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Population structure and local adaptations in marine fishes

PhD Thesis

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October 2006

Preface

This thesis presents the main results from my PhD conducted at the Departments of Marine Ecology and Ecology & Genetics, University of Aarhus (AU) and at the Department of Inland Fisheries at the Danish Institute for Fisheries Research (DIFRES) in Silkeborg. The work has been focused on population structuring forces in marine fishes in general and on the genetic basis of local adaptations in particular. It's been a lot of fun and hard work, and there are many people, who have contributed significantly to a positive outcome of these last years. I may forget some; please forgive me.

First and foremost I thank my family. This may not be the official order of doing things, but they do belong up here. Thanks Sidsel for always being there for me, to support and inspire me and for being the World's best mom. Jonathan and Lærke, just because you are here!

Anyway, I would also like to thank all the people at the three departments where I have spent quite some time during the last years. Especially my supervisors Katherine Richardson, Volker Loeschcke, Peter GrønkJær and Einar Eg Nielsen who have all been very helpful; needless to say that I would most probably still be drifting around somewhere, had they not guided me with great academic and personal insight. I have especially been bothering Volker, Peter and Einar who have devoted a lot of time and energy to me - Einar in particular during the last hectic months. Thank you all very much!

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Århus, October 2006

Jakob Hemmer-Hansen

Contents

General introduction	Population structure and local adaptations in marine fishes - Current knowledge and perspectives for future developments	1
Manuscript I	Evolutionary factors shaping the genetic population structure of marine fishes; lessons from the European flounder (<i>Platichthys flesus</i> L.)	39
Manuscript II	Adaptive divergence in a high gene flow environment: <i>Hsc70</i> variation in the European flounder (<i>Platichthys flesus</i> L.)	91
Manuscript III	Evidence of genetic introgression from plaice (<i>Pleuronectes platessa</i> L.) to European flounder (<i>Platichthys flesus</i> L.) populations	121
Manuscript IV	Ecological and genetic interactions between flounder (<i>Platichthys flesus</i> L.) populations in the Baltic Sea	151
Summary		173

Population structure and local adaptations in marine fishes

- Current knowledge and perspectives for future developments

It is now well established that the World is facing its 6th mass extinction, believed to be driven mainly by human disturbance (Pimm *et al.* 1995; Thomas *et al.* 2004; Pimm *et al.* 2006). While terrestrial systems have been subject to attention from conservation biology and conservation genetics for many decades, the marine environment has only recently received increased attention in conservation contexts (Awise 1998), despite the fact that it is covering more than 70 % of the World's surface. It is now clear that marine ecosystems are as vulnerable to human disturbance as are terrestrial (e.g. Malakoff 1997; Solan *et al.* 2004) and that several marine species are now already extinct or at the brink of extinction (Powles *et al.* 2000, Dulvy *et al.* 2003, Reynolds *et al.* 2005). The increased awareness of the need for marine conservation has also led to the acknowledgement of a need to conserve intra-specific biodiversity, especially in relation to harvestable resources (Ryman *et al.* 1995; Nielsen and Kenchington 2001; Smedbol and Stephenson 2001; Ruzzante *et al.* 2006). Thus, there is an urgent need for knowledge of how selective forces operate in the sea to structure marine species into discrete population units. Because local populations may harbor a unique portion of the species' genetic material allowing it to adapt to specific environmental components in present as well as in future times (e.g. Hilborn *et al.* 2003), the study of the genetic basis of local adaptations has been highlighted as worth special attention (Moritz 2002; van Tienderen *et al.* 2002). Studies of the genetic structuring of populations of marine fishes have benefited tremendously from the advent of highly variable neutral genetic markers in recent years (e.g. Carvalho and Hauser 1998), which has facilitated an exploration of structuring forces in this high

gene flow environment (Waples 1998). However, while our knowledge of the interplay of demographical processes (gene flow, drift and historical processes) is improving rapidly these years, we still know very little about how selection may act to structure marine fish population, because the commonly applied markers are believed to be selectively neutral and hence not applicable for studies of adaptive population divergence.

Although technological developments have advanced our ability to study evolutionary processes at still finer scales, it should be remembered that processes at the species and population levels are highly interconnected. For instance, species specific physiological constraints may limit the ability of populations to adapt to environmental changes such as increasing temperatures (e.g. Somero 2005). Similarly, large scale climatic cycles will affect the levels of genetic diversity and sub-structuring within species observed today (Grant and Bowen 1998; Hewitt 2000). Furthermore, the degree of species integrity is likely to affect such processes, since continuous gene flow between species could result in genetic homogenization within species and might eventually lead to loss of local adaptations (e.g. Taylor *et al.* 2006). On the other hand, it is becoming increasingly clear that species are not homogenous genetic units and that it is only through an understanding of processes at the population level that we are able to predict the ability of species to adapt to future environmental changes.

Until recently it was relatively complicated, expensive and time consuming to demonstrate the genetic footprints of selection in non-model organisms such as marine fishes. While marine fishes still present researchers with many challenges in these types of studies,

recent increases in information about the organization and structure of marine fish genomes as well as technological developments in molecular biology have all provided new opportunities. Hence, it no longer appears an impossible task to examine adaptive population divergence in the marine environment.

This thesis

The overall theme of this PhD thesis is population structure and local adaptations in marine fishes. The purpose has been to elucidate the interplay of evolutionary forces in the sea at different hierarchical levels ranging from inter-specific hybridization to macro- and micro-geographical neutral structuring as well as adaptive population divergence within species. The aim has been to elucidate the scale and magnitude of population structure and local adaptations in marine fishes. It is only through an understanding of these interacting evolutionary processes on different hierarchical levels that we may gain a better understanding of how selection operates in this high gene flow environment.

We have used the European flounder (*Platichthys flesus* L.) as a model species for these studies, since several aspects of the species' biology make it a suitable model to study the action of evolutionary forces in the marine environment. First of all, it shares many characteristics with other marine species, such as pelagic egg and larval stages and, presumably, large effective population sizes believed to result in limited structuring of marine fish populations. On the other hand, it is relatively stationary and coastal, which could lead to the prediction that structuring should be more pronounced in this species compared to other more oceanic species. Hence, this species may actually represent a case where true signals are clearly larger than random noise (cf. Waples 1998). Secondly, its wide distribution exposes

it to diverse environmental conditions. As a coastal species spending much of its life in shallow waters, it is expected to be affected by spatial and temporal differences in environmental parameters making it a suitable model for studies of adaptive population divergence. Thirdly, as a euryhaline species, it represents an important contrast to other marine fishes studied so far. This facilitates comparative and contrasting analytical set-ups and should prove useful for validating earlier results from other species. And finally, local adaptations have actually been suggested in populations in the Baltic Sea, which are believed to have adapted their spawning strategy to life at low salinities (Aro 1989; Nissling *et al.* 2002), but the genetic signatures of these presumed adaptations have never been investigated.

The purpose of this general introduction is to review current knowledge of population structure and local adaptations in marine fishes to set the stage for a discussion of recent developments which could improve our understanding of the genetic basis of local adaptations in marine fishes. By evaluating available techniques in a marine fish perspective, I will try to highlight the approaches most likely to be useful for disclosing the genetic basis as well as the scale and magnitude of local adaptations in marine fishes in the coming years. This field has got the potential to contribute significantly to our understanding of evolution in general and of the outcome of the interplay of evolutionary forces in the sea in particular. Throughout this introduction I will integrate the results from my own PhD and highlight the areas where I believe that the results have contributed significantly to increase our understanding of these processes.

Population structure in marine fishes

The genetic structuring of populations of marine fishes has only received intense attention within the last 10-20 years. Historically, studies of the genetic structure of marine fish populations have followed technological developments, but have tended to lag somewhat behind studies in terrestrial environments (Avise 1998).

Molecular markers

Studies on haemoglobin polymorphism in Atlantic cod in the 1960s (Sick 1965a, 1965b) were among the first applications of biochemical markers in fishes. The advent of enzyme electrophoresis (Hubby and Lewontin 1966; Lewontin and Hubby 1966) brought with it an explosion of studies characterising genetic variation in natural populations of fishes, demonstrating genetic population structure for many species (e.g. Christiansen *et al.* 1976, Winans 1980, Grant and Utter 1980, Kornfield *et al.* 1982). Although allozymes are often complimented with DNA based markers today (e.g. González-Wangüemert *et al.* 2004; Maes *et al.* 2006; Pampoulie *et al.* 2004) they are occasionally applied separately to infer population structure in marine fishes (e.g. Stefanni *et al.* 2003; Cimmaruta *et al.* 2005). However, several studies have found allozymes to be under selection (Hilbish and Koehn 1985; DiMichele *et al.* 1991; Schmidt and Rand 1999) and they should hence be used as neutral markers with caution. Modern DNA based techniques, particularly highly variable markers such as microsatellites, have revolutionized studies of marine fish population structure. These markers have been very useful for disclosing the often low levels of structure found in marine fishes (Waples 1998; DeWoody and Avise 2000) because of their associated high statistical power (Ryman *et al.* 2006; Waples and Gaggiotti 2006). Although

still in its infancy, single nucleotide polymorphisms (SNPs) have been highlighted as a potential marker of choice in future studies of neutral population divergence (Morin *et al.* 2004). However, since these markers are often bi-allelic, more loci will be required in order to achieve the same power as in microsatellite studies (Kalinowski 2002), and the choice of marker should always be based on both theoretical and technical considerations.

Statistical developments

The development of highly variable genetic markers has brought with it a suite of useful statistical techniques for handling the wealth of information from these markers (e.g. Luikart and England 1999; Excoffier and Heckel 2006). This has allowed population genetic studies to move beyond the purely descriptive stage and has also resulted in interesting applications in marine fishes.

Assignment and individual admixture analyses can provide important information on interactions between populations by disclosing migration and hybridization (e.g. Hansen *et al.* 2001; Manel *et al.* 2005). Bayesian methods have been developed to assess the most likely number of populations represented by the data by minimizing levels of Hardy Weinberg and linkage disequilibrium within groups (e.g. Pritchard *et al.* 2000; Corander *et al.* 2003). These methods have also been applied in marine fishes (e.g. Nielsen *et al.* 2003; Nielsen *et al.* 2004; Manuscript IV), but it should be kept in mind that the power of these approaches for correctly identifying both true genetic structure and hybrid individuals decreases dramatically at the low levels of structuring (Latch *et al.* 2006; Vähä and Primmer 2006) often observed in marine fishes. Hence, in marine fishes individual admixture analyses have mostly been applied at the species level to identify species and hybrid individuals (e.g.

eels, Albert *et al.* 2006 and Maes *et al.* 2006; flatfish, Manuscript III).

Even though hybrid individuals can be difficult to identify with statistical certainty *within* species, these methods can provide important information about the genetic interaction among marine fish populations. For instance, they have been used to examine the distribution of individual admixture proportions in cod (Nielsen *et al.* 2003; Nielsen *et al.* 2005), turbot (Nielsen *et al.* 2004) and flounder (Manuscript IV) populations with the aim of examining evolutionary and ecological interactions among genetically differentiated populations. Hence, although the specific hybrid class of individuals could not be identified with high certainty, the *distribution* of individual admixture proportions gave important insights into the interactions of differentiated populations of these species.

Recently, the increased statistical power provided by genetic markers has been combined with other information in integrative approaches which hold great promise for providing increased understanding of population structure and ecological processes in nature. For instance, demographic and genetic information has been combined in a method aiming at identifying colonization events and processes (Gaggiotti *et al.* 2004), and the emerging field of landscape genetics combines geographical and genetic information to identify the largest genetic breaks among interconnected populations (Manel *et al.* 2003), which has also found its applications in marine fishes (e.g. Bekkevold *et al.* 2005; Jørgensen *et al.* 2005; Manuscript I). Future developments of these approaches could include information of oceanographic currents as both potential barriers to gene flow and as dispersal mechanisms (Galindo *et al.* 2006) and could be very useful in marine fishes exposed to such physical forces. A recently published method aims at combining geographic information with the power of Bayesian clustering algorithms to

identify populations and migrants/hybrids in a geographical context (Guillot *et al.* 2005; Coulon *et al.* 2006). This could prove valuable for disclosing cryptic population structure in marine fishes, but, although this particular method has not been evaluated at low levels of structuring, it most likely suffers from the same problems as other clustering methods in these scenarios. Environmental parameters have been incorporated with genetic information in partial Mantel tests to examine if patterns of genetic structuring are best explained by geographic or environmental differences between populations (Bekkevold *et al.* 2005; Jørgensen *et al.* 2005; Manuscript I). This approach thus points to potential environmental factors, such as salinity and temperature, which could be important for shaping genetic structure in marine fishes, but do not show if populations are locally adapted since the markers applied are not subject to selection. Future developments of integrative approaches should be useful for studies of population structure in marine fishes, thereby moving this research area further by gaining important new knowledge of the interplay of evolutionary forces in the marine environment.

Population structure

The emerging picture from the studies conducted so far has been one of limited genetic structuring of populations. This is thought to be caused by the particular characteristics of the marine environment and its inhabitants such as few physical barriers to gene flow, large effective population sizes and high dispersal capabilities, both as adults and as pelagic eggs and larvae. Such features are generally seen as important for promoting high levels of gene flow (Ward *et al.* 1994; Waples 1998), which in turn decreases the signal:noise ratio (Waples 1998) and complicates studies of population subdivision in marine fishes (see Box 1).

Box 1. Statistical and biological significance in studies of marine fish population structure

The high basal levels of gene flow and the high statistical power of highly variable markers have resulted in concerns about the coupling between biological and statistical significance in high gene flow scenarios, such as in marine fishes (Waples 1998, Hedrick 1999). First of all, the high levels of gene flow often result in minute signals which could be difficult to detect with genetic markers, resulting in Type2 errors (not rejecting H_0 ="no structure" when it is in fact false). This problem is particularly important in relation to less variable markers, such as allozymes, but could also present a problem in microsatellite studies, e.g. if too few loci are used. For instance, we have found that populations of European flounder in the Baltic Sea are highly structured between different life history forms (Manuscript I). However, detailed analyses of samples from a particular locality revealed important biological signals most likely reflecting seasonally migrating individuals. These signals did not, however, translate into significant tests for population subdivision and could have gone undetected in a genetic study if less information about the biology of the species had been available (Manuscript IV).

Type1 errors (rejecting H_0 ="no structure" when it is actually true) is a more common problem with microsatellite markers; i.e. that tests are statistically significant but not reflecting true biological signals (Waples 1998, Hedrick 1999). Because the true signal is often very small, noise could bias conclusions seriously (Waples 1998). Noise in microsatellite studies is most often generated or inflated by non-random sampling (Waples 1998) or genotyping errors (e.g. Pompanon *et al.* 2005). However, unrecognized biological sources of noise could also bias results even if studies are perfectly designed. For instance, inter specific hybridization could result in biased results, particularly if introgression is unevenly distributed throughout the sampled range. Although hybridization between marine fish species is relatively sparsely studied, evidence is emerging that it could be relatively common between some species (Gardner 1997). While there was little evidence that introgression from plaice to flounder could have biased the results from our study of population structure in European flounder (Manuscript III), other studies have found high levels of introgression in some parts of a species' distributional area (Roques *et al.* 2001; Buonaccorsi *et al.* 2005; Albert *et al.* 2006). If not recognized, such introgression could seriously bias surveys of population structure.

Thus, from the above it should be clear that an arbitrary significance cut-off (such as an alpha-level of 0.05) makes little sense without a thorough evaluation of the signals and patterns of structuring in the data set. Furthermore, results will often be easier to interpret if sampling is temporally replicated (Waples 1998). Our own studies on European flounder serve to illustrate these points.

However, despite the general picture of high levels of gene flow, recent studies have identified significant genetic structuring of marine fish populations. Several evolutionary mechanisms have been proposed to be important for generating the small but significant levels of genetic structuring in an environment without any obvious physical barriers to gene flow.

First of all, genetic structuring may be affected by processes not related to contemporary physical or environmental factors

directly. In some cases, patterns of structuring have been found to be influenced by levels of inter-specific hybridization (e.g. Roques *et al.* 2001; Roques *et al.* 2002; Albert *et al.* 2006). We used individual admixture analyses of real and simulated data to identify hybrids of first and later generations between plaice and European flounder (Manuscript III). We found that introgression from plaice to European flounder did occur beyond the first generation in flounder populations in nature, but at a relatively modest rate, implying efficient

selection against hybridization between the two species in nature, so the effects of introgressive hybridization obviously depends on the specific setting.

The importance of including a species' history when studying patterns of genetic variation in contemporary samples has repeatedly been stressed (e.g. Grant and Bowen 1998; Hickerson and Cunningham 2005) because any pattern observed today is the result of historical as well as contemporary processes. It has been suggested that many classical marine fishes (i.e. species with large effective population sizes, high migration potential and pelagic eggs and larvae, see Nielsen and Kenchington 2001) have shallow population histories in the Northern Atlantic because of recent colonization after the last glaciation (Grant and Bowen 1998; Pogson *et al.* 2001). In most species examined in detail, shallow histories have indeed been confirmed (e.g. Árnason 2004; Hoarau *et al.* 2004) implying young population ages and potential non-equilibrium between migration and drift. The effect of these factors could be to depress levels of structuring, thereby leading to an underestimate of the true level of population structure (e.g. Pogson *et al.* 2001). However, colonization processes also leave behind signatures which can be detected in contemporary populations, such as decreased levels of variability in populations in the extreme parts of the distributional area associated with founder events when new habitat was colonized (Hewitt 2000; Widmer and Lexer 2001). Such patterns have also been observed in marine fishes (Wilson 2006; Manuscript I).

Differences in life-history strategies have long been known to result in significant genetic structuring of salmonid populations (e.g. Taylor 1991), but few examples of populations with different life histories are found in marine fishes. One such example is Atlantic herring, which has been shown to be

structured among population components with different spawning strategies (Bekkevold *et al.* 2005). The European flounder is another example with populations in the Baltic Sea exhibiting different life history characteristics. We found a sharp genetic break between these life histories, indicating that this is an effective barrier to gene flow in marine fishes (Manuscript I). We also found indications of a mixing of different populations at some times of the year (Manuscript IV), suggesting significant ecological interactions among these differentiated populations.

A number of studies have also found contemporary physical drivers to be important for shaping the distribution of genetic variation between populations. Thus, geographic distance *per se* has been suggested to be important for creating patterns of isolation by distance in some instances (Pogson *et al.* 2001) and current systems have been found to be important for shaping both temporally stable (e.g. Ruzzante *et al.* 1999) and variable (e.g. Knutsen *et al.* 2004) genetic structure. Finally, environmental transitions have often been found to be associated with significant genetic structuring (Riginos and Nachman 2001; Nielsen *et al.* 2003; Nielsen *et al.* 2004; Bekkevold *et al.* 2005; Johanneson and André 2006) implicating barriers to gene flow associated with adaptations to local environments. Thus, there appears to be numerous factors capable of structuring marine fish populations, but it has rarely been possible to evaluate the relative importance of these factors because often only a single factor has been investigated, or sampling schemes have not been extensive enough to allow the investigation of several factors. In a few cases, however, several mechanisms have been assessed simultaneously. These studies have found that several mechanisms are indeed interacting to structure populations within the same species (Riginos and Nachmann 2001; Roques *et al.* 2002). In the study of population structuring n

European flounder (Manuscript I), we also found evidence of the simultaneous operation of several factors, such as population history, life-history and current systems, demonstrating that the outcome of this interplay depends strongly on the specific setting. The effect of environmental differences has been evaluated specifically in relation to geographical distance in some cases (Bekkevold *et al.* 2005; Jørgensen *et al.* 2005; Manuscript I). These studies have found that environmental factors rather than geographical distance *per se* seem to be significantly associated with genetic structure. While, these results suggest that environmental parameters are responsible for generating genetic structure they do not prove that the associations are caused by restrictions in gene flow between locally adapted populations because the markers applied are believed to be selectively neutral.

Local adaptations in marine fishes

It is evident from the section above that we are now fairly sure that many species of marine fish are structured into discrete units. Often the genetic difference between these units are not large, but they are consistent (e.g. when comparing different species in the same area) and, importantly, appear to be temporally stable in many cases (e.g. Poulsen *et al.* 2006; Ruzzante *et al.* 2006). Conversely, we know relatively little about what this structure means in terms of adaptive population divergence, because very few studies have addressed this question specifically. The main reason for this lack of knowledge is probably the general time-lag between studies on marine and terrestrial conservation genetics (Avice 1998), but the general picture of high levels of gene flow in marine fishes has most likely also resulted in limited interest in this field, since gene flow is expected to hamper local adaptations. However, it is important to recognize that local

adaptations arise and are maintained as a result of interplaying evolutionary forces. The large effective population sizes of many marine fishes tend to increase the importance of selection over the random process of genetic drift. The level of adaptive divergence is furthermore affected by environmental differences (i.e. the selection differential) between populations, so basically any outcome is possible, even in situations of high levels of gene flow. Since marine species often have very wide distributions it is likely that fish in different parts of the range may experience very different selection regimes (Palumbi 1994), thereby increasing the potential importance of natural selection.

Evidence for local adaptations

In fishes, local adaptations have mainly been investigated in freshwater and anadromous species where research has been devoted at identifying local populations, mainly for conservation purposes. In salmonids, for example, the marked genetic structuring found between different river or tributary populations is thought to reflect that many of these populations are also locally adapted (Taylor 1991; Adkison 1995; Church and Taylor 2002). Even though it is now widely accepted that genetically distinct salmonid populations are also locally adapted at the genetic level, it has only been thoroughly evaluated and demonstrated in a few cases that the genetic structure at neutral markers also translates into differences in the genes subject to selection and thereby directly involved in local adaptations (e.g. Koskinen *et al.* 2002; McGinnity *et al.* 2003; McGinnity *et al.* 2004). As is the case in marine fishes, this is mainly because such a demonstration is not straightforward. But unlike the case in many marine species, salmonids have traditionally been kept in aquaculture and have thus been easier to manipulate in large scale ecological and genetic studies.

Furthermore, the focus of the aquaculture industry on this group of fishes has led to an increased effort to map and understand the genetic architecture behind adaptive traits.

Only few studies have examined genetically based differences in adaptive traits between populations of marine fishes. One such example is found in the common killifish, *Fundulus heteroclitus*. Here, the molecular basis for changes in both enzyme transcription and activity has been identified (e.g. Schulte *et al.* 2000; Schulte 2001). For example, a single base mutation in the promoter region of the *Ldh-B* gene results in adaptive differences between Northern and Southern populations in the regulation in this gene. *F. heteroclitus* probably represents the most well studied marine species with respect to local adaptations. As a model species in comparative physiology and evolutionary genetics, most *Fundulus* research has compared Northern and Southern subpopulations (sometimes referred to as subspecies). These are believed to have diverged between 0.5 and 1 Million years ago (Bernardi *et al.* 1993). Combined with stationary behaviour, this translates into very high levels of population structuring at neutral markers (Pairwise $F_{ST} \approx 24\%$; Whitehead and Crawford 2006). Hence, while the research on *Fundulus* has provided exceptional insights into the action of natural selection in the marine environment, results may not necessarily be easily transferred to classical marine fishes, which often have younger population ages and lower levels of population structure/higher levels of gene flow, as evidenced in the sections above.

Studies of counter-gradient variation in life history traits have found that population differences are maintained by selection in several species (e.g. Conover 1998; Marcil *et al.* 2006). These results suggest that local adaptations may in fact be common in the marine environment despite phenotypic similarity between individuals from different

populations (Conover 1998). However, while demonstrating local adaptations, these studies have not revealed anything about its genetic architecture.

In order to elucidate the genetic footprints of local adaptations, we applied a candidate gene approach to study local adaptations in European flounder (Manuscript II). We found strong indications of local adaptations to the Baltic Sea environment by comparing genetic differentiation at neutral microsatellite loci with that at a candidate gene locus for adaptive divergence. However, besides these few examples, there is a marked shortage on studies investigating the genetic basis of adaptive population divergence in marine fishes.

Studies on *Fundulus heteroclitus* and countergradient variation in general have been concentrated on latitudinal environmental gradients, which illustrates that temperature has been the most extensively studied environmental component in relation to local adaptations in marine fishes. However, many marine species inhabit other environmental gradients as well. Estuaries and lagoons represent interesting study systems with respect to temperature, salinity and also often pollutants. Salinity has been found to be an important selective force in many studies of allozyme polymorphisms in mussels (Koehn *et al.* 1980; Hilbish and Koehn 1985), and hence these systems could be good places to start searching for local adaptations in marine fishes. Other gradients, such as photoperiod, depth, degree of disturbance etc all represent interesting environmental parameters worth future investigations, but have so far not been investigated.

Methods to detect local adaptations

The complicated interplay between genetic and environmental components of phenotypic variation makes studies of the genetic basis of

local adaptations in non-model organisms very challenging (e.g. Falconer and Mackay 1996). Various approaches have been and can be applied, ranging from demonstrating the presence of local adaptations at the phenotypic level to indirect quantification of its genetic component and investigations of the causing polymorphisms at the level of DNA itself.

Neutral genetic markers

It has been debated if neutral markers can be used to predict the level of population divergence at loci under adaptive evolution (e.g. Merilä and Crnokrak 2001; Reed and Frankham 2001; McKay and Latta 2002). However, even if such a correlation should exist, it is most likely not perfect because different selective forces are affecting the two types of loci. Therefore, most researchers who are interested in local adaptation of fishes and employ neutral genetic markers are restricted to make inferences about the *potential* for local adaptations. Valuable insights into the conditions permitting local adaptations have been obtained by incorporating genetic and ecological or environmental information in models to predict the scenarios in which local adaptations are most likely to occur. For example, if populations are small, genetic drift will be more important than local selection and hence local adaptations are not predicted at the population level. However, if several populations are connected through some gene flow and furthermore experience the same selective regimes, then adaptations to the common environmental conditions are much more likely (e.g. Hansen *et al.* 2002). However, this particular approach could be difficult to apply in many marine fishes, which are often exposed to clinal rather than discrete environmental variation, complicating a separation into distinct environmental groupings.

Important information have been gained with the ability to retrieve DNA from archived

samples, since it is now possible to assess the temporal stability of population structure without the need for a long-term sampling effort (e.g. Hutchinson *et al.* 2003, Poulsen *et al.* 2006). Even though the period assayed may be quite short on evolutionary time scales, this approach does offer some unique possibilities, since local adaptations are clearly more likely to arise if population structure is temporally stable. Furthermore, and as noted earlier, some studies have demonstrated a significant correlation between genetic structure and environmental parameters, indicating that the specific variables may be involved in driving adaptive population divergence (Bekkevold *et al.* 2005; Jørgensen *et al.* 2005; Manuscript I). Clearly temporally stable population structure associated with drastic environmental transition indicates local adaptation, but does not prove it, since the markers applied are not directly affected by natural selection. It is therefore expected that research on local adaptations in marine fishes will apply other methods targeted at answering these questions more specifically in the future.

Transplantations and common gardens

A quite simple, but often logistically complicated, method to assess the degree of adaptation to different habitats is to conduct (reciprocal) transplants and compare fitness of introduced and native populations in the respective environments (Endler 1977). This is most easily done in model or laboratory held species even though some large scale experiments have been conducted on salmon (Altukhov and Salmenkova 1990). In an extensive study, Hendry *et al.* (2002) also used transplantations to assess the importance of selection and gene flow for creating genetic differences between lake and stream ecomorphs of sticklebacks. In marine fishes, this approach has rarely been applied, and although transplants have been conducted in some

species (e.g. Schmidt 1917) its applicability seems fairly limited in most species.

An alternative to transplants is to bring individuals from different populations under common environmental conditions. This strategy has been applied in Atlantic silversides and other species to study countergradient variation in life history traits. One of the best known examples is growth rate in Atlantic silverside, where populations show adaptive and clinal variation in growth rates (reviewed in Conover 1998). The findings from silversides have been confirmed in other traits and species (e.g. Conover et al. 1997; DiMichele and Westerman 1997; Salvanes et al. 2004; Marcil et al. 2006), indicating that this may in fact be a general phenomenon.

Outbreeding depression

An assessment of the severity of outbreeding depression is another approach, which does not necessarily employ genetic markers. Hybrids from crosses between presumed adaptively diverged populations are expected to show reduced fitness when compared to the original populations. This reduction is mainly caused by the break-up of coevolved gene complexes, and hence may first become assessable in F_2 hybrids (Gharrett and Smoker 1991). Many studies have concentrated on salmonids, where some have assessed outbreeding depression in crosses between different natural populations (e.g. Gharrett et al. 1999; Gilk et al. 2004), while other studies concerns the presumed detrimental effects that non-native fish may have on wild conspecific populations. A recent study showed that both hybrids from farm x wild crosses, backcrosses to both kinds of parents and the F_2 s had a marked reduction in lifetime fitness in nature, underlining the potential devastating effects that farmed fish could have on wild fish populations (McGinnity et al. 2003).

The long generation times of many marine fishes makes the methods based on

common gardens reviewed here logistically difficult to employ in this group of species. Furthermore, while these methods demonstrate that populations may be locally adapted, they reveal little about the genetic architecture underlying these adaptations. Other approaches must be taken to achieve this goal.

Q_{ST} vs. F_{ST}

The magnitude of adaptive divergence can be assessed by comparing the differentiation of presumed neutral loci (F_{ST}) with the differentiation at the genetic component of the quantitative genetic variance (Q_{ST}). This approach was first proposed by Spitze (1993), who used it to demonstrate adaptive divergence of *Daphnia* populations, but has since been applied in a large range of species.

The approach requires the separation of the various components of the quantitative phenotypic variance (Falconer and Mackay 1996). Only the additive genetic variance (V_A) responds to selection, so a Q_{ST} vs. F_{ST} comparison will need a fairly good estimate of V_A , which is often assayed in common garden experiments. Once an estimate of V_A is obtained, the comparison with the variance of the neutral markers is straightforward, the idea being that a Q_{ST} larger than the F_{ST} would implicate adaptive differentiation, while $Q_{ST}=F_{ST}$ signals differentiation due to neutral evolutionary forces (genetic drift) only. A Q_{ST} smaller than F_{ST} implicates the operation of stabilizing selection on the traits studied (Spitze 1993).

This approach is obviously most useful in species which are easily kept in common gardens. Hence, it has also primarily been applied in plants or, for instance, *Drosophila* or *Daphnia* among animals (reviewed in Merilä and Crnokrak 2001; McKay and Latta 2002). Few studies have applied the approach in fishes. Koskinen et al. (2002) showed that Q_{ST} for many life history traits in small grayling populations only isolated a few (10-20)

generations ago greatly exceeded the F_{ST} estimates from microsatellites, implying that natural selection had been responsible for the rapid divergence observed between these populations. Likewise, Rogers *et al.* (2002) found that Q_{ST} for behaviour related traits was significantly larger than F_{ST} between whitefish ecomorphs.

There are, however, several potential problems with this approach. One is that Q_{ST} may in fact not equal F_{ST} under neutrality, for instance in situations with high mutation rates and low migration rates. Furthermore, different conclusions could be reached depending on the types of neutral markers applied, because different markers have very different mutation rates and mechanisms (Hendry 2002). Another problem is that Q_{ST} estimates have been found to be quite imprecise under most study designs realistically carried out in marine fishes (O'Hara and Merilä 2005). Finally, V_A estimates are environment and population specific (Falconer and MacKay 1996) making generalisations from such studies difficult.

To our knowledge, this approach has never been applied in marine fishes, and although it could relatively easily be done in aquaculture species, the approach seems to have lost some of its popularity in recent years, probably because of some of the problematic issues mentioned above. Furthermore, logistical constraints will seriously limit the number of marine fish species, where this approach could be applied.

Gene expression

A change in gene expression is regarded as an important component of an organism's ability to acclimate and adapt to novel or changed environmental conditions (Ferea *et al.* 1999; Schulte 2004; Whitehead and Crawford 2006b).

Hence, the analyses of expression of specific target genes have been studied in individuals from different populations in common environments to investigate adaptive

differences. A very illustrative example among marine fishes is the common killifish (*Fundulus heteroclitus*), which has been found to show consistent differences between Northern and Southern populations in gene expression for a number of genes of adaptive importance (e.g. Schulte *et al.* 2000, Fangue *et al.* 2006). Furthermore, a large number of genes have been investigated in other marine fishes, primarily those of interest to aquaculture (e.g. Deane and Woo 2005; Olsvik *et al.* 2006; Hall *et al.* 2003)

Techniques to study global gene expression are now also available in species of little relevance for drug discovery or as models for development (Gibson 2002). Studies in non-model species offer unique possibilities for dissecting the evolution of gene transcription between and within species (Ranz and Machado 2006), thereby making it possible to explore the genetic basis of adaptive divergence in gene expression (e.g. Whitehead and Crawford 2006b).

Microarrays have been developed for a few fishes (e.g. Atlantic salmon, Rise *et al.* 2004; killifish, Oleksiak *et al.* 2002; carp, Gracey *et al.* 2004; zebrafish, Ton *et al.* 2002; catfish, Li and Waldbieser 2006; medaka, Kimura *et al.* 2004), but unfortunately only for very few marine species (European flounder, Williams *et al.* 2003; Japanese flounder, Kurobe *et al.* 2005).

Until now, microarrays have only been used on natural populations in *Fundulus heteroclitus* among marine fishes. These studies have contributed significantly to an understanding of how genetic variation in gene expression is distributed within and between natural populations (Whitehead and Crawford 2006b). Oleksiak and colleagues (2002) thus found a correlation between variation in gene expression within and between populations for most genes, when comparing Northern and Southern populations of *Fundulus heteroclitus*; a pattern also seen in primates and believed to

reflect neutral evolution of gene expression (Khaitovich *et al.* 2006). However, Oleksiak *et al.* (2002) also identified interesting outlier loci, which were not influenced by genetic drift alone. Some of these genes furthermore showed similar expression patterns in Southern populations of a sister species of *F. heteroclitus*, while being different from Northern *F. heteroclitus*. These expression patterns further support the conclusion they are under adaptive evolution. Similarly, Whitehead and Crawford (2006a) have recently identified differentially expressed genes, which could not be explained by drift alone, along a latitudinal temperature gradient in *F. heteroclitus*, supporting the conclusions from earlier studies of local adaptations via gene expression in the species.

However, analysis of global gene expression is not without its problems either. First of all, studies rely on the assumption that gene expression is heritable, but this is rarely tested in the specific setting (Gibson and Weir 2005), because studies in non-model organisms are almost always carried out in one generation only. Hence, it is difficult to rule out non additive genetic effects such as maternal effects, epistatic interactions and gene by environment interactions (Gibson and Weir 2005).

Furthermore, it has been argued that there may be little coherence between mRNA expression levels and actual activity of the protein in the cells, which is the resulting phenotype (Feder and Walser 2005). A correlation between mRNA and protein activity is often an assumption not tested in microarray studies, but if it proves to be the case that the two levels are not correlated, gene expression data should be interpreted with great caution. Finally, it should be noted that measures of gene expression produce a phenotype of an individual. Hence, to be useful for demonstrating local adaptations in marine fishes, a controlled environment is required,

much like any of the other phenotype based approaches discussed above.

It is doubtful, if microarrays will be available for many marine species in the near future. This shortage on resources could limit the method's applicability as a general tool for studying local adaptations in marine fishes. However, cross species hybridization to cDNA arrays has been reported to be successful (Renn *et al.* 2004), so this technical obstacle may be overcome in species where arrays are available in close relatives. Alternatively, new methods to study quantitative gene expression at a global level have recently been developed, and these could prove to be very valuable alternatives to microarrays in non model organisms. Although differential display (Liang and Pardee 1992) and cDNA-AFLP (Bachem *et al.* 1996) have been available for some years, they have primarily been used to identify specific candidate genes which are differentially expressed. However, it was recently suggested that these techniques could also be used to study quantitative gene expression (Breyne *et al.* 2003; Venkatesh *et al.* 2005), and they have in fact been shown to produce data of equal quality to those obtained from microarrays (Breyne *et al.* 2003). Since differential display and cDNA-AFLP do not require specialized equipment and could be applied to all species relatively easily, these latest developments appear very promising for marine fish. Results from *Fundulus* are promising for future applications of this approach in marine fishes, provided that technical resources, such as non-array based techniques to study gene expression, become available for studies in non-model organisms.

Genome scans

Recently, much attention has been centred on ways to identify markers potentially under selection in genome scans (reviews in Luikart *et al.* 2003; Storz 2005) applying microsatellites (Kauer *et al.* 2003, Vasemägi *et*

al. 2005), AFLPs (Campbell and Bernatchez 2004) or single nucleotide polymorphisms (SNPs) (Akey *et al.* 2002). The principle behind these approaches is genetic hitch-hiking, where a marker linked to a mutation under selection shows non-neutral patterns of variation (Maynard Smith and Haigh 1974). The classical Lewontin-Krakauer test is based on the assumption that loci under positive selection will show increased levels of population divergence (Lewontin and Krakauer 1973). Thus, based on the observed distribution of F_{ST} values across the panel of loci studied, outliers can be detected based on comparisons to expectations under neutrality. This particular test has now been almost totally abandoned because of heavy criticism over its potential sensitivity to population structure (reviewed in Guinand *et al.* 2004; Vasemägi and Primmer 2005). However, new developments (e.g. Beaumont and Nichols 1996; Vitalis *et al.* 2001) have revived this analytical framework by introducing simulations to assess departures from neutral expectations taking population structure into account. An alternative strategy also based on principles of genetic hitch-hiking is to search for loci showing reduced variability in certain populations. Thus, the LnRH and LnRV tests (Schlötterer 2002; Kauer *et al.* 2003) were developed specifically for microsatellites to identify loci showing abnormal variance in heterozygosity or repeat lengths when conducting pairwise population comparisons.

The usefulness of genome scans has recently been questioned, particularly because of potential problems with ascertainment bias, population sub-structure and large numbers of false positives (Kelley *et al.* 2006; Teshima *et al.* 2006) and hence they should be applied with caution, and results should be verified by alternative analyses. For example, similar signatures from independent population comparisons strongly suggest a true signal rather than any of the alternative explanations

(e.g Storz and Dubach 2004; Vasemägi *et al.* 2005; Bonin *et al.* 2006).

To our knowledge, genome scans have never been conducted on marine fishes, probably because fairly dense genome coverage is required for these approaches to be useful. Even though fish genomes are unlikely to reach the marker resolution currently applied in human genome scans (e.g. ~ 1.5 Million SNPs, Kelley *et al.* 2006), these approaches could be useful for studying local adaptations in marine fish populations if other types of markers are applied. Thus, Campbell and Bernatchez (2004) applied AFLPs in genome scans in whitefishes and identified several loci potentially undergoing adaptive evolution. Alternatively, microsatellite markers linked to EST sequences were applied in a genome scan of salmon populations to show that some of the loci had apparently diverged adaptively (Vasemägi *et al.* 2005). These resources should provide short cuts to cover a large part of marine fish genomes at relatively modest time and economic costs.

QTL mapping

If marker locations within the genome are known, the genome scan approach can be further refined to identify specific genomic regions involved in adaptive divergence. QTL mapping identifies genomic regions that are influencing a given quantitative trait and is hence a first step in understanding the genetic architecture of complicated phenotypic traits. Classic QTL mapping strategies requires an elaborate breeding programme in combination with adequate genetic markers such as microsatellites, AFLPs or SNPs (Mackay 2001). The successful identification of QTL allows inferences about the genetic architecture of local adaptations. QTL mapping has been combined with mRNA expression data, so-called “genetical genomics” (Jansen and Nap 2001), to construct eQTL (expression QTL) maps. These have recently been highlighted as

a useful tool for studying adaptive divergence (Vasemägi and Primmer 2005) by revealing the genetic architecture of transcriptional regulation. However, eQTL mapping has until now only been applied in model organisms (e.g. Yang *et al.* 2006).

In fishes, linkage maps have been constructed for well studied model species, such as sticklebacks (Peichel *et al.* 2001), cichlids (e.g. Albertson *et al.* 2003; Streelman *et al.* 2003) and whitefish (Rogers *et al.* 2001). In non-model fishes, single QTL have mostly been identified in species of relevance to aquaculture, and the traits involved have primarily been closely related to production, e.g. heat or cold resistance (Perry *et al.* 2001; Cnaani *et al.* 2003), spawning time (Sakamoto *et al.* 1999; O'Malley *et al.* 2003), growth (Cnaani *et al.* 2003; Reid *et al.* 2005) or disease resistance (Fuji *et al.* 2006). Once identified these QTL may be very useful for studies of the action of selection in natural populations of these or closely related species (Reid *et al.* 2005; Somorjai *et al.* 2003).

However, it is unknown if QTL identified under laboratory conditions are also important in natural populations experiencing very different environments (Malmberg *et al.* 2005; Slate 2005). In principle, it is possible to estimate relatedness based on molecular markers in wild populations (Ritland 2000) so the requirement for a known pedigree can be met under these conditions and hence QTL mapping can be done in wild populations (Slate 2005). This approach has successfully been applied in e.g. red deer (Slate *et al.* 2002). However, the very large effective population sizes and high levels of within population genetic variance make this an almost impossible task in most marine fishes.

Furthermore, even if QTL studies are successful, there is still a very long way to identifying the genes and nucleotides involved in local adaptations in marine fishes, since markers are often anonymous and single QTL

may cover several thousand bases as well as several genes or regulatory regions (e.g. Flint and Mott 2001; Erickson *et al.* 2004). Thus, the progress from QTL to QTN (Quantitative Trait Nucleotide, Mackay 2001) is an overwhelming task in species other than e.g. human and model organisms, for which genetic markers are abundant and/or which are easy to manipulate genetically (e.g. Glazier *et al.* 2002; Flint *et al.* 2005; Salvi and Tuberosa 2005). Even in these species, certain QTL, such as regulatory regions and other non-coding regions have practically been impossible to isolate at the nucleotide level (Salvi and Tuberosa 2005). Since these regions are in fact targeted by the eQTL strategy, it could be especially problematic to get all the way from eQTL to eQTN in these cases. Furthermore, strategies aiming at dissecting QTL into genes and gene interactions have only recently been applied in model species, where they have shown that the genetic architecture of quantitative traits may be even more complex than previously thought (e.g. Kroymann and Mitchell-Olds 2005; Malmberg *et al.* 2005). These findings are not encouraging for the applicability of the methods in non-model species.

As is the case with some of the approaches mentioned earlier, QTL-based strategies require common garden set-ups. Furthermore, it is required that the species can be kept and bred in the laboratory and that a dense set of markers is available. These are the main obstacles preventing the strategies reviewed here from becoming a general tool for dissecting the genetic basis of local adaptations in marine fishes.

There does not seem to be any marine fishes used as model species for systematic QTL mapping at the moment, but valuable resources may be generated in e.g. Japanese flounder (Fuji *et al.* 2006) or some gadoids in the years to come. AFLP markers, in particular, should be useful for mapping of both QTL and

eQTL (Vuylsteke *et al.* 2006) in marine fishes if these approaches are applied.

Admixture mapping

An alternative to QTL mapping is admixture mapping in wild admixed populations (Rieseberg and Buerkle 2002; Darvasi and Shifman 2005; Vasemägi and Primmer 2005). Admixture mapping removes the dependency on a known pedigree and takes advantage of linkage disequilibrium built up during many generations (Rieseberg and Buerkle 2002), but it still requires a dense marker map in the species under investigation and therefore suffers from some of the same potential problems as the QTL mapping approach in relation to marine fishes.

Until now, admixture mapping has primarily been applied in humans (Darvasi and Shifman 2005) and plants. One of the best known examples from plants involves admixture mapping in hybrid zones between two sunflower species (Rieseberg *et al.* 1999), but hybrid zones in forest trees have also been proposed to be suitable for admixture mapping (Lexer *et al.* 2004; Lexer *et al.* 2006). Hybrid zones constitute very suitable settings for admixture mapping because a large variety of hybrid categories can be found in a narrow zone of contact between genetically distinct populations (Rieseberg and Buerkle 2002).

Intra-specific hybrid zones have recently been described in marine fishes (Nielsen *et al.* 2003; Nielsen *et al.* 2004), and this setting should provide a very valuable resource for admixture mapping in several marine fish species, since many species are found to be significantly structured across the same environmental gradient (Nielsen *et al.* 2003; Nielsen *et al.* 2004; Bekkevold *et al.* 2005; Johanneson and André 2006), providing good opportunities to conduct comparative studies.

Genetic variation in candidate genes

The study of variation in candidate genes is the most direct way of studying complex quantitative traits (Mackay 2001). Genetic variation in candidate genes is subject to a number of different analytical strategies, depending on the kind of variation or marker assessed.

Variation can be assessed on the allele frequency or nucleotide level, examples of these types of tests are the Ewens-Watterson test (Watterson 1977) and Tajima's D-test (Tajima 1989) respectively. However, these tests are very sensitive to population demography and population subdivision and consequently have limited power for detecting the footprints of selection (Nielsen 2001). Alternative and more powerful tests are based on comparisons of synonymous to non-synonymous substitutions (reviewed in e.g. Skibinski 2000; Yang and Bielawski 2000). These tests are not confounded by population demographics and structure because they are focusing on a specific and short segment of the genome (Nielsen 2001). Refinements of these methods have increased power significantly by allowing specific sites to be tested rather than the entire gene (e.g. Swanson *et al.* 2001; Ross and Rodrigo 2002), thereby allowing functional interpretations of observed patterns of genetic variation (e.g. Swanson *et al.* 2001). Although originally developed and typically used for interspecies comparisons, also in fishes (see e.g. Bargelloni *et al.* 1998; Ford 2001), these tests could potentially be used to detect adaptive sequence divergence between populations within a species. However, this has only very rarely been attempted in fishes (e.g. Ford 2000), but with the continued efforts to refine and increase statistical power of these methods, they should be useful in the future.

Another classical way of inferring natural selection is the association of certain gene products with environmental parameters (Endler 1977). Studies on enzyme polymorphisms have illustrated the power of

this approach for inferring natural selection (Berry and Kreitman 1993; Gardner and Kathiravetpillai 1997; Gardner and Palmer 1998; Storz and Dubach 2004), but despite its potential it has not received much attention in marine fishes. A noteworthy exception is the haemoglobin 1 locus, which has been found to be non-randomly distributed between populations of cod (Sick 1965a,b), and especially temperature selection has been proposed to act on the haemoglobin genotypes (Karpov and Novikov 1981; Brix *et al.* 1998; Petersen and Steffensen 2003). Many of the above studies have identified allele frequency clines along latitudinal clines, illustrating that environmental parameters associated with latitude may have been important for shaping the observed distribution of genetic variation in the candidate genes.

We used a candidate gene approach to study adaptive divergence of European flounder populations (Manuscript II). We compared genetic variation in nine microsatellite loci with that at a marker linked to *Hsc70*, a heat shock cognate gene, and found that variation at the latter marker was non-neutrally distributed between populations. This strongly indicated adaptive divergence at this locus and therefore suggested local adaptations in the flounder populations.

The usefulness of the candidate gene approach for finding genes involved in specific diseases has been questioned (reviewed in Tabor *et al.* 2002). Since phenotypes often have a very complex genetic basis, single studies have had difficulties in identifying specific genes significantly associated with a given phenotype (e.g. Aguirre-Hernández and Sargan 2005). However, it should be emphasized that there is a fundamental difference between the classical candidate gene approach in disease research and the approach suggested here. The main difference is that in the latter case we need not be focused on elucidating the genetic basis of specific phenotypes, but can focus on

identifying abnormal behavior of the genetic markers themselves. Hence, by choosing genes expected to be important for local adaptations without focusing on specific phenotypes (which are probably not even known), we may increase our ability to identify patterns of genetic variation which is non-neutral.

A major problem with the candidate gene approach is ascertainment bias, which has been a major concern in genome wide surveys of genetic diversity in humans (Clark *et al.* 2005). Because SNPs are often identified in a sub-sample of individuals, rare alleles could be missed with consequences for estimation of parameters such as diversity and population differentiation (Nielsen 2004). Ascertainment bias can potentially be a problem when choosing candidate markers for local adaptations if markers are not chosen from a random and representative sub-sample of the total sample (van Tienderen *et al.* 2002; Morin *et al.* 2004). However, ascertainment bias cannot be totally eliminated unless all individuals are sequenced for all candidate genes. Since this is not a realistic strategy for the coming years, ascertainment bias should be taken into account when designing candidate gene studies. The good thing is, however, that experiences from the human genome projects have illustrated the severity of the problem as well as its impact on various downstream analyses under different ascertainment schemes (Nielsen 2004). Therefore, it is now possible to design sampling and screening for genetic variation based on acceptable levels of ascertainment bias. In general, it is important that markers are chosen based on a random sample of individuals from many populations in the survey (Morin *et al.* 2004). Furthermore, critical evaluation of results in relation to environmental components suspected to be involved in shaping the observed genetic variation should aid in assessing if observed patterns are indeed real or artefacts.

Another problem similar to QTL approaches is that even if a particular gene can be identified as a very likely candidate for being responsible for the genetic basis of local adaptations, there may still be a long way to understanding the true functional and physiological meaning of the observed genetic variation (e.g. Schulte 2001). Since not all amino acid substitutions are equally important to the function of a protein, knowledge of sequence differences may still be quite far away from the functional importance of these differences (Golding and Dean 1998). Moreover, the signal of selection may not stem from variations in the genes themselves but from variation in regulatory elements, which would obviously require very detailed knowledge about the genes' regulatory machinery. However, even when complete knowledge about the functional relationships between gene variants is not available, suitable candidate genes can still provide valuable information as a first step towards an understanding of adaptive divergence of populations (Ford 2002).

Even though this approach has only been applied in a few marine fishes, it does appear promising for future studies for several reasons. First of all, it can be applied to all species, provided that they can be accessed for sampling. Secondly, there appear to be plenty of suitable candidate genes to choose from (see e.g. Ford 2002, Moran 2002 and Box 2). Thirdly, tools are now also available for discovering genetic polymorphism in natural populations (e.g. Till *et al.* 2006) and these strategies should provide researchers with access to genetic resources also in non-model fishes. And finally, the high level of gene flow often observed between populations of marine fishes (Ward *et al.* 1994; Waples 1998) should actually provide good opportunities for detecting outlier loci based on the distribution of genetic variation within or between populations.

The importance of a neutral background

Regardless of the methodology adopted for the study of local adaptation it is of major importance to be able to rule out the possibility that an observed pattern is simply caused by random evolutionary forces (Landry and Bernatchez 2001; Moran 2002). This problem will obviously not affect all strategies to the same degree, but is particularly important to have in mind in candidate gene studies and studies of gene expression. Genome scans and QTL/admixture mapping approaches should take random forces into account by searching for deviations from the neutral signals in the data set.

The simplest way to ascertain that observed patterns are truly caused by natural selection is to generate a neutral background from markers expected to behave neutrally. The choice of marker for the neutral background is however not without its difficulties either. Even though microsatellites may be the neutral genetic marker of choice these years, they may not be particularly well suited as a neutral background for candidate gene studies applying other marker types such as SNPs. This is because different mutation rates and processes of the two types of markers could make interpretations of results difficult, unless results are very clear and supported by evidence other than different signals from the two marker types.

