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Review

A Darwinian Laboratory of Multiple Contact Zones

Kerstin Johannesson ^{1,*}, Alan Le Moan,^{1,2} Samuel Perini,¹ and Carl André¹

Barriers to gene flow between divergent populations result in contact (hybrid) zones. Locations where multiple contact zones overlap can be used in comparative studies asking: what mechanisms maintain barriers; what is the origin of the genetic variation involved; and do differences in life history affect the nature of barriers? In a review of 23 marine species' genetic divergence over a postglacial salinity gradient, many showed steep genetic clines supported by divergent selection and/or temporal or spatial segregation. Contacts were primary or secondary and shaped by ancestral variation sometimes involving inversions. The dispersal potential of species seemed less important in shaping clines. Studies of multi-species contact zones will increase our understanding of speciation, but we need to address the taxonomic bias and focus more on postzygotic isolation.

Contact Zones Are Speciation Laboratories

Contact zones (see [Glossary](#)) are laboratories for studies of the nature of barriers to gene flow between divergent populations or taxa [1–3]. Genome-wide sequencing and genetic maps have powered the identification of candidates for **barrier loci** and investigations of the **genomic landscape** shaping divergence and speciation [4]. Divergence is usually considered to evolve either *in situ* as a result of different selection regimes acting on either side of the contact (primary contact) or after secondary contact of populations that have already accumulated differences by selection and/or drift [5–8] ([Box 1](#)). In both types of contact, identification of the origin and mechanisms of the genetic barriers are central to our understanding of the evolution and maintenance of the divergence [4,7,9]. For example, what are the roles of divergent ecological selection, assortative mating (whether due to spatial, temporal, or behavioural isolation), and postzygotic isolation mechanisms caused by intrinsic genetic incompatibilities or extrinsic selection against hybrids?

Studies of single contact zones generate important details of mechanisms involved but give less indication of their quantitative importance. Moreover, single-species studies cannot inform us about the role of traits that are invariable within a species (e.g., many life-history traits, large genomic traits). Studies of **multispecies contact zones**, where the contacts of different species overlap under the same external conditions, allow assessments of the general importance of different patterns of origin, ancestral variation, and **genomic architectures** in barrier formation. They also enable us to investigate how life-history traits such as dispersal, generation time, and reproductive biology contribute to gene-flow barriers, and the role of prezygotic and postzygotic isolation mechanisms. Multispecies contact zones (sometimes referred to as '**suture zones**') are present in both terrestrial [10,11] and marine [5,12–17] environments. Compiling recent data from a marine multispecies contact zone at the entrance of the Baltic Sea, we here highlight questions that a comparative approach of such zones can address, to support an increased understanding of the mechanisms involved in divergence and speciation.

Highlights

Barriers to gene flow are best studied where divergent populations are in contact, and studies of single-taxon hybrid zones have generated important knowledge about the nature of reproductive barriers.

Marine environments, earlier considered to host unstructured species due to high connectivity, offer multispecies contact zones structured by simple physical gradients (e.g., salinity) ideal for comparative studies of divergence and speciation.

Overlapping contact zones offer possibilities for comparison of barriers among species of various taxa, life histories, and demographic backgrounds and to test the role of species-specific traits in the formation and function of barriers.

Combining genome scans and demographic modelling, barrier regions in the genome can be located and barrier origin traced. With genetic maps, inversions that affect recombination rate (and hence gene flow) can be identified.

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The Baltic Sea Multispecies Contact Zone

The **transition zone** between the marine North Sea (North-East Atlantic) and the brackish Baltic Sea is shaped by a salinity gradient with its steepest part in the Danish Straits (Figure 1, Key Figure). Importantly, low salinity imposes strong physiological stress on most marine organisms and so this gradient impacts heavily on the distribution and adaptation of marine species in this area. After its formation 8000 years ago by the opening of a postglacial freshwater lake into the Atlantic, the Baltic Sea was subsequently colonised by a subset of the marine species living in the North Sea. A few of these established populations along the entire environmental gradient while the majority colonised only the outer part of the gradient [18]. Many of the marine species established in the Baltic Sea show strong plasticity in phenotypic traits [19–22]; in addition, early genetic studies had already shown examples of strong genetic clines over the salinity gradient [23,24].

