the pheromone/receptor (PR) locus. The mating loci extend over several kb of repressed recombination and are expanding in a manner similar

to the evolution of sex chromosomes. Whole genomes will enable an analysis of linkage disequilibrium and the comparison of genomes from the same location and timepoints will enable a detailed study of population-level variation in the architecture of mating types. Comparison of genomes from different populations and timepoints can shed light on the evolution of mating type genes, including the origin of new HD and PR alleles.
Comparing native to invasive populations may reveal genomic changes in response to invasion. *A. thiersii* fits the more classic paradigm of a low diversity invasive population that spreads rapidly, whereas *A. phalloides*, which in California appears to be very genetically diverse, is spreading more slowly over a smaller area. This may be explained by the fact that *A. thiersii* is a free-living saprotroph while *A. phalloides* must find compatible mycorrhizal hosts. *A. thiersii*'s near clonality may be related to anomalies in the mating loci that were found in the first sequenced *A. thiersii* genome. Multiple genomes from these populations will allow the study the intersection of mating systems, symbiosis, and range expansion in unprecedented resolution.

605T The important root rot pathogen *Thielaviopsis basicola* shown to be heterothallic. *W.J. Nel*[1], T.A. Duong[2], B.D. Wingfield[2], M.J. Wingfield[1], A. Hammerbacher[1], Z.W. de Beer[1] 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Gauteng, ZA; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Gauteng, ZA.

The ascomycete fungus, *Thielaviopsis basicola* (Microascales: Ceratocystidaceae), was described in 1850 and is a serious, mainly root-infecting plant pathogen of many important crop and ornamental plant species. Despite multiple unsuccessful attempts to induce mating, nothing is known regarding the mating strategy of *T. basicola* and it has often been referred to as an asexual fungus. The aim of this study was to determine the mating strategy of the pathogen. DNA, extracted from a culture derived from a single conidium, was sent for genome sequencing using a combination of next generation sequencing platforms. The draft genome was assembled and the mating locus identified by BLAST searches against known mating type genes available for other species in the Ceratocystidaceae. The results showed that the sequenced isolate harbours a *MAT1-2* idiomorph with a *MAT 1-2-1* gene, as well as a gene with strong similarity to *MAT 1-2-7* previously reported from species in the related genus *Huntiella.* The absence of the *MAT 1-1-1* gene suggests that *T. basicola* is most likely heterothallic. Ongoing research is focussed on attempts to locate a *MAT1-1* idiomorph in a large collection of cultures and to further understand whether sexual reproduction might be occurring in this important pathogen.

606F Recombination is suppressed on collinear chromosomes in *Neurospora tetrasperma*. *Jesper Svedberg*[1], Yu Sun[2], Markus Hiltunen[1], Pádraic Corcoran[3], Hanna Johannesson[1] 1) Department of Organismal Biology, Uppsala University, Uppsala, SE; 2) Guangdong Provincial Key Laboratory of Protein Function and Regulation in Agricultural Organisms, College of Life Sciences, South China Agricultural University, CN; 3) Department of Animal and Plant Sciences, University of Sheffield, UK.

In many eukaryotic genomes meiotic recombination is locally suppressed as a response to various selective pressures. The most well-known examples are non-recombining sex chromosomes, where recombination is hypothesised to be suppressed in response to sexually antagonistic selection, but similar ”supergenes” have also been implicated in driving local adaptation and speciation in other systems. The causes of the suppression of recombination are in many of these cases not well understood; structural rearrangements – such as inversions – have often been implicated, but they may also be a consequence of suppression, rather than a cause. In the pseudohomothallic ascomycete *Neurospora tetrasperma* the proper packaging of the two mating types in is ensured by suppressing recombination between the mating-type locus and the centromere. Cytological and crossing data, as well as data on nucleotide divergence and linkage disequilibrium have further shown that recombination is absent in 60-80 % of the chromosome. In an earlier genome study the chromosome carrying one of the mating types in one specific strain was also shown to carry a complex of overlapping inversions, and these inversions were hypothesized to maintain the suppression of recombination in the species. By generating high quality genome assemblies from eight other *N. tetrasperma* strains we can here show that the majority of *N. tetrasperma* strains are collinear, that the previously identified inversions are a derived feature and that the suppression of recombination has to be maintained through non-structural means. We also identify several other derived inversions on the chromosome, supporting the hypothesis that suppression of recombination can relax selection against inversions. These results establish the existence of large-scale, long-term recombination suppression in the absence of structural rearrangements and caution against interpreting the presence of such rearrangements as being causative. The identified mode of suppression of recombination is a new phenomenon that potentially has significant effects on genome evolution and how we interpret signals of divergence.

607W Return of the mitochondrial DNA. *B. Brankovics*[1,2], P. van Dam[3], M. Rep[3], S. de Hoog[1,2], T. van der Lee[4], C. Waalwijk[4], A. van Diepeningen[1,4] 1) CBS Fungal Biodiversity Centre, KNAW, Utrecht, NL; 2) Institute for Biodiversity and Ecosystem Dynamics, UvA, Amsterdam, NL; 3) Swammerdam Institute for Life Sciences, UvA, Amsterdam, NL; 4) Wageningen Plant Research, WUR, Wageningen, NL.

The mitochondrial genome is present in high copy number within cells, making it easier to access than single copy nuclear sites. In many organisms mitochondrial sequences are uniparentally inherited, hence non-recombining, making them ideal for phylogenetic reconstructions. This is the reason why mitochondrial markers have been widely used in population genetic studies. In the '90s the mitochondrial encoded COI (*cox1*) was used as the first barcoding marker. However, amplifying the sequence proved problematic in many fungal groups, because the frequent insertion of introns into the region made universal primer design difficult. Hence, the mitochondrial marker was abandoned and the barcoding community choose the ITS region as the official barcode for fungi. Also this barcode proved to have insufficient resolution in many closely related species. Thus, multi-locus analysis became the new standard, most of which included at least one mitochondrial marker. There has been no consensus on which mitochondrial loci to include.
With next generation sequencing and new assembly tools it is possible to assemble the complete mitochondrial genome of the isolates, which provide all the benefits that are associated with using mitochondrial markers. In addition, using the complete mitochondrial genome offers better resolution for phylogenetic analyses and with sufficient sampling, it can be placed in the context of previous works done on mitochondrial barcoding markers.
In order to prove the usefulness and applicability of this whole mitochondrial genome analysis approach, we have assembled the complete mitogenomes of 61 *Fusarium oxysporum* strains. Multiple NGS platforms were used and a new assembly pipeline was developed to

reconstruct the mitochondrial genomes. Our findings concurred that complete mitogenomes are a rich source of phylogenetic information that confirm earlier phylogenies and add more resolution. Furthermore, the analysis has revealed the presence of a cryptic parasexual cycle in *F. oxysporum* as revealed by mitochondrial recombination. In conclusion: complete mitochondrial genome sequences offer a stable basis and reference point for phylogenetic and population genetic studies, while still offering compatibility with earlier studies.

608T Genome-wide identification of horizontal gene transfer in *Fusarium verticillioides*. *S. Gao*[1], J Wisecaver[2], Y Zhang[3], L.-J Ma[3], A Rokas[2], S Gold[4], A Glenn[4] 1) University of Georgia, Athens, Georgia; 2) Vanderbilt University, Nashville, Tennessee; 3) University of Massachusetts, Amherst, Massachusetts ; 4) Richard B. Russell Research Center, USDA-ARS, Athens, Georgia.

Horizontal gene transfer (HGT), the exchange and stable integration of genetic material between different lineages, breaks species boundaries and generates new biological diversity. In eukaryotes, despite potential barriers, like the nuclear envelope and multicellularity, HGT may be facilitated by the intimate contact between organisms and other strategies. Intrigued by the potential role of HGT in the evolution and adaptation of the maize pathogen *Fusarium verticillioides* (*Fv*), we applied a phylogenomic pipeline to identify potential HGT candidates in the genome of *Fv*. From an initial output of 1801 genes, manual curation revealed 117 strong HGT candidates, which tend to be intronless, non-plastid-derived, and bacteria-acquired. Functional category analysis suggested several enriched metabolic pathways (e.g. lysine biosynthesis and nitrogen metabolism) compared to the general frequency of such genes within the *Fv* genome. Interestingly, among the five candidates that were categorized as being involved in lysine biosynthesis, FVEG_09873 has no orthologs in other fungi. This gene was acquired from plant-associated Proteobacteria and putatively encodes a diaminopimelate epimerase in the diaminopimelic acid pathway employed by most bacteria but not commonly found in fungi, further supporting the bacterial origin of this candidate. Another promising HGT candidate acquired from bacteria is FVEG_10494 that encodes a putative class-III aminotransferase, functioning in nitrogen metabolism. Additional analyses will focus on other HGT candidates having limited fungal distribution. Transcriptional profiling and functional characterization are underway to determine the significance of select HGT candidates on the fitness, virulence, and physiological responses of *Fv*.

609F Repeat Induced Point Mutations: Driving genome divergence of the *Fusarium fujikuroi* species complex. *S. van Wyk*[1], E. T. Steenkamp[1], B. D. Wingfield[2], L. De Vos[2], N. A. van der Merwe[2] 1) Department of Microbiology, University of Pretoria, Forestry and Agricultural Biotechnology Institute , Pretoria, Gauteng, ZA; 2) Department of Genetics, University of Pretoria, Forestry and Agricultural Biotechnology Institute, Pretoria, Gauteng, ZA.

The *Fusarium fujikuroi* species complex (FFSC) is a monophyletic assemblage of plant pathogens that threaten sustainable agriculture and forestry. Despite the growing number of genomes available for this complex, the genetic basis and genomic processes underlying species differentiation amongst its lineages remain poorly understood. In this study, we considered genome differentiation by investigating Repeat Induced Point mutation (RIP), which is generally regarded as an irreversible genome defense mechanism acting on duplicated sequences and transposable elements. For this purpose, we investigated the frequency, effect, distribution and genomic context of hyper-mutation associated with RIP in the genomes of four representatives of the FFSC (i.e., *F. circinatum*, *F. temperatum, F. fujikuroi* and *F. verticillioides*). The results of our in *silico* analyses showed that hyper-mutation patterns, indicative of RIP-like genome defense, were present in all the genomes analysed, although the extent and frequencies of mutation varied among genomes. Variable genomic regions such as telomeres and dispensable chromosomes also seemed to be more highly affected by RIP than the so-called core genomic regions. Here, the RIP targeted regions appeared to be distributed throughout core genomic regions. Comparison of the four genomes also revealed that signatures of RIP were more highly pronounced in *F. temperatum* and *F. fujikuroi*, to such an extent that the RIP affected regions mostly did not contain genes. This was in contrast to the situation in *F. circinatum* and *F. verticillioides*, suggesting that rapid sequence divergence and modification of codon usage allowed for the occurrence of genes in their RIP affected regions. Future research will seek to investigate the genetic and biological aspects contributing to this variation within this species complex.

610W Population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen, *Fusarium culmorum*, in Algeria, Italy, Australia and the United States. *I. Laraba*[1], H. Boureghda[1], N. Abdallah[1], F. Obanor[2], A. Moreti[3], D. M. Geiser[4], A. C. Kelly[5], S. McCormick[5], R. H. Proctor[5], T. J. Ward[5], K. O'Donnell[5] 1) Laboratoire de phytopathologie et de biologie moléculaire, Département de botanique, Ecole Nationale Supérieure Agronomique, Algiers, Algeria; 2) Grains Research and Development Corporation, Canberra, Australia; 3) Institute of Sciences of Food Production, CNR, Bari, Italy; 4) Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, Pennsylvania; 5) NCAUR-USDA-ARS, Peoria, Illinois.

Surveys for crown rot (FCR) and head blight (FHB) of Algerian wheat conducted during 2014 and 2015 revealed that Fusarium culmorum producing 3-acetyldeoxynivalenol (3ADON) and nivalenol (NIV) were the primary causal agents of these economically important diseases. Morphological identification of the F. culmorum isolates (n FCR = 91, n FHB = 15) was confirmed by sequencing a portion of TEF1. To assess mating type idiomorph composition, trichothecene chemotype potential and global population genetic structure, the Algerian strains were compared with F. culmorum collections from Italy (n = 27), Australia (n = 30) and the United States (n = 28). A PCR assay for MAT idiomorph revealed that MAT1-1 and MAT1-2 strains were segregating in nearly equal proportions, except within Algeria where two-thirds of the strains were MAT1-2. An allele-specific PCR assay indicated that the 3ADON trichothecene genotype was predominant globally (83.8% 3ADON) and in each of the four countries sampled. Moreover, while significant differences in trichothecene genotype frequencies were observed in Algeria, Australia and the United States, none was detected in Italy. Fusarium culmorum strains produced 3ADON (n = 65), NIV (n = 14), or culmorin (n = 79) in vitro; however, 9 strains failed to produce detectable levels of toxins. Global population genetic structure of 191 strains was assessed using 9 microsatellite markers (SSRs). AMOVA of the clone corrected data indicated that 89% of the variation was within populations. Bayesian clustering analysis of the SSR data, using the admixture model with independent allele frequencies, identified two globally distributed, sympatric populations within which both trichothecene chemotypes and mating types were represented.

611T Experimental evolution of *Fusarium oxysporum* reveals genome rearrangements as the major evolution mechanism governing short-term evolution. *D.H. Ayhan*[1], C. López Díaz[2], A. Di Pietro[2], L-J. Ma[1] 1) Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA; 2) Department of Genetics, University of Córdoba, Spain.

Natural selection is a fundamental evolutionary process that acts on changes in a genome and results in the adaptation of an organism. The most common forms of genome changes range from single nucleotide polymorphism (SNP), small insertion/deletion, segmental duplication, chromosomal rearrangement, to whole genome duplication. How do these different changes influence the evolutionary processes under different selection pressure? To explore answers to these questions, this study takes an experimental approach to observe evolving processes using a model organism *Fusarium oxysporum*, a highly adaptive species complex containing the mobile and lineage-specific (LS) chromosomes that are rich for transposons and determines host-specific pathogenicity. The same starting population (*F. oxysporum* f. sp*. lycopersici* strain 4287, a tomato pathogenic isolate) was passaged ten times through two distinct serial transfers in its host or on rich media plates. We have sequenced 5 evolved populations from each serial transfer at the end of the experiment. Comparative genomics revealed the presence of segmental duplications and deletions on parts of LS regions, suggesting that genome rearrangements are the major forces that shape short-term evolution. Retaining a relatively constant mutation rate, there is a much lower possibility to accumulate beneficial SNPs in short time frame. As expected, we observed few SNPs among these evolving populations. Interestingly, the populations that evolved in rich media tended to lose parts of or entire LS chromosomes, suggesting the dispensable nature and energy cost associated with these chromosomes when growing on a petri dish. These results highlight that different evolutionary constraints determine the outcome when populations are adapting to different environments.

612F Chromosome plasticity during experimental evolution of the cross-kingdom pathogen *Fusarium oxysporum*. *C Lopez-Diaz*[1], D Hazal Ayhan[2], L.J Ma[2], A Di Pietro[1] 1) Department of Genetics, University of Córdoba, Cordoba, Spain; 2) University of Massachusetts, Amherst, USA.

Filamentous plant pathogens undergo rapid evolution, leading to expansions or shifts in host range. The *Fusarium oxysporum* species complex collectively causes vascular wilt disease in more than a hundred different crops. Meanwhile, clinical isolates of the fungus cause life-threatening systemic infections in humans. Remarkably, a single strain of *F. oxysporum, Fol* 42-87, is able to kill tomato plants, immunodepressed mice and larvae of the invertebrate animal model *Galleria*. The evolutionary mechanisms underlying host range dynamics remain poorly understood. Here we followed an experimental evolution approach involving serial passages of *Fol* 42-87 through tomato plants, axenic media plates, or *Galleria* larvae. Independently evolved populations obtained after ten consecutive passages through plants or rich media displayed notable phenotypic differences with respect to the initial clonal isolate, with four of the five plate-passaged populations showing significantly reduced virulence on tomato plants. Resequencing of the evolved populations revealed segmental duplications and deletions on transposon-rich accessory regions of the genome, including loss of entire chromosomes. These findings suggest that chromosome plasticity acts as a major evolutionary driver in *F. oxysporum*, and provide new insights into the genetic mechanisms underlying host adaptation in this cross-kingdom fungal pathogen.

613W Adaptive Zn tolerance is associated with high expression of a CDF family transporter in the ectomycorrhizal fungus *Suillus luteus*. *J. Ruytinx*, L. Coninx, H. Nguyen, J. Colpaert Hasselt University, Diepenbeek, BE.

Suillus luteus is a cosmopolitan fungal species, symbiotically associated with pine trees. In particular, in primary successions of pines this species is abundant and involved in seedling establishment. On severely metal-contaminated soils, Zn-tolerant *S. luteus* populations evolved by natural selection. Tolerant individuals effectively protect their host tree from Zn toxicity on these soils. However, the molecular-genetic mechanism underlying adaptive Zn tolerance in *S. luteus* is unknown. We hypothesize that the tolerance phenotype is due to an adaptation in the common Zn homeostasis network. By comparative and functional genetics we identified several *S. luteus* genes encoding transporters involved in Zn homeostasis. One of these transporters, SlZnT2, a CDF family transporter exhibits a differential gene expression among Zn-tolerant and Zn-sensitive phenotypes. Zn-tolerant *S. luteus* isolates show a high expression whereas Zn-sensitive isolates show a near to background expression of the gene. The difference in expression level seems to be partly caused by an extensive gene multiplication, partly by differences is cis-regulation. *SlZnT2* copy number in Zn-tolerant isolates ranges between two and seven. Zn-sensitive isolates have one copy. Three different promoter genotypes were identified; one of these was exclusively found in Zn-tolerant isolates. SlZnT2 is predicted to be localized on the tonoplast and to move Zn from the cytoplasm into the vacuole. We could not confirm this function by heterologous expression in yeast. In conclusion, Zn-tolerant *S. luteus* isolates are selected for high expression of *SlZnT2*, a CDF family transporter of unknown function. Further functional analyses are required to define the role of high *SlZnT2* expression in Zn tolerance.

614T Genomic analysis reveals multiple endemic and pandemic lineages of rice blast and gene flow into recombining populations from southeast continental Asia. *Pierre GLADIEUX*[1], Maud THIERRY[2], Adrien RIEUX[3], Sebastien RAVEL[2], Sandrine CROS-ARTEIL[1], Henri ADREIT[2], Joëlle MILAZZO[2], Elisabeth FOURNIER[1], Ryohei TERAUCHI[4], Didier THARREAU[2] 1) BGPI Research Unit, INRA, Montpellier, FR; 2) BGPI Research Unit, CIRAD, Montpellier, FR; 3) PVBMT Research Unit, CIRAD, La Réunion, FR; 4) Iwate Biotechnology Research Center, Kitakami, Iwate, JP.

The rice blast fungus *Magnaporthe oryzae* is the most damaging rice pathogen, and a textbook example of widely distributed, rapidly adapting pathogen, despite limited genetic diversity. The aim of our study was to elucidate the factors and evolutionary changes underlying the emergence, diversification and spread of *M. oryzae* in rice agrosystems. Analyses of population structure based on Infinium-genotyping of 5300 SNPs for 970 isolates collected on five continents identified three main pandemic lineages on *indica* and *japonica* rice, coexisting with multiple lineages with more restricted distributions in Asia and sub-saharan Africa. Analyses of recombination based on resequencing data for a subset of 89 isolates revealed that most lineages are highly clonal, although we found evidence for recombination in a widely distributed lineage infecting japonica and hybrid rice in Yunnan, Laos and Thailand. Because the sequenced isolates were collected between 1973 and 2009 and recombination is limited, we could use a phylogenetic approach to date the emergence and global dispersal of *M. oryzae* using dated tips to calibrate tree nodes. Our analysis provided an accurate estimate of the nucleotide substitution rate. Rice- and Setaria-infecting major lineages diverged ~10000 years before present, matching the oldest archaeological evidence for human exploitation of rice, and rice-infecting lineages radiated ~1200 years ago. Probabilistic and non-parametric 'chromosome painting' revealed gene flow

into multiple non-pandemic lineages -including the recombining lineages from southeast continental Asia- from multiple source populations. Gene sets matching putatively migrant mutations were functionally enriched in genes involved in non-self recognition and programmed cell death, suggesting a role of fungal immunity in increasing the genomic diversity of rice blast populations. Despite the general lack of polymorphism and the accumulation of deleterious mutations in rice-infecting lineages, we could identify regions with signatures of balancing selection (increased functional variation) that were functionally enriched in small secreted proteins and proteins involved in oxidation-reduction processes. Our work provides a population-level genomic framework for defining molecular markers to assist in the control of rice blast and for investigating the molecular underpinnings of phenotypic and fitness differences between divergent lineages.

615F PgtSNP 3.0k chip: A high-throughput SNP genotyping array for *Puccinia graminis* f. sp. *tritici*. *Les J. Szabo*[1], Jerry L. Johnson[1], Pablo Olivera[2], Yue Jin[1] 1) Cereal Disease Laboratory, USDA- ARS, St. Paul, MN; 2) Department of Plant Pathology, University of Minnesota, St. Paul, MN.

Understanding the genetic diversity and population structure of the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, has been hampered by the obligate, dikaryotic nature of this fungus and the lack of a robust high-throughput genotyping tools. A custom Illumina Infinium SNP array (PgtSNP 3.0k chip) was developed based on a *P. graminis* f. sp. *tritici* reference genome (U.S. isolate 75-36-700) and NGS sequence data from selected isolates. A balanced selection scheme was used to identify 3,000 candidate SNP loci evenly distributed (average 27 kb) along the assembled supercontigs, covering greater than 90% of the genome. These loci are partitioned between genic (84.1%) and intergenic (15.9%). This new array represents an expansion of the original PgtSNP 1.5k chip, based on GoldenGate technology. To test the performance of the PgtSNP 3.0k chip a set of 100 *P. graminis* f. sp. *tritici* isolates, derived from wheat stem rust collections from Africa, Asia, Europe, Middle East and North America, were used. In general, the *Puccinia graminis* f. sp. *tritici* isolates were grouped geographically, with the North American isolates forming two genetic clusters and the isolates from Africa, Asia, Europe and Middle East forming three genetic clusters. In addition, DNA extracted from ethanol-killed *P. graminis* f. sp. *tritici* infected wheat tissue (single pustule) was tested and shown to give similar results to DNA extracted from fresh samples (collected spores and infected tissue), thus allowing genotyping of a wider variety of samples. The PgtSNP 3.0k chip provides the wheat rust community a powerful genotyping tool for mapping the genetic landscape of *P. graminis* f. sp. *tritici*.

616W Molecular analysis of killer DNA from *Neurospora Spore killer-2* . *N. Rhoades*[1], A. Harvey[1], D. Samarajeewa[1], P. Manitchotpisit[1], J. Svedberg[2], D. Brown[3], P. Shiu[4], H. Johannesson[2], T. Hammond[1] 1) School of Biological Sciences, Illinois State University, Normal, IL; 2) Department of Organismal Biology, Uppsala University, Uppsala, Sweden; 3) National Center for Agricultural Utilization Research USDA-ARS, Peoria, IL; 4) Division of Biological Sciences, University of Missouri, Columbia, MO.

In standard Mendelian inheritance, each allele in a sexual cross has an equal probability of being transmitted to the next generation. However, there are certain "selfish" genes that are able to propagate themselves at a higher frequency than others in a population. Examples include the *Neurospora Spore killers* (*Sk*): *Spore killer-1* (*Sk-1*), *Spore killer-2* (*Sk-2*), and *Spore killer-3* (*Sk-3*). The work presented here focuses on the *Sk-2* element. Crosses of *Sk-2* x Sk^S (*Spore killer*-sensitive) produce asci with four black, viable ascospores and four white, inviable ascospores. The four surviving ascospores inherit the *Sk-2* element, resulting in a nearly 100% transmission of *Sk-2* to the surviving population. Because of this, *Sk-2* is transmitted in a non-Mendelian manner. Previous work has identified one gene and one locus involved in the spore killing mechanism, *rsk* (resistance to *Spore killer)* and *rfk-1* (required for killing). Here, we report the identification of a 1500 bp segment of DNA (designated *AH36*) from the *rfk-1* locus that correlates with a peculiar ascus abortion phenotype. Genetic evidence suggests that *AH36* encodes a transcript that must be expressed for ascus abortion to occur. Preliminary *in-silico* analyses suggests that AH36 may encode a 39 amino acid protein that causes the ascus abortion phenotype. Site-directed mutagenesis and sub-cloning assays are in progress as part of an effort to test this hypothesis.

617T Genomic diversity of *Malassezia* yeasts: implications for epidemiology and the clinic. *Teun Boekhout*[1,2], Anna Kolecka[1], Bart Theelen[1], Claudia Cafarchia[3], Roberta Iatti[3], Thomas Dawson[4], the Malassezia Genome Consortium 1) CBS Fungal Biodiversity Centre, Utrecht, NL; 2) Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, NL ; 3) Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, 70010 Valenzano, Bari, Italy ; 4) A*STAR Institute of Medical Biology (IMB), Singapore.

Malassezia is a genus of lipid-dependent basidiomycetous yeast species belonging to Ustilaginomycotina. *Malassezia* spp. are a major component of the human skin mycobiome and may be implicated in several skin disorders, such as pityriasis versicolor, seborrheic dermatitis and atopic dermatitis. Some species may also causes sepsis, especially in neonates. Here we present results from a comparative genomics study of all species of the genus with the aim to contribute to our understanding of the biodiversity, disease potential and genetic processes such as hybridization.

Methods.

The genomes of all species were sequenced by Illumina technology, and for some species more than one isolate using Illumina and Pacific BioSciences technology. Bioinformatics analysis included de novo assembly, annotation and gene family analysis. Additional analysis also included MultiLocus sequencing, MALDI-TOF MS and Amplified Fragment length Polymorphism (AFLP) applied to a greater set of isolates, including outbreak isolates.

Results.

Malassezia have among the smallest free-living eukaryotic genomes, ranging between 7-9 Mb, with some exceptions having ca. 14Mb genomes. Three phylogenetic clusters can be discerned, largely confirming previous insight obtained by the analysis of rDNA. The compact genomes differ from those of related Ustilaginomycotina by major gene loss and limited gene gain processes. Interestingly, the larger genomes all belonged to the *M. furfur* complex with three distinct clades, with one representing potential aneuploids as hypothesized based on previous MLST, AFLP and PFGE data. Strains belonging to one of the potential *M. furfur* parental clades seem to be more prevalent in causing invasive infection. Detailed comparative genomics analysis of the *M. furfur* strains may shed light on the molecular mechanisms. In addition, MLST revealed a significant genetic heterogeneity of outbreak isolates suggesting that patients can be infected by multiple isolates.

Conclusions: All 17 hitherto described species of *Malassezia* belong to three major clades that differ in gene loss/gain patterns. The

genomes of all species are small, except for those isolates that seem to represent hybrids in the *M. furfur* complex. Invasive isolates of *M. furfur* mainly belong to a specific parental group but also show considerable diversity. It remains to be seem how this relates to the *Malassezia* diversity present on skin of neonates, parents and hospital staff.

618F Population structure of *Aspergillus flavus* in soil and corn kernels from four different states. *M.S. Molo*[1], R. Singh[1], J.B. White[1], T. Isakeit[2], K.A. Wise[3], C.P. Woloshuk[3], B.H. Bluhm[4], B.W. Horn[5], R. Heiniger[1], I. Carbonw[1] 1) North Carolina State University, Raleigh, NC; 2) Texas A&M University, College Station, TX; 3) Purdue University, West Lafayette, IN; 4) University of Arkansas, Fayetteville, AR; 5) United States Department of Agriculture, Agriculture Research Service, Dawson, GA.