Strategies for separating global gene expression data into adaptive and neutrally evolving components have also been developed. Whitehead and Crawford (2006a) used a neutral baseline generated from microsatellites to estimate the variation between populations accounted for by phylogenetic relationships (genetic drift) before moving on to consider outliers associated with specific environmental parameters.

Box 2. How to find the needle in the haystack

A very critical step in any candidate gene strategy is to choose suitable candidates. Different approaches can be taken to find candidate genes for adaptive population divergence in marine fishes. First of all, since several studies have shown that allozymes may in fact not be selectively neutral, the known or suspected function of some allozymes makes them a very useful tool to infer the action of natural selection. Furthermore, the function of many allozymes is relatively well described, which makes these markers a good starting point for candidate gene studies. The studies on adaptive divergence in Ldh-B genes in *Fundulus heteroclitus* is probably the best example of this approach. In this species, several enzymes, including Ldh-B, show clear allele frequency clines with latitude (Powers and Place 1978), suggesting that the locus is under selection. Through a series of studies, the genetic basis of differences in enzyme activity has been dissected to identify specific responsible mutations (Schulte *et al.* 2000; Schulte 2001). While this example illustrates the power of the candidate gene approach, it also highlights the complexities involved in demonstrating the genetic basis of observed physiological differences, since the studies have been conducted over a period of more than 20 years.

An alternative strategy is to isolate novel candidate genes in the species under study. Two different strategies could be applied for this purpose. One strategy is to focus on genes with a central position in signalling pathways. These so-called “hub-genes” integrate signals from many different pathways/environmental components and have therefore been suggested to be good candidate genes for human disease (Lehner *et al.* 2006). Lehner *et al.* (2006) identified six genes with chromatin modelling function to be important in *C. elegans* and suggested that the same genes may well be important in other animals as well. Alternative hub-genes could be Heat shock protein genes or other stress hormones, since these genes are also integrating signals from many pathways (Rutherford and Lindquist 1998; Feder and Hofmann 1999; Queitsch *et al.* 2002; Sørensen *et al.* 2003). The newly discovered microRNAs (reviewed in e.g. He and Hannon 2004) have also been found to be both conserved between species (Pasquinelli *et al.* 2000; Berezikov *et al.* 2005) and to have central roles as regulators of the expression of many genes and pathways (e.g. Lim *et al.* 2005). Hence, these genes, or their target sites (Plasterk 2006), could also be interesting candidates in this category. Since these genes seem to be so essential for cellular functions they would also be expected to be under heavy selective constraints, allowing little variation within species. While this may be a discouraging fact, it also means that any genetic variation detected has a higher likelihood of having a phenotypic consequence.

Another strategy could be to apply candidate genes with a well defined and understood function in relation to specific environmental parameters. MHC in salmonids (e.g. Miller *et al.* 2001) is a classical example of this category. Moran (2002) and Ford (2002) suggest numerous other candidate genes which could be studied in natural populations of fishes (primarily salmonids). Alternatively, genetic polymorphisms with known phenotypic effects isolated in aquaculture species could be applied in natural populations, since many genes of interest to aquaculture, such as growth (e.g. Tao and Boulding 2003) and maturity and spawning (e.g. Leder *et al.* 2006), would also be useful for studies of local adaptations in marine fishes. Although relatively few marine fishes are subject to intense aquaculture efforts, candidate gene sequences should be applicable for primer design in closely related species (Ford 2002), especially if the chosen candidate genes are well conserved through evolution. However, if a specific marker is not already available, it could be difficult to identify causing polymorphisms, even if good candidate genes for a specific phenotype are available (e.g. Aguirre-Hernández and Sargan 2005), maybe because phenotypic effects of genetic variation in these genes are buffered by hub-genes, such as heat shock proteins (Rutherford and Lindquist 1998), thereby reducing selective constraints on the genes.

Alternatively, variation within and between populations can be compared for the expression of each gene separately. Generally, it is expected that neutral evolution will result in a correlation between the two measures, and outliers can then be identified as either showing more or less variation between populations than expected under neutrality (Oleksiak *et al.* 2002; Whitehead and Crawford 2006a). These strategies have been proposed as standard tools for separating gene expression data into neutral versus selected components (Whitehead and Crawford 2006a).

Complicating factors

As evidenced by the sections above, studies of the genetic basis of adaptive population divergence are very complicated and time consuming in most animals. However, marine fishes present researchers with several specific challenges, which deserve elaboration before moving on to discuss future developments within the field.

First of all, it will be difficult if not impossible to keep many marine species under controlled environmental conditions for several generations, as required by the phenotype based approaches discussed above. The reasons for this are that we know relatively little about the biology of many species and that many marine fishes have long generation times and special space or habitat requirements, which complicates studies in common garden environments. However, the few marine fishes already kept in aquaculture could be good candidates for these types of approaches (see next section).

Secondly, there is a general lack of genomic resources for marine fish species. Only few species are subject to systematic gene sequencing (Cossins and Crawford 2005), and classical marine species are not among the targeted species. Moreover, no marine fish genomes have yet been mapped to any

reasonable resolution (Clark 2003; Cossins and Crawford 2005). We therefore know relatively little about the organization and structure of fish genomes in general and marine fish genomes in particular.

Thirdly, all fish species are believed to have gone through at least one full round of genome duplications besides several independent and lineage specific duplications (Volf 2005). While many paralogous gene copies have since been lost, several hundreds are still functional, some having acquired new functions (Volf 2005). Several gene copies make both gene expression and sequence polymorphism based strategies more complicated because it may be difficult to separate different copies from different alleles within a copy. Na⁺K⁺-ATPase genes may be an interesting case of acquisition of new functions by gene duplications in fishes (Cutler and Cramb 2001). For instance, recent studies have shown that different isoforms may be important under different environmental conditions (Richards *et al.* 2003; Bystriansky *et al.* 2006; Morrison *et al.* 2006), illustrating one of the many complex aspects of gene regulation, which may be important for adaptations to local environments (Schulte 2004). Obviously, such complexity could pose serious technical and interpretational problems if undetected.

Future perspectives

Undoubtedly, future studies of population structure in marine fishes will apply more neutral markers, since these are getting still cheaper and faster to develop and genotype (e.g. Brumfield *et al.* 2003; Morin *et al.* 2004). However, while this will increase statistical power, it will not change the basic signal of little structure in many marine fish species. Since research the last few years has confirmed that there *is* genetic structuring among marine fish populations, it now seems to be the time to

move on to try and understand what this structure means. To achieve this goal, integrative approaches, such as combinations of neutral genetic and demographic, geographic or environmental information seem very promising. Furthermore, research specifically targeting the genetic basis of local adaptations is warranted, since we know very little about the scale and magnitude of adaptive population divergence in this high gene flow environment. For studies of the genetic basis of adaptive divergence in marine fishes to be successful, it would obviously be worthwhile focusing on resources already available as well as the special attributes of marine fishes, which provide good opportunities to study local adaptations, instead of trying to adopt strategies not easily applicable to marine fishes.

As emphasized above, several aspects of the biology of marine fishes and our understanding of this, makes it difficult to keep many of the species in captivity for several generations. This limits the number of species for which common garden set-up can be applied. However, a few marine species are subject to increasing aquaculture efforts (e.g. gadoids, Rosenlund and Skretting 2006), providing valuable settings to conduct these studies. As advocated by Vasemägi and Primmer (2005) there should be great advantages in combining some of the techniques discussed in the sections above in integrative approaches. For example, the application of EST linked markers in mapping studies could aid in identifying specific candidate genes within the QTL (Erickson *et al.* 2004). Alternatively, specific candidate gene markers could be used in combination with QTL mapping (Leder *et al.* 2006), thereby increasing the candidate status of specific QTL and genes. Rogers and Bernatchez (2005) have integrated traditional QTL analyses with a genome scan to identify QTL most likely to be associated with the ecological differentiation of dwarf and normal ecomorphs of whitefishes. In

another integrative strategy, Wayne and McIntyre (2002) combined mapping with microarray analyses to narrow the range of potential candidate genes for avariole numbers in *Drosophila*. In cases where microarrays are not available, alternative techniques, such as cDNA-AFLP, hold great promise for future applications in marine fishes. These techniques could also be used for discovering genes important for local adaptations (e.g. Cui *et al.* 2006). While these integrative strategies are promising for narrowing the range of candidates responsible for complex phenotypes and local adaptations in general it is doubtful if they will be applicable to most marine fishes in the nearest future because of time, space and economical constraints. However, less ambitious aims, such as studying the genetic architecture of local adaptations on a more coarse resolution (e.g. Rogers and Bernatchez 2005), should be feasible in some marine fishes in the near future.

In contrast to common garden based strategies, natural populations can be studied in all species, provided that they can be accessed for sampling. Furthermore, since several attributes of wild fish populations makes them ideal for applying some of the strategies outlined in the sections above, these settings hold great promise for future studies.

Since many marine fishes are distributed over very large geographical areas they often also experience diverse environmental conditions, which should provide excellent opportunities to study the effect of different environments on genetic structuring. To exploit this opportunity first and foremost requires that sampling is truly targeting a significant part of the environmental distribution of the species. If properly designed genome scan and candidate gene approaches should be very useful for disclosing adaptive population divergence in samples from natural populations.

Normally a dense distribution of markers over the entire genome is required for genome scans to be efficient. In most marine fishes such a map is not readily available, making a complete genome scan problematic. However, the newly developed AFLP markers (Vos *et al.* 1995) appear to be a particularly promising in non model organisms, since they are fast, reliable and provide reasonable genome coverage (Bensch and Åkeson 2005). While, until now, only applied in a few cases in marine fishes (e.g. eels, Albert *et al.* 2006), the advantages of AFLPs should make these markers preferable in marine fishes in the future (see e.g. Campbell and Bernatchez 2004). Other methods for identifying markers of potential interest have been proposed. For example, Vasemägi *et al.* (2005) used EST-linked microsatellites in a genome scan of Atlantic salmon populations and identified several potential candidate genes for adaptive population divergence. Since EST resources are building up quite rapidly in a number of fish species or can be generated relatively easily (e.g. Chen *et al.* 2005; Cossins and Crawford 2005) this strategy could be very useful for identifying candidates under selection in genome scans.

An alternative approach is to adopt a hybrid candidate gene/multi-locus approach by selecting specific candidate genes suspected to be important for adaptive population divergence and then survey these specific genes as well as putatively neutral markers for genetic variation in natural populations. This method would provide hints on genes and processes directly involved in adaptation and aid in finding specific nucleotides responsible. Such a strategy was recently used by Anderson *et al.* (2005) to study adaptive divergence of malaria parasites in response to drug treatments. The authors compared putatively neutral SNPs with SNPs in known genes for drug resistance and found that the latter clearly exhibited non-neutral behavior, strongly indicating adaptive

divergence. We used a similar approach for studying adaptive divergence in European flounder by comparing genetic structuring at neutral microsatellite markers to structuring at a *Hsc70* gene, which was chosen as a candidate gene for adaptive divergence (Manuscript II), and found strong indications of local adaptations in flounder populations. The results from the study suggest that this approach has a great potential in non-model species such as many marine fishes, since relatively few markers are required. In future studies, it is expected that marker development will proceed at increased speeds, making it relatively easy to isolate and characterize genetic markers such as SNPs in non-model species (e.g. Till *et al.* 2006). These techniques should be very useful, since specific genes of interest can be targeted in combination with putative neutral regions.

Furthermore, it would be logical to take advantage of the neutral baselines which have already been generated for a large number of species. These data sets represent valuable information which could be applied in combination with candidate gene variation. In this case, neutral markers and markers in candidate genes should preferably be comparable with respect to mutational properties and mutation rates. Since many studies of population structure have applied microsatellites, it would be preferable to use this marker type linked to candidate genes, for instance applying the EST based strategy proposed by Vasemägi *et al.* (2005). It should, however, be kept in mind that the original studies were not necessarily designed to capture the total environmental heterogeneity experienced by the species. Original sample material may therefore need to be supplemented with new samples from poorly covered areas in order for this strategy to be successfully applied.

Another excellent setting available in marine fishes is found in some of the hybrid zones already described (e.g. Schulte 2001;

Nielsen *et al.* 2003; Nielsen *et al.* 2004). These areas and the species living in them should provide a good opportunity for applying an admixture mapping strategy in wild populations. Furthermore, the fact that hybrid zones may be shared between several species (Nielsen *et al.* 2004; Johanneson and André 2006) will be a very good setting for conducting comparative studies which could further strengthen the evidence that genetic divergence is truly adaptive (see e.g. Schulte 2001) and which could shed important light on the generality versus species specificity of the genetic architecture underlying local adaptations. The previously mentioned advantages of AFLPs make these markers well suited for admixture mapping strategies in marine fishes.

Finally, it would be very interesting to add a temporal dimension to studies of local adaptation in marine fishes, for instance in relation to human induced evolutionary forces such as climate change and fishery induced selection. In this case, only DNA based techniques would be useful, since archived material (such as scales, otoliths and bones) should be used for comparisons to contemporary samples. This opportunity has not previously been explored in marine fishes, but it could relatively easily be applied, if markers are designed to amplify in samples of partly degraded DNA. Earlier studies using microsatellites (Ruzzante *et al.* 2001; Hutchinson *et al.* 2003; Poulsen *et al.* 2006) indicate that this should be a feasible approach applied to candidate genes as well.

Thus, there appears to be plenty of opportunities for designing high-quality studies of the genetic basis of local adaptations in marine fishes. Particularly studies in natural populations appear promising, since these strategies are in principle feasible in any species. Such studies would gain considerable power if the same signals are detected in independent population comparisons (e.g. Storz and Dubach 2004; Bonin *et al.* 2006), and marine fishes do provide several opportunities for designing comparative studies within and across species. But regardless of the strategy adopted, there is a need for a deeper physiological understanding of local adaptations. In order to achieve this goal, it will be necessary to initialize cross disciplinary projects which are integrating population genetics with evolutionary and physiological genetics. These research fields have largely been asking and answering similar questions, but have tended to communicate very little across disciplines, for instance by publishing in different scientific journals. An integration of these areas would improve our understanding of the genetic and physiological basis of local adaptations from individual cells to systems in the marine environment (Pörtner 2002). By providing new insights into how selection operates in marine fishes, these studies will further our understanding of evolution in this high gene flow environment and should provide indispensable tools for management of marine biodiversity in the future.

References

- Adkison MD. 1995. Population differentiation in Pacific salmon: Local adaptation, genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences* 52: 2762-2777.
- Aguirre-Hernández J, Sargan DR. 2005. Evaluation of candidate genes in the absence of positional information: A poor bet on a blind dog! *Journal of Heredity* 96: 475-484.
- Akey JM, Zhang G, Zhang K, Jin L, Shriver MD. 2002. Interrogating a high-density SNP map for signatures of natural selection. *Genome Research* 12: 1805-1814.
- Albert V, Jónsson B, Bernatchez L. 2006. Natural hybrids in Atlantic eels (*Anguilla anguilla*, *A. rostrata*): evidence for successful reproduction and fluctuating abundance in space and time. *Molecular Ecology* 15: 1903-1916.
- Albertson RC, Streebman JT, Kocher TD. 2003. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proceedings of the National Academy of Sciences of the USA* 100: 5252-5257.
- Altukhov YP, Salmenkova EA. 1990. Introductions of distinct Stocks of chum salmon, *Oncorhynchus-Keta* (Walbaum), into natural populations of the species. *Journal of Fish Biology* 37: 25-33.
- Anderson TJC, Nair S, Sudimack D, Williams JT, Mayxay M, Newton PN, Guthmann JP, Smithuis FM, Hien TT, van den Broek IVF, White NJ, Nosten F. 2005. Geographical distribution of selected and putatively neutral SNPs in Southeast Asian malaria parasites. *Molecular Biology and Evolution* 22: 2362-2374.
- Árnason E. 2004. Mitochondrial cytochrome b DNA variation in the high-fecundity Atlantic cod: Trans-Atlantic clines and shallow gene genealogy. *Genetics* 166: 1871-1885.
- Aro E. 1989. A review of fish migration patterns in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer* 190: 72-96.
- Awise JC. 1998. Conservation genetics in the marine realm. *Journal of Heredity* 89: 377-382.
- Bachem CWB, vanderHoeven RS, deBruijn SM, Vreugdenhil D, Zabeau M, Visser RGF. 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. *Plant Journal* 9: 745-753.
- Bargelloni L, Marcato S, Patarnello T. 1998. Antarctic fish hemoglobins: Evidence for adaptive evolution at subzero temperature. *Proceedings of the National Academy of Sciences of the USA* 95: 8670-8675.
- Beaumont MA, Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London B* 263: 1619-1626.
- Bekkevold D, Andre C, Dahlgren TG, Clausen LAW, Torstensen E, Mosegaard H, Carvalho GR, Christensen TB, Norlinder E, Ruzzante DE. 2005. Environmental correlates of population differentiation in Atlantic herring. *Evolution* 59: 2656-2668.
- Bensch S, Åkeson M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology* 14: 2899-2914.
- Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RHA, Cuppen E. 2005. Phylogenetic shadowing and computational identification of human microRNA. *Genes. Cell* 120: 21-24.
- Bernardi G, Sordino P, Powers DA. 1993. Concordant mitochondrial and nuclear-DNA phylogenies for populations of the teleost fish *Fundulus heteroclitus*. *Proceedings of the National Academy of Sciences of the USA* 90: 9271-9274.
- Berry A, Kreitman M. 1993. Molecular analysis of an allozyme cline - alcohol-dehydrogenase in *Drosophila melanogaster* on the East coast of North America. *Genetics* 134: 869-893.
- Bonin A, Taberlet P, Miaud C, Pompanon F. 2006. Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). *Molecular Biology and Evolution* 23: 773-783.

- Breyne P, Dreesen R, Cannoot B, Rombaut D, Vandepoele K, Rombauts S, Vanderhaeghen R, Inze D, Zabeau M. 2003. Quantitative cDNA-AFLP analysis for genome-wide expression studies. *Molecular Genetics and Genomics* 269: 173-179.
- Brix O, Forås E, Strand I. 1998. Genetic variation and functional properties of Atlantic cod hemoglobins: Introducing a modified tonometric method for studying fragile hemoglobins. *Comparative Biochemistry and Physiology - A* 119: 575-583.
- Brumfield RT, Beerli P, Nickerson DA, Edwards SV. 2003. The utility of single nucleotide polymorphisms in inferences of population history. *Trends in Ecology & Evolution* 18: 249-256.
- Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD. 2005. Limited realized dispersal and introgressive hybridization influence genetic structure and conservation strategies for brown rockfish, *Sebastes auriculatus*. *Conservation Genetics* 6: 697-713.
- Bystriansky JS, Richards JG, Schulte PM, Ballantyne JS. 2006. Reciprocal expression of gill Na⁺/K⁺-ATPase alpha-subunit isoforms alpha 1a and alpha 1b during seawater acclimation of three salmonid fishes that vary in their salinity tolerance. *Journal of Experimental Biology* 209: 1848-1858.
- Campbell D, Bernatchez L. 2005. Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution* 21: 945-956.
- Carvalho GR, Hauser L. 1998. Advances in the molecular analysis of fish population structure. *Italian Journal of Zoology* 65: Suppl. 21-33.
- Chen SL, Liu YG, Xu MY, Li J. 2005. Isolation and characterization of polymorphic microsatellite loci from an EST-library of red sea bream (*Chrysophrys major*) and cross-species amplification. *Molecular Ecology Notes* 5: 215-217.
- Christiansen FB, Frydenberg O, Hjorth JP, Simonsen V. 1976. Genetics of *Zoarces* populations. 9. Geographic variation at 3 phosphoglucose loci. *Hereditas* 83: 245-255.
- Church SA, Taylor DR. 2002. The evolution of reproductive isolation in spatially structured populations. *Evolution* 56: 1859-1862.
- Cimmaruta R, Bondanelli P, Nascetti G. 2005. Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). *Molecular Ecology* 14: 2577-2591.
- Clark AG, Hubisz MJ, Bustamante CD, Williamson SH, Nielsen R. 2005. Ascertainment bias in studies of human genome-wide polymorphism. *Genome Research* 15: 1496-1502.
- Clark MS. 2003. Genomics and mapping of Teleostei (bony fish). *Comparative and Functional Genomics* 4: 182-193.
- Cnaani A, Hallerman EM, Ron M, Weller JI, Indelman M, Kashi Y, Gall GAE, Hulata G. 2003. Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid. *Aquaculture* 223: 117-128.
- Conover DO, Brown JJ, Ehtisham A. 1997. Countergradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2401-2409.
- Conover DO. 1998. Local adaptation in marine fishes: Evidence and implications for stock enhancement. *Bulletin of Marine Science* 62: 477-493.
- Corander J, Walmann P, Sillanpaa MJ. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* 163: 367-374.
- Cossins AR, Crawford DL. 2005. Opinion - Fish as models for environmental genomics. *Nature Reviews Genetics* 6: 324-333.
- Coulon A, Guillot G, Cosson JF, Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM. 2006. Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology* 15: 1669-1679.
- Cui JZ, Shen XY, Gong QL, Yang GP, Gu QQ. 2006. Identification of sex markers by cDNA-AFLP in *Takifugu rubripes*. *Aquaculture* 257: 30-36.

- Cutler CP, Cramb G. 2001. Molecular physiology of osmoregulation in eels and other teleosts: the role of transporter isoforms and gene duplication. *Comparative Biochemistry and Physiology Part A* 130: 551-564.
- Darvasi A, Shifman S. 2005. The beauty of admixture. *Nature Genetics* 37: 118-119.
- Deane EE, Woo NYS. 2005. Cloning and characterization of the hsp70 multigene family from silver sea bream: Modulated gene expression between warm and cold temperature acclimation. *Biophysical Research Communications* 333: 776-783.
- DeWoody JA, Avise JC. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of fish biology* 56: 461-473.
- DiMichele L, Paynter KT, Powers DA. 1991. Evidence of lactate dehydrogenase-B allozyme effects in the Teleost, *Fundulus heteroclitus*. *Science* 253: 898-900.
- DiMichele L, Westerman ME. 1997. Geographic variation in development rate between populations of the teleost *Fundulus heteroclitus*. *Marine Biology* 128: 1-7.
- Dulvy NK, Sadovy Y, Reynolds JD. 2003. Extinction vulnerability in marine populations. *Fish and Fisheries* 4: 25-64.
- Endler JA. 1977. *Natural selection in the wild*. Princeton University Press, Princeton.
- Erickson DL, Fenster CB, Stenoien HK, Price D. 2004. Quantitative trait locus analyses and the study of evolutionary process. *Molecular Ecology* 13: 2505-2522.
- Excoffier L, Heckel G. 2006. Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics* 7: 745-758.
- Falconer DS, Mackay TFC. 1996. *Introduction to Quantitative Genetics*. Longman, England.
- Fangue NA, Hofmeister M, Schulte PM. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *Journal of Experimental Biology* 209: 2859-2872.
- Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review in Physiology* 61: 243-282.
- Feder ME, Walser JC. 2005. The biological limitations of transcriptomics in elucidating stress and stress responses. *Journal of Evolutionary Biology* 18: 901-910.
- Ferea TL, Botstein D, Brown PO, Rosenzweig RF. 1999. Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proceedings of the National Academy of Sciences of the USA* 96: 9721-9726.
- Flint J, Mott R. 2001. Finding the molecular basis of quantitative traits: Successes and pitfalls. *Nature Reviews genetics* 2: 437-445.
- Flint J, Valdar W, Shifman S, Mott R. 2005. Strategies for mapping and cloning quantitative trait genes in rodents. *Nature Reviews Genetics* 6: 271-286.
- Ford MJ. 2000. Effects of natural selection on patterns of DNA sequence variation at the transferrin, somatolactin, and p53 genes within and among chinook salmon (*Oncorhynchus tshawytscha*) populations. *Molecular Ecology* 9: 843-855.
- Ford MJ. 2001. Molecular evolution of transferrin: Evidence for positive selection in salmonids. *Molecular Biology and Evolution* 18: 639-647.
- Ford MJ. 2002. Applications of selective neutrality tests to molecular ecology. *Molecular Ecology* 11: 1245-1262.
- Fuji K, Kobayashi K, Hasegawa O, Coimbra MRM, Sakamoto T, Okamoto N. 2006. Identification of a single major genetic locus controlling the resistance to lymphocystis disease in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 254: 203-210.
- Gaggiotti OE, Brooks SP, Amos W, Harwood J. 2004. Combining demographic, environmental and genetic data to test hypotheses about colonization events in metapopulations. *Molecular Ecology* 13: 811-825.
- Galindo HM, Olson DB, Palumbi SR. 2006. Seascape Genetics: A coupled oceanographic-genetic model predicts population structure of

- Caribbean corals. *Current Biology* 16: 1622–1626.
- Gardner JPA. 1997. Hybridization in the sea. *Advances in Marine Biology* 31: 1-78.
- Gardner JPA, Kathiravetpillai G. 1997. Biochemical genetic variation at a leucine aminopeptidase (LAP) locus in blue (*Mytilus galloprovincialis*) and greenshell (*Perna canaliculus*) mussel populations along a salinity gradient. *Marine Biology* 128: 619-625.
- Gardner JPA, Palmer NL. 1998. Size-dependent, spatial and temporal genetic variation at a leucine aminopeptidase (LAP) locus among blue mussel (*Mytilus galloprovincialis*) populations along a salinity gradient. *Marine Biology* 132: 275-281.
- Gharrett AJ, Smoker WW, Reisenbichler RR, Taylor SG. 1999. Outbreeding depression in hybrids between odd-and even-broodyear pink salmon. *Aquaculture* 173: 117-129.
- Gharrett AJ, Smoker WW. 1991. 2 Generations of hybrids between even-year and odd-year pink salmon (*Oncorhynchus-Gorbuscha*) - A test for outbreeding depression. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1744-1749.
- Gibson G, Weir B. 2005. The quantitative genetics of transcription. *Trends in Genetics* 21: 616-623.
- Gibson G. 2002. Microarrays in ecology and evolution: a preview. *Molecular Ecology* 11: 17-24.
- Gilk SE, Wang IA, Hoover CL, Smoker WW, Taylor SG, Gray AK, Gharrett AJ. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environmental Biology of Fishes* 69: 287-297.
- Glazier AM, Nadeau JH, Aitman TJ. 2002. Finding Genes That Underlie Complex Traits. *Science* 298: 2345-2349.
- Golding GB, Dean AM. 1998. The structural basis of molecular adaptation. *Molecular Biology and Evolution* 15: 355-369.
- González-Wangüemert M, Pérez-Ruzafa Á, Marcos C, García-Charton JA. 2004. Genetic differentiation of *Diplodus sargus* (Pisces: Sparidae) populations in the south-west Mediterranean. *Biological Journal of the Linnean Society* 82: 249–261.
- Gracey AY, Fraser EJ, Li W, Fang Y, Taylor RR, Rogers J, Brass A, Cossins AR. 2004. Coping with cold: An integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. *Proceedings of the National Academy of Sciences of the USA* 101: 16970–16975.
- Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, 89: 415-426.
- Grant WS, Utter FM. 1980. Biochemical genetic variation in walleye pollock, *Theragra chalcogramma* – population structure in the Southwestern Bering Sea and the Gulf of Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 1093-1100.
- Guillot G, Mortier F, Estoup A. 2005. GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes* 5: 712-715.
- Guinand B, Lemaire C, Bonhomme F. 2004. How to detect polymorphisms undergoing selection in marine fishes? A review of methods and case studies, including flatfishes. *Journal of Sea Research* 51: 167-182.
- Hall TE, Cole NJ, Johnston IA. 2003. Temperature and the expression of seven muscle-specific protein genes during embryogenesis in the Atlantic cod *Gadus morhua* L. *Journal of Experimental Biology* 206: 3187-3200.
- Hansen MM, Kenchington E, Nielsen EE. 2001. Assigning individual fish to populations using microsatellite DNA markers. *Fish and Fisheries* 2: 93-112.
- Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg KLD. 2002. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown

- trout (*Salmo trutta*) populations. *Molecular Ecology* 11: 2523-2535.
- He L, Hannon GJ. 2004. MicroRNAs: Small RNAs with a big role in gene regulation. *Nature Reviews Genetics* 5: 522- 531.
- Hedrick PW. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution* 53: 313-318.
- Hendry AP, Taylor EB, McPhail JD. 2002. Adaptive divergence and the balance between selection and gene flow: Lake and stream stickleback in the misty system. *Evolution* 56: 1199-1216.
- Hendry AP. 2002. $QST \neq FST$? *Trends in Ecology and Evolution* 17: 502.
- Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907-913.
- Hickerson MJ, Cunningham CW. 2005. Contrasting quaternary history in an ecologically divergent sister pair of low-dispersing intertidal fish (*Xiphister*) revealed by multilocus DNA analysis. *Evolution* 59: 344-360.
- Hilbish TJ, Koehn RK. 1985. The physiological-basis of natural-selection at the LAP Locus. *Evolution* 39: 1302-1317.
- Hilborn R, Quinn TP, Schindler DE, Rogers DE. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the USA* 100: 6564–6568.
- Hoarau G, Piquet AMT, van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL. 2004. Population structure of plaice (*Pleuronectes platessa* L.) in northern Europe: a comparison of resolving power between microsatellites and mitochondrial DNA data. *Journal of Sea Research* 51: 183-190.
- Hubby JL, Lewontin RC. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. I. The number of different alleles at different loci in *Drosophila pseudoobscura*. *Genetics* 54: 577-594.
- Hutchinson WF, van Oosterhout C, Rogers SI, Carvalho GR. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London B* 270: 2125–2132.
- Jansen RC, Nap JP. 2001. Genetical genomics: the added value from segregation. *Trends in Genetics*, 17; 388-391.
- Johannesson K, André C. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Molecular Ecology* 15: 2013-2029.
- Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V. 2005. Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Molecular Ecology* 14: 3219-3234.
- Kalinowski ST. 2002. How many alleles per locus should be used to estimate genetic distances? *Heredity* 88: 62–65.
- Karpov AK, Novikov GG. 1981. Hemoglobin alloforms in cod, *Gadus morhua* (Gadiformes, Gadidae), their functional characteristics and occurrence in populations. *Journal of Ichthyology*: 45-49.
- Kauer MO, Dieringer D, Schlotterer C. 2003. A microsatellite variability screen for positive selection associated with the "Out of Africa" habitat expansion of *Drosophila melanogaster*. *Genetics* 165: 1137-1148.
- Kelley JL, Madeoy J, Calhoun JC, Swanson W, Akey JM. 2006. Genomic signatures of positive selection in humans and the limits of outlier approaches. *Genome Research* 16: 980-989.
- Khaitovich P, Enard W, Lachmann M, Pääbo S. 2006. Evolution of primate gene expression. *Nature Reviews Genetics* 7: 693-702.
- Kimura T, Jindo T, Narita T, Naruse K, Kobayashi D, Shin-I T, Kitagawa T, Sakaguchi T, Mitani H, Shima A, Kohara Y, Takeda H. 2004. Large-scale isolation of ESTs from medaka embryos and its application to medaka developmental genetics. *Mechanisms of Development* 121: 915-932.
- Knutsen H, Andre C, Jorde PE, Skogen MD, Thuroczy E, Stenseth NC. 2004. Transport of North Sea cod larvae into the Skagerrak coastal populations. *Proceedings of the Royal Society of London B* 271: 1337-1344.

- Koehn RK, Newell RIE, Immermann F. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proceedings of the National Academy of Sciences of the USA* 77: 5385-5389.
- Kornfield I, Sidell BD, Gagnon PS. 1982. Stock definition in the Atlantic herring (*Clupea harengus harengus*) – Genetic evidence for discrete fall and spring spawning populations. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 1610-1621.
- Koskinen MT, Haugen TO, Primmer CR. Contemporary fisherian life-history evolution in small salmonid populations. *Nature* 419: 826-830.
- Kroymann J, Mitchell-Olds T. 2005. Epistasis and balanced polymorphism influencing complex trait variation. *Nature* 435: 95-98.
- Kurobe T, Yasuike M, Kimura T, Hirono I, Aoki T. 2005. Expression profiling of immune-related genes from Japanese flounder *Paralichthys olivaceus* kidney cells using cDNA microarrays. *Developmental and Comparative Immunology* 29: 515–523.
- Landry C, Bernatchez L. 2001. Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology* 10: 2525-2539.
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* 7: 295–302.
- Leder EH, Danzmann RG, Ferguson MM. 2006. The candidate gene, Clock, localizes to a strong spawning time quantitative trait locus region in rainbow trout. *Journal of Heredity* 97: 71-80.
- Lehner B, Crombie C, Tischler J, Fortunato A, Fraser AG. 2006. Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nature Genetics* 38: 896-903.
- Lewontin RC, Hubby JL. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics* 54: 595-609.
- Lewontin RC, Krakauer J. 1973. Distribution of gene frequency as a test of theory of selective neutrality of polymorphisms. *Genetics* 74: 175-195.
- Lexer C, Buerkle CA, Joseph JA, Heinze B, Fay MF. 2006. Admixture in European Populus hybrid zones makes feasible the mapping of loci that contribute to reproductive isolation and trait differences. *Heredity*, advance online publication, September 20; doi:10.1038/sj.hdy.6800898.
- Lexer C, Heinze B, Alia R, Rieseberg LH. 2004. Hybrid zones as a tool for identifying adaptive genetic variation in outbreeding forest trees: lessons from wild annual sunflowers (*Helianthus* spp.). *Forest Ecology and Management* 197: 49–64.
- Li RW, Waldbieser GC. 2006. Production and utilization of a high-density oligonucleotide microarray in channel catfish, *Ictalurus punctatus*. *BMC Genomics* 7: article 134.
- Liang P, Pardee AB. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257: 967-971.
- Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433: 769-773.
- Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics* 4: 981-994.
- Luikart G, England PR. 1999. Statistical analysis of microsatellite DNA data. *Trends in Ecology & Evolution* 14: 253-256.
- Mackay TFC. 2001. The genetic architecture of quantitative traits. *Annual review of Genetics* 35: 303-339.

- Maes GE, Pujolar JM, Hellemans B, Volckaert FAM. 2006. Evidence for isolation by time in the European eel (*Anguilla anguilla* L.). *Molecular Ecology* 15: 2095-2107.
- Maes GE, Pujolar JM, Raeymaekers JAM, Dannewitz J, Volckaert FAM. 2006. Microsatellite conservation and Bayesian individual assignment in four *Anguilla* species. *Marine Ecology-Progress Series* 319: 251-261.
- Malakoff D. 1997. Extinction on the high seas. *Science* 277: 486-488.
- Malmberg RL, Held S, Waits A, Mauricio R. 2005. Epistasis for fitness-related quantitative traits in *Arabidopsis thaliana* grown in the field and in the greenhouse. *Genetics* 171: 2013-2027.
- Manel S, Gaggiotti OE, Waples RS. 2005. Assignment methods: matching biological questions with appropriate techniques. *Trend in Ecology & Evolution* 20: 136-142.
- Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* 18: 189-197.
- Marcil J, Swain DP, Hutchings JA. 2006. Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). *Biological Journal of the Linnean Society* 88: 351-365.
- Maynard Smith J, Haigh J. 1974. Hitch-hiking effect of a favorable gene. *Genetical Research* 23: 23-35.
- McGinnity P, Prodohl P, Ferguson K, Hynes R, O'Maoileidigh N, Baker N, Cotter D, O'Hea B, Cooke D, Rogan G, Taggart J, Cross T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proceedings of the Royal Society of London B* 270: 2443-2450.
- McGinnity P, Prodohl P, Maoileidigh NO, Hynes R, Cotter D, Baker N, O'Hea B, Ferguson A. 2004. Differential lifetime success and performance of native and non-native Atlantic salmon examined under communal natural conditions. *Journal of Fish Biology* 65 Suppl. A: 173-187.
- Mckay JK, Latta RG. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution* 17: 285-291.
- Merilä J, Crnokrak P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14: 892-903.
- Miller KM, Kaukinen KH, Beachham TD, Withler RE. 2001. Geographic heterogeneity in natural selection on an MCH locus in sockeye salmon. *Genetica* 111: 237-257.
- Moran P. 2002. Current conservation genetics: building an ecological approach to the synthesis of molecular and quantitative genetic methods. *Ecology of Freshwater Fish* 11: 30-55.
- Morin PA, Luikart G, Wayne RK. 2004. SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution* 19: 208-216.
- Moritz C. 2002. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* 51: 238-254.
- Morrison JF, Guynn SR, Scofield MA, Dowd FJ, Petzel DH. 2006. Warm acclimation changes the expression of the Na⁺,K⁺-ATPase α subunit isoforms in Antarctic fish gills. *Journal of Experimental Marine Biology and Ecology* 333: 129-139.
- Nielsen EE, Gronkjaer P, Meldrup D, Paulsen H. 2005. Retention of juveniles within a hybrid zone between North Sea and Baltic Sea Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2219-2225.
- Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology* 12: 1497-1508.
- Nielsen EE, Kenchington E. 2001. A new approach to prioritizing marine fish and shellfish populations for conservation. *Fish and Fisheries* 2: 328-343.
- Nielsen EE, Nielsen PH, Meldrup D, Hansen MM. 2004. Genetic population structure of

- turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology* 13: 585-595.
- Nielsen R. 2001. Statistical tests of selective neutrality in the age of genomics. *Heredity* 86: 641-647.
- Nielsen R. 2004. Population genetic analyses of ascertained SNP data. *Human Genomics* 1: 218-224.
- Nissling A, Westin L, Hjerne O. 2002. Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. *ICES Journal of Marine Science* 59: 93-108.
- O'Hara RB, Merila J. 2005. Bias and Precision in QST Estimates: Problems and Some Solutions. *Genetics* 171: 1331-1339.
- Oleksiak MF, Churchill GA, Crawford DL. 2002. Variation in gene expression within and among natural populations. *Nature Genetics* 32: 261-266.
- Olsvik PA, Kristensen T, Waagbo R, Tollefsen KE, Rosseland BO, Toften H. 2006. Effects of hypo- and hyperoxia on transcription levels of five stress genes and the glutathione system in liver of Atlantic cod *Gadus morhua*. *Journal of Experimental Biology* 209: 2893-2901.
- O'Malley KG, Sakamoto T, Danzmann RG, Ferguson MM. 2003. Quantitative trait loci for spawning date and body weight in rainbow trout: Testing for conserved effects across ancestrally duplicated chromosomes. *Journal of Heredity* 94: 273-284.
- Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* 25: 547-572.
- Pampoulie C, Gysels ES, Maes GE, Hellemans B, Leentjes V, Jones AG, Volckaert FAM. 2004. Evidence for fine-scale genetic structure and estuarine colonisation in a potential high gene flow marine goby (*Pomatoschistus minutus*). *Heredity* 92: 434-445.
- Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kurodak MI, Maller B, Hayward DC, Ball EE, Degan B, Müller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E, Ruvkun G. 2000. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 408: 86-89.
- Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* 414: 901-905.
- Perry GML, Danzmann RG, Ferguson MM, Gibson JP. 2001. Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). *Heredity* 86: 333-341.
- Petersen MF, Steffensen JF. 2003. Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin types at normoxia and moderate hypoxia. *Journal of Experimental Biology* 206: 359-364.
- Pimm S, Raven P, Peterson A, Çağan HS, Şekercioğlu, Ehrlich PR. 2006. Human impacts on the rates of recent, present, and future bird extinctions. *Proceedings of the National Academy of Sciences of the USA* 103: 10941-10946.
- Pimm SL, Russell GJ, Gittleman JL, Brooks TM. 1995. The future of biodiversity. *Science* 269: 347-350.
- Plasterk RHA. 2006. Micro RNAs in animal development. *Cell* 124: 877-881.
- Pogson GH, Taggart CT, Mesa KA, Boutilier RG. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. *Evolution*, 55, 131-146.
- Pompanon F, Bonin A, Bellemain E, Taberlet P. 2005. Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics* 6: 847-859.
- Pörtner HO. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals.

- Comparative Biochemistry and Physiology A 132: 739–761.
- Poulsen NA, Nielsen EE, Schierup MH, Loeschcke V, Gronkjaer P. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology* 15: 321–331.
- Powers DA, Place AR. 1978. Biochemical Genetics of *Fundulus heteroclitus* (L.). I. Temporal and spatial variation in gene frequencies of Ldh-B, Mdh-A, Gpi-B, and Pgm-A. *Biochemical Genetics* 16: 593–607.
- Powles H, Bradford MJ, Bradford RG, Doubleday WG, Innes S, Levings CD. 2000. Assessing and protecting endangered marine species. *ICES Journal of Marine Science* 57: 669–676.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- Queitsch C, Sangster TA, Lindquist S. 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417: 618–624.
- Ranz JM, Machado CA. 2006. Uncovering evolutionary patterns of gene expression using microarrays. *Trends in Ecology & Evolution* 21: 29–37.
- Reed DH, Frankham R. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution* 55: 1095–1103.
- Reid DP, Szanto A, Glebe B, Danzmann RG, Ferguson MM. 2005. QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity* 94: 166–172.
- Renn SCP, Aubin-Horth N, Hofmann HA. 2004. Biologically meaningful expression profiling across species using heterologous hybridization to a cDNA microarray. *BMC Genomics*, 5, article no. 42.
- Reynolds JD, Dulvy NK, Goodwin NB, Hutchings JA. 2005. Biology of extinction risk in marine fishes. *Proceedings of the Royal Society of London B* 272: 2337–2344.
- Richards JG, Semple JW, Bystriansky JS, Schulte PM. 2003. Na⁺/K⁺-ATPase (alpha-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *Journal of Experimental Biology* 206: 4475–4486.
- Rieseberg LH, Buerkle CA. 2002. Genetic mapping in hybrid zones. *American Naturalist* 158: S36–S50.
- Rieseberg LH, Whitton J, Gardner K. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* 152: 713–727.
- Riginos C, Nachman MW. 2001. Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology* 10: 1439–1453.
- Rise ML, von Schalburg KR, Brown GD, Mawer MA, Devlin RH, Kuipers N, Busby M, Beetz-Sargent M, Alberto R, Ross Gibbs A, Hunt P, Shukin R, Zeznik JA, Nelson C, Jones SRM, Smailus DE, Jones SJM, Schein JE, Marra MA, Butterfield YSN, Stott JM, Ng SHS, Davidson WS, Koop BF. 2004. Development and application of a salmonid EST database and cDNA microarray: Data mining and interspecific hybridization characteristics. *Genome Research* 14: 478–490.
- Ritland K. 2000. Marker-inferred relatedness as a tool for detecting heritability in nature. *Molecular Ecology* 9: 1195–1204.
- Rogers SM, Bernatchez L. 2005. Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (*Coregonus clupeaformis*). *Molecular Ecology* 14: 351–361.
- Rogers SM, Campbell D, Baird SJE, Danzmann RG, Bernatchez L. 2001. Combining the analyses of introgressive hybridisation and linkage mapping to investigate the genetic architecture of population divergence in the lake whitefish (*Coregonus clupeaformis*, Mitchill). *Genetica* 111: 25–41.

- Rogers SM, Gagnon V, Bernatchez L. 2002. Genetically based phenotype-environment association for swimming behavior in lake whitefish ecotypes (*Coregonus clupeaformis* Mitchell). *Evolution* 56: 2322-2329.
- Roques S, Sévigny J-M, Bernatchez L. 2001. Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. *Molecular Ecology* 10: 149-165.
- Roques S, Sévigny J-M, Bernatchez L. 2002. Genetic structure of deep-water redfish, *Sebastes Mantella*, populations across the North Atlantic. *Marine Biology* 140: 297-307.
- Rosenlund G, Skretting M. 2006. Worldwide status and perspective on gadoid culture. *ICES Journal of Marine Science* 63: 194-197.
- Ross HA, Rodrigo AG. 2002. Immune-mediated positive selection drives human immunodeficiency virus type 1 molecular variation and predicts disease duration. *Journal of Virology* 76: 11715-11720.
- Rutherford SL, Lindquist S. 1998. Hsp90 as a capacitor for morphological evolution. *Nature* 396: 336-342.
- Ruzzante DE, Mariani S, Bekkevold D, Andre C, Mosegaard H, Clausen LAW, Dahlgren TG, Hutchinson WF, Hatfield EMC, Torstensen E, Brigham J, Simmonds EJ, Laikre L, Larsson LC, Stet RJM, Ryman N, Carvalho GR. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. *Proceedings of the Royal Society of London B* 273: 1459-1464.
- Ruzzante DE, Taggart CT, Cook D. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. *Fisheries Research* 43: 79-97.
- Ruzzante DE, Taggart CT, Doyle RW, Cook D. 2001. Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics* 2: 257-269.
- Ryman N, Palm S, Andre C, Carvalho GR, Dahlgren TG, Jorde PE, Laikre L, Larsson LC, Palme A, Ruzzante DE. 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. *Molecular Ecology* 15: 2031-2045.
- Ryman N, Utter F, Laikre L. 1995. Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries* 5: 417-446.
- Sakamoto T, Danzmann RG, Okamoto N, Ferguson MM, Ihssen PE. 1999. Linkage analysis of quantitative trait loci associated with spawning time in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 173: 33-43.
- Salvanes AGV, Skjaeraasen JE, Nilsen T. 2004. Sub-populations of coastal cod with different behaviour and life-history strategies. *Marine Ecology-Progress Series* 267: 241-251.
- Salvi S, Tuberosa R. 2005. To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Science* 10: 297-304.
- Schlötterer 2002: Towards a molecular characterization of adaptation in local populations. *Current Opinion in Genetics & Develoelopment* 12: 683-687.
- Schmidt J. 1917. Racial investigations I. *Zoarces viviparus* L. and local races of the same. *Comptes rendus des travaux du laboratoire Carlsberg* 13: 277-397.
- Schmidt PS, Rand DM. 1999. Intertidal microhabitat and selection at MPI: Interlocus contrasts in the Northern Acorn Barnacle, *Semibalanus balanoides*. *Evolution* 53; 135-146.
- Schulte PM, Glemet HC, Fiebig AA, Powers DA. 2000. Adaptive variation in lactate dehydrogenase-B gene expression: role of a stress-responsible regulatory element. *Proceedings of the National Academy of Sciences of the USA* 97: 6597-6602.
- Schulte PM. 2001. Environmental adaptations as windows on molecular evolution. *Comparative Biochemistry and Physiology Part B* 128: 597-611.
- Schulte PM. 2004. Changes in gene expression as biochemical adaptations to environmental

- change: a tribute to Peter Hochachka. *Comparative Biochemistry and Physiology B* 139: 519–529.
- Sick K. 1965a. Haemoglobin polymorphism of cod in Baltic and Danish Belt Sea. *Hereditas* 54: 19-48.
- Sick K. 1965b. Haemoglobin polymorphism of cod in North Sea and North Atlantic Ocean. *Hereditas* 54: 49-69.
- Skibinski DOF. 2000. DNA tests of neutral theory: applications in marine genetics. *Hydrobiologia* 420: 137-152.
- Slate J, Visscher PM, MacGregor S, Stevens D, Tate ML, Pemberton JM. 2002. A genome scan for quantitative trait loci in a wild population of red deer (*Cervus elaphus*). *Genetics* 162: 1863-1873.
- Slate J. 2005. Quantitative trait locus mapping in natural populations: progress, caveats and future directions. *Molecular Ecology* 14: 363–379.
- Smedbol RK, Stephenson R. 2001. The importance of managing within-species diversity in cod and herring fisheries of the north-western Atlantic. *Journal of Fish Biology* 59: 109-128.
- Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS. 2004. Extinction and ecosystem function in the marine benthos. *Science* 306: 1177-1180.
- Somero GN. 2005. Linking biogeography to physiology: Evolutionary and acclamatory adjustments of thermal limits. *Frontiers in Zoology* 2: no. 1.
- Somorjai IML, Danzmann RG, Ferguson MM. 2003. Distribution of temperature tolerance quantitative trait loci in Arctic charr (*Salvelinus alpinus*) and inferred homologies in rainbow trout (*Oncorhynchus mykiss*). *Genetics* 165: 1443-1456.
- Sørensen JG, Kristensen TN, Loeschcke V, 2003: The evolutionary and ecological role of heat shock proteins. *Ecology Letters* 6: 1025-1037.
- Spitze K. 1993. Population-structure in *daphnia obtusa* - quantitative genetic and allozymic variation. *Genetics* 135: 367-374.
- Stefanni S, Gysels ES, Volckaert FAM, Miller PJ. 2003. Allozyme variation and genetic divergence in the sand goby, *Pomatoschistus minutus* (Teleostei: Gobiidae). *Journal of the Marine Biological Association of the United Kingdom* 83: 1143-1149.
- Storz JF, Dubach JM. 2004. Natural selection drives altitudinal divergence at the albumin locus in deer mice, *Peromyscus maniculatus*. *Evolution* 58: 1342–1352.
- Storz JF. 2005. Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology* 14: 671-688.
- Streelmann JT, Albertson RC, Kocher TD. 2003. Genome mapping of the orange blotch colour pattern in cichlid fishes. *Molecular Ecology* 12: 2465-2471.
- Swanson WJ, Zhang ZH, Wolfner MF, Aquadro CF. 2001. Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals. *Proceedings of the National Academy of Sciences of the USA* 98: 2509-2514.
- Tabor HK, Risch NJ, Myers RM. 2002. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nature Reviews Genetics* 3: 1-7.
- Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.
- Tao WJ, Boulding EG. 2003. Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). *Heredity* 91: 60–69.
- Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Molecular Ecology* 15: 343-355.
- Taylor EB. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* 98: 185-207.
- Teshima KM, Coop G, Przeworski M. 2006. How reliable are empirical genomic scans for selective sweeps? *Genome Research* 16: 702-712.