We scanned published studies describing genetic variation in species present over the North Sea–Baltic Sea transition and retrieved useful data for 23 species. These species represent a wide range of taxa (15 fish, five invertebrates, two macroalgae, and one microalga) and a wide range of life histories (Table 1). With only one exception, the recently invaded bay barnacle (*Balanus improvisus*) [25], all species show genetic differentiation over the transition zone. In most species, the separation is between a Baltic Sea and a North Sea population, while in European flounder (*Platichthys* spp.) there is an additional subdivision inside the Baltic Sea (Figure 1). Importantly, all species form contact zones between divergent populations. Original descriptions indicated that 16 of 22 contact zones roughly overlapped (Figure 1), and after fitting clines to the species with enough data (14 species) this shows that the overlap coincides with the steepest part of the salinity gradient, although some clines are slightly shifted towards lower salinities (Figure 2). In 12 species, the available data allowed a more formal analysis of the presence of genetic clines. This test supports stepped or segmented clines in ten species, while in the isopod *Idotea baltica* and the Baltic clam [*Limicola (Macoma) balthica*] divergence tends to be linear rather than stepped (Table 1).

A Taxonomic Bias

We found genetic data for only two macroalgae (*Fucus vesiculosus* and *Saccharina latissima*) and one diatom species (*Skeletonema marinoi*) and none of them were genome-wide data. Nevertheless, all show divergence over the salinity ecotone (Table 1), with the contact zone of *Saccharina* shifted 300 km outside the steepest part of the transition. In both macroalgae, divergence is weak

Box 1. Primary and Secondary Contact

A contact zone originates in one of two ways: (i) secondary contact is formed between two populations that have diverged by genetic drift or selection during a period of isolation prior to contact; whereas (ii) primary contact is formed *in situ* when a population expands across an environmental transition under divergent selection and gene flow. Genomic patterns resulting from hybridisation will initially be very different between secondary and primary contact zones [7], and one such difference is the haplotype structure. Secondary contact will produce an introgression pattern characterised by successively smaller ‘pieces’ of the original background haplotypes with increasing distance from the contact [87]. This is because repeated recombination during backcrossing breaks apart large introgressed pieces of genomic material (Figure 1, ‘young’ secondary contact). Primary contact, by contrast, will emerge from a common background and hybridisation will occur between haplotypes sharing a common origin. In both secondary and primary zones, ancestral genetic variation (green and brown loci/alleles in Figure 1) are likely to play a key role: in secondary zones, ancient adaptive differences will remain important for divergence after contact, while in primary zones ancient polymorphisms can segregate under divergent selection. Over time, new variation (dark blue and red in Figure 1), from new mutations or from outside the zones, will establish under selection and contribute to divergence and barriers. Secondary contact can initially form wherever the two earlier isolated populations happen to meet. However, both neutral and selected genetic clines that form at first contact will move until they become trapped in a density trough and/or at a steep environmental shift [1,5]. Primary hybrid zones will evolve at steep environmental shifts. Positions of secondary and primary zones might overlap sometime after establishment [7], modelling the demographic history using nonadmixed populations outside the contact zone can, however, help to resolve their origin. Also, primary zones might have components of secondary origin; for instance, if primary contact zones form from standing genetic variation that evolved during earlier periods of isolation [85].

Glossary

Barrier loci: loci involved in reproductive isolation and in reducing gene flow between populations.

Chromosomal arrangement: here used for the two chromosome sequences – the ancestral and the derived – with mirrored orders of nucleotides that are the result of a chromosomal inversion.