Aspergillus flavus is a cosmopolitan soil-borne fungus that contaminates oil seed crops by producing carcinogenic aflatoxins (AFs). Ingesting AF-contaminated grains has been linked to hundreds of deaths in Africa. Due to the health risks AFs pose, the FDA has strict regulations on grain contaminated with AFs. Therefore, AFs have a huge economic impact on corn growers through management practices, yield loss, and adverse health effects. Previous reports indicate that certain *A. flavus* genotypes exist in the soil and others are specialized to colonize corn kernels, but this is based on sequence data from a relatively small number of genetic markers. Understanding the genetic differences that separate corn and soil isolates could lead to insights into the development of the next generation of biological control agents. We sampled *A. flavus* from soil early in the growing season and from kernels at harvest in Texas, North Carolina, Arkansas, and Indiana in 2013. This allowed us to examine genetic structure between populations from different states as well as between soil and kernel substrates. We sequenced a total of 246 isolates using double digest restriction-site associated DNA sequencing, which represents the genome in a manageable size by providing markers across each chromosome and the mitochondria. A preliminary analysis revealed that each state had native strains of *A. flavus* as well as shared evolutionary lineages. Further work will examine the distribution of genetic variation between soil and kernel populations.

619W Influence of farming practices on the genetic diversity of *Cercospora zeina* in South Africa. *DL. Nsibo*[1], N. Kunene[1], I. Barnes[2], DK. Berger[1] 1) Plant and Soil Sciences, FABI, University, Pretoria, Gauteng, ZA; 2) Genetics, FABI, University, Pretoria, Gauteng, ZA.

Grey leaf spot disease (GLS) is an important foliar disease of maize and a threat to global food security by causing up to 65 % yield losses. The increasing prevalence of GLS in Africa, Asia and the Americas is driven in part by increased conservation agriculture. *Cercospora zeina* is the causal agent of GLS in Africa, and the disease is prevalent on both commercial and smallholder maize farms. Since the latter are more similar to conservation agriculture, with no chemical control and a wider diversity of genotypes within a field, we hypothesized that smallholder farming practices would select for distinct pathogen populations with greater diversity than large-scale commercial farms. Genotyping with 13 microsatellite markers was performed on 347 isolates from seven smallholder farms, each within a 50 km radius of commercial production areas. Only 61% of the 75 alleles obtained for these markers were shared with those identified from commercial farms. Although no population differentiation was observed between the farming systems, a slightly greater gene diversity was observed from smallholder farms (0.44 compared to 0.35) in KwaZulu Natal province, a local hotspot of GLS. Both mating type idiomorphs (*MAT1-1* and *MAT1-2)* were isolated from each field for both farming systems, and complied with the 1:1 mating type frequency at six out of seven sites screened. It is likely that dispersal mechanisms such as wind and the possibility of sexual recombination, influences the admixture of haplotypes into one large mega population across the region. Although a higher genetic diversity was observed under conservation agriculture conditions, the current data did not provide strong support for our hypothesis. Results from this study indicate that farming practices do not have an influence on the genetic diversity of *C. zeina* on maize in South Africa and that the same disease management strategies can be used for both smallholder and commercial farming systems.

620T Evidence for sexual reproduction: identification, frequency and spatial distribution of *Fusicladium effusum* (pecan scab) mating type idiomorphs. *Carolyn A. Young*[1], Clive H. Bock[2], Nikki D. Charlton[1], Chakradhar Mattupalli[1], Kim M. Plummer[3], Johanna K. Bowen[4], Bruce W. Wood[2] 1) Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401, U.S.A; 2) USDA-ARS-SEFTNRL, 21 Dunbar Rd, Byron, GA 31008, U.S.A; 3) Department of Animal, Plant and Soil Sciences, La Trobe University, Melbourne, Australia; 4) The New Zealand Institute for Plant & Food Research Ltd, New Zealand.

Fusicladium effusum is the causal agent of pecan scab, the most prevalent disease of pecan (*Carya illinoinensis*) in the southeastern USA. Infection by the pathogen can result in serious and even catastrophic yield loss when conditions are favorable for an epidemic. *Fusicladium effusum* is currently known only by its asexual (conidial) stage. However, the degree and distribution of genetic diversity observed within and among populations of *F. effusum* are typical of a sexually reproducing fungal pathogen, and comparable to other dothideomycetes with a known sexual stage, including the closely related apple scab pathogen, *Venturia inaequalis*. Using the mating type loci from V. inaequalis we identified a single mating-type gene, mtAA (mat1-1, containing alpha-box) idiomorph, in a draft genome of F. effusum flanked by two conserved genes encoding a DNA lyase (apnB) and an uncharacterised PH domain-containing protein. The mating type locus, spanning the flanking genes, was amplified and sequenced in 14 isolates, collected from different geographic locations and cultivars. Only seven of these samples contained the mtAA idiomorph, whereas the remaining samples contained the mtBA (mat1-2, containing HMG-box) idiomorph. A multiplex PCR screen was developed to amplify a conserved housekeeping gene (tubB), mtAA and mtBA, and used to screen 1203 F. effusum isolates collected from 13 pecan populations across the southeastern USA. A hierarchical sampling protocol representing region, orchard, tree and leaflet was followed at all sites so the mating type structure at different spatial scales could be assessed. Analysis of this collection revealed the frequency of the mating type idiomorphs is in a 1:1 equilibrium of *MTA*:*MTB*. In vitro paired matings resulted in the formation of pseudothecia only between isolates of the opposite mating types indicating that F. effusum is heterothallic. The apparent equilibrium of the mating type idiomorphs and *in vitro* mating provides impetus to a renewed effort to search for a sexual stage of *F. effusum.* Identification of a sexual stage will provide a fuller understanding of the disease epidemiology and pathogen population genetics, and eventually a basis for better management of existing resistance genes in pecan.

621F Mating System and Mating Genes Evolution in *Suillus luteus*. *Y.-H. Ke*[1], R. Debray[1], K. Hameed[1], A. Senchenkov[1], A. Rojas[1], H.-L. Liao[2], N. Nguyen[3], P. Kennedy[3], T. Bruns[4], R. Vilgalys[1] 1) Duke University, Durham, NC; 2) University of Florida, Quincy, FL; 3) University of Minnesoda, Minneapolis, MN; 4) University of California, Berkeley, CA.

The bolete genus *Suillus* is a highly diverse lineage of worldwide distributed ectomycorrhizal fungi (over 50 spp) genus with a worldwide distribution which that has emerge as a model to study ectomycorrhizal symbiosis with different host trees in the Pinaceae system. Mating pattern is an important character in fungi that deeply impacts affectingits population structure, ecology and evolution. Previous study of mating system studies in *Suillus* have been largely restricted to small scale mating experiments that arePrevious works indicate *S. luteus* and *S. granulatus* possessing tetrapolar mating systems. However, the traditional method for mating system is difficult to conduct in *Suillus* because basidiospores hardly germinate poorly on artificial media, and because dikaryotic mycelia don't do not produce clamp connections after successful mating. Based on observed changes in mycelial morphology and nuclear cytology, Fries in the 1980's suggest that two species *S. luteus* and *S. granulatus* each have a tetrapolar mating system governed by two mating loci (A and B). However, sThus only mass morphology and cytology are used to conclude mating results before. Strong supports from microscopic and molecular-based information are were not available. In the other hand, former mating system studies in Suillus are restricted to small scale mating experiments from a few fruiting bodies. Since *S. luteus* had a rapid habitat expansion within hundreds of years by human activities, by mating gene polymorphism sampling across worldwide populations, we are able to identify gene evolution and allele frequency changes along the process.

InAs an alternative to traditional approaches that rely on pairing of single-spore isolates this research, we introduced developed a new sequencingsequence-based method to easily obtainfor determining mating gene haplotypes and mating system inS. luteus based on next-gen sequencing. From By comparing genome data for the A mating locus from 6 species (available at JGI mycocosm website), we designed six primer pairs to that successfully amplify 900-1300 bp fragments spanning the A mating locus. separate mating A loci to 900-1300 bp long fragments. By employingUsing NGS techniques, we are able to rebuild reconstruct the two haplotypes of mating gene haplotypessi, and infer patterns of mating allele segregation among sibling progeny and fruit bodieseach individual. In the future, we willThese data are being used use them to study the life history and mating biology in of *S. luteus and other Suillus species*. In the first part, we will inoculateinoculated basidiospores from each spore prints onto one pine seedling roots and allow them to germinate and form mycorrhizae with their pine host through random mating. random mating. Dikaryotic mycelia forming on roots are sequenced to determine genotypes then and to infer inferring mating system. In the second part, we will survey population level mating gene diversity of *S. luteus* to indicate mating gene evolution and allele frequency change during its rapid expansion historyacross different geographic populations.

622W *wtf* causes infertility via a poison-antidote system. Maria A. Bravo Nunez[1], Nicole L. Nuckolls [1], Michael T. Eickbush[1], Janet M. Young[2], Gerald R. Smith[2], Harmit S. Malik[2,3], *SaraH E. Zanders*[1] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Howard Hughes Medical Insitute, Chevy Chase, MD.

Meiotic drivers are genomic parasites that act selfishly to promote their own transmission into more than half of the gametes (e.g. sperm) generated by heterozygotes. In this work, we identify *Schizosaccharomyces kambucha wtf3* as the first described meiotic drive gene in a yeast species. *wtf3* belongs to the class of 'ultra-selfish' meiotic drivers because it acts by destroying the gametes that fail to inherit the gene. This gamete destruction increases *wtf3* transmission into up to 96% of gametes generated by a heterozygote, while cutting fertility of by nearly 50%. To enact drive, *wtf3* encodes two largely overlapping proteins. The short protein is a trans-acting gamete-killing poison that fills the cytoplasm surrounding and can enter gametes. The long protein is an antidote that becomes enriched only in the gametes that encode *wtf3*, thereby rescuing them from destruction. *wtf3* is a member of the previously uncharacterized large *wtf* gene family. Gamete killing ability is shared by other *wtf* genes, suggesting meiotic drive is the ancestral function of the family. This work provides insight into how meiotic drivers function, shape genome evolution and cause rapid speciation.

623T Population genomic and transcriptomic analysis of the brown rot fungus Fomitopsis pinicola. *Nils Högberg*, Prakash Kancherla, Jan Stenlid, Mikael Brandström Durling Forest mycology & plant pathology, Forest mycology & plant pathology, Uppsala, SE.

The basidomycete Fomitopsis pinicola is an effective wood decomposer and one of the most common brown rot fungi. It is part of a species complex with several closely related species. We have sequenced 28 haploid isolates of this fungus in order to use population genomic methods to reveal footprints of selection especially with respect to wood decay mechanisms. One isolate was sequenced with a combination of Illumina and SOLiD sequencing technology. The resulting genome sequence was 45MB and 13,888 gene models were predicted. A comparison with a previously sequenced *F. pinicola* isolate showed that their CAZyme profiles were relatively similar while the secondary metabolite clusters differed substantially between the two isolates. When *F. pinicola* transcriptomes were compared between a glucose medium and wood decomposition several CAZymes, oxidative enzymes, transporters, lipases and proteases were among the groups of genes which were up-regulated during wood decomposition. The most highly up regulated gene was a copper-dependent lytic polysaccharide monooxygenase. The gene expression pattern was similar to that of other brown rot fungi but differed in important aspects for example with respect to iron reduction and oxalate metabolism.

SNPs from a total of 28 Swedish *F. pinicola* genomes were used to identify selection and for genome wide association of markers linked to the phenotypic trait wood decomposition which differed among the sequenced isolates. Footprints of selected sweeps with elevated levels of linkage disequilibrium were predominately found around genes involved in secondary metabolite production, transportation and chitin production. When the phenotypic trait wood decay was associated with the genome wide SNP-matrix, markers close to a quinate permease and genes involved in oxidoreductase activity, transportation, alcohol metabolism were linked to this trait.

In conclusion, our study found that several groups of genes which were highly up-regulated during wood decomposition also were associated to the wood decay trait in a GWAS analysis, among these were genes which may be linked to the Fenton reaction. Footprints of selected sweeps were mostly found for genes involved in secondary metabolism. Secondary metabolites have been suggested to act as competitive weapons used against other fungi and their rapid evolution may be a factor behind the success of the *F. pinicola* species complex in conifer forests.

624F Mycosarcoma, a resurrected generic name for corn smut (Ustilago maydis). *D. Begerow*[1], A. McTaggart[2], R. Shivas[3] 1) Geobotany, Ruhr-Universität Bochum, Bochum, DE; 2) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 3) Plant Pathology Herbarium, Biosecurity Queensland, Department of Agriculture and Fisheries, Queensland, Australia.

Ustilago is a polyphyletic genus of smut fungi found mainly on Poaceae. The development of a taxonomy that reflects phylogeny requires subdivision of *Ustilago* into smaller monophyletic genera. Several separate systematic analyses have determined that *Macalpinomyces mackinlayi*, *M. tubiformis*, *Tolyposporella pachycarpa*, *Ustilago bouriquetii* and *U. maydis*, occupy a unique phylogenetic position within the Ustilaginaceae. A previously introduced monotypic generic name typified by *U. maydis*, *Mycosarcoma*, is available to accommodate these species, which resolves one component of polyphyly for *Ustilago* s. lat. in Ustilaginaceae. An emended description of *Mycosarcoma* is provided to reflect the morphological synapomorphies of this monophyletic group. A specimen of *Ustilago* maydis that has had its genome sequenced is designated as a neotype for this species. Taxonomic stability will further be provided by a forthcoming proposal to conserve the name *Uredo maydis* over *Lycoperdon zeae*, which has priority by date, in order to preserve the well-known epithet *maydis*.

625W Population structure and drivers of genetic diversity in *Candida glabrata*. *E. Shor*[1], K. Healey[1], C. Jimenez-Ortigosa[1], S. Lockhart[2], V. Loparev[2], D. Perlin[1] 1) Public Health Research Institute, Rutgers University, Newark, NJ; 2) Centers for Disease Control, Atlanta, GA.

The prevalence of *Candida glabrata* infections has been rising for several decades, and it now accounts for approximately 25% of all Candida bloodstream infections in the U.S. and can in some settings predominate as the principal bloodstream fungal pathogen. Furthermore, *C. glabrata* exhibits elevated intrinsic resistance to triazoles and readily acquires resistance to echinocandins. It also becoming increasingly apparent that *C. glabrata* has a highly genetically diverse population structure. However, how this diversity arises and whether it contributes to the virulence and drug resistance of *C. glabrata* is unknown. A multi-locus sequence typing (MLST) scheme has thus far identified over 80 different *C. glabrata* sequence types (STs); however, our preliminary analysis indicates that this number is likely much higher. We also found that different STs carry different alleles of DNA repair gene *MSH2*, some of which cause elevated rates of drug resistance *in vitro*, indicating that different STs have different propensity towards mutability and acquiring drug resistant gene variants. *C. glabrata* also displays a high degree of variability at the level of chromosomal architecture. For instance, it has been reported that clinical *C. glabrata* isolates show great diversity in terms of chromosomal number and structure, displaying a high number of chromosomal rearrangements relative to the reference laboratory strain. This observation has led to the hypothesis that emergence of new chromosomes is a virulence mechanism in *C. glabrata* and may underlie emergence of drug resistance. However, we find that strains of the same ST have similar chromosomal patterns, suggesting that specific chromosomal configurations may pre-exist emergence of virulence and may be a feature of commensal *C. glabrata* populations. To identify new determinants of virulence and/or drug resistance in *C. glabrata*, we are performing optical mapping and long read genome sequencing (PacBio) of several STs that are prevalent in the U.S. We are also investigating the importance of the DNA replication checkpoint in facilitating chromosomal rearrangements in *C. glabrata*.

626T Comparative genomics of hybrids reveal potential host specificity genes in *Microbotryum*. Britta Büker[1], Michael E. Hood[2], Andreas Brachmann[3], Sven Rahmann[4], *Dominik Begerow*[1] 1) Geobotanik, Ruhr-Universität Bochum, Bochum, DE; 2) Department of Biology, Amherst College, Amherst, MA, USA ; 3) Genetics, Faculty of Biology, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany; 4) Genominformatik, Institut für Humangenetik, Medizinische Fakultät, Universität Duisburg-Essen, Essen, Germany.

The evolution of obligate plant pathogens like smut fungi is often characterized by lineage tracking, resulting in host specificity and one-to-one relationships. Therefore, adaptation and specialization to the host plant seems to be crucial and should involve genes or regulatory pathways governing host specificity. To identify genes relevant for host specificity of *Microbotryum* species, we produced artificial hybrids between the two host-specific species *M. lychnidis-dioicae* and *M. silenes-acaulis* and applied strong experimental selection on different host plants to identify genes necessary for successful infections.

Genome comparison of the two species revealed that most gene families are shared and the majority of genes are conserved, indicating very similar biological features of both species, including host adaptation and infection processes. Lower nucleotide identity of genes encoding for secreted proteins might indicate their importance for host specific interaction, as it is known from other plant pathogens. Moreover, we identified 211 candidate genes that occur in each hybrid and backcross genome that were posed under host-driven selection and might therefore play a crucial role in host specialization. The analysis of hybrid genomes also demonstrates the effect of genetic homogeneity on the fitness of hybrid individuals including the occurrence of species-specific mating type chromosomes.

In conclusion, the combination of comparative genomics with experimental selection and hybridization is a promising way to identify potential host specificity factors. Our data suggest that only a limited set of genes is required for successful infection, but also demonstrate the strong influence of intra-genomic conflicts on the viability of hybrids.

Synthetic Biology

627F Functional reconstitution of the Trypacidin Gene Cluster in *Aspergillus fumigatus* by Advanced Gene Editing. *J. Weber*[1,2], V. Valiante[3], C.S. Nødvig[4], D.J. Mattern[1,2], R.A. Slotkowski[1,2], U.H. Mortensen[4], A.A. Brakhage[1,2] 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) –, Jena, Germany; 2) Institute of Microbiology, Friedrich Schiller University Jena, Germany; 3) Leibniz Research Group – Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 4) Eukaryotic Molecular Cell Biology, Section for Eukaryotic Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Kongens Lyngby, Denmark.

The human pathogenic fungus *Aspergillus fumigatus* is known to produce various spore-borne natural products. One of these is the polyketide trypacidin which has been shown to be involved in the interactions with alveolar macrophages as well as with the amoeba *Dictyostelium discoideum*. Even though recent studies could elucidate the corresponding gene cluster in *A. fumigatus*, it still remained elusive why several isolates do not produce trypacidin. We addressed this question employing a CRISPR/Cas9-based gene editing strategy. Thus, we could link a single nucleotide insertion in the polyketide synthase of the trypacidin biosynthetic pathway and also

reconstitute its production in a nonproducing strain. In addition, we developed a split-marker approach for the selection of edited strains, since the selectable marker could not be directly linked to the target site. The here established tool could be useful in next generation fungal genetics *e.g.* for the investigation of single nucleotide polymorphism, or amino acid substitutions.

628W Identification of gene targets for improved heterologous enzyme production in *Aspergillus niger*. *M.C. Reilly*[1], J. Kim[1], J. Lynn[1], J.M. Gladden[1,2], J.K. Magnuson[1,3], S.E. Baker[1,3] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Sandia National Laboratory, Livermore, CA; 3) Pacific Northwest National Laboratory, Richland, WA.

Efficient and economical deconstruction of biomass is critical for the success of lignocellulosic biorefineries. The use of ionic liquids (ILs) in the pretreatment of biomass leads to increased biomass saccharification efficiency at lower cellulose loadings. However, some ILs can inhibit the activity of commercial cellulases and must be removed from the biomass prior to the application of the lignocellulosic enzyme cocktail, a costly additional step in the conversion process. To overcome this issue, cellulolytic enzymes that can maintain function in the presence of ILs have been identified. Many of these enzymes originate from bacterial species that are difficult to cultivate in the laboratory. One possible solution are filamentous fungi, which have been widely utilized for enzyme production in industry. Here, a forward genetics approach was utilized to increase heterologous enzyme production in *Aspergillus niger*: a strain engineered to secrete an IL-tolerant bacterial beta-glucosidase (BG) was mutagenized and the resulting progeny screened for increased BG activity. Subsequent full-genome sequencing revealed several loci associated with the hyper-production of the heterologous enzyme. Deletion of one of these, a putative sugar transporter, results in a doubling of heterologous BG enzyme activity detected in culture supernatants. Future studies will further characterize the role of this sugar transporter in heterologous enzyme production.

629T An *in-silico* reconstructed gene regulation network for *Aspergillus niger* for the prediction of protein functions. *Sascha Jung*, Norman Paege, Paul Schäpe, Vera Meyer Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany.

The genome of *A. niger* belongs to the best annotated genomes among *Aspergillus* species; however, only 2% of its ~14,000 genes are functionally verified and 50% of the predicted open reading frames encode for hypothetical proteins. Hence, the genetic basis for almost all cellular processes in *A. niger* and its physiological peculiarities is unknown. On the other hand, hundreds of post-genomic data including transcriptomic and proteomic data are available for *A. niger* for more than 150 different growth conditions. This holistic dataset can be scrutinized and used to predict gene functions and gene interactions. For a proof-of-concept, we have focused on the *anafp* gene encoding the antifungal protein AnAFP known to selectively inhibit the growth of filamentous fungi. As more than 50 AnAFP orthologs have been identified in many different genera of the *Ascomycota* tree of life, we wished to understand which regulatory systems control expression of the *anafp* gene.

Our analysis uncovered that *anafp* displays a highly coordinated temporal and spatial transcriptional profile which is concomitant with key nutritional and developmental processes. Its expression profile coincides with early starvation response and parallels with genes involved in nutrient mobilization and autophagy. Using fluorescence- and luciferase reporter strains we could demonstrate that the *anafp* promoter is indeed under control of CreA and FlbA as predicted by the *in silico* data. A co-expression network analysis further predicted that *anafp* expression is embedded in several cellular processes including allorecognition, osmotic and oxidative stress survival, development, secondary metabolism and autophagy, and predicted StuA and VelC as additional regulators (1). We currently prove these predictions by respective wet-lab experiments. First results match the expected knock out phenotype in *A. niger*.

(1) Paege N, Jung S, et al. A Transcriptome Meta-Analysis Proposes Novel Biological Roles for the Antifungal Protein AnAFP in *Aspergillus niger*. PlosOne 2016; DOI:10.1371/journal.pone.0165755.s009

630F Genome wide consequences of the deletion of the Aspergilli non-homologous end joining (NHEJ) DNA repair mechanism. I. Álvarez-Escribano[1], C. Sasse[2], J. Woo Bok[3], A. Lipzen[4], W. Schackwitz[4], J. Marin[4], K. Barry[4], I. Grigoriev[4], A.T. Marcos[1], N.P. Keller[3], G.H. Braus[2], *D. Canovas*[1] 1) Department of Genetics, Faculty of Biology, University of Seville, Spain; 2) Department of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University, Göttingen, Germany; 3) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, USA; Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA; 4) DOE Joint Genome Institute, Walnut Creek, California, USA.

The relevance of *Aspergillus* for human daily life is immeasurable: not only because of its industrial applications but also because of its clinical implications. Most *Aspergillus* laboratories world-wide use mutants in the non-homologous end joining (NHEJ) pathway (*KU70* or *KU80*) to do genetic modifications in the strains under study due to a higher frequency of homologous integrations after transformation of DNA constructs. *KU70* is involved in a DNA repair mechanism highly conserved in eukaryotes, and genetic manipulations are much faster in NHEJ mutant (*?KU70*) strains in many fungal species.

Three *Aspergillus* species were selected for this study to allow for comparative genetics and genomics: *A. flavus, A. fumigatus* and *A. nidulans*. Wild-type and NHEJ mutants were grown on solid media for 60 growth passages using asexual spores (ca. 3,000 mitosis). In the case of *A. nidulans* ten passages with sexual cleistothecia were also independently performed. Genome sequences were obtained and analyzed. In both *A. flavus* and *A. fumigatus*, the *KU70* mutants accumulated on average more mutations than the wild type strains (8.37 +/- 1.80 vs 6.12 +/- 1.76 in *A. flavus*, and 5.75 +/- 2.33 vs 4.12 +/- 1.76 in *A. fumigatus*). The higher number of non-synonymous mutations in *A. fumigatus* and of mutations in the intergenic regions in *A. flavus* in the *KU70* mutants was statistically significant. None of the sexual passages of the *A. nidulans KU70* strain could be completed due to the lack of cleistothecia formation, while the majority of the wild type passages reached ten passages. Collectively here we provide an assessment of the effects of the NHEJ pathway in the genomic stability in Aspergilli.

631W CRISPR/Cas9 systems can be used to manipulate gene function in the thermally dimorphic fungus *Histoplasma capsulatum*. *Saori Amaike Campen*, Sinem Beyhan J Craig Venter Institute, La Jolla, CA.

The thermally dimorphic fungus, *Histoplasma capsulatum* is a causal agent of the severe pulmonary and systemic human disease histoplasmosis. *H. capsulatum* can cause disease in both immunocompromised and immunocompetent individuals and is endemic in the

U.S. It estimated that over twenty-five thousand individuals acquire life-threatening infections each year with up to 50% mortality rates. The pathogen grows in the filamentous form in soil. Upon inhalation by a mammalian host, the fungus switches its morphology from filamentous form to yeast form, and continues to grow intracellularly within host macrophages. Despite its high impact on human health, there is limited research done in *H. capsulatum* due to the limited reverse genetic tools available for this organism. Targeted allelic replacements have been performed in *H. capsulatum* but their success varies greatly among *H. capsulatum* strains. Importantly, the highly virulent North American G217B strain remains largely refractory to gene knockouts by targeted allelic replacements. To study gene function, we frequently utilize RNA interference and generate knockdown mutants in the highly virulent G217B strain; however, the available tools often result in unstable phenotypes over time due to insufficient knockdown levels, or integration or eventual loss of the knockdown plasmid. In this study, our goal is to develop robust gene editing technologies to manipulate gene function in *H. capsulatum*. Recently, cutting-edge gene editing technology, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated proteins (Cas) system have been used successfully to manipulate gene function in filamentous fungi. Here, we employ this technology to target two well-characterized genes in *H. capsulatum*: *URA5*, encoding an orotidine-5'-monophosphate pyrophosphorylase that is required to grow in the absence of uracil and *RYP1*, encoding a transcription factor that is required for yeast-phase growth in *H. capsulatum*. Our preliminary results show that the transformants carrying a Cas9-*URA5* construct exhibit uracil auxotrophy and the transformants carrying a Cas9-*RYP1* construct exhibit filamentous phenotype at 37°C. We are currently quantifying the frequency of these phenotypes and optimizing the efficiency of the CRISPR/Cas9 systems in *H. capsulatum*. Overall our results suggest that CRISPR/Cas9 systems can be used to manipulate gene function in *H. capsulatum*.

632T A Universal Expression System for Protein Production. *Mari Valkonen*, Anssi Rantasalo, Salla Koskela, Marika Vitikainen, Jussi Jäntti, Christopher Landowski, Dominik Mojzita Industrial Biotechnology, VTT Technical Research Centre of Finland Ltd., Espoo, Finland.

Trichoderma reesei is a filamentous fungus that is used world-wide as a host for industrial enzyme production. The enzymes produced are used, for example, in pulp and paper production, in the food and feed industries, and in the textile industry. *T. reesei* enzymes are also increasingly important because of their ability to turn lignocellulosic biomass into sugars that can be used to produce biofuels and chemicals. *Pichia pastoris* is another well-known protein production organism important for industrial enzymes and biopharmaceuticals. The strongest promoters used in both of these systems typically require the use of inducing molecules, which create limitations and generate extra cost in the process. To simplify and improve the production process we have employed a novel orthogonal expression system based upon a synthetic transcription factor (sTF) that regulates expression of the target gene via a sTF-dependent promoter. The great benefit is that new expression allows for high level, constitutive expression in diverse growth conditions and growth stages. Multiple sTF-binding sites can be designed to enable selection of a wide range of target gene expression levels, from very low to extremely high. The same system functions in a wide spectrum of eukaryotic microorganisms providing a simpler genetic toolbox for host strain construction. We have taken advantage of this unique expression system to improve native and heterologous protein production in the industrial microbes *T. reesei* and *P. pastoris*. We have been able to produce high levels of secreted proteins without the use of inducing molecules, which are typically used for these production organisms. For instance, with *T. reesei* we could produce the native CBH1 cellulase at high levels when grown in glucose medium. The new universal expression system allows for simpler media and bioprocesses to be used for industrial protein production.

633F Adapting filamentous fungi to an automated environment for the application of a Design-Build-Test-Analyze-Learn approach to strain optimization. *K.S. Bruno*, E. Szewczyk, K Rothschild-Mancinelli, P.J. Westfall Zymergen, Inc, Emeryville, CA.