- Thomas JA, Telfer MG, Roy DB, Preston CD, Greenwood JJD, Asher J, Fox R, Clarke RT, Lawton JH. 2004. Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science* 303: 1879-1881.
- Till BJ, Zerr T, Bowers E, Greene EA, Comai L, Henikoff S. 2006. High-throughput discovery of rare human nucleotide polymorphisms by Ecotilling. *Nucleic Acids Research* 34: e99.
- Ton C, Stamatiou D, Dzau VJ, Liew CC. 2002. Construction of a zebrafish cDNA microarray: gene expression profiling of the zebrafish. *Biochemical and Biophysical Research Communications* 296: 1134-1142.
- Vähä JP, Primmer CR. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology* 15: 63-72.
- van Tienderen PH, de Haan AA, van der Linden CG, Vosman B. 2002. Biodiversity assessment using markers for ecologically important traits. *Trends in Ecology & Evolution* 17: 577-582.
- Vasemägi A, Nilsson J, Primmer CR. 2005. Expressed sequence tag-linked microsatellites as a source of gene-associated polymorphisms for detecting signatures of divergent selection in Atlantic salmon (*Salmo salar* L.). *Molecular Biology and Evolution* 22: 1067-1076.
- Vasemägi A, Primmer CR. 2005. Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Molecular Ecology* 14: 3623-3642.
- Venkatesh B, Hettwer U, Koopmann B, Karlovsky P. 2005. Conversion of cDNA differential display results (DDRT-PCR) into quantitative transcription profiles. *BMC Genomics* 6: no. 51.
- Vitalis R, Dawson K, Boursot P. 2001. Interpretation of variation across marker loci as evidence of selection. *Genetics* 158: 1811-1823.
- Volff J-N. 2005. Genome evolution and biodiversity in teleost fish. *Heredity* 94: 280-294.
- Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP — a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407-4414.
- Vuylsteke M, Van Den Daele H, Vercauteren A, Zabeau M, Kuiper M. 2006. Genetic dissection of transcriptional regulation by cDNA-AFLP. *The Plant Journal* 45: 439-446.
- Waples RS, Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* 15: 1419-1439.
- Waples RS. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89: 438-450.
- Ward RD, Woodward M, Skibinski DOF. 1994. A comparison of genetic diversity levels in marine, freshwaters and anadromous fishes. *Journal of Fish Biology* 44: 213-232.
- Watterson GA. 1977. Heterosis or neutrality. *Genetics* 85: 789-814.
- Wayne ML, McIntyre LM. 2002. Combining mapping and arraying: An approach to candidate gene identification. *Proceedings of the National Academy of Sciences of the USA* 99: 14903-14906.
- Whitehead A, Crawford DL. 2006a. Neutral and adaptive variation in gene expression. *Proceedings of the National Academy of Sciences of the USA* 103: 5425-5430.
- Whitehead A, Crawford DL. 2006b. Variation within and among species in gene expression: raw material for evolution. *Molecular Ecology* 15: 1197-1211.
- Widmer A, Lexer C. 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends in Ecology & Evolution* 16: 267-269.
- Williams TD, Gensberg K, Minchin SD, Chipman JK. 2003. A DNA expression array to detect toxic stress response in European

- flounder (*Platichthys flesus*). Aquatic Toxicology 65: 141-157.
- Wilson AB. 2006. Genetic signature of recent glaciation on populations of a near-shore marine fish species (*Syngnathus leptorhynchus*). Molecular Ecology 15: 1857-1871.
- Winans GA. 1980. Geographic variation in the milkfish *Chanos chanos*. 1. Biochemical evidence. Evolution 34: 558-574.
- Yang X, Schadt EE, Wang S, Wang H, Arnold AP, Ingram-Drake L, Drake TA, Lusis AJ. 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. Genome Research 16: 995-1004.
- Yang ZH, and Bielawski JP. 2000. Statistical methods for detecting molecular adaptation. Trends in Ecology & Evolution 15: 496-503.

Manuscript I

**Evolutionary factors shaping the genetic population structure of marine fishes;
lessons from the European flounder (*Platichthys flesus* L.)**

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21 **Key words:** Genetic population structure, landscape genetics, marine fish, barriers to gene flow,
22 European flounder, environmental gradients
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29 Running title: Population structure in European flounder

1 **Abstract**

2
3 A number of evolutionary mechanisms have been suggested for generating low but significant genetic
4 structuring among marine fish populations. We used microsatellites and recent developments in
5 landscape genetics and coalescence based estimation of historic gene flow and effective population
6 sizes to assess temporal and spatial dynamics of the population structure in European flounder
7 (*Platichthys flesus* L). We found highly significant genetic differentiation among samples covering a
8 large part of the species' range (global $F_{ST} = 0.024$, $P < 0.0001$). In addition to historical processes, a
9 number of contemporary evolutionary drivers were found to be associated with genetic structuring.
10 Physical drivers, such as oceanographic and bathymetric barriers, were most likely related with the
11 extreme isolation of the island population at the Faroe Islands. A sharp genetic break was associated
12 with the change in life-history from pelagic to benthic spawners in the Baltic Sea. Furthermore, partial
13 Mantel tests showed that environmental transitions rather than geographic distances *per se* were
14 associated with genetic structuring among Atlantic populations (latitude controlling for geography:
15 $r=0.764$, $P=0.002$, geography controlling for latitude: NS). These results support the hypothesis of
16 environmental differences as a major structuring force in marine fishes and highlight that the
17 magnitude and scale of structuring generated by a specific driver depend critically on its interplay with
18 other structuring forces. We suggest evaluating all potential evolutionary drivers simultaneously,
19 emphasizing the importance of investigating species with wide geographical and ecological
20 distributions, such as the European flounder, to increase our understanding of evolution in the marine
21 environment.

1 Introduction

2
3 Understanding evolution in marine organisms requires knowledge of the genetic population structure as
4 well as the biotic and a-biotic drivers responsible for creating patterns of neutral and adaptive genetic
5 divergence. During the last decades the availability of highly variable genetic markers has facilitated
6 the gathering of large quantities of data on the scale and magnitude of population structuring in marine
7 fishes. These studies have generally confirmed that population structure in marine fish species is best
8 explained by a low structure-high gene flow scenario (Ward *et al.* 1994; Waples, 1998; DeWoody &
9 Avise 1999; Hutchingson *et al.* 2001; Hoarau *et al.* 2002a; Mariani *et al.* 2005). Still, small but
10 significant, and usually temporally stable, genetic structuring has been demonstrated for many species
11 of marine fishes (Ruzzante *et al.* 1999; Nielsen *et al.* 2003; Nielsen *et al.* 2004; Bekkevold *et al.* 2005;
12 Jørgensen *et al.* 2005).

13 A number of potential mechanisms have been suggested to explain how population structure
14 can evolve in an environment without any obvious physical boundaries to gene flow. Historical
15 processes associated with colonization of new habitat after the last glaciation has been recognized as
16 important for shaping present day genetic structure (Grant & Bowen 1998; Gysels *et al.* 2004;
17 Hickerson & Cunningham 2005; Wilson 2006) and certain life-history characteristics, particularly
18 restricted dispersal of egg and larval stages, have been found to be associated with increased structuring
19 of populations (Waples 1987; Bernardi 2000). Physical structuring factors, such as geographical
20 distance, oceanic current systems and bathymetric barriers to gene flow have also been proposed to be
21 important (Ruzzante *et al.*, 1999; Lundy *et al.*, 2000; Pogson *et al.* 2001; Knutsen *et al.* 2004).
22 Furthermore, environmental transitions have been found to be associated with genetic structuring of
23 populations (Lundy *et al.* 1999; Riginos & Nachman 2001; Nielsen *et al.* 2003; Nielsen *et al.* 2004;
24 Bekkevold *et al.* 2005; Johanneson & André 2006), implicating that populations are locally adapted to
25 their native environment. Although many mechanisms have been proposed, it has been difficult to
26 assess the relative importance of these evolutionary forces, since mechanisms have often been
27 investigated on a case by case basis. Important exceptions to this general picture can be found. Thus,
28 Riginos & Nachman (2001) investigated the effects of several potential evolutionary drivers among
29 highly structured populations of *Axoclinus nigricaudus* in the Gulf of California and found interacting
30 effects from biogeography, geographic distance and habitat quality. Similarly, Roques *et al.* (2002)
31 found evidence for the effects of both historical and contemporary forces in structuring redfish
32 (*Sebastes mantilla*) populations in the Northern Atlantic. Hence, until now only few studies have
33 identified multiple mechanisms - and their relative importance - for shaping population structure in a
34 “classical” marine fish species, i.e. a species with a wide distribution, large population sizes, high
35 fecundity and high dispersal potential for juveniles and adults (Nielsen & Kenchington 2001).

36 The European flounder has a very wide geographical and ecological distribution. It inhabits
37 coastal areas throughout the North Eastern Atlantic from the White Sea to the Mediterranean Sea and
38 as one of only a few marine fish species, it is also found in the inner parts of the brackish Baltic Sea.
39 The flounder is very tolerant to low salinities and is often found migrating to rivers and lakes for
40 extended periods before returning to the sea to spawn. Accordingly, it is uniquely suited for studying
41 the simultaneous action of several evolutionary drivers for shaping population structure in a potential
42 high gene flow species.

43 The flounder offers a very good opportunity for evaluating the effect that different life-history
44 strategies may have on the genetic structuring of populations because different strategies are found
45 within the same species in different parts of the distributional area. While the “normal” form spawn

1 pelagic eggs at off-shore spawning grounds, the so-called “Bank-flounders” (sometimes referred to as a
2 sub-species of *P. flesus*) in the innermost parts of the Baltic Sea appear to have adapted to the brackish
3 environment by spawning benthic eggs (Solemdal 1973; Aro 1989; Drevs *et al.* 1999). This adaptation
4 assures that eggs are not exposed to anoxic conditions in the deeper parts of the Baltic Sea. The
5 distributions of the two types of flounders overlap around the island of Gotland in the central Baltic
6 Sea, but the level of interbreeding between the two types is not known. This setting permits studying
7 genetic differentiation between and within two life-history forms, which has only rarely been possible
8 in marine fish species.

9 The life-history of the flounder also allows studying the effect of geographic distance *per se* on
10 genetic structuring. Adults are relatively stationary and are primarily migrating locally between feeding
11 and spawning grounds (Aro 1989). Juveniles are sedentary in brackish or in fresh water. Eggs are only
12 pelagic for a few weeks and larvae have been shown to be able to utilize estuarine currents for selective
13 tidal stream transport (Campos *et al.* 1994; Bos 1999) enabling them to reach their preferred nursery
14 grounds even before metamorphosis. It therefore seems reasonable to assume that the potential life time
15 dispersal of a single individual does not include the full distributional area of the species, which
16 provides a good opportunity for evaluating the effect of geographical distance on genetic structuring.

17 Oceanic current systems and bathymetric features could also be expected to affect the
18 structuring among populations of flounder. European flounder is a coastal species and rarely migrates
19 large distances over deep seas (Aro 1989), and hence it is expected that island populations should be
20 particularly isolated or even missing. Therefore, the inclusion of the Faroe Island population, isolated
21 by deep Atlantic waters and a circular current system (Jákupsstovu & Reinert 1994; Joensen *et al.*
22 2000) allows an assessment of the importance of these potential barriers to gene flow in the marine
23 environment. In particular, they facilitate an assessment of the strength of these barriers compared to
24 sharp environmental clines and geographic distance *per se*.

25 The flounder is exposed to a number of highly pronounced but very different environmental
26 gradients following south-north and east-west transects, thereby allowing an evaluation of the relative
27 importance of environmental differences for shaping its genetic structure. Along a latitudinal transect in
28 the Atlantic part of the distributional area, several environmental parameters vary, the most obvious
29 being related to temperature, light and seasonal cycles. Another important gradient is the Atlantic-
30 Baltic Sea transition which has been studied intensively in other species. Flounders in the central and
31 western Baltic Sea show several special physiological features thought to reflect adaptations to the
32 brackish environment. For instance egg shell thickness and sperm mobility appear to be adapted to
33 allow efficient reproduction at the lower salinities in these regions (Solemdal 1967; Solemdal 1973;
34 Nissling *et al.* 2002). Similar adaptations have also been described in other species in this region
35 (Vallin *et al.* 1999; Ojaveer & Kalejs 2005), however, with its unique tolerance to low salinities the
36 flounder represents an important contrast to the marine species studied in the North Eastern Atlantic so
37 far.

38 The aims of the present study of population structure in the European flounder were to assess the
39 relative importance of several evolutionary drivers acting to shape the genetic structure of marine
40 fishes. We combine a method for indirect estimation of gene flow and effective population size with
41 landscape genetic analysis and an assessment of the temporal and spatial dynamics of the population
42 structure. In particular, we first of all evaluate the relative importance of historical versus contemporary
43 processes. We then assess the levels of structuring within as well as between life-history strategies
44 before moving on to study the importance of physical forces, such as geographical distance and
45 oceanographic currents. Finally, we concentrate on selective forces associated with environmental

1 differences in two different scenarios from the species' distribution. Understanding the outcome of the
2 interplay of these structuring forces in a high gene flow setting is central to gain a better understanding
3 of the magnitude and scale of population structuring in the marine environment and will hence improve
4 our ability to manage marine biodiversity efficiently.

7 **Materials and Methods**

9 *Sampling*

10 Samples of approximately 50 European flounders were collected by research vessels or local fishermen
11 (see Figure 1 and Table 1 for sampling locations) both in 2003 and 2004. Among the sampled locations
12 were two secluded Danish fjords and Lake Pulmanki in Northern Norway and Finland, which is
13 connected to the Barents Sea via the river Teno. Most samples were collected during the spawning
14 season and primarily comprised of mature or spawning individuals (Table 1). In some cases, however,
15 samples consisted of immature adults or juveniles. Gill filaments or fin clips (app. 0.5*0.5 cm) were
16 collected from each individual and stored in 96% ethanol for subsequent DNA extraction.

17 European flounder is known to hybridise with plaice (*Pleuronectes platessa* L.) in some parts of
18 the distributional area. The frequency of hybrids varies between localities but is generally believed to
19 be highest in the western part of the Baltic Sea (Sick *et al.* 1963). A large quantity of these hybrids
20 could potentially bias the results of the population genetic analyses conducted here. Hence, a sample of
21 18 North Sea plaice collected in 2003 was included to aid in the identification of hybrid individuals.

23 *DNA analyses*

24 DNA was extracted with standard HotSHOT (Truett *et al.* 2000), Chelex (Estoup *et al.* 1996) or
25 DNeasy (Qiagen) techniques. PCR was performed on DNA extracted by all three methods from fifteen
26 randomly chosen individuals in order to test for discrepancies between genotypes obtained with the
27 different extraction methods.

28 Nine microsatellite loci were employed for the genetic analyses. LIST1001 (Watts *et al.* 1999,
29 GenBank accession number: AF149831), PL142 and PL167 (Hoarau *et al.* 2002b, accession numbers:
30 AF406750 and AF406751), originally developed for plaice but worked well on flounder following
31 modifications of PCR conditions. StPf1001, StPf1002, StPf1004, StPf1005, StPf1015 and StPf1022
32 (Dixon *et al.* unpublished, accession numbers: AJ315970, AJ315975, AJ315973, AJ315974,
33 AJ538313, AJ538320) were all developed for European flounder. PCR was applied with standard
34 reagents and thermal cyclers and PCR products were analyzed on a Pharmacia ALFexpress automated
35 sequencer following the manufacturer's recommendations. Standard size ladders and individuals of
36 known genotypes were run on each gel to minimize scoring error.

38 *Statistics*

39 The programme MICRO-CHECKER (van Oosterhout *et al.* 2004) was used to check for potential
40 technical problems such as null alleles, stuttering and large allele drop-out and null-allele frequencies
41 were estimated according to Chakraborty *et al.* (1992). Exact tests for departure from HW equilibrium
42 (Guo & Thompson 1992) were performed per locus and sample in GENEPOP (Raymond & Rousset
43 1995). F_{ST} (Weir & Cockerham's (1984) θ), their confidence intervals and significances were
44 estimated using the program FSTAT (Goudet 1995), which was also used to estimate allelic richness
45 (ElMousadik & Petit 1996).

1 A hybrid neighbour joining tree was constructed with the PHYLIP package (Felsenstein 2004).
2 The tree topology was based on Cavalli-Sforza's chord distance (Cavalli-Sforza & Edwards 1967) with
3 statistical support from 1000 bootstrapped data sets. Branch lengths were based on $(\delta\mu)^2$ (Goldstein *et*
4 *al.* 1995) calculated with the program POPULATIONS (Distributed by Olivier Langella, available
5 from http://bioinformatics.org/project/?group_id=84). These genetic distance measures have been
6 proposed to be particularly suitable to represent tree topology and evolutionary distances between
7 populations based on microsatellite markers (Takezaki & Nei 1996).

8 The program BARRIER (Manni *et al.* 2004) was used to assess the largest breaks in the genetic
9 structure across the species' range. BARRIER identifies barriers to gene flow based on geographic and
10 genetic relationships among populations. In order to assess the robustness of barriers, 100 matrices of
11 Weir & Cockerham's θ (1984) were generated by bootstrapping over loci. The robustness of barriers is
12 proportional to the number of times each barrier is supported by the 100 data sets. Each data set
13 supports a number of barriers of decreasing order, reflecting the relative strength of the detected
14 barriers.

15 We used simple linear regression to examine a potential association between genetic diversity
16 and latitude. Partial Mantel tests were performed in order to test if the genetic structure observed in a
17 subset of the samples is best explained by the geographic or environmental distance between samples.
18 Geographic distance was calculated as the shortest coastal distance between samples. It has been shown
19 that it can be very difficult to separate the effects of different environmental parameters if they are
20 highly correlated and potentially interacting (Sarup *et al.* 2006). Hence, we used differences in latitude
21 and longitude from the sample positions as proxies for environmental differences between samples,
22 since these measures are expected to capture much of the environmental variation among the samples
23 included in the partial Mantel tests. Partial Mantel tests were done controlling for latitude and longitude
24 separately and significance was assessed with 1000 permutations. The partial Mantel tests were
25 conducted with the software IBDWS (Jensen *et al.* 2005).

26 The program MIGRATE (Beerli & Felsenstein 2001) was used to estimate population specific
27 Θ ($=4N_e\mu$, where μ is the mutation rate across loci) and pairwise scaled migration rates, M ($=m/\mu$,
28 where m is the migration rate). This program uses a coalescence based likelihood and MCMC method
29 to estimate Θ and M values and allows for the assessment of asymmetric rates of migration. We stress
30 that MIGRATE estimates a scaled historic effective population size and migration rate. Hence, this
31 approach will pick up any signal affecting these parameters in the history of the populations and we do
32 therefore not attempt to take the estimates at face value. Neither do we find it reasonable to multiply
33 the estimates with some arbitrarily chosen mutation rate to reach estimates of actual effective
34 population size and migration rate, but are rather comparing patterns and relative values throughout this
35 paper.

36 We applied the "heating scheme", which runs parallel Markov chains at different temperatures.
37 A cold chain is sampling locally, while hotter chains move more freely and can therefore explore more
38 of the genealogy space. This scheme should allow for a more efficient exploration of the genealogy
39 space by swapping states between chains. This approach should be useful in situations with flat
40 likelihood surfaces (Beerli 2004) such as would be expected in marine fishes. Initial trial runs indicated
41 that a plateau in likelihood values of chains was reached after approximately 10 short chains. To be
42 conservative, 12 short and 3 long chains were run in the subsequent analyses. Short and long chains
43 were run for 500 and 5000 sampled genealogies or until 50 and 500 new genealogies had been found in
44 cases with low acceptance ratios. The only exception to this general setting was for locus StPf1001,
45 which was run until 25 and 250 new trees were accepted for short and long chains respectively, because

1 the algorithm found very few acceptable genealogies for this locus. The two years were kept separate in
2 order to assess the reliability of the method in the case of marine fish species showing relatively little
3 evidence of genetic divergence across the range of samples. Three runs, each consisting of a number of
4 sub-runs, were conducted for each year. Parameter estimates in the first sub-run were based on F_{ST}
5 estimates, while subsequent sub-runs had estimates from the preceding sub-run as starting values. The
6 runs were stopped when the majority of estimates were found to be within 95% confidence intervals in
7 successive sub-runs, and final parameter estimates were then obtained by combining the long chains in
8 the final sub-run.

10 **Results**

12 *Genetic variatio*

13 DNA extractions with the three different methods yielded consistent genotypes in 14 of the 15 chosen
14 individuals; the 15th individual was discarded from further analyses because of DNA of poor quality
15 not giving consistent PCR amplification. In total 5 out of 1086 individuals (0.46%) were discarded
16 from analyses because of poor quality DNA. No locus or population had more than 4% missing
17 genotypes, indicating that genotyping success was high in the remaining individuals. The MICRO-
18 CHECKER analyses provided little evidence of technical problems with only 7 of 198 tests conducted
19 (3.5%) showing evidence of minor null allele problems. Potential null alleles were found for locus
20 StPf1004 in Bor03 (null-allele frequency: 0.08), Aer03 (frequency 0.06) and Aer04 (frequency: 0.07),
21 for locus List1001 in Bor04 (frequency: 0.17), for StPf1022 in Irs04 (frequency: 0.07), StPf1001 in
22 Bor03 (frequency: 0.06) and P1142 in Rin03 (frequency: 0.04). All loci amplified well under standard
23 conditions in the sample of North Sea plaice. In this sample potential null alleles were identified in loci
24 StPf1004, StPf1005 and StPf1022 (frequencies of 0.31, 0.15 and 0.15 respectively), which were all
25 developed for European flounder. These potential null-alleles were not affecting our ability to detect
26 hybrids between the two species. Hybridisation with plaice did not present a significant problem for the
27 analyses conducted in this study, since rates of introgression from plaice to flounder were found to be
28 low and relatively evenly distributed throughout most of the flounder's distributional range. Only one
29 out of 1063 flounders genotyped was identified as a potential F1 hybrid. This individual was discarded
30 from the data set.

31 When conducting the exact test for deviations from Hardy-Weinberg equilibrium, we found 13
32 significant tests among the total 198 tests conducted (Appendix A). The apparent deficiency of
33 heterozygotes in a few loci in the western Baltic Sea samples is most likely not indicative of a Wahlund
34 effect, since a simulated sample consisting of a mechanical mixture of pure North Sea and inner Baltic
35 Sea flounders did not deviate significantly from Hardy Weinberg expectations. This reflects a general
36 lack of statistical power for detecting true Wahlund effects in marine fishes and suggests that the
37 heterozygote deficiencies in these samples are caused by technical problems as indicated by the
38 MICRO-CHECKER analysis rather than by mechanical mixing of individuals from divergent
39 populations. However, since such potential problems are not substantial and furthermore not biasing
40 the analyses conducted here, we retained all loci for further analyses.

41 The allelic richness was highest in the North Sea/Irish Sea samples and tended to decline in the
42 extreme parts of the distributional area. The Faroe Island samples had exceptionally low levels of
43 genetic diversity (Appendix A). Overall there was a weak but significant association between allelic
44 richness and latitude ($r^2=0.19$, $P=0.04$). However, this association was no longer significant when the
45 extreme Faroe Island population was excluded from analysis ($r^2=0.13$, $P=0.12$).

Temporal stability

No pairwise comparisons involving temporal samples from the same locality were significant. F_{ST} ranged from -0.003 between the Bay of Biscay and Faroe Islands samples to 0.003 between the Thyborøn samples (Appendix B), thus indicating that the signals of genetic differentiation detected in this study were temporally stable over the short time period covered. In spite of this overall similarity, temporal samples were kept separate for two main reasons. First of all, it allowed for an assessment of the reliability of the results obtained from the different analyses. Since the 2004 data set covered a much larger part of the distributional area it was also expected to capture more of the total genetic variation in the species compared to the narrower sampling scheme in 2003. By keeping the years separate it was possible to evaluate the effect of this difference in sample coverage between the two years. This is expected to be particularly important in the MIGRATE analyses, since this approach has been shown to be relatively sensitive to “low signal” data sets (Abdo *et al.* 2004), such as expected in the present case. Secondly, small but non significant differences were detected between temporal replicates, for example between the Thyborøn and Gotland samples. By pooling these samples, important information about the short term temporal dynamics could be lost. Hence while the overall conclusion is that the population structure detected here is temporally stable, we find it more useful for analytical purposes to keep temporal samples separate.

Population structure

The hybrid tree (Figure 2) showed a strong partitioning of samples into distinct population groups. The Faroe Islands, Bay of Biscay and benthic spawning flounders in the inner Baltic Sea were clearly isolated from the remaining populations, which grouped together with lower bootstrap support. Additionally, the evolutionary distances (branch lengths of $(\delta\mu)^2 \approx 6$ and 14 respectively) to the inner Baltic and Lake Pulmanki populations were very large compared to the distances among the remaining populations.

The overall F_{ST} was 0.024 (95 % CI: 0.018-0.031) and highly significant ($P < 0.0001$). Examining the pairwise F_{ST} estimates (Appendix B) it is evident that some contributed much more to this overall pattern than others. The highest pairwise F_{ST} values were from comparisons involving the Faroe Islands, the Bay of Biscay, Lake Pulmanki and the benthic spawners in the inner Baltic Sea. Although the differences between populations within the area from the North and Irish Seas to the western Baltic Sea were fairly small (F_{ST} ranged from 0 to app. 0.01), most of these comparisons were still statistically significant. In comparison, the pairwise F_{ST} between the plaice and flounder samples was around 0.2.

The landscape genetic analysis identified four barriers with high bootstrap support in 2004 (Figure 1 and Table 2). All data sets except one supported the barrier around the Faroe Islands as the most important one. This barrier was followed by barriers of decreasing importance around Lake Pulmanki, Bay of Biscay and the benthic spawning populations at Gotland and Turku (Table 2). In addition, a barrier separating Trondheim and the western Baltic Sea could be detected with lower bootstrap support. Computation of additional barriers resulted in a random scatter of barriers on the map. In 2003 the barrier around the Faeroe Islands was also identified as the strongest barrier supported as the first order barrier by 96 of 100 data sets. This barrier was followed by the barrier isolating the benthic spawning flounder populations in the Baltic Sea. A third barrier with less support was identified north of the Bay of Biscay and between the Thyborøn and Limfjord/Ærø samples. The apparent isolation of the Limfjord population could potentially be an artefact resulting from an intense

1 supportive breeding program in this area using a limited number of parent fish to produce a large
2 number of juveniles (J. Støttrup, Danish Institute for Fisheries Research, personal communication).

3 Partial Mantel tests were conducted on a subset of samples excluding samples from the Faroe
4 Islands, Gotland and Turku. This was done since these samples showed clear evidence of structuring
5 caused by factors other than geographical distance *per se* (see discussion). Excluding these samples
6 allowed an in-depth analysis of potential structuring forces in the remaining set of samples. Among
7 these samples there was a clear and highly significant pattern of isolation by distance in 2004 ($r=0.705$,
8 $P=0.002$) but not in 2003 ($r=0.008$, $P=0.495$) (Table 3). There was also a significant association
9 between both environmental variables and genetic distance in 2004 (latitude: $r=0.886$, $P=0.001$,
10 longitude: $r=0.703$, $P=0.009$). When controlling for environmental variability in 2004, geographic
11 distance was no longer significant, while latitude was still highly significant when controlling for
12 geography ($r=0.764$, $P=0.002$). Likewise, longitude was not significant when controlling for geography
13 (Table 3).

14 *Estimates of scaled population sizes and migration rates*

15 The consistency between different runs of the program MIGRATE was markedly better in 2004 than in
16 2003, probably because of a better coverage of the total distributional area and hence a larger signal in
17 the data set. Because of the uncertain estimates from the 2003 data, this data set was not used for
18 further analyses and the reported estimates are from the 2004 data set only. These results stress the
19 importance of a broad coverage of the distributional area of the species under investigation.

20 Furthermore, they illustrate that the application of this particular method may be problematic in studies
21 of genetic structure in marine fishes, which often exhibit even lower levels of structuring than the ones
22 reported here. At the very least, validation by several independent runs of MIGRATE should be
23 conducted in order to check the reliability of estimates.

24 Estimates of scaled long term effective population size (Θ) (Table 4 for a summary of all three
25 runs and supplementary tables S1-S3 for individual runs with estimates and 95% confidence intervals)
26 show that the Faroe Islands and Lake Pulmanki populations are clearly the smallest in the data set.
27 Trondheim is relatively small, while the populations in the North Sea (Thy) and western/central Baltic
28 Sea (Aer and Bor) represent larger populations. The three different runs gave inconsistent estimates of
29 the sizes of Ringkøbing Fjord, Turku, Gotland, Bay of Biscay and Irish Sea. Turku, Ærø and Thyborøn
30 were always net donors of migrants, while the Faroe Islands and Lake Pulmanki populations were
31 consistently net receivers. There also appeared to be asymmetrical migration from the large North Sea
32 and western Baltic Sea populations into the southernmost population in the Bay of Biscay.

33 When examining specific population pairs, some additional signals were common to all three
34 runs. One was an asymmetrical migration from Thyborøn in the North Sea into the central Baltic Sea at
35 Bornholm, while the western Baltic Sea population (Ærø) appeared to donate migrants both into and
36 out of the Baltic Sea. Gotland also consistently received more migrants from the western Baltic and
37 North Sea populations than it donated.

38 **Discussion**

39
40 Overall, there is clearly large and highly significant genetic structuring among the samples of European
41 flounder used in this study, which is supporting the occurrence of local populations for the species.
42 However, the level of structuring is unevenly distributed throughout the geographical range, and the
43
44

1 analyses conducted indicate that different factors may be responsible for generating these signals of
2 genetic structuring.

3 4 *History and founder events*

5 In general, populations from the extreme parts of the distributional area showed signs of reduced allelic
6 richness, which could be the result of founder events when new habitat was colonised after the last
7 glaciation (Hewitt 2000; Widmer & Lexer 2001). Results from the MIGRATE analysis clearly
8 corroborate a signal of founder events in the Faroe Islands and Lake Pulmanki by estimating these
9 populations to be significantly smaller than the remaining populations in the data set. The larger $(\delta\mu)^2$
10 distances to Lake Pulmanki as well as to the benthic spawning populations in the inner Baltic Sea are
11 also consistent with episodes of elevated genetic drift, possibly reflecting founder events. While the
12 Baltic Sea populations appear to have reduced genetic diversity compared to their Atlantic neighbours,
13 these populations do not, however, seem to be particularly small as based on estimates of Θ from
14 MIGRATE.

15 Although the large evolutionary distances to these populations are consistent with founder
16 events, they could possibly also reflect an alternative evolutionary history, such as a colonisation from
17 different glacial refuges. Some studies have proposed a possible glacial refuge in the southern North
18 Sea in addition to the well established refuge near the Iberian peninsular (e.g. brown trout, García-
19 Marín *et al.* 1999; Atlantic salmon, Verspoor *et al.* 1999; common goby, Gysels *et al.* 2004), and it is
20 not impossible that such ice free areas in the southern North Sea could have allowed both freshwater
21 and coastal marine species, including European flounder, to sustain local populations during the last ice
22 age.

23 The $(\delta\mu)^2$ distances of app. 8 and 13 between the bank-flounder populations and Lake Pulmanki
24 and their closest neighbours would imply an average mutation rate of app. $1 - 2 \cdot 10^{-3}$, assuming a linear
25 relationship between $(\delta\mu)^2$ and time for microsatellites (Goldstein *et al.* 1995), a generation time of
26 three years and a divergence time of 10.000 years since the last glaciation. This estimate seems to be
27 high compared to other estimates for teleost microsatellites ($2 \cdot 10^{-4}$ in zebrafish, Shimoda *et al.* 1999;
28 $\sim 4 \cdot 10^{-3}$ in pink salmon, Steinberg *et al.* 2002), implying that these populations could have diverged
29 before the last glaciation. However, the proposed founder events may well have inflated the genetic
30 distances to these populations, thereby inflating the above mutation rate estimates. Furthermore,
31 classical marine fishes, such as Atlantic cod (Árnason 2004) and plaice (Hoarau *et al.* 2004), show
32 shallow mtDNA genealogies, providing little evidence for different glacial lineages in the North
33 Eastern Atlantic in these species. Hence, while we cannot exclude different glacial refugia as
34 responsible for generating parts of the signals detected here, it seems highly unlikely that this should be
35 the only factor involved in generating the signals of three (and potentially four if the Faroe Islands are
36 included) glacial lineages in the flounder. It appears more plausible that the large genetic distances to,
37 and marked genetic isolation of, these populations were generated by relatively recent events rather
38 than by different glacial histories.

39 40 *Life-history strategy*

41 We did not find elevated levels of structuring among populations spawning benthic eggs, which is
42 expected to reduce gene flow relative to a “pelagic egg” life history strategy. Waples (Waples 1987)
43 found a close relationship between dispersal capacity of eggs and larvae and levels of genetic
44 structuring in a large range of coastal species, but so far it has not been possible to compare levels of
45 divergence between life-history strategies with different dispersal capacities within the same species. In

1 the present case, the largest levels of structuring are found among populations with the high gene flow
2 life-history characteristics. While this finding may be contradictory to expectations it does illustrate
3 that life-history strategy *per se* may not be a good predictor of the expected levels of population
4 structure when several evolutionary drivers are considered simultaneously. On the other hand, the
5 known differences in life-history strategies do seem to be a very potent structuring force in European
6 flounder. The sharp break in genetic constitution among Baltic Sea populations is found between the
7 two different forms and indeed it is no surprise that a change from pelagic to benthic spawning habitat
8 would result in considerable reductions in gene flow between populations. Different life-histories are
9 also found in Atlantic herring, where population-components spawn during different times of the year.
10 The genetic and environmental base of this complex life-history switching is largely unknown, but
11 recently Ruzzante *et al.* (2006) have shown that these spawning components remain genetically distinct
12 despite extensive seasonal mixing at feeding grounds, implicating strong homing behaviour and
13 temporally stable population structure. Bekkevold *et al.* (in press) demonstrated that sympatric winter
14 and spring spawning components may in fact have different origins. Some winter spawning groups
15 were found to be genetically identical to the sympatric spring spawners, implying a plastic response in
16 a local population, while others were genetically more similar to winter spawning herring from distant
17 localities. In the latter case, the sympatric spawning components apparently have different geographical
18 origins, further illustrating the complexity of the Atlantic herring population structure in the North
19 Eastern Atlantic. While the degree of geographical overlap between the two life-history strategies in
20 European flounder is not known in detail, the genetic break observed in the inner Baltic Sea is
21 substantial and implicates significant reductions in gene flow between the two forms.

22 The evidence of ecological differentiation in European flounder demonstrates that rapid
23 adaptive divergence might also be possible under a high gene flow scenario in the marine environment.
24 If the splitting of “pelagic” and “benthic” flounder in the Baltic Sea happened after the initial
25 colonisation of the Baltic Sea it must have happened within the last app. 8.000 years, after the brackish
26 state of the Baltic Sea was established by salt water intrusion through the Danish straits (Bjorck 1995).
27 Similar examples of rapid evolution based on ecological differentiation (Orr & Schmidt 1998) have
28 also been found in cichlids (e.g. Galis & Metz 1998; Albertson *et al.* 2003), sticklebacks (McKinnon &
29 Rundle 2002) and whitefish (e.g. Lu & Bernatchez 1999). If the “benthic spawning” life-history did
30 evolve in response to the low saline environment, the selective forces would have acted on reproductive
31 strategies and generated reproductive isolation directly. Alternatively, reproductive isolation could have
32 acted as a reinforcement mechanism after an initial divergence in response to selection for other traits
33 (Schluter 2001), such as different migration patterns and/or delayed spawning time in response to
34 longer and more severe winters in the innermost Baltic Sea. Sub-species of European flounder have
35 also been described in the Mediterranean Sea, where *P. flesus luscus* and *P. flesus italicus* are believed
36 to have diverged from *P. flesus* about 2 My ago (Galleguillos & Ward 1982; Borsa *et al.* 1997), but so
37 far no detailed study has included the Bank-flounders in the Baltic Sea, which have apparently diverged
38 much more recently.

39 *Geographical distance*

40 Geographical distance *per se* does not seem to be very important for structuring populations of
41 European flounder, since we find no indication of isolation by distance when controlling for
42 environmental differences between samples. This result is caused mainly by the fact that populations
43 all the way from the western Baltic Sea to the Irish Sea are very similar genetically but still some of
44 them are separated by large geographic distances. The effects of other evolutionary drivers as well as
45

1 random noise are overriding any structuring effect of geographic distance in the present case. Genetic
2 structuring has also been found to be significantly associated with environmental parameters rather
3 than geographical distance in Atlantic herring (Bekkevold *et al.* 2005; Jørgensen *et al.* 2005). In these
4 cases structuring is associated with the marked environmental differences between the Baltic Sea and
5 the North Sea rather than with transitions within one of the two areas. Several species do show a
6 pattern of genetic isolation by distance in the North Eastern Atlantic. Geographic distance has been
7 found to be significantly associated with genetic structure in cod (Hutchinson *et al.* 2001), plaice
8 (Haorau *et al.* 2002a) and Atlantic herring (Mariani *et al.* 2005). These signals were mainly driven by
9 single divergent populations in the Barents Sea (cod), Iceland (plaice) and English Channel and Norway
10 (herring). Structuring among the remaining populations was low and only weakly associated with
11 geographic distance. A more detailed analysis of potential structuring along a latitudinal cline has not
12 been possible in these species because of a lack of samples along the cline.

13 *Oceanography and bathymetry*

14 The marked genetic isolation of the Faroe Islands population is in agreement with the expectations
15 based on oceanographic and bathymetric conditions in this area. Deep trenches and anti-cyclonic
16 circulation systems surrounding the islands (Jákupsstovu & Reinert 1994; Joensen *et al.* 2000) suggest
17 retention of egg and larvae and limited adult migration and thus function as effective barriers to gene
18 flow. Hoarau *et al.* (2002a) found that the Icelandic plaice (*P. platessa* L.) population was isolated from
19 all other populations in the North Eastern Atlantic and found no evidence of a separate
20 phylogeographic lineage at Iceland (Hoarau *et al.* 2004). Both European flounder and plaice are near
21 the margin of their distributions in these cases. Combined with limited gene flow across the deep areas
22 in the Atlantic, this pronounced physical isolation of island populations of flatfishes is the most
23 parsimonious explanation for their genetic isolation.

24 *Environmental differences*

25 We found that environmental differences coincided with population structure in two different cases,
26 highlighting the importance of these drivers for generating genetic structure in marine fish species. One
27 case was the abrupt change in genetic constitution in the inner Baltic Sea, while the other was a more
28 general association between environmental change and genetic structuring along a latitudinal cline in
29 the Atlantic. In both cases the effect of geographical distance was found to be of minor importance.

30 The clear isolation of the benthic spawning populations in the inner Baltic Sea is of a similar
31 magnitude to what has been observed at the transition zone to the Baltic Sea in other marine species
32 (Nielsen *et al.* 2003; Nielsen *et al.* 2004; Bekkevold *et al.* 2005; Johanneson & André 2006) and
33 suggests that the levels of divergence observed in these species represent significant levels of local
34 adaptation to the Baltic Sea environment. As an euryhaline species the flounder appears to tolerate the
35 steep salinity gradient at the transition zone and, accordingly, it does not present a major barrier to gene
36 flow. Instead, the major break is found at even lower salinities, where the gradient is less steep and is
37 clearly related to the marked change in life-history strategy believed to be an adaptation to the
38 environment in the inner Baltic Sea. Migration rate estimates from MIGRATE place the bank flounder
39 population sampled at Turku as a net donor of migrants, which further supports the status of this
40 population as a self-sustaining local population rather than a sink population.

41 The association between latitude and genetic structuring, when excluding populations at the
42 Faroe Islands, Gotland and Turku (benthic spawners) provide further evidence in support of
43 environmental differences as an important evolutionary force in marine fishes. This suggests that some
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1 environmental differences associated with latitude are more important than geographical distance *per*
2 *se* in shaping the genetic relationships among these populations. We can only hypothesize about
3 potential factors of importance, since we do not know the specific environmental agents involved. The
4 temperature gradient, both with respect to mean and variance in annual temperatures, is the most
5 obvious latitudinal gradient in this area. This gradient has been proposed to be important for generating
6 observed patterns of counter gradient variation in fitness related traits found in several marine fish
7 species inhabiting latitudinal clines (Conover *et al.* 1997; Dimichele & Westerman 1997; Conover
8 1998; Salvanes *et al.* 2004). However, other environmental components, such as day length and
9 seasonal cycles, could also be of importance for individual reproductive success.

10 It should be noted that the observed pattern of isolation by latitude is highly influenced by Lake
11 Pulmanki. This population is divergent from the remaining populations both with respect to genetic and
12 latitudinal differences. The apparent founder event in Lake Pulmanki could bias these analyses.
13 Exclusion of Lake Pulmanki from the partial Mantel tests does, however, not affect the conclusion of
14 latitude being more important than geography (geography, controlling for latitude: $r=0.1025$, $P=0.339$.
15 Latitude, controlling for geography: $r=0.7926$, $P=0.033$). Furthermore, there is also well supported
16 evidence of a barrier to gene flow to the Bay of Biscay, and hence the pattern of isolation by latitudinal
17 gradients rather than by geographical distance seems to be well supported.

18 *Subtle structure of potential biological significance*

19 The subtle structuring between the North Sea and the central Baltic Sea (Bor) populations appears to be
20 temporally stable and could hence be of evolutionary significance. Migration seems to be asymmetric
21 from the North Sea (Thy) to Bornholm, while Ærø is a net donor of migrants like the large population
22 in the North Sea. This may indicate that Bornholm represents the extreme edge of the Atlantic/North
23 Sea population-complex. Significant structuring indicate some reduction in gene flow between the
24 North Sea and central Baltic Sea, but it is unknown if this reduction is sufficient to allow adaptations to
25 local environments in these areas. However, unpublished results from ongoing work on gene
26 expression and genetic variation in candidate gene loci indicate that the populations of European
27 flounder in the western and central Baltic Sea are subject to different selective pressures and may
28 indeed be genetically adapted to local environments (P. Foged Larsen, Danish Institute for Fisheries
29 Research, personal communication; J. Hemmer-Hansen *et al.* unpublished results).

30 As is the case with most studies of genetic structure in marine fish species, it is unknown if the
31 system has yet reached drift-migration equilibrium. Hence, a pattern of little genetic differentiation
32 between populations could simply be the result of a non equilibrium situation rather than a signal of
33 high levels of gene flow. In this case, we would overestimate the levels of gene flow to the central
34 Baltic Sea. However, this study also clearly illustrates that sufficient time has passed to attain very high
35 levels of structuring among other populations of European flounder, so it seems reasonable to conclude
36 that gene flow from the Atlantic into the western and central Baltic Sea is relatively high in European
37 flounder compared to other species in this region.

38 *Conclusions*

39 We found evidence within one single species of marine fish for the simultaneous action of many of the
40 evolutionary drivers which have so far been reported in independent studies for different species. In
41 general, we found the largest genetic distances and reductions in gene flow to populations in the
42 extreme parts of the species' distributional area. While historical processes associated with colonization
43 following the last glaciation have most likely affected this signal, it is evident that several other
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1 evolutionary drivers are also acting to structure populations of European flounder. The genetic
2 signature of each driver is not easily predicted but rather seems to be dependent on its interplay with
3 other drivers in each specific scenario. Interestingly, we do not find any effect of geographical distance
4 *per se*. We do, however, find that physical forces such as oceanographic currents and bathymetric
5 obstacles to migration are coinciding with marked levels of genetic structuring. Moreover, we find that
6 environmental factors associated with environmental transitions are potent structuring forces in the
7 marine environment. These drivers are capable of generating patterns of structuring of similar or larger
8 magnitude as observed between populations with different life-history strategies, supporting the
9 evidence for the importance of these factors for driving population sub-structuring in marine fish
10 species.

References

- Abdo Z, Crandall KA, Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration. *Molecular Ecology*, **13**, 837-851.
- Albertson CA, Streebman JT, Kocher TD (2003) Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proceedings of the National Academy of Sciences of the USA*, **100**, 5252-5257.
- Árnason E (2004) Mitochondrial cytochrome b DNA variation in the high-fecundity Atlantic cod: Trans-atlantic clines and shallow gene genealogy. *Genetics*, **166**, 1871-1885.
- Aro E (1989) A review of fish migration patterns in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, **190**, 72-96.
- Beerli (2004) MIGRATE Documentation, Version 2.2. Available with the program package from <http://popgen.csit.fsu.edu>.
- Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of the USA*, **98**, 4563-4568.
- Bekkevold D, Andre C, Dahlgren TG *et al.* (2005) Environmental correlates of population differentiation in Atlantic herring. *Evolution*, **59**, 2656-2668.
- Bekkevold D, Clausen LAW, Mariani S *et al.* (2006) Divergent origins of sympatric herring population components determined using genetic mixture analysis. Marine Ecology Progress Series, in press.
- Bernardi G (2000) Barriers to gene flow in *Embiotoca jacksoni*, a marine fish lacking a pelagic larval stage. *Evolution*, **54**, 226-237.
- Bjorck S (1995) A Review of the History of the Baltic Sea, 13.0-8.0 Ka Bp. *Quaternary International*, **27**, 19-40.
- Borsa P, Blanquer A, Berrebi P (1997) Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, **129**, 233-246.
- Bos AR (1999) Tidal transport of flounder larvae (*Pleuronectes flesus*) in the Elbe River, Germany. *Archive of Fishery and Marine Research*, **47**, 47-60.
- Campos WL, Kloppmann M, von Westernhagen H (1994) Inferences from the horizontal distribution of dab *Limanda limanda* (L.) and floundre *Platichthys flesus* (L.) larvae in the southeastern North Sea. *Netherlands Journal of Sea Research*, **32**, 277-286.
- Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis - models and estimation procedures. *Evolution*, **21**, 550-570.
- Chakraborty R, De Andrade M, Daiger SP, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics*, **56**, 45-57.
- Conover DO (1998) Local adaptation in marine fishes: Evidence and implications for stock enhancement. *Bulletin of Marine Science*, **62**, 477-493.
- Conover DO, Brown JJ, Ehtisham A (1997) Countergradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2401-2409.
- DeWoody JA, Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of fish biology*, **56**, 461-473.
- Dimichele L, Westerman ME (1997) Geographic variation in development rate between populations of the teleost *Fundulus heteroclitus*. *Marine Biology*, **128**, 1-7.

- 1 Drevs T, Kadakas V, Lang T, Møllergaard S (1999) Geographical variation in the age/length
2 relationship in Baltic flounder (*Platichthys flesus*). *Ices Journal of Marine Science*, **56**, 134-137.
- 3 ElMousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among
4 populations of the argan tree (*Argania spinosa* (L) Skeels) endemic to Morocco. *Theoretical and*
5 *Applied Genetics*, **92**, 832-839.
- 6 Estoup A, Largiadere CR, Perrot E, Chourrou D (1996) Rapid one-tube DNA extraction for reliable
7 PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and*
8 *Biotechnology*, **5**, 295-298.
- 9 Felsenstein J (2004) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author.
10 Department of Genome Sciences, University of Washington, Seattle.
- 11 Galleguillos RA, Ward RD (1982) Genetic and morphological divergence between populations of the
12 flatfish *Platichthys flesus* (L.) (Pleuronectidae). *Biological Journal of the Linnean Society*, **17**, 395-
13 408.
- 14 Gallis F, Metz JAJ (1998) Why are there so many cichlid species? *Trends in Ecology & Evolution*, **13**,
15 1-2.
- 16 García-Marín JL, Utter FM, Pla C (1999) Postglacial colonization of brown trout in Europe based on
17 distribution of allozyme variants. *Heredity*, **82**, 46-56.
- 18 Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) Genetic absolute dating based on
19 microsatellites and the origin of modern humans. *Proceedings of the National Academy of Sciences of*
20 *the USA*, **92**, 6723-6727.
- 21 Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of*
22 *Heredity*, **86**, 485-486.
- 23 Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine
24 fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**,
25 415-426.
- 26 Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple
27 alleles. *Biometrics*, **48**, 361-372.
- 28 Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004) Phylogeography of the common goby,
29 *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the
30 North Sea. *Molecular Ecology*, **13**, 403-417.
- 31 Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907-913.
- 32 Hickerson MJ, Cunningham CW (2005) Contrasting quaternary history in an ecologically divergent
33 sister pair of low-dispersing intertidal fish (*Xiphister*) revealed by multilocus DNA analysis.
34 *Evolution*, **59**, 344-360.
- 35 Hoarau G, Cook D, Stam WT, Olsen JL (2002b) New microsatellites primers for plaice, *Pleuronectes*
36 *platessa* L. (Teleostei : Pleuronectidae). *Molecular Ecology Notes*, **2**, 60-61.
- 37 Hoarau G, Piquet AMT, van der Veer HW *et al.* (2004) Population structure of plaice (*Pleuronectes*
38 *platessa* L.) in northern Europe: a comparison of resolving power between microsatellites and
39 mitochondrial DNA data. *Journal of Sea Research*, **51**, 183-190.
- 40 Hoarau G, Rijnsdorp AD, Van der Veer HW, Stam WT, Olsen JL (2002a) Population structure of
41 plaice (*Pleuronectes platessa* L.) in northern Europe: microsatellites revealed large-scale spatial and
42 temporal homogeneity. *Molecular Ecology*, **11**, 1165-1176.
- 43 Hutchinson WF, Carvalho GR, Rogers SI (2001) Marked genetic structuring in localised spawning
44 populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by
45 microsatellites. *Marine Ecology Progress Series*, **223**, 251-260.

- 1 Jákupsstovu SH í, Reinert J (1994) Fluctuations in the Faroe Plateau cod stock. *ICES Marine Science*
2 *Symposium*, **198**, 194-211.
- 3 Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13.
4 <http://phage.sdsu.edu/~jensen/>.
- 5 Joensen H, Steingrund P, Fjallstein I, Grahl-Nielsen O (2000) Discrimination between two reared
6 stocks of cod (*Gadus morhus*) from the Faroe Islands by chemometry of the fatty acid composition in
7 the heart tissue. *Marine Biology*, **136**, 573-580.
- 8 Johanneson K, André C (2006) Life on the margin: genetic isolation and diversity loss in a peripheral
9 marine ecosystem, the Baltic Sea. *Molecular Ecology*, **15**, 2013-2029.
- 10 Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes
11 and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Molecular*
12 *Ecology*, **14**, 3219-3234.
- 13 Knutsen H, André C, Jorde PE *et al.* (2004) Transport of North Sea cod larvae into the Skagerrak
14 coastal populations. *Proceedings of the Royal Society of London, Series B - Biological Sciences*, **271**,
15 1337-1344.
- 16 Lu G, Bernatchez L (1999) Correlated trophic specialization and genetic divergence in sympatric lake
17 whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis.
18 *Evolution*, **53**, 1491-1505.
- 19 Lundy CJ, Moran P, Rico C, Milner RS, Hewitt GM (1999) Macrogeographical population
20 differentiation in oceanic environments: a case study of European hake (*Merluccius merluccius*), a
21 commercially important fish. *Molecular Ecology*, **8**, 1889-1898.
- 22 Lundy CJ, Rico C, Hewitt GM (2000) Temporal and spatial genetic variation in spawning grounds of
23 European hake (*Merluccius merluccius*) in the Bay of Biscay. *Molecular Ecology*, **9**, 2067-2079.
- 24 Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic)
25 variation: How barriers can be detected by using Monmonier's algorithm. *Human Biology*, **76**, 173-
26 190.
- 27 Mariani S, Hutchinson WF, Hatfield EMC (2005) North Sea herring population structure revealed by
28 microsatellite analysis. *Marine Ecology Progress Series*, **303**, 245-257.
- 29 McKinnon JA, Rundle HD (2002) Speciation in nature: the threespine stickleback model systems.
30 *Trends in Ecology & Evolution*, **17**, 480-488.
- 31 Nielsen EE, Kenchington EA (2001) A new approach to prioritizing marine fish and shellfish
32 populations for conservation. *Fish and Fisheries*, **3**, 328-343.
- 33 Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P (2003) Evidence of a hybrid-zone in
34 Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture
35 analysis. *Molecular Ecology*, **12**, 1497-1508.
- 36 Nielsen EE, Nielsen PH, Meldrup D, Hansen MM (2004) Genetic population structure of turbot
37 (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the
38 transition zone between the Baltic Sea and the North Sea. *Molecular Ecology*, **13**, 585-595.
- 39 Nissling A, Westin L, Hjerne O (2002) Reproductive success in relation to salinity for three flatfish
40 species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*),
41 in the brackish water Baltic Sea. *ICES Journal of Marine Science*, **59**, 93-108.
- 42 Ojaveer E, Kalejs M (2005) The impact of climate change on the adaptation of marine fish in the Baltic
43 Sea. *ICES Journal of Marine Science*, **62**, 1492-1500.
- 44 Orr MR, Smith TB (1998) Ecology and speciation. *Trends in Ecology & Evolution*, **13**, 502-506.