Contact zone: a geographic area where two divergent populations or closely related species overlap in distribution and potentially hybridise; the term ‘contact zone’ is more appropriate than ‘hybrid zone’ unless it is clear that hybrids are produced.

Demographic inference/modelling: approaches that use DNA sequence information to test historical scenarios of population expansions and bottlenecks, isolation, and gene flow, and which can suggest the timing of various population events.

Genomic landscape/architecture: how genes and other functional elements of the genome are physically arranged on the chromosomes.

Haplotype block: a part of the genome that is inherited together without being broken up by recombination.

Introgression: incorporation of genetic material from one species or population into the genome of another species or population by hybridisation followed by repeated backcrossing.

Multispecies contact zone: a geographic area where several species form overlapping contact zones (see also ‘suture zone’)

Reciprocal transplant: translocation of individuals between two divergent populations living in different habitats to assess how much of their phenotypic difference is induced by the environment and how much is inherited difference. Transplant experiments can be done in the wild, but often (for practical reasons) they are conducted in the laboratory, where the two environments are simulated.

Suture zone: a boundary area where two separate organism assemblages meet. In biology a suture zone is similar to a multispecies contact zone, but in geology it has a completely different meaning and is therefore avoided here.

Transition zone: in the setting of this study, used to describe the geographic area over which the environmental gradient (here salinity) changes.