Identifying, understanding and ultimately engineering the complex set of interacting genes responsible for any cellular phenotype is extremely difficult and traditionally requires years of study. For example, optimizing metabolic flux to a native or non-native small molecule, or increasing the diversity of carbon sources that a microbe can use are well studied topics and yet very few novel compounds have made it through the high risk path to commercialization. To address this problem, Zymergen has built a platform for automated and high-throughput microbial strain improvement that is focused on improving the economics of large-scale fermentation processes and accelerating the industrialization of novel bio-products. The platform has been designed to be flexible, highly reliable, and host-agnostic.

Filamentous fungi are sought after as production hosts for their ability to tolerate a variety of feedstocks as well as their capacity for high production of enzymes and other small compounds. However, adapting fungi to modern automation platforms for high throughput manipulation poses some unique challenges. Large scale, routine preparation of protoplasts, automated liquid handling during transformation, reduction of heterokaryon formation and minimizing cross-contamination with volatile spores are just some of the engineering challenges that need to be overcome to ensure successful strain improvement outcomes. Here we describe the adaptation of Aspergillus niger to an automated liquid handling and data capture environment that allows large scale implementation of a Design-Build-Test-Analyze-Learn cycle for strain improvement and pathway engineering.

634W A CRISPR-Cas9 platform for Genetic Engineering of Diverse Filamentous Fungi. Matthew Entler[1,2], Peter Meidl[1,2], *Jessy Labbé*[1,2] 1) Biosciences Division, ORNL, Oak Ridge, TN; 2) University of Tennessee, Knoxville, TN 37996.

Fungi represent one of the most essential groups of organisms on the planet, playing vital roles in the biosphere. The development of effective methods for genetic engineering of fungi over the past two decades has demonstrated the importance of such tools to alter both the detrimental and beneficial activities and metabolic processes of fungi. Nonetheless, detailed knowledge into the molecular biology and biochemistry is only available for a few fungal models. Full genome sequencing efforts (e.g., 1KFG project) are rapidly increasing the ability to explore the fungal kingdom beyond the handful that are currently available. However, the sparsity of Basidiomycete and Zygomycete biochemical knowledge can be partly explained by a lack of well-developed genetic tools which are hampered by low gene targeting frequencies. Therefore, we established an easy and versatile fungal genetic engineering platform that works for all major phyla (i.e., Ascomycetes, Basidiomycetes and Zygomycetes) and based on the CRISPR-Cas9 system that has been successfully adapted to a wide range of organisms. We created modular CRISPR-Cas9 vectors for directed mutagenesis across multiple fungal phyla and optimized versatile fungal CRISPR-Cas9 gene-editing methods. Development of these tools is critically necessary to position ORNL at the cutting edge of systems and synthetic biology. The versatility of our system of assembled vectors, which contain a cas9 gene codon optimized for

fungi, three promotors, different selections markers and a GFP-reporter, is being tested into four fungal species representatives of Ascomycetes (*Trichoderma reesei*), Basidiomycetes (*L. bicolor* and *Lactarius psammicola*) and Zygomycetes (*Mortierella elongata*) phyla. Here, we will show that it is possible to introduce defined point mutations in specific genes using this versatile system in several genes and discuss efficiencies.

635T *Kluyveromyces marxianus* as a robust systems and synthetic biology platform. *R. Estrela*[1,3], P. Cernak[1,3], C. Zhang[1,3], V. Yu[1,3], J. Cate[1,2,3] 1) Molecular and Cell Biology Department, UC Berkeley, Berkeley, CA; 2) Department of Chemistry, UC Berkeley, Berkeley, CA; 3) Energy Biosciences Institute, Berkeley, CA.

Among "non-standard" yeast, there is increasing interest in studying *Klyuveromyces marxianus (Km)* given its combination of industry-appealing traits: broad range of carbon source utilization, ability to grow at temperatures up to 52ºC and ferment at 45ºC and one of the highest growth rates among eukaryotes. It is not surprising, however, that most of the studies published on *Km* up to date exploits its general biotechnological potential rather than studying the basic biology underlying its interesting traits. The lack of genetic tools has hampered in-depth genetics and biochemistry focused studies in *Km* and in many other non-model organisms. Our goal is to tackle this problem and develop *K. marxianus* into a robust platform for systems biology studies and synthetic biology applications in industrial microbiology. We also established CRISPR-Cas9 genome editing in *Km* and used this system to engineer several strains to use in classical genetics. We have been using these synthetic biology tools to probe the genetic basis for thermotolerance in *Km* and also to use *Km* to produce value-added chemicals. We are successfully using CRISPR-Cas9 to knockout genes of interest and identify ones that might have an important role in thermotolerance. Our synthetic biology tools are also being used to explore the potential of *Km* to produce renewable fuels and chemicals.

Other

636F Molecular mechanisms of demethylase inhibitor fungicide resistance in the barley pathogen *Pyrenophora teres* f. sp. *teres*. *Wesley Mair*[1], Weiwei Deng[1], Jonathan Mullins[2], Samuel West[2], Penghao Wang[3], Naghmeh Besharat[1], Simon Ellwood[1], Richard Oliver[1], Francisco Lopez-Ruiz[1] 1) Department of Environment and Agriculture, Centre for Crop and Disease Management, Curtin University, Bentley, WA, Australia; 2) Institute of Life Science, School of Medicine, Swansea University, Swansea, UK; 3) School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia.

Pyrenophora teres f. sp. *teres* is a necrotrophic fungal pathogen and the causative agent of net form of net blotch (NFNB) that, together with spot form of net blotch, is one of the most important diseases of barley (*Hordeum vulgare*). Net blotch diseases are mainly controlled using site-specific systemic fungicides. Unfortunately, disease management has been compromised by the emergence of fungicide resistance. Although resistance to succinate dehydrogenase inhibitors and quinone outside inhibitors has been addressed before in net blotches, mechanisms controlling demethylation inhibitor (DMI) resistance have not yet been reported at the molecular level. Here we report the isolation of strains of NFNB in Australia since 2013 resistant to a range of DMI fungicides. A novel mutation in the gene encoding the DMI target enzyme CYP51A was present only in resistant strains. This amino acid substitution F489L was orthologous to F495I in *Aspergillus fumigatus* and F506I in *Penicillium digitatum*. Structural *in silico* modelling of CYP51A proteins docked with different DMI fungicides showed how the interaction of F489L within the heme cavity produced a localized constriction of the region adjacent to the docking site that is predicted to result in lower binding affinities. Resistant strains also displayed enhanced induced expression of the two *Cyp51A* paralogs and of the *Cyp51B* gene. Under fungicide induction, expression of *Cyp51A1*, *Cyp51A2* and *Cyp51B* was higher in the resistant isolate compared to the wild type. These increased levels of expression were not supported by changes in the promoters of any of the three genes. Analysis of simple-sequence repeat markers indicated that the allele derives from a single mutational event, with the mutant allele subsequently recombining into different genetic backgrounds. A digital PCR assay has been developed for the high-throughput detection of mutant populations in the field.

637W *NSG2 (ORF19.273)* encoding protein controls sensitivity of *Candida albicans* to azoles through regulating the toxic C14-methylated sterols synthesis. Q.Z. Lv, *L. Yan*, Y.Y. Jiang School of Pharmacy, Second Military Medical University, Shanghai, China.

Candida albicans is the major opportunistic pathogen that causes serious candidemia, candiduria or organic abscesses in the immunocompromised individuals. Currently, azoles are the most common used antifungal drugs in clinic which is targeted the rate-limiting enzyme of the ergosterol biosynthesis, Erg11p (lanosterol demethylase). But as a consequence of prophylactic use and prolonged treatment regimens, the azoles resistance in *C. albicans* has become a thorny problem. Regardless of mammals or yeast, cells regulate sterol homeostasis by multiple feedback mechanisms through transcriptional and posttranscriptional mechanisms. The transcriptional regulation of the enzymes involved in the biosynthesis of ergosterol in *C. albicans* is mainly dependent on the zinc cluster transcriptional factor Upc2p, which is induced by the ergosterol depletion. On the other hand, the level of sterol could be controlled by insulin-induced genes (INSIGs) interacting directly with proteins containing sterol-sensing domains (SSDs) in a posttranscriptional manner in *Saccharomyces cerevisiae*. But the post-transcriptiona*l regulation of the enzymes in C. albicans has been demonstrated rarely. Through homologous comparison, we identified that C3_02820C_A (*ORF19.273), containing the INSIG domain, is homologous to *NSG2* in *S. cerevisiae* and therefore we designated it as *CaNSG2*. Using the strategy of fusion PCR and auxotrophic markers, we obtained the null mutant of *NSG2*. In contrast with the functions of NSGs in *S. cerevisiae*, *NSG2*-deficient *C. albicans* strain only improved the sensitivity to azoles and showed no difference to other stresses compared with its parental strain SN152. Hypersensitivity of *NSG2Δ/Δ* to azoles was caused by the imbalance of sterols, especially the increase of toxic eburicol and obtusifoliol. Finally, we concluded that *NSG2* is responsible for reducing the aberrant sterols with a C-14-methyl group and maintaining its resistance to azloes in *C. albicans*. Our study gives a new insight to understand the toxic sterols synthesis in *C. albicans* and the roles of sterols homoeostasis in resistance to azoles stress.
Methods: *NSG2* in *C. albicans* is disrupted by the strategy of HIS1-ARG4-LEU2 auxotrophic markers and two alleles were disrupted with the *Candida maltosa LEU2* and *Candida dubliniensis ARG4* marker**.** To determine the drug sensitivity, we used the spot assay method that plated serial concentrations of different strains on the YPD agars containing the indicated agents. The transmission electron microscopy was used to observe the membrane changes of yeast cells in response to fluconazole. The obtained cell pellets were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C for 24 h. Quantitation of the percentage of ergosterol was determined by the gas

chromatography mass spectrometer (GC-MS) analysis. The total intracellular ergosterol amount was quantified in the presence and absence of fluconazole and the percentage of different sterols was calculated. The yeast cells were treated with solutions that contains 15% NaOH dissolved in 90% ethanol and reacted in 80? for 1h. Petroleum ether (boiling range 30 ~ 60 °C) was added to the extract for 3 times, and the combined extractions was washed with etroleum ether, ether layer was then evaporated in the 60 °C water bath. Systemic murine candidiasis model was used to evaluate the virulence of different strains. The BALB/C mices were injected with 5×10^5 *C. albicans* cells through tail vein.

Results:

Verification of ORF19.273 disruption in *C. albicans*

C. albicans ORF19.273 is the only ortholog of the mammalian INSIG genes, which is homologous to *NSG2* in *S. cerevisiae*. Although *ORF19.273* is significantly diverged from *S. cerevisiae NSG2* with only 27% identity throughout, we designated it as *NSG2* and predicted it to be a sterol-biosynthetic-process-related gene. So we used the nutritional markers of HIS1-ARG4-LEU2 and fusion PCR method to acquire homologous recombination fragments. Replacements of the *NSG2* alleles with fusion PCR fragments were verified by PCR. The *nsg2* null mutant strain (*NSG2Δ/Δ*) and the re-introduction strain (*NSG2Δ/Δ*+ *NSG2*) were then obtained.

We then investigated the growth rate, hyphae and biofilm formation of *nsg2* null mutant. Our results indicated that *NSG2* has no influence on the proliferation and morphological transformation of *C. albicans*.

The deficiency of *NSG2* specifically increased sensitivity of *C. albicans* to azoles

In *S. cerevisiae*, the disruption of *NSG1* and *NSG2* leads to an increased sensitivity to lovastatin. Statins are widely used drugs in the treatment of human hyperlipemia, which reduce the cholesterol levels through a competitive inhibition of HMG-CoA reductase. Lovastatin (8μg/ml) strongly inhibited the growth of SN152 and *NSG2*Δ/Δ equally. This is a significant difference between the functions of INSIGs in *S. cerevisiae* and *C. albicans*. There are the other three classes of antifungal drugs targeted to the sterol synthetic pathway: allylamines (terbinafine, butenafine) targeted to the squalene epoxidase (Erg1p), triazoles (fluconazole, ketoconazole, etc) targeted to the lanosterol 14-alpha-demethylase (Erg11p) and morpholines (amorolfine, fenpropimorph) targeted to C-8 sterol isomerase (Erg2p) and C-14 sterol reductase (Erg24p). So we examined the sensitivity of *NSG2Δ/Δ* to these drugs. In contrasted with the stains of *NSG2 Δ/Δ* in *S. cerevisiae*, there are no differences in the sensitivity to lovastatin between the NSG2-dificient stain and SN152. However, the sensitivities of azole drugs were significantly different between the NSG2-disrupted and SN152. [a1] Using two different clones *NSG2Δ/Δ*-1 and *NSG2Δ/Δ*-2, we concluded that *NSG2* is essential in *C. albicans* in maintaining the resistance to azoles specifically. The sensitivities to the upstream or downstream inhibitors of Erg11p (terbinafine, fenpropimorph) showed no difference between the wild type and *NSG2Δ/Δ* strains. From this point, it is possible that *NSG2* interacts with Erg11p in response to azoles.

The depletion of *NSG2* destructed the cell membrane integrity of *C. albicans*

Ergosterol plays an important role in cell membrane integrity, permeability and cell polarization. To verify the integrity of plasma membrane, transmission electron microscopy was performed to observe the ultra-structure changes of SN152 and *NSG2Δ/Δ* strains in the presence or absence of fluconazole. In the absence of fluconazole, the integrant *NSG2Δ/Δ* displayed slightly damage in plasma membrane. This effect was amplified in the presence of 8 μg/ml of fluconazole. The cell membranes of the SN152 strain modestly damaged and a small amount of cell contents leaked into the gap between cell membrane and the cell wall when treated with 8 μg/ml of fluconazole. In contrast, the membranes of *NSG2Δ/Δ* treated with 8 μg/ml of fluconazole seriously collapsed and bulks of the cell contents leaked into the gap. These results indicated that the major function of *NSG2* is to maintain the integrity of the plasma membrane, especially in response to azoles.

The biosynthesis of ergosterol effects the polarization of *C. albicans* directly. We stained the initial hyphae of *NSG2Δ/Δ* with filipin, which combines with ergosterol specifically. It is indicaed that *NSG2* polarizes normally with an ergosterol-rich domain between the yeast cell and hyphae.

The deficiency of *NSG2* increased the accumulation of the toxic C14-methylated sterols

The fungistatic function of azoles is caused by the reduction of ergosterol in the membranes and the accumulation of the toxic sterols, which disturb the fluidity and permeability of the plasma membrane. We measured the compositions of sterols by gas chromatography-mass spectrometer (GC-MS) analysis. In the absence of fluconazole,the major sterol is still ergosterol in both SN152 and *NSG2Δ/Δ* strains. But the percentage of sterols located in the upstream of Erg11p was a little higher in *NSG2Δ/Δ*,which included obtusifoliol and 14a-methylfecosterol. What's more, these sterols accumulated in *NSG2Δ/Δ* always contained a 14α-methyl group and this structure is fungistatic for yeast. These data suggest that *NSG2* plays a role in regulating the balance of different sterols and this function is more obvious in *C. albicans* in response to fluconazole. When treated with 8μg/ml of fluconazole, the percentage of ergosterol in SN152 and *NSG2Δ/Δ* has decreased to about 4% and some sterols with a C14-methyl group increased obviously. There is a significant difference in the distribution of sterols with 14α-methylation. Fluconazole inhibited the activity of lanosterol 14-alpha-demethylase (Erg11p) and induced the accumulation of lanosterol obviously in SN152. When *NSG2* is disrupted, the proportion of eburicol, obtusifoliol and 14α-methylfecosterol significantly enhanced. In consistent with this, the percentage of lanosterol in *NSG2Δ/Δ* is lower compared with SN152. When using the squalene epoxidase inhibitor terbinafine, the distinctions of distribution and compositions of sterols in either SN152 or *NSG2Δ/Δ* was not obvious. Finally, we concluded that the specific hypersensitivity of *NSG2Δ/Δ* to azoles is attributed to the slight reduction of ergosterol and the accumulations of toxic sterols with14α-methyl group.

The regulation of *NSG2* on the content of sterols is not dependent on the ERG genes transcription, as the transcriptional levels of *ERG1*, *ERG7*, *ERG11*, *ERG2*, and *ERG3* had no change in the *NSG2Δ/Δ* strain.

The disruption of *NSG2* in *C. albicans* increased efficacy of fluconazole treatment in a murine systemic candidiasis.

To investigate the effects of *NSG2* in maintaining the virulence of *C. albicans* in murine, we compared the survival rate and the renal fungal burden in BALB/C mice. Without treatment with fluconazole, the survival curve of *NSG2Δ/Δ* is similar with that of SN152 and the reintroduction strain. In order to evaluate the in vivo sensitivities of *NSG2Δ/Δ*, the BALB/C mice was delivered with 2mg/kg of fluconazole for 7 days following intraperitoneal injection after 2 hours infected with 3×10^5 cells of SN152, *NSG2Δ/Δ* or *NSG2Δ/Δ*+NSG2. We measured the fungal burden in kidney after stopping the fluconazole delivery in 2 days. The results showed that *NSG2Δ/Δ* was almost all cleared in kidney and the survival outcome of fluconazole treatment was about 90% during the 50 days observation. Corresponding to this, only 30%-40% of the mice infected with SN152 or *NSG2* reintroduction strain was alive after 50 days. Overall, *NSG2* maintained the *C. albicans* resistance to azoles no matter in vitro or in vivo.

638T Purification and characterization of killer toxin from *Debaryomyces hansenii* isolated from cheese. *N. Banjara*, H.E. Hallen-Adams Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE.

Some yeasts produce toxic proteins or glycoproteins called killer toxins, which can kill sensitive yeast isolates. The yeast *Debaryomyces hansenii* Dh-237 (CBS 14264) isolated from cheese secretes killer toxin which has inhibitory activity in the lab against pathogenic *Candida* spp. including *C. albicans, C. pelliculosa,* and *C. tropicalis*. In order to gain more information on the nature of *D. hansenii* killer toxin, we purified and characterized the killer toxin. To purify and characterize the toxin, *D. hansenii* was grown in liquid YPD media, pH 4.5, at 25 C for 72 hours and culture supernatant was concentrated by ultrafiltration with 3 kDa membrane and purified by chromatographic separations, which includes one anion exchange and two size exclusion chromatography steps. Every purified and concentrated product was tested to ensure it retained killer activity against *C. albicans* and *C. tropicalis*. SDS-PAGE of the purified killer toxin indicated a protein with apparent molecular mass of 11 kDa. The purified killer toxin was active at acidic pH, up to 5.0, and killing activity was highest at lower temperatures (25 C and below). However, pure toxin retained stability at 37 C for 3 hours. The ability to act at 37 C, even transiently, coupled with human gut mycobiome data suggest both a negative correlation between foodborne *D. hansenii* and gut commensal *Candida* levels, and a mode of action for such correlation.

639F Determinants of pheromone-independent dimorphism in the human pathogen *Cryptococcus neoformans*. *J. Lin*, Y. Zhao, X. Lin Department of Biology, Texas A&M University, College Station, TX.

The human pathogen *Cryptococcus neoformans* infects more than a million people worldwide, resulting in annual deaths of over 600,000. This highlights the pressing need to better understand cryptococcal pathogenesis. Like many fungal pathogens, morphological transition from yeast to hypha is associated with virulence shift in *Cryptococcus.* Although mating is historically associated with dimorphism in *Cryptococcus*, the pheromone pathway has minimum impact on virulence. We previously identified transcription factor Znf2 that directs switch to the hyphal form independent of the pheromone cascade. As a potent anti-virulence factor, $ZNF2^{oe}$ can also serve as a vaccine agent against cryptococcosis in animal models. To understand how Znf2 directs morphogenesis and virulence, we decided to identify its downstream targets critical for morphogenesis.

We designed a T-DNA insertional mutagenesis screen in a parental strain where mCherry-Znf2 is under the control of an inducible promotor. We generated and screened 88,000 mutants and isolated 84 filamentation-defective mutants under Znf2 inducing condition. Secondary screens for the examining yeast cell morphology and the presence of fluorescence under Znf2 inducing condition narrowed the candidates to 25. We further confirmed 8 mutants with single T-DNA insertion that is linked to the nonfilamentous. Sequencing of pooled genomic DNA identified the affected genes in these 8 mutants. Four genes were found disrupted by insertions in the 8 mutants, with two of the genes being independently inserted twice. One gene is *SNF5*, which was previously shown to be critical for filamentation. The other gene *ZAF1* is predicted to encode a transcriptional corepressor. Targeted deletion of *ZAF1* recapitulated the non-hyphal phenotype without impairing the pheromone response, like the *znf2*Δ mutant. Thus, our screen has identified key genes required for Znf2 to drive filamentation. Currently, we are characterizing the genetic relationship between our candidates and Znf2. Understanding the Znf2-regulatory network will advance our knowledge of cryptococcal pathogenesis and guide new drug/vaccine development.

640W Investigation of Meiosis in *Cryptococcus*. *Y. Zhao*[1], S. Upadhyay[1], C. Xue[2], X. Lin[1] 1) Biology, Texas A&M University, College Station, TX; 2) Public Health Research Institute, Rutgers Biomedical and Health Sciences, Newark, NJ.

Cryptococcus neoformans claims ~600,000 people's lives each year, mostly in regions where AIDS are prevalent. This ubiquitous environmental fungus is acquired by humans through inhalation and it can stay dormant in the lungs for decades. Reactivation of latent infections occurs primarily in immunocompromised individuals (e.g. due to HIV infection), resulting in fatal systemic diseases. This fungus typically grows in a haploid state, but polyploid *Cryptococcus* cells are observed in infected lungs and they are proposed to promote cryptococcal latency and persistence. These polyploid cells give rise to haploid cryptococcal populations. What triggers polyploidization and how ploidy reduction is achieved during infection are unclear.

We recently found that *Cryptococcus* increased cell size when cultured in serum, and blocking meiosis by deleting conserved meiosis-specific genes *SPO11* or *DMC1* resulted in larger proportion of cells with elevated size. Remarkably, severe genotoxic stress efficiently induced polyploidization in *Cryptococcus*. Interestingly, we observed meiosis-like features during ploidy reduction *in vitro*. Consistently, meiosis mutants showed delayed kinetics in ploidy reduction *in vitro* and these mutants showed reduced fungal burden in the lungs compared to WT when polyploid cells were used as inoculum. Most excitingly, using the recombination-based *in vivo* expression technology that couples the transient expression of the meiosis-specific gene *DMC1* with a permanent genetic change that yields a drug-resistance phenotype, we detected the activation of *DMC1* in *Cryptococcus* during infection in the mouse model of cryptococcosis. We believe *Cryptococcus* could complete sexual cycle after increased ploidy by meiosis to return to the haploid state.

641T Evolution of the ascomycete specific sex pheromone receptor Ste2. *B.P. Johnathan*[1], B.D. Wingfield[2], G. Fourie[2], E.T. Steenkamp[1] 1) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 2) Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

Seven Transmembrane proteins (7TMPs) are an important group of proteins that act as transporters or response mediators to environmental factors. The low sequence similarity between the various classes of 7TMPs has made it difficult to obtain support for hypotheses regarding their origin(s). Although the availability of genome sequence information and sensitive alignment tools have allowed for the detection of homologues even above the superfamily level, the overall evolution of fungal 7TMPs remains unclear and understudied. One such group of fungal 7TMPs is the ascomycete specific α-sex pheromone receptor commonly annotated as Ste2 or Pre-2. Although previous research has successfully established a link between the a-sex pheromone receptor (Ste3 or Pre-1) and the cyclic AMP receptor, no close relative has been determined for Ste2. In this study, we examine a possible link between Ste2 and the fungal pH sensor PalH. This link was initially discovered using hidden Markov model profiles to search protein databases for possible Ste2 homologues. Further analysis was conducted to investigate the possible relationship between these two proteins which included sequence alignments, motif searches as

well as structure comparisons. Contrary to the mechanisms described for Ste2, there is evidence to suggest that PalH functions similarly to mammalian arrestin regulated receptors and does not appear to be coupled to a G-protein. This research thus raises further interesting questions regarding the origin and evolution of such mechanisms. Furthermore, any relationship between these two classes of receptors and other non-fungal proteins remain to be elucidated. Our findings also raise interesting questions regarding the apparently rapid evolution that must have occurred after the emergence of the Ascomycota to have allowed for the development of these two proteins.

642F Gene function studies in *Malassezia*: elucidating the mechanisms of action of calcineurin inhibitors in *Malassezia Sympodialis* and *M. Furfur*, and insights into their sexual cycle. *Giuseppe Ianiri*[1], Alex Idnurm[2], Joseph Heitman[1] 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, 27710, USA; 2) School of BioSciences, University of Melbourne, VIC 3010, Australia.

The genus *Malassezia* includes yeasts that are commonly found on the skin or hair of animals and humans as commensals, and are associated with a number of skin disorders. Genome sequencing of 14 known *Malassezia* species highlighted a reduction in genome size (~7-9 Mb) and multiple gene losses and gain events. We have developed an *Agrobacterium tumefaciens* transformation system effective for both forward and reverse genetics in *M. furfur* and *M. sympodialis.* Using these resources we aimed to characterize i) the immunophilin FKBP12 as target of calcineurin inhibitors, 2) the structure of the *M. furfur MAT* locus, and 3) the function of a *Malassezia* flavohemoglobin-encoding gene acquired by horizontal gene transfer from Actinobacteria. Tacrolimus (FK506), ascomycin, and pimecrolimus are calcineurin inhibitors that in some cases are used as topical drug treatments on the skin. While *M. furfur* and *M. sympodialis* showed in vitro sensitivity to these agents, *fkb1?* mutants displayed full resistance to all three, indicating that FKBP12 is the target of calcineurin inhibitors and it is essential for their activity. In accord, spontaneous *M. sympodialis* isolates resistant to FK506 had mutations in the *FKB1* gene, with the majority of them found in a TTTTCCCCC homopolymer repeat. No sexual cycle for any *Malassezia* species has as yet been identified. Analysis of the PacBio genome assemblies of *M. furfur* isolates identified a pseudobipolar mating type system with the pheromone/pheromone receptor (*A*) and the homeodomain protein (*B*) loci partially linked as they are on the same chromosome, but distant enough (~590 kb) to enable recombination. *A1B1*, *A2B2*, *A1B2* and *A2B1* combinations were identified, consistent with recombination between the *A* and *B MAT* loci. These findings combined with the availability of developed genetic markers will facilitate studies that aim to define the sexual cycle of *Malassezia*.

643W Genetics in support of geomycology: melanin-deficient fungus is less capable of adhesion, but promotes mineral weathering nevertheless. Ruben Gerrits[1,3], Rasesh Pokharel[2], Nicole Knabe[1], Jan A. Schüssler[2], Ines Feldmann[1], Friedhelm von Blanckenburg[2,3], *Anna A. Gorbushina*[1,3] 1) Materials and the Environment, Federal Institute of Materials Research and Testing, Berlin, Germany; 2) GFZ German Research Centre for Geosciences, 3.4 Earth Surface Geochemistry, Potsdam, Germany; 3) Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy & Department of Earth Sciences, Berlin, Germany.

Soil formation on rock surfaces is intrinsically coupled to primary microbial colonisation of the atmosphere-lithosphere interface. Rock-inhabiting microbial life is ubiquitous but the mechanisms of biofilm establishment and more importantly, quantification of its geological input are so far only possible to be studied in simplified, well-controlled laboratory experiments. In a previous study [1] a laboratory biofilm consisting of the phototrophic cyanobacterium *Nostoc punctiforme* ATCC 29133 and the rock-inhabiting ascomycete *Knufia petricola* A95 was tested for its mineral weathering potential. Mineral dissolution was enhanced in biotic experiments as compared to abiotic ones. Here the influence of *K. petricola* strain A95 and a recently constructed melanin deficient mutant (A95ΔPKS) were used to study the influence of fungal pigments on weathering of forsteritic olivine. The olivine with fungal biomass was submerged in a nutrient solution (pH 6.2) in batch-reactor flasks that were incubated for 90 d at 25°C and 90 µmol photons.m^2.sec^{-1}, while shaken at 150 rpm. qPCR was used to quantify the growth of fungi. Mineral dissolution was quantified by ICP-OES analysis of the liquid medium while SEM-EDX analysis of the solid phase was used to determine secondary mineral formation and visualise growth behaviour. Wild type and mutant accelerated dissolution of the mineral: over time both release more Mg and Si from olivine than the abiotic control. Also SEM revealed a closer physical contact of the wild type cells to the mineral and a higher production of EPS of the melanin mutant A95ΔPKS. This important difference in the ability of the wild type strain to adhere to the mineral surface might be crucial in maintaining a biologically modified environment. This biologically engineered habitat serves as a place where mineral dissolution as well as deposition of metabolic products (EPS + pigments) can impact the rock surface. We expect this study to increase the awareness on the impact of microbiology, and more specifically, rock-inhabiting fungi on mineral weathering.