- 1 Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic cod,
2 *Gadus morhua*, at large and small geographic scales. *Evolution*, **55**, 131-146.
- 3 Raymond M, Rousset F (1995) Genepop (Version-1.2) - population-genetics software for exact tests
4 and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- 5 Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of
6 biogeography, geographical distance and discontinuous habitat to genetic differentiation in a
7 blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology*, **10**, 1439-1453.
- 8 Roques S, Sévigny J-M, Bernatchez L (2002) Genetic structure of deep-water redfish, *Sebastes*
9 *Mantella*, populations across the North Atlantic. *Marine Biology*, **140**, 297-307.
- 10 Ruzzante DE, Mariano S, Bekkevold D et al. (2006) Biocomplexity in a highly migratory pelagic
11 marine fish, Atlantic herring. *Proceedings of the Royal Society of London, Series B - Biological*
12 *Sciences*, **273**, 1459-1464.
- 13 Ruzzante DE, Taggart CT, Cook D (1999) A review of the evidence for genetic structure of cod
14 (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off
15 Newfoundland and the Gulf of St. Lawrence. *Fisheries Research*, **43**, 79-97.
- 16 Salvanes AGV, Skjaeraasen JE, Nilssen T (2004) Sub-populations of coastal cod with different
17 behaviour and life-history strategies. *Marine Ecology-Progress Series*, **267**, 241-251.
- 18 Sarup P, Sørensen JG, Dimitrov K, Barker JSF, Loeschcke V (2006) Climatic adaptation of *Drosophila*
19 *buzzatii* populations in southeast Australia. *Heredity*, **96**, 479-486.
- 20 Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**, 372-380.
- 21 Shimoda N, Knapik EW, Ziniti J et al. (1999) Zebrafish genetic map with 2000 microsatellite markers.
22 *Genomics*, **58**, 219-232.
- 23 Sick K, Frydenberg O, Nielsen JT (1963) Haemoglobin patterns of plaice, flounder, and their natural
24 and artificial hybrids. *Nature*, **198**, 411-412.
- 25 Solemdal P (1967) The effect of salinity on buoyancy, size and development of flounder eggs. *Sarsia*,
26 **29**, 431-442.
- 27 Solemdal P (1973) Transfer of Baltic flatfish to a marine environment and long-term effects on
28 reproduction. *Oikos*, **15**, 268-276.
- 29 Steinberg EK, Lindner KR, Gallea J et al. (2002) Rates and patterns of microsatellite mutations in pink
30 salmon. *Molecular Biology and Evolution*, **19**, 1198-1202.
- 31 Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from
32 microsatellite DNA. *Genetics*, **144**, 389-399.
- 33 Truett GE, Heeger P, Mynatt RL et al. (2000) Preparation of PCR-quality mouse genomic DNA with
34 hot sodium hydroxide and TRIS (HotSHOT). *BioTechniques*, **29**, 52-54.
- 35 Vallin L, Nissling A, Westin L (1999) Potential factors influencing reproductive success of Baltic cod,
36 *Gadus morhua*: A review. *Ambio*, **28**, 92-99.
- 37 van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for
38 identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-
39 538.
- 40 Verspoor E, McCarthy EM, Knox D (1999) The phylogeography of European Atlantic salmon (*Salmo*
41 *salar* L.) based on RFLP analysis of the ND1/16sRNA region of the mtDNA. *Biological Journal of*
42 *the Linnean Society*, **68**, 129-146.
- 43 Waples RS (1987) A Multispecies approach to the analysis of gene flow in marine shore fishes.
44 *Evolution*, **41**, 385-400.

- 1 Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene
2 flow species. *Journal of Heredity*, **89**, 438-450.
- 3 Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity levels in marine,
4 fresh-water, and anadromous fishes. *Journal of Fish Biology*, **44**, 213-232.
- 5 Watts PC, Nash RDM, George SG, Kemp SJ (1999) Isolation and characterization of microsatellite loci
6 in the European plaice, *Pleuronectes platessa* L. (Teleostei: Pleuronectidae). *Molecular Ecology*, **8**,
7 2151-2152.
- 8 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population-structure.
9 *Evolution*, **38**, 1358-1370.
- 10 Widmer A, Lexer C (2001) Glacial refugia: sanctuaries for allelic richness, but not for gene diversity.
11 *Trends in Ecology & Evolution*, **16**, 267-269.
- 12 Wilson AB (2006) Genetic signature of recent glaciation on populations of a near-shore marine fish
13 species (*Syngnathus leptorhynchus*). *Molecular Ecology*, **15**, 1857-1871.

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23 presentations and to Mikkel Heide Schierup for computer assistance for the MIGRATE analyses.

27 Author Information Box

28
29 This work represents a part of Jakob Hemmer-Hansen's PhD thesis. He is interested in population
30 structure and evolution of marine fishes in general and the genetic basis of local adaptations in
31 particular. The focus of Einar Eg Nielsen's research is on population structure and evolution of marine
32 and anadromous fishes. Peter Grønkjær is a fisheries and fish biologist working on population biology
33 and recruitment in marine fishes and Volker Loeschcke has a broad interest in evolutionary genetics
34 and has been working on a number of species from fruit flies to fishes.

1 **Figure legends**

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3 **Figure 1.** Map of sampling locations and major barriers to gene flow. Numbers refer to sample names
4 in Table 1 and barrier letters to barriers identified in Table 2.

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6 **Figure 2.** Hybrid tree based on Cavalli-Sforza's chord distance for topology and $(\delta\mu)^2$ for branch
7 lengths. The tree is rooted with the North Sea plaice sample. The distance to the plaice sample is
8 truncated to enhance graphical presentation of the remaining flounder populations. The $(\delta\mu)^2$ distance
9 between the plaice and flounder samples is approximately 1700.

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1 **Table 1** Samples of European flounder. Location numbers refer to Figure 1. Mature/maturing individuals are
 2 ripe and/or running.

Location	Sample name	Date	App. position	Sample size	Spawning strategy	Proportion maturing and mature
1. Turku 2003	Tur03	May	22° E, 60° N	54	Benthic	100
2. Turku 2004	Tur04	June	22° E, 60° N	50	Benthic	24
3. Gotland 2003	Got03	April	18.5° E, 57° N	46	Benthic	App. 50
4. Gotland 2004	Got04	June	19.5° E, 58° N	48	Benthic	0
5. Bornholm 2003	Bor03	March	16° E, 55.1° N	55	Pelagic	100
6. Bornholm 2004	Bor04	March	16° E, 55.1° N	53	Pelagic	100
7. Ærø 2003	Aer03	Feb-Mar	10° E, 55° N	52	Pelagic	100
8. Ærø 2004	Aer04	Mar	10° E, 55° N	52	Pelagic	100
9. Thyborøn 2003	Thy03	Feb	8° E, 57° N	55	Pelagic	100
10. Thyborøn 2004	Thy04	March	8° E, 57° N	59	Pelagic	19
11. Ringkøbing Fjord 2003	Rin03	Jan	8.3° E, 55.96° N	39	Pelagic	90
12. Ringkøbing Fjord 2004	Rin04	Feb	8.3° E, 55.96° N	50	Pelagic	10
13. The Limfjord 2003	Lim03	April	8.59° E, 56.5° N	55	Pelagic	0
14. Irish Sea 2003	Irs03	March	-4° E, 54° N	49	Pelagic	69
15. Irish Sea 2004	Irs04	March	-4° E, 54° N	49	Pelagic	98
16. Westerschelde estuary 2003	Wes03	May-Sept	3.7° E, 52.4° N	47	Pelagic	0
17. Bay of Biscay 2003	Bis03	Sept	-2.3° E, 47.20° N	49	Pelagic	0
18. Bay of Biscay 2004	Bis04	Okt	-2.3° E, 47.20° N	39	Pelagic	0
19. Trondheim 2004	Tro04	Sept	11° E, 65° N	49	Pelagic	Juveniles
20. Lake Pulmanki 2004	Pul04	Jun-Jul	28.02° E, 70.01° N	34	Pelagic	0
21. Faeroe Islands 2003	Far03	April	-6.45° E, 62° N	44	Pelagic	84
22. Faeroe Islands 2004	Far04	Feb-Jul	-6.45° E, 62° N	34	Pelagic	56
23. Plaice 2003	Pla03	March	9° E, 57.3° N	18	Pelagic	0

1 **Table 2** Location of major barriers to gene flow identified by the program BARRIER. The barrier
 2 order reflects the strength of the barrier. The numbers given are the number of bootstrapped F_{ST}
 3 matrices supporting a barrier. Note that numbers do not always add up to 100 because some rare
 4 barriers are supported by a few matrices. Barrier letters refer to Figure 1. Barrier e) refers
 5 particularly to the separation of Trondheim and western Baltic Sea; fewer matrices support the
 6 separation of Trondheim and the North Sea. Barrier f) is a barrier between the Thyborøn and
 7 Limfjord/Ærø and samples.

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2003	1 st order	2 nd order	3 rd order	4 th order
Barrier a	96	4		
Barrier b	4	90	6	
Barrier d		5	27	
Barrier f		1	57	
2004				
Barrier a	99	1		
Barrier c	1	51	34	10
Barrier d		33	39	16
Barrier b		10	18	38
Barrier e		5	9	34

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1 **Table 3** Mantel and partial Mantel tests on a subset of samples, excluding samples from Turku, Gotland and the Faroe Islands.
 2 Significance was assessed with 1000 permutations.
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		Indicator								
		Z	r	P	Z	Latitude r	P	Z	Longitude r	P
2003										
Geographic distance		203.60	0.008	0.495						
	- controlling for indicator					-0.073	0.602		-0.143	0.652
Indicator					0.6537	0.168	0.390	1.3189	0.067	0.428
	- controlling for geography					0.183	0.316		0.157	0.404
2004										
Geographic distance		939.27	0.705	0.002						
	- controlling for indicator					0.161	0.241		0.268	0.138
Indicator					4.5689	0.886	0.001	6.0680	0.703	0.009
	- controlling for geography					0.764	0.002		0.260	0.132

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1 **Table 4.** Final estimates of Θ and M from the three MIGRATE runs. Results with 95% confidence intervals from individual runs
 2 are given in supplementary tables S1-S3.

<i>Receiving population</i>		Θ	M	<i>Donor population</i>									
				Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs
Turku	First run	0.238		17.67	13.10	13.21	10.71	8.86	11.83	17.93	7.80	14.53	8.81
	Second run	0.332		14.53	11.07	16.08	11.04	9.97	14.65	18.92	6.18	10.10	9.00
	Third run	0.321		16.04	11.54	12.79	12.28	6.70	11.82	14.63	7.27	9.09	5.88
Bornholm	First run	0.308	16.67		15.61	19.37	15.51	10.62	16.22	12.26	9.86	14.54	13.27
	Second run	0.370	16.25		13.75	20.02	14.04	6.76	14.83	12.13	9.47	13.93	9.56
	Third run	0.308	24.72		16.33	19.64	13.80	10.30	16.33	16.67	11.59	15.67	12.47
Ærø	First run	0.354	15.28	14.73		14.12	13.71	8.09	15.33	9.80	7.90	15.98	9.99
	Second run	0.350	12.22	13.89		16.19	14.92	7.09	13.32	15.97	7.65	12.36	11.54
	Third run	0.349	12.60	14.94		14.35	10.28	6.63	12.62	10.24	9.74	12.71	12.04
Thyborøn	First run	0.417	10.93	14.39	15.04		13.82	8.54	17.92	11.67	11.14	14.35	9.63
	Second run	0.359	12.95	15.29	15.13		17.31	9.75	11.93	15.45	8.50	14.00	12.50
	Third run	0.419	11.72	15.82	17.66		12.23	8.81	16.55	11.12	9.04	15.18	11.08
Ringkøbing Fjord	First run	0.402	11.26	16.04	16.47	21.34		9.32	17.51	10.79	7.95	10.27	11.97
	Second run	0.298	15.26	21.05	24.39	22.61		9.29	21.61	8.54	11.71	19.25	17.24
	Third run	0.399	14.43	12.90	12.91	18.07		10.10	14.95	8.91	7.00	13.71	11.48
Faroe Islands	First run	0.188	11.61	12.64	12.23	12.98	13.12		9.84	7.93	4.45	8.95	10.04
	Second run	0.172	17.79	13.49	14.79	17.39	13.81		13.97	12.10	6.50	10.57	12.68
	Third run	0.170	12.09	12.72	14.62	13.98	12.45		12.51	9.50	9.14	10.47	8.90
Trondheim	First run	0.284	9.933	12.73	13.79	16.45	16.45	6.65		11.26	7.85	10.33	8.96
	Second run	0.285	14.18	16.25	16.93	21.60	15.27	11.57		13.42	8.62	13.69	12.24
	Third run	0.269	11.89	10.93	13.76	19.25	13.94	7.62		13.21	7.12	10.37	10.03

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1 **Table 4 continued**

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<i>Receiving population</i>		θ	M	<i>Donor population</i>									
			Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs	Bis
Gotland	First run	0.272	17.64	17.23	13.02	18.27	16.20	5.13	14.41		7.95	10.38	8.82
	Second run	0.310	16.75	15.79	19.33	19.55	13.40	7.54	15.97		8.65	11.49	11.22
	Third run	0.224	28.82	18.63	16.57	16.50	14.37	15.29	13.51		13.08	13.80	14.36
Lake Pulmanki	First run	0.189	11.34	15.20	16.76	17.33	14.05	5.75	16.51	11.91		12.24	10.28
	Second run	0.189	20.45	22.15	20.24	19.19	15.18	7.17	19.71	18.45		15.92	13.50
	Third run	0.179	9.653	10.83	15.20	16.32	10.83	5.98	12.16	14.80		9.59	7.50
Irish Sea	First run	0.294	18.61	15.19	10.90	19.04	13.30	8.30	12.75	11.72	5.64		8.08
	Second run	0.317	15.89	12.17	15.32	18.62	16.28	6.98	11.33	13.00	8.44		10.85
	Third run	0.383	8.947	14.48	15.17	16.11	11.83	8.29	11.87	9.60	6.99		9.45
Bay of Biscay	First run	0.325	12.49	11.98	13.90	16.87	18.03	12.04	11.27	13.46	8.31	9.66	
	Second run	0.224	13.74	15.26	17.19	18.44	14.27	11.70	14.67	10.84	10.18	12.00	
	Third run	0.307	12.25	13.94	13.56	15.07	16.00	8.99	14.56	9.79	9.67	14.36	

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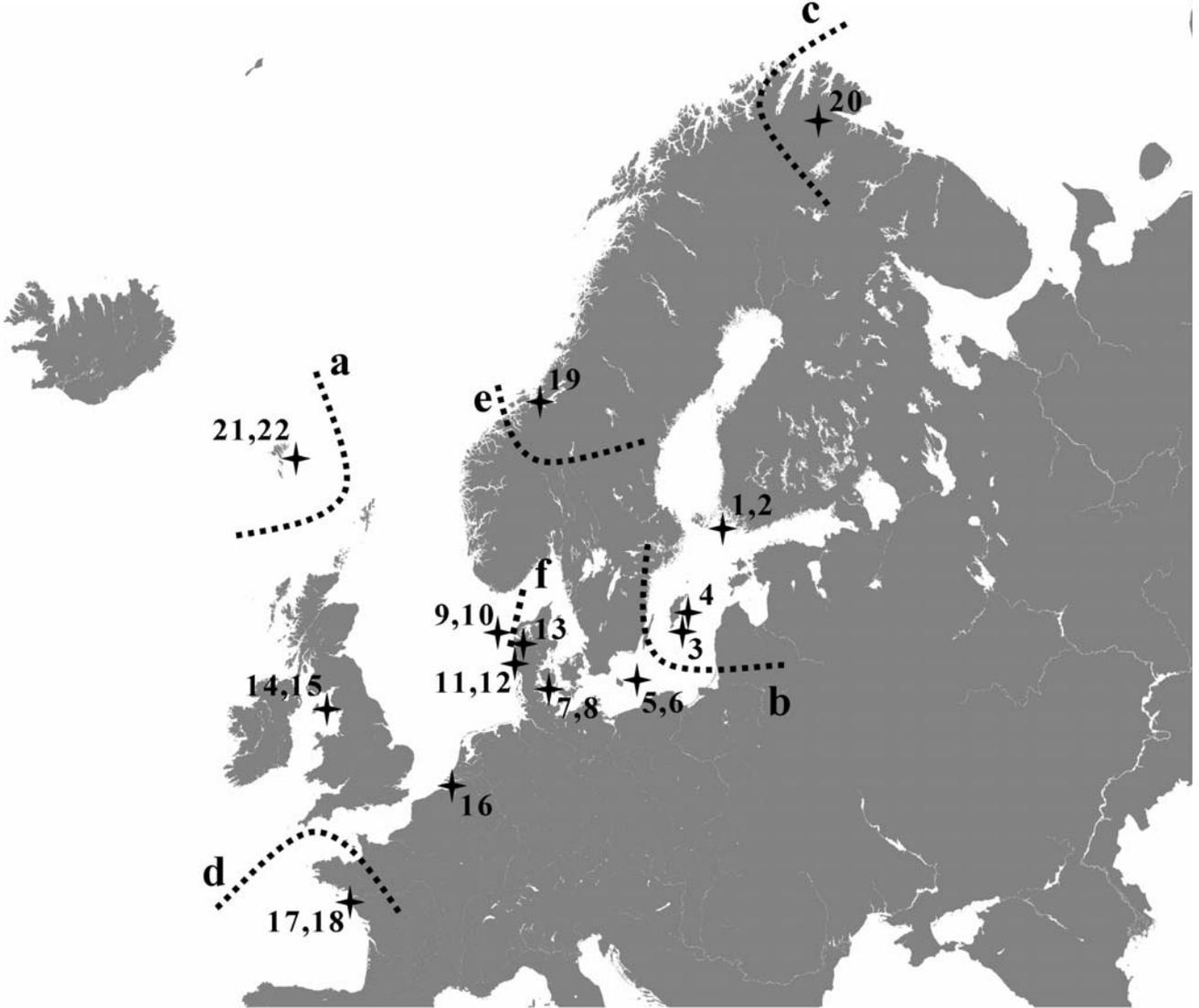
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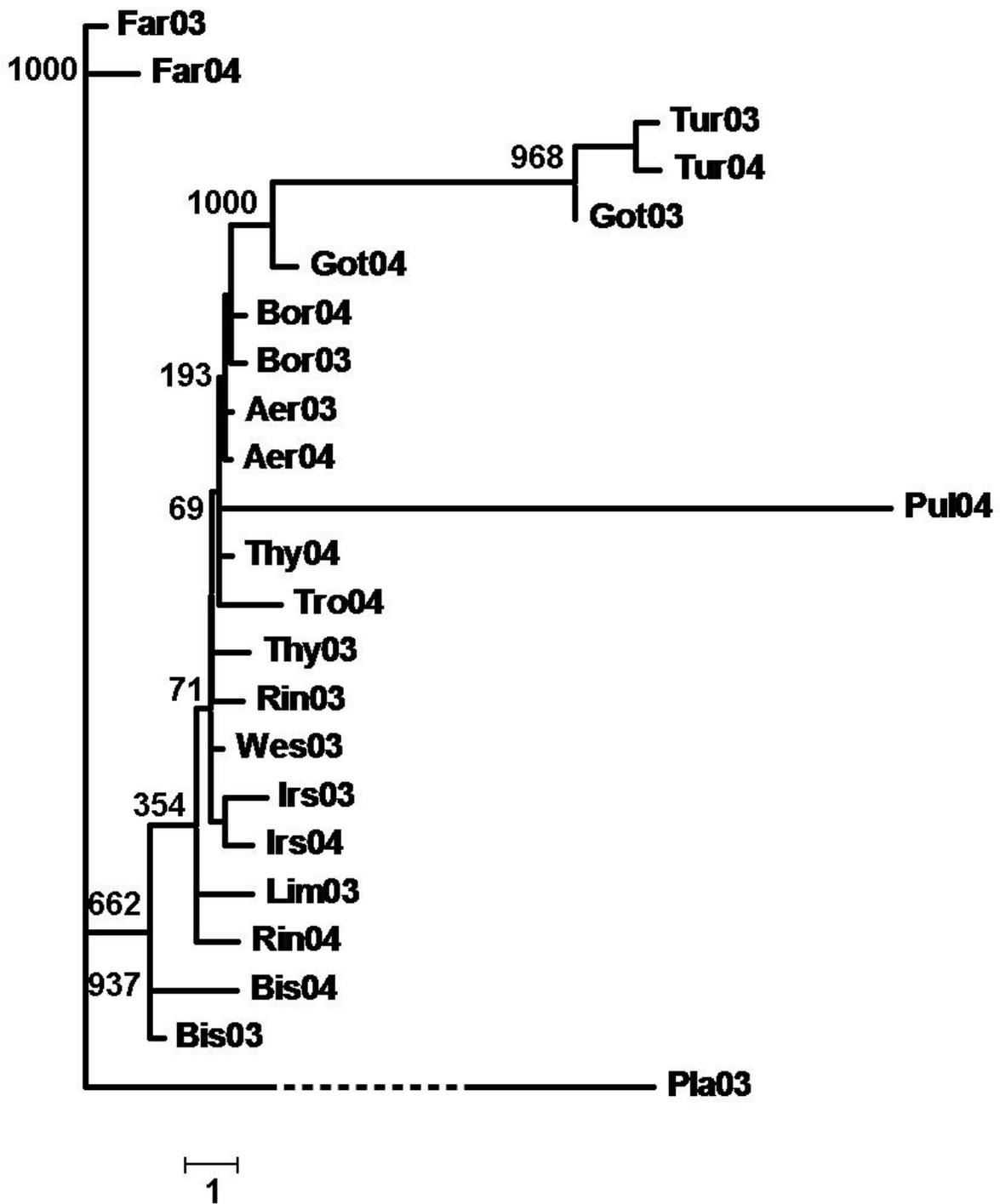
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Figure 1.



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2 Figure 2.
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1 **Appendix A** Estimates of genetic diversity and exact tests for deviation from Hardy Weinberg proportions

2

Locus P1142

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	17	21	17	16	20	19	22	22
Allelic richness	13.873	17.257	15.156	14.813	16.852	16.209	18.401	18.421
Size range	159-207 bp	153-207 bp	161-209 bp	139-203 bp	143-195 bp	147-191 bp	147-203 bp	145-203 bp
H _o	0.889	0.88	0.87	0.976	0.891	0.887	0.922	0.904
H _e	0.887	0.902	0.90	0.91	0.928	0.9	0.917	0.922
HWE exact test	0.8889	0.2869	0.0679	0.9536	0.5732	0.9172	0.8956	0.5374
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	21	18	18	18	21	24	19	21
Allelic richness	18.021	15.841	17.167	16.110	18.231	21.114	17.424	18.47
Size range	141-203 bp	137-195 bp	151-191 bp	155-197 bp	155-203 bp	147-229 bp	147-195 bp	147-203 bp
H _o	0.981	0.898	0.846	0.96	0.927	0.959	0.867	0.841
H _e	0.93	0.92	0.937	0.928	0.934	0.94	0.927	0.927
HWE exact test	0.6545	0.4657	0.0043	0.9449	0.0102	0.7821	0.0303	0.4279
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	19	17	22	13	9	10		14
Allelic richness	16.830	15.959	18.428	12.967	8.664	9.817		
Size range	139-193 bp	139-191 bp	139-199 bp	147-189 bp	159-185 bp	155-185 bp		137-193 bp
H _o	0.898	0.833	0.959	0.903	0.864	0.848		0.889
H _e	0.912	0.896	0.933	0.901	0.821	0.797		0.894
HWE exact test	0.6148	0.2541	0.7079	0.9499	0.6074	0.2114		0.4971

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1 **Appendix A continued.**

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Locus StPf1004

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	11	14	10	14	11	16	18	17
Allelic richness	10.001	11.474	9.394	11.889	9.412	12.716	14.206	14.180
Size range	138-188 bp	132-180 bp	144-188 bp	144-188 bp	142-188 bp	138-192 bp	132-200 bp	140-180 bp
H _o	0.778	0.8	0.891	0.872	0.618	0.774	0.68	0.712
H _e	0.779	0.841	0.816	0.833	0.728	0.831	0.782	0.832
HWE exact test	0.6729	0.7464	0.2666	0.8538	0.0065	0.3215	0.0161	0.4156
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	17	18	18	15	11	19	16	18
Allelic richness	13.621	13.698	16.181	11.476	8.252	16.084	14.041	14.322
Size range	138-190 bp	138-190 bp	132-198 bp	138-194 bp	142-184 bp	136-188 bp	140-188 bp	138-192 bp
H _o	0.855	0.763	0.737	0.66	0.545	0.816	0.773	0.804
H _e	0.835	0.744	0.82	0.727	0.602	0.797	0.801	0.763
HWE exact test	0.9989	0.3504	0.188	0.356	0.5482	0.5504	0.5611	0.9703
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	17	14	15	15	5	8		10
Allelic richness	13.908	13.050	12.829	14.356	5.000	7.870		
Size range	134-188 bp	136-166 bp	132-192 bp	144-192 bp	138-188 bp	138-192 bp		144-190 bp
H _o	0.776	0.842	0.857	0.878	0.795	0.765		0.444
H _e	0.777	0.809	0.809	0.882	0.725	0.789		0.884
HWE exact test	0.25	0.5355	0.3474	0.0717	0.4352	0.4667		0.0000

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1 **Appendix A continued.**

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Locus List1001

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	3	3	3	3	3	4	3	3
Allelic richness	2.964	2.939	2.965	2.982	2.982	3.533	2.995	2.970
Size range	82-86 bp	80-90 bp	82-86 bp	82-86 bp				
H _o	0.352	0.28	0.267	0.229	0.236	0.226	0.327	0.327
H _e	0.316	0.307	0.257	0.306	0.232	0.323	0.401	0.313
HWE exact test	0.4109	0.6001	0.3879	0.0511	0.6117	0.0215	0.103	1
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	3	3	3	4	3	4	3	4
Allelic richness	2.964	2.988	2.949	3.597	2.545	3.557	2.852	3.827
Size range	82-86 bp	82-86 bp	82-86 bp	80-86 bp	82-86 bp	80-86 bp	82-86 bp	80-86 bp
H _o	0.37	0.424	0.41	0.44	0.327	0.388	0.347	0.362
H _e	0.363	0.438	0.446	0.438	0.348	0.368	0.336	0.419
HWE exact test	1	0.9111	0.0229	0.1349	0.7515	0.7395	0.5652	0.2793
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	3	4	3	3	3	3		5
Allelic richness	2.980	3.769	2.993	2.997	2.991	2.988		
Size range	82-86 bp	80-86 bp	82-86 bp	82-86 bp	82-86 bp	82-86 bp		88-94 bp
H _o	0.388	0.436	0.49	0.563	0.477	0.235		0.786
H _e	0.39	0.492	0.495	0.478	0.422	0.324		0.706
HWE exact test	1	0.0956	1	0.4848	0.6641	0.0965		0.3964

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1 **Appendix A continued.**

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Locus P1167

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	11	11	10	10	17	14	13	12
Allelic richness	9.523	9.622	8.662	9.035	13.101	11.843	11.044	10.132
Size range	168-208 bp	168-208 bp	168-210 bp	168-208 bp	168-232 bp	168-232 bp	168-232 bp	168-224 bp
H _o	0.778	0.7	0.778	0.886	0.873	0.774	0.769	0.769
H _e	0.743	0.701	0.762	0.809	0.878	0.858	0.853	0.805
HWE exact test	0.4276	0.5452	0.6861	0.3017	0.0245	0.0668	0.356	0.006
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	14	13	12	14	15	13	16	14
Allelic richness	11.880	11.914	10.842	11.648	12.920	11.687	13.321	11.668
Size range	164-224 bp	168-214 bp	168-220 bp	166-212 bp	168-210 bp	168-208 bp	168-224 bp	168-224 bp
H _o	0.8	0.842	0.795	0.78	0.836	0.735	0.841	0.851
H _e	0.822	0.843	0.799	0.804	0.83	0.815	0.813	0.787
HWE exact test	0.0542	0.8788	0.4106	0.444	0.1059	0.396	0.402	0.9881
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	13	11	14	9	7	8		22
Allelic richness	11.201	10.693	11.313	8.931	6.235	7.634		
Size range	168-224 bp	168-224 bp	168-208 bp	168-308 bp	184-226 bp	168-206 bp		132-228 bp
H _o	0.816	0.771	0.714	0.781	0.707	0.588		1
H _e	0.798	0.841	0.761	0.792	0.641	0.691		0.97
HWE exact test	0.5417	0.4237	0.7524	0.9422	0.5612	0.0672		1

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1 **Appendix A continued.**

2

Locus StPf1005

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	5	4	4	5	3	4	4	4
Allelic richness	4.540	3.977	3.987	4.633	3.000	3.566	3.588	3.577
Size range	99-117 bp	99-117 bp	99-117 bp	99-117 bp	99-115 bp	97-115 bp	99-115 bp	99-117 bp
H _o	0.648	0.74	0.696	0.638	0.473	0.623	0.647	0.673
H _e	0.677	0.696	0.669	0.702	0.574	0.625	0.599	0.605
HWE exact test	0.6678	0.1754	0.8897	0.5982	0.2255	0.2496	0.328	0.1442
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	4	3	4	3	4	4	4	3
Allelic richness	3.545	3.000	3.769	3.000	3.545	3.612	3.625	3
Size range	99-115 bp							
H _o	0.564	0.61	0.692	0.5	0.745	0.49	0.604	0.596
H _e	0.595	0.613	0.66	0.611	0.606	0.57	0.661	0.607
HWE exact test	0.3509	0.6084	0.5377	0.2492	0.109	0.2991	0.6643	0.4478
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	5	5	5	3	3	2		6
Allelic richness	4.224	4.538	4.557	3.000	2.970	2.000		
Size range	99-117 bp	97-115 bp	97-115 bp	99-115 bp	99-115 bp	99-105 bp		101-111 bp
H _o	0.531	0.564	0.714	0.667	0.318	0.118		0.588
H _e	0.55	0.523	0.678	0.615	0.295	0.112		0.827
HWE exact test	0.5562	0.8031	0.9591	0.4018	0.5014	1		0.0269

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1 **Appendix A continued.**

2

Locus StPf1022

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	19	15	16	16	15	15	17	16
Allelic richness	15.857	13.786	14.235	13.989	13.316	13.546	14.212	14.013
Size range	164-234 bp	166-226 bp	166-226 bp	162-228 bp	164-210 bp	164-214 bp	164-226 bp	164-214 bp
H _o	0.887	0.82	0.957	0.872	0.836	0.887	0.865	0.942
H _e	0.857	0.865	0.872	0.857	0.886	0.879	0.898	0.894
HWE exact test	0.7917	0.259	0.195	0.3594	0.2045	0.5106	0.8132	0.8102
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	15	17	17	17	14	14	15	14
Allelic richness	13.133	14.853	15.627	14.818	12.493	12.605	13.196	12.865
Size range	164-214 bp	164-222 bp	166-226 bp	164-222 bp	166-210 bp	158-206 bp	162-226 bp	166-210 bp
H _o	0.833	0.864	0.897	0.86	0.945	0.857	0.771	0.867
H _e	0.886	0.907	0.895	0.909	0.891	0.893	0.891	0.904
HWE exact test	0.1927	0.0604	0.9784	0.2243	0.0177	0.8516	0.0176	0.2253
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	16	16	17	10	9	10		12
Allelic richness	13.671	15.212	14.951	9.856	8.235	9.517		
Size range	166-222 bp	166-218 bp	164-214 bp	164-194 bp	166-214 bp	166-214 bp		262-326 bp
H _o	0.938	0.872	0.918	0.853	0.886	0.912		0.667
H _e	0.891	0.923	0.886	0.833	0.814	0.827		0.926
HWE exact test	0.624	0.2471	0.8271	0.4611	0.9708	0.1737		0.0381

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1 **Appendix A continued.**

2

Locus StPf1015

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	5	4	5	6	6	6	6	5
Allelic richness	4.909	3.999	4.598	5.487	5.811	5.486	5.354	4.958
Size range	116-136 bp	124-136 bp	116-136 bp	116-140 bp	116-140 bp	116-140 bp	116-140 bp	116-136 bp
H _o	0.463	0.44	0.4	0.5	0.509	0.434	0.549	0.423
H _e	0.494	0.459	0.376	0.541	0.503	0.369	0.498	0.462
HWE exact test	0.1843	0.2973	0.3983	0.5335	0.7495	0.5101	0.8486	0.2007
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	5	6	6	6	5	6	6	6
Allelic richness	4.527	5.855	5.756	5.477	4.988	5.443	5.646	5.829
Size range	116-136 bp	116-140 bp	116-140 bp	116-140 bp	116-136 bp	116-140 bp	116-140 bp	116-140 bp
H _o	0.4	0.508	0.692	0.5	0.473	0.408	0.367	0.630
H _e	0.447	0.52	0.648	0.484	0.468	0.442	0.374	0.551
HWE exact test	0.4342	0.8214	0.5435	0.5389	0.5703	0.2461	0.2014	0.8566
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	5	5	6	4	3	3		4
Allelic richness	4.985	4.718	5.824	3.988	2.997	3.000		
Size range	116-136 bp	116-136 bp	116-140 bp	116-136 bp	124-136 bp	124-136 bp		96-132 bp
H _o	0.673	0.513	0.531	0.588	0.364	0.471		0.308
H _e	0.642	0.611	0.488	0.619	0.414	0.45		0.397
HWE exact test	0.1745	0.6023	0.3218	0.2536	0.5254	0.5591		0.523

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1 **Appendix A continued.**

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Locus StPf1002

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	6	6	6	6	5	5	7	6
Allelic richness	5.876	5.516	5.598	5.613	4.906	4.843	6.441	5.807
Size range	169-185 bp							
H _o	0.648	0.58	0.756	0.688	0.764	0.679	0.692	0.635
H _e	0.734	0.716	0.683	0.723	0.705	0.674	0.669	0.702
HWE exact test	0.4337	0.2849	0.6218	0.3893	0.8935	0.9335	0.4622	0.2667
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	7	7	7	8	6	7	7	6
Allelic richness	6.066	6.208	6.667	6.678	5.324	6.444	6.912	5.699
Size range	137-185 bp	169-185 bp	169-185 bp	137-185 bp	169-185 bp	169-185 bp	137-185 bp	169-185 bp
H _o	0.691	0.678	0.538	0.68	0.636	0.714	0.688	0.702
H _e	0.679	0.71	0.649	0.626	0.648	0.705	0.703	0.642
HWE exact test	0.6042	0.5992	0.0685	0.9653	0.3955	0.3904	0.0459	0.3871
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	8	6	6	4	4	3		4
Allelic richness	7.062	5.933	5.444	3.999	3.682	3.000		
Size range	163-185 bp	169-185 bp	169-185 bp	169-177 bp	171-185 bp	171-177 bp		177-189 bp
H _o	0.755	0.649	0.714	0.765	0.523	0.636		0.444
H _e	0.664	0.634	0.679	0.622	0.606	0.576		0.529
HWE exact test	0.7388	0.887	0.614	0.5929	0.5113	0.7687		0.2617

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1 **Appendix A continued.**

2

Locus StPf1001

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04
Na	19	17	15	17	21	21	20	25
Allelic richness	15.214	14.043	13.535	13.787	15.596	15.532	16.138	18.148
Size range	233-337 bp	233-343 bp	233-345 bp	233-329 bp	233-339 bp	233-337 bp	233-337 bp	221-323 bp
H _o	0.907	0.88	0.884	0.723	0.704	0.717	0.74	0.75
H _e	0.879	0.865	0.848	0.771	0.801	0.774	0.789	0.807
HWE exact test	0.8737	0.183	0.4166	0.0869	0.0828	0.1621	0.0899	0.2888
Population	Thy03	Thy04	Rin03	Rin04	Lim03	Irs03	Irs04	Wes03
Na	22	21	20	24	15	16	16	18
Allelic richness	18.070	17.131	18.118	18.416	12.836	13.250	13.571	15.667
Size range	243-345bp	233-329 bp	233-293 bp	233-349 bp	233-291 bp	233-291 bp	233-295 bp	233-337 bp
H _o	0.918	0.875	0.838	0.88	0.808	0.694	0.796	0.800
H _e	0.876	0.895	0.892	0.897	0.821	0.779	0.816	0.856
HWE exact test	0.9725	0.8688	0.9419	0.7265	0.4565	0.2251	0.1368	0.5138
Population	Bis03	Bis04	Tro04	Pul04	Far03	Far04		Pla03
Na	11	13	18	15	6	8		21
Allelic richness	10.234	11.881	15.493	13.904	5.999	7.875		
Size range	233-273 bp	233-297 bp	233-337 bp	243-301 bp	233-291 bp	231-291 bp		209-201 bp
H _o	0.898	0.684	0.898	0.735	0.762	0.719		0.944
H _e	0.817	0.778	0.89	0.717	0.82	0.811		0.97
HWE exact test	0.3498	0.0309	0.3741	0.7196	0.0478	0.4249		0.177

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1 **Appendix B** Pariwise F_{STS} with 95% confidence intervals (above diagonal) and P-values for their significance (below diagonal)

	Tur03	Tur04	Got03	Got04	Bor03	Bor04
Turku 2003		-0.002 (-0.005 – 0.001)	-0.002 (-0.004 – 0.00)	0.003 (-0.003 – 0.008)	0.026 (0.015 – 0.038)	0.025 (0.008 – 0.046)
Turku 2004	0.93600		-0.002 (-0.005 – 0.001)	-0.002 (-0.004 – 0.009)	0.027 (0.015 – 0.041)	0.023 (0.005 – 0.048)
Gotland 2003	0.86550	0.88770		0.001 (-0.003 – 0.006)	0.027 (0.013 – 0.043)	0.023 (0.005 – 0.045)
Gotland 2004	0.40490	0.27740	0.5371		0.013 (0.005 – 0.022)	0.010 (0.001 – 0.020)
Bornholm 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.001 (-0.003 – 0.003)
Bornhom 2004	< 0.0001	< 0.0001	< 0.0001	0.0002	0.4507	

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	Aer03	Aer04	Thy03	Thy04	Rin03	Rin04
Turku 2003	0.035 (0.019 – 0.052)	0.035 (0.014 – 0.059)	0.039 (0.020 – 0.059)	0.037 (0.015 – 0.064)	0.043 (0.017 – 0.072)	0.044 (0.016 – 0.074)
Turku 2004	0.032 (0.016 – 0.049)	0.033 (0.013 – 0.062)	0.036 (0.020 – 0.055)	0.037 (0.016 – 0.067)	0.041 (0.015 – 0.073)	0.041 (0.016 – 0.070)
Gotland 2003	0.035 (0.016 – 0.061)	0.033 (0.012 – 0.058)	0.040 (0.021 – 0.065)	0.039 (0.019 – 0.061)	0.049 (0.020 – 0.086)	0.046 (0.018 – 0.082)
Gotland 2004	0.017 (0.006 – 0.031)	0.015 (0.004 – 0.026)	0.025 (0.015 – 0.036)	0.025 (0.013 – 0.035)	0.027 (0.010 – 0.048)	0.028 (0.013 – 0.047)
Bornholm 2003	0.001 (-0.003 – 0.007)	0.001 (-0.004 – 0.006)	0.006 (0.001 – 0.013)	0.007 (-0.001 – 0.016)	0.014 (0.003 – 0.029)	0.007 (-0.001 – 0.015)
Bornhom 2004	0.001 (-0.003 – 0.004)	-0.001 (-0.005 – 0.003)	0.005 (0.001 – 0.009)	0.007 (-0.001 – 0.014)	0.013 (0.003 – 0.025)	0.007 (0 – 0.015)

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	Lim03	Irs03	Irs04	Wes03	Bis03	Bis04
Turku 2003	0.048 (0.020 – 0.077)	0.040 (0.020 – 0.060)	0.036 (0.014 – 0.059)	0.048 (0.020 – 0.078)	0.055 (0.033 – 0.079)	0.061 (0.041 – 0.081)
Turku 2004	0.048 (0.023 – 0.076)	0.039 (0.019 – 0.062)	0.033 (0.010 – 0.059)	0.045 (0.019 – 0.078)	0.055 (0.032 – 0.081)	0.062 (0.041 – 0.086)
Gotland 2003	0.051 (0.022 – 0.081)	0.042 (0.020 – 0.066)	0.036 (0.012 – 0.065)	0.052 (0.25 – 0.083)	0.061 (0.034 – 0.092)	0.067 (0.042 – 0.101)
Gotland 2004	0.030 (0.014 – 0.049)	0.022 (0.010 – 0.035)	0.017 (0.006 – 0.031)	0.029 (0.014 – 0.046)	0.033 (0.021 – 0.049)	0.038 (0.024 – 0.057)
Bornholm 2003	0.010 (0.004 – 0.015)	0.003 (-0.002 – 0.009)	0.005 (0 – 0.011)	0.007 (0 – 0.016)	0.014 (0.007 – 0.022)	0.020 (0.011 – 0.032)
Bornhom 2004	0.011 (0 – 0.022)	0.006 (-0.003 – 0.015)	0.005 (-0.002 – 0.014)	0.008 (0.003 – 0.014)	0.016 (0.010 – 0.024)	0.019 (0.013 – 0.027)

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	Tro04	Pul04	Far03	Far04	Pla03
Turku 2003	0.039 (0.016 – 0.071)	0.060 (0.039 – 0.085)	0.082 (0.054 – 0.113)	0.091 (0.053 – 0.145)	0.222 (0.114 – 0.348)
Turku 2004	0.034 (0.010 – 0.071)	0.056 (0.019 – 0.091)	0.081 (0.051 – 0.122)	0.091 (0.047 – 0.159)	0.220 (0.108 – 0.352)
Gotland 2003	0.039 (0.017 – 0.066)	0.064 (0.039 – 0.093)	0.090 (0.059 – 0.128)	0.100 (0.055 – 0.166)	0.236 (0.112 – 0.380)
Gotland 2004	0.028 (0.013 – 0.044)	0.041 (0.020 – 0.061)	0.077 (0.048 – 0.113)	0.091 (0.047 – 0.154)	0.212 (0.108 – 0.336)
Bornholm 2003	0.019 (0.006 – 0.038)	0.035 (0.019 – 0.053)	0.052 (0.033 – 0.070)	0.061 (0.038 – 0.090)	0.218 (0.101 – 0.357)
Bornhom 2004	0.014 (0.005 – 0.024)	0.027 (0.011 – 0.045)	0.065 (0.35 – 0.093)	0.073 (0.040 – 0.113)	0.210 (0.099 – 0.345)

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1	Tur03	Tur04	Got03	Got04	Bor03	Bor04
Ærø 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1167	0.5081
Ærø 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6799	0.7795
Thyborøn 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0055	0.0138
Thyborøm 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0079	0.0348
Ringkøbing Fjord 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0088	0.0083
Ringkøbing Fjord 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1794	0.1661

2	Aer03	Aer04	Thy03	Thy04	Rin03	Rin04
Ærø 2003		0.002 (-0.002 – 0.002)	0.003 (-0.002 – 0.008)	0.003 (-0.003 – 0.010)	0.008 (0 – 0.017)	0.001 (-0.005 – 0.008)
Ærø 2004	0.5783		-0.001 (-0.005 – 0.004)	0.001 (-0.004 – 0.007)	0.006 (-0.002 – 0.016)	0.00 (-0.005 – 0.006)
Thyborøn 2003	0.0201	0.7615		0.003 (-0.001 – 0.008)	0.004 (-0.001 – 0.012)	0.00 (-0.003 – 0.003)
Thyborøm 2004	0.0618	0.1652	0.0581		0.002 (-0.002 – 0.006)	-0.002 (-0.006 – 0.002)
Ringkøbing Fjord 2003	0.2747	0.871	0.5712	0.1913		-0.002 (-0.006 – 0.005)
Ringkøbing Fjord 2004	0.289	0.9721	0.7755	0.6986	0.9775	

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	Lim03	Irs03	Irs04	Wes03	Bis03	Bis04
Ærø 2003	0.005 (-0.001 – 0.011)	0.002 (-0.003 – 0.008)	0.003 (0 – 0.007)	0.002 (-0.002 – 0.006)	0.005 (0 – 0.010)	0.006 (0.001 – 0.011)
Ærø 2004	0.006 (-0.003 – 0.018)	-0.001 (-0.004 – 0.002)	-0.002 (-0.004 – 0.001)	0.00 (-0.002 – 0.003)	0.006 (0 – 0.015)	0.010 (0.003 – 0.020)
Thyborøn 2003	0.011 (-0.001 – 0.027)	0.003 (-0.003 – 0.008)	0.001 (-0.003 – 0.006)	0.00 (-0.005 – 0.006)	0.010 (-0.001 – 0.022)	0.013 (0.006 – 0.022)
Thyborøm 2004	0.005 (-0.001 – 0.011)	0.006 (0 – 0.012)	0.004 (0.001 – 0.007)	0.00 (-0.002 – 0.001)	0.009 (0.004 – 0.014)	0.013 (0.006 – 0.020)
Ringkøbing Fjord 2003	0.012 (0.002 – 0.023)	0.001 (0 – 0.024)	0.004 (-0.006 – 0.020)	-0.001 (-0.004 – 0.002)	0.007 (-0.001 – 0.015)	0.012 (-0.001 – 0.026)
Ringkøbing Fjord 2004	0.003 (-0.003 – 0.010)	0.003 (-0.002 – 0.011)	-0.001 (-0.004 – 0.004)	-0.003 (-0.006 - 0.00)	0.003 (-0.002 – 0.009)	0.007 (0.001 – 0.015)

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	Tro04	Pul04	Far03	Far04	Pla03
Ærø 2003	0.011 (0.004 – 0.019)	0.027 (0.011 – 0.043)	0.043 (0.022 – 0.063)	0.056 (0.030 – 0.090)	0.201 (0.096 – 0.325)
Ærø 2004	0.009 (0.001 – 0.019)	0.024 (0.010 – 0.034)	0.005 (0.028 – 0.074)	0.062 (0.034 – 0.100)	0.209 (0.096 – 0.347)
Thyborøn 2003	0.008 (0.002 – 0.015)	0.029 (0.015 – 0.041)	0.045 (0.025 – 0.063)	0.053 (0.032 – 0.084)	0.201 (0.088 – 0.338)
Thyborøm 2004	0.003 (0 – 0.007)	0.027 (0.014 – 0.037)	0.049 (0.022 – 0.078)	0.061 (0.031 – 0.102)	0.196 (0.096 – 0.315)
Ringkøbing Fjord 2003	0.005 (-0.003 – 0.017)	0.027 (0.013 – 0.042)	0.053 (0.025 – 0.087)	0.065 (0.029 – 0.125)	0.180 (0.086 – 0.289)
Ringkøbing Fjord 2004	0.007 (-0.001 – 0.019)	0.036 (0.020 – 0.053)	0.044 (0.021 – 0.067)	0.057 (0.029 – 0.099)	0.197 (0.093 – 0.321)

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1	Tur03	Tur04	Got03	Got04	Bor03	Bor04
Limfjord 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Irish Sea 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0163	0.0281
Irish Sea 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0741	0.0125
Westerschelde estuary 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0809	0.0808
Bay of Biscay 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Bay of Biscay 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

2	Aer03	Aer04	Thy03	Thy04	Rin03	Rin04
Limfjord 2003	< 0.0001	0.0003	< 0.0001	< 0.0001	0.0002	0.019
Irish Sea 2003	0.006	0.3032	0.0139	0.0002	0.0024	0.0218
Irish Sea 2004	0.0011	0.5453	0.3381	0.0156	0.4446	0.7491
Westerschelde estuary 2003	0.1581	0.7732	0.7078	0.2357	0.9576	0.9992
Bay of Biscay 2003	< 0.0001	0.0027	< 0.0001	< 0.0001	< 0.0001	0.0487
Bay of Biscay 2004	< 0.0001	0.0008	< 0.0001	< 0.0001	0.0002	0.0019

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	Lim03	Irs03	Irs04	Wes03	Bis03	Bis04
Limfjord 2003		0.004 (-0.002 – 0.011)	0.005 (0 – 0.012)	0.003 (-0.001 – 0.006)	0.011 (0.002 – 0.022)	0.016 (0.007 – 0.028)
Irish Sea 2003	0.0002		-0.001 (-0.005 – 0.006)	-0.001 (-0.003 – 0.002)	0.008 (-0.001 – 0.023)	0.011 (0.003 – 0.026)
Irish Sea 2004	< 0.0001	0.1958		0.00 (-0.003 – 0.005)	0.013 (0.001 – 0.031)	0.017 (0.005 – 0.035)
Westerschelde estuary 2003	0.0047	0.6372	0.7885		0.003 (-0.002 – 0.010)	0.007 (0.002 – 0.014)
Bay of Biscay 2003	< 0.0001	< 0.0001	< 0.0001	0.0315		-0.003 (-0.003 – 0.002)
Bay of Biscay 2004	< 0.0001	< 0.0001	< 0.0001	0.0002	0.3824	

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	Tro04	Pul04	Far03	Far04	Pla03
Limfjord 2003	0.017 (0.007 – 0.027)	0.038 (0.021 – 0.056)	0.052 (0.029 – 0.075)	0.069 (0.041 – 0.104)	0.222 (0.109 – 0.352)
Irish Sea 2003	0.013 (0.008 – 0.018)	0.029 (0.017 – 0.039)	0.047 (0.029 – 0.063)	0.059 (0.037 – 0.085)	0.211 (0.101 – 0.343)
Irish Sea 2004	0.010 (0.001 – 0.022)	0.034 (0.018 – 0.053)	0.052 (0.028 – 0.086)	0.069 (0.033 – 0.127)	0.210 (0.093 – 0.354)
Westerschelde estuary 2003	0.007 (0.003 – 0.011)	0.026 (0.015 – 0.034)	0.043 (0.018 – 0.069)	0.054 (0.025 – 0.092)	0.2 (0.1 – 0.316)
Bay of Biscay 2003	0.021 (0.011 – 0.032)	0.033 (0.019 – 0.049)	0.040 (0.024 – 0.056)	0.051 (0.032 – 0.073)	0.199 (0.101 – 0.306)
Bay of Biscay 2004	0.027 (0.014 – 0.041)	0.036 (0.016 – 0.058)	0.048 (0.031 – 0.065)	0.060 (0.042 – 0.079)	0.189 (0.095 – 0.293)

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1	Tur03	Tur04	Got03	Got04	Bor03	Bor04
Trondheim 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lake Pulmanki 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Plaice 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

2	Aer03	Aer04	Thy03	Thy04	Rin03	Rin04
Trondheim 2003	< 0.0001	0.0029	0.0097	0.009	0.0993	0.1278
Lake Pulmanki 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Plaice 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

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1	Lim03	Irs03	Irs04	Wes03	Bis03	Bis04
Trondheim 2003	< 0.0001	< 0.0001	0.0002	0.0401	< 0.0001	< 0.0001
Lake Pulmanki 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Faroe Islands 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Plaice 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

2	Tro04	Pul04	Far03	Far04	Pla03
Trondheim 2003		0.020 (0.006 – 0.034)	0.054 (0.026 – 0.089)	0.067 (0.019 – 0.123)	0.198 (0.101 – 0.316)
Lake Pulmanki 2004	< 0.0001		0.083 (0.053 – 0.114)	0.092 (0.057 – 0.132)	0.204 (0.114 – 0.306)
Faroe Islands 2003	< 0.0001	< 0.0001		-0.003 (-0.006 – 0.002)	0.258 (0.152 – 0.381)
Faroe Islands 2004	< 0.0001	< 0.0001	0.4387		0.269 (0.153 – 0.404)
Plaice 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

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1 **Supplementary table S1.** Final estimates and 95% confidence intervals of Θ and M from the first MIGRATE run.