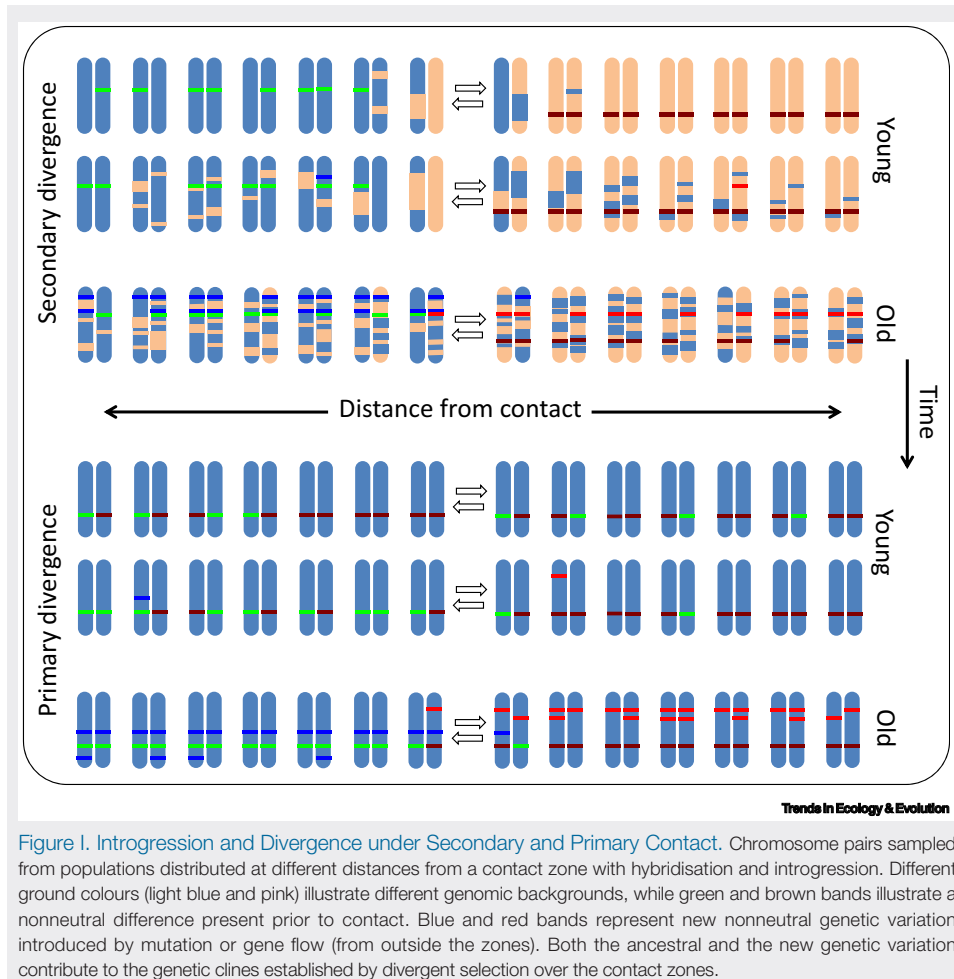


Figure 1. Introgression and Divergence under Secondary and Primary Contact. Chromosome pairs sampled from populations distributed at different distances from a contact zone with hybridisation and introgression. Different ground colours (light blue and pink) illustrate different genomic backgrounds, while green and brown bands illustrate a nonneutral difference present prior to contact. Blue and red bands represent new nonneutral genetic variation introduced by mutation or gene flow (from outside the zones). Both the ancestral and the new genetic variation contribute to the genetic clines established by divergent selection over the contact zones.

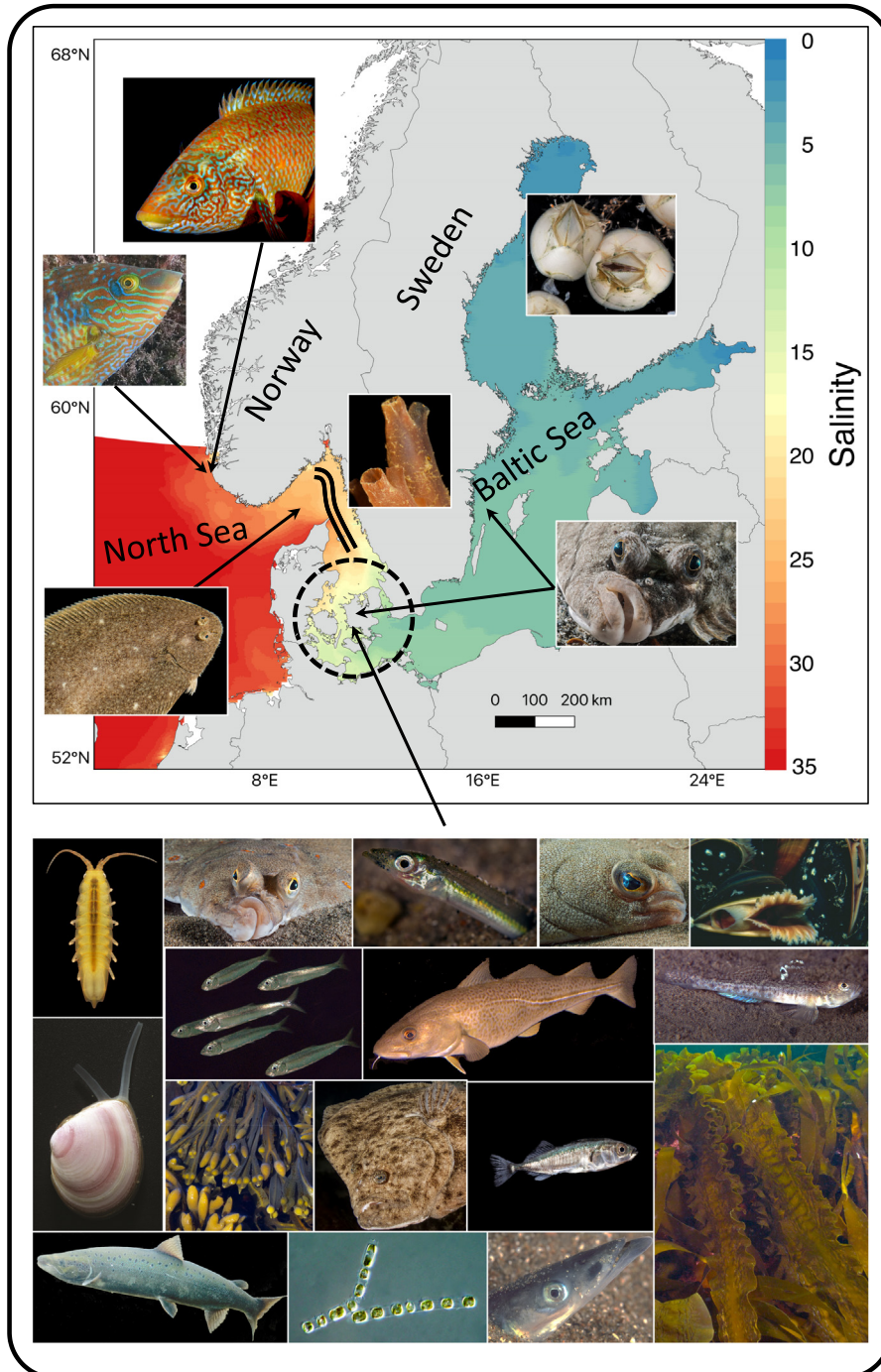
and might simply be the result of isolation by distance [26,27]. *Skeletonema* represents a pelagic microscopic species with an enormous population size (1 million cells per litre during spring blooms [28]) and a high potential for long-distance dispersal. Still, divergence over the salinity transition is clearly stepped in this species (Figure 2 and Table 1). Divergent selection, anchoring of local populations by a benthic resting stage, and oceanographic connectivity patterns have been suggested to contribute to the clinal genetic structure [29]. This unique example from a diatom species shows that clinal divergence over contact zones can also be informative in microorganisms, stressing the need to reduce the current taxonomic bias in future comparative studies.