644T Identification of specific Targets for early Diagnosis of Mucormycosis. *C. Baldin*[1], S. Soliman[1,2], T. Gebremariam[1], V. Bruno[3,4], J. Edwards[1,5], A. Ibrahim[1,5] 1) Division of Infectious Diseases, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA; 2) Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharaja, UAE; 3) Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD; 4) Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD; 5) David Geffen School of Medicine at UCLA, Los Angeles, CA.

Mucormycosis is a life-threatening infection caused by fungi in the order Mucorales. Among them, *Rhizopus spp*. are responsible for almost 70% of all cases of mucormycosis, followed by *Mucor spp*. and *Lichteimia spp*. The major predisposing risk factor for mucormycosis is an impaired immune system caused by neutropenia, corticosteroid treatment, and uncontrolled diabetes with or without ketoacidosis. Patients with severe trauma and burns, without prior immune deficiency, are also at increased risk of developing mucormycosis. Standard treatment of mucormycosis involves both surgical removal of infected tissue and antifungal therapy. However, mortality rates of all forms of mucormycosis are approximately 50%. In patients with prolonged neutropenia, disseminated disease or cerebral involvement, mortality can reach 90-100%. The rapid progression of the disease and the current lack of early and effective diagnostic methods (current methods rely on histopathological, microbiological and radiological methods) contribute to the high mortality rates.

Up to date, few alternatives have been proposed for early identification of Mucorales in biological samples and all rely on PCR-based detection of specific fragments of the 18S and 28S genes. In the present study, we suggest a novel target for our PCR-based approach: the spore coating protein homolog encoding *CotH* genes. These Mucorales-specific genes encode cell surface proteins that are involved in mucormycosis pathogenesis. We performed bioinformatic analyses to identify short consensus sequences present in most *CotH* gene sequences to be used as PCR primers. Several candidates were tested in PCR reactions using gDNA extracted from biological samples

spiked with *R. delemar*, *R. oryzae*, *M. circinelloides*, *L. corymbifera* and *Cunninghamella bertholletiae*. Different primer sets showed diverse specificity and sensitivity. Some of our candidate primers were able to amplify the specific sequence from more than one Mucorales species and allowed us to differentiate between Mucorales and closely related filamentous fungus, e.g. *Aspergillus fumigatus*. Some of these primers successfully amplified their targeted fragment from biological samples taken from mice infected with different Mucorales as early as 24 h post infection. These encouraging results warrant further development of *CotH* and their protein targets for early diagnostics of mucormycosis.

645F Functional Study of Protein Kinases in The Fruiting Development of Model Mushroom—*Coprinopsis Cinerea*. *k. Chan*, J. Chang, H. Kwan school of life science, the chinese university of hong kong, hong kong, CHINA.

Introduction: Phosphorylation between different protein kinases triggers activation or inhibition of downstream targets affecting their activities and mediating their developmental responses to growth factors, environmental signals, internal processes, and vital roles in signal transduction pathways. Protein kinases not only are crucial to the signal transduction, but also are master switches of development. Some protein kinases are proposed to be important in mushroom development processes. Activation or inhibition of putatively important kinases involved in signaling transduction pathway for fruiting would affect the fruiting development. *Coprinopsis cinerea* is a model mushroom for studies of developmental processes in homobasidiomycetous fungi. The fruiting development of *C. cinerea* depends on the sensing of environment conditions, such as light, temperature, humidity and nutrients. The signal transduction pathways from the environmental condition sensors to the regulators and reproduction has been an important research topic.
Aims: This study investigated identified kinases involved in *C. cinerea* fruiting body development. Through the functional study of selected putative regulatory kinases, we would have a better understanding of the pathway between external stimuli and fruiting body initiation and development.
Methods: Small interfering RNA(siRNA) of different candidate genes, such as Cc.frg and Cc.neg, was applied to *C. cinerea* culture externally to transiently knockdown potential regulators for fruiting development. With or without kinase inhibitors siRNA was applied to knockdown particular candidate genes under different unfavourable conditions. The efficacy of siRNA was tested by real-time PCR assays.
Results: The transient knockdown with Cc.frg siRNA cultures produced deformed fruiting body and some showed retarded fruiting body development. The culture with Cc.neg siRNA and without inhibitor under excessive glucose, on the other hand, continued fruiting body development gradually, when compared with the control.
Discussion: By gene knockdown, we confirmed that Cc.frg gene is involved in the fruiting body initiation and control further development of fruiting body. Besides, there was signal transduction pathway between Cc.frg and Cc.neg that regulates the fruiting of *C. cinerea,* as Cc.neg responded to nutritional stimuli.

646W Proteomics and transcriptomics of interacting mycelia of the brown rot fungi *Postia placenta* and *Gloeophyllum trabeum* in in-tact aspen wood. *G.N. Presley*[1], E. Panisko[2], J. S. Schilling [1] 1) Bioproducts and Biosystems Engineering , University of Minnesota, St. Paul, MN; 2) Chemical and Biological Processes Development Group, Pacific Northwest National Laboratory, Richland, WA.

Brown rot fungi differentially express proteins at the leading hyphal front of fungal cultures during wood decay to spatially segregate incompatible chemical oxidants and hydrolytic enzymes. However, many upregulated gene products do not appear to be involved in wood catabolism and may mediate other processes such as interspecific combat. In this study we identified transcripts and proteins that mediate hyphal interactions between two well studied brown rot fungi, *Postia placenta* and *Gloeophyllum trabeum* by describing the transcriptome and secretome of their interacting mycelia. *Postia placenta* and *Gloeophyllum trabeum* were grown in opposition to one another on 60 x 25 x 3 mm aspen wood wafers until the two hyphal fronts encountered one another and formed a boundary line. Total RNA was isolated from the interaction zone and 5 mm behind on either side of the interaction zone and was subjected to RNA sequencing. Extracellular protein was extracted from equivalent sections from pools of 15 replicate interaction wafers and subjected to LC-MS/MS analysis and select enzyme assays. This work identifies elements of the brown rot transcriptome and secretome that may mediate interspecific interactions during wood decay and provides the groundwork for identifying the biological function of otherwise uncharacterized basidiomycete proteins.

647T Effects of light on sporulation and gene expression in the oomycetes *Phytophthora infestans* and *Phytophthora capsici*. *Andrea Vu*, Howard Judelson Department of Plant Pathology and Microbiology, University of California, Riverside, CA.

Light plays a role in the life and disease cycles of *Phytophthora*, and is particularly important relative to sporangium production. Within this genus of important plant pathogens, different species exhibit distinct responses to light. For example, while sporulation is repressed or delayed by light in species such as the potato blight pathogen *P. infestans,* other species such as *P. capsici* require light for sporulation. This is not believed to be related to a photoentrainable circadian rhythm. Our goal is to understand which genes are regulated by light, and how these genes are integrated into the sporulation program. Preliminary experiments identified the wavelengths and light intensities that affected development in *P. infestans* strain 1306 and *P. capsici* strain LT1534. Using Illumina technology, RNA-seq was performed using *P. infestans* grown under constant light, constant dark, and a 12-hour light/dark cycle, and *P. capsici* under similar conditions. Differentially expressed genes in the sporulation time-courses were classified based on whether they were expressed prior to or after the light-regulated stage. In the early pre-sporulation timepoint, about 430 genes were up-regulated and 80 down-regulated in *P. infestans*. In contrast, only about 80 genes were up-regulated and 180 genes down-regulated in *P. capsici.* The differentially expressed genes included those involved in signal transduction (especially transcription factors and protein kinases), metabolism, and forming the structural components of sporangia and zoospores.

648F *Saccharomyces* Genome Database: Outreach and online training services. *K.A. MacPherson*, K.S. Dalusag, O. Lang, S.T. Hellerstedt, S.R. Engel, J.M. Cherry, The SGD Project Department of Genetics, Stanford University, Palo Alto, CA.

The *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org) is the leading community resource for the budding yeast *S. cerevisiae*. SGD provides high-quality, manually curated information on the yeast genome and offers a wide variety of tools and features that have made it an indispensable resource for many researchers. To inform our user community about new developments at SGD, improve familiarity with SGD features and tools, and increase public awareness of the importance of yeast to biological and biomedical research, SGD has engaged in a variety of online training services and outreach efforts. Here we present the SGD Webinar Series, a series

of interactive webcasts aimed at demonstrating the SGD website and the value of yeast as a model organism, and the SGD YouTube channel, which currently provides over 30 useful help videos and quick tutorials (http://www.youtube.com/SaccharomycesGenomeDatabase). We will continue to develop these services to provide outreach to students and scientists on the significance and beauty of biology, and facilitate greater use and understanding of the resources made available by SGD. This work is supported by a grant from the NHGRI (U41 HG001315).

649W The *Saccharomyces* Genome Database Variant Viewer. O. Lang, T. Sheppard, B. Hitz, S. Engel, G. Song, *T. Jackson*, J.M. Cherry Genetics, Stanford University, Palo Alto, CA.

The complete genome sequence of the budding yeast *Saccharomyces cerevisiae*, and its annotation, is maintained by the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org), as a public resource for researchers. Until recently, the genome sequence displayed at SGD has been derived solely from the S288C strain background. We have now incorporated 11 additional *S. cerevisiae* genomes into SGD, as well as providing their annotation. We have also developed a new Variant Viewer to further support the community's analysis of these new data. Variant Viewer allows visualization and comparison of sequences from multiple strains. Users can quickly scan the entire genome, or a subset of genes, for overviews of both slight and significant sequence differences. SGD's Variant Viewer also includes a more detailed comparison of sequence differences between strains that highlights insertions, deletions, and SNPs that is available for both viewing and download. The Variant Viewer offers yet another way to explore the genomic sequence data available at SGD, as part of our continuing mission to educate students, enable bench researchers and facilitate scientific discovery. This work is supported by a grant from the NHGRI (U41 HG001315).

650T Production of Folipastatin and Unguinol in Talaromyces virginiae, *sp. nov.* correlates with its ability to inhibit growth and aflatoxin synthesis of *Aspergillus parasiticus*. *A. Chanda*, Phani M. Gummadidala, Virginia E Hopkins, Chandrani Mitra Environmental Health Sciences, University of South Carolina, Columbia, SC.

The bioactive secondary metabolites, Folipastatin and Unguinol have been reported mostly from the genus Aspergillus *and* Penicillium. In this study we report a novel strain, *Talaromyces virginiae sp. nov.,* discovered from sediment samples from a salt pond on San Salvador, The Bahamas (24°01′N, 74°27′W). The strain produced a red colored metabolite fraction that primarily consisted of the secondary metabolites folipastatin and unguinol when grown in oatmeal agar and potato dextrose agar but not in yeast extract agar. Scanning electron micrographs of *T. virginiae* colonies grown on potato dextrose agar revealed its morphological characteristics that are typical of the red pigment producing species within the genus Talaromyces. Multigene phylogenic analysis (ITS and β-tubulin) confirmed its unique nature. Finally, we show that this novel Taloromyces strain has the ability to inhibit growth and synthesis of the hepatocarcinogen, aflatoxin of *Aspergillus parasiticus*, a popular plant pathogen, which correlates with its ability to synthesize these secondary metabolites. This is the first evidence of biosyntheses of folipastatin and unguinol by the genus Talaromyces and highlights the ecological and public health significance of the genus Talaromyces.

651F Transcriptional profiling of the *Phytophthora infestans* life cycle reveals stage-specific transcription factors. *Wiphawee Leesutthiphonchai*, Audrey Ah-Fong, Howard Judelson Department of Plant Pathology and Microbiology, University of California Riverside, Riverside, CA, USA.

Our goal is to characterize the regulatory networks that control life stage transitions in *Phytophthora infestans,* the oomycete that causes the late blight diseases of potato and tomato. About 194 sequence-specific transcription factors have been annotated in the *P. infestans* genome. In this work, we used RNA-seq to monitor the expression of transcription factors in developmental stages including nonsporulating mycelia, sporangia, sporangia chilled to induce zoosporogenesis, zoospores, germinated cysts, and plant infection. Robust expression calls were made for 114 transcription factor genes and 75 percent are predominantly expressed in sporangia, cleaving sporangia, zoospores, and germinated cysts compared to hyphae. Genes encoding HLH, bZIP, and TUB transcription factors were over-represented among the spore-induced genes. To understand the roles of such proteins, we selected transcription factors specific to the sporangia and zoosporogenesis stages and generated knockdown strains using transgene-mediated silencing. We found that strains knocked-down for three transcription factors exhibited little or no sporulation. In some cases, however, this may have been due to off-target silencing caused by heterochromatin spreading from the target locus to adjoining sequences. A subset of transformants were silenced for the transcription factor gene with no effect on adjoining loci. While showing reduced sporulation, these were not impaired in growth on media or in plant pathogenesis.

652W *Puccinia striiformis* f. sp. *tritici* isolates recovered from rusted barberry in the western Shaanxi in 2016 and its pathogenicity on Chinese and *Yr*-gene line differentials. J. Zhao, Y. Y. Zhao, S. X. Zuo , Z. M. Du, *Z. S. Kang* College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, CN.

Wheat stripe (or yellow) rust, caused by biotrophic fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive diseases on wheat worldwide. Severe epidemic of the disease resulted in huge yield loss. The resistance of wheat cultivars often was often overcome by new races of *Pst* within a few years after their release. Generally, mutation, heterokaryosis, and sexual recombination are considered as major approaches for emerge of new races resulting from virulence variation. Sexual reproduction of *Pst* has not been known for centuries until the finding of barberry (*Berberis*) serving as alternate host for *Pst*. Focus on roles of barberry in occurrence of stripe rust on wheat and virulence variation of *Pst* were made. In this study, we obtained 103 isolates of *Pst* from aecial pustules of diseased barberry (*B. shensiana* Ahrendt) in spring in Baoji, Shaanxi by inoculation on susceptible wheat (cv. Mingxian 169). Each of all *Pst* isolates was tested pathogenicity on Chinese differentials consisting of 19 wheat cultivars and 27 single *Yr* gene line differentials, respectively, and Mingxian 169 was used as control in all tests. The results of all *Pst* isolates on Chinese differentials indicated that 58 of the 103 isolates were current known races and 35 were new races. The former were grouped into four race groups including Hy46 race group (CYR32, Hy-103, and Hy-30), G22 race group (G22-14, 68, 72, 9, and 91), Su11 race group (Su11-14-3, 24, and 68), and ZS race group (ZS-6). All of *Pst* isolates on 27 *Yr*-gene line differentials produced 76 various virulence patterns, presenting a high diversity in virulence. Our studies here provide more sufficient evidences, combined with results of the past three individual years, for supporting the fact that sexual cycle of *Pst* occurs in

nature in areas where susceptible barberry and wheat coexist in China, which plays important roles in virulence variation through sexual recombination and in providing inoculums to cause stripe rust infection on wheat plants in field.

653T Improving STEM student retention via early research engagement: a pilot. *Michael Watters*, Patrice Bouyer Dept Biol, Valparaiso Univ, Valparaiso, IN.

We describe here the results of a pilot study, the goal of which was to expose freshman to an ongoing research project during the academic year to promote student growth and improve retention in the STEM disciplines. Freshmen worked with a faculty mentor and were also chaperoned by a more senior student researcher in order that they learn lab techniques and the capacity to work independently. Participants were fully engaged in a research project (performing experiments, analyzing and discussing results), not a classic classroom projects, but discovery based projects. By bringing students into the research lab at this early stage, our aim was to improve retention by allowing science students to actually *act* as scientists, providing an enhanced experience over the usual freshman survey course content. Of the 6 students who joined the program as freshmen, 5 are still in their major, 4 are still actively engaged in research with a faculty member and have co-authored 11 different papers and conference presentations as of their junior year.

654F EPIC: Establishing Practices for Integrating Commuter students – Year 0. *Michael Watters*[1], Kristi Bugajski[1], Mindi Capaldi[3], Karl Schmitt[3], Jon Schoer[2], Bonnie Dahlke[4] 1) Dept Biol, Valparaiso Univ, Valparaiso, IN; 2) Dept Chem, Valparaiso Univ, Valparaiso, IN; 3) Dept Math & Stats, Valparaiso Univ, Valparaiso, IN; 4) Valparaiso Univ, Valparaiso, IN.

Commuter students face challenges which distinguish them from resident students. Commuters are more likely to work off campus (of necessity). Commuters are less likely to avail themselves of academic opportunities which are not required for their degrees. Commuters are also less likely to be socially engaged with the wider campus community. These factors have all been shown individually to have a detrimental impact on student success, causing overall lower graduation rates among commuters.

We report here the outline of a program, currently funded by the NSF S-STEM program, designed to address these issues and to assess the impact of the planned interventions on commuter students. The progress and assessment will be reported as available.

655W Effects of a dsRNA mycovirus (PoV-ASI2792) on the vegetative growth of the edible mushroom *Pleurotus ostreatus*. *H Song*[1], D Kim[2], J Kim[1] 1) Bio-Environmetnal Chemistry Dept, WKU, South Korea; 2) Molecular Biology Dept, JBNU, South Korea.

A double-stranded RNA (dsRNA) mycovirus was detected in malformed fruiting bodies of Pleurotus *ostreatus* strain ASI2792, one of bottle cultivated commercial strains of the edible oyster mushroom. The partial RNA-dependent RNA polymerase (RdRp) gene of the *P. ostreatus* ASI2792 mycovirus (PoV-ASI2792) was cloned, and a cDNA sequences alignment revealed that the sequence was identical to the RdRp gene of a known PoSV found in the *P. ostreatus* strain. To investigate the symptoms of PoV-ASI2792 infection by comparing the isogenic virus-free *P. ostreatus* strains with a virus-infected strain, isogenic virus-cured *P. ostreatus* strains were obtained by the mycelial fragmentation method for virus curing. The absence of virus was verified with gel electrophoresis after dsRNA-specific virus purification and Northern blot analysis using a partial RdRp cDNA of PoV-ASI2792. The growth rate and mycelial dry weight of virus-infected *P. ostreatus* strain with PoV-ASI2792 mycovirus were compared to those of three virus-free isogenic strains on 10 different media. The virus-cured strains showed distinctly higher mycelial growth rates and dry weights on all kinds of experimental culture media, with at least a 2.2-fold higher mycelial growth rate on mushroom complete media (MCM) and Hamada media, and a 2.7-fold higher mycelial dry weight on MCM and yeast-malt-glucose agar media than those of the virus-infected strain. These results suggest that the infection of PoV mycovirus has a deleterious effect on the vegetative growth of *P. ostreatus.*

Numbers with an asterisk * after the number indicate they are the presenting author.

A

Abbruscato, P.,234F
Abdallah, N.,.....610W
Abe, K.,..... 54F*, 86T
Abou-Mansour, E., ...241W
Ackapinar, Gunseli., ..115W
Acosta, Wilson,. 98T, 208W
Acquadro, A.,.....234F
Adamiak, Holger,.....477F
Adams, C. A., ...41T*
Adams1, Rachel,.....325W
Adenigba, O. O.,..... 595W*
Adpressa, D.,.....570F
ADREIT, Henri,.....614T
Aebi, M., ...582F
Aebi, Markus, ...555F
Affeldt, K. J., ...449T
Ág, N.,.....585F
Agrawal, N.,.....488T*
Aguilar Pontes, M. V.,.....
.....289W, 366F, 453F
Aguilar-Pontes, M. V., .26T*
Aguiletta, G.,.....229W
Ah-Fong, Audrey, ...651F
Ahren, Dag, ...494T
Ahrendt, S.,.....200T*
Ahrendt, Steven,.....294F
Ahrens, Lisa-Marija, ...182T
Ahuja, Manmeet, ...70W
Aime, M. C.,.....347T
Akagi, Y., ...344T
Akai, K., ...344T
Akers, Natalie, ...356T
Al Abdallah, Q., ...84F*
Alam, Md Ashiqul, ...363F
Alanazi, Ahlam, ...318F*
Alawfi, Sami,.....17T
Alazi, E., ...453F*
Albert, Ryan,.....246F
Alder, A.,.....570F
Alenezi, M. A. K.,.....544W
ALFURAIJI, N.,.....204F*
Al-Furaji, N.,.....83T
Alharthi, Amani, ...198F*
Allen, Katie, ...421W
Allen-Vercoe, E., ...463W
Almeida, M. C.,.....99F*
Alonso Monroy,
Alberto, ...432F*
Al-Salihi, Suhad,.....299T*
Altamirano, D.Sophie.,108F*
Altmüller, J.,.....504F
Álvarez-Escribano, I., .630F
Amaike Campen,
Saori, ... 631W*
Amaike-Campen, Saori,50T
Amano, Hitoshi, ...52W
Ambrosio, A.,.....582F
Ament-Velasquez,
S. L., ...598W, 603F*
AMSELEM, J., ...551T
Amses, K. R., ... 346W*
Anasontzis, G., ...469W
Andersen, L. N., ... 33F
Andersen, M., ...308T
ANDERSEN, M. R.,....243F
Andersen, M. R., ...230T,
..... 277W, 278T
Anderson, Marilyn, ...21F
Andika, Ida Bagus, ...438F
Andreopoulos, W.,.....232W
Andreopoulos, Wiliam,294F
Andrianopoulos, A., ...571W
Andrianopoulos, Alex
,.....146T*
Anh, D. V., ...244W
Anta, Francisco, 98T, 208W
Ao, Jie,.....101T
Apken, L., ...20T
Archana, A.,.....376W
Arechiga-Carvajal, E.,187W
Arentshorst, M., ...453F
Argotti, Eduardo, ... 131T
Arie, T.,.....344T*
Arita, T.,..... 54F
Ariyawansa, S., ... 137T
Arkin, Adam,..... 238W
Arkin, Adam P., ... 66F
Arndt, B., ... 20T
Arnold, Jonathan, ... 424W
Arnold, Stephanie, ... 7W
Arredondo, Felipe, ... 507F
Arshed, Saadiah, ...97W*
Asai, S.,.....122T*
Asamura, Taro, ... 52W
Asaria, Iman,..... 419T
Asch, David,.....421W*
Asfaw, Y., ... 357F
Ashton, P. M.,.....244W*
Åsman, Anna,..... 123F
Assa, D.,.....417F*
Aßmann, D.,..... 504F
Atanasova, Lea, ...581T*
Au, Chun Hang, ... 255F
Aubin, Yves,..... 518T
Audeon, C., ... 180F
Auvinen, Petri,..... 74T
Avalos, J.,..... 399F
Avalos, Javier,..... 404T
Avila, Cristina, ... 535W
Ayhan, D. H.,.....611T*
Ayliffe, M., ...493W
Ayukawa, Y., ... 344T
Azimova, D. R., ...490W*

B

Baccile, J.,..... 6F
Bachellier-Bassi, S.,... 147F
Bachleitner, S.,.....431T*
Badis, Y.,.....324F*
Badouin, H., ...229W
Baek, M., ...379W
Baermann, G.,.....520W*
Báez-Ojeda, Liszeth,. 523W
Bahadoor, Adilah, ... 518T
Baidoo, Edward,..... 66F
Bailey, Andrew M., ... 69F
Bailey, Andy, ... 299T
Bajaj, R.,.....436W*
Baker, L.G, ... 377T
Baker, S., ...244W, 308T,
..... 331W
Baker, S. E., ...51F, 199W,
..... 230T, 278T, 628W
BAKER, S. E., ... 243F
Bakker, L. V.,..... 242T
Bakonyi, J.,.....334W, 335T
Bal, Jyotiranjan, ... 211W,
... 270F, 285F, 288F, 321F
Baldin, C.,.....644T*
Baldrich, P.,..... 226W
Baldwin, Thomas, ...460W*
Balesdent, Marie-Hélène,
..... 484W, 495F, 592W
Balesdent, M.-H., ... 506T
Balesdent, M. H., ... 498F
Balico, L. L. L., ...4W*
Bálint, B.,..... 228F
Banani, H., ... 234F
Banerjee, S., ... 407T
Bange, G., ... 566T
Bango, Zackary,. 23T, 134T
Banjara, N., ...468F, 638T*
Barad, S., ...381F, 442W
Barda, O.,..... 462F
Barkla, B. J.,..... 271W
Barnes, I.,..... 619W
Barnes, Irene,..... 302T
Baroncelli, R.,.....212T*
Barrales, Ramon, ... 567F
Barrios Gozalez, J.,.... 443T
Barron Gutierrez, A., ... 71T
Barry, K.,..... 559W, 630F
Barry, Kerrie,..... 145W,
.....250W, 380T, 562W
Bartholomai, Bradley,104T*
Bartnicki-Garcia,
Salomon,..... 156F*
Bary, Vinay, ...18F
Basenko, Evelina,..... 203T*
Bassik, M. C.,.....543F
Basso, V., ...147F
Bastiaans, E.,.....603F
Battaglia, E., ...410T
Baudo, C. D.,199W*, 316W*
Baudo, Charles D., ...198F
Baum, T., ...499W
Baumann, L., ... 20T, 390F
Baumann, Sebastian, 190W
Bawa, P., ...407T
Bayon, C., ...503T
Bayram Akcapinar,
Gunseli,..... 78F*
Beardsley, J., ...244W
Beattie, S., ... 456F*
Beattie, S. R.,.....459F
Beaudet, D.,.... 265W, 296T
Bebber, Dan,.....336F
Beccari, G., ...30F
Becker, K., ... 177F, 433W,
..... 435F
Becker, Y., ...561F
Beckerman, Janna,.....300F
Bedekovic, T., ... 100W*
Beever, Ross, ...97W
Begerow, D., ... 624F*
Begerow, Dominik, ...326T,
..... 626T*
Beier, A., ... 120F*
Belay, Kassaye, ...569T
Bell-Pedersen, D.,379W, 420F
Belova, O., ...57F
Benfield, A. H.,.....30F
Benito, Ernesto P.,.....98T,
.....131T, 208W, 523W
Bennett, Jared, ...509T
Bennett, Joan, W., ...304W
Bennett, R. J., ...209T
Benocci, T., ... 289W*
Benoit-Gelber, Isabelle, 11T
Benucci, GMN.,.....559W
Berger, Dave,..... 302T*
Berger, DK., ...619W
Berman, J., ...587T
Berne, S., ... 491T*
Berrin, JG,.....469W
Bertazonni, Stefania, ..510F
Bertazzoni, S., ... 293T*
Bertolini, Maria Célia, 425T*
Bertolini, M. C., ...379W
Bertsch, C., ...241W
Besharat, Naghmeh,...636F
Betz, R., ...489F
Beyhan, S., ... 414F*
Beyhan, Sinem,418W, 631W
Bhatnagar, D.,.. 194T, 545T
Bian, Z., ... 394W*
Bibi, S., ...525F
Bidard, F., ...73W
Bidard, Frederique, ...80T
Biega, A., ...253W
Biermann, A. R., ...320T
Bilanenko, E. N., ...214W
Billmyre, B.,.....402F
Billmyre, Blake, ...382W
Billmyre, R. Blake, ...588F,
..... 589W*
Bilton, Z.,.....392T
Bitencourt, T. A.,401T*, 524T
Black, C., ...456F
Blackwell, Barbara, ...518T
Blackwell, Meredith,....248T
Blackwood, C.,.....253W
Blaise, F.,.....498F
Blaise, Françoise, ...495F
Blank, L. M., ...76W
Blaschke, C., ... 392T
Blaxter, M., ... 546F
Bleackley, Mark,..... 21F
Bliss, J. M., ... 209T
Blondeau, K.,..... 498F
Blondeau, Karine,..... 495F
Bluhm, B.,..... 267F
Bluhm, B. H., .. 282F*, 618F
Bluhm, Burton,..... 302T
Blum, Ailisa,..... 164T
Blum, C.,..... 570F
Bock, Clive H.,..... 620T
Boddy, L., ... 215T
Bodie, E.,..... 65T
Boekhout, Teun,588F, 617T*
Boelker, M., ... 189F
Boenisch, Marike,..... 37W*,
..... 164T*, 169W*
Boenisch, M. J.,..... 519F
Boesch, K.,.....549F*
Boevink, P., ... 497T
Böhmdorfer, Stefan, ... 432F
Bok, J., ...413T, 449T
Bok, Jin-Woo,..... 70W
Bölker, M., ... 45F
Bolton, Melvin,..... 556W*
Bon, E. P. S.,.....58W
Bonazza, Klaus, ... 78F
Bonito, G., ... 349W, 559W
Bonito, Gregory, ... 249F,
..... 354F, 562W*
Bonner, C., ...130W
Bonner, Christopher, .395T*
Boppidi, K., ...364W
Boppidi, Karthik, ...145W
Borhan, M. H., ... 220W*
Bormann, J., ... 519F, 570F*
Bosman, Saskia, ... 509T
Bosnich, Whynn, ... 518T
Bosnjak, Natasa, ...419T*
Bougnoux,
Marie-Elisabeth, ... 197T
Bougnoux, M. E.,..... 147F
Boulware, David, ... 213F
Boureghda, H., ...610W
Bourras, Salim,.....484W
Bouwmeester, Klaas, 526W
Bouyer, Patrice,..... 653T
Bowen, J.,..... 530T
Bowen, Joanna,.....97W,
..... 245T, 509T*
Bowen, Johanna K., ... 620T
Bowyer, P., ... 204F
Bowyer, Paul, ... 318F,
.....359T, 458T
Boyd, A., ... 386T
Bracher, F.,..... 360F
Brachmann, Andreas, 626T
Bradley, E. L.,.....558F*
Bradshaw, R.,..... 558F
Bradshaw, R. E., ... 137T
Bradshaw, Rosie, ...508W
Bragg, Paige,..... 7W
Brakhage, A.,..... 408F
Brakhage, A.A, ... 360F
Brakhage, A. A., ... 627F
Branco, S.,....223W, 229W*
Brand, A., ...100W
Brand, A. C.,..... 99F
Brandenbourg, J.-T., .229W
Brandström Durling,
Mikael, ... 623T
Brandt, P., ...32T*
Brandt, U., ...140T, 158T
Brankovics, B., ... 607W*
Brankovics, Balázs, ... 295W
Brauer, Elizabeth,..... 518T
Braus, G., ...161T, 192F
Braus, G. H., 9F, 88W, 92T,
..... 582F, 630F
Braus-Stromeyer, S. A.,582F
Bravo Nunez, M. A., ..622W