<i>Receiving population</i>	Θ	<i>Donor population</i>										
	<i>M</i>	Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs	Bis
Turku	0.238		17.67	13.10	13.21	10.71	8.86	11.83	17.93	7.80	14.53	8.81
Lower percentile	0.224		15.84	11.53	11.63	9.30	7.58	10.34	16.08	6.60	12.88	7.53
Upper percentile	0.252		19.63	14.80	14.92	12.26	10.27	13.45	19.91	9.13	16.32	10.21
Bornholm	0.308	16.67		15.61	19.37	15.51	10.62	16.22	12.26	9.86	14.54	13.27
Lower percentile	0.288	14.93		13.93	17.49	13.83	9.25	14.50	10.77	8.54	12.92	11.72
Upper percentile	0.329	18.54		17.42	21.38	17.32	12.13	18.06	13.87	11.33	16.29	14.94
Ærø	0.354	15.28	14.73		14.12	13.71	8.09	15.33	9.80	7.90	15.98	9.99
Lower percentile	0.332	13.69	13.17		12.59	12.20	6.94	13.73	8.54	6.77	14.35	8.71
Upper percentile	0.379	16.99	16.41		15.77	15.33	9.35	17.04	11.18	9.15	17.73	11.38
Thyborøn	0.417	10.93	14.39	15.04		13.82	8.54	17.92	11.67	11.14	14.35	9.63
Lower percentile	0.393	9.678	12.94	13.56		12.40	7.45	16.30	10.37	9.87	12.90	8.46
Upper percentile	0.444	12.29	15.94	16.62		15.34	9.75	19.65	13.07	12.51	15.90	10.91
Ringkøbing Fjord	0.402	11.26	16.04	16.47	21.34		9.32	17.51	10.79	7.95	10.27	11.97
Lower percentile	0.375	9.89	14.39	14.80	19.43		8.08	15.78	9.45	6.81	8.97	10.56
Upper percentile	0.432	12.75	17.81	18.26	23.37		10.68	19.35	12.25	9.21	11.70	13.51
Faroe Islands	0.188	11.61	12.64	12.23	12.98	13.12		9.84	7.93	4.45	8.95	10.04
Lower percentile	0.174	9.95	10.90	10.53	11.22	11.35		8.32	6.57	3.45	7.50	8.50
Upper percentile	0.202	13.45	14.55	14.11	14.91	15.06		11.54	9.46	5.62	10.57	11.75
Trondheim	0.284	9.933	12.73	13.79	16.45	16.45	6.65		11.26	7.85	10.33	8.96
Lower percentile	0.267	8.688	11.31	12.31	14.83	14.83	5.64		9.93	6.75	9.06	7.78
Upper percentile	0.302	11.29	14.26	15.38	18.18	18.18	7.77		12.71	9.06	11.72	10.25
Gotland	0.272	17.64	17.23	13.02	18.27	16.20	5.13	14.41		7.95	10.38	8.82
Lower percentile	0.255	15.73	15.35	11.40	16.33	14.38	4.14	12.70		6.70	8.94	7.49
Upper percentile	0.292	19.69	19.26	14.80	20.35	18.16	6.27	16.27		9.35	11.97	10.29

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1 **Table S1 continued.**
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<i>Receiving population</i>	θ	<i>Donor population</i>										
	<i>M</i>	Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs	Bis
Lake Pulmanki	0.189	11.34	15.20	16.76	17.33	14.05	5.75	16.51	11.91		12.24	10.28
Lower percentile	0.174	9.555	13.11	14.57	15.10	12.04	4.51	14.33	10.08		10.38	8.58
Upper percentile	0.206	13.34	17.49	19.17	19.78	16.26	7.20	18.90	13.96		14.31	12.18
Irish Sea	0.294	18.61	15.19	10.90	19.04	13.30	8.30	12.75	11.72	5.64		8.08
Lower percentile	0.276	16.71	13.48	9.47	17.12	11.70	7.05	11.19	10.22	4.63		6.85
Upper percentile	0.315	20.65	17.04	12.48	21.10	15.03	9.69	14.45	13.35	6.80		9.45
Bay of Biscay	0.325	12.49	11.98	13.90	16.87	18.03	12.04	11.27	13.46	8.31	9.66	
Lower percentile	0.300	10.81	10.34	12.13	14.91	16.00	10.40	9.68	11.71	6.95	8.20	
Upper percentile	0.352	14.33	13.79	15.84	18.99	20.22	13.85	13.02	15.36	9.82	11.29	

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1 **Supplementary table S2.** Final estimates and 95% confidence intervals of Θ and M from the second MIGRATE run.

<i>Receiving population</i>	Θ	<i>M</i>	<i>Donor population</i>									
		Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs	Bis
Turku	0.332		14.53	11.07	16.08	11.04	9.97	14.65	18.92	6.18	10.10	9.00
Lower percentile	0.312		12.99	9.73	14.45	9.69	8.69	13.09	17.14	5.19	8.82	7.79
Upper percentile	0.354		16.20	12.53	17.83	12.48	11.35	16.32	20.81	7.29	11.50	10.32
Bornholm	0.370	16.25		13.75	20.02	14.04	6.76	14.83	12.13	9.47	13.93	9.56
Lower percentile	0.346	14.63		12.27	18.21	12.54	5.74	13.29	10.74	8.25	12.43	8.33
Upper percentile	0.397	17.98		15.35	21.94	15.66	7.90	16.49	13.64	10.82	15.54	10.91
Ærø	0.350	12.22	13.89		16.19	14.92	7.09	13.32	15.97	7.65	12.36	11.54
Lower percentile	0.328	10.86	12.44		14.61	13.42	6.07	11.90	14.41	6.58	11.00	10.22
Upper percentile	0.374	13.69	15.45		17.87	16.54	8.23	14.85	17.64	8.82	13.84	12.96
Thyborøn	0.359	12.95	15.29	15.13		17.31	9.75	11.93	15.45	8.50	14.00	12.50
Lower percentile	0.339	11.58	13.80	13.65		15.73	8.57	10.62	13.96	7.40	12.57	11.16
Upper percentile	0.381	14.42	16.89	16.71		19.01	11.03	13.35	17.06	9.70	15.52	13.95
Ringkøbing Fjord	0.298	15.26	21.05	24.39	22.61		9.29	21.61	8.54	11.71	19.25	17.24
Lower percentile	0.279	13.43	18.89	22.05	20.36		7.87	19.42	7.19	10.11	17.18	15.22
Upper percentile	0.319	17.25	23.38	26.88	25.02		10.86	23.97	10.05	13.46	21.47	19.36
Faroe Islands	0.172	17.79	13.49	14.79	17.39	13.81		13.97	12.10	6.50	10.57	12.68
Lower percentile	0.159	15.54	11.54	12.74	15.16	11.84		11.99	10.26	5.18	8.86	10.72
Upper percentile	0.186	20.25	15.65	17.04	19.82	15.99		16.17	14.15	8.03	12.49	14.78
Trondheim	0.285	14.18	16.25	16.93	21.60	15.27	11.57		13.42	8.62	13.69	12.24
Lower percentile	0.268	12.53	14.48	15.12	19.54	13.55	10.08		11.81	7.34	12.06	10.73
Upper percentile	0.304	15.97	18.17	18.89	23.80	17.13	13.20		15.16	10.03	15.45	13.91
Gotland	0.310	16.75	15.79	19.33	19.55	13.40	7.54	15.97		8.65	11.49	11.23
Lower percentile	0.289	14.96	14.05	17.40	17.61	11.80	6.36	14.23		7.38	10.02	9.77
Upper percentile	0.333	18.68	17.66	21.40	21.62	15.13	8.85	17.86		10.05	13.10	12.81

1 **Table S2 continued**

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<i>Receiving population</i>	θ	M	<i>Donor population</i>									
			Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs
Lake Pulmanki	0.189	20.45	22.15	20.24	19.19	15.18	7.17	19.71	18.45		15.92	13.50
Lower percentile	0.175	17.71	19.29	17.51	16.53	12.83	5.60	17.02	15.85		13.51	11.30
Upper percentile	0.206	23.47	25.28	23.24	22.11	17.80	9.01	22.68	21.32		18.60	15.98
Irish Sea	0.317	15.89	12.17	15.32	18.62	16.28	6.98	11.33	13.00	8.44		10.85
Lower percentile	0.295	14.17	10.67	13.63	16.75	14.54	5.86	9.88	11.45	7.21		9.44
Upper percentile	0.340	17.75	13.80	17.14	20.62	18.16	8.23	12.91	14.69	9.82		12.40
Bay of Biscay	0.224	13.74	15.26	17.19	18.44	14.27	11.70	14.67	10.84	10.18	12.00	
Lower percentile	0.209	11.96	13.38	15.18	16.36	12.45	10.06	12.82	9.26	8.66	10.34	
Upper percentile	0.241	15.70	17.32	19.36	20.68	16.26	13.51	16.68	12.58	11.88	13.84	

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1 **Supplementary table S3.** Final estimates and 95% confidence intervals of Θ and M from the third MIGRATE run.

<i>Receiving population</i>	Θ	<i>Donor population</i>										
	M	Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs	Bis
Turku	0.321		16.04	11.54	12.79	12.28	6.70	11.82	14.63	7.27	9.09	5.88
Lower 95 percentile	0.301		14.50	10.24	11.42	10.93	5.72	10.50	13.16	6.25	7.95	4.96
Upper 95 percentile	0.342		17.69	12.94	14.27	13.73	7.79	13.25	16.21	8.40	10.35	6.89
Bornholm	0.308	24.72		16.33	19.64	13.80	10.30	16.33	16.67	11.59	15.67	12.47
Lower 95 percentile	0.288	22.50		14.54	17.67	12.16	8.89	14.54	14.86	10.09	13.92	10.91
Upper 95 percentile	0.330	27.08		18.26	21.75	15.58	11.85	18.27	18.62	13.23	17.57	14.17
Ærø	0.349	12.60	14.94		14.35	10.28	6.63	12.62	10.24	9.74	12.71	12.04
Lower 95 percentile	0.327	11.23	13.44		12.88	9.05	5.65	11.24	9.01	8.54	11.34	10.70
Upper 95 percentile	0.373	14.09	16.54		15.93	11.62	7.72	14.10	11.58	11.04	14.20	13.49
Thyborøn	0.419	11.72	15.82	17.66		12.23	8.81	16.55	11.12	9.04	15.18	11.08
Lower 95 percentile	0.395	10.39	14.26	16.01		10.87	7.66	14.96	9.82	7.87	13.66	9.78
Upper 95 percentile	0.445	13.16	17.49	19.42		13.71	10.07	18.25	12.53	10.31	16.82	12.48
Ringkøbing	0.399	14.43	12.90	12.91	18.07		10.10	14.95	8.91	7.00	13.71	11.48
Lower 95 percentile	0.372	12.84	11.40	11.40	16.28		8.78	13.33	7.68	5.91	12.16	10.07
Upper 95 percentile	0.428	16.14	14.53	14.53	19.98		11.54	16.70	10.27	8.21	15.38	13.02
Faroe Islands	0.170	12.09	12.72	14.62	13.98	12.45		12.51	9.50	9.14	10.47	8.90
Lower 95 percentile	0.157	10.37	10.96	12.73	12.13	10.70		10.76	7.99	7.66	8.88	7.44
Upper 95 percentile	0.184	13.98	14.66	16.70	16.02	14.37		14.44	11.19	10.80	12.24	10.54
Trondheim	0.269	11.89	10.93	13.76	19.25	13.94	7.62		13.21	7.12	10.37	10.03
Lower 95 percentile	0.253	10.47	9.58	12.23	17.43	12.40	6.49		11.71	6.04	9.05	8.73
Upper 95 percentile	0.286	13.43	12.41	15.41	21.19	15.61	8.86		14.83	8.32	11.81	11.44
Gotland	0.224	28.82	18.63	16.57	16.50	14.37	15.29	13.51		13.08	13.80	14.36
Lower 95 percentile	0.210	26.11	16.47	14.53	14.47	12.48	13.34	11.68		11.28	11.94	12.47
Upper 95 percentile	0.240	31.72	20.98	18.79	18.71	16.44	17.42	15.52		15.07	15.83	16.44

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1 **Table S3 continued**

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<i>Receiving population</i>	θ	M	<i>Donor population</i>									
			Tur	Bor	Aer	Thy	Rin	Far	Tro	Got	Pul	Irs
Lake Pulmanki	0.179	9.653	10.83	15.20	16.32	10.83	5.98	12.16	14.80		9.59	7.50
Lower 95 percentile	0.166	8.136	9.22	13.28	14.32	9.22	4.80	10.44	12.90		8.08	6.18
Upper 95 percentile	0.193	11.35	12.62	17.31	18.49	12.62	7.33	14.05	16.88		11.28	9.01
Irish Sea	0.383	8.947	14.48	15.17	16.11	11.83	8.29	11.87	9.60	6.99		9.45
Lower 95 percentile	0.358	7.741	12.93	13.58	14.47	10.43	7.13	10.48	8.35	5.93		8.21
Upper 95 percentile	0.411	10.27	16.15	16.88	17.87	13.34	9.57	13.39	10.97	8.16		10.81
Bay of Biscay	0.307	12.25	13.94	13.56	15.07	16.00	8.99	14.56	9.79	9.67	14.36	
Lower 95 percentile	0.284	10.61	12.18	11.83	13.24	14.12	7.60	12.77	8.34	8.22	12.57	
Upper 95 percentile	0.332	14.05	15.85	15.45	17.05	18.05	10.54	16.52	11.41	11.28	16.30	

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Manuscript II

Adaptive divergence in a high gene flow environment: *Hsc70* variation in the European flounder (*Platichthys flesus* L.)

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1 **Adaptive divergence in a high gene flow environment: *Hsc70* variation in the**
2 **European flounder (*Platichthys flesus* L.)**

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4 **Research Article**

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23 **Key words:** Local adaptation, adaptive evolution, candidate genes, marine fish, European flounder,
24 Heat shock cognate gene.

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29 Running head: Adaptive divergence in European flounder

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Abstract

Understanding local adaptations and their genetic basis in marine organisms is central to improve our knowledge of how evolution operates in the sea. Marine fishes have recently been subject to a number of studies applying highly variable genetic markers. These studies have often found weak genetic differentiation between local populations. However, only rarely have marine fishes been subject to studies of adaptive population divergence by applying markers potentially under selection. Hence, the extent to which marine fishes are adapted to local environmental conditions is presently unknown. In this study, we applied a candidate gene approach to investigate adaptive population divergence in the European flounder (*Platichthys flesus* L.), a coastal flatfish distributed throughout the Northeastern Atlantic. We contrasted patterns of genetic variation in a presumably neutral microsatellite baseline to patterns from a heat shock cognate protein gene, *Hsc70*, which was chosen as a candidate gene for adaptive population divergence. Using two different neutrality tests, we found that the microsatellite data set most likely did represent a neutral baseline. In contrast, *Hsc70* strongly deviated from neutral expectations, indicating that this locus was under selection or linked to a locus under selection. When estimating standardized levels of population divergence, the discrepancies between the two data sets were particularly striking in pairwise comparisons involving populations in the Western and central Baltic Sea. These results strongly indicate the presence of flounder populations under local selective pressures and they suggest that the populations under study are locally adapted. To our knowledge, this is the first demonstration of the genetic basis of adaptive population divergence in a marine fish species, illustrating that adaptive evolution is possible in marine fishes despite high levels of gene flow. Our results also suggest that the candidate gene approach is potentially very useful for demonstrating local selection in non model organisms such as most marine fish species.

1 Introduction

2
3 Marine fishes have been subject to an increasing number of population genetic studies applying
4 neutral genetic markers in recent years. These studies have generally confirmed that gene flow
5 between populations of many marine fishes is high (Ward et al. 1994; DeWoody and Avise 1999;
6 Waples, 1998). Since gene flow is expected to hamper adaptive population divergence, these results
7 indicate that local adaptations should be rare or absent in marine fishes. On the other hand, the wide
8 distributions over diverse environments and large effective population sizes of many marine fish
9 species would tend to favor the effects of natural selection over the random effect of drift and the
10 homogenizing effect of gene flow (Palumbi 1994). Despite the high levels of gene flow, a number of
11 studies have identified significant, albeit small, levels of genetic structuring among populations of
12 marine fishes, primarily with the aid of highly variable genetic markers such as microsatellites
13 (Ruzzante et al. 1999; Nielsen et al. 2003; Nielsen et al. 2004; Bekkevold et al. 2005; Jørgensen et
14 al. 2005; Ruzzante et al. 2006). However, because these markers are presumable neutral any
15 inferences about the evolutionary significance of the results in terms of adaptive population
16 divergence have been based on evaluating the likely conditions under which populations would be
17 allowed to diverge adaptively. Thus, evolutionary scenarios with temporally stable genetic
18 structuring among populations could permit relatively strong local selection to override the effects
19 of drift and gene flow, resulting in temporally stable adaptive population divergence, i.e. local
20 adaptations.

21 Only rarely have marine fishes been subject to studies of population differences at loci
22 believed to be directly affected by selection. One classical example of a selected gene in marine
23 fishes is the hemoglobin locus in Atlantic cod (*Gadus morhua*). This locus shows clinal allele
24 frequency variation along a latitudinal cline in the Northeastern Atlantic as well as differences
25 between the North Sea and the Baltic Sea (Sick 1965a; Sick 1965b), and physiological experiments
26 strongly suggest that natural selection is maintaining this variation (Brix et al. 1998; Petersen and
27 Steffensen 2003). Few other examples of genes suggested to be under selection in marine fishes can
28 be found (reviewed in ICES 2006) such as the vesicular membrane protein gene Pantophysin, which
29 also shows non-neutral patterns of variation in Atlantic cod (Pogson 2001; Pogson and Fevolden
30 2003). None of these studies have, however, attempted to compare patterns of population divergence
31 for genes of presumed adaptive value to a background of neutral genetic markers, which is
32 important since it allows for a separation of the effects of natural selection from demographic
33 effects, i.e. migration and genetic drift (Landry and Bernatchez 2001; Moran 2002).

34 In this study we examine genetic variation in a candidate gene for adaptive divergence of
35 European flounder (*Platichthys flesus* L.) populations in combination with a neutral baseline from
36 genomic microsatellite loci. The European flounder is a wide ranging euryhaline flatfish species
37 inhabiting a large part of the Northeastern Atlantic including the brackish Baltic Sea. As a coastal
38 species inhabiting shallow areas the flounder is more exposed to spatial and temporal environmental
39 differences in parameters such as temperature, salinity and light compared to other species found in
40 deeper and more stable marine habitats. Furthermore, the flounder shows an unusual tolerance to
41 low salinities; e.g. it is often found migrating to rivers for long periods before returning to the sea to
42 spawn. In European waters flounders are exposed to two major environmental gradients. One
43 gradient is a gradual environmental transition with latitude in the Atlantic parts of the distributional
44 area. The other is a more abrupt change between the marine North Sea and brackish Baltic Sea.
45 Flounders in the innermost Baltic Sea are believed to have adapted to the local environment by
46 changing from the normal pelagic spawning strategy to benthic spawning near the coast (Aro 1989).

1 This adaptation assures that eggs are not exposed to oxygen poor water in the deeper parts of the
2 Baltic Sea. Furthermore, and as seen in other marine fishes in the Baltic Sea, such as cod (Vallin et
3 al. 1999), the flounders in the Western Baltic Sea show distinct physiological characteristics. Thus,
4 increased egg volumes and higher sperm mobility at lower salinities compared to neighboring
5 marine populations in the North Sea are believed to reflect local adaptations to the brackish
6 environment in the Baltic Sea (Nissling et al. 2002; Ojaveer and Kalejs 2005). Accordingly, due to
7 the flounder's unique geographical and ecological distribution it is very well suited for studying the
8 potential effects of environmental parameters on adaptive divergence among populations.

9 In this study we use a heat shock protein gene as a candidate gene for local adaptations in the
10 European flounder. Heat shock cognate 70 (*Hsc70*, sometimes denoted *Hsc71*) is a member the heat
11 shock protein 70 (*Hsp70*) gene family of molecular chaperones. These genes have been found in
12 every organism from bacteria and plants to humans and play a central role in the cellular stress
13 response system by assuring correct transport and folding of damaged proteins (Feder and Hofmann
14 1999). *Hsp70s* have been found to be expressed in response to a large variety of stressors in fishes,
15 such as elevated and lowered temperature, osmotic stress, radiation and heavy metals (reviewed in
16 Iwama 1998; Basu et al. 2002), illustrating their ubiquitous role in the cellular stress response. The
17 function of *Hsc70* is believed to be similar to that of *Hsp70* except that these genes are primarily
18 constitutively expressed (Freeman and Morimoto 1996). The few genomic sequences available from
19 fishes show that the *Hsp70* genes are highly conserved through fish evolution (Basu et al. 2002),
20 which indicates that they are under heavy selective constraints, probably because of their central role
21 in maintaining cellular homeostasis.

22 Our aims were to study local adaptations in marine fishes by investigating adaptive genetic
23 divergence among European flounder populations covering the majority of the species distribution.
24 We compared standardized levels of genetic differentiation for a *Hsc70* linked indel and a set of
25 neutral microsatellite markers. Despite the great potential for this candidate gene vs. neutral
26 variation approach to disclose the genetic basis of adaptive population divergence among natural
27 populations it has, to our knowledge, never been applied in marine fish species.

30 **Materials and Methods**

32 **Sampling and DNA extraction**

33 Samples of European flounder were collected in 2003 and 2004 covering most of the species'
34 distributional range (Fig. 1 and Table 1). We collected samples from the Baltic Sea (Turku, Gotland,
35 Bornholm and Ærø), Danish Fjords (Ringkøbing Fjord and the Limfjord) and the Atlantic/North Sea
36 (Thyborøn, Irish Sea, Bay of Biscay, the Faroe Islands and Trondheim). Finally, we collected
37 flounders from Lake Pulmanki which is connected to the Barents Sea via app. 100 km of the river
38 Teno, through which flounders presumable need to travel to the sea to spawn. DNA was extracted
39 from ethanol stored fin or gill tissue by Chelex (Estoup et al. 1996), DNeasy (Qiagen) and hotSHOT
40 (Truett et al. 2000) methods.

42 **Genome walking and screen for genetic variation**

43 A fragment of the gene coding the European flounder *Hsc70* gene was obtained by genome walking
44 using the DNA walking SpeedUp™ kit (Seegene). Primers were designed to walk upstream from
45 aligned sequences of the 5' end of other fish *Hsp70* genes. Obtained sequences were subsequently
46 used to design primers for additional walks upstream and downstream from the known sequence.

1 Between two and four individuals from different populations were used for an initial screen
2 for genetic variation. These individuals were chosen to cover the total distributional area of the
3 species. All fragments were cloned in the TOPO TA cloning kit (Invitrogen) prior to sequencing.

4 PCR and genotyping

5 Nine highly polymorphic microsatellites developed for plaice (LIST1001, GenBank Acc. no.
6 AF149831, Watts et al. 1999; PL142 and PL167, Acc. no's AF406750 and AF406751, Hoarau et al.
7 2002a) and European flounder (StPf1001, StPf1002, StPf1004, StPf1005, StPf1015 and StPf1022,
8 Acc. no's AJ315970, AJ315975, AJ315973, AJ315974, AJ538313, AJ538320, Dixon et al.
9 unpublished) were amplified under standard PCR conditions and genotyped on an ALFExpress
10 automated sequencer (Amersham Biosciences). Other primers were designed to amplify a fragment
11 containing a 21 bp. indel in intron1 of the flounder *Hsc70* gene (Forward 5' - GAG ACA TGT GAG
12 GGA TCC CTC C - 3'; Reverse 5' - CAT CAT TCT TGC TGG AAA CAA GC - 3'). These
13 fragments were amplified under standard PCR conditions and genotyped on agarose gels.
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15 Statistical analysis

16 Departures from Hardy-Weinberg equilibrium were tested with the exact test implemented in the
17 software GENEPOP (Raymond and Rousset 1995) for microsatellites and *Hsc70* genotypes
18 separately. Temporal stability was assessed by estimating pairwise F_{ST} , their confidence intervals
19 (by bootstrapping over loci) and significance between temporal samples within localities in the
20 program FSTAT (Goudet 1995). We calculated G'_{ST} (Hedrick 2005) to facilitate comparisons of
21 population structuring between the microsatellite and *Hsc70* data sets. G'_{ST} is a standardized
22 measure of population divergence independent of levels of heterozygosity (Hedrick 1999, 2005).
23 This standardized measure was used because the markers applied in this study (microsatellites
24 versus indel) are expected to differ markedly in levels of heterozygosity and hence in maximum
25 levels of population divergence attainable (see Hedrick 2005). Multidimensional scaling (MDS)
26 plots of G'_{ST} were constructed with the program Vista 5.6.3. (Young 1996) to visualize the
27 relationships among populations. MDS plots were made for microsatellites and *Hsc70* separately.
28 Pairwise comparisons of G'_{ST} for microsatellites and *Hsc70* were conducted to examine which
29 population pairs showed the largest discrepancy between the two marker types, i.e. where selection
30 could be inferred. For this purpose, we calculated 95% confidence intervals for microsatellite G'_{ST}
31 by bootstrapping over loci. Point estimates of G'_{ST} from *Hsc70* were then evaluated in relation to
32 these confidence intervals.
33

34 We used two different tests to assess the assumption of microsatellite neutrality, since
35 conclusions regarding the outlier status of particular microsatellite loci are more robust when
36 supported by different analytical approaches (e.g. Vasemägi and Primmer 2005). The simulation
37 based test by Beaumont and Nichols (1996) implemented in the program FDIST2 and the LnRH test
38 by Schlötterer (Schlötterer 2002; Kauer et al. 2003) were both developed to identify outlier loci in
39 genome scans, but differ in their approaches and assumptions in a number of ways. The FDIST2 test
40 is based on the assumption that outlier loci will show increased levels of population structuring if
41 they are under diversifying selection or closely linked to a locus which is, i.e. genetic hitch-hiking
42 (Maynard Smith and Haigh 1974). The method compares actual levels of differentiation at
43 individual loci in relation to heterozygosity to a simulated distribution of loci generated from
44 observed levels of population differentiation. We carried out the simulation under both the Stepwise
45 Mutation Model (SMM), which should be well suited for microsatellites, and the Infinite Alleles
46 Model (IAM), which should describe the mutational process of an indel better. Beaumont and

1 Nichols (1996) have shown that the type of marker applied (i.e. mutational mechanisms involved)
2 has little effect on the simulated distribution under neutrality. Consequently, this method was used
3 both to evaluate the neutrality of microsatellites as well as the outlier status of *Hsc70*. *Hsc70* was
4 not, however, used to generate the expected neutral distribution, since we expected this locus *a*
5 *priori* to be under selection.

6 The LnRH test assumes that microsatellite loci which are linked to a gene of adaptive
7 importance subject to a selective sweep will show reduced levels of diversity within the populations
8 subject to selection (Schlötterer 2002, Kauer et al. 2003). The LnRH test therefore compares relative
9 levels of heterozygosity between loci in pairwise population comparisons. Since the LnRH test was
10 specifically developed for microsatellite loci it has not been evaluated in situations where mutational
11 mechanisms vary between loci and, accordingly, it was only used to assess the assumption of
12 neutrality of the microsatellite baseline.

15 Results

17 Genome walking and screen for genetic variation

18 The genome walking produced a sequence of 1586 bp. This sequence showed the highest similarity
19 to Japanese flounder (*Paralichthys olivaceus*) *Hsc70* mRNA followed by other *Hsc70/Hsc71* and
20 *Hsp70* genes in a Blast search (Altschul et al. 1997). Based on an alignment with the Japanese
21 flounder *Hsc70* mRNA, we assume that the European flounder sequence contains part of intron1,
22 the entire exon1 and a part of intron2 of a *Hsc70* gene. Other genomic sequences from fish contain
23 an untranslated exon0 upstream of the translational start site. This exon is located app. 1640 and
24 1270 bp upstream in two *Hsc70* genes from *Rivulus marmoratus* (Park et al. 2001; Lee 2004) and
25 app. 1830 bp upstream in rainbow trout (Zafarullah et al. 1992). Based on comparisons with these
26 few available fish genomic sequences and the fact that gene prediction softwares failed to identify
27 any transcriptional start sites in the European flounder sequence (results not shown), we find it
28 unlikely that we have reached this potential untranslated exon0 by the genome walking in European
29 flounder. The partial sequence of European flounder *Hsc70* has been deposited in GenBank under
30 accession number XXXXXX. The initial screen for genetic variation identified a 21 bp deletion in
31 intron1 in individuals from the Irish Sea, Turku and Lake Pulmanki. This indel was subsequently
32 genotyped in all individuals.

34 Genetic variation

35 Exact tests showed that no locus or population exhibited consistent deviations from Hardy-
36 Weinberg equilibrium (Appendix A). Only one pairwise comparison between temporal samples was
37 statistically significant (Thy03 versus Thy04 for microsatellites, $F_{ST}=0.006$, $P=0.04$). However, this
38 is not more than expected by chance in sixteen tests and it does not change the overall picture of
39 temporal stability over the two years studied. Temporal samples from the same localities were
40 therefore pooled for the remaining analyses.

42 Population structure

43 The overall F_{ST} for microsatellites was 0.024 (95% CI 0.018-0.031) and highly significant
44 ($P<0.0001$), while the overall F_{ST} for *Hsc70* was 0.139 and also highly significant ($P<0.0001$). As
45 evidenced by Figure 1, the genetic variation at *Hsc70* seemed to be highly unevenly distributed
46 throughout the distributional range. Populations in the Baltic Sea and Lake Pulmanki had very low

1 frequencies of the shortest allele, while the remaining populations had higher and more variable
2 frequencies of this allele. Examining the relationships between populations, it was evident that the
3 two marker types showed highly discordant patterns of structuring.

4 The microsatellite MDS plot mirrored the geographical relationship between the populations
5 and revealed a clear separation of populations in the inner Baltic Sea (benthic spawners), Faroe
6 Islands, the Bay of Biscay and Lake Pulmanki (Fig. 2). The Western and central Baltic Sea samples
7 were positioned between the Atlantic and inner Baltic Sea samples, while the remaining populations
8 were grouping together. The MDS plot based on *Hsc70* (Fig. 3) identified the sample from
9 Bornholm in the central Baltic Sea as very distinct, reflecting the extremely low frequency of the
10 short allele at this locality (see Fig. 1). Furthermore, there was a clear grouping of populations in the
11 Baltic Sea with Lake Pulmanki. The Faroe Island population was similar to the North Sea
12 populations, while Ringkøbing Fjord, Trondheim and the Bay of Biscay were all quite unique.

13 The pairwise comparisons in Figure 4 revealed the largest discrepancies between the two
14 data sets in comparisons involving the Western and central Baltic Sea populations showing
15 particular high levels of divergence from the Atlantic and North Sea populations for *Hsc70*, but high
16 similarity employing microsatellites.

17 When populations were divided into two major groups based on environmental similarity
18 (Baltic Sea samples and Lake Pulmanki versus all remaining Atlantic Sea samples), there was a
19 highly significant difference in allele frequencies between the two groups (Mann-Whitney U-test,
20 $P=0.006$). Moreover, allele frequencies were more variable among the populations in the Atlantic
21 group as evidenced by larger standard deviations in this group (0.09 for the Atlantic group versus
22 0.05 for the Baltic Sea/Lake Pulmanki samples).

23 24 Neutrality tests

25 We found little difference between the results obtained by simulations under the two mutation
26 models (SMM versus IAM) as also noted by Beaumont and Nichols (1996) when analyzing
27 simulated data. Consequently, we report only results obtained under the SMM. Results based on the
28 estimated mean F_{ST} of 0.024 (see above) showed that only one microsatellite locus (P1167) fell
29 slightly outside the upper 95% confidence level, while the *Hsc70* locus showed much higher levels
30 of population differentiation given its level of heterozygosity (Fig. 5). We also conducted the
31 simulations for the lower bound on F_{ST} (lower confidence level of 0.018), resulting in one additional
32 locus (StPf1002) falling slightly outside the upper 95% confidence interval of the simulated loci.
33 When the simulations were done with the upper bound on F_{ST} (0.031), no microsatellite loci were
34 outliers while *Hsc70* was still identified as an extreme outlier (results not shown). The LnRH test on
35 all 594 pairwise comparisons (66 population comparisons in 9 loci) revealed no loci as conspicuous
36 outliers (Fig. 6). As expected, some loci were in the outer 5 % of the distribution of Standardized
37 LnRH values. These data points were primarily from loci StPf1004, StPf1001, P1167 and StPf1005
38 in different pairwise population comparisons.

39 40 41 **Discussion**

42
43 We found that the *Hsc70* linked marker was clearly identified as an outlier locus in terms of levels
44 of population divergence when compared to a presumed neutral baseline. The clear discrepancy
45 between signals from the microsatellite baseline and *Hsc70* strongly suggests that natural selection
46 is affecting the distribution of genetic variation at *Hsc70*. In contrast, we found no convincing

1 evidence for non-neutrality of any of the microsatellites used to represent the neutral baseline in this
2 study. These results imply adaptive population divergence of the *Hsc70* gene in European flounder
3 or at a closely linked locus. Importantly, it is evident that such adaptations are possible even in a
4 background of very high levels of gene flow as inferred by the population similarity at microsatellite
5 loci

6 Whitehead and Crawford (2006) recently identified adaptive differences in gene expression
7 among highly structured populations of the intertidal *Fundulus heteroclitus* based on comparisons to
8 a neutral baseline from microsatellites. *F. heteroclitus* is the most well studied marine fish species
9 with respect to adaptive population divergence (e.g. Schulte et al. 2000; Schulte 2001) but it is not a
10 typical marine species in many respects. There is a deep phylogeographic split between Northern
11 and Southern populations of *F. heteroclitus*, which are believed to have diverged between 0.5 and 1
12 Million years ago (Bernardi et al. 1993) and consequently show very large levels of population
13 structuring at neutral markers (Pairwise $F_{ST} \approx 0.24$; Whitehead and Crawford 2006). In contrast,
14 many classical marine fish species (i.e. species with very large effective population sizes, pelagic
15 eggs and larvae and highly mobile adults; Nielsen and Kenchinton 2001) have younger population
16 ages (Grant and Bowen 1998, Árnason 2004, Hoarau et al. 2004) and show much lower levels of
17 structuring between populations (Pairwise F_{ST} typically not above 0.05, e.g. Ruzzante et al. 1999;
18 Haorau et al. 2002b; Nielsen et al. 2003; Nielsen et al. 2004; Bekkevold et al. 2005). To our
19 knowledge this very large group of marine fishes has until now never been subject to studies aiming
20 at disclosing the genetic basis of local adaptations.

21 The signal of local selection at *Hsc70* is particularly strong in the Western and central Baltic
22 Sea and Lake Pulmanki populations. This may suggest that these populations are locally adapted at
23 *Hsc70*, or alternatively at a locus closely linked to *Hsc70*. The populations in the Western and
24 central Baltic Sea (Ærø and Bornholm) are very similar to the North Sea and Irish Sea populations
25 when assessed with neutral microsatellites, but markedly different at the *Hsc70* linked marker. The
26 grouping of Lake Pulmanki with the Baltic Sea populations with respect to *Hsc70*, but not
27 microsatellites, further supports the conclusion that selection is involved in shaping the inter-
28 population relationships at *Hsc70* since Lake Pulmanki share many environmental characteristics
29 with the Baltic Sea, such as low winter temperatures, fluctuating seasonal temperatures and low
30 salinity. Finally, we note that variation in *Hsc70* allele frequencies is much larger among the
31 Atlantic Sea samples than among the Baltic Sea and Lake Pulmanki samples. This further supports
32 the conclusion that directional selection is stronger at the latter localities, thereby reducing the
33 overall variation in allele frequencies among populations. The apparent reduction in selective
34 pressures in the Atlantic Sea would thus allow allele frequencies to vary more freely around 0.5.

35 Based on the results from this study we are not able definitively to point to a specific
36 environmental variable driving selection at *Hsc70*. However, variation in a number of environmental
37 parameters can be found within the studied area. In the narrow zone connecting the marine Atlantic
38 and the brackish Baltic Sea there is a drastic environmental transition with respect to temperature
39 and salinity in particular. Generally, both annual and intra-annual temperature variations are larger
40 and annual minimum temperatures are lower and in the Baltic Sea than in the nearby North Sea (e.g.
41 Becker and Pauly 1996; Siegel et al. 2006). Therefore, temperature fluctuations could be an
42 important selective agent driving adaptive divergence at *Hsc70*, since both *Hsp70* and *Hsc70* have
43 been found to be expressed in response to temperature changes in fishes (Basu et al. 2002; Deane
44 and Woo 2005; Fangue et al. 2006). An alternative selective driver could be annual minimum
45 temperatures. Although the majority of studies on heat shock gene expression conducted so far have
46 concentrated on elevated temperatures, heat shock proteins have also been shown to be induced by

1 cold stress in fishes (Iwama et al. 1998; Ali et al. 2003, Place et al. 2004; Place and Hofmann
2 2005a). Studies done in some Antarctic notothenoids suggest that these species may have a very
3 high need for cellular protection against denatured proteins, since it appears that their *Hsp70s* have
4 shifted from an induced to a constitutive expression pattern (Place et al. 2004). Furthermore, the
5 *Hsc70s* from the same group of species have been found to retain chaperoning activity at lower
6 temperatures compared to temperate species in the same genera (Place and Hofmann 2005b; Place
7 and Hofmann 2005c), suggesting adaptation to cold environmental conditions at the molecular level.
8 While these species inhabit extremely cold environments of sub-zero temperatures, less extreme
9 conditions have also been found to induce *Hsc70* in other species. Ali et al. (2003) have, for
10 instance, shown that expression of *Hsc70* was increased 7.5 – 10 fold in carp muscle when fish were
11 exposed to a temperature drop from 12°C to 5°C, indicating that *Hsc70s* may also have an important
12 role in protecting cells against cold stress in species exposed to less extreme temperatures in nature.

13 Ambient salinity is a second and very important environmental parameter distinguishing the
14 brackish Baltic Sea and freshwater Lake Pulmanki populations from the remaining marine
15 populations in this study. Osmotic stress has been found to induce heat shock protein expression in
16 fish (Smith et al. 1999; Deane and Wo 2004). Thus, Sea bream (*Sparus sarba*) has been found to
17 induce *Hsc70* expression in response to both hypo – and hyperosmotic conditions indicating a
18 central role for *Hsc70* in the response to osmotic stress in fishes (Deane and Wo 2004).

19 A third potentially important environmental component is aquatic pollution, since heat shock
20 proteins have also been found to be induced in response to elevated levels of e.g. heavy metals
21 (Basu et al. 2002; Ali et al. 2003). However, while the Baltic Sea is quite heavily polluted this is
22 highly unlikely to apply to Lake Pulmanki, since this lake is located far from any major urban areas.
23 We therefore regard it as more likely that temperature and/or salinity is the selective agent involved.
24 Since these parameters are highly correlated among the samples in the present study, we are not able
25 to differentiate between them. Indeed they may not be mutually exclusive but could both be
26 involved in generating the observed distribution of genetic variation at *Hsc70*.

27 Interestingly, the distribution of genetic variation at *Hsc70* is not perfectly correlated with
28 temperature and/or salinity parameters, since the innermost Baltic Sea populations (Gotland and
29 Turku), which experience the most extreme environmental conditions, i.e. lowest temperatures and
30 salinities, do not have the most extreme *Hsc70* allele frequencies. These populations are believed to
31 have adapted to the extreme environment in the inner Baltic Sea by changes in spawning strategy
32 from spawning pelagic to benthic eggs (Aro 1989; Drevs et al. 1999). Hence, the Bornholm sample
33 represents the most extreme Baltic Sea sample of populations with the “normal” life-history
34 strategy. These results suggest that selection at or near *Hsc70* is very strong in the pelagic spawning
35 populations in the Western and central Baltic Sea, while less intense in the benthic spawning
36 populations. This could indicate that *Hsc70* acts as a “first defense” against the stressful conditions
37 at low/fluctuating temperatures and/or low salinities, while more complex biochemical adaptations
38 in addition to the known behavioral adaptations could be found in populations at the more extreme
39 environmental conditions.

40 Other studies conducted on fishes have found relatively high levels of structuring at neutral
41 markers in the transition zone between the North and Baltic Seas (Nielsen et al. 2003; Nielsen et al.
42 2004; Bekkevold et al. 2005; Johanneson and André 2006). These results have generally been
43 believed to reflect restrictions in gene flow between populations. The results from the present study
44 differ from previous findings in that levels of neutral structuring across this transition zone are
45 relatively low, implying higher levels of gene flow between flounder populations in the two regions.
46 Still, we have demonstrated that populations of European flounder in the Western and central Baltic

1 Sea are under heavy local selective pressures at a candidate locus for environmental adaptation and
2 consequently probably locally adapted. These results indicate that the high levels of structuring at
3 neutral markers observed in this region in other species are indeed indicative of strong barriers to
4 gene flow caused by local adaptations.

5 While the *Hsc70* allele frequencies in Lake Pulmanki are as extreme as in the Baltic Sea, it is
6 more difficult to interpret this signal. Since these fish are most likely a component of the Barents
7 Sea population, the signal could be reflecting allele frequencies in the Barents Sea or be specific to
8 Lake Pulmanki, i.e. reflecting selective constraints on fish migrating to the lake. However, without a
9 Barents Sea reference population it is not possible to know where the signal was generated.

10 The use of different marker types in the present study could introduce statistical and
11 interpretational problems, particularly related to downward bias of microsatellite divergence
12 between the North and Baltic Seas caused by e.g. size homoplasy (Estoup et al. 2002) and departure
13 from migration-drift equilibrium (see e.g. Pogson et al. 2001). However, it appears highly unlikely
14 that these mechanisms should have had a major impact on the patterns observed for the following
15 reasons. First of all, it is clear that some populations of flounder are strongly differentiated (e.g.
16 pairwise microsatellite G'_{ST} values between 0.1 and 0.2), which shows that enough time has elapsed
17 to allow populations to diverge significantly. Furthermore, the fact that many marine organisms are
18 highly structured at neutral markers across the same transition zone imply that time has been
19 sufficient to structure populations of other species in this specific environmental setting. The
20 diverging pattern observed for European flounder could thus be explained by either unique
21 mutational properties for flounder microsatellites (e.g. extreme size homoplasy and/or mutation
22 rates) or simply higher levels of gene flow in flounder compared to other marine fishes. The first
23 explanation would require very extreme mutational properties for flounder microsatellites, for which
24 there is no evidence. On the contrary, observed levels of variability, numbers of alleles and allele
25 size ranges are similar to what has been found in other marine fishes. It is, however, well known that
26 European flounder displays a unique tolerance to low salinities compared to other marine species.
27 This would explain why flounders are less structured across the steep salinity gradient in the
28 transition zone compared to other species in the area. It therefore seems likely that the similarity of
29 the North Sea and Western/central Baltic Sea flounder populations with respect to microsatellites
30 truly reflects high levels of gene flow rather than non-equilibrium situations or microsatellite
31 mutational mechanisms. Finally, it should be noted that ascertainment bias could have influenced
32 the observed pattern of variation in *Hsc70*. However, the *Hsc70*-polymorphism was initially
33 identified in several individuals from different populations. Furthermore, as evidenced by the
34 microsatellite data set, the populations which are grouping with respect to *Hsc70* apparently belong
35 to very different population components within the species. Thus, the grouping with environmental
36 similarity rather than with geographic or historical proximity strongly suggest that a biological or
37 evolutionary explanation is more likely responsible for generating the observed pattern than is
38 ascertainment bias.

39 In summary, we cannot rule out that the potentially confounding mechanisms mentioned
40 above may have influenced the patterns of structuring observed in this study. However, their effects
41 are most likely very modest and they cannot account for the very clear picture of a discordant
42 distribution of genetic variation between the two marker types. Local selection remains the single
43 most likely explanation for the observed patterns, which is suggesting local adaptations in the
44 populations under study.

45 Our study thus strongly suggests adaptive evolution even in a background of high gene flow.
46 Since many marine fishes demonstrate very low levels of genetic differentiation, local adaptations

1 may be much more widespread in the marine environment than previously believed from evaluating
2 the distinctness of marine fish populations from neutral markers alone. Still, there is a need for
3 further studies confirming the generality of the patterns observed here. Such knowledge of the scale
4 and magnitude of local adaptations in marine fishes is central to improve our understanding of how
5 evolution operates in the sea. Furthermore, knowledge of the genetic basis of local adaptation will
6 add to our ability to manage biodiversity efficiently in relation to human impact such as exploitation
7 and global warming by improving our ability to predict how distribution and abundance of species
8 will change in response to human mediated evolutionary forces.

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21 **Literature Cited**

- 23 Ali KS, Dorgai L, Ábrahám M, Hermes E. 2003. Tissue – and stressor-specific differential
24 expression of two *hsc70* genes in carp. *Biochem Bioph Res Co* 307: 503-509.
- 25 Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped
26 BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids*
27 *Res* 25: 3389-3402.
- 28 Árnason E. 2004. Mitochondrial cytochrome b DNA variation in the high-fecundity Atlantic cod:
29 Trans-Atlantic clines and shallow gene genealogy. *Genetics* 166: 1871-1885.
- 30 Aro E. 1989. A review of fish migration patterns in the Baltic. *Rapp P-v Reún Cons Int Explor Mer*
31 190: 72-96.
- 32 Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK. 2002. Heat
33 shock protein genes and their functional significance in fish. *Gene* 295: 173-183.
- 34 Beaumont MA, Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population
35 structure. *P Roy Soc Lond B Bio* 263: 1619-1626.
- 36 Becker GA, Pauly M. 1996. Sea surface temperature changes in the North Sea and their causes.
37 *ICES J Mar Sci* 53: 887-898.
- 38 Bekkevold D, Andre C, Dahlgren TG, Clausen LAW, Torstensen E, Mosegaard H, Carvalho GR,
39 Christensen TB, Norlinder E, Ruzzante D. 2005. Environmental correlates of population
40 differentiation in Atlantic herring. *Evolution* 59: 2656-2668.
- 41 Bernardi G, Sordino P, Powers DA. 1993. Concordant mitochondrial and nuclear DNA phylogenies
42 for populations of the teleost fish *Fundulus heteroclitus*. *P Natl Acad Sci USA* 90: 9271-9274.
- 43 Brix O, Forås E, Strand I. 1998. Genetic variation and functional properties of Atlantic cod
44 hemoglobins: Introducing a modified tonometric method for studying fragile hemoglobins. *Comp*
45 *Biochem Physiol A* 119: 575–583.

- 1 Deane EE, NYS Woo. 2005. Cloning and characterization of the *hsp70* multigene family from silver
2 sea bream: Modulated gene expression between warm and cold temperature acclimation. *Biochem*
3 *Bioph Res Co* 333: 776-783.
- 4 Deane EE, Wo NYS. 2004. Differential gene expression associated with euryhalinity in sea bream
5 (*Sparus sarba*). *Am J Physi – Reg I* 287: 1054-1063.
- 6 DeWoody JA, Avise JC. 2000. Microsatellite variation in marine, freshwater and anadromous
7 fishes compared with other animals. *J Fish Biol* 56: 461-473.
- 8 Drevs T, Kadakas V, Lang T, Mellergaard S. 1999. Geographical variation in the age/length
9 relationship in Baltic flounder (*Platichthys flesus*). *ICES J Mar Sci* 56: 134-137.
- 10 Estoup A, Jarne P, Cornuet JM. 2002. Homoplasy and mutation model at microsatellite loci and
11 their consequences for population genetics analysis. *Mol Ecol* 11: 1591-1604.
- 12 Estoup A, Largiader CR, PerrotE, Chourrout D. 1996. Rapid one-tube DNA extraction for reliable
13 PCR detection of fish polymorphic markers and transgenes. *Mol Mar Biol Biotech* 5: 295-298.
- 14 Fangué NA, Hofmeister M, Schulte PM. 2006. Intraspecific variation in thermal tolerance and heat
15 shock protein gene expression in common killifish, *Fundulus heteroclitus*. *J Exp Biol* 209: 2859-
16 2872.
- 17 Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response:
18 Evolutionary and ecological physiology. *Annu Rev Physiol* 61: 243-282.
- 19 Freeman BC and Morimoto RI. 1996. The human cytosolic molecular chaperones *hsp90*, *hsp70*
20 (*hsc70*) and *hdj-1* have distinct roles in recognition of a non-native protein and protein refolding.
21 *EMBO J* 15: 2969-2979.
- 22 Goudet J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *J Hered* 86:
23 485-486.
- 24 Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine
25 fishes: Insights from sardines and anchovies and lessons for conservation. *J Hered* 89: 415-426.
- 26 Hedrick PW. 1999. Perspective: Highly variable genetic loci and their interpretation in evolution
27 and conservation. *Evolution* 53: 313-318.
- 28 Hedrick PW. 2005. A standardized genetic differentiation measure. *Evolution* 59: 1633-1638.
- 29 Hoarau G, Cook D, Stam WT, Olsen JL. 2002a. New microsatellites primers for plaice,
30 *Pleuronectes platessa* L. (Teleostei : Pleuronectidae). *Mol Ecol Notes* 2: 60-61.
- 31 Hoarau G, Piquet AMT, van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL. 2004. Population
32 structure of plaice (*Pleuronectes platessa* L.) in northern Europe: a comparison of resolving power
33 between microsatellites and mitochondrial DNA data. *J Sea Res* 51: 183-190.
- 34 Hoarau G, Rijnsdorp AD, Van der Veer HW, Stam WT, Olsen JL. 2002b. Population structure of
35 plaice (*Pleuronectes platessa* L.) in northern Europe: microsatellites revealed large-scale spatial
36 and temporal homogeneity. *Mol Ecol* 11: 1165-1176.
- 37 ICES. 2006. Report of the Working Group on the Application of Genetics in Fisheries and
38 Mariculture (WGAGFM), 24–27 March 2006, Newport, Ireland. ICES CM 2006/MCC:04. 59 pp,
39 available from www.ices.dk.
- 40 Iwama GK, Thomas PT, Forsyth RB, Vijayan MM. 1998. Heat shock protein expression in fish. *Rev*
41 *Fish Biol Fisher* 8: 35-56.
- 42 Johanneson K, André C. 2006. Life on the margin: genetic isolation and diversity loss in a
43 peripheral marine ecosystem, the Baltic Sea. *Mol Ecol* 15: 2013-2029.
- 44 Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V. 2005. Marine landscapes
45 and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Mol Ecol* 14:
46 3219-3234.