Primary and Secondary Contacts

The origin of contact zones is of fundamental interest. With thousands of genetic markers, it is possible to reconstruct the divergence history of populations using **demographic inference**, in which one compares different scenarios of evolution ('models') with the observed patterns of divergence. From divergence under simple allopatry, primary, or secondary contact, these models have been increasingly tuned to represent more complex demographic scenarios involving past and/or present variation in effective population size [30,31]. In addition, these

Key Figure

The North Sea–Baltic Sea Multispecies Contact Zone



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(See figure legend at the bottom of the next page.)

models can account for the genomic heterogeneity involved in divergence and speciation; for example, the semipermeability of gene flow along the genome [32–34] or the interactions between recombination rate and selection at linked sites [8,30,35]. Integrating such model-based demographic inference under a comparative framework represents a way to understand the origin of contact zones, although the development of these models remains in its infancy and earlier conclusions might have to be revisited [31].

Phylogeographic studies of the Baltic clam (*L. balthica*) and the blue mussel (*Mytilus edulis/trossulus*) show that, following repeated invasions from the Pacific to the Atlantic, the Baltic Sea populations are the result of recent invasions while the lineages present in the North Sea have an old relationship with the Pacific ancestors [36,37]. By contrast, recent demographic inference suggests that the Baltic Sea population of Atlantic herring (*Clupea harengus*) was formed by a recent niche expansion from the eastern Atlantic, followed by divergence under gene flow [38]. Moreover, a study on five flatfish species (Pleuronectiformes) with similar adult migratory patterns and with passive drift of large numbers of eggs and larvae during several weeks found both primary and secondary contacts of the Baltic Sea populations based on demographic inferences using models that included genomic heterogeneity [39–41] and variation in effective population sizes [31]. This suggests that the origin of clines might be independent of dispersal capacity. That the majority of secondary clines overlap with the steepest part of the salinity gradient supports the **trapping hypothesis**; that is, clines in both neutral and selected alleles are trapped at environmental ecotones and density troughs [1,5]. Trapping is also illustrated by the position of the contact zones in ballan wrasse and corkwing wrasse (see below).

Studying multispecies contact zones offers a possibility to disentangle the separate and combined roles of primary and secondary divergence over many species. Using relatively young contact zones increases the power of these analyses since patterns of primary and secondary divergence converge over time in the presence of gene flow (Box 1).

The Nature