Breen, S., ... 19W
Breen, S. A.,..... 28W
Bregitzer, Phil,..... 460W
Breitenbach, Romy, .. 103W
Brem, Rachel, 238W, 600F*
Brennan, R. G., ... 467T
Brizuela, C., ... 322W
Brock, M., ... 72F
Brockmann, A., ... 570F
Broglie, K. E., ... 319W
Bromley, M., ... 83T, 204F
Bromley, Michael, ... 458T
Bromley, Mike, ... 359T*
Bromley, M. J., ... 360F
Brommonschenkel, S. H., ... 319W
Bross, Tanner, ... 239T
Brown, D., ... 616W
Brown, Daren W.,..... 391W*
Brown, D. W., ... 279F
Brown, J., ... 475W
Brown, J. C. S., ... 474F
Brown, Kayla, ... 421W
Brown, N. A., ... 517W*
Broz, Karen, ... 37W, 46W, ... 164T, 169W
Brueggeman, R., ... 218T
Brueggeman, Robert, 569T
Bruix, M., ... 133W
Brun, S., ... 173T, 176T*
Brun, Sylvain, ... 538W*
Bruno, K. S., ... 633F*
Bruno, V., ... 644T
Bruns, T., ... 223W, 621F
Buda de Cesare, Giuseppe, ... 17T
Buecher, Debbie, ... 353T
Bugajski, Kristi, ... 654F
Büker, Britta, ... 626T
Bulik, C., ... 333F
Bulik-Sullivan, E., ... 333F
Bulla, I., ... 582F
Bulone, Vincent, ... 21F
Buntzman, A. S., ... 553W
Bunzel, M., ... 57F
Bus, Vincent, ... 245T
Bushley, Kathryn E., . 276F*
Bushley, K. E., 345F, 436W
Busot, G. Y., ... 452T
Butchko, R. A. E., ... 449T
Byrne, K. P., ... 227T
Byun, J.,262W, 292W, 306F

C

Cabrera-Ponce, J.,.... 187W ..... , 389T
Cafarchia, Claudia, ... 617T
Caffrey, A. K., ... 459F
Caffrey, Alayna, ...3F
Cahill, C., ... 541W
Cai, Feng, ... 115W
Caimi, Nicole, ... 353T
Callejas-Negrete, O.A,154W*
Callejas-Negrete, O. A.,159F
Callejas-Negrete, Olga,156F
Calvey, Christopher H.,248T
Calvo, Ana M., ... 362T
Campanella, Jonatas Erick, ... 425T
Campen, S. A., ... 51F
Candy, A. V., ... 185T*
Canessa, Paulo, ... 378F*
Canovas, D., ... 8T*, 630F*
Capaldi, Mindi, ... 654F
Capilla, J., ... 360F, 577W
Carbone, I., ... 194T, 586W
Carbonw, I., ... 618F
Carella, G., ... 335T
Carere, J., ... 30F
Carlier, F., ... 174F*
Carlson, A., ... 114F
Carman, B., ... 541W
Carr, E., ... 560T*
Carr, P. D., ... 360F
Carrasco, U., ...166W
Carrasco Navarro, U., ... 271W*, 443T
Carreras-Villaseñor, N., ... 55W*
Carroll, I., ...333F
Carsten, Alexander,....564F
Carter, Helen, ...312F
Carter-Wientjes, C., ...42F, ... 545T
Carver, A., ... 223W*
Cary, J., ..42F*, 194T, 545T
Casado-del Castillo, Virginia, ...523W
Casado Lopez, S., ... 331W*
Cascant-Lopez, E., ... 471F*
Caster, S. Z., ...420F
Castillo, K. D., ... 420F*
Catalán-Dibene, J., ...322W
Cate, J., ...635T
Cavinder, B., ...284T
Cavinder, Brad, ...300F
Cernak, P., ...635T
Cerqueira, G. C., ...585F
Cevik, V., ...122T
Chaillot, J., ... 210F*
Champion, C., ...469W
Chan, A. K., ...244W
Chan, k., ... 645F*
Chan, W.-Y., ...233T
Chanda, A., ... 650T*
Chang, Guo-An, ...290T
Chang, J., ...412W*, 441F, ... 645F
Chang, Jinhui, ...255F
Chang, P., ... 385W*
Chang, T., ...454W
Chang, Xiao Lan, ...23T
Chang, Y., ...349W*, 559W
Chang, Yun, ...107T
Chang, Z., ... 402F*
Chan Ho Tong, L., ... 73W*
Chan Ho Tong, Laetitia, ...127W
Chapeland-Leclerc, F., ... 171F*, 173T
Charlton, Nikki D.,.....620T
Chau, T. T., ...244W
Chaverri, P., ...317T
Chelius, C., ...364W
Chen, Chia-Ling, ... 290T*, ... 307W
Chen, Chung-Yu, ...290T
Chen, Daipeng, ...434T
Chen, Chun-Hung., . 265W*
Chen, Jay, ...339F
Chen, Ko-Hsuan, ... 479T*
Chen, L., ...394W
Chen, Li-Hung, ...480F
Chen, N., ...114F
Chen, P., ...376W
Chen, She, ...409W
Chen, X., ... 492F*, 505W*
Chen, Y., ...442W
CHEN, Y., ... 373W*
CHEN, Yingying, ... 363F*
Chen, Z., ...65T
Cheng, C., ...456F
Cheng, Jan-Fang, ...294F
Cheng, J.-F., ...200T
Cheng, Jiasen, ...437T
Cheng, Xuanjin, ...255F
Cheong, K., ...260T
Cherry, J. M., .. 648F, 649W
Cheung, Man Kit, ...255F
Chiang, Yi-Ming, ...70W
Chichester, A., ...163W
Chierakul, W., ...244W
Chinnaswarmy, Kala, .198F
Chng, S., ...313W
Choera, T., ...449T
Choi, G., ... 260T, 534F*
Choi, I., 262W, 292W, 306F
Choi, I.-G., ...214W
Choi, Jaeyoung, ...285F
Choi, So Yeun, .287T, 288F
Christakou, Eleni, ... 75F
Chuang, Yu-Chien, ... 307W
Chun, Jeesun, ... 25W, 211W*, 285F, 287T, 568W*
Chun, JeeSun, ... 270F
Ciobanu, D., ... 200T
Ciobanu, Doina, ...294F*
Clairet, Colin, ... 538W
Clake, Helen, ... 312F
Clancey, Shelly, ...589W
Clarke, D., ... 439W
Clum, A., ... 200T
Clum, Alicia, ... 294F
Coelho, M., ... 229W
Coetzee, M. P. A., ...222F*
Cohen, A. L., ...543F*
Cohen, M. J., ...163W*
Cohrs, K. C., ...141F*
Cohrs, Kim, ... 372F
Coleman, J., ...232W, 512T
Collard, L., ...530T*
Collard, Linda, ... 509T
Colombo, A. L., ... 209T
Colpaert, J., ... 613W
Commer, B., ...90F*
Commere, Pierre-Henri, ... 197T
COMPAIN, J., ... 551T
Conant, G., ... 263T
CONFAIS, J., ... 551T
Coninx, L., ... 613W
Connolly, Lanelle,23T, 134T
Connolly, Lanelle R., .. 311T
Constantin, Maria-Ermioni, ...521T*
Contamine, V., ... 174F
Cook, David E., ...268W*
Cook, D. E., ...387F, 511W
Cook, N. M., ...313W*
Cooperstein, Isabelle, 117F
Coppin, E., ... 173T
Coradetti, Samuel, ...238W*
Coradetti, S. T., ... 405F
Corcoran, Pádraic, ... 606F
Cornejo, O., ...505W
Corradi, N., ...265W, ... 296T, 489F
Corrales-Escobosa, Alma, ... 557T
Corran, A., ... 16W
Correll, J., ... 259W
Corrochano, LM, ... 428T
Corrochano Pelaez, Luis M., ... 148W
Corsaro, D., ... 296T
Cosetta, Casey, ... 478W
Coslett, Morgan, ... 239T
Costa, M. D., ... 264F
Cotty, Peter, ... 309F
Couger, B., ... 254T
Coughlan, Aisling Y., .. 248T
Courtney, A., ... 450F
Courville, K., ... 549F
Covarelli, L., ... 30F
Covo, Shay, ...18F*
Cowen, Leah, ... 466W
Cox, Murray, ... 97W
Cox, Russell J., ... 69F
Cramer, R., ... 456F
Cramer, R. A., ... 459F
Cramer, Robert, ..3F, 415W
Crampton, BG, ... 590T
Crampton, B. G., ... 258F
Crampton, Bridget, ... 302T
Cravador, A., ... 335T
Craven, J., ... 99F
Craven, M., ... 590T
Creamer, J., ...253W
Crenshaw, Nicole, ... 514W
Crocker, A. W., ... 320T
CROLL, D., ... 551T
Croll, Daniel, ...486F, 552F
Cromie, G., ... 587T
CROS-ARTEIL, Sandrine, ... 614T
Crous, Pedro, ... 246F, 302T
Crowell, Alexander,.....380T
Cruaud, C., ...506T
Csukai, Michael, ... 312F*
Cubba, Caitlyn, ... 124W
Cuc, N. T. K., ... 244W
Cuddy, W., ...305T
Culley, D., ...315F
Cunha, Anderson Ferrira da, ... 457W
Cunningham, G.,.....89T
Cuomo, C., ...209T
Cuomo, C. A., .. 257T, 465F
Cuomo, Christina A.,....588F
Cuzick, Alayne, 201F, 266T

D

Dafa-Berger, A., ...462F
Daguerre, Y., ... 469W
Dahlke, Bonnie, ...654F
Dahlmann, T. A.,68T, 435F*
Dai, Ziyu, ...77T
Daigneault, MC, ... 463W
Dalle, F., ...147F
Dalusag, K. S., ...648F
Dam, S., ...546F
Damann, K. E., Jr., ... 310W
Damaso, M. C. T., ... 58W
Dang, Yunkun, 409W, 437T
Dannebaum, R. O., ... 451W
DASKALOV, A. I., ... 183F*
Daugherty, J. M., ...474F
Davanture, M., ...429F
Davis, Laura, ... 496W
Davis, M., ... 439W
Dawe, Angus, ... 202W
Dawson, Thomas, ...617T
Day, J. N., ... 244W
Dean, R., ... 532W
Dean, Ralph, ...261F
de Beer, Z. W., . 602T, 605T
Debets, A., ... 583W
Debets, A. J. M., ... 214W, ... 584T, 603F
Debray, R., ...621F
Debuchy, R., ...173T
de Freitas Pereira, M., 264F
de Groot, P. W. J., ...102F
de Hoog, S., ... 607W
Deinzer, T., ...45F
Delulio, G., ... 273F*
de Jonge, Ronnie, ... 556W
de Koster, C., ...102F
de Lamo, Francisco, ...521T
Delaruelle, Christine, ..500T
Deller, Siân, ...312F
Delorey, T., ...465F
Delph, L., ... 226W
de Mattos-Shipley, Kate M. J., ... 69F*
deMello, A. J., ...582F
Dementyeva, Polina,. 103W
Demers, E., ... 320T*
d'Enfert, C., ... 147F*
d'Enfert, Christophe, ...197T
Deng, C., ...530T
Deng, Cecilia, ...509T
Deng, F., ... 375F*
Deng, S., ... 547W*
Deng, Weiwei, ...636F
DENG, Y.Z, ...105F
Deng, Zhaojie, ... 424W
Denham, S. T., ... 474F*
Dentinger, Allison, ...447F
Depotter, J., ... 511W
Depotter, Jasper., ... 593T*
de Reus, P. E., ... 601W*
Derevnina, Lida, ... 529W
De Saeger, S., ...42F
De Silva, Nishadi, ...266T
Desirò, A., ... 349W, 559W*
Desiro, Alessandro, ... 249F, 562W
Desjardin, D. E., ...327F

Desveaux, D., . 130W, 269T
Deutsch, Sam, ... 50T
Deveau, A., ... 469W
de Vires, R. P., ... 410T
De Vos, L., ... 233T, 609F
De Vries, R., ... 289W
de Vries, R., ... 331W
de Vries, Ronald, ... 11T
De Vries, Ronald, ... 366F
DE VRIES, R. P., ... 243F
de Vries, R. P., ...26T, 56T, ... 57F, 214W, 453F
Dhillon, B., ... 267F, 282F
Dhingra, S., ... 456F
Dhingra, Sourabh, ... 3F
Diana Di Mavungu, J. , ... 42F, 385W
Díaz Mínguez, José María, ... 208W*
Díaz-Mínguez, J. M., ...98T*, 131T*, 523W*
Dietrich, Fred, ... 354F
Dietrich, Fred S., ... 275T*
Dietrich, R., ... 16W
Di Falco, M., ... 453F
Dijk, K., ... 583W
Dilks, T., ... 517W
Dilokpimol, A., ... 56T, 57F*
Dilokpimol, Adiphol, ... 11T
Dinkins, Randy D., ... 528F
Di Pietro, A., .. 132F, 133W, ... 181W, 611T, 612F
Di Pietro, Antonio, ... 43W
Dixelius, Christina, ... 123F
Djamei, A., ... 566T
Dodds, P., ... 303F
Dodds, P. N., ... 493W
Doehlemann, Gunther, 476T
Dohnalkova, A., ... 331W
DONG, Liguo, ... 363F
Dong, Liguo, ...186F, 365T
Dong, Yanhong, ... 46W
Donlin, M.J, ... 377T
Dörnte, Bastian, 135F, 196W
Dorter, I., ... 87F*
dos Reis, Thaila, ... 145W
Dovzhenok, A., ... 379W
Downes, D. J., ... 452T
Drechsler, F., ... 488T
Driessen, Arnold, ... 40W
Druzhinina, Irina S., .. 61W*, ... 78F, 115W*
Dry, Ian, ... 496W
Du, Z. M., ... 652W
Duah, Kwamaa, ... 466W
Dubey, A., ... 442W
Dubey, A. K., ... 381F
Dubey, M., ... 221T
DUCASSE, A., ... 551T
Dudley, A., ... 587T
Dudley, E., ... 215T
DuFour, Mitchell, ... 569T
Dundon, Samantha E. R., ... 104T
Dunlap, Christopher, .. 353T
Dunlap, J., ... 152T
Dunlap, Jay, ... 380T, 415W
Dunlap, Jay C., ... 104T
Duong, T. A., ...595W, ... 602T*, 605T
Duplessis, Sebastien, 207F*
Duplessis, Sébastien, . 500T
Dupont, P., ... 137T
Dupont, PY., ...185T, 558F
Durán, A., ... 334W, 335T
Dussart, F., ... 39F*
Dutken, N. M., ... 199W
Dutta, Tanmoy, ... 66F
Duval, L., ... 80T
Dvir, Hay, ... 535W
Dyer, P., ... 315F
Dzierzon, Helge, ... 245T

E

Eastwood, D. C., ... 215T* ....., 544W*
Eaton, C.J, ... 185T
Eaton, C. J., ...137T, 502W, ... 558F
Ebert, Malaika, ... 556W
Edel, D., ... 16W
Edwards, J., ... 644T
Eickbush, Michael T., 622W
Ellis, Shannon, ... 239T
Ellwood, Simon, ... 636F
Elmore, M. Holly, ...604W*
El Mounadi, K., 536T, 537F*
Emerson, J., ... 152T
Endo, S., ... 143T
Ene, I. V., ... 209T*
Engel, S., ... 649W
Engel, S. R., ... 648F
English, B. C., ... 490W
Entler, Matthew, ... 634W
Erchinger, P., ...565W*
Eric, Gelhaye, ... 29T
Erlendson, Allyson, ... 134T
Escalante, A. E., ... 322W
Eschenbrenner, C. J. , ... 311T, 477F, 599T*
Espeso, E. A., ... 381F
Esposito, Madison, ... 23T
E. Stanley, Claire, ... 555F
Estrela, R., ... 635T*

F

Fabri, João Henrique Tadini Marilhano, ... 457W
Faino, L., ... 511W
Fajardo, S., ...334W, 335T
Falkoski, D. L., ... 214W
Fan, Yumeng, ...383T*
Fang, D., ... 108F
Fang, Y., ... 165F*
Fargo, Corinne, ... 23T
Farmer, A., ... 16W
Fávaro, L. C. L., ... 58W
Fejes, B., ... 10W
Fekete, E., ... 10W, 585F*
Feldbrügge, M., .76W, 549F
Feldbrügge, Michael, 190W
Feldmann, Ines, ... 643W
Fenstermacher, K., ... 41T
Feri, Adeline, ... 197T
Fernanado, W. G. D., 220W
Fernandez, F. J., ... 166W
Fernandez Perrino, F., . 71T
Ferrari, R., ... 171F
Fierro, F., ...166W, 271W
Fierro Fierro, F., . 71T, 443T
Figueroa, M., ...22W, 206T, ... 303F, 493W*
Fill, Taicia Pacheco, .. 457W
Filler, S., ... 587T
Fillinger, S., ... 180F, 429F
Fillinger, Sabine, ... 372F
Finkernagel, F., ... 448W
Finkernagel, Florian, .. 476T
Finlayson, M., ... 102F
Fischer, G., ... 82W
Fischer, G. J., ... 449T
Fischer, M. S., ...149T*
Fischer, R., ..34W, 35T, 48F
Fischer, Reinhard, ... 93F
Fisher, Andrew, ... 480F
Fitzsimons, H. L., ... 561F
Flaherty, J., ...392T*
Flajšman, M., ... 491T
Fleissner, A., ..139W, 140T, ... 157W*, 158T
Flipphi, M., ... 585F
Foged Lyngkjær, M., .. 546F
Fogelqvist, Johan, ... 123F
Fokina, O., ... 48F*
Fokkens, Like, ... 295W
Fones, Helen, ...336F
Fontaine, F., ...241W
Forche, A., ... 587T*
Ford, C. B., ...465F
Forester, Natasha, ...563T
Fortenbacher, J., ...448W
Fortwendel, JR, ...84F
Foster, Gary, ...299T
Fourie, G., ...641T
FOURNIER, Elisabeth, 614T
Frandsen, Rasmus John Normand, ...40W
Frandsen, R. J. N., ...601W
Franssen, H. J., ...214W
Frantzeskakis, L., ...549F
Fraser, J., ...439W
Free, Stephen, ... 101T*
Freitag, J., ...189F
Freitag, M., ... 433W, 570F
Freitag, Michael, ...23T, ... 134T, 311T
Freitas, Fernanda, ...425T
Frelinger, J. A., ...553W
Frey, Pascal, ... 207F, 500T
Friesen, T. L., ...293T
Frisvad, J., ... 351F*
Frisvad, J. C., ...230T, ... 277W, 278T
Frisvad, Jens C., ...40W
Fu, Ci, ... 172W*
Fu, E., ...209T
Fu, Jingjing, ...409W
Fudal, I., ... 498F, 506T
Fudal, Isabelle, 484W*, 495F
Fuller, Kevin, ... 415W*
Fulton, D., ...26T
Furnish, Madison, ...447F
Furukawa, T., ...360F
Furukawa, Takanori, ...359T
Furzer, O. J., ...122T

G

Gabriel, Raphael, ...77T
Gacek-Matthews, A., ..431T
Gachon, c., ...324F
Galgiani, J. N., ...553W
Gallagher, J., ... 253W*
Gallay, I., ...498F
Gallay, Inès, ...495F
Galuszka, P., ...574W
Gan, P., ...473T
Gao, M., ... 513F*
Gao, S., ... 608T*
Gao, X., ...578T
Gao, Xiaolei, ... 93F*
Garbelotto, M. M., ... 594F*
Garcia, J. F., ... 430W*
García-Altares, M., ...32T
Garcia Bastidas, F., ...483F
Garcia-Rangel, C. E., 463W*
García-Rico, R. O., ...271W
Gardiner, D. M., ... 30F*
Gardiner, Donald, ...164T
Garre, V., ... 573F, 577W
Garzon, C. D., ...254T
Gauthier, Gregory, ... 117F*
Gautier, Valérie, ...127W
Gazis, R., ...317T
Gdanetz MacCready, Kristi, ...355W
Gebremariam, T., ...644T
Geib, E., ... 72F*
Geiser, D., ...41T
Geiser, D. M., ...610W
Geiser, E., ...76W
Geislman, Gina, ...238W
Geißel, Bernadette, ...81F
Gelhaye, Eric, ...13W
Gell, R. M., ... 586W*
Geranios, P., ... 83T*
Gerke, J., ...88W*, 92T
Gerrits, Ruben, ...643W
Gerstein, Aleeza, ... 213F*
Gervais, J., ... 498F, 506T*
Gervais, Julie, ... 484W
Ghanbarnia, K., ... 220W
Gibeaux, R., ... 112W
Gilmore, Sarah, ... 418W
Gil Sanchez, M. del Mar, ... 148W*
Giordano, L., ... 594F
Giorgi, J., ... 265W
Giraud, T., ... 229W
Gladden, J. M., ..51F, 628W
Gladden, John, ... 50T
Gladden, John M., ... 66F
Gladfelter, Amy S., ... 104T
GLADIEUX, Pierre, ...614T*
Gladyshev, E., ... 386T
Gladyshev, Eugene, ..422T*
Glasco, A., ... 338T
Glasenapp, A., ... 519F
Glass, N. L., ...149T, 155T, ... 405F, 444F
GLASS, N. L., ... 183F
Glatter, T., ...504F, 566T
Gleddie, Steve, ... 518T
Glenn, A., ...513F, 608T
Glienke, Chirlei, ... 219F
Gluck-Thaler, E., ...216F*
Göhre, V., ... 549F
Gold, S., ...513F, 608T
GOLD, Scott, ... 514W*
Goldman, G., ... 456F
Goldman, Gustavo, ..145W, ... 281T
Goldman, Gustavo Henrique, ...362T, 457W
Goldshmidt-Tran, Orit, ... 184W
Goldstein, Allen, ... 354F
Gomes, Eriston, ...426F*
Gomi, K., ...53T, 367W, ... 369F*, 370W
Gomi, Katsuya, ... 371T
Gondal, A. S., ...348F*
Gong, Z., ... 393F
Gonthier, P., ... 594F
González, M., ..334W, 335T
Gorbushina, Anna A., ...103W, 643W*
Goryachkin, Aleksandr , ... 555F
Gottschalk, L. M. F., ... 58W
Goulin, Eduardo, ... 60F*
Gouzy, J., ... 229W
Govers, Francine, ... 526W
Gow, N. A., ... 137T
Gow, N. A. R., ... 99F
GOYEAU, H., ... 551T
Grace, C. L., ... 327F
Graf Grachet, N., ...254T*
Grahl, N., ... 320T
Grandaubert, Jonathan, ... 477F
Granek, Joshua A., ... 588F
Greco, Claudio, ... 69F
Green, K. A., ...561F*
Green, Kimberly, ...127W
Green, R., ... 454W
Grego, Jonathan, ... 275T
Grenville-Briggs, L., ... 497T
Grenville-Briggs, Laura J., ...252F*
Griffith, James, ... 424W
Grigoriev, I., 114F, ... 200T, 207F, 212T, 214W, 223W, 230T, 232W, 243F, 248T, 264F, 277W, 278T, 292W, 308T, 315F, 347T, 451W, ...559W, 562W, 630F
Grigoriev, Igor, ...145W, ... 246F, 250W, 294F, 380T
Grijseels, Sietske, ... 40W*
Grognet, Pierre, ... 127W
Gronquist, M., ... 580W
Grothe, Hinrich, ... 78F
Gruber, Sabine, ... 581T
Grum-Grzhimaylo,

A. A., ... 214W*
Gryganskyi, A., ... 451W
Gsaller, F., ... 360F*
Gsaller, Fabio, ... 359T
Gu, Biao, ... 507F
Gu, X., ... 513F
Güldener, U., ... 519F
Gullino, M. L., ... 234F
Gummadidala, Phani M., ... 650T
Gunde-Cimerman, N., .. 44T
Guo, J., ... 554T*
Guo, Shuhui, ... 365T*
Gupta, Y. K., ... 319W*
Gurr, Sarah, ... 336F*
Guskins, Verneta, ... 304W
Gutierrez Ruiz, M., ... 443T