- 1 Kauer MO, Dieringer D, Schlotterer C. 2003. A microsatellite variability screen for positive
2 selection associated with the "Out of Africa" habitat expansion of *Drosophila melanogaster*.
3 Genetics 165: 1137-1148.
- 4 Landry C, Bernatchez L. 2001. Comparative analysis of population structure across environments
5 and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic
6 salmon (*Salmo salar*). Mol Ecol 10: 2525-2539.
- 7 Lee JS. 2004. Genomic cloning of a heat shock cognate 71-1 gene (*HSC71-1*) from the
8 hermaphroditic fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae). DNA Sequence 15: 33-
9 38.
- 10 Maynard Smith J, Haigh J. 1974. Hitch-hiking effect of a favorable gene. Genet Res 23: 23-35.
- 11 Moran P. 2002. Current conservation genetics: building an ecological approach to the synthesis of
12 molecular and quantitative genetic methods. Ecol Freshw Fish 11: 30-55.
- 13 Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P. 2003. Evidence of a hybrid-zone
14 in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual
15 admixture analysis. Mol Ecol 12: 1497-1508.
- 16 Nielsen EE, Kenchington EA. 2001. A new approach to prioritizing marine fish and shellfish
17 populations for conservation. Fish and Fisheries 3: 328-343.
- 18 Nielsen EE, Nielsen PH, Meldrup D, Hansen MM. 2004. Genetic population structure of turbot
19 (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in
20 the transition zone between the Baltic Sea and the North Sea. Mol Ecol 13: 585-595.
- 21 Nissling A, Westin L, Hjerne O. 2002. Reproductive success in relation to salinity for three flatfish
22 species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes*
23 *flesus*), in the brackish water Baltic Sea. ICES J Mar Sci 59: 93-108.
- 24 Ojaveer E, Kalejs M. 2005. The impact of climate change on the adaptation of marine fish in the
25 Baltic Sea. ICES J Mar Sci 62: 1492-1500.
- 26 Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu Rev
27 Ecol Syst 25: 547-572.
- 28 Park JH, Lee JJ, Yoon S, Lee JS, Choe SY, Choe J, Park EH, Kim CG. 2001. Genomic cloning of
29 the *Hsc71* gene in the hermaphroditic teleost *Rivulus marmoratus* and analysis of its expression in
30 skeletal muscle: identification of a novel muscle-preferred regulatory element. Nucleic Acids Res
31 29: 3041-3050.
- 32 Petersen MF, Steffensen JF. 2003. Preferred temperature of juvenile Atlantic cod *Gadus morhua*
33 with different haemoglobin types at normoxia and moderate hypoxia. J Exp Biol 206: 359-364.
- 34 Place SP, Hofmann GE. 2005a. Constitutive expression of a stress-inducible heat shock protein
35 gene, *hsp70*, in phylogenetically distant Antarctic fish. Polar Biol 28: 261-267.
- 36 Place SP, Hofmann GE. 2005b. Comparison of *Hsc70* orthologs from polar and temperate
37 notothenioid fishes: differences in prevention of aggregation and folding of denatured proteins.
38 Am J Physiol – Reg I 288: 1195-1202.
- 39 Place SP, Hofmann GE. 2005c. Temperature differentially affects adenosine triphosphatase activity
40 in *Hsc70* orthologs from Antarctic and New Zealand notothenioid fishes. Cell Stress Chaperon 10:
41 104-113.
- 42 Place SP, Zippay ML, Hofmann GE. 2004. Constitutive roles for inducible genes: evidence for the
43 alteration in expression of the inducible *hsp70* gene in Antarctic notothenioid fishes. Am J Physiol –
44 Reg I 287: 429-436.

- 1 Pogson GH, Fevolden SE. 2003. Natural selection and the genetic differentiation of coastal and
2 Arctic populations of the Atlantic cod in northern Norway: a test involving nucleotide sequence
3 variation at the pantophysin (*Pan I*) locus. *Mol Ecol* 12: 63–74.
- 4 Pogson GH, Taggart CT, Mesa KA, Boutilier RG. 2001. Isolation by distance in the Atlantic cod,
5 *Gadus morhua*, at large and small geographic scales. *Evolution* 55: 131-146.
- 6 Pogson GH. 2001. Nucleotide polymorphism and natural selection at the pantophysin (*Pan I*) locus
7 in the Atlantic cod, *Gadus morhua* (L.). *Genetics* 157: 317–330.
- 8 Raymond M, Rousset F. 1995. Genepop (Version-1.2) - Population-genetics software for exact tests
9 and ecumenicism. *J Hered* 86: 248-249.
- 10 Ruzzante DE, Mariano S, Bekkevold D et al. (17 co-authors). 2006. Biocomplexity in a highly
11 migratory pelagic marine fish, Atlantic herring. *P Roy Soc Lond B Bio* 273: 1459-1464.
- 12 Ruzzante DE, Taggart CT, Cook D. 1999. A review of the evidence for genetic structure of cod
13 (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off
14 Newfoundland and the Gulf of St. Lawrence. *Fish Res* 43: 79-97.
- 15 Schlötterer C. 2002. A microsatellite-based multilocus screen for the identification of local selective
16 sweeps. *Genetics* 160: 753-763.
- 17 Schulte PM, Glémet HC, Fiebig AA, Powers DA. 2000. Adaptive variation in lactate
18 dehydrogenase-B gene expression: role of a stress-responsive regulatory element. *P Natl Acad Sci*
19 *USA* 97: 6597-6602.
- 20 Schulte PM. 2001. Environmental adaptations as windows on molecular evolution. *Comp Biochem*
21 *Phys B* 128: 597-611.
- 22 Sick K. 1965a. Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. *Hereditas*
23 54: 19–48.
- 24 Sick K. 1965b. Haemoglobin polymorphism of cod in the North Sea and the North Atlantic Ocean.
25 *Hereditas* 54: 49–69.
- 26 Siegel H, Gerth M, Tschersich G. 2006. Sea surface temperature development of the Baltic Sea in
27 the period 1990–2004. *Oceanologia* 48: 118-131.
- 28 Smith TR, Tremblay GC, Bradley TM. 1999. *Hsp70* and a 54 kDa protein (*Osp54*) are induced in
29 Atlantic salmon (*Salmo salar*) in response to hyperosmotic stress. *J Exp Zool* 284: 286-298.
- 30 Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. 2000. Preparation of PCR-
31 quality mouse genomic DNA with hot sodium hydroxide and TRIS (HotSHOT). *Biotechniques*
32 29, 52-54
- 33 Vallin L, Nissling A, Westin L. 1999. Potential factors influencing reproductive success of Baltic
34 cod, *Gadus morhua*: A review. *Ambio* 28: 92-99.
- 35 Vasemägi A, Nilsson J, Primmer CR. 2005. Expressed sequence tag-linked microsatellites as a
36 source of gene-associated polymorphisms for detecting signatures of divergent selection in
37 Atlantic salmon (*Salmo salar* L.). *Mol Biol Evol* 22: 1067-1076.
- 38 Waples RS. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high
39 gene flow species. *J Hered* 89: 438-450.
- 40 Ward RD, Woodwark M, Skibinski DOF. 1994. A comparison of genetic diversity levels in marine,
41 fresh-water, and anadromous fishes. *J Fish Biol* 44: 213-232.
- 42 Watts PC, Nash RDM, George SG, Kemp SJ. 1999. Isolation and characterization of microsatellite
43 loci in the European plaice, *Pleuronectes platessa* L. (Teleostei: Pleuronectidae). *Mol Ecol* 8:
44 2151-2152.
- 45 Whitehead A, Crawford DL. 2006. Neutral and adaptive variation in gene expression. *P Natl Acad*
46 *Sci USA* 103: 5425-5430.

1 Young FW. 1996. ViSta: the visual statistics system. Research Memorandum 94-1(b) 2d. ed. L.L.
2 Thurstone Psychometric Laboratory, University of North Carolina, Chaper Hill, NC.
3 Zafarullah M, Wisniewski J, Schworak NW, Schieman S, Misra S, Gedamu L. 1992. Molecular
4 cloning and characterization of a constitutively expressed heat-shock-cognate *Hsc71* gene from
5 rainbow trout. Eur J Biochem 204: 893-900.
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1 **Figure legends**

2
3 Figure 1. Map of sampling locations and *Hsc70* allele frequencies. Sample numbers refer to sample
4 names in Table 1. Black proportion in pie charts denotes frequencies of the long allele.

5
6 Figure 2. Multidimensional scaling plot of G'_{ST} (Hedrick 2005) calculated based on the
7 microsatellite data set. Sample names refer to Table 1.

8
9 Figure 3. Multidimensional scaling plot of G'_{ST} (Hedrick 2005) calculated based on the *Hsc70* data
10 set. Sample names refer to Table 1.

11
12 Figure 4. Pariwise point estimates of G'_{ST} for microsatellites (◆) with confidence intervals calculated
13 by bootstrapping over loci and point estimates of G'_{ST} for *Hsc70* (□).

14
15 Figure 5. Results from the simulations with the FDIST2 program under the Stepwise Mutation
16 Model. Shown is the distribution of F_{ST} values (mean and 95% confidence intervals) from 500000
17 simulated loci as well as the actual values of individual microsatellite loci (○) and *Hsc70* (□).

18
19 Figure 6. Results from the LnRH tests. Shown are all pairwise population comparisons by
20 microsatellite loci. Locus Pl42 (■), StPf1004 (◇), List1001 (◆), Pl167 (▲), StPf1015 (△), StPf1005
21 (□), StPf1022 (○), StPf1002 (x) and StPf1001 (●). Dashed lines represent $\pm 1.96\sigma$.

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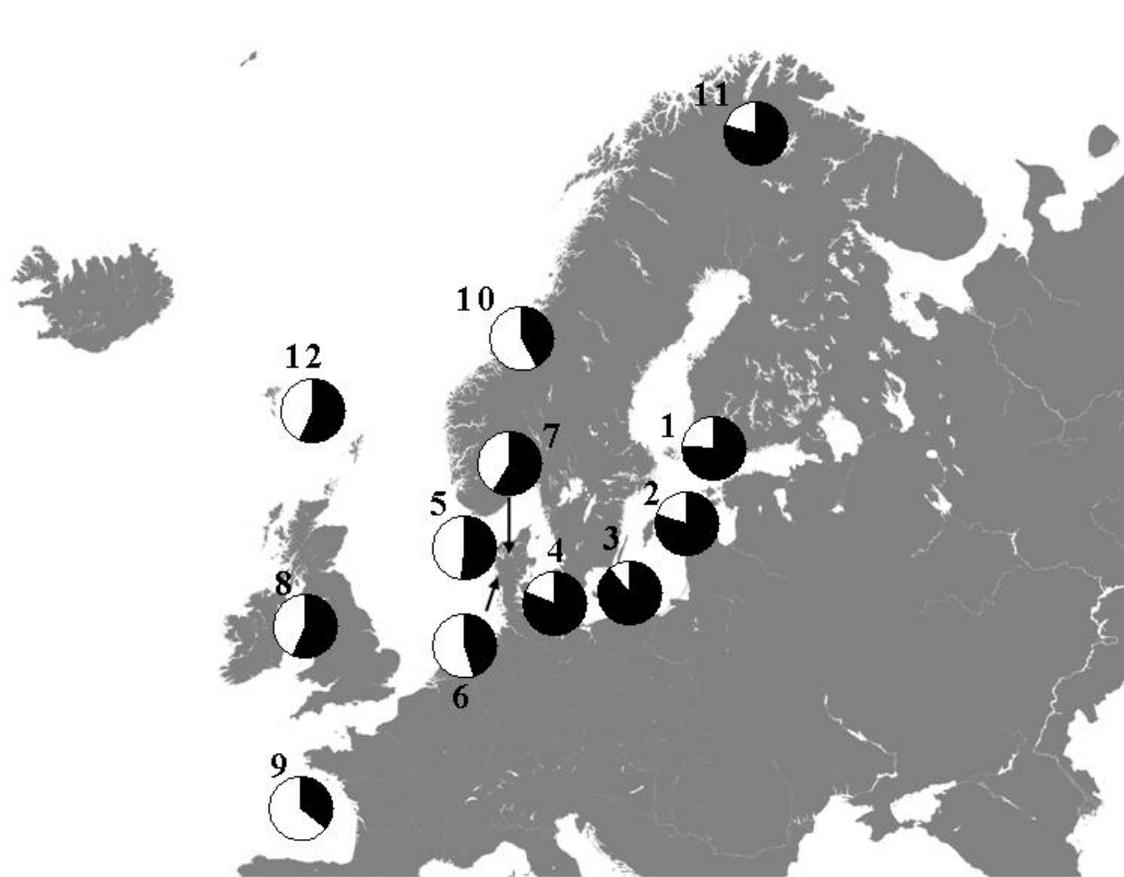
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Table 1. Location and sample size of sampled populations.

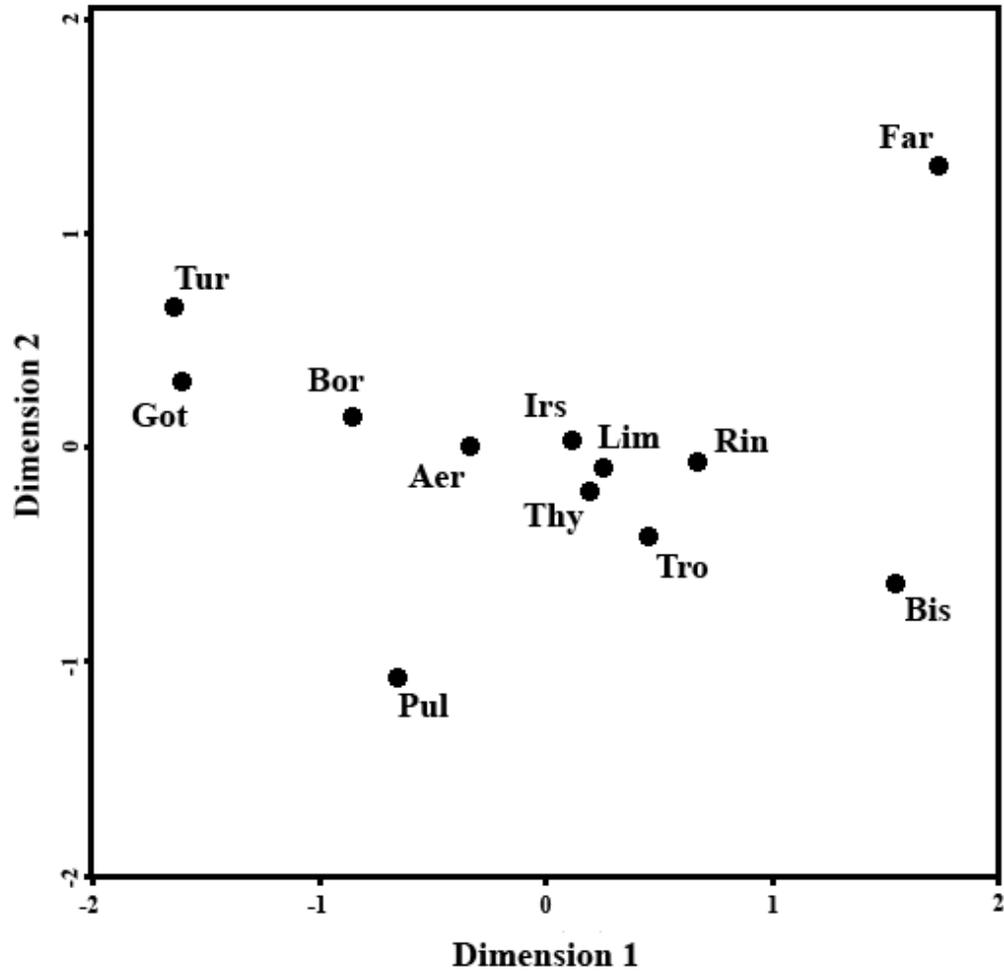
Locality	Sample year	Approximate position	Total sample size
1. Turku (Tur)	2003 and 2004	22° E, 60° N	79
2. Gotland (Got)	2003 and 2004	19 ° E, 57.5 ° N	94
3. Bornholm (Bor)	2003 and 2004	16° E, 55° N	108
4. Ærø (Aer)	2003 and 2004	10° E, 55° N	103
5. Thyborøn (Thy)	2003 and 2004	8° E, 57° N	80
6. Ringkøbing Fjord (Rin)	2004	8.3° E, 55.96° N	50
7. The Limfjord (Lim)	2003	8.59° E, 56.5° N	34
8. Irish Sea (Irs)	2003 and 2004	-4° E, 54° N	73
9. Bay of Biscay (Bis)	2003 and 2004	-2.3° E, 47.20° N	55
10. Trondheim (Tro)	2004	11° E, 65° N	49
11. Lake Pulmanki (Pul)	2004	28.02° E, 70.01° N	34
12. Faroe Islands (Far)	2003 and 2004	-6.45° E, 62° N	50

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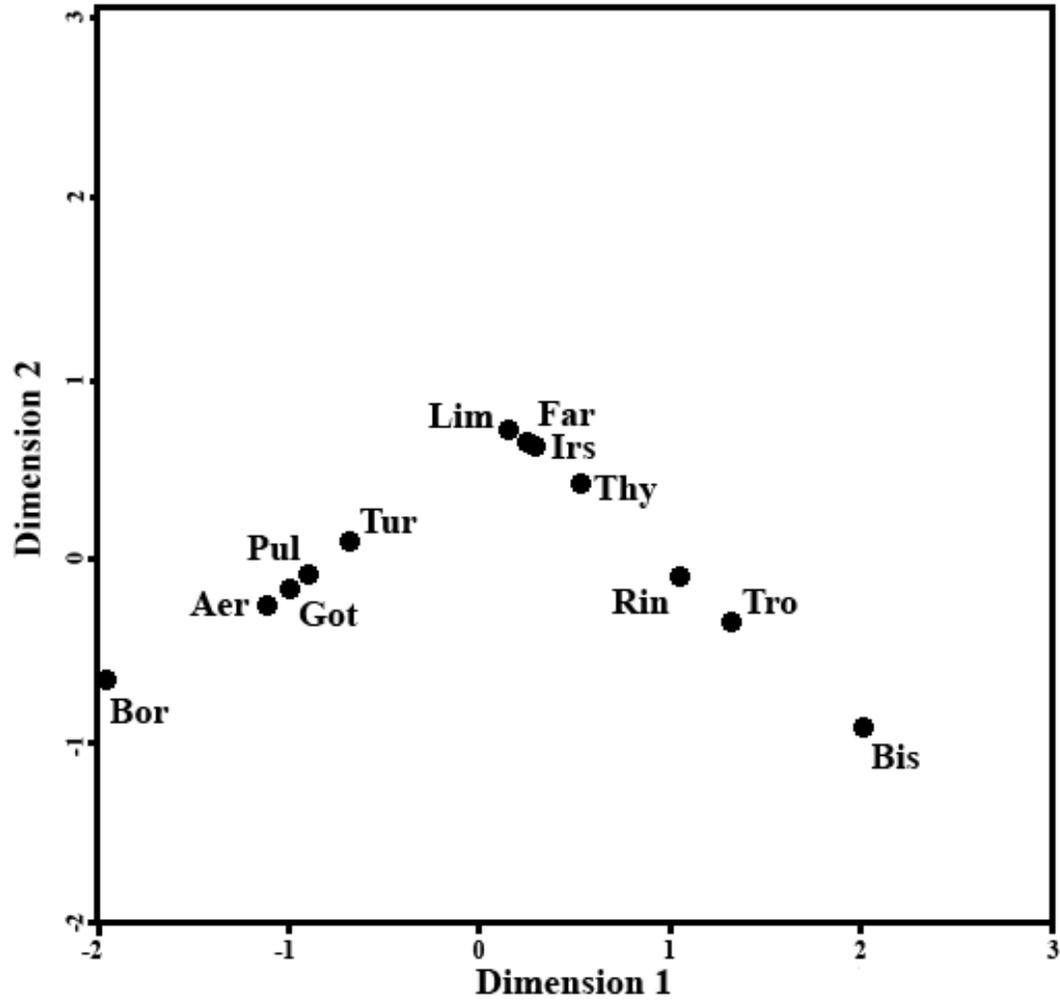
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Figure 3

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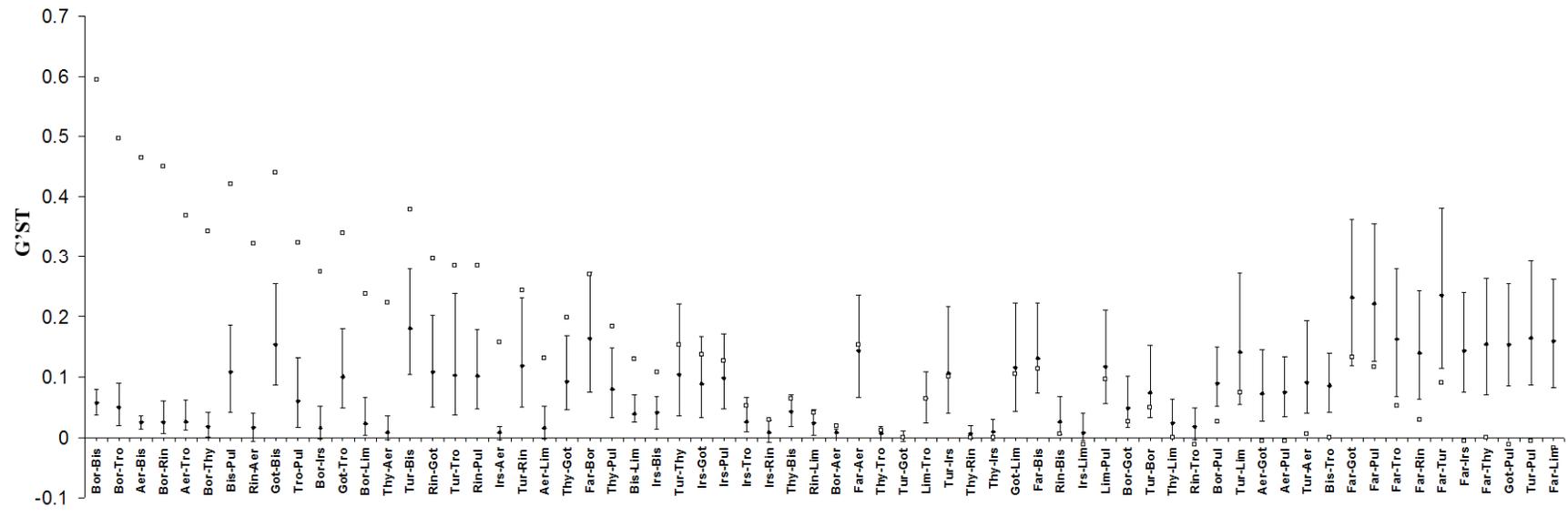
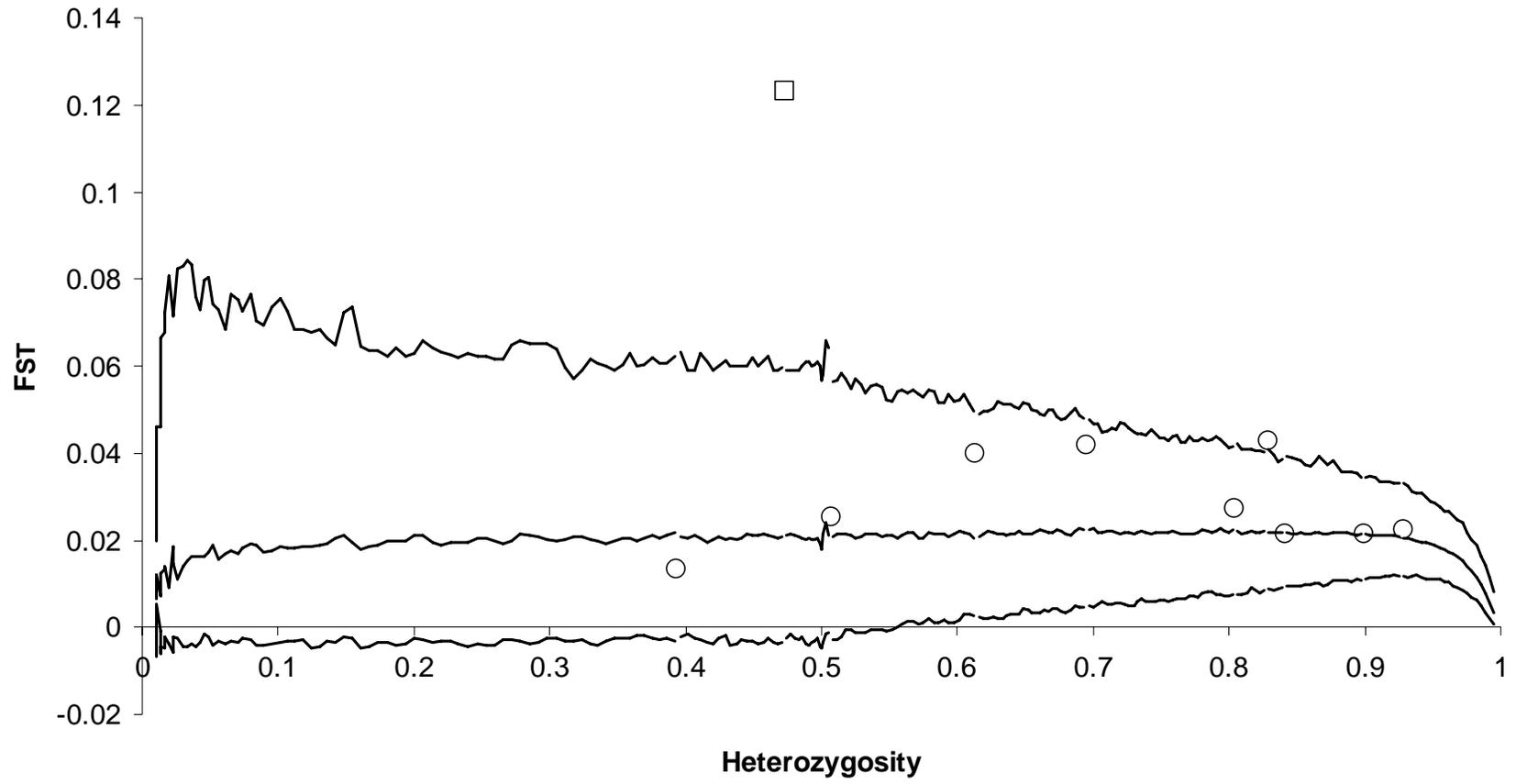


Figure 4

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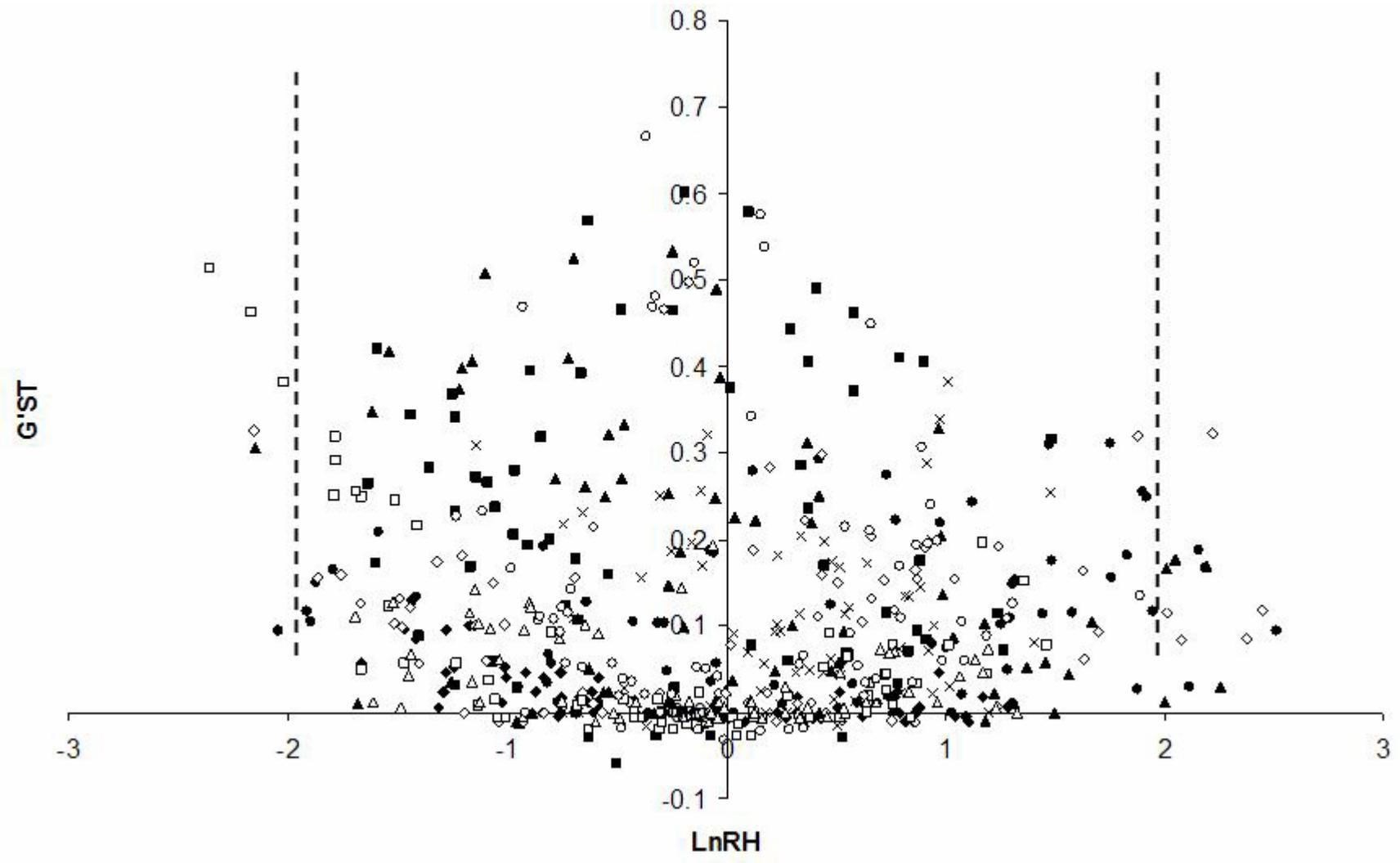
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Figure 5

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Appendix A.

Locus P1142						
Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.886	0.920	0.889	0.912	0.912	0.960
He	0.896	0.902	0.916	0.921	0.925	0.928
HWE exact test	0.4531	0.4012	0.6735	0.8601	0.4874	0.8974
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.941	0.899	0.827	0.959	0.903	0.857
He	0.937	0.929	0.894	0.933	0.901	0.809
HWE exact test	0.0514	0.1666	0.2777	0.6213	0.9373	0.5848
Locus StPf1004						
Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.810	0.882	0.694	0.693	0.813	0.660
He	0.817	0.824	0.781	0.809	0.793	0.727
HWE exact test	0.8429	0.4891	0.0133	0.0089	0.7850	0.4299
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.559	0.779	0.815	0.857	0.879	0.800
He	0.580	0.794	0.790	0.809	0.882	0.776
HWE exact test	0.4988	0.5263	0.1083	0.4350	0.0868	0.9118

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Appendix A continued

Locus List1001						
Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.367	0.247	0.231	0.320	0.412	0.440
He	0.355	0.281	0.277	0.352	0.424	0.438
HWE exact test	1	0.0591	0.0988	0.2537	0.7585	0.1377
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.265	0.397	0.400	0.490	0.563	0.320
He	0.314	0.379	0.468	0.495	0.478	0.388
HWE exact test	0.5685	0.2626	0.0624	1	0.4855	0.2155
Locus P1167						
Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.759	0.831	0.824	0.767	0.846	0.780
He	0.734	0.786	0.870	0.829	0.840	0.804
HWE exact test	0.6640	0.1230	0.1818	0.0361	0.9223	0.3893
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.794	0.765	0.765	0.714	0.781	0.580
He	0.811	0.798	0.831	0.761	0.792	0.669
HWE exact test	0.3488	0.3619	0.2133	0.7053	0.9279	0.0291

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Appendix A continued

Locus StPf1005

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.722	0.667	0.546	0.667	0.637	0.500
He	0.693	0.684	0.598	0.602	0.622	0.611
HWE exact test	0.4114	0.9337	0.2051	0.0912	0.3292	0.2508

Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.735	0.542	0.618	0.714	0.667	0.180
He	0.600	0.634	0.543	0.678	0.615	0.185
HWE exact test	0.2385	0.3307	0.3896	0.9617	0.4011	0.1977

Locus StPf1022

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.846	0.914	0.861	0.903	0.838	0.860
He	0.869	0.863	0.884	0.895	0.906	0.909
HWE exact test	0.1544	0.0586	0.5513	0.9743	0.0588	0.1880

Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.941	0.819	0.909	0.918	0.853	0.920
He	0.892	0.887	0.915	0.886	0.833	0.820
HWE exact test	0.1036	0.0344	0.2568	0.8531	0.4852	0.1488

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Appendix A continued

Locus StPf1015

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.456	0.452	0.472	0.490	0.438	0.500
He	0.497	0.466	0.486	0.483	0.475	0.484
HWE exact test	0.1628	0.2742	0.8695	0.5743	0.5873	0.5029

Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.441	0.356	0.527	0.531	0.588	0.420
He	0.467	0.399	0.603	0.488	0.619	0.471
HWE exact test	0.2505	0.0811	0.5271	0.3261	0.2605	0.3796

Locus StPf1002

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.595	0.720	0.722	0.670	0.713	0.680
He	0.727	0.707	0.687	0.687	0.709	0.627
HWE exact test	0.0926	0.4680	0.8964	0.5970	0.5836	0.9699

Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.706	0.708	0.660	0.714	0.765	0.633
He	0.640	0.702	0.615	0.679	0.622	0.585
HWE exact test	0.5721	0.0695	0.9745	0.6196	0.6078	0.8751

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Appendix A continued

Locus StPf1001

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.873	0.800	0.710	0.743	0.883	0.880
He	0.867	0.810	0.787	0.791	0.894	0.897
HWE exact test	0.1256	0.2848	0.1835	0.0229	0.8039	0.5493
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.839	0.781	0.704	0.898	0.735	0.750
He	0.821	0.815	0.779	0.890	0.717	0.811
HWE exact test	0.4717	0.0389	0.0627	0.2983	0.7109	0.4937

Locus Hsc70

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing Fjord
Ho	0.351	0.364	0.194	0.356	0.506	0.540
He	0.367	0.327	0.191	0.307	0.502	0.500
HWE exact test	0.7563	0.5097	1	0.1867	1	0.7757
Population	The Limfjord	Irish Sea	Bay of Biscay	Trondheim	Lake Pulmanki	Faroe Islands
Ho	0.529	0.580	0.463	0.578	0.364	0.580
He	0.492	0.495	0.466	0.493	0.339	0.495
HWE exact test	0.7337	0.2155	1	0.3610	1	0.2522

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Manuscript III

Evidence of genetic introgression from plaice (*Pleuronectes platessa* L.) to European flounder (*Platichthys flesus* L.) populations

Jakob Hemmer-Hansen and Einar Eg Nielsen

In preparation

1 **Evidence of genetic introgression from plaice (*Pleuronectes platessa* L.) to**
2 **European flounder (*Platichthys flesus* L.) populations**

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21 **Key words:** inter-specific hybridization, genetic introgression, F1 hybrids, flatfish, microsatellites,
22 individual admixture analyses.

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1 Introduction

2
3 Inter-specific hybridization and genetic introgression has been documented and analyzed in detail in a
4 large number of terrestrial animals (e.g. Goodman *et al.* 1999; Beaumont *et al.* 2001; Payseur and
5 Nachman 2005; Verardi *et al.* 2006) and plants (e.g. Rieseberg 1999; Rieseberg *et al.* 2003, review in
6 Arnold *et al.* 1999). Classical hybrid zone theory typically regard hybridization as an evolutionary dead
7 end because different models of stable hybrid zones assume that hybrids are either less fit than both
8 parental species (e.g. tension zones, Barton and Hewitt 1985) or only more fit at intermediate
9 environments in restricted geographic regions (e.g. bounded hybrid superiority, Moore 1977). Recently,
10 a number of examples have illustrated that hybridization may be an important source of evolutionary
11 novelty (e.g. Arnold *et al.* 1999; Salzburger *et al.* 2002; Rieseberg *et al.* 2003; Seehausen 2004,) and
12 hence hybridization is no longer by default regarded as a detrimental process (Arnold *et al.* 1999). In
13 contrast, the outcome of hybridization events appears to be highly dependent on ecological and
14 evolutionary contexts (e.g. Arnold *et al.* 1999; Barton 2001). However, one potential outcome of
15 intensive genetic introgression could be the break down of species barriers (e.g. Taylor *et al.* 2006) or
16 the prevention or prolongation of complete species' splits (because of continuous events of gene flow),
17 since introgression by definition involves an integration of species' genomes.

18 Although several marine fishes have characteristics, such as wide distributions, mass spawning
19 and external fertilization, which should make this group of species good models to study hybridization
20 in the marine environment, quite few marine fishes have been subject to detailed studies (Gardner
21 1997). Even fewer studies have examined genetic introgression among marine fish species (Gardner
22 1997), probably because backcrosses are often difficult to identify based on morphological characters
23 alone. However, the advent of molecular markers suitable for identifying hybridization beyond the first
24 generation has resulted in a number of recent studies confirming hybridization and introgression
25 between marine fish species in nature. Interesting examples include redfish (Roques *et al.* 2001),
26 rockfish (Seeb 1998; Buonaccorsi *et al.* 2005), eels (Albert *et al.* 2006) and flatfish (She *et al.* 1987). In
27 most examples, hybridization was found to be geographically restricted, reflecting the contact zones
28 between species with different geographical or ecological distributions. Apart from the interesting case
29 of hybridization between European and American eels (Albert *et al.* 2006), introgressive hybridization
30 in marine fishes has only rarely been investigated in species which are potentially able to hybridize
31 throughout most of their reproductive ranges.

32 In this study, we examine genetic introgression between two closely related flatfish species,
33 European flounder (*Platichthys flesus* L.) and plaice (*Pleuronectes platessa* L.), sharing a large part of
34 their geographical distribution. Specifically, we are investigating introgression from plaice to European
35 flounder populations throughout most of the flounder's distributional range in order to assess the spatial
36 dynamics of introgression from plaice to flounder.

37 European flounder and plaice are both coastal Pleuronectid flatfishes inhabiting the
38 Northeastern Atlantic - overlapping distributions. The major difference between their distributions is in
39 the Baltic Sea, where flounders are found in the innermost parts while plaice are only found in the
40 westernmost parts of the brackish sea. Although closely related, there is a major biological difference
41 in the ability of the species to tolerate reduced levels of salinity; while plaice is a typical marine species
42 European flounder tolerates brackish or even fresh water for long periods of time and particularly
43 juvenile stages are often found in fresh water. Spawning localities and times have been investigated
44 rather extensively in North Sea plaice by egg and larval surveys (Harding *et al.* 1978) and tagging
45 experiments (Hunter *et al.* 2003) revealing that major spawning ground are located in the Southern
46 North Sea and that plaice seem to home to specific spawning areas. Early larval stages of European

1 flounder are found at the same locations in the North Sea and English Channel (Campos *et al.* 1994,
2 Grioche *et al.* 1999) indicating that flounder and plaice may use the same spawning habitats. Both
3 species spawn in early spring but peak spawning of flounder seems to be delayed a few months
4 compared to plaice. The same patterns have been observed in the Danish Belt Sea connecting the North
5 Sea to the Baltic Sea (Heegaard 1947). Thus, it seems reasonable to conclude that at least time and
6 place of reproduction in the two species are overlapping to some extent, providing opportunities for
7 hybridization.

8 Naturally occurring hybrids between the two species have been known for more than hundred
9 years (Gottsche 1835 reviewed in Ubisch 1953), and they appear to be easily recognized based on their
10 intermediate phenotypes (e.g. Pape 1935; Sick *et al.* 1963). Both F1 and backcrosses have been
11 produced in the laboratory (Pape 1935; Ubisch 1953; Sick 1973), which shows that the species are at
12 least capable of hybridizing beyond the first generation. In an early study, Sick *et al.* (1963)
13 investigated hemoglobin patterns in both parental species as well as in putative hybrids from the
14 Danish Belt Sea. They found distinct differences between the three classes, confirming the presence of
15 F1 hybrids in nature and also identified a single aberrant individual which could have been a later
16 generation hybrid. Sick *et al.* (1963) suggested that hybridization is most frequent in the Western Baltic
17 Se and in the Danish Belt Sea, but that F1 individuals may reproduce inefficiently in nature resulting in
18 limited genetic introgression between the two species. However, the geographical extend of
19 hybridization has so far not been thoroughly investigated. Furthermore, the degree of introgression in
20 natural populations has not been investigated, and hence it is generally unknown if the species
21 hybridize beyond the first generation in nature.

22 In this study, our aim was to assess the magnitude and geographical distribution of genetic
23 introgression from plaice to European flounder. Specifically, our purpose was to search for potential
24 later-generation hybrids among putative flounders caught throughout the distributional range of the
25 species. We applied highly variable microsatellite markers, which are well suited for detecting later
26 generation hybrids, and a combination of individual admixture analysis and data simulations to detect
27 signals of introgressive hybridization between the two species.

28 29 **Materials and methods**

30 31 *Samples*

32 A total of 1093 European flounder were collected in 2003 and 2004 from localities covering most of
33 the distributional range of the species. Detailed population genetic analysis of these population samples
34 have been submitted for publication. In order to assess the level of introgression from plaice to
35 flounder, the flounder samples were supplemented with a sample of 49 plaice from the North Sea
36 collected in 2003 and a sample of 9 putative hybrids collected in the Bay of Århus in 2006 (Figure 1
37 and Table 1). Fin clips or gill tissue was stored in 95% ethanol before DNA extraction with DNeasy
38 (Qiagen), Chelex (Estoup *et al.* 1996) or HotSHOT (Truett *et al.* 2000) methods.

39 40 *Microsatellite analyses*

41 We used three microsatellite loci developed for plaice (List1001, Watts *et al.* 1999 and P1142 and
42 P1167, Hoarau *et al.* 2002a) and five developed for European flounder (StPf1001, StPf1002, StPf1004,
43 StPf1005, StPf1022, Dixon *et al.* unpublished) in the present study. These loci worked well in both
44 species with minor modifications of PCR conditions. PCR products were analyzed on an AlExpress
45 automated sequencer (Amersham Biosciences).

1 *Statistical analyses*

2 Genetic diversity within samples was assessed by observed (H_o) and expected (H_e) levels of
3 heterozygosity, number of alleles, allele size ranges and allelic richness (ElMousadik and Petit 1996)
4 estimated with the program FSTAT (Goudet 1995). Conformances to Hardy-Weinberg expectations
5 were tested for each locus in ARLEQUIN (Excoffier *et al.* 2005) and null allele frequencies according
6 to Chakraborty *et al.* (1992) were estimated with the program MICROCHECKER (van Oosterhout *et*
7 *al.* 2004). The distribution of genetic variance within and between species was assessed by the Analysis
8 of MOlecular VAriance (AMOVA) framework implemented in ARLEQUIN. Pairwise estimates of
9 Weir and Cockerham's (1984) θ were estimated in FSTAT.

10 We used the model based Bayesian clustering algorithm implemented in the software
11 STRUCTURE (Pritchard *et al.* 2000) to assess the population and individual levels of genetic
12 introgression, specifically focusing on introgression from plaice to flounder. We first ran
13 STRUCTURE on the total data set representing all sampled individuals in order to estimate the most
14 likely number of populations. This was done by estimating the likelihood under assumptions of the
15 number of populations (K) ranging from 1 to 8 in 10 independent runs for each K. Subsequently, the
16 flounder samples were grouped into corresponding populations based on their population level
17 admixture proportions. Throughout we will refer to the groups identified by STRUCTURE as
18 "populations" and the sampled plaice and flounder individuals as "samples".

19 Other studies have compared levels of introgression in populations in allopatry and in sympatry
20 in order to separate signal from noise, i.e. assess if the signals detected reflect true introgression rather
21 than random noise or population sub-structure (see Borge *et al.* 2005 for a recent example). However,
22 this approach was not possible in the case of plaice-flounder introgression because the two species are
23 found at the same locations throughout most of the geographical range covered. The inner Baltic Sea is
24 the only place, where allopatric flounder populations could be expected, but these populations of
25 flounder are highly differentiated from the populations in the remaining study area (see results) and
26 hence they could not be used as an unbiased non-introgressed baseline. Therefore, we approached the
27 question through comparisons of real and simulated data as outlined below. First, we simulated 1000
28 individuals from each of the four populations (three flounders and one plaice, see results) identified by
29 STRUCTURE. This was done by randomly drawing alleles from the average allele frequency
30 distributions estimated by the first ten program runs. Subsequently, these simulated populations were
31 used to generate simulated F1 and backcrossed hybrids in all three flounder populations (100
32 individuals in each category) in the program HYBRIDLAB (Nielsen *et al.* 2006). These simulated
33 individuals (4600 in total) were then included with the real sample data in a second round of ten
34 STRUCTURE runs in order to compare admixture proportions between real and simulated data. The
35 number of populations was fixed at four, since this was the number originally identified by
36 STRUCTURE. If the original data set was truly structured, and if STRUCTURE was able to pick up
37 the signal, we would expect the simulated data to show higher levels of population structure (i.e. less
38 introgression) than the original data set, because the program would have identified immigrants and
39 introgressed individuals as belonging to a different population than the one in which they were placed.
40 Therefore, we assumed that a smaller level of introgression in a simulated population (for instance from
41 simulated plaice to simulated Atlantic flounders) indicated that the signals detected among the real data
42 (i.e. levels of plaice-introgression in the real Atlantic samples) were of biological significance rather
43 than just random noise. Averages over the ten runs were also used to compare individual admixture
44 proportions in all flounder samples in a boxplot, allowing an assessment of the distribution of outliers
45 (i.e. high plaice admixture proportions) within and between populations. We chose to focus our
46 analyses on F1 and backcross hybrid categories only, since these categories represent realistic targets

1 given the levels of genetic divergence between the plaice and flounder samples and the fact that a
2 relatively modest number of loci were used in this study (Vähä and Primmer 2006).

3 To assess the power of the approach for detecting individual hybrids, we evaluated the
4 distribution of individual level admixture proportions in the simulated parental and backcrossed
5 individuals as suggested by Vähä and Primmer (2006). This was done in order to set a q-value
6 threshold above which individuals could reliably be considered hybrids rather than miss-classified
7 parentals. The thresholds were chosen in order to balance the false positive rate (i.e. parentals wrongly
8 identified as hybrids) with the expected number of missed backcrosses at the threshold.

11 **Results**

13 *Genetic diversity*

14 Overall genetic diversity was high in both flounder and plaice samples (H_e between 0.277 and 0.934 in
15 flounder and between 0.562 and 0.960 in plaice). The plaice sample tended to be more variable than
16 most flounder samples for the majority of the eight loci as also evidenced by higher allelic richness in
17 the plaice sample (Appendix A). No flounder population deviated systematically from Hardy Weinberg
18 expectations, while the plaice sample deviated significantly in three (StPf1001, StPf1004 and
19 StPf1005) and was nearly significant in two (P1142 and StPf1002) of the eight loci (Appendix A).
20 These deviations from Hardy Weinberg expectations were caused by heterozygote shortage potentially
21 because of null alleles. The deviation was particularly pronounced at locus StPf1004 with a null allele
22 frequency of 0.2268. Locus StPf1005 also showed evidence of null alleles with frequencies of 0.0384.
23 However, while null alleles may lower power to detect hybrids, they may not necessarily be a major
24 problem for the purpose of hybrid identification, since their effects were distributed over all alleles. We
25 therefore retained all microsatellite loci for further analyses.

26 Plaice and flounder had overlapping allele size ranges for all except one locus. Thus, alleles at
27 locus StPf1022 were roughly 100 bp longer in plaice than in flounder. Although size ranges were
28 overlapping among the remaining loci, the two groups did have different allele frequency distributions
29 for the majority of loci (Appendix B).

31 *Population structure*

32 Results from the AMOVA showed that a much larger proportions of the genetic variation was
33 distributed between species (16.06%, $P=0.0000$) than between samples of European flounder (2.12%,
34 $P=0.0000$). These results thus also show a significant genetic structuring of flounder samples, albeit of
35 minor magnitude than the structuring between species.

36 Results from the 10 independent STRUCTURE runs were highly consistent and all returned
37 $K=4$ as the most likely number of populations represented by the data. These groups corresponded to
38 one plaice and three flounder populations (Faroe Islands, Turku and Gotland in the inner Baltic Sea and
39 all remaining Atlantic/Western Baltic Sea samples), confirming the structuring of flounder populations
40 detected by the AMOVA. We refer to these three sample groups as “Faroe Islands”, “Atlantic” and
41 “Baltic”.

43 *Power analyses*

44 Evaluating the different false positive rates with respect to power of detecting hybrid individuals (Table
45 2) it was evident that power to detect F1 individuals in all populations was very high. Among the
46 backcrosses, the proportion of missed individuals was only reduced substantially when moving from a

1 false positive rate of 0.1% to 5% in the Atlantic and Baltic populations. However, at a false positive
2 rate of 5%, the q-value cut-off for designating individuals as hybrids was very low in these populations
3 (about 0.015). Since we were more interested in examining geographical differences in the level of
4 introgression than in identifying every hybrid present in the data set, we chose a q-value cut-off for
5 designating individuals as introgressed at the more restrictive false positive rate of 1%. This was done
6 because this cut-off represented a reasonable compromise between power to detect hybrids and
7 expected number of false positives (i.e. about 8 individuals among the samples in the Atlantic
8 population, 2 in the Baltic population and below one in the Faroe Islands population).

9 *Introgression*

10 STRUCTURE identified all presumed F1 individuals as admixed between the flounder and plaice
11 groups, having a plaice admixture proportion of approximately 0.5. The nine individuals were primarily
12 identified as hybrids between plaice and Atlantic flounders, but some also had a contribution from both
13 Baltic and Faroe flounders (Figure 2). The apparent split of the flounder proportion into three
14 populations most likely reflects a lack of statistical power than true introgression among the flounder
15 groups.