H

Haas, H., ... 360F
Hackley, Rylee, ... 600F
Haddadi, P., ... 220W
Hagen, F., ... 583W
Hagerty, Christina, ... 548T
Hagiwara, D., ... 369F
Haj Hammadeh, H., .. 139W
Hajovsky, Heather, ... 70W
Hallen-Adams, H., ... 343W
Hallen-Adams, H. E., ... 468F*, 638T
Hamann, A., ...110T*, 175W
Hameed, K., ... 621F
Hameed, Khalid, ... 342F
Hamilton, J., ... 392T
Hamm, Paris, ... 353T*
Hammel, K., ... 22W
Hammerbacher, A., ... 605T
Hammond, T., 386T*, 616W
Hammond-Kosack, K., ... 482T, 517W, 576F
Hammond-Kosack, Kim, ... 201F*, 266T
Hampel, Martin, ... 476T
Han, Kap-Hoon, ... 91W*, 361W*
Han, X., ... 566T*
Hane, James, ... 510F*
Hane, J. K., ... 293T
Hanson, S. J., ... 227T*
Hao, Z., ... 559W
Haon, M., ... 469W
Harb, Omar, ... 203T
Haridas, S., ... 212T, ... 246F*, 308T
Haridas, Sajeet, ... 248T
Harkess, A., ... 226W*
Harris, Linda, ... 518T*
Harris, S., ... 244W, 560T
Harris, S. D., ... 364W
Harris, Steven, ... 145W*
Harris, Steven D., ... 240F
Harrison, R. J., ... 471F
Harth, Simon, ... 77T
Harting, R., ... 582F*
HARTMANN, F., ... 551T
Hartmann, Fanny, ... 486F
Hartung, J., ... 570F
Hartung, L., ... 157W
Hartwig, F., ... 566T
Harvey, A., ... 616W
Harvey, J. W., ... 259W
Hasegawa, F., ... 54F, 86T
Hassing, B., ... 502W*
Hatakeyama, S., ... 150F
Haueisen, J., ... 599T
Haueisen, Janine, ... 477F*
Havis, N., ... 546F
Havis, N. D., ... 39F
Hawkins, W., ... 267F
Hazal Ayhan, D., ... 612F
He, Z., ... 413T
Healey, K., ... 625W
Heard, David, ... 69F
Hecker, Arnaud, ... 500T
Heeger, Felix, ... 103W
Heimel, K., ... 446T, 448W
Heimel, Kai, ... 476T*
Heiniger, R., ... 618F
Heinze, C., ... 570F
Heitman, J., ... 402F
Heitman, Joseph, ... 106W, 172W, 382W, 588F, ... 589W, 642F
Helfer, H. P., ... 113T
Helk, Bernhard, ... 64W
Heller, J., ... 155T*
Hellerstedt, S. T., ... 648F
Henke, C., ... 217W
Henrissat, B., ... 212T, ... 214W, 469W, 519F
Herbert, E., ... 171F
Hernández-Aparicio, Francisco J., ... 523W
Hernandez-Cervantes, A., ... 147F
Herrera-Estrella, A., ... 55W
Herrera-Estrella, L., ... 55W
Hertweck, C., ... 32T
Herzka, S., ... 322W
Herzog, S., ... 158T*
Hess, Jaqueline, ... 604W
Hevia, Montserrat, ... 378F
Hibbett, D., ... 114F, 231F
Higuchi, H., ... 54F
Hijne, Ruben, ... 542T
Hildén, K., ... 56T, 331W
Hildén, K. S., ... 57F
Hilgart, Amelia, ... 332T
Hiltunen, Markus, ... 606F
Hinsch, J., ... 540F, 574W
Hirai, C., ... 14T
Hirai, Chihiro, ... 15F
Hirsch, A., ... 158T
Hirsch, C., ... 303F
Hiruma, K., ... 472W*
Hiscox, J., ... 215T
Hitchings, M., ... 544W
Hittinger, Chris Todd, . 248T
Hitz, B., ... 649W
Hjortshøj, R., ... 546F
Hoebe, P. N., ... 39F
Hoefer, A., ... 582F
Hoff, K., ... 582F
Hoffmeister, D., ...32T, 408F
Hofstad, Beth, ... 77T
Hogan, D. A., ... 320T
Högberg, Nils, ... 623T*
Holland, Linda, ... 146T
Holm, D. K., ... 33F
Homan, T. G., ... 453F
Hong, C. I., ... 379W*
Hoo, S., ... 376W*
Hood, M., ... 229W
Hood, Michael E., ... 626T
Hoof, J. B., ... 33F*
Hopkins, Virginia E., ...650T
Horev, Guy, ... 535W
Hori, Juliana Issa, ... 457W
Horn, B. W., ... 618F
Horta Jung, M., ... 334W*, 335T*
Hortschansky, P., ... 360F
Horwitz, Benjamin, ... 184W*
Hosseini, S., ... 597F
Houterman, Petra M., 295W
Howell, Holly, ... 121W
Hsueh, Y., ... 247W, 580W*
Hu, Haifei, ... 510F
Hu, Y., ... 305T
Hua, Chen-lei, ... 526W
Huang, Chien-Hao, ... 290T, 307W
Huang, L., ... 400W, 578T
Huang, M., ... 62T
Huang, Qianli, ... 255F
Huang, Y., ... 376W
Huberman, L. B., ... 405F*
Hudson, C., ... 263T
Huey, E., ... 109W
Huh, E., ... 333F
Huh, Jin Hoe, ... 285F
Hull, Brooke, ... 424W*
Hulse-Kemp, Amanda, ... 529W
Hulvey, J., ... 454W
Human, MP., ... 590T
Humpf, H.-U., ... 20T
Hunt, C., ... 22W
Hunter, C., ... 439W*
Hur, J. S., ... 79W*
Hurlburt, Nicholas, ... 480F
Hutchinson, M. I., ... 329T, 337W*
Hynes, M., ... 439W

I

Ianiri, Giuseppe, ... 106W*, 642F*
Iatti, Roberta, ... 617T
Ibarra, O., ... 379W
Ibe, Chibuike, ... 17T
Ibeas, Jose, ... 567F*
Ibrahim, A., ... 644T
Ichinose, S., ... 367W*
Idnurm, Alex, ...106W, 642F
Iguchi, N., ... 125T
Illgen, J., ... 140T
Inoue, Taishi, ... 371T*
Iqbal, M., ... 221T*
Irshad, G., ... 348F
Isaac, Dervla, ... 550W
Isakeit, T., ... 618F
Ishaque, N., ... 122T
Ishida, Hiroki, ... 52W
Ishii, Hideo, ... 245T
Islam, K., ... 537F
Ito, K., ... 297F
Ito, Masakazu, ... 66F
Itoh, Hiroya, ... 280W
Ivanoff, D., ... 80T
Iwashita, K., ... 368T

J

J., J., ... 79W
Jackson, T., ... 649W*
Jacobs, Abigail, ... 356T
Jacobs, Jon, ... 50T
Jacobson, Dan, ... 250W
Jaenisch*, J., ... 14T
Jaenisch, Johan, ... 15F
Jahng, Kwang Yeop, . 286W
Jahng, Kwang-Yeop, ... 91W
Jain, Sachin, ... 94W*
Jamal, Atif, ... 438F
James, E., ... 546F
James, T., 200T, 296T, 338T
James, Timothy, ... 294F
James, T. Y., ... 301W, ... 346W, 451W
Janbon, G., ... 445W*
Janevska, S., ... 20T*, 390F*
Jang, K., ... 262W
Jänsch, L., ... 139W
Jäntti, J., ... 67W
Jäntti, Jussi, ... 632T
Jarczynska, Z. D., ... 63F
Javornik, B., ... 491T
Jayantibhai Parsania, Chirag, ... 365T
J. Cabrera-Ponce, ... 389T
J. DeMello, Andrew, ... 555F
Jeffery, E., ... 587T
Jeffree, Chris, ... 176T
Jeffries, Thomas W., .. 248T
Jensen, D. F., ... 221T
Jeon, J., ... 260T, 534F
Jeon, Junhyun, ... 285F
Jeong, M. H., ... 79W
Jia, Leijie, ... 516F
Jiang, Cong, .. 397W*, 434T
Jiang, Y. Y., ... 637W
Jiao, M., ... 554T
Jimenez-Ortigosa, C., 625W
Jin, Yue, ... 615F
Jiwan, D., ... 505W
Jo, Myeongjin, . 211W, 287T
Johannesson, H., ... 597F, ... 603F, 616W
Johannesson, Hanna, ... 598W, 606F
John, Tricia, ... 49W
Johnathan, B. P., ... 641T*
Johnson, Jerry L., ... 615F
Johnson, Linda, ... 508W*, 563T*
Johnson, L. J., ... 471F
Johnson, R.D, ... 36F
Johnson, Shakira, ... 245T*
Joly, D., ... 242T
Jones, Dan, ... 245T
Jones, J. D. G., ... 122T
Joosten, M. H. A. J., . 319W
Josephson, B., ... 570F
Josephson, Brian, 23T, 134T*
Jourdier, E., ... 80T
Judelson, Howard, ... 647T, 651F
Judelson, H. S., ... 291F
Judge, Michael, ... 424W
Jun, Sang-Cheol, ... 91W
June, Kwon-Chung, ...107T
Jung, A., ... 110T
Jung, A. T., ... 116T*
Jung, Elke-Martina, ... 118W, 182T*
Jung, G., ... 454W
Jung, Sascha, ... 629T*
Jung, T., ... 334W, 335T
Junker, Klara, ... 572T
Jurgenson, J. E., ... 515T*
Jurick II, Wayne, W., . 304W
Juvvadi, P. R., ... 357F
J. Velez-Haro, ... 188T

K

Kabbage, Mehdi, ... 94W
Kaemper, J., ... 390F
Kaever, A., ... 582F
Kahmann, R., ... 504F, ... 565W, 566T
Kahmann, Regine, ... 564F
Kakade, P., ... 374T*
Kale, Shiv D., ... 507F
Kalyandurg, P. B., ... 497T
Kamakura, T., . 142W, 143T
Kamble, M., ... 546F
Kamei, M., ... 450F*
Kamisuki, S., ... 143T
Kamoun, Sophien, ... 500T
Kämper, J., ... 192F*, 448W
Kampinga, G., ... 583W
Kancherla, Prakash, ...623T
Kang, Ji Young, ... 25W*
Kang, S. E., ... 455T*
Kang, Z. S., ... 652W*
Kang, ZS, ... 554T
Kanyuka, K., ... 503T
Kanzi, A. M., ... 251T
Karaffa, L., ... 10W*, 585F
Karimi Aghcheh, R., .. 161T*
Kariyawasam, G. K., . 579F*
Karki, Shanta, ... 423F*
Karlsson, M., ... 221T
Kasahara, S., ... 86T
Kastman, Erik, ... 478W
Kaur, Baljinder, ... 95T*
Kavalecz, N., ... 585F
Kawamoto, S., ... 369F
Kaymakci, D., ... 571W*
Kazuhiro, I., ... 12F
Ke, X., ... 578T
Ke, Y.-H., ... 621F*
Keasling, Jay D., ... 66F
Keller, N., ..6F, 192F, 442W
Keller, Nancy, ... 94W, 413T
Keller, Nancy P., 70W, 82W

Keller, N. P.,10W, 449T, 630F
Kelly, A. C.,.... 237F*, 610W
Kelly, Joan,.....363F
Kelly, Steve,.....359T
KEMA, G. H. J.,.....551T
Kema, G. H.J,.....483F
Kemler, Martin,.....326T*
Kempken, F., 153F*, 328W*
Kemppainen, M., ...469W
Kemuriyama, K.,.....125T
Ken, O., ...12F
Kennedy, P.,.....621F
Kennell, J. C., 199W, 316W
Kennell, John C.,.....198F
Kersey, Paul, ...266T
Kershaw, M. J.,259W, 487W
Kettles, G. J.,.....503T*
Khan, Izhar, ...518T
Khanal Lamichhane,
Ami, ...107T*
Khang, C. H.,.....416T
Khateb, Aiah,.....458T*
Khonsuntia, Weeradej,.....
..... 195F, 196W
Khosravi, C.,... 366F*, 453F
Kianian, S., ...303F
Kianian, S. F.,.....493W
Kibengo, F. M.,.....244W
Kijpornyongpan, T., ...347T
Kikuchi, S.,.....344T
Kilani, J.,.....180F*, 429F*
Kilani, Jaafar,.....372F
Kilaru, Sreedhar, ...576F
Kim, D.,..... 314T, 655W
Kim, Dae-Hyuk, ...211W,
270F, 285F, 286W, 287T,
.....288F, 321F*, 568W
Kim, Da-Woon,236T*, 396F*
Kim, D. S., ...122T
Kim, D. W., ...416T
Kim, g.h, ...324F
Kim, H.,.....260T*
Kim, Hee-Kyoung, ...236T,
..... 396F
Kim, H.-S., ...279F*
Kim, J.,..... 51F*, 314T,
..... 628W, 655W
Kim, J. A., ...79W
Kim, Jong-Hwa,91W, 361W
Kim, Jung-Mi, ..270F, 285F,
....286W, 287T, 288F, 321F
Kim, K. T.,.....260T
Kim, W.,.....283W*, 284T*
Kind, S.,.....540F*, 574W*
Kindel, Stefanie, ...43W
King, Robert,.....576F
Kirby, James,.....66F
Kirchner, F. H., ...63F*
Kiss, B., ...228F
Kiss, E., ...225F*
Kistler, H. C., ...483F
Kistler, H. Corby, ...37W,
.....46W, 164T, 169W
Kitagawa, N., ...472W
Kjaerboelling, I.,..... 230T,
..... 277W*
Kjærbølling, I., ...278T
Kleckner, Nancy, ...422T
Klein, Joël, ... 481W*
Klimes, A., ...525F*
Klis, F. M.,.....102F
Klosterman,
Steven J.,..... 529W*
Kluge, J.,.....119T*
Knabe, Nicole,103W*, 643W
Knox, B. P.,.....449T
Knudson, A., ...114F*
Ko, Yo-Han, ...211W,
.270F*, 285F, 287T*, 288F*
Kobayashi, T., ...96F*
Kobe, B.,.....19W
Kodama, M., ...344T
Koehler, A. M.,.....9F*
Kogler, H.,.....30F
Kohler, A.,..... 264F*, 469W
Kohler, Amanda,.....480F
Ko¨hler, JR, ... 463W
Koike, Steven T.,..... 529W
Kojima, Y.,..... 150F
Kolecka, Anna, ... 617T
Kollath-Leiß, K., ... 153F
Komatsu, K.,..... 344T
Kondo, Hideki,..... 438F
Konings, Michiel,..... 224T
Konte, T.,..... 44T
Koolaard, J., ... 137T
Kornitzer, Daniel, ...535W*
Korotkin, Hailee B., ... 479T
Korzeniewski, F.,..... 308T
Koskela, Salla, ... 632T
Kothe, E., ... 138F, 217W*
Kothe, Erika,....118W, 182T
Kowalczyk, J. E.,.....410T*
Kowalski, C. H.,.....459F*
Kozubowski, L.,108F, 109W
Kramer, M., ... 387F*, 511W
Kramer, Martin, ... 268W
Kramer, P., ... 110T
Krause, K., ...138F, 217W
Kreil, David, ... 581T
Krevet, S., ...177F, 435F
Krishnan, Parvathy,.... 486F
Krizsan, K., ... 225F
Krizsán, K.,..... 114F, 228F
Ku, J., ... 319W
Kubicek, Christian P.,... 78F
Kubicek, C. P., ... 161T
Kück, U., 68T, 120F, 175W,
..... 177F*, 433W*, 435F
Kueck, U.,..... 119T
Kües, Ursula,..126F, 135F*,
..... 195F*, 196W*, 272T*
Kuijper, E.,..... 583W
Kuivanen, J., ... 67W
Kumakura, N., ... 473T
Kumar, A., ...328W, 599T
Kumar, D., ...381F, 442W
Kumar, J.,..... 364W
Kumar, Jyothi, ...240F*
Kumar, R., ... 407T
Kunene, N., ... 619W
Künzler, M., ... 582F
Künzler, Markus, ... 555F
Kuo, A., ...223W, 264F,
..... 308T*, 315F*
Kuo, Alan,..... 250W
Kuo, Hsiao-Che,..... 176T
Kuo, R. C.,..... 451W
kuppireddy, V.S,.....541W*
Kuroki, M., ...142W*
Kurre, R.,..... 113T
Kurtzman, Cletus P.,.. 248T
Kusch, H.,..... 582F
Kusumoto, Ken-Ichi,.... 52W
Kuuskeri, Jaana, ... 74T
Kwan, H.,..... 205W,
..... 645F
Kwan, H. S., . 255F*, 256W,
412W
Kwan, H.S, ... 388W
Kwan, H.-S., ... 441F

L

Labadie, K., ... 506T
Labbé, Jessy, ...250W*,
..... 339F, 634W*
Labbe, Jessy, ... 354F
Labeda, David, ... 353T
LaButti, K.,..... 114F
LaButti, Kurt, ...207F, 562W
Laine, Pia, ... 74T
Lalucque, H., ... 173T, 561F
Lalucque, Herve, ...127W*
Lam, W.C, ... 377T
Lam, Y. C., ... 256W
Lamb, T., ... 379W
Lambert, K.,..... 392T
Lamont, Iain, ... 563T
Lamour, Kurt H.,..... 252F
Landesfeind, M., ... 582F
Landowski, C., ...67W
Landowski, Christopher, ...
..... 64W, 632T
Lane, Geoffrey, ...563T
Lane, T. E., ...474F
Lang, E. A. S.,. 403W, 524T
Lang, O., ... 648F, 649W
LAPALU, N., ...551T
Laraba, I.,..... 610W*
Larignon, P., ...241W
Larkan, N., ...220W
Larrondo, Luis,.....378F
Larsen, Thomas O,.....40W
Larsen, T. O.,.... 33F, 230T,
..... 277W, 278T
Lau, Y.T, ... 388W*
Lauder, Rebecca, ...576F
Lazar, N., ...498F
Lazar, Noureddine, ...495F
Lebar, M.,. 42F, 194T, 545T
Lebrun, M.-H.,.....519F
Lebrun, MH., ...241W
Lee, James, ...97W
Lee, Mi-Kyoung, ...361W
Lee, Mi Yeon,.....15F
Lee, M. Y., ...14T
Lee, S., ...333F
Lee, S. C.,..... 402F
Lee, Song Hee,.....568W
Lee, W.-S.,..... 482T
Lee, Y. H.,79W, 260T, 534F
Lee, Yong-Hwan,.....285F,
..... 533T
Leesutthiphonchai,
Wiphawee,..... 651F*
Le Floch, G., ...212T
Legrand, Melanie,.....197T
Lemcke, R.,.....546F
Le Moigne, T.,.....498F
Le Moigne, Théo,.....495F
Lenassi, M., ...44T
Lentz, K.,.....433W
Leon-Ramirez, C., ... 389T*
León-Ramírez, C., ...187W
Leslie, John F., ... 235W*
Levesque, C. A., ...242T
Levy, M., ... 462F*
Lewis, L.,.....553W
Lewis, Z., ...450F
Leyva-González, M.,....55W
Li, A.,..... 452T*
Li, B.,.....442W
Li, F.,.....493W
Li, G., ... 181W*
Li, Guofen, ...43W
Li, J., ...511W
LI, JIMING,..... 522F*
Li, Jingtao, ... 47T*
Li, Lei, ...255F
Li, Linhan, ...496W
Li, R.,..... 385W, 596T
Li, Wan-Chen, 290T, 307W*
Li, Y.,..... 394W, 492F
Li, Yang,.....434T
Li, Z.,.....578T
Liang, L.,.....504F
Liao, H.-L., ...621F
Liao, Hui-Ling,.....479T
Liao, Sunny,.....339F
Lichius, A., ...140T
Lichius, Alexander, ...581T
Lim, F.,..... 6F*
Lim, F. Y., ...449T
Lim, S.,.....379W
Lim, You-Jin,..... 533T*
Limon, M. C., ...399F
Limón, M. Carmen, ...404T
Lin, C., .373W, 375F, 376W
Lin, J., ... 639F*
Lin, L., ...547W
Lin, S.,..... 247W*
Lin, X.,...384F, 639F, 640W
Lin, Xiaorong,..... 5T*, 383T
Lin, Yao-Cheng,.....302T
Lin, Zeran,.....575T
Linconl, S., ...364W
Lind, A., ... 6F
Lind, Abigail,..... 1W, 281T*
Linglin, J., ...506T
Link, T.,.....499W
Linne, U., ... 45F
Lipke, P., ...163W
Lipzen, A., ...114F, 630F
Lipzen, Anna, ..145W, 207F
Lisong, M., ...220W
Little, Alan,..... 21F
Littlejohn, G. R.,..... 144F,
..... 259W, 487W
Liu, H., ... 223W, 394W
Liu, Huiquan, ...397W
Liu, Huquan, ...434T*
Liu, Lin, ...409W
Liu, NN, ...463W
Liu, X., ... 341T
Liu, Yi,.....409W*, 437T*
Liu, Z.,..... 579F
Liversage, J., ...258F*
Liwanag, April J. M.,....11T*
Lo, Ko-Yun, ... 290T
Lockhart, S., ...625W
Lodge, J.K, ... 377T
Loesgen, S., ... 570F
Lohmar, Jessica M., ... 362T
Lonergan, Z., ...253W
Looney, Brian, .250W, 339F
Loparev, V., ...625W
Lopes, L.,..... 403W*
Lopes, Mariana R., ... 248T
Lopez, J.-B., ... 546F
López-Arredondo, D.,...55W
López Díaz, C.,..... 611T
Lopez-Diaz, C.,.....612F*
López-Fernández, L., 577W
Lopez-Ruiz, Francisco,636F
Lorang, Jenny,.....548T*
Lord, K., ... 83T
Lorenz, Michael, ...464T*
Loros, J.,..... 152T
Loros, Jennifer,380T, 415W
Loros, Jennifer J.,..... 104T
Lorrain, C.,.....500T*
Lorrain, Cecile, ... 207F
Lou, Y., ...129F*
Lowe, Rohan, ...21F*
Lu, L.,.....358W
Lu, Ling,..... 85W
Lu, X., ...205W
Lubbers, R. J. M., ... 410T
Ludwig, N., ...504F*
Luebeck, Mette, ... 75F
Luebeck, Peter, ...75F*
Lueschow, Shiloh, ... 353T
Lugones, Luis, ... 128T
Luis, J. M., ...194T*
Lundell, Taina,..... 74T
Luo, S. Q., ...547W
Luo, X., ...442W, 449T
Luque Fobelo,
Eva M., ...148W
Luria, N., ...381F
Lutomski, M., ...175W
Lutz, Matthias, ... 326T
Lutzoni, F.,..... 315F
Lutzoni, Francois, ... 479T
Lv, Q. Z.,.....637W
Lyer, Revel, ... 332T
Lynn, J.,.....628W

M

Ma, L.,..... 273F
Ma, Lay-Sun, ...564F*
Ma, L.-J.,232W, 608T, 611T
Ma, L.J,..... 612F
Ma, W., ...492F
Mabey, J.,..... 204F
Mace, W.J, ... 36F
Mach, Robert,..... 440T
Machado, A. K.,.....482T*
Machado, Marcos,..... 60F,

..... 219F
Mach-Aigner, Astrid, .. 440T
Mach-Aigner, Astrid R., 78F
Machida, Masayuki, .280W*
Mack, B., ... 42F, 545T
MacLean, D.,..... 319W
Maclean, P., ... 137T
Maclean, Paul, 508W, 563T
MacPherson, K. A.,... 648F*
Maeda, H., ... 96F
Maeda, Hiroshi,..... 52W
Magnuson, J., .315F, 451W
Magnuson, J. K.,51F, 628W
Magnuson, Jon, ... 50T, 77T
Magnuson, Jon K., ... 66F
Maheswari, Uma,..... 266T
Maia, C.,.....334W, 335T
Maintz, J.,..... 313W
Maio, Yi, ... 467T
Mair, Wesley, ...636F*
Majcherczyk, Andrzej, 272T
Majoor, Eline, ... 366F
Majumdar, R., ...42F, 545T*
Mäkelä, M., ... 331W
Mäkelä, M. R.,..... 56T, 57F
Mäkinen, Mari, ... 74T*
Malagnac, F., ... 174F
Malagnac, Fabienne, 127W
Malassezia Genome
Consortium,..... 617T
Malavazi, Iran,.....457W*
Malik, Harmit S.,..... 622W
Malone, T., ... 386T
Mandel, M. A.,.....553W*
Manitchotpisit, P.,.....386T,
..... 616W
Mansouri, S.,..... 57F
Manspeaker, R.,..... 392T
Manzanilla, Victor, ...59T,
..... 121W
Mao, Leidong, ... 424W
Marais, C.,.....498F*
MARCEL, T. C., ...551T*
Marcel, Thierry, ... 552F
Marcos, A. T.,..... 8T, 630F
Marcos, J. F., ...8T
Marek, S.,..... 254T
Margeot, A., ...73W, 80T
Marian, Ioana, ... 128T
Marin, J., ... 630F
Marinovic, M.,.....56T*
Mark, K.,..... 456F
Märker, R., ... 175W
Marroquin-Guzman,
Margarita,..... 461T
Marschall, R.,...38T*, 140T,
..... 160W*
Marten, Mark,..... 145W
Marten, M. R., ... 364W
Martin, F.,.....263T, 264F,
..... 265W, 469W
Martin, Francis, ... 250W
Martinez, A.,..... 102F
Martínez-del-Pozo, A.,133W
Martínez-García, P., .. 573F
Martínez Rocha, A.-L.,519F
Martinez-Rossi, Nilce, 426F
Martinez-Rossi, N. M.,401T,
..... 403W, 524T
Martinez-Soto, D.,.....188T*
Martínez-Soto, D.,..... 389T
Martins, Maíra,..... 426F
Martin Urdiroz, M., ... 487W
Martin-Urdiroz, M., ... 144F
Marton, K., ... 491T
Marton, Timea,.....197T*
Marty, Amber, ... 117F
Maryush, Z., ... 597F
Masai, K., ... 113T
Maskarinec, S. A.,..... 467T
Masonjones, S., ... 320T
Masuda, R.,..... 125T
Matana, N. G.,..... 442W
Mateos, Pablo Acera, 425T
Matheny, P. Brandon, 479T
Matson, M. E. H., ... 291F*
Matsui, Makoto, ...280W
Matsushima, K.,.....297F
Mattern, D. J., ...627F
Mattupalli,
Chakradhar,.....620T
Maufrais, Corinne, ...197T
Mayxay, M., ...244W
Mazzoni, Camila, ...103W
McCluskey, K.,.....199W
McCormack, E., ...109W
McCormick, S., ...610W
McCormick, Susan P.,391W
McDonald, B. A.,.....293T
McDonald, Bruce,.....486F,
..... 552F
McDonald,
Megan C., ... 427W, 494T*
McDonnell, E., ...26T
McGrann, G. R. D.,.....39F
McGreal, Brogan, ...509T
McKean, Lauren, ...356T
McNeal, K., ...253W
McRae*, A., ... 14T*
McRae, Amanda,.....15F
McTaggart, A., ...624F
Mead, O. L.,..... 28W*
Mehalow, Adrienne,.. 380T*
Mehreteab, Alexander, 59T,
.....121W
Meidl, Peter, ...634W
Meijer, Harold, ... 526W*
Meile, Lukas, ...486F
Meis, J., ...583W
Mela, Alexander,..... 162F*
Melangath, G.,.....407T
Melanson, Jeremy, ...518T
Melchers, W.,.....583W
Melchers, W. J. G.,.....584T
Mellado, Laura,..... 362T*
Mello-de-Sousa,
Thiago,.....78F, 440T
Mendes, T. D.,.....58W
Mendoza-Espinoza, J.,.71T
Mendoza-Mendoza,
Artemio,.....564F
Meng, X., ...358W
Meng, Yunfang, ...383T
Mentges, M.,... 519F*, 570F
Mercier, Alex,.....538W
Mesarich, Carl, 245T, 508W
Mesarich, C. H.,.....502W
Metzger, Alex,.....356T
Meunier, C., ... 597F*
Meya, David,.....213F
Meyer, M.,.....506T
Meyer, Michel, ...484W
Meyer, Vera, ...629T
Meyers, B., ...226W
Miao, Z.,.....452T
Miazzi, M., ...180F
Michel, R.,.....296T
Michelmore, Richard, ...
.....529W
Mideros, Santiago,.....575T
Midorikawa, G. E. O., ..58W
Miguel Rojas, Cristina,
..... 274W*, 300F*
MILAZZO, Joëlle,.....614T
Milgate, Andrew,.....494T
Miller, M., ... 303F*
Miller, T. A., ... 36F*
Min, B.,.....214W, 262W,
..... 292W*, 306F*
Minh, C. N.,..... 334W, 335T
Minocha, S.,.....545T
Miralles Durán, A., ... 428T*
Mirmiran, A., ... 269T*
Mirsiaghi, Mona, ...66F
Misar, Christopher, ...569T
Misztal, Pawel,.....354F
Mitchell, A. P.,..... 62T*
Mitchell, T., ...259W
Mitema, Alfred, ... 332T*
Mitra, Chandrani, ...650T
Mitrousia, Georgia,... 539T*
Mituyama, T.,.....297F
Miyazawa, K.,.....86T*
Miyuki, U.,..... 12F
Mizutani, O., ... 368T
Moeller, Mareike,..... 311T
Moerschbacher, B.,.... 470T
Moffat, Caroline,..... 501F
Mogg, C.,.....130W, 269T
Mohamed Nor,
Nik Mohd Izham, ... 235W
Mojzita, D., ... 67W
Mojzita, Dominik,..... 632T
Molnár, Á.P., ... 10W
Molo, M. S.,.....618F*
Momany, M., ...87F, 455T
Momany, Michelle, ... 162F
Mondo, S.,..... 308T
Mondo, S. J., 347T*, 451W*
Mondo, Stephen,..... 562W
Mondo, Stephen J.,.... 207F
MONTIBUS, M., ... 398T
Moody, Benjamin, ...508W
Moody, S. C., ... 215T
Moolhuijzen, Paula,.... 510F
Moore, G. G., ... 194T
Moore, R.,..... 56T
Moore, Shauna,..... 70W
Morais, David, ...538W
Moran, G., ... 147F
Mora Sala, B., ... 335T
Morel-Rouhier, M., ...29T*
Morel-Rouhier,
Mélanie,..... 13W
Moreno-Sanchez,
Ismael,..... 567F
Moreti, A.,..... 610W
Moretti, Antonio, ...391W
Moretti, Marino, ... 564F
Moricca, S., ... 335T
Morin, E., ...264F, 265W
Morin, Emmanuelle, ... 207F
Morrison, E. M., ... 99F
Mortensen, U. H., ... 33F,
..... 277W, 278T, 627F
Mortensen, U. M.,..... 63F
Mosbach, A., ...16W*
Mosca, S., ...334W, 335T
Moscou, M.,.....493W
Moseley, M. A., ... 357F
Mosher, Anthony, ... 240F
Mouriño-Pérez, R., ... 89T
Mouriño-Pérez, Rosa.,156F
Mouriño-Pérez, R.R, . 154W
Mouriño.Pérez, R. R.,159F*
Mousaviderazmahalleh,
Mahsa,..... 510F
Moye-Rowley, Scott, .. 359T
Muddiman, David, ... 261F
Mueller, K., ...333F*
Mueller, O.,..... 315F
Mulholland, Nicholas,... 69F
Müller, C., ... 360F
Müller-Esparza,
Hanna,..... 378F
Mullins, Jonathan, ... 636F
Münch, Karin, ... 564F
Munoz, J. F., ...465F*
Munro, Carol, ...17T*
Münsterkötter, M., ... 519F
Muraguchi, H.,.....125T*
Murillo-Corona, I.,.... 154W,
..... 159F
Murithi, H. M.,..... 319W
Murua, P.,..... 324F
Mutiga, S., ...259W
Mwongera, D., ... 259W
Myint, Anthony, ... 418W