16
17 When including both simulated and real data in the same STRUCTURE runs (ten independent
18 runs), there was a consistent difference in the levels of population admixture between the simulated and
19 real data sets (Table 3). When examining the introgression between the three inferred populations of
20 flounder it was evident that the real data set contains considerable more admixture than the simulated
21 data set, reflecting genetic introgression among the real flounder samples. With respect to introgression
22 from plaice to flounder, a signal of introgression was only present in the Atlantic population, since this
23 population showed less introgression in the simulated compared to the real data set. In contrast, both
24 Faroe and Baltic flounder appeared to show little signs of introgression from plaice. Furthermore, there
25 was no signal of introgression from any of the flounder groups to plaice, despite the fact that absolute
26 levels of population admixture from flounder to plaice were larger than from plaice to flounder (Table
27 3).

28 Apparent levels of population introgression from plaice to the Atlantic flounders was low,
29 averaging 0.9% over the ten STRUCTURE runs (Table 2). Among the putative flounders, one
30 individual from the Westerschelde estuary (Wes_2) had a plaice q-value of app. 0.5 indicating that this
31 individual was a hybrid. The multi-locus genotype of this individual (PI142: 147/165, StPf1004:
32 154/166, List1001: 84/86, PI167: 170/190, StPf1005: 99/103, StPf1022: 194/262, StPf1002: 177/177,
33 StPf1001: 251/251) was fully compatible with having one allele from each parental species, suggesting
34 that it was most likely a first generation hybrid. In addition to this single individual, a number of
35 flounders showed signs of having a portion of their genome of plaice ancestry. This signal was
36 particularly evident among the Atlantic samples as evidenced by the number and magnitude of outliers
37 in these samples (Figure 3). Among the Atlantic samples only the Thyborøn and the Limfjord samples
38 did not show signs of significant outlier individuals, while very few apparent outliers were present in
39 the Baltic Sea samples (Gotland and Turku) and in the Faroe Islands population. The differences in
40 individual admixture proportions between populations was statistically significant (Atlantic versus
41 Baltic, $P=0.0001$; Atlantic versus Faroe, $P=1.1E-30$, Baltic versus Faroe, $P=5.6E-11$, Mann-Whitney
42 U-tests). These findings are in accordance with results from the population level admixture proportions
43 (Table 3).

44 Table 4 lists all individuals with q-values above the q-value threshold corresponding to a false
45 positive rate of 1%. There were no individuals above the threshold in either of the Faroe or Baltic
46 samples, reflecting the absence or very limited extend of introgression in these populations. Besides the

1 putative F1 hybrid, Wes_2, twenty individuals from the Atlantic populations had q-values above the
2 threshold. These were distributed among several of the populations in this group, mirroring results from
3 the boxplot in Figure 3. Approximately eight of these outlier individuals were expected to be false
4 positives at a false positive rate of 1%, which indicates that 1.5% (12/817) of the individuals in the
5 Atlantic population had a portion of their genome of plaice ancestry.
6
7

8 **Discussion**

9

10 The results from this study have documented the presence of first generation hybrids between plaice
11 and European flounder as well as of backcrosses to flounder in natural populations of flounder.
12 Comparisons of simulated and real data and the geographical distribution of introgression suggest that
13 the signal of introgression is most likely a true biological signal rather than random noise. Likewise did
14 introgression not simply seem to reflect the genetic distance between flounder and plaice samples or
15 the level of genetic variation within flounder samples, since samples closest to plaice (Trondheim,
16 Thyborøn, Westerschelde) were also among the most variable and did not show signs of increased
17 levels of introgression (Appendix A and Figure 3). Furthermore, increased variation in these samples
18 would be expected to increase the overall level of population introgression (i.e. all individuals
19 appearing more admixed) rather than resulting in an increased number of outliers which was actually
20 observed. Hence, these results show that genetic introgression does occur from plaice to European
21 flounder in nature. Importantly, evidence of introgression was found through most of the geographical
22 range surveyed in this study. This suggests that hybridization is occurring wherever the two species co-
23 occur and not specifically in e.g. the Western Baltic Sea which has previously been identified as a
24 hybridization hot-spot because of the observation of relatively large numbers of first generation hybrids
25 (Sick *et al.* 1963). It should be noted that our results can not be taken as an estimate of the frequency of
26 first generation hybrids in nature, since sampling was specifically targeting presumed pure flounders
27 and may hence have avoided collecting F1 hybrids. In contrast, they are expected to reflect true levels
28 of introgression beyond the first generation, because backcrosses are generally impossible to separate
29 from pure individuals based on phenotypes alone. Our results add to the increasing number of recent
30 studies confirming that hybridization may be a common phenomenon in marine fishes (Gardner 1997;
31 Seeb 1998; Roques *et al.* 2001; Bounaccorsi *et al.* 2005; Albert *et al.* 2006; Wilson 2006; Yaakub *et al.*
32 2006), but differ from most previous studies by investigating introgression between species which are
33 potentially able to hybridize throughout most of their distributional ranges.

34 The rate of introgression from plaice to flounder seems to be relatively low as evidenced by low
35 levels of population admixture (0.9%) as well as the relatively few individuals which were identified as
36 significantly introgressed (1.5%) among the flounders. These results are in contrast to some of the
37 studies of introgression between marine fish species conducted so far. Thus Roques *et al.* (2001) found
38 levels of introgression of 15% between redfish species in the Northwestern Atlantic. Similarly,
39 extensive hybridization and introgression beyond the first generation has recently been found between
40 American and European eels at Icelandic localities (Albert *et al.* 2006).

41 The relatively low introgression from plaice to European flounder may be surprising given that
42 time and place of spawning is presumably largely overlapping and that the species are fully capable of
43 producing viable and fertile offspring as evidenced by both field and laboratory studies (Pape 1935;
44 Sick *et al.* 1963; This study). This indicates that selection is preventing extensive introgression from
45 plaice to flounder thereby maintaining species barriers. Selection against hybridization can occur at
46 both pre and postzygotic stages. Although time and place of spawning may be overlapping, spawning

1 behavior could limit the extent of hybridization between the two species. Flatfishes are known to
2 exhibit courtship behavior preceding spawning, often involving pairing of male and female followed by
3 ventral mounts (Forster 1953; Stoner 1999). Some studies have also suggested assortative mating by
4 female choice (Carvalho *et al.* 2003). Although such barriers to gene flow have not been reported
5 specifically with respect to plaice-flounder hybridization, it is clear that spawning behavior in flatfish is
6 complex and could hence serve as an important barrier to gene flow between species.

7 However, F1 hybrids have been reported to constitute a relatively large part of flounder catches
8 in some areas (Pape 1935; Sick *et al.* 1963), indicating that these pre-zygotic barriers, if present, are far
9 from complete in nature. Since frequent hybridization has been reported in these areas for more than
10 hundred years, there is little reason to believe that these patterns should have changed to a lower
11 frequency of F1 hybrids today. Nevertheless, we find little evidence of large levels of introgression in
12 these same areas which indicates reduced reproductive fitness of first generation hybrids in nature (e.g.
13 McGinnity *et al.* 2003). An alternative explanation, not excluding the first, could be outbreeding
14 depression caused by the break-up of co-adapted gene complexes in later generation hybrids (Burton *et*
15 *al.* 1999; Edmands 1999; Gharrett *et al.* 1999). The most pronounced difference between the life
16 histories of the two species is the ability of flounders to tolerate fresh water for prolonged periods of
17 time. Juvenile flounders prefer sites with fresh-water run off and are often migrating to rivers and
18 lagoons where they spend the first few years of their lives (Kerstan 1991; Bos 1999), whereas juvenile
19 plaice are found in coastal marine nursery areas (Zijlstra 1972). It is plausible that selection would act
20 against plaice genotypes not adapted to fresh water at this life stage, thereby preventing their
21 introgression in the flounder genome. However, since the fitness of hybrids have never been studied in
22 detail it is difficult to point to specific mechanisms responsible. Nevertheless, it appears highly likely
23 that selection must be involved in preventing extensive gene flow between the two species.

24 It should be noted that the estimates of the number of introgressed individuals are most likely
25 underestimates, since we expect to miss app. 10% of the simulated backcrosses in the Atlantic
26 populations at a q-value threshold of 0.036. It is clear from simulation studies (e.g. Vähä and Primmer
27 2006) that the eight loci applied here represents close to the minimum required for efficient hybrid
28 identification at levels of divergence between the parent populations of app. 0.16-0.2 (see Figure 3).
29 Hence more loci with discriminating power could be applied in future studies to increase power of
30 hybrid detection. However, the results also show that power is sufficient to detect first generation
31 hybrids with great certainty and backcrosses with reasonable success, and we find it unlikely that
32 introgression should be considerably larger than observed here. This is supported by the fact that we
33 find only one plaice-specific allele from locus StPf1022 among the putative flounders (Wes_2), which
34 is unexpected if introgression is truly pervasive. On the other hand more than one allele could be
35 expected if introgression occurs at the rate suggested here, and it cannot be excluded that a few
36 additional plaice specific alleles were lost due to technical problems such as large allele drop-out in
37 hybrids. Differential amplification success of plaice and flounder alleles in hybrids was observed at a
38 locus which was omitted from the study. However, locus StPf1022 amplified well in all known hybrids
39 indicating reasonable amplification success across species.

40 The apparent lack of introgression from flounder to plaice could suggest asymmetrical rates of
41 introgression between the two species. However, it should be noted that the single plaice sample may
42 not have been sufficient to thoroughly investigate introgression in this direction. There was a tendency
43 for STRUCTURE to underestimate the discreteness of the plaice sample by assigning alleles not
44 present in the real plaice sample (such as flounder alleles at locus StPf1022) non-zero allele
45 frequencies. This may have resulted in the detected signal of more introgression in the simulated data.
46 At the individual level, there was in fact a single individual (Pla_18) which may have been a backcross

1 to plaice (plaice q-value of app. 0.63, results not shown). This may indicate that backcrossing occur
2 both ways in nature.

3 In this study, we were able to take the overall population structuring among the flounder
4 samples into account, but could not do so for plaice. It is unlikely, however, that missing plaice
5 samples are biasing the results regarding introgression from plaice to Atlantic flounder, because plaice
6 has been found to show very low levels of population structure throughout the range of the Atlantic
7 samples in this study (Hoarau *et al.* 2002b). Faroe plaice, however, appears to be significantly different
8 from the plaice in the North Sea (Hoarau *et al.* 2002b) and it cannot be excluded that a Faroe plaice
9 sample could have changed conclusions regarding introgression in Faroe flounders slightly. However,
10 the very low levels of genetic diversity in Faroe Island flounders is not compatible with substantial
11 levels of introgression from plaice since this would be expected to increase levels of diversity by
12 introducing new alleles to the population.

13 To summarize, we find evidence of hybridization and genetic introgression from plaice to
14 European flounder in nature. Results suggest that introgression occurs wherever the two species co-
15 occur supporting the conclusion from other studies that hybridization and introgression may be
16 widespread in marine fishes. However, despite the long known ability for these species to hybridize
17 introgression seems to be of limited magnitude. Notably, there was not an increased signal of
18 introgression in areas where intensive hybridization has been reported to occur. This is strongly
19 suggesting that selection against hybrids at some stages of the life histories of the species are involved
20 in maintaining species barriers.

1 **References**

- 2
- 3 Albert V, Jonsson B, Bernatchez L. 2006. Natural hybrids in Atlantic eels (*Anguilla anguilla*, *A.*
4 *rostrata*): evidence for successful reproduction and fluctuating abundance in space and time. *Molecular*
5 *Ecology* 15: 1903-1916.
- 6
- 7 Arnold ML, Bulger MR, Burke JM, Hempel AL, Williams JH. 1999. Natural hybridization: How low
8 can you go and still be important? *Ecology* 80: 371-381.
- 9
- 10 Barton NH, Hewitt GM. 1985. Analysis of hybrid zones. *Annual reviews of Ecology and Systematics*
11 16: 113-148.
- 12
- 13 Barton NH. 2001. The role of hybridization in evolution. *Molecular Ecology* 10: 551-568.
- 14
- 15 Beaumont M, Barratt EM, Gottelli D, Kitchener AC, Daniels MJ, Pritchard JK, Bruford MW. 2001.
16 Genetic diversity and introgression in the Scottish wildcat. *Molecular Ecology* 10: 319-336.
- 17
- 18 Borge T, Lindroos K, Nadvornik P, Syvanen AC, Saetre GP. 2005. Amount of introgression in
19 flycatcher hybrid zones reflects regional differences in pre and post-zygotic barriers to gene exchange.
20 *Journal of Evolutionary Biology* 18: 1416-1424.
- 21
- 22 Bos AR. 1999. Tidal transport of flounder larvae (*Pleuronectes flesus*) in the Elbe River, Germany.
23 *Archive of Fishery and Marine Research* 47: 47-60.
- 24
- 25 Buonaccorsi VP, Kimbrell CA, Lynn EA, Vetter RD. 2005. Limited realized dispersal and
26 introgressive hybridization influence genetic structure and conservation strategies for brown rockfish,
27 *Sebastes auriculatus*. *Conservation Genetics* 6: 697-713.
- 28
- 29 Burton RS, Rawson PD, Edmands S. 1999. Genetic architecture of physiological phenotypes:
30 Empirical evidence for coadapted gene complexes. *American Zoologist* 39: 451-462.
- 31
- 32 Campos WL, Kloppmann M, von Westernhagen H. 1994. Inferences from horizontal distribution of
33 dab *Limanda limanda* (L.) and flounder *Platichthys flesus* (L.) larvae in the southeastern North Sea.
34 *Netherlands Journal of Sea Research* 32: 277-286.
- 35
- 36 Carvalho N, Afonso P, Santos RS. 2003. The harem mating system and mate choice in the wide-eyed
37 flounder, *Bothus podas*. *Environmental Biology of Fishes* 66: 249-258.
- 38
- 39 Chakraborty R, De Andrade M, Daiger SP, Budowle B. 1992. Apparent heterozygote deficiencies
40 observed in DNA typing data and their implications in forensic applications. *Annals of Human*
41 *Genetics*, 56, 45-57.
- 42
- 43 Edmands S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide
44 range of divergence. *Evolution* 53: 1757-1768.
- 45

1 ElMousadik A, Petit RJ. 1996. High level of genetic differentiation for allelic richness among
2 populations of the argan tree (*Argania spinosa* (L) Skeels) endemic to Morocco. Theoretical and
3 Applied Genetics 92: 832-839.
4

5 Estoup A, Largiader CR, PerrotE, Chourrout D. 1996. Rapid one-tube DNA extraction for reliable PCR
6 detection of fish polymorphic markers and transgenes. Molecular Marine Biology and Biotechnology
7 5: 295-298.
8

9 Excoffier, L. G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for
10 population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.
11

12 Forster GR. 1953. The spawning behaviour of plaice. Journal of the Marine Biological Association of
13 the United Kingdom 32: 319-319.
14

15 Gardner JPA. 1997. Hybridization in the sea. Advances in Marine Biology 31: 1-78.
16

17 Gharrett AJ, Smoker WW, Reisenbichler RR, Taylor SG. 1999. Outbreeding depression in hybrids
18 between odd-and even-broodyear pink salmon. Aquaculture 173: 117-129.
19

20 Goodman SJ, Barton NH, Swanson G, Abernethy K, Pemberton JM. 1999. Introgression through rare
21 hybridization: A genetic study of a hybrid zone between red and sika deer (Genus *Cervus*) in Argyll,
22 Scotland. Genetics 152: 355-371.
23

24 Goudet J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of
25 Heredity 86: 485-486.
26

27 Grioche A, Koubbi P, Harlay X. 1999. Spatial patterns of ichthyoplankton assemblages along the
28 Eastern English Channel French coast during spring 1995. Estuarine, Coastal and Shelf Science 49:
29 141-152.
30

31 Harding D, Nichols JH, Tungate DS. 1978. The spawning of plaice (*Pleuronectes platessa* L.) in the
32 Southern North Sea and English Channel. Rapports et Procés-Verbaux des Réunions du Conseil
33 International pour l'Exploration de la Mer 172: 102-113.
34

35 Heegaard P. 1947. Investigations on the breeding season and the quantities of eggs of the food-fishes of
36 the Kattegat and the Northern Belt Sea 1929-1941. Meddelelser fra Kommissionen for Danmarks
37 Fiskeri – og Havundersøgelser XI, nr. 4: 1-22.
38

39 Hoarau G, Cook D, Stam WT, Olsen JL. 2002a. New microsatellites primers for plaice, *Pleuronectes*
40 *platessa* L. (Teleostei : Pleuronectidae). Molecular Ecology Notes 2: 60-61.
41

42 Hoarau G, Rijnsdorp AD, Van der Veer HW, Stam WT, Olsen JL. 2002b. Population structure of
43 plaice (*Pleuronectes platessa* L.) in northern Europe: microsatellites revealed large-scale spatial and
44 temporal homogeneity. Molecular Ecology 11: 1165-1176.
45

- 1 Hunter E, Metcalfe JD, Reynolds JD. 2003. Migration route and spawning area fidelity by North Sea
2 plaice. Proceedings of the Royal Society of London - Series B 270: 2097-2103.
3
- 4 Kerstan M., 1991: The importance of rivers as nursery grounds for 0 and 1-group flounder (*Platichthys*
5 *flesus* L.) in comparison to the Wadden Sea. Netherlands Journal of Sea Research 27: 353-366.
6
- 7 McGinnity P, Prodohl P, Ferguson K, Hynes R, O'Maoileidigh N, Baker N, Cotter D, O'Hea B, Cooke
8 D, Rogan G, Taggart J, Cross T. 2003. Fitness reduction and potential extinction of wild populations of
9 Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the
10 Royal Society of London - Series B 270: 2443-2450.
11
- 12 Moore WS. 1977. Evaluation of narrow hybrid zones in vertebrates. Quarterly Review of Biology 52:
13 263-277.
14
- 15 Nielsen EE, Bach LA, Kotlicki P. 2006. Hybridlab (version 1.0): a program for generating simulated
16 hybrids from population samples. Molecular Ecology Notes, published online 20-07-2006.
17
- 18 Pape A. 1935. Beiträge zur Naturgeschichte von *Pleuronectes pseudoflesus*. Wissenschaftliche
19 Meeresuntersuchungen Abteilung Kiel 22: 53-88.
20
- 21 Payseur BA, Nachman MW. 2005. The genomics of speciation: investigating the molecular correlates
22 of X chromosome introgression across the hybrid zone between *Mus domesticus* and *Mus musculus*.
23 Biological Journal of The Linnean Society 84: 523-534.
24
- 25 Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus
26 genotype data. Genetics 155: 945-959.
27
- 28 Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL,
29 Schwarzbach AE, Donovan LA, Lexer C. 2003. Major ecological transitions in wild sunflowers
30 facilitated by hybridization. Science 301: 1211-1216.
31
- 32 Rieseberg LH, Whitton J, Gardner K. 1999. Hybrid zones and the genetic architecture of a barrier to
33 gene flow between two sunflower species. Genetics 152: 713-727.
34
- 35 Roques S, Sevigny JM, Bernatchez L. 2001. Evidence for broadscale introgressive hybridization
36 between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. Molecular
37 Ecology 10: 149-165.
38
- 39 Salzburger W, Baric S, Sturmbauer C. 2002. Speciation via introgressive hybridization in East African
40 cichlids? Molecular Ecology 11: 619-625.
41
- 42 Seeb LW. 1998. Gene flow and introgression within and among three species of rockfishes, *Sebastes*
43 *auriculatus*, *S. caurinus*, and *S. maliger*. Journal of Heredity 89: 393-403.
44
- 45 Seehausen O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Genetics 19: 198-
46 207.

- 1
2 She JX, Autem M, Kotulas G, Pasteur N, Bonhomme F. 1987. Multivariate analysis of genetic
3 exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). Biological Journal of
4 the Linnean Society 32: 357-371.
5
6 Sick K, Frydenberg O, Nielsen JT. 1963. Haemoglobin patterns of plaice, flounder and their natural
7 and artificial hybrids. Nature 198: 411–412.
8
9 Sick K, Frydenberg O, Nielsen JT. 1973. Hemoglobin patterns of second-generation hybrids between
10 plaice and flounder. Heredity 30: 244-245.
11
12 Stoner AW, Bejda AJ, Manderson JP, Phelan BA, Stehlik LL, Pessutti JP. 1999. Behavior of winter
13 flounder, *Pseudopleuronectes americanus*, during the reproductive season: laboratory and field
14 observations on spawning, feeding, and locomotion. Fishery Bulletin 97: 999-1016.
15
16 Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006. Speciation in
17 reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus*
18 *aculeatus*) species pair. Molecular Ecology 15: 343-355.
19
20 Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman M. 2000. Preparation of PCR-
21 quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques 29: 52-
22 54.
23
24 Ubisch von LV. 1953. Über die Zahl der Flossenstrahlen bei *Pleuronectes plates*, *Pl. flesus* und der
25 Bastarde und Rückkreuzungen zwischen beiden Arten. Zoologischer Anzeiger 151: 75-87.
26
27 Vähä JP, Primmer CR. 2006. Efficiency of model-based Bayesian methods for detecting hybrid
28 individuals under different hybridization scenarios and with different numbers of loci. Molecular
29 Ecology 15: 63-72.
30
31 van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for
32 identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-
33 538.
34
35 Verardi A, Lucchini V, Randi E. 2006. Detecting introgressive hybridization between free-ranging
36 domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. Molecular
37 Ecology 15: 2845–2855
38
39 Watts PC, Nash RDM, George SG, Kemp SJ. 1999. Isolation and characterization of microsatellite loci
40 in the European plaice, *Pleuronectes platessa* L. (Teleostei: Pleuronectidae). Molecular Ecology 8:
41 2151-2152.
42
43 Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population-structure.
44 Evolution 38: 1358-1370.
45

1 Wilson AB. 2006. Interspecies mating in sympatric species of *Syngnathus* pipefish. *Molecular Ecology*
2 15: 809-824.
3
4 Yaakub SM, Bellwood DR, van Herwerden L, Walsh FA. 2006. Hybridization in coral reef fishes:
5 Introgression and bi-directional gene exchange in *Thalassoma* (family Labridae). *Molecular*
6 *Phylogenetics and Evolution* 40: 84-100.
7
8 Zijlstra JJ. 1972. On the importance of the Waddensea as a nursery area in relation to the conservation
9 of the southern North Sea fishery resources. *Symposium Zoological Society of London* 29: 233–258.
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1 **Figure legends**

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4 **Figure 1.** Sampling locations for samples of flounders, plaice and hybrids. Sample abbreviations refer
5 to positions in Table 1.

6
7 **Figure 2.** Individual level admixture proportions of nine putative hybrids collected in the Bay of Århus
8 in 2006.

9
10 **Figure 3.** Boxplot of individual level admixture proportion (plaice proportion) in flounder samples.
11 Samples are arranged from left to right with increasing F_{st} to the plaice sample. Population labels are 1:
12 Trondheim ($F_{st}=0.159$), 2: Thyborøn ($F_{st}=0.161$), 3: Westerschelde ($F_{st}=0.161$), 4: Ringkøbing Fjord
13 ($F_{st}=0.164$), 5: Bay of Biscay ($F_{st}=0.167$), 6: Ærø ($F_{st}=0.169$), 7: Lake Pulmanki ($F_{st}=0.169$), 8: Irish
14 Sea ($F_{st}=0.170$), 9: Bornholm ($F_{st}=0.177$), 10: The Limfjord ($F_{st}=0.182$), 11: Turku ($F_{st}=0.186$), 12:
15 Gotland ($F_{st}=0.186$), 13: Faroe Islands ($F_{st}=0.225$). The F1 hybrid from Westerschelde has been
16 removed to enhance graphical presentations of the remaining individuals.

1 Table 1. Samples of flounder, plaice and hybrids with approximate position and sample sizes.
 2

Locality	Sample year	Approximate position	Total sample size
1. Turku (Tur)	2003 and 2004	22° E, 60° N	104
2. Gotland (Got)	2003 and 2004	19 ° E, 57.5 ° N	94
3. Bornholm (Bor)	2003 and 2004	16° E, 55° N	108
4. Ærø (Aer)	2003 and 2004	10° E, 55° N	104
5. Thyborøn (Thy)	2003 and 2004	8° E, 57° N	114
6. Ringkøbing Fjord (Rin)	2003 and 2004	8.3° E, 55.96° N	119
7. The Limfjord (Lim)	2003	8.59° E, 56.5° N	55
8. Westerschelde estuary	2003	3.7° E, 52.4° N	48
9. Irish Sea (Irs)	2003 and 2004	-4° E, 54° N	98
10. Bay of Biscay (Bis)	2003 and 2004	-2.3° E, 47.20° N	88
11. Trondheim (Tro)	2004	11° E, 65° N	49
12. Lake Pulmanki (Pul)	2004	28.02° E, 70.01° N	34
13. Faroe Islands (Far)	2003 and 2004	-6.45° E, 62° N	78
14. Plaice (Pla)	2003	9° E, 57.2° N	49
15. Hybrids (Hyb)	2006	10.30° E, 56.30° N	9

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 2 Table 2. Estimated q-value cut-offs and missed backcrosses in the simulated
 3 datasets under different false positive rates. Shown are the average values
 4 from ten STRUCTURE runs.
 5

False positives	0.5%	1%	5%
<i>q-value cut-off</i>			
Atlantic	0.048	0.036	0.015
Faroe	0.082	0.059	0.028
Inner Baltic	0.082	0.052	0.014
<i>Missed F1 individuals at threshold</i>			
Atlantic	0%	0%	0%
Faroe	0%	0%	0%
Inner Baltic	0%	0%	0%
<i>Missed backcrosses at threshold</i>			
Atlantic	11%	9%	7%
Faroe	9%	5%	3%
Inner Baltic	13%	11%	5%

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 2 Table 3. Population level admixture proportions for simulated and real data sets (mean and standard deviation from
 3 ten STRUCTURE runs).
 4

Populations identified by STRUCTURE					
Population	Number of individuals	Atlantic	Faroe	Baltic	Plaice
Simulated Atlantic	1000	0.875 (0.006)	0.051 (0.003)	0.067 (0.004)	0.007 (0.001)
Atlantic	817	0.838 (0.006)	0.066 (0.003)	0.087 (0.004)	0.009 (0.001)
Simulated Faroe	1000	0.044 (0.001)	0.930 (0.002)	0.018 (0.001)	0.007 (0)
Faroe	78	0.049 (0.001)	0.923 (0.002)	0.024 (0.001)	0.004 (0.001)
Simulated Baltic	1000	0.088 (0.003)	0.026 (0.002)	0.880 (0.004)	0.006 (0)
Baltic	198	0.210 (0.006)	0.053 (0.002)	0.731 (0.006)	0.006 (0)
Simulated Plaice	1000	0.022 (0)	0.013 (0.001)	0.014 (0.001)	0.951 (0.001)
Plaice	49	0.016 (0.001)	0.010 (0.001)	0.009 (0.001)	0.966 (0.001)

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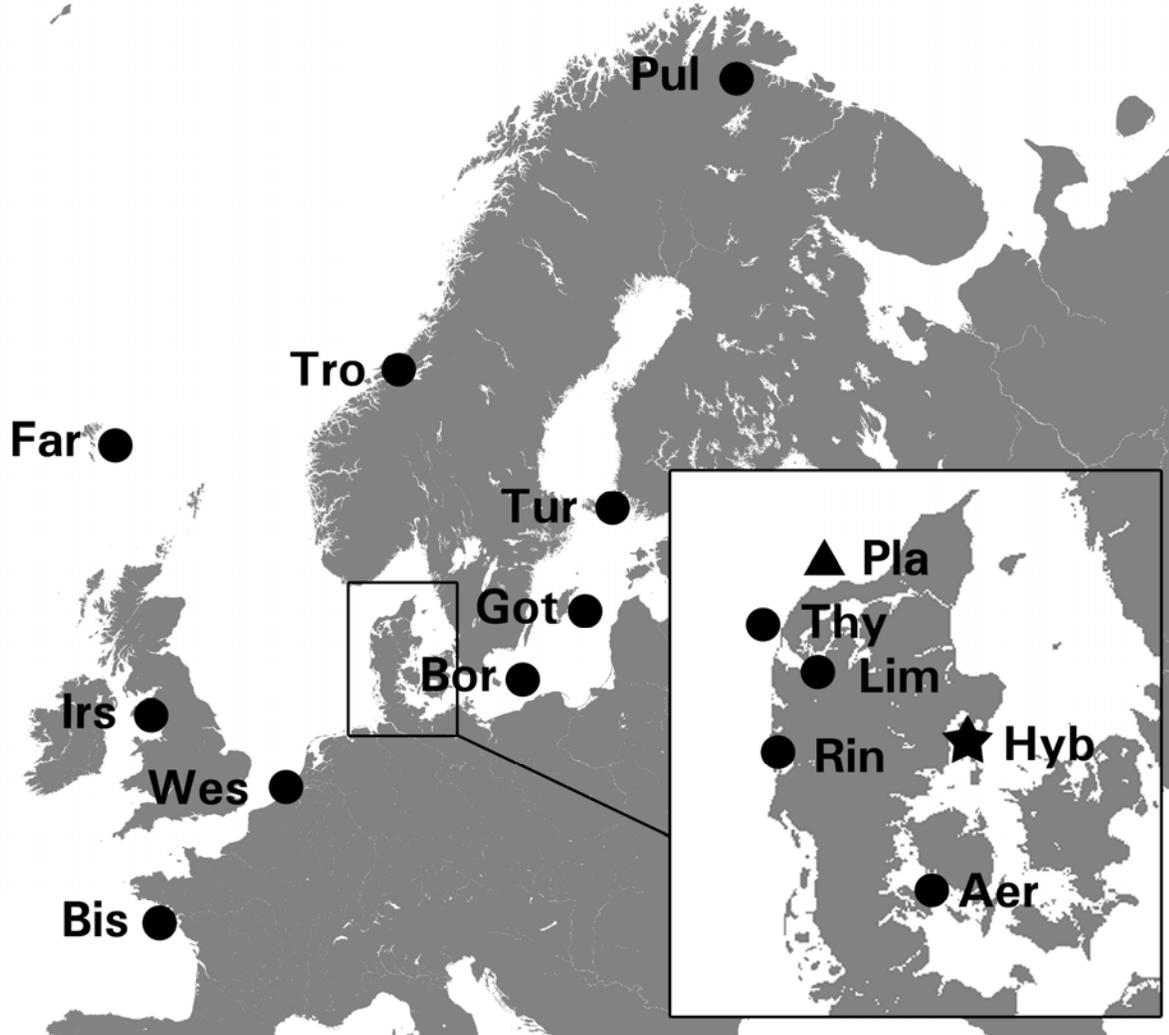
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Table 4. Mean individual flounder q-values from 10 STRUCTURE runs. Shown are all individuals from the samples belonging to the Atlantic population with q values above the population specific q value threshold corresponding to a false positive rate of 1%. Broken line indicates 0.5% threshold. The simulated individuals from the Atlantic population with q-values above the 1% threshold are also presented. No individuals from the Faroe, Gotland and Turku samples had individual q values above the 1% threshold.

Rank	Atlantic population	q values	Simulated Atlantic
1	Wes_2	0.4826	0.1476
2	Irs_20	0.2113	0.0845
3	Rin_39	0.1848	0.0575
4	Irs_65	0.1433	0.0496
5	Bis_36	0.143	0.0478
6	Bis_9	0.1406	0.0437
7	Bor_60	0.0847	0.0423
8	Tro_48	0.0836	0.039
9	Irs_6	0.0821	0.0384
10	Rin_14	0.0806	0.0361
11	Aer_71	0.0781	
12	Aer_55	0.0719	
13	Irs_59	0.0707	
14	Pul_31	0.0696	
15	Rin_41	0.0474	
16	Pul_25	0.0445	
17	Rin_89	0.0418	
18	Pul_28	0.0402	
19	Rin_59	0.038	
20	Bor_105	0.0369	
21	Bor_18	0.0365	

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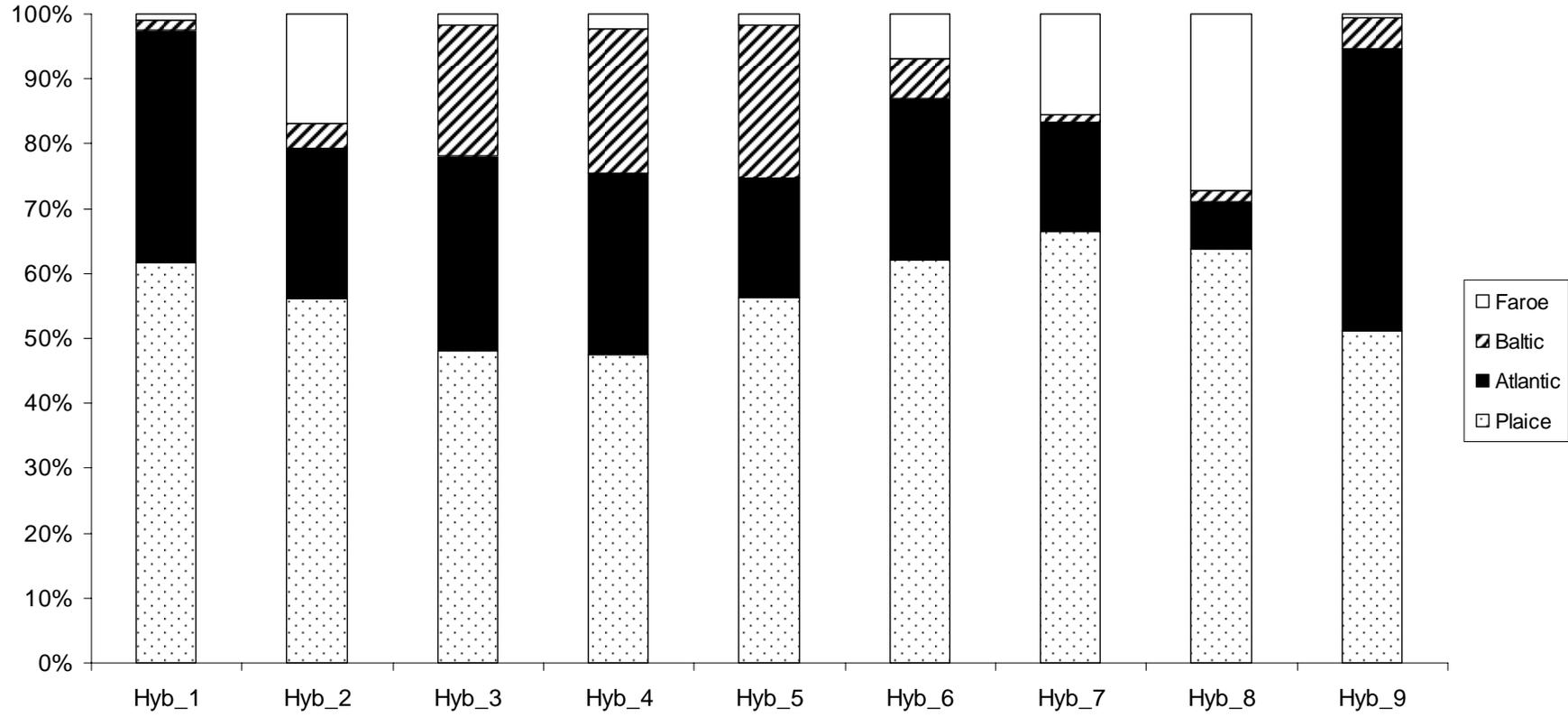
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Figure 1

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Figure 2.

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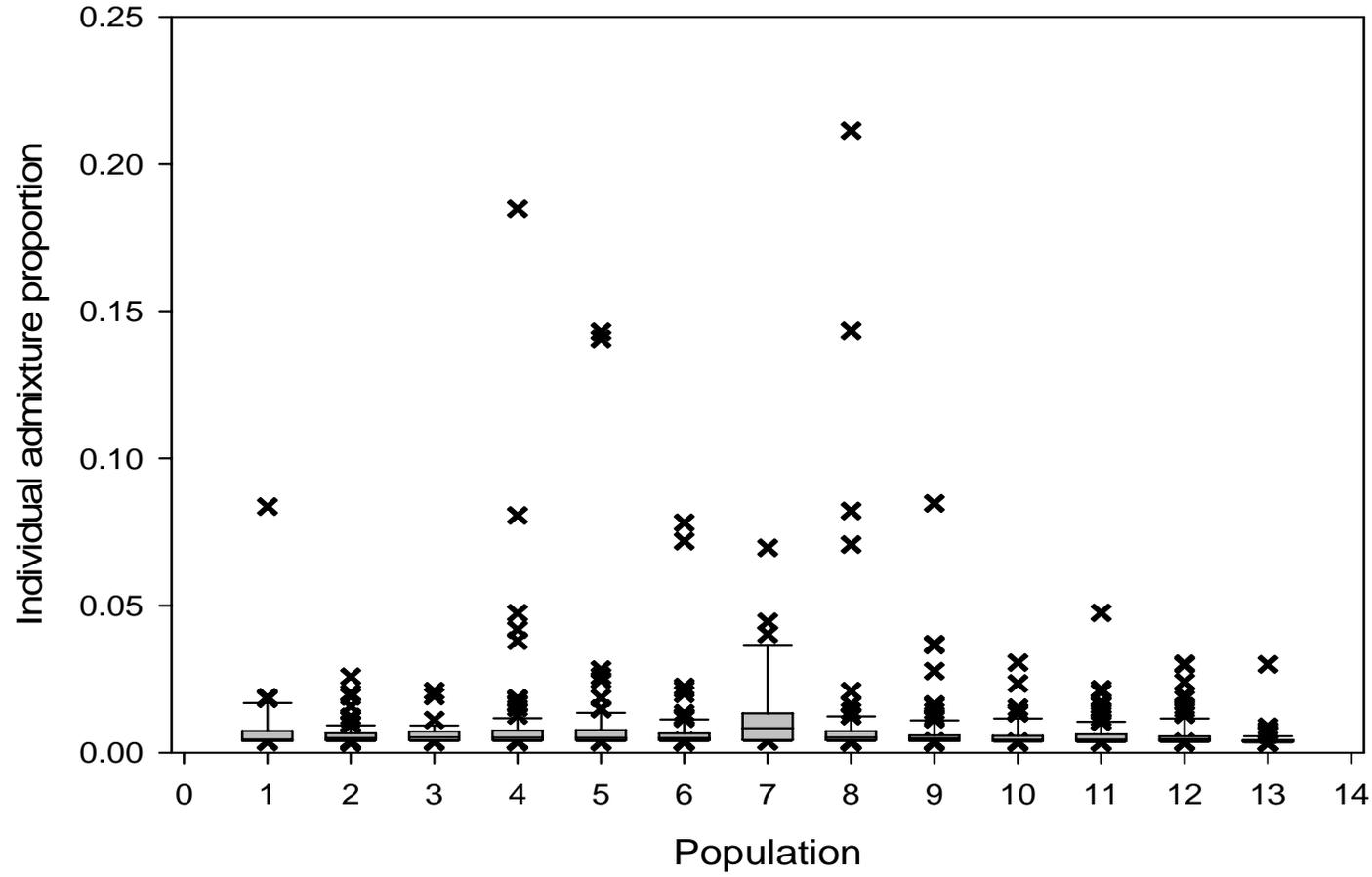


Figure 3.

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1 Appendix A. Summary statistics and tests for Hardy Weinberg Equilibrium

2

Locus PI142

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	23	21	21	25	23	21	21
Allelic richness	15.23	15.07	16.28	18.49	17.00	16.83	18.23
Size range	153-207	139-209	143-195	147-203	137-203	139-197	155-203
Ho	0.885	0.920	0.889	0.913	0.937	0.933	0.927
He	0.893	0.902	0.916	0.921	0.923	0.928	0.934
HWE exact test	0.706	0.369	0.725	0.845	0.715	0.550	0.015

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	21	24	22	22	13	11	19
Allelic richness	18.55	19.46	17.40	18.43	12.97	9.12	15.99
Size range	147-203	147-229	139-193	139-199	147-189	155-185	129-193
Ho	0.844	0.915	0.871	0.959	0.903	0.857	0.833
He	0.927	0.933	0.906	0.933	0.901	0.808	0.887
HWE exact test	0.367	0.164	0.636	0.732	0.956	0.623	0.055

Locus StPf1004

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	16	14	17	24	23	26	11
Allelic richness	10.79	10.61	11.06	14.63	13.74	13.80	8.25
Size range	132-188	144-188	138-192	132-200	138-190	132-198	142-184
Ho	0.788	0.882	0.694	0.696	0.807	0.695	0.545
He	0.811	0.824	0.781	0.808	0.794	0.758	0.602
HWE exact test	0.706	0.538	0.000	0.011	0.867	0.262	0.544

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	18	23	18	15	15	8	17
Allelic richness	14.40	15.54	13.33	12.83	14.36	6.87	15.95
Size range	138-192	136-188	134-188	132-192	144-192	138-192	144-194
Ho	0.809	0.796	0.805	0.857	0.879	0.782	0.486
He	0.770	0.799	0.790	0.809	0.882	0.752	0.914
HWE exact test	0.984	0.427	0.129	0.415	0.093	0.564	0.000

1 Appendix A continued

2

Locus List1001

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	3	3	4	3	3	4	3
Allelic richness	2.91	2.94	3.23	2.97	2.96	3.39	2.55
Size range	82-86	82-86	82-90	82-86	82-86	80-86	82-86
Ho	0.317	0.247	0.231	0.327	0.398	0.412	0.327
He	0.310	0.281	0.277	0.357	0.402	0.442	0.348
HWE exact test	1.00	0.061	0.110	0.285	0.728	0.167	0.752

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	4	4	4	3	3	3	6
Allelic richness	3.84	3.15	3.32	2.99	3.00	2.95	5.59
Size range	80-86	80-86	80-86	82-86	82-86	82-86	84-94
Ho	0.375	0.367	0.409	0.490	0.563	0.372	0.702
He	0.427	0.350	0.436	0.495	0.478	0.380	0.636
HWE exact test	0.396	0.495	0.185	1.000	0.485	0.740	0.582

Locus Pl167

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	11	13	21	16	18	17	15
Allelic richness	9.21	8.94	12.46	10.65	12.03	11.08	12.92
Size range	168-208	168-210	168-232	168-232	164-224	166-220	168-210
Ho	0.740	0.831	0.824	0.769	0.821	0.748	0.836
He	0.721	0.786	0.870	0.831	0.834	0.783	0.830
HWE exact test	0.551	0.149	0.304	0.026	0.638	0.077	0.130

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	15	16	14	14	9	10	33
Allelic richness	12.20	12.25	10.81	11.31	8.93	7.08	29.15
Size range	168-224	168-224	168-224	168-208	168-208	168-226	132-228
Ho	0.854	0.785	0.798	0.714	0.781	0.654	1.000
He	0.788	0.811	0.814	0.761	0.792	0.660	0.965
HWE exact test	0.995	0.347	0.532	0.725	0.934	0.105	0.984

1 Appendix A continued

2

Locus StPf1005

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	5	5	4	5	4	5	4
Allelic richness	4.25	4.30	3.28	3.58	3.26	3.34	3.55
Size range	99-117	99-117	97-115	99-117	99-119	99-115	99-115
Ho	0.692	0.667	0.546	0.660	0.588	0.597	0.745
He	0.687	0.684	0.598	0.599	0.605	0.632	0.606
HWE exact test	0.441	0.932	0.198	0.086	0.333	0.377	0.109

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	4	5	7	5	3	3	6
Allelic richness	3.63	3.62	4.36	4.56	3.00	2.77	6.00
Size range	99-115	99-115	97-117	97-115	99-115	99-115	101-111
Ho	0.604	0.546	0.545	0.714	0.667	0.231	0.612
He	0.619	0.622	0.536	0.678	0.615	0.219	0.802
HWE exact test	0.357	0.296	0.689	0.960	0.404	0.425	0.001

Locus StPf1022

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	19	19	17	17	18	19	14
Allelic richness	14.47	13.96	13.35	13.73	14.08	14.37	12.49
Size range	164-234	162-228	164-214	164-226	164-222	164-226	166-206
Ho	0.854	0.914	0.861	0.904	0.850	0.874	0.945
He	0.861	0.863	0.884	0.894	0.903	0.900	0.891
HWE exact test	0.222	0.058	0.565	0.981	0.111	0.884	0.016

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	15	18	18	17	10	11	19
Allelic richness	13.46	13.01	14.45	14.95	9.89	8.65	17.53
Size range	166-262	158-226	166-222	164-214	164-194	166-214	262-358
Ho	0.870	0.823	0.919	0.918	0.879	0.897	0.848
He	0.907	0.890	0.905	0.886	0.841	0.817	0.929
HWE exact test	0.225	0.168	0.386	0.874	0.478	0.824	0.240

1 Appendix A continued

2

Locus StPf1002

Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	6	6	5	7	8	9	6
Allelic richness	5.70	5.60	4.83	6.22	6.06	6.09	5.32
Size range	169-185	169-185	169-185	169-185	137-185	137-185	169-185
Ho	0.615	0.720	0.722	0.663	0.684	0.653	0.636
He	0.723	0.707	0.687	0.684	0.694	0.637	0.648
HWE exact test	0.230	0.463	0.893	0.559	0.248	0.824	0.385

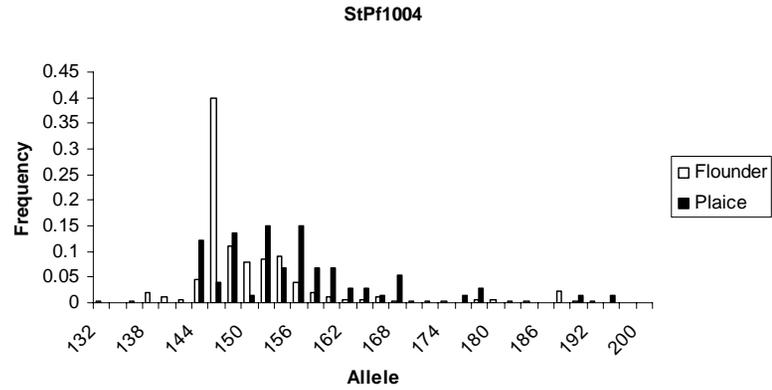
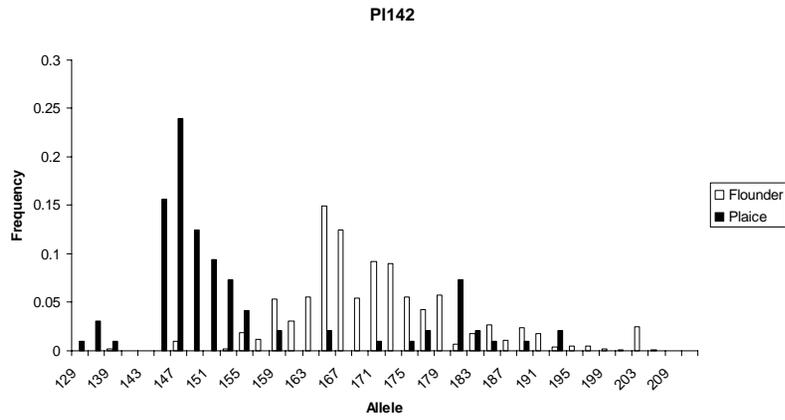
Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	6	8	8	6	4	4	6
Allelic richness	5.67	6.90	6.47	5.44	4.00	3.39	4.93
Size range	169-185	137-185	163-185	169-185	169-177	171-185	177-191
Ho	0.688	0.701	0.709	0.714	0.765	0.571	0.490
He	0.635	0.703	0.648	0.679	0.622	0.591	0.562
HWE exact test	0.419	0.118	0.905	0.598	0.594	0.902	0.060

Locus StPf1001

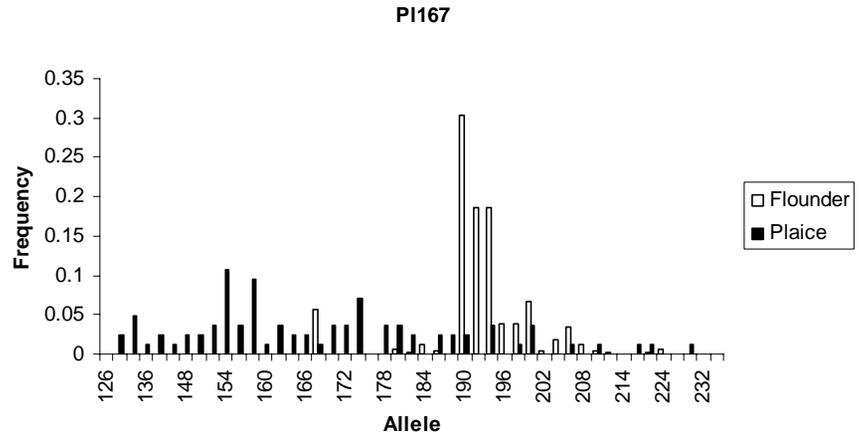
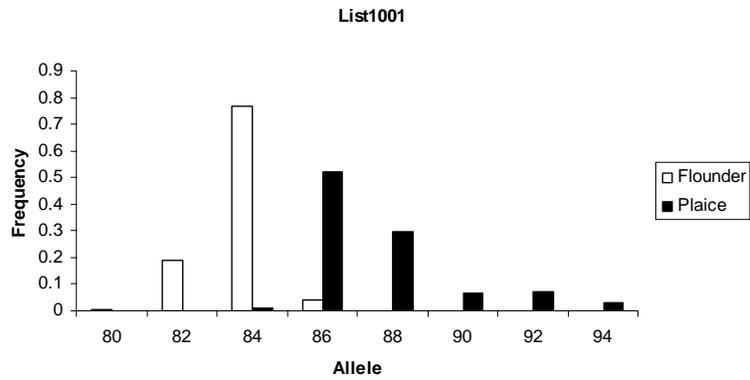
Population	Turku	Gotland	Bornholm	Ærø	Thyborøn	Ringkøbing	The Limfjord
Na	25	21	30	28	26	29	15
Allelic richness	14.87	13.51	15.82	16.63	17.40	18.15	12.84
Size range	233-343	233-345	233-339	221-337	233-345	233-349	233-291
Ho	0.894	0.800	0.710	0.745	0.895	0.871	0.808
He	0.871	0.810	0.787	0.795	0.886	0.895	0.821
HWE exact test	0.459	0.253	0.098	0.094	0.878	0.732	0.441

Population	Westerschelde	Irish Sea	Bay of Biscay	Trondheim	Pulmankijärvi	Faroe Islands	Plaice
Na	18	21	16	18	15	6	27
Allelic richness	15.49	13.90	11.62	15.49	13.90	5.99	24.17
Size range	233-337	233-295	233-297	233-337	243-301	231-291	209-291
Ho	0.780	0.745	0.805	0.898	0.735	0.740	0.898
He	0.856	0.799	0.801	0.890	0.717	0.813	0.960
HWE exact test	0.544	0.086	0.251	0.392	0.689	0.134	0.002

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2 Appendix B. Allele frequency distributions of plaice and pooled flounder samples.
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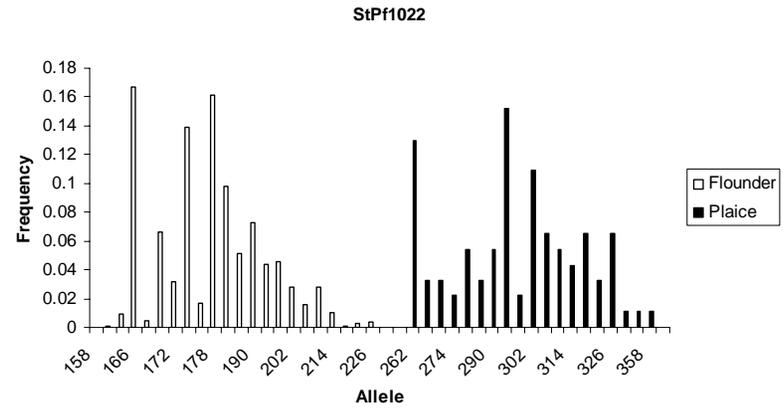
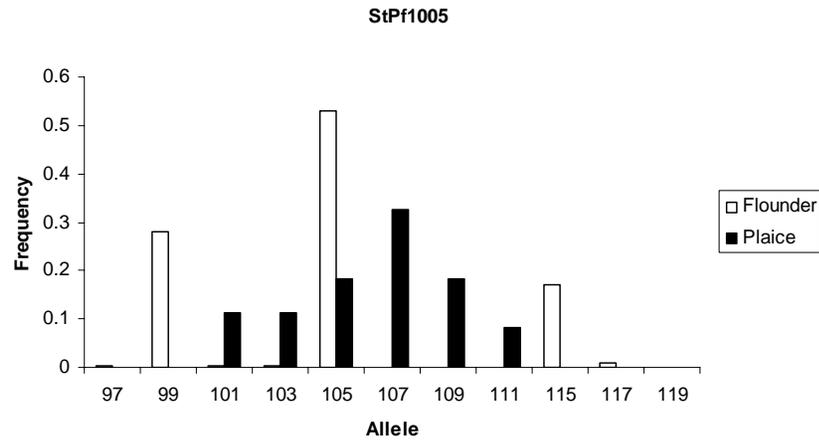


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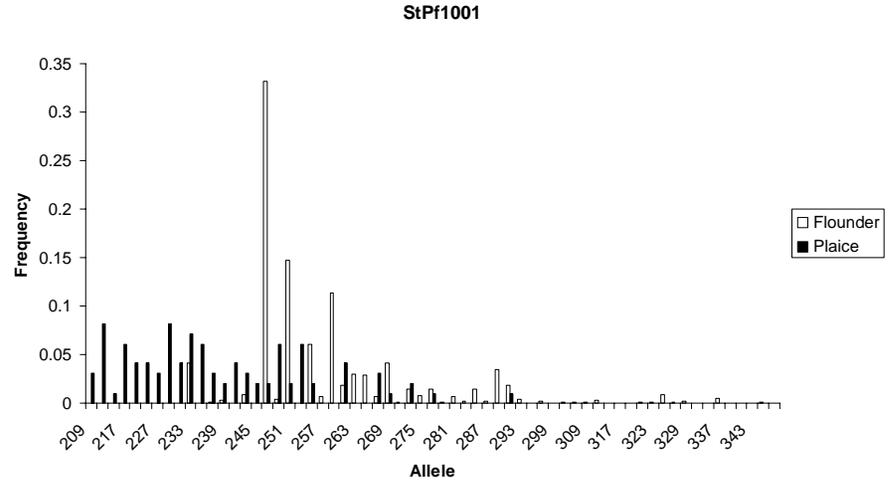
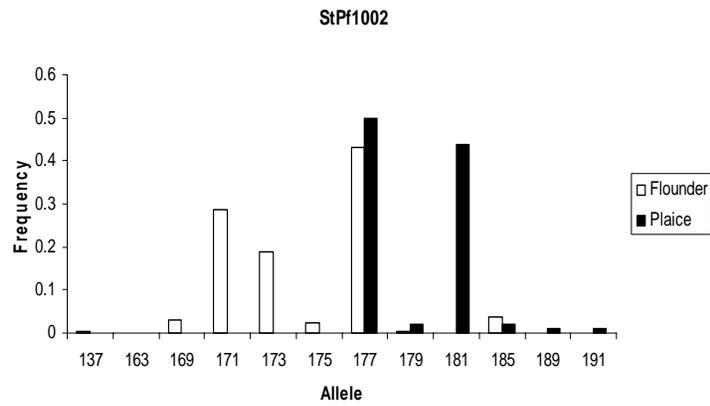


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Manuscript IV

Ecological and genetic interactions between flounder (*Platichthys flesus* L.) populations in the Baltic Sea

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In preparation

1 **Ecological and genetic interactions between flounder (*Platichthys flesus* L.)**
2 **populations in the Baltic Sea**

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1 **Introduction**

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3 The advent of highly variable genetic markers, such as microsatellites, has resulted in the
4 demonstration of highly significant genetic structuring among marine fish populations (e.g. Ruzzante et
5 al. 1999; Nielsen et al. 2001; Nielsen et al 2003; Knutsen et al. 2003; Bekkevold et al. 2005). These
6 findings have resulted in increased efforts to understand the evolutionary mechanisms responsible for
7 generating and maintaining significant genetic differences between populations in an environment
8 without any obvious barriers to gene flow. Hence, several physical forces, such as current systems and
9 habitat suitability have been found to be important for maintaining genetic structure in marine fishes
10 (e.g. Ruzzante et al. 1999; Riginos and Nachmann 2001; Taylor and Hellberg 2003). Furthermore,
11 environmental transitions have been highlighted as an important structuring force, potentially because
12 gene flow is limited between locally adapted populations (Nielsen et al. 2003; Nielsen et al. 2004;
13 Bekkevold et al. 2005).

14 Recently, the interaction between genetically differentiated populations has also been
15 investigated in more detail. For instance it has been found that populations of cod and turbot apparently
16 interact in hybrid zones in an area of sharp environmental transition between the marine North Sea and
17 the brackish Baltic Sea (Nielsen et al. 2003; Nielsen et al. 2004). These findings and the fact that many
18 other organisms show high levels of genetic structuring across the same environmental gradient
19 (Johanneson and André 2006) indicate a role for natural selection in maintaining these population
20 differences.

21 Another interesting case of interactions between genetically differentiated populations has been
22 described in Atlantic herring (Bekkevold et al. 2006; Ruzzante et al. 2006), where sympatric feeding
23 components utilize different spawning areas and spawn at different times of the year. The genetic
24 component of these life-history differences is largely unknown, but it has been shown that, at least in
25 some instances, the divergence between spawning populations are maintained despite intense mixing at
26 feeding grounds (Ruzzante et al. 2006), suggesting very complex population structure, intra-annual
27 migration patterns and interactions among genetically differentiated populations.

28 The European flounder is one of a few marine fishes also found in the innermost parts of the
29 Baltic Sea. As is the case for many other fishes in the Baltic Sea (Nissling and Westin 1997; Nissling et
30 al. 1999, Nissling et al. 2002; Ojaveer and Kalajs 2005), populations in the Western and central parts
31 have decreased egg shell thickness resulting in eggs being able to stay buoyant at lower salinities
32 compared to populations in the highly saline North Sea (Nissling et al. 2002). However, in the
33 innermost parts of the Baltic Sea, eggs are smaller and heavier. This physiological change is associated
34 with a change in spawning strategy to benthic spawning in coastal habitats, which are the only areas
35 where ambient oxygen levels allow survival of the eggs (Solemdal 1967; Nissling et al. 2002), and
36 hence this life-history change is regarded as an important adaptation to local environmental conditions
37 in the inner Baltic Sea. The distributions of the “pelagic” and “benthic” types of flounder overlap
38 around the island of Gotland in the central Baltic Sea. Tagging studies have shown that flounder
39 populations are relatively stationary in the area, but that seasonal migrations between spawning and
40 feeding areas do occur (Aro 1989).

41 We have previously identified highly significant genetic structuring of flounder populations
42 with different life-history strategies in the Baltic Sea (Manuscript in preparation). However, that study
43 also indicated temporal differences between samples from the island of Gotland. In this study, we
44 examine the spatial and temporal population structure of flounder populations in the Baltic Sea with a
45 view to investigate potential interactions among populations exhibiting very different life-history
46 strategies. We apply highly variable microsatellite markers, which are well suited to disclose minute

1 levels of genetic structuring, in combination with both population and individual level admixture
2 analyses to examine potential interaction among populations at a local scale.

3 4 **Materials and Methods**

5 6 *Sample information*

7 A total of 524 adult flounders from five locations along a transect from the North Sea to the inner
8 Baltic Sea were caught for genetic analyses (Figure 1). Most fish were sampled in the spawning season.
9 However, some samples were taken near the end of the spawning season (e.g. Thy04, Tur03 and
10 Got03) and were therefore not solely comprised of mature individuals. A single sample from Gotland
11 2004 was collected after the spawning season and consisted entirely of individuals which had already
12 spawned (Table 1). Samples of gill tissue were stored in ethanol prior to DNA extraction using Chelex
13 (Estoup *et al.* 1996), HotShot (Truett *et al.* 2000) or DNeasy (Qiagen) techniques.

14 15 *Molecular markers*

16 Nine microsatellite loci were employed for the genetic analyses. LIST1001 (Watts *et al.* 1999,
17 GenBank accession number: AF149831), PL142 and PL167 (Hoarau *et al.* 2002b, accession numbers:
18 AF406750 and AF406751), originally developed for plaice but worked well on flounder following
19 modifications of PCR conditions. StPf1001, StPf1002, StPf1004, StPf1005, StPf1015 and StPf1022
20 (Dixon *et al.* unpublished, accession numbers: AJ315970, AJ315975, AJ315973, AJ315974,
21 AJ538313, AJ538320) were all developed for European flounder. PCR was applied with standard
22 reagents and thermal cyclers and PCR products were analyzed on an ALFexpress (Amersham
23 Biosciences) automated sequencer following the manufacturer's recommendations. Standard size
24 ladders and individuals of known genotypes were run on each gel to minimize scoring error.

25 26 *Statistical analyses*

27 Exact tests for departure from Hardy-Weinberg equilibrium (Guo & Thompson 1992) were performed
28 per locus and sample in GENEPOP (Raymond & Rousset 1995). F_{ST} (Weir and Cockerham's (1984)
29 θ), their confidence intervals and significances were estimated using the program FSTAT (Goudet
30 1995), which was also used to estimate allelic richness (ElMousadik & Petit 1996). Temporal stability
31 was assessed by comparing temporal samples within localities. Population relationships were
32 visualized by a multidimensional scaling plot of Nei's D_A (1983) created in Vista 5.6.3. (Young 1996).
33 Furthermore, differences between years in the pairwise F_{ST} were examined by estimating pairwise F_{ST}
34 from Thyborøn to the Baltic Sea samples for the two years separately. We used the Bayesian model
35 based clustering algorithm implemented in the program STRUCTURE (Pritchard *et al.* 2000) to
36 estimate the most likely number of populations in the data set and in a sub-set consisting of samples
37 from Bornholm, Gotland and Turku. The program Admix1.0 (Bertorelle & Excoffier 1998) was used to
38 estimate population level admixture proportions according to Roberts and Hiorns (1965) in populations
39 of intermediate geographical position between the two most extreme populations (Thyborøn and
40 Turku), which were used as baselines. Subsequently, we used STRUCTURE to estimate individual
41 level admixture proportions in all samples using simulated Thyborøn and Turku samples (each of 1000
42 individuals) as fixed baselines. Simulated samples were generated by randomly drawing alleles
43 according to the estimated allele frequency distributions in the two samples in the program
44 HYBRIDLAB (Nielsen *et al.* 2006). Individual admixture proportions were subsequently compared by
45 Mann Whitney *U*-tests to examine spatial as well as temporal differences.

Results

Genetic variation

Genetic variation was high in all samples, expected levels of heterozygosity ranging from app. 0.3 at locus List1001 to between 0.8 and 0.9 in many of the other loci. The populations in the innermost parts of the Baltic Sea tended to have lower levels of genetic diversity as evidenced by lower levels of allelic richness at these localities (Appendix). No sample or locus deviated systematically from Hardy Weinberg expectations.

Population structure

The F_{ST} among all samples was 0.016 (95% CI: 0.008 – 0.025) and highly significant ($P < 0.0001$). The structuring of samples was also evident in the MDS plot (Figure 2) where samples grouped in two major aggregations according to their spawning strategy. Most of the variation (72%) was explained by dimension one along which samples were distributed largely corresponding to their geographical relationships. The geographically extreme Thyborøn and Turku samples were positioned near the ends of this axis with the remaining Baltic Sea populations in between. Along axis one, all temporal samples from the same locality were grouping together except for the Gotland samples which were relatively far apart. Dimension two explained 8 % of the variance.

No temporal samples from the same locality were significantly differentiated. Among the spatial samples, the largest differences were found in comparisons of samples in the two groups comprised of the different spawning strategies, although significant differentiation was also found between samples in the Western and central Baltic Sea and samples in the North Sea (Table 2). When estimating pairwise F_{ST} between Thyborøn and samples in the Baltic Sea, there was a large shift between Bornholm and Gotland, mirroring the picture from the MDS plot. However, this shift was less extreme in 2004, where the transition to the inner Baltic Sea sample at Turku appeared more gradual (Figure 3).

The clustering algorithm in STRUCTURE returned the highest posterior probability of two populations in the total data set. These two populations corresponded to the same structure identified in the MDS plot and in spatial estimates of population structuring, but with some introgression between samples in the two populations. Individuals in the Thyborøn and Turku samples were found to assign most strongly to the two populations identified by STRUCTURE. Hence temporal samples from these localities were pooled to represent baseline populations for calculations of admixture proportions in intermediate samples in the Baltic Sea. We also ran STRUCTURE using only samples from Bornholm, Gotland and Turku to examine potential population interactions at Gotland in more detail, but in this case, the most likely number of populations was one, reflecting decreased power of this approach at low levels of structure and a modest number of loci (Latch et al. 2006). Hence, we carried out all analyses with the original data set.

Population and individual admixture

Using pooled samples from Thyborøn and Turku as baselines for population admixture analyses, the intermediate samples were all admixed to some degree, the proportion from Turku in the samples increased from the Western to the central Baltic Sea samples (Table 3). Results were highly consistent for temporal samples from Ærø and Bornholm, while the Gotland samples showed a somewhat different pattern; here the 2003 sample appeared almost as non-admixed (i.e. pure Turku), while the

1 2004 sample had a significant Thyborøn proportion. The Gotland samples were the only samples where
2 standard deviations did not overlap between the temporal samples.

3 When estimating individual level admixture proportions, it was also evident that the samples
4 from Ærø and Bornholm were very similar, both within and between localities while the distribution of
5 individual admixture proportions at Gotland were significantly different between years (Table 4). At
6 Gotland the distribution of individual level admixture proportions were more sigmoid in 2004 than in
7 2003, reflecting an increased number of fish with extremely low Turku admixture proportions in 2004
8 (Figure 4).

9 10 **Discussion**

11
12 We found strong indications that the two temporal samples from Gotland deviated significantly, while
13 all other temporal samples were very similar. The analyses conducted indicate that the sample from
14 Gotland from 2004 contained a significant input of flounder genotypes from the Southern and/or
15 central Baltic Sea, while the sample from 2003 was very similar to Turku in the inner Baltic Sea. This
16 result is unlikely to be an artifact by sampling at different locations in the two years, since the samples
17 in 2004 were actually collected further to the North than the 2003 sample (see Figure 1). Hence, these
18 results indicate relatively strong temporal shifts at the Gotland locality, as evidenced by both
19 population level admixture and the distribution of individual level admixture proportions, despite the
20 fact that the two Gotland samples were not significantly differentiated when estimating population
21 divergence with Weir and Cockerham's θ .

22 These temporal differences could be explained either by differences between years, i.e. a
23 temporally unstable population structure, or by temporal changes within years, for instance caused by
24 migrating individuals. Interestingly, a recent study on Baltic Sea turbot (Florin and Höglund 2006) also
25 found temporal changes among Gotland samples, but otherwise found little evidence of population
26 structure in the Baltic Sea. The authors suggested that population structure is highly unstable and
27 largely dependent on the frequency of high saline inflows and the length of intervening stagnation
28 periods. Furthermore, they noted that the minor changes at Gotland could be caused by subtle temporal
29 differences in larval dispersal or different contribution of cohorts in the three years under study.
30 However, this study only had temporal replicates of samples from Gotland and hence it was not
31 possible to assess if the temporal shifts was a general phenomenon as suggested by the authors. In the
32 case of European flounder, temporal changes were only found at Gotland. If large scale movements,
33 e.g. following a major inflow in 2003, would have allowed "pelagic" populations to move further into
34 the Baltic Sea, we would have expected to see the shifts in the other samples as well. This was clearly
35 not the case, since all other temporal samples were very stable. Hence, while we cannot exclude the
36 possibility of a local environmental shift near Gotland, we believe that the results point to an alternative
37 explanation, i.e. variation within years for instance in caused by feeding migrations. This theory is
38 supported by the fact that the sample from Gotland in 2003 was the only one without mature or
39 spawning individuals. Since this sample was collected later in the year, it is plausible that it could have
40 contained individuals on feeding migrations. Tagging studies have shown that adult flounders do
41 perform annual migrations along the Swedish and Russian coasts (Aro 1989), a picture supporting the
42 proposed intra-annual movements seen in this study.

43 Results indicate that a significant part of the sample at Gotland in 2004 is of "pelagic" origin
44 (e.g. the individuals with very low Turku admixture proportions in Figure 4), which could suggest
45 mixing of flounders with different life-history strategies at feeding grounds. This was indeed suggested
46 by Solemdal (1973) who caught two flounders with physiological characteristics matching each of the

1 two flounder types near Stockholm in October. This locality is also near the proposed overlap of the
2 two flounder types and Solemdal suggested that the two types could mix during feeding seasons. We
3 attempted to model the distribution of simulated hybrid versus mechanically mixed Gotland
4 populations applying the method outlined in Nielsen et al. (2003). However, our power was not
5 sufficiently high (too few loci and relatively modest levels of differentiation) to statistically
6 differentiate between the expected distributions under the two scenarios, i.e. both mechanically mixed
7 and hybrid populations had similar distributions of individual admixture proportions. Hence, it was
8 difficult to determine with statistical certainty that the individuals with very low Turku admixture
9 proportion in our study truly were immigrants from a “pelagic” population further to the south, or
10 alternatively from the Gotland basin, which may also support some spawning of the “pelagic” type.
11 However, the mixing of different life-history forms is supported by the observations by Solemdal and
12 by tagging studies showing migrations in the directions proposed here (Aro 1989).

13 Different life-history strategies are well known among salmonids where differences in run-time
14 and migration patterns are believed to result in high levels of structuring among populations (e.g.
15 Taylor 1991). Among marine fishes intra specific life-history variation has been described in Atlantic
16 herring, where complex spawning components utilize different spawning areas and seasons (e.g.
17 Bekkevold et al. 2006; Ruzzante et al. 2006). Ruzzante et al. (2006) found a temporally stable pattern
18 of structuring of spawning components exhibiting different spawning behaviors, despite the seasonal
19 mixing of these components in feeding areas. On the other hand, comparable spawning groups have
20 been found to be both genetically homogenous and genetically structured across life-history breaks in
21 other areas (Bekkevold et al. 2006), suggesting very complex demographic and evolutionary scenarios
22 in Atlantic herring. While the degree of geographical overlap between the two life-history strategies in
23 European flounder is not known in detail, and the genetic component of the differences has never been
24 investigated, the genetic break observed in the inner Baltic Sea appears to be relatively sharp,
25 suggesting significant reductions in gene flow between the two forms.

26 If the results from the present study do indeed indicate seasonal mixing of genetically different
27 life-history forms, then there must be strong pre – or post-zygotic barriers to hybridization between the
28 two forms, since their distributions are apparently overlapping, at least at some times of the year. In the
29 present case, the most obvious barrier to gene flow is migration patterns and spawning habitat, since
30 the two types are apparently utilizing different spawning habitat. However, other mechanisms, such as
31 assortative mating (Carvalho et al. 2003), has also been described in flatfishes and could be operating
32 in order to prevent interbreeding. However, even if interbreeding should occur, a number of post-
33 zygotic mechanisms could reduce fitness of hybrids. For instance, interbreeding of locally adapted
34 populations has been shown to result in outbreeding depression and the subsequent loss of local
35 adaptations (Gharrett et al. 1999; Edmands 1999; Taylor et al. 2006). Such effects could easily be
36 imagined, for instance in relation to well described physiological adaptations, such as egg buoyancy
37 and sperm mobility (Solemdal 1967, 1973; Nissling et al. 2002).

38 However, while we cannot conclude that there is a mechanical mix of different life-history
39 forms in 2004, the results from this study most likely show a considerable seasonal mixing of fish from
40 different areas in the Baltic Sea. If population structure is truly stable as suggested by the temporal
41 stability of all remaining samples, this would indicate significant homing of adults to spawning
42 grounds, a also suggested in Atlantic herring (Ruzzante et al. 2006) and as seen from tagging studies
43 applying electronic data in, for instance, plaice (Hunter et al. 2003). These results are largely in
44 agreement with earlier tagging studies, which have also demonstrated seasonal movements of flounders
45 in the Baltic Sea (Aro 1989, Bagge and Steffensen 1989). However, these studies have concluded that
46 flounders in the Baltic Sea are relatively stationary, implicating that these movements are not

1 substantial. This does not seem to be the case in the present study, since the genetic shift observed
2 between years is substantial and seemingly involves many individuals.

3 In order to elucidate the sharpness of the genetic break between the two life-history forms in the
4 Baltic Sea, future studies should aim at a more detailed sampling scheme around the island of Gotland.
5 Preferably, samples from both coastal and deep waters should be collected in the spawning season to
6 examine fine scale differences at the time of spawning. In addition, temporal sampling throughout the
7 season could help considerably in establishing if the pattern of intra-annual movements suggested here,
8 is a general phenomenon. Future studies could also gain substantial power by combining information
9 from genetic markers with physical and physiological measures of e.g. egg buoyancy, egg shell
10 thickness and sperm mobility (Solemdal 1967, 1973; Nissling et al. 2002) on the individual level.

11 In conclusion, we believe that this study has demonstrated significant intra-annual movements
12 of flounder individuals and populations in the Baltic Sea. These results suggest that ecological and
13 genetic interactions between these populations may be quite considerable despite substantial life-
14 history differences between them.

References

- Aro E. 1989. A review of fish migration patterns in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer* 190: 72-96
- Bagge O, Steffensen E. 1989. Stock identification of demersal fish in the Baltic. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer* 190: 3-16.
- Bekkevold D, Andre C, Dahlgren TG *et al.* (2005) Environmental correlates of population differentiation in Atlantic herring. *Evolution*, **59**, 2656-2668.
- Bekkevold D, Clausen LAW, Mariani S *et al.* (2006) Divergent origins of sympatric herring population components determined using genetic mixture analysis. *Marine Ecology Progress Series*, in press.
- Bertorelle G, Excoffier L. 1998. Inferring admixture proportions from molecular data. *Molecular Biology and Evolution* 15: 1298–1311.
- Carvalho N, Afonso P, Santos RS. 2003. The harem mating system and mate choice in the wide-eyed flounder, *Bothus podas*. *Environmental Biology of Fishes* 66: 249-258.
- Edmands S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53: 1757-1768.
- El Mousadik A, Petit RJ. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics* 92: 832–839.
- Estoup A, Largiader CR, PerrotE, Chourrout D. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* 5: 295-298
- Florin A-B, Höglund J. 2006. Absence of population structure of turbot (*Psetta maxima*) in the Baltic Sea. *Molecular Ecology*, published online 25-09-2006.
- Gharrett AJ, Smoker WW, Reisenbichler RR, Taylor SG. 1999. Outbreeding depression in hybrids between odd-and even-broodyear pink salmon. *Aquaculture*, vol. 173; 117-129.
- Goudet J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* 86: 485-486.
- Hunter E, Metcalfe JD, Reynolds JD. 2003. Migration route and spawning area fidelity by North Sea plaice. *Proceedings of the Royal Society of London Series B* 270: 2097-2103
- Knutsen H, Jorde PE, Andre C, Stenseth NC. 2003. Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Molecular Ecology* 12: 385-394.

- 1 Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE. 2006. Relative performance of Bayesian
2 clustering software for inferring population substructure and individual assignment at low levels of
3 population differentiation. *Conservation Genetics* 7: 295–302.
4
- 5 Nei M, Tajima F, Tatano Y. 1983. Accuracy of estimated phylogenetic trees from molecular data.
6 *Journal of Molecular Evolution* 19: 153-170.
7
- 8 Nielsen EE, Bach LA, Kotlicki P. 2006. Hybridlab (version 1.0): a program for generating simulated
9 hybrids from population samples. *Molecular Ecology Notes*, published online 20-07-2006.
10
- 11 Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P. 2003. Evidence of a hybrid-zone in
12 Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture
13 analysis. *Molecular Ecology* 12: 1497-1508.
14
- 15 Nielsen EE, Hansen MM, Schmidt C, Meldrup D, Gronkjaer P. 2001. Fisheries - Population of origin
16 of Atlantic cod. *Nature* 413: 272.
17
- 18 Nielsen EE, Nielsen PH, Meldrup D, Hansen MM. 2004. Genetic population structure of turbot
19 (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the
20 transition zone between the Baltic Sea and the North Sea. *Molecular Ecology* 13: 585-595.
21
- 22 Nissling A, Westin L, Hjerne O. 2002. Reproductive success in relation to salinity for three flatfish
23 species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in
24 the brackish water Baltic Sea. *ICES J Mar Sci* 59: 93-108
25
- 26 Ojaveer E, Kalejs M. 2005. The impact of climate change on the adaptation of marine fish in the Baltic
27 Sea. *ICES Journal of Marine Science* 62: 1492-1500.
28
- 29 Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus
30 genotype data. *Genetics* 155: 945–959.
31
- 32 Raymond M, Rousset F. 1995. Genepop (Version-1.2) - Population-genetics software for exact tests
33 and ecumenicism. *Journal of Heredity* 86: 248-249.
34
- 35 Riginos C, Nachman MW. 2001. Population subdivision in marine environments: the contributions of
36 biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid
37 fish, *Axoclinus nigricaudus*. *Molecular Ecology* 10: 1439-1453
38
- 39 Roberts DF, Hiorns RW. 1965. Methods of analysis of the genetic composition of a hybrid population.
40 *Human Biology* 37: 38–43.
41
- 42 Ruzzante DE, Mariani S, Bekkevold D, Andre C, Mosegaard H, Clausen LAW, Dahlgren TG,
43 Hutchinson WF, Hatfield EMC, Torstensen E, Brigham J, Simmonds EJ, Laikre L, Larsson LC, Stet
44 RJM, Ryman N, Carvalho GR. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic
45 herring. *Proceedings of the Royal Society of London Series B* 273: 1459-1464.
46

- 1 Ruzzante DE, Taggart CT, Cook D. 1999. A review of the evidence for genetic structure of cod (*Gadus*
2 *morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and
3 the Gulf of St. Lawrence. *Fisheries Research* 43: 79-97.
4
- 5 Solemdal P. 1967. The effect of salinity on buoyancy, size and development of flounder eggs. *Sarsia*
6 29: 431-442
7
- 8 Solemdal P. 1973. Transfer of Baltic flatfish to a marine environment and long term effects on
9 reproduction. *Oikos* 15: 268-276
10
- 11 Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006. Speciation in
12 reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus*
13 *aculeatus*) species pair. *Molecular Ecology* 15: 343-355.
14
- 15 Taylor EB. 1991. A Review of Local Adaptation in Salmonidae, with Particular Reference to Pacific
16 and Atlantic Salmon. *Aquaculture* 98: 185-207.
17
- 18 Taylor MS, Hellberg ME. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean
19 reef fish. *Science* 299: 107-109.
20
- 21 Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman M. 2000. Preparation of PCR-
22 quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29: 52-
23 54.
24
- 25 Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population-structure.
26 *Evolution* 38: 1358-1370.
27
- 28 Young FW. 1996. ViSta: the visual statistics system. Research Memorandum 94-1(b) 2d. ed. L.L.
29 Thursone Psychometric Laboratory, University of North Carolina, Chaper Hill, NC.
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1 **Figure legends**

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Figure 1. Map of sample sites

Figure 2. MDS plot of Nei's D_A (1983)

Figure 3. Pairwise F_{ST} from Thyborøn in the North Sea to all Baltic Sea samples in 2003 (▲) and 2004 (■).

Figure 4. Individual Turku admixture proportions in the Gotland samples from 2003 (▲) and 2004 (■). Note that there are 46 individuals in Got03 while there are 48 in Got04. The two populations are presented together for comparative purposes

1 Table 1. Sampling locations. Sample names correspond to geographical presentation in Figure 1.
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Location	Sample name	Date	App. position	Sample size	Spawning strategy	Proportion maturing and mature
1. Turku 2003	Tur03	May	22° E, 60° N	54	Benthic	100
2. Turku 2004	Tur04	June	22° E, 60° N	50	Benthic	24
3. Gotland 2003	Got03	April	18.5° E, 57° N	46	Benthic	App. 50
4. Gotland 2004	Got04	June	19.5° E, 58° N	48	Benthic	0
5. Bornholm 2003	Bor03	March	16° E, 55.1° N	55	Pelagic	100
6. Bornholm 2004	Bor04	March	16° E, 55.1° N	53	Pelagic	100
7. Ærø 2003	Aer03	Feb-Mar	10° E, 55° N	52	Pelagic	100
8. Ærø 2004	Aer04	Mar	10° E, 55° N	52	Pelagic	100
9. Thyborøn 2003	Thy03	Feb	8° E, 57° N	55	Pelagic	100
10. Thyborøn 2004	Thy04	March	8° E, 57° N	59	Pelagic	19

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Table 2. Pairwise F_{ST} with 95% CI above diagonal and their associated P values for the permutation test for significance below diagonal.

	Turku 2003	Turku 2004	Gotland 2003	Gotland 2004	Bornholm 2003	Bornholm 2004
Turku 2003		-0.002 (-0.005 – 0.001)	-0.002 (-0.004 – 0.00)	0.003 (-0.003 – 0.008)	0.026 (0.015 – 0.038)	0.025 (0.008 – 0.046)
Turku 2004	0.93600		-0.002 (-0.005 – 0.001)	-0.002 (-0.004 – 0.009)	0.027 (0.015 – 0.041)	0.023 (0.005 – 0.048)
Gotland 2003	0.86550	0.88770		0.001 (-0.003 – 0.006)	0.027 (0.013 – 0.043)	0.023 (0.005 – 0.045)
Gotland 2004	0.40490	0.27740	0.5371		0.013 (0.005 – 0.022)	0.010 (0.001 – 0.020)
Bornholm 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001		0.001 (-0.003 – 0.003)
Bornholm 2004	< 0.0001	< 0.0001	< 0.0001	0.0002	0.4507	
Ærø 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1167	0.5081
Ærø 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6799	0.7795
Thyborøn 2003	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0055	0.0138
Thyborøn 2004	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0079	0.0348

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Table 2 continued

	Ærø 2003	Ærø 2004	Thyborøn 2003	Thyborøn 2004
Turku	0.035	0.035	0.039	0.037
2003	(0.019 – 0.052)	(0.014 – 0.059)	(0.020 – 0.059)	(0.015 – 0.064)
Turku	0.032	0.033	0.036	0.037
2004	(0.016 – 0.049)	(0.013 – 0.062)	(0.020 – 0.055)	(0.016 – 0.067)
Gotland	0.035	0.033	0.040	0.039
2003	(0.016 – 0.061)	(0.012 – 0.058)	(0.021 – 0.065)	(0.019 – 0.061)
Gotland	0.017	0.015	0.025	0.025
2004	(0.006 – 0.031)	(0.004 – 0.026)	(0.015 – 0.036)	(0.013 – 0.035)
Bornholm	0.001	0.001	0.006	0.007
2003	(-0.003 – 0.007)	(-0.004 – 0.006)	(0.001 – 0.013)	(-0.001 – 0.016)
Bornholm	0.001	-0.001	0.005	0.007
2004	(-0.003 – 0.004)	(-0.005 – 0.003)	(0.001 – 0.009)	(-0.001 – 0.014)
Ærø		0.002	0.003	0.003
2003		(-0.002 – 0.002)	(-0.002 – 0.008)	(-0.003 – 0.010)
Ærø	0.5783		-0.001	0.001
2004			(-0.005 – 0.004)	(-0.004 – 0.007)
Thyborøn	0.0201	0.7615		0.003
2003				(-0.001 – 0.008)
Thyborøn	0.0618	0.1652	0.0581	
2004				

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Table 3. Population level admixture proportions (Roberts and Hiorns 1965) with standard deviations.

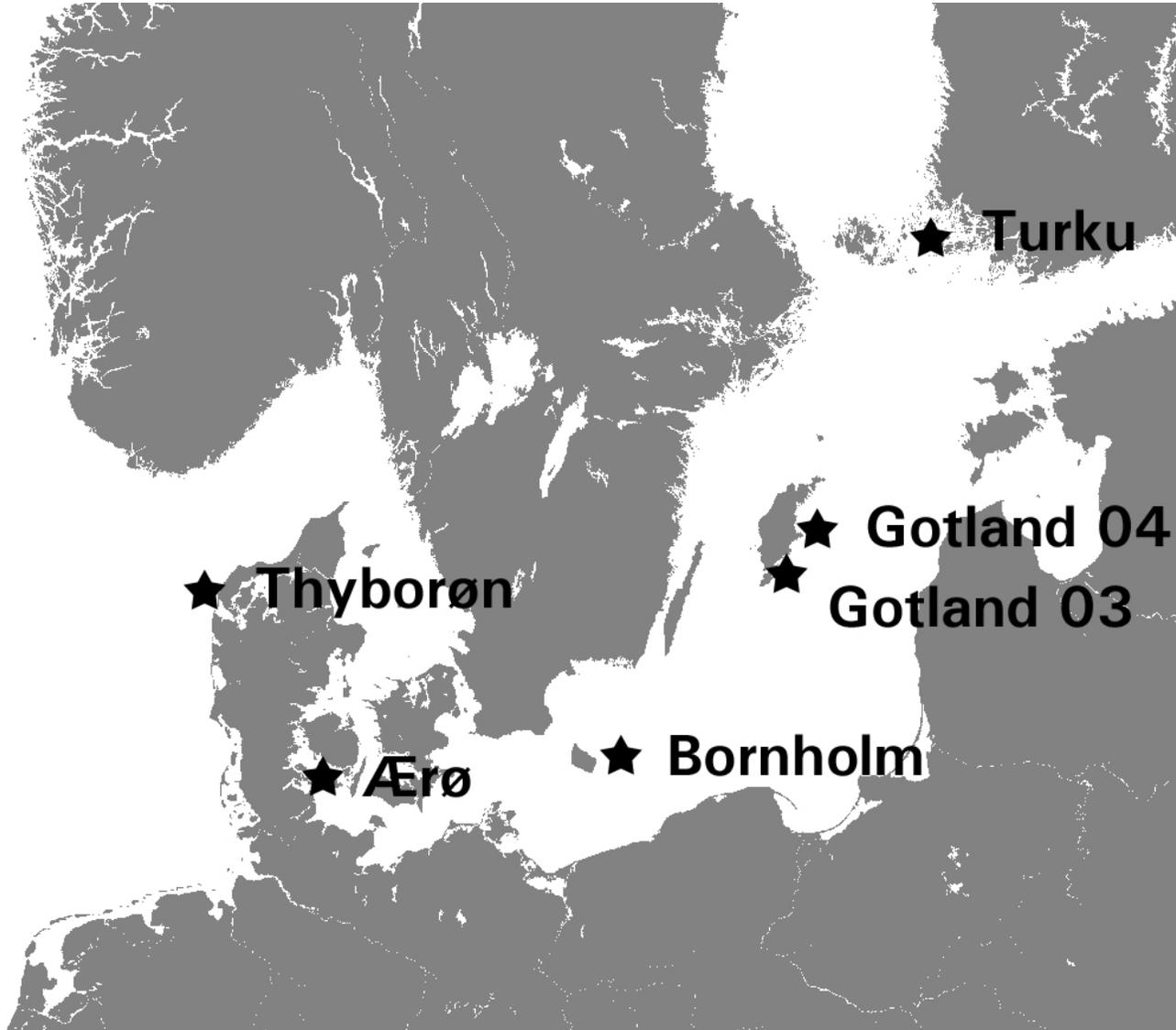
Population	Turku proportion
Gotland 2003	0.92 (0.06)
Gotland 2004	0.73 (0.07)
Bornholm 2003	0.27 (0.06)
Bornholm 2004	0.29 (0.06)
Ærø 2003	0.16 (0.06)
Ærø 2004	0.13 (0.06)

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Table 4. P values from pairwise Mann Whitney U-tests on individual admixture proportions. Simulated Thyborøn and Turku populations were used as fixed baselines.

	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Tur03										
Tur04	0.8249									
Got03	0.1019	0.0397								
Got04	0.0001	< 0.0001	0.0217							
Bor03	< 0.0001	< 0.0001	< 0.0001	< 0.0001						
Bor04	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.4085					
Aer03	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.9876	0.2962				
Aer04	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.2413	0.0344	0.1732			
Thy03	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0045		
Thy04	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0095	0.6583	

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Figure 1

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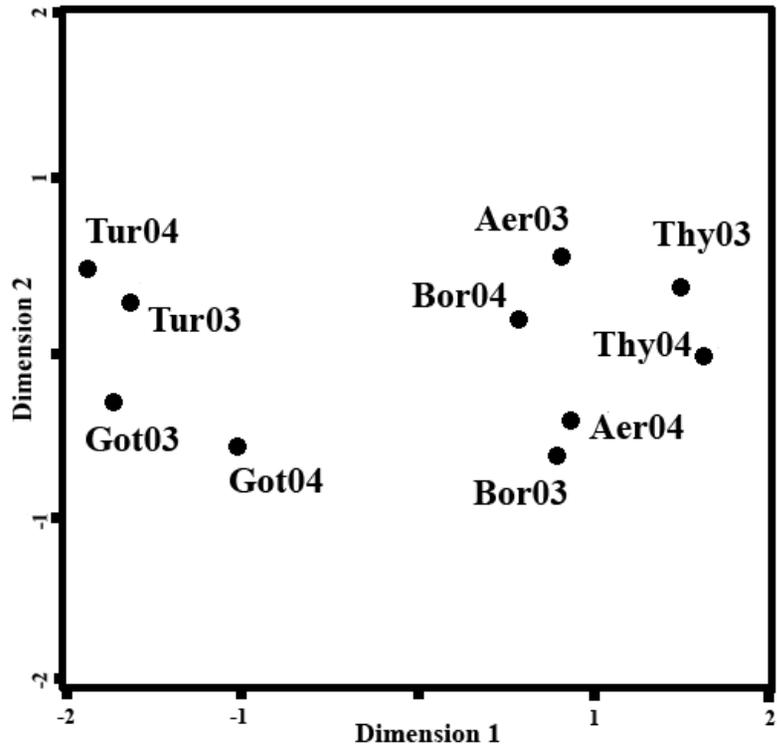
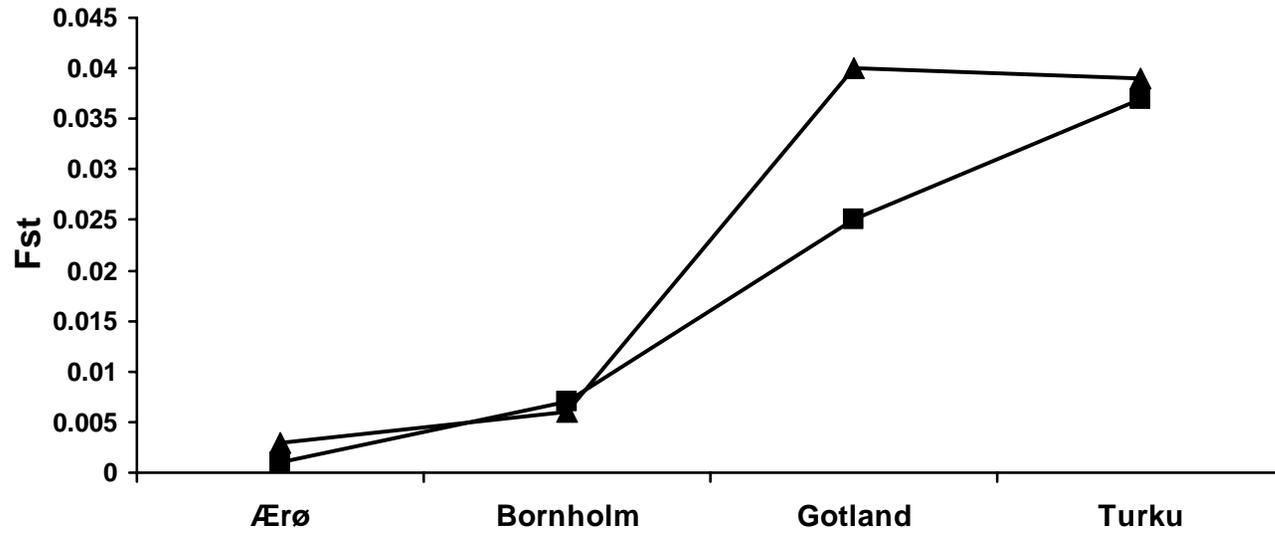


Figure 2

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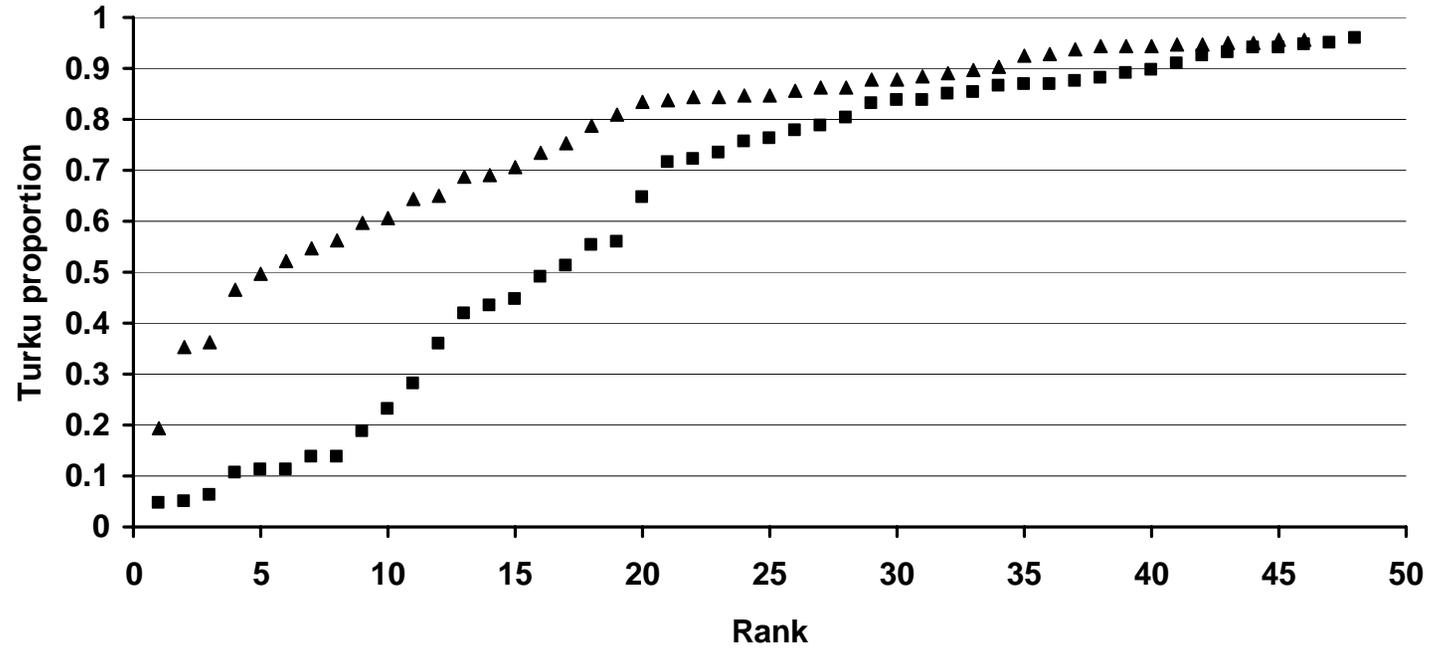
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Figure 3

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Appendix

Locus P1142

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	15.686	19.751	16.631	16.000	18.509	17.880	20.757	20.678	19.798	16.982
H _o	0.889	0.88	0.87	0.976	0.891	0.887	0.922	0.904	0.981	0.898
H _e	0.887	0.902	0.90	0.91	0.928	0.9	0.917	0.922	0.93	0.92
HWE exact test	0.8889	0.2869	0.0679	0.9536	0.5732	0.9172	0.8956	0.5374	0.6545	0.4657

Locus StPf1004

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	10.668	13.033	9.900	13.447	10.236	14.504	16.552	15.919	15.446	15.762
H _o	0.778	0.8	0.891	0.872	0.618	0.774	0.68	0.712	0.855	0.763
H _e	0.779	0.841	0.816	0.833	0.728	0.831	0.782	0.832	0.835	0.744
HWE exact test	0.6729	0.7464	0.2666	0.8538	0.0065	0.3215	0.0161	0.4156	0.9989	0.3504

Locus List1001

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	2.998	2.997	3.000	3.000	2.999	3.791	3.000	2.999	2.998	3.000
H _o	0.352	0.28	0.267	0.229	0.236	0.226	0.327	0.327	0.37	0.424
H _e	0.316	0.307	0.257	0.306	0.232	0.323	0.401	0.313	0.363	0.438
HWE exact test	0.4109	0.6001	0.3879	0.0511	0.6117	0.0215	0.103	1	1	0.9111

Locus P1167

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	10.321	10.512	9.733	9.864	15.052	13.085	12.189	11.217	13.510	12.583
H _o	0.778	0.7	0.778	0.886	0.873	0.774	0.769	0.769	0.8	0.842
H _e	0.743	0.701	0.762	0.809	0.878	0.858	0.853	0.805	0.822	0.843
HWE exact test	0.4276	0.5452	0.6861	0.3017	0.0245	0.0668	0.356	0.006	0.0542	0.8788

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1 Appendix continued

Locus StPf1005

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	4.777	4.000	4.000	4.894	3.000	3.792	3.824	3.808	3.764	3.000
H _o	0.648	0.74	0.696	0.638	0.473	0.623	0.647	0.673	0.564	0.61
H _e	0.677	0.696	0.669	0.702	0.574	0.625	0.599	0.605	0.595	0.613
HWE exact test	0.6678	0.1754	0.8897	0.5982	0.2255	0.2496	0.328	0.1442	0.3509	0.6084

Locus StPf1022

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	17.782	14.735	15.637	15.540	14.349	14.582	15.808	15.303	14.225	15.980
H _o	0.887	0.82	0.957	0.872	0.836	0.887	0.865	0.942	0.833	0.864
H _e	0.857	0.865	0.872	0.857	0.886	0.879	0.898	0.894	0.886	0.907
HWE exact test	0.7917	0.259	0.195	0.3594	0.2045	0.5106	0.8132	0.8102	0.1927	0.0604

Locus StPf1015

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	4.990	4.000	4.933	5.861	5.976	5.784	5.789	4.999	4.763	5.976
H _o	0.463	0.44	0.4	0.5	0.509	0.434	0.549	0.423	0.4	0.508
H _e	0.494	0.459	0.376	0.541	0.503	0.369	0.498	0.462	0.447	0.52
HWE exact test	0.1843	0.2973	0.3983	0.5335	0.7495	0.5101	0.8486	0.2007	0.4342	0.8214

Locus StPf1002

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	5.988	5.836	5.933	5.875	4.988	4.984	6.893	5.964	6.527	6.624
H _o	0.648	0.58	0.756	0.688	0.764	0.679	0.692	0.635	0.691	0.678
H _e	0.734	0.716	0.683	0.723	0.705	0.674	0.669	0.702	0.679	0.71
HWE exact test	0.4337	0.2849	0.6218	0.3893	0.8935	0.9335	0.4622	0.2667	0.6042	0.5992

Locus StPf1001

Population	Tur03	Tur04	Got03	Got04	Bor03	Bor04	Aer03	Aer04	Thy03	Thy04
Allelic richness	17.200	15.873	14.906	16.127	18.567	18.583	18.730	22.147	20.037	19.352
H _o	0.907	0.88	0.884	0.723	0.704	0.717	0.74	0.75	0.918	0.875
H _e	0.879	0.865	0.848	0.771	0.801	0.774	0.789	0.807	0.876	0.895
HWE exact test	0.8737	0.183	0.4166	0.0869	0.0828	0.1621	0.0899	0.2888	0.9725	0.8688

Summary

This thesis consists of five main parts; a general introduction to knowledge and future perspectives for studies of population structure and local adaptations in marine fishes, and four manuscripts presenting the major findings from my PhD.

The first manuscript describes the analyses of population structure in European flounder (*Platichthys flesus*) on both large and local geographical scales. Using microsatellite genetic markers, we found high levels of genetic structuring between different flounder populations. Importantly, these differences apparently had very different causes, some being driven by changes in life-history characteristics, while others were more likely to be associated with physical oceanographic forces or gradual environmental changes. This study thus adds to our understanding of how evolutionary forces may interact in the sea to structure species of marine fishes into discrete and reproductively isolated units.

The second manuscript focuses specifically on adaptive population divergence. By applying a candidate gene approach we found strong indications of adaptive population divergence of flounder populations despite seemingly high levels of gene flow between populations. This approach has not previously been applied in marine fishes, and the results indicate that adaptive divergence and local adaptations are indeed possible in the high gene flow marine environment. A result which is encouraging for future studies of local adaptations in marine fishes, of which we know very little.

In the third manuscript, we are investigating potential genetic introgression from plaice (*Pleuronectes platessa*) to flounder populations in different parts of the distributional area of the flounder. We found strong indications of significant introgression throughout the areas where the two species have overlapping distributions. However, levels of introgression were low, indicating relatively strong selection against hybridization between the two species. Hence, in contrast to other studies which have demonstrated high levels of introgression in hybrid zones between other species, there seems to be considerable selective constraints on plaice-flounder hybridization. This could in turn allow the two species to remain genetically separate despite apparently sharing distributional and spawning areas.

The fourth manuscript examines the stability of population structure on a local scale in the Baltic Sea. Specifically, we are investigating interactions between populations with different life-history characteristics; some populations are spawning pelagic eggs, while others are spawning benthic eggs. We found relatively strong temporal shifts at the same locality between samples collected in two different years and at different times of the year (one close to time of spawning, the other in the feeding season). Hence, the results indicate substantial intra-annual movements and potential mechanical mixing at feeding grounds of individuals from populations exhibiting very different life-history characteristics. These findings thus add to our understanding of how genetically differentiated populations may interact genetically and ecologically.