N

Nagabhyru, Padmaja, 528F
Nagaraja, V., ... 374T
Nagayama, M.,..... 54F
Nagoshi, T.,..... 125T
Nagy, I.,..... 228F
Nagy, L.,.....231F
Nagy, L. G.,..... 114F, 225F,
.....228F
Nakajima, M.,.....143T
Naquin, D.,..... 73W
NAQVI, N. I., ... 105F*
Nargang, Frank,.....419T
Narukawa, M.,. 142W, 143T
Narusaka, M., ...473T
Narusaka, Y., ...473T
Nasser, Lena, ... 535W
Nath, R.,..... 580W
Natvig, D. O., .. 329T, 337W
Nava, Jacob,..... 121W
Navarrete, K.,.....209T
Navarro-Mendoza, M. I.,
.....573F, 577W*
Navarro-Rodríguez,
P.,..... 577W
Naz, F., ...348F
Nazir, Natasha, ...198F
Ndikumana, S., ... 265W
Nedelec, F., ... 112W
Nel, W. J., ... 605T*
Nelliat, A.,.....596T
Nelson, Berlin, ...569T
Nelson, Rebecca, ...575T
Németh, Z., ... 10W
Nesemann, K., ...582F
Nett, M., ...32T
Newman, D. S.,.....327F
Newton, A., ...546F
Nguyen, H., ... 242T, 613W
Nguyen, N.,.....621F
Nguyen, T. Q., ...525F
Nicely, N. L., ...357F
Nicolás, F. E., .573F, 577W
Nielsen, Jens, ... 40W
Nielsen, Jens
Christian,..... 40W
Nielsen, K. F., ... 30F, 278T
Nielsen, K. F. N.,..... 601W
Nielsen, Kirsten,.....213F
Nielsen, Kristian Fog,.. 40W
Nielsen, M. L.,.....33F
Nieuwoudt, A., ... 590T*
Niño-Sánchez,
Jonathan, ... 523W
Niu, M.,..... 82W*
Noble, Luke,.....146T
Nødvig, C. D., ...63F
Nødvig, C. S., ... 33F, 627F
Noel, J.,..... 265W
Nong, W.,..... 205W
Nong, Wenyan, ...255F
Nong, W. Y., ... 256W
Noorifar, N.,..... 470T*
Nordmann, D., ...113T
Nordzieke, Steffen, ... 404T*
Noronha, E. F., ... 58W
Northen, T.,.....14T
Northen, Trent,.....15F
Northup, Diana,.....353T
Norton, TA,.....84F
Nousiainen, P., ...56T
Novák, L.,..... 10W
Nowrousian, M.,..... 175W
Nowrousian, Minou,....588F
Nozaka, A., ... 143T*
Nsibo, DL., ...619W*
Nuckolls, Nicole L., ... 622W
Nuñez-Moreno,
Fernando,.....156F
Nybo, J. L.,.....230T*, 243F,
..... 277W, 278T
Nygård, Yvonne,..... 40W

O

Oakley, Berl R.,..... 70W
Oakley, C. Elizabeth, .. 70W
Obanor, F.,..... 610W
Obar, J. J., ...459F
Obar, Josh, ...3F
Obhof, T.,.....192F

Oda, K., ...368T*
O'Donnell, K., . 284T, 610W
O'Donnell, Kerry, ...249F
Oh, Baek Rock, ...25W
Oh, Yeonyee, ...261F*
Ohba-Tanaka, A., ...369F
Ohkura, Mana,.....309F*
Ohm, R., ...292W
Ohm, R. A.,.....114F
Ohm, Robin, ..128T*, 224T*
Ohyama, Akira,.....280W
Oiwa, T., ...96F
Ojiambo, P. S., ...194T
Okamoto, Ayako,.....52W
Okauchi, K.,.....142W
Okoth, Sheila,.....332T
Okuda, M.,.....368T
Olarte, R. A.,.....345F*
Oliveira, Tiago, ...219F
Oliveira, V. M.,..401T, 524T
Oliveira Garcia, E., ...485T*
Oliver, Richard,..... 501F, ...510F, 636F
Oliver, R. P., ...293T
Olivera, Pablo,.....615F
Olivier, Nicholas, ...302T
Ollivier, B., ...498F, 506T
Olmedo-Monfil, Vianey,.....557T*
Olmez, F.,.....482T
O'Malley, M. A., ...451W
O'Mara, Sean P.,..... 46W*
O'Meara, Teresa,..... 466W*
Omidvar, V.,.... 303F, 493W
Oostlander, A., ...139W
Orbach, Marc,.....309F
Orbach, M. J.,.....553W
Ordonez, N., ...483F
Ornelas, Roberto, ...529W
Orr, G.,.....331W
Ory, Jeramia, ...239T*
Oses-Ruiz, M.,144F*, 487W
Osiewacz, H. D.,..... 110T, ... 116T, 175W
Osmani, A.,.....111F
Osmani, S.,.....111F
Ouédraogo, I., ...259W
Ouedraogo, Jean Paul,..... 49W*
Outram, M.A, ...19W
Ozturk, A.,.....137T*

P

Paege, Norman, ...629T
Paietta, J. V., ... 406W*
Pakula, Tiina,.....411F
Palkova, Z.,.....168F
PALMA-GUERRERO, J.,.....551T
Palma-Guerrero, Javier, ...552F*
Palmer, J. M., ...449T
Pan, Hongyu,.....47T
Panaccione, Daniel, ... 7W*
Pang, Guan, ...115W
Panisko, E., ... 22W, 646W
Pape, L., ...141F
Pardo, A.,.....469W
Park, C. H., ...79W
Park, H.,262W, 292W, 306F
Park, R.,.....303F, 305T
Park, R. F., ... 257T, 493W
Park, Seung-Moon, ... 270F
Park, S. Y., ...79W
Parker, Jennifer, ...261F
Parker, Josie, ...359T
Parker, R., ...430W
Parra-Rivero, O., ...399F*
Parsania, C.,.....527T
Partida-Hanon, A.,.....133W
Pasa-Tolic, Ljiljana, ...50T
Patel, Pryank, ...539T
Pathiraja, D.,.262W*, 292W
Paulin, Lars,.....74T
Payne, G. A.,..... 194T
Pearson, Michael, ... 97W
Pedersoli, Wellington, 426F
Pedro, Helder, .201F, 266T*
Pelkmans, Jordi, ... 128T
Pena-Centeno, T.,..... 582F
Peng, Hui, ... 304W
Peng, M.,..... 453F
Pennachio, C., ... 114F
Pennington, Helen, ... 496W
Pensec, F., ... 212T
Peres, N. T. A.,403W, 524T*
Perez, Eli,..... 507F
Pérez-Arques, C., ...573F*, ... 577W
Perez Perez, W.,71T*, 443T*
Perez-Rodriguez, F.,. 191T*
Pérez-Sierra, A.,334W, 335T
Perfect, J. R., ... 467T
Perlin, D., ... 625W
Perlin, M., ... 226W
Perlin, M.H, ... 541W
Perlin, Michael H., ... 447F
Perotto, S., ... 263T
Perrot, Thomas, ... 29T
Perry, B. A., ... 327F
Petersen, L. M.,..... 33F
Petersen, T. I., ... 33F
Petit, Y.,..... 498F
Petit-Houdenot, Yohann,.....495F*
Petre, Benjamin, ... 500T
Petroswky, Jérémy,.... 500T
Pfannenstiel, B. T.,....449T*
Phan, Huyen, ... 501F
Philippsen, P., ...102F*, ...112W*, 113T*
Phule, P.,..... 328W
Pierce, Brett, ... 23T, 134T
Pinel, Dominic, ... 238W
Pinter, N., ...192F, 446T*
Piombo, E.,.....234F*
Pirayre, A., ...80T*
Pires, J. C.,..... 263T
Plemenitas, A., ... 44T*
Plett, J. M., ... 264F
Plissonneau, C., ... 506T
Plissonneau, Clémence, ... 486F, 495F, ...552F, 592W
Plitzko, B., ... 570F
Plummer, K., ... 530T
Plummer, Kim,.. 245T, 509T
Plummer, Kim M., ... 620T
Poetschner, J., ...138F*
Pohl, Carsten, ... 40W
Pokharel, Rasesh,..... 643W
Politi, A. Z.,..... 112W
PONTS, N., ...398T*
Porcel, Betina,..... 588F
Porras-Alfaro, Andrea, 353T
Porras-Troncoso, Daniela, ... 557T
Potgieter, L.,..... 222F
Powell, G.,..... 215T
Powell, J.,..... 30F
Powers, R. A., ...301W*
Prahl, Jan-Philip,..... 77T
Prasanna N., A.,..... 225F, 228F*
Pray, Todd,..... 66F
Presley, G., ... 22W
Presley, G. N.,31W*, 646W*
Priest, Shelby,382W*, 589W
Pringle, A.,..... 41T
Pringle, Anne,..... 604W
Proctor, R. H., . 279F, 284T, 610W
Proctor, Robert H., ... 391W
Progar, V., ... 491T
Prooyen, N. V.,..... 543F
Prusky, D.,..... 381F*, 442W
Przydacz, Michal, ... 496W
Przylucka, Agnes, ... 78F
Przylucka, Agnieszka,115W
Punekar, Narayan S.,... 95T
Purvine, S., ...331W
Pyne, R., ...273F

Q

Qi, M., ... 499W*
Qin, L., ... 444F*
QU, Z., ...105F
Quandt, A.,.....296T*, 338T*
Quandt, Alisha, ...294F
Quandt, C. A.,.....200T

R

Raaymakers, Tom, ...542T
Rabinovich, R., ...65T
Radebner, Theresa,....581T
Radutoiu, S.,.....546F
Rafudeen, Suhail, ...332T
Rahimi, Mohammad, .115W
Rahmann, Sven,.....626T
Rai, M. N.,..... 527T*
Rai, R.,.....527T
Rajasekaran, K., ...545T
Raley, C., ...303F
Ram, A.,..... 185T, 558F
Ram, A. F. J.,.....453F
Ramegowda, Y., ...267F
Ramírez-Valdespino, Claudia,.....557T
Ramšak, B., ...120F
Rantasalo, A., ... 67W*
Rantasalo, Anssi,.....632T
Rao, M., ...341T
Rao, R. P., ...465F
Rash, B., ...360F
Rassinger, Alice,..... 440T*
Rath, Manisha,.....514W
Rathjen, J.,..... 303F, 305T
Raudaskoski, Marjatta,..... 118W*
Rauf, A.,.....348F
RAVEL, Sebastien,.....614T
Ravishankar, A., ...253W
Read, N.,..... 83T, 140T
Read, N. D.,.....137T
Read, Nick, ...176T
Reed, T.,.....386T
Rees, H.,..... 334W, 335T
Reese, Amy, ... 356T*
Rego, C.,.....241W
Rehner, Stephen A., ...276F
Reilley, M.,.....444F
Reilly, M. C., ... 628W*
Reimhult, Erik, ...78F
Reis, L.,.....456F
Reißmann, S.,.....504F
Reissmann, Stefanie, .564F
Ren, Di,..... 202W*
Rep, M., ... 483F, 607W
Rep, Martijn, ... 295W, ...508W, 521T, 522F
Requena, N.,.....489F
Rey, P., ...241W
Reyes-Chin-Wo, Sebastian,.....529W
Reynolds, R., ...474F
Rhoades, N.,..386T, 616W*
Ribeiro, L. F. C., ... 364W*
Richards, J.,.....218T
Richardson, Kirsten, ...447F
Richardson, Malcolm, .318F
Rico Ramirez, A.,..... 151W*
Rico-Ruiz, G., ...55W
Ridenour, J., ... 267F, 282F
Ridout, Christopher,....539T
Ries, Laure, ...145W
RIEUX, Adrien, ...614T
Rikkerink, E.,.....530T
Rikkerink, Erik,.....509T
Riley, R., ... 114F, 230T, ... 292W, 308T
Riley, Robert, ... 248T*
Ringelberg, C.,.....152T
Riquelme, M., 151W, 322W
Riquelme, R.,.....340W
Rischatsch, R., ...102F
Risulainen, Netta,..... 74T
Robbertse, B., ...317T*
Robert, G.,..... 241W*
Robertson, Suzanne, . 261F
Robinson, A. J.,329T*, 337W
Robinson, E.,.....331W
Robison, Sara,..... 323T
Rocha, Marina Campos, ...457W
Rocha, Raquel,.....24F*
Rocha-Olivares, A., ...322W
Rodenburg, Alexander Y.,.....178W
Rodnay, Sormani, ... 29T
Rodrigues, Carol, ... 219F
Rodrigues, Fernando, 281T
Rodriguez, Carmen, .. 424W
Rodriguez, Lauren,.. 418W*
Rodriguez, Rosa, ... 550W*
Rodriguez de la Vega, R., ...229W
Rojas, A.,..... 621F
Rojas, J.Alejandro., ...339F*, 342F*
Rokas, A.,..... 6F, 608T
Rokas, Antonis, ...1W, 281T
Rollins, Jeffrey A., ... 47T
Romero-Olivares, A. L., ...322W
Romich, M., ... 488T
Rong-Mullins, X.,..... 253W
Roop, Jeremy, ... 600F
Roos, David,..... 203T
Ropars, J.,..... 147F
Rose, Eric,.....121W
Rossi, A.,401T, 403W, 524T
Rossi, Antonio, ... 426F
Rosso, MN,..... 469W
Rothschild-Mancinelli, K., ... 633F
Rotich, F.,.....259W
Rottschaefer, S. E., ...493W
Rouxel, T., ...498F, 506T
Rouxel, Thierry,.....484W, ... 495F, 592W*
Rovenich, H.,.....511W
Rowland, Owen,..... 395T
Roy, Udita,.....535W
Rubin, A.,.....109W
Rudd, Jason, ...576F*
Rudd, J. J., ...503T
Ruger-Herreros, Macarena, ...404T
Ruiz-Herrera, J.,.....187W, 188T, 191T, 193W, 389T
Ruiz-Vázquez, R., ...577W
Ruprich-Robert, G., ... 171F, ... 173T*
Rutherford, Kim, ... 201F
Ruytinx, J.,..... 613W*
Rybak, Kasia, ...501F
Ryder, L. S., ...487W
Ryota, S.,.....12F*
Ryu, S.,.....199W

S

Saarinen, Juhani, ...64W
Saijo, Y., ...472W
Saitoh, K.,.....142W
Saitou, R.,.....368T
Sakthikumar, S., ... 257T
Sakulkoo, W., ... 144F
Salamov, A., ...200T, 230T, . 232W*, 277W, 278T, 308T
Salamov, Asaf, ... 246F, ... 248T, 294F
Sale, Kenneth,..... 66F
Saloheimo, Markku, ... 64W*, 411F
Salvucci, Anthony,..... 480F
Samarajeewa, D.,..... 616W

Sammer, D., ... 217W
Sanches, Pablo, ... 426F
Sanches, P. R., ... 401T
Sanchez, J.N, ... 483F
Sánchez-Arreguín, J., 389T
Sanchez-Arreguin, J., 187W*
Sanchez-Garcia, M., . 231F*
Sánchez-Romero, MA, 428T
Sánchez-Vallet, Andrea, 486F*
Sanchis, M., ... 577W
Sandrock, B., ... 45F*
Sanfuentes
von Stowasser,
E., ... 334W, 335T
Sang, H., ...165F, 454W*
San Segundo, Pedro, .. 98T
Santamaría, Rodrigo, ... 98T
Santander, Daniela, ... 98T
Santos, E. S., ... 4W
Santos, Paulo, ... 219F*
Sanyal, K., ... 374T
Sanyal, Kaustuv, ... 588F
Sardar, P., ... 153F
Sasse, C., .88W, 92T, 630F
Sato, A., ... 297F*
Sauer, Ursula, ... 43W
Saunders, D. G. O., .. 313W
Saunders, Diane, ... 500T
Saupe, S. J., ... 603F
Savenkov, E. I., ... 497T
Savoian, M. S., ... 470T
Savoury, M., ... 215T
Scalliet, G., ... 16W
Scanu, B., ...334W, 335T
Scazzocchio, C., ... 585F
Schackwitz, W., 212T, 630F
Schadt, Chris, ... 354F
Schadt, Christopher, .. 591F
Schaefer, W., ..519F, 520W
Schäfer, W., ... 570F
Schakwitz, W., ... 114F
Schäpe, Paul, ... 629T
Schardl,
Christopher L., ... 528F*
Scharfenstein, L., ... 385W
Schena, L., ...334W, 335T
Schilling, J., ...22W*, 206T
Schilling, J. S., .31W, 646W
Schinko, T., ...8T
Schipper, K., ...76W*, 504F
Schirawski, J., ... 488T
Schmid, Marjorie, ... 93F
Schmitt, Karl, ... 654F
Schmitz, H. P., ... 113T
Schmitz, Lara, ... 476T
Schmoll, M., ... 181W
Schmoll,
Monika, ...43W*, 432F
Schneider, K., ... 192F
Schneiderman,
Danielle, ... 518T
Schoch, C., ... 317T
Schoer, Jon, ... 654F
Schoina, Charikleia, .. 526W
Schoonbeek,
Henk-Jan, ... 539T
Schotanus, Klaas, ... 311T*
Schoustra, S., ... 583W
Schoustra, S. E., ... 584T
Schroeckh, V., ... 408F
Schroeder, F., ... 6F, 580W
Schuerg, Timo, ... 77T*
Schuhmacher, J., ... 566T
Schuhmacher, Rainer, 432F
Schultzhaus, Z., . 90F, 159F
Schultzhaus, Z. S., ... 89T*
Schulze-Lefert, P., ... 472W
Schumacher, J., ... 141F
Schumacher, Julia, ... 372F*
Schumann,
M. R., ... 139W*, 157W
Schurack, S., ... 540F
Schüssler, Jan A., ... 643W
Schuster, Andre, ... 432F
Schütz, Gerhard, ... 581T
Schwarz, E., ...580W
Schwessinger,
B., ... 303F, 305T*
Schwessinger,
Benjamin, ... 480F
Scott, B., . 36F, 185T, 470T,
..... 502W, 558F, 561F
Scott, Barry, ...127W
Scott, Eileen, ...312F
Scott, S., ...152T
Sedlacek, T., ...158T
See, D., ...505W
See, Pao Theen, ...501F
Seiboth, B., ...289W
Seidl, M. F., ... 387F, 511W
Seidl, Michael F., ...268W,
.....593T
Seiler, S., ...561F
Seitz, Nicholas, ...121W
Sellam, A., ... 210F, 463W
Sen, T., ...407T
Senchenkov, A., ...621F
Seress, D., ... 334W, 335T
Serrano, A., ... 140T*
Serrano, S., ...133W
Sertour, N., ...147F
Sertour, Natacha, ...197T
SGD Project, ...648F
Shah, D., ...536T, 537F
Shahi, Shermineh, ...522F
Shalaby, Samer, ...184W
Shanmugasundram,
Achchuthan, ...203T
Sharma, S., ...282F
Sharma Poudel, R., .. 218T*
Shaw, B., ...90F
Shaw, B. D., ...89T, 159F
Shaw, B.D, ...154W
Shay, J. E., ... 327F*
Shelest, E., ...408F
Shen, Qirong, ...115W
Shen, W. C., ... 352W*
Sheng, G., ...492F
Sheppard, T., ...649W
Sherlock, Gavin, ...197T
Shibata, Takashi, ...280W
Shiga, Y., ...142W
Shi-Kunne, X., ...511W
Shimamoto, K., ...368T
Shin, H., ...306F
Shintani, T., ... 53T, 367W,
369F, 370W
Shintani, Takahiro, ...371T
Shirasu, K., ...122T, 473T
Shirley, Neil, ...21F
Shiu, P., ...616W
Shivas, R., ...624F
Shor, E., ... 625W*
Shrestha, S., ...218T
Shrivastava, J., ...291F
Shubitz, L. F., ...553W
Shukla, N., ... 111F*
Shwab, E. K., ... 357F*
Siciliano, I., ...234F
Sickler, C., ...545T
Siguenza, S., ...229W
Sil, A., ...414F, 417F,
..... 490W, 543F
Sil, Anita, ... 418W, 550W
Silar, P., ... 73W, 171F,
.....173T, 561F
Silar, Philippe, 127W, 538W
Sillo, F., ...594F
Silva, A. C., ...4W
Silva, A. S., ...58W
Silva, L., ...14T
Silva, Leslie, ...15F
Silva, Lilian Pereira, ...457W
Silverstein, K., ...206T
Simaan, Hiba, ...184W
Simmons, B., ...308T
Simmons, B. A., ...51F
Simmons, Blake, ...50T
Simmons, Blake A., ... 66F
Simmons, C., ...108F
Simon, Adeline, ...372F
Simon, J., ... 273F
Simpfendorfer, Steven, 494T
Simpson, Thomas J., ... 69F
Singan, V., ... 264F
Singer, Steven, ... 77T
Singer, Steven W., ... 66F
Singh, D., ... 493W
Singh, R., ... 618F
Singh, Seema, ... 66F
Sipilä, J., ... 56T
Sipos, G., ... 228F
Sjökvist, E., ...546F*
Skaar, I., ...341T*
Skerker, Jeffrey, 238W, 600F
Skerker, Jeffrey M., ... 66F
Sklenar, Jan, ... 500T
Slot, J., ... 216F
Slotkowski, R. A., ... 627F
Smith, D. S., ... 242T
Smith, Gerald R., ... 622W
Smith, Kristina, ...23T*
Smith, Kristina M., ... 311T
Smolander, Olli-Pekka, 74T
Snelders, E., .. 583W, 584T*
Snodgrass, Z.Allen., ..536T*
So, Kum-Kang, ...211W,
..... 270F, 285F*, 286W*,
..... 287T, 288F
Soanes, D. M., 259W, 487W
Sobczyk, M. K., ... 471F
Soberanes-Gutierrez,
C., ...193W*
Soderblom, E. J., ... 357F
Solanki, S., ... 218T
Soliman, S., ... 644T
Solomon, Peter S., ... 427W,
..... 494T
Solomon, P.S, ...19W
Solomon, P. S., ...28W
Sondergaard, T. E., ... 30F
Song, G., ... 649W
Song, H., ...314T*, 655W*
Song, Letian, ... 49W
Sørensen, J. L., ... 30F
Souciet, Jean-Luc, ... 588F
Soukup, A. A., ... 449T
Sousa, L. O., ... 4W
Spadaro, D., ... 234F
Spanu, Pietro, ...496W*
Sparker, J., ... 413T
Sparks, C. A., ... 503T
Spatafora, J. W., ... 207F,
..... 246F, 276F, 349W,
..... 451W, 559W
Specht, C.A, ... 377T
Spilak, Michal, ...325W
Spoel, S. H., ... 39F
Spraker, J. E., ... 449T
Sreenivasaprasad, S., 212T
Srinivasan, S., ... 407T
Srivastava, R., ...364W
S. Schmieder, Stefanie, 555F
Stajich, Jason, ... 249F
Stajich, J. E., ... 320T,
.....451W, 459F
Stanley, C. E., ... 582F
Stappler, Eva, ...43W, 432F
Starke, M., ... 582F
Steenkamp, Emma, ... 170T
Steenkamp, Emma T., 136W
Steenkamp, E. T., ...233T*,
.... 251T, 595W, 609F, 641T
Stefanko, Kayla, ... 124W
Steffens, S., ... 177F
Stehlik, T., ...189F*
Steinbach, W. J., ... 357F
Steinberg, Gero, ... 576F
Stempinski, P., ...109W*
Stenlid, Jan, ... 623T
Stergiopoulos,
Ioannis, ...480F*
Sternberg, P. W., ... 580W
Stillman, K., ... 223W
Stöckli, M., ... 582F
Stoffel, Kevin, ... 529W
Stoffels, P., ... 76W
Stotz, Henrik, ...539T
Stoychev, S. H., ...258F
Strasser, K., ...26T
Strauss, J., . 8T, 161T, 431T
Strittmatter, M., ...324F
Strope, P., ...317T
Studt, L., ...431T
Stukenbrock, E. H., ...599T
Stukenbrock,
Eva H., ... 311T, 477F
Sturm, Laura, ...81F
Su, Ching-Hua, ...290T
Subandiyah, S., ...483F
Subba, Shanta, ... 126F*
Subramaniam,
G., ...130W*, 269T
Subramaniam,
Rajagopal, ...395T
Sugawara, F., ...143T
Sugiyama, K., ...369F
Sukno, S. A., ...212T
Sukno, Serenella, ...131T
Sullivan, D., ...147F
Sulyok, M., ... 341T, 431T
Sulyok, Michael, ...432F
Sun, Congcong, ... 85W
Sun, G., ... 2T*
Sun, Jian, ...66F
Sun, P., ...393F
Sun, Shen, ... 106W
Sun, Sheng, ... 588F*
Sun, Xianyun, ...437T
Sun, Yu, ...606F
Sundin, George, ...300F
Sundstrom, Eric R., ...66F
Susca, Antonella, ... 391W
Suzuki, Nobuhiro, ... 438F*
Svedberg, J., ... 603F, 616W
Svedberg, Jesper, ... 598W,
..... 606F*
Swart, Velushka, ...302T
Sweany, R. R., ...310W*
Sylvain, Iman, ... 325W
Syme, R. A., ...293T
Syme, Robert, .. 501F, 510F
Szabo, Les J., ... 615F*
Szewczyk, E., ...633F

T

Tabor, G., ... 319W
Tae-Hong, L., ... 472W
Taguchi, H., ...143T
Takama, Mitsuru, ...371T
Takano, Y., ...473T
Takemoto, D., ...561F
Takeuchi, M., ...96F
Takeuchi, Michio, ... 52W
Takita, Marco, ... 60F, 219F
Takken, Frank, ...521T
Takyu, Yoko, ... 52W
Talbot, N. J., .. 144F, 259W,
..... 487W
Tami, O., ...12F
Tamietti, M. S., ...58W*
Tamura, S., ...344T
Tan, Kar-Chun, ... 501F*
Tan, K. C., ...293T
Tanabe, H., ...54F
Tanaka, A., ...561F
Tanaka, Chihiro, ...27F
Tanaka, M., ... 53T, 367W,
..... 369F, 370W*, 472W
Tanaka, Mizuki, ...371T
Tanaka, N., ...143T
Tanaka, S., ...150F
Tanaka, T., ...54F
Tang, B., ... 487W
Tang, Weihua, ... 516F*
Tanjore, Deepti, ...66F
Tannous, J., ... 442W*
Taranto, Adam P., ... 427W*
Tatham, C. T., ... 179T*
Tauber, J., ... 408F*
Taylor, J., ...341T

Taylor, John,..... 325W*
Tayyrov, Annageldi,...555F*
Teagarden, Jocelyn,... 239T
Tebbji, F.,.....463W
Teichert, I.,..... 120F,
.....175W*, 177F
Templeton, M., ...530T
Templeton, Matthew,..97W,
.....509T
Tenor, J. L., ...467T*
Teraoka, T., ...142W
TERAUCHI, Ryohei,... 614T
Terauchi, Y., ...54F
Terfehr, D., ...68T*
Terhem, Razak,.....178W
ter Horst, Anneliek,....295W
Testa, Alison,.....510F
Thakur, J.,.....330F*
Thammahong, Arsa,.....3F*
Thanh, L. T., ...244W
THARREAU, Didier, ... 614T
Theelen, Bart,..... 617T
Theobald, S., . 230T, 277W,
..... 278T*
THEOBALD, S.,.....243F
Thieme, K. G., ... 88W, 92T
Thieme, N.,..... 140T
Thieme, S., ... 88W, 92T*
THIERRY, Maud,..... 614T
Thomma, Bart,..... 268W,
..... 556W, 593T
Thomma, B. P. H. J.,. 387F,
..... 511W*
Thompson, D. A., ...465F
Thon, Michael,.....208W
Thon, M. R.,.....212T
Throckmorton, K.,..... 449T
Thu, P. Q., ... 334W, 335T
Thwaites, G. E.,.....244W
Tian, S., ...442W
Timpner, C.,..... 582F
Tirabassi, Dana, ...421W
Todd, R.,.....439W
Todd, R. B., ...452T
Toffaletti, D. L.,.....467T
Toji, Hiroki,.....371T
Toomajian,
Christopher,.....235W
Torres-Martínez, S., ..577W
Townsend,
Jeffrey P., ...124W*, 274W,
..... 300F
Townsend, J. P.,.....284T
Trail, F., ... 283W, 284T
Trail, Frances, ...124W,
..... 274W, 300F, 355W*
Trieu, P. H., ...244W
Trieu, T. A.,.....577W
Trinh, H. T.,.....553W
Trippel, Christine, ...564F
Tsai, C., ...269T
Tsai, H.-J., ...596T*
Tsai, M.C, ...541W
Tsang, A., ...26T, 453F
Tsang, Adrian, ...49W
Tsang, K. S. W., ... 256W*
Tschaplinski, Tim,..... 354F
Tsukuta, K., ...125T
Tsushima, A., ...473T*
Tudzynski, B.,.....20T, 390F
Tudzynski, P.,....38T, 140T,
..... 160W, 540F, 574W
Tung, Shu-Yun, ...307W
Turgeon, B. Gillian,....575T*
Turo, Chala,.....510F
Turrà, D.,132F*, 133W, 181W
Turrà, David,.....43W
Tyler, B. M., ...165F
Tyler, Brett M., ...507F*
Tzelepis, George, ...123F*

U

Uehling, Jessie, ...354F*,
..... 562W
Ullmann, Steffen, ... 564F
Ulrich, J., ... 390F, 448W*
Underwood, William, .569T*
Uniyal, P.,..... 330F
Unruh, S.,.....263T*
Upadhya, R., ...377T*
Upadhyay, S.,..... 640W
Upadhyay, Srijana,..... 5T
Urban, M., ... 482T
Urban, Martin, ... 201F,
.....266T, 576F
Uyemura, S. A.,..... 4W

V

Vachova, L., ...168F*
Valdez, Ernest,..... 353T
Valent, B.,..... 485T
Valerius, O.,88W, 92T, 192F
Valette, N., ...13W*
Valette, Nicolas, ... 29T
Valiante, V.,..... 627F
Valkonen, Mari,411F*, 632T*
Vallet, J., ... 241W
Valverde-Bogantes,
E.,.....343W*
Van Butselaar, Tijmen,542T
van Campe, Ulrich, ... 195F
van Dam, P., ...483F, 607W
van Dam, Peter, ...295W*
van de Geest, H. C., .. 242T
Van den Ackerveken,
Guido,..... 481W, 542T*
van den Berg, G. C., . 511W
van den Berg, Grardy
C. M.,..... 593T
van den Heuvel, J., ... 214W
van den Hondel,
Cees A. M. J. J.,..... 81F
Van de Peer, Yves, ... 302T
Vande Pol, N.,..... 559W
Vande Pol, Natalie, ..249F*,
562W
van der Does, H.C, ... 483F
van der Gragt,
Michelle, ... 295W
van der Lee, T., ... 607W
van der Lee, T. A. J.,.. 242T
van der Merwe, N. A.,251T*,
..... 595W, 609F
van der Nest, M.,167T, 298W*
van der Nest, M. A., .. 179T,
.....222F, 233T
van de Vossenberg,
B. T. L. H.,.....242T*
Van Deynze, Allen, ... 529W
van Diepeningen, A.,. 607W
van Esse, H.P, ... 319W
Vang, Jesper, ... 75F
van Gent Pelzer, M., .. 242T
van Kan, J. A. L.,..... 214W
van Kan, Jan A. L.,..178W*,
..... 208W
Van Prooyen, Nancy, 550W
van Tilbeurgh,
Herman, ...495F, 498F
van Verk, Marcel, ... 481W
van Wyk, S., ...233T, 609F*
Vargas Gastelum, L.,322W*
Varjosalo, Markku, ... 74T
Vega Gutierrez,
Sarath,.....323T*
Vela-Corcia, D.,..... 462F
Velez, Jessica, ...591F*
Velez, P.,.....340W*
Velez-Haro, J., ... 188T
Veneault-Fourrey, C., 469W
Vera-Estrella, R.,..... 271W
Verma, S., ... 474F
Verweij, P.,..... 583W
Verweij, P. E.,..... 584T
Vesth, T. C., ...230T, 243F*,
.....277W, 278T
Vetukkuri, Ramesh,.... 123F
Vetukuri, Ramesh R., .252F
Vetukuri, R. R., ... 497T*
Viaud, Muriel,.....372F
Vijayakumar, V., ...216F
Vijaykrishna, N.,.....407T
Vijayraghavan, U., ... 407T*
Viketoft, M.,.....221T
Vilgalys, R.,.....621F
Vilgalys, Rytas, ...339F,
..... 342F, 354F
Villani, Alessandra, ...391W
Vincent, F.,.....147F
Vincent, Jason L., ...69F
Virag, A., ... 65T*
Virgilio, S.,.....379W
Visel, A.,.....451W
Visentin, S.,.....546F
Visser, J., ...453F
Visser, R. G. F., ...242T
Vital, Nuria, ...18F
Vitale, S., ...132F,
.....133W*
Vitikainen, Marika, ...411F,
..... 632T
Vlaardingerbroek, Ido, 522F
Voegele, R.,.....499W
Voegeli, S., ...102F
Vogan, A., ...603F
Vogan, A. A.,..... 598W*
Voisey, Christine,.....508W
Voisey, C. R.,.....137T
Volk, H., ...491T
von Blanckenburg,
Friedhelm,.....643W
Voorhies, M.,.... 414F, 417F
Voorhies, Mark, ...418W
Voss, B., ... 34W*
Vossen, J.H, ...242T
Vu, Andrea,..... 647T*
Vylkova, S.,.....464T

W

Waalwijk, C.,.....607W
Wada, Y.,.....368T
Wagener, Johannes, .. 81F*
Wagner, K.,.....217W
Waiger, Daniel, ...18F
Waitt, G.,.....357F
Walker, Gary,.....421W
Walker, Louise,.....17T
Walker, N.,.....254T
Wallen, R. Margaret,. 447F*
Walochnik, J., ...296T
Walter, S., ... 489F*
Walters, Nichole, ...121W
Wambaugh, M., ... 475W*
Wang, B.,.....152T
Wang, Chenfang,.....397W
Wang, Clay C. C.,..... 70W*
Wang, C. Y., ...547W
Wang, Fang, ...186F*, 363F
Wang, G.,..... 393F*
Wang, G.-L., ...259W
Wang, Jianhua,.....397W
Wang, Lei,.....564F
Wang, Liuyang,.....588F
Wang, M., ...505W, 532W*
Wang, Penghao,.....636F
Wang, Q.,.....547W
Wang, Qinhu,.....434T
Wang, Qunqing,.....507F
Wang, Shu-tong,.....526W
Wang, Ting-Fang,.....290T,
.....307W
Wang, Y., ...333F
Wang, Z., . 89T, 284T, 393F
Wang, Zheng, 124W, 274W
Ward, M., ...65T
Ward, T. J., ...610W
Ward, Todd J., ...237F
Waring, Michael,.....325W
Warris, S., ...242T
Wasil, Zahida,.....40W
Waterstraat, M., ...57F
Watson, G., ...165F
Watters, Michael, ...59T*,
.....121W*, 653T*, 654F*
Wawra, Stefan,..... 564F
Weber, J.,.....627F*
Wegulo, S.,.....343W
Weichert, M., ...140T
Weikert, T., ... 470T
Weiss, Carly, ... 600F
Weissman, Ziva,..... 535W
Wellapilli, D.,..... 165F
Wendell, L., ...512T*
Wenderoth, M.,.....35T*
Wendland, Jürgen, ...572T*
Were, V. M., ... 259W*
West, Samuel,..... 636F
Westerholm-Parvinen,
Ann, ...64W
Westfall, P. J., ... 633F
Westhus, E., ...316W
Westphal, K., ... 30F
Whisson, S. C.,..... 497T
White, J. B., ... 618F
Whitham, S.,.....499W
Widdison, S., ...16W
Widinugraheni, Sri,....483F*
Wiemann, P.,.....6F, 449T
Wierckx, N., ...76W
Wilcox, Andrew,..... 275T
Wildermuth, Mary, ...15F*
Wildermuth, M. C., ... 14T
Wilken, Markus,..... 170T
Wilken, P. M., ... 167T
Wilken, P.M, ... 179T
Wilken, P. Markus, .. 136W*
Williams, S.J,..... 19W*
Willis, Christine L.,..... 69F
Wilson, A., ...167T*
Wilson, R., ... 2T
Wilson, Richard, 24F, 461T*
Wimmer, R., ... 30F
Win, Joe, ... 500T
Winandy, L., ... 48F
Winans, Matthew,.....350T*
Wincker, Patrick, ... 588F
Wingfield, B. D.,..... 167T,
...170T*, 179T, 222F, 251T,
..... 595W, 602T, 605T,
.....609F, 641T
Wingfield, Brenda D., ...
..... 136W, 298W
Wingfield, Michael J., ...
.....136W, 170T, 298W
Wingfield, M. J.,..... 167T,
... 179T, 595W, 602T, 605T
Wingfiled, B. D.,..... 233T
Winter, Ara, ... 353T
Wirth, Sophia,..... 182T
Wise, K. A.,..... 618F
Wisecaver, J.,..... 608T
Wisecaver,
Jennifer,..... 1W*, 281T
Wissing, J., ...139W
Wolfe, Benjamin, ... 478W*
Wolfe, Kenneth H., ... 248T
Wolfe, K. H., ... 227T
Wolff, P. P., ... 33F
Wollenberg, R. D.,..... 30F
Woloshuk, C. P.,..... 618F
Wolpert, Thomas,..... 548T
Won, T.,..... 6F
Wong, Chris Koon Ho, ...
.....186F, 365T
Wong, C. K., ...256W
Wong, K.,..... 452T
Wong, K. H., ...527T
Wong, Koon Ho, ... 363F
Wong, Man Chun, ... 255F
Woo Bok, J., ... 630F
Wood, Bruce W., ... 620T
Wood, Thomas A., ... 593T
Workman, Mhairi, ... 40W
Wösten, Han,..... 128T
Woyke, T., ... 200T
Wrobel, Kazimierz, ... 557T
Wu, B.,..... 114F

Wu, Dongliang, ... 575T
Wu, Guangxi, ... 304W
Wu, J., ... 565W
Wu, J. Q., ... 257T*
Wu, V., ... 444F
Wu, Y., ... 400W*

X

Xia, C., ... 505W
Xiao, Yang, ... 255F
Xie, N., ... 171F, 173T
Xie, Y.,..... 441F*
Xing, L., ... 205W
Xing, Y., ... 492F
Xu, J.,.. 241W, 393F, 394W
Xu, Jin-Rong, ... 434T
Xu, Jin-rong,..... 397W
Xu, JR, ... 531F
Xu, L.,..... 400W
Xu, Xinping,.....5T
Xu, Z.,..... 114F
Xue, C., ... 640W

Y

Yadav, Vikas, ... 588F
Yaegashi, Junko, ... 66F*
Yagtu, Civan, ... 115W
Yaish, Shir,..... 535W
Yamagata, Y., ... 96F
Yamagata, Youhei, ...52W*
Yamashino, T., ... 30F
Yan, L.,.....637W*
Yan, X., ... 259W, 487W*
Yang, Han-Eul,..... 568W
Yang, Lei, ... 75F
Yang, Moon-Sik, ... 270F
Yang, Q., ... 153F
Yang, Wen-Der, ... 290T
Yang, Yi, ... 290T
Yano, S., ... 86T
Yeh, Hsu-Hua, ... 70W
Yeh, Zong-Ming, ... 290T
Yeo, Soo-Hwan, ... 321F
Yin, Guohua, ... 304W
Yin, Tao, ... 397W
Yin, Z.,..... 578T*
Yokoi, T., ... 150F*
Yokoyama, Tatsuya, . 280W
Yoon, Olivia,..... 70W
Yoshida, Hiroshi,..... 27F*
Yoshimi, A., ... 86T
Young, Carolyn A.,.... 620T*
Young, Janet M.,..... 622W
Yu, Chien-Hung, ... 409W
Yu, Jae-Hyuk, ... 361W
Yu, Jiujiang, ...304W*
Yu, V., ... 635T
Yuan, Tinglu,..... 516F
Yuan Yin, Q.,..... 102F
Yue, Wei, ... 235W
Yun, Suk-Hyun,321F, 568W
Yun, Sung-Hwan,236T, 396F

Z

Zaccaron, M., ...267F*
Zander, Sabrina, ...190W*
Zanders, SaraH E., ..622W*
Zawil, D., ...53T*
Zechmann, Bernd, ... 564F
Zegans, Mike, ... 415W
ZEHRAOUI, E., ... 398T
Zeilinger, Susanne, ... 581T
Zettler, L., ... 263T
Zhai, Y.,..... 492F
Zhang, C., ...358W*, 635T
Zhang, Dong, ... 516F
Zhang, F.,.....469W*
Zhang, H., ... 333F
Zhang, J., ...22W, 206T*,
..... 583W*, 584T
Zhang, Jinxiang,.....50T*
Zhang, Qiang,.....397W
Zhang, Summer,.....496W
Zhang, X., ...531F*, 547W
Zhang, Y.,303F, 341T, 608T
Zhang, Yanhua, ...47T
Zhang, Ying, ...93F
Zhang, Yuanchen, ...478W
Zhang, Yuanwei,..... 85W*
Zhao, J.,.....652W
Zhao, X., ...19W
zhao, x., ... 413T*
Zhao, Y., ... 384F*, 639F,
..... 640W*
Zhao, Y. Y.,.....652W
Zheng, W., ...89T
Zheng, Yun, ...49W
Zhong, Y., ... 205W*
ZHONG, Z., ...551T
Zhong, Ziming,.....552F
Zhou, B., ...259W
Zhou, Mian,.....409W
Zhou, X., ... 152T*
Zhou, Zhipeng, 409W, 437T
Zhu, J., ... 416T*
Zhu, Zhaojun, ...81F
Zia, Amjad, ...272T
Zielinski, J.,.....109W
Ziemons, S., ...433W
Zimmerman, K., ...41T
Zink, E.,.....331W
Zink, Erika,.....50T
Zivy, M., ...429F
Znaidi, S., ...147F
Zoll, J., ...583W
Zuniga, J. E., ... 166W*
Zuo, S. X.,.....652W
Zwaan, Bas,.....583W
Zwaan, B. J.,.....584T

The following index is composed of keywords selected by presenting authors from a list on the Abstract Submission Site. Abstract program numbers follow each keyword.

annotation
1W 200T 201F 202W 203T 222F 242T 266T 293T 317T 649W

antifungal drug resistance
584T

appressorium
2T 24F 47T 142W 143T

ashbya gossypii 275T

asperfillus oryzae
12F 52W 53T 54F 96F 205W 367W 368T 369F 370W 371T 586W

aspergillus
308T

aspergillus flavus
194T 309F 310W 332T

aspergillus fumigatus
3F 4W 5T 6F 7W 81F 82W 83T 84F 85W 204F 318F 357F 358W 359T 360F 455T 456F 457W 458T 459F 583W 584T 627F

aspergillus nidulans
8T 9F 10W 48F 70W 111F 86T 87F 88W 89T 85W 90F 91W 92T 93F 94W 145W 361W 362T 363F 364W 365T 431T 439W 585F

aspergillus niger
26T 11T 49W 50T 51F 57F 95T 243F 366F 629T 628W

automation
633F

barley
218T 460W 636F

biocontrol
582F

bioinformatics
510F

biomass
29T 13W 66F 76W 55W 56T 58W 59T 57F 171F 289W 206T 628W

biotrophic
14T 15F 207F 324F 319W 540F 461T

botrytis cinerea
16W 97W 98T 180F 208W 372F 429F 462F

candida albicans
17T 62T 99F 100W 197T 209T 210F 373W 374T 375F 376W 463W 464T 465F 466W 467T 468F 587T 637W 638T

cell cycle
144F 210F

cell death
193W

cell wall
17T 21F 3F 101T 102F 103W 86T 151W 211W 377T 469W 470T 480F

cellulases
77T

centromere
311T

chemical genetics
312F

chromatin structure
398T

circadian clock
104T 105F 152T 258F 378F 379W 380T 420F 424W 471F

colletotrichum
60F 212T 381F 472W 473T

communication
149T

comparative genomics
347T

comparative mitochondrial genomics
316W

conidiation
14T 15F 101T 141F 211W 274W 392T 455T

coprinopsis cinerea
195F 196W

cryptococcus neoformans
106W 107T 108F 109W 172W 213F 244W 377T 382W 383T 384F 445W 474F 475W 588F 639F 640W

database
203T

development
110T 112W 113T 114F 115W 116T 117F 118W 111F 119T 120F 131T 132F 134T 82W 137T 87F 88W 89T 146T 147F 148W 153F 181W 186F 195F 196W 385W 414F 476T 525F

diagnostic
644T

diversity
200T 294F 320T 321F 322W 323T 325W 326T 327F 328W 329T 330F 331W 332T 333F 334W 335T 324F 336F 337W 338T 339F 340W 342F 318F 350T 352W 353T 477F 478W 521T 587T 589W 590T 615F 619W 624F

DNA repair
18F 386T 589W 630F

early diverging fungi
451W

ecology
121W 214W 215T 216F 217W 223W 317T 325W 336F 337W 338T 339F 340W 341T 463W 478W 479T 560T 591F 593T 594F 643W

ectomycorrhiza
217W

education
653T 654F

effector
19W 122T 123F 219F 220W 218T 245T 259W 295W 387F 416T 476T 480F 481W 482T 483F 484W 485T 486F 487W 488T 489F 490W 491T 492F 493W 494T 495F 496W 497T 498F 499W 500T 501F 502W 503T 504F 505W 506T 507F 508W 509T 510F 516F 541W 542T 555F 557T 565W 566T 569T 579F 592W

epigenetics
450F

epigenomics
451W

evolution
41T 61W 102F 112W 113T 122T 124W 221T 222F 223W 224T 225F 226W 227T 228F 229W 230T 231F 233T 246F 247W 248T 249F 276F 277W 278T 296T 297F 298W 309F 311T 326T 327F 342F 481W 511W 580W 588F 592W 593T 594F 595W 596T 597F 598W 599T 600F 601W 602T 603F 604W 605T 606F 607W 608T 609F 611T 612F 616W 621F 622W 641T

expression system
632T

fruiting body
114F 124W 125T 126F 127W 128T 173T 187W 231F 388W 389T 645F

functional genomics
452T

fungal diversity
356T

fungicide resistance
313W

fusarium
20T 232W 233T 234F 235W 236T 279F 390F 391W 404T 512T 513F 514W 515T 595W 607W 608T 609F 610W

fusarium graminearum
21F 129F 130W 237F 343W 392T 393F 394W 395T 396F 397W 398T 482T 516F 517W 518T 519F 520W 537F 570F 460W

fusarium oxysporum
18F 131T 132F 133W 344T 399F 483F 521T 522F 523W 611T 612F

gene deletion
4W 46W 47T 62T 49W 58W 107T 115W 116T 163W 96F 238W 239T 373W 400W 368T 524T 531F 543F 571W 596T 631W 637W 642F

gene editing technology development
631W

gene expression
22W 23T 27F 60F 63F 64W 65T 67W 66F 50T 72F 75F 117F 134T 135F 174F 194T 240F 280W 205W 386T 387F 401T 402F 403W 404T 405F 406W 407T 408F 409W 410T 411F 412W 361W 362T 417F 425T 426F 432F 369F 440T 441F 447F 484W 485T 486F 525F 526W 527T 528F

gene expression
529W 532W 544W 613W 623T

gene/genome editing
634W

genome editing
635T

genome organization
268W

genome sequencing
136W 175W 208W 212T 214W 219F 224T 241W 242T 244W 245T 246F 247W 248T 249F 250W 251T 243F 252F 253W 254T 255F 256W 257T 263T 315F 207F 328W 329T 345F 346W 347T 363F 473T 530T 630F

GFP
118W 90F 154W 95T 299T 393F

heterochromatin
422T

hydrophobins
78F

hyphae
99F 100W 83T 137T 138F 182T 225F 348F 643W

hyphal fusion

97W 139W 140T 155T 348F 413T 561F

inducer
453F

industrial biotechnology
633F

lentinula edodes
314T

lichen
315F

lichen, symbiosis, resynthesis, fungi, algae
79W

light
141F 240F 258F 372F 414F 415W 428T

loss of heterozygosity
197T

magnaporthe oryzae
2T 24F 144F 142W 143T 259W 260T 261F 416T 487W 531F 532W 533T 534F 461T 614T

maize
355W 488T 545T

malassezia
316W

mass spectrometry
36F

metabolism
16W 22W 25W 27F 28W 26T 8T 31W 43W 71T 135F 234F 238W 262W 415W 456F 535W

metabolomics
354F

microarray
536T 615F

microbiome
468F

mitochondria
150F 198F 199W

morphogenesis
119T 121W 125T 126F 146T 147F 145W 156F 383T 417F 418W 572T 581T 639F

multidrug resistance
454W

mycobiome, fungi in the gut
333F

mycorrhiza
250W 263T 264F 265W 330F 349W 489F 613W

mycovirus
655W

nematode-trapping fungi
580W

neurospora
59T 104T 148W 149T 150F 176T 379W 380T 405F 419T 420F 421W 422T 434T 437T 536T 597F 598W 616W

neurospora crasse
151W 152T 153F 154W 155T 156F 157W 158T 159F 183F 406W 423F 424W 425T 426F 444F 450F 537F

nuclear mutation
199W

oxidative stress
29T 13W 103W 538W

pathogenicity
28W 30F 61W 108F 109W 160W 188T 201F 220W 235W 266T 267F 268W 313W 344T 381F 427W 442W 462F 464T 471F 474F 475W 490W 491T 492F 493W 494T 495F 496W 497T 511W 512T 517W 522F 524T 526W 527T 530T 539T 541W 542T 543F 544W 545T 546F 547W 548T 549F 550W 551T 552F 553W 540F 554T 567F 617T 626T 644T 652W

pectinase
453F

pheromone
133W

phytophthora
334W 335T

plant pathogen
306F

pleurotus ostreatus
655W

population
168F 209T 251T 281T 319W 590T 591F 599T 610W 614T 618F 619W 620T

protein degradation
520W

protein kinase
44T 120F 91W 204F 357F 364W 554T 645F

protein localizatoin
5T 138F 139W 93F 157W 158T 159F 162F 163W 164T 165F 169W 185T 189F 190W 370W 500T

protein production
77T

protein-protein interactions
9F 54F 92T 161T 260T 269T 394W 428T 498F 499W 533T 578T

proteomics
31W 37W 74T 166W 215T 261F 270F 271W 272T 421W 429F 646W

receptor
641T

rhodotorula
356T

RNA-seq
51F 68T 80T 167T 226W 236T 252F 273F 274W 300F 314T 407T 366F 430W 513F 546F 555F 573F 646W 647T

RNAi
577W

saccharomyces cerevisiae
130W 168F 253W 275T 350T 430W 600F 648F 649W

secondary metabolism
20T 23T 6F 7W 32T 33F 34W 35T 36F 37W 38T 39F 42F 45F 68T 69F 71T 70W 169W 216F 276F 277W 278T 279F 280W 281T 390F 395T 399F 432F 431T 433W 443T 449T 556W 563T 574W 601W

secondary metabolite
30F 40W 41T 42F 12F 72F 282F 341T 351F 408F 413T 518T 457W 557T 575T 627F 650T

sexual development
73W 123F 127W 136W 94W 167T 170T 172W 173T 174F 175W 176T 177F 178W 179T 171F 191T 283W 284T 301W 345F 352W 396F 434T 435F 602T 583W 620T 621F 622W 586W 640W

signal transduction
38T 43W 44T 105F 140T 160W 166W 180F 181W 182T 183F 184W 271W 285F 286W 287T 288F 310W 384F 358W 501F 547W

single nucleotide polymorphism
458T 623T

stress
184W

stwintron
585F

symbiosis
185T 346W 353T 354F 436W 470T 502W 528F 558F 559W 560T 561F 562W

toxin
10W 164T 302T 351F 397W 503T 548T 638T

transcription
67W 186F 409W 433W 371T 437T 438F

transcription factor
128T 165F 177F 267F 289W 374T 391W 410T 411F 359T 360F 365T 418W 435F 438F 440T 441F 439W 442W 443T 448W 452T 454W 563T 632T 651F

transcription profiling
1W 39F 283W 375F 376W 427W 444F 465F 477F 529W 534F 651F

transcriptome
74T 98T 178W 227T 228F 254T 255F 264F 284T 206T 331W 412W 445W 479T 519F 629T

translation
388W

transmembrane proteins
581T

transport
75F 192F 535W

trichoderma reesei
73W 80T 307W

trichoderma virens
78F

ustilago maydis
45F 76W 187W 188T 189F 190W 191T 192F 193W 389T 446T 447F 448W 504F 549F 564F 565W 566T 567F 624F

vaccine
553W

verticillium
582F

virulence
46W 84F 256W 257T 269T 303F 304W 400W 466W 467T 505W 506T 507F 514W 515T 523W 459F 550W 568W 569T 570F 571W 572T 573F 574W 575T 576F 577W 579F 652W

wheat
19W 305T 343W 355W 508W 551T 552F 576F

whole genome sequencing
179T 202W 213F 229W 230T 237F 239T 265W 290T 291F 292W 293T 294F 295W 296T 297F 298W 299T 300F 301W 302T 303F 304W 305T 306F 307W 308T 349W 449T 509T 562W 578T 603F 604W 605T 606F 617T 625W 626T

wood decay
272T

zymoseptoria
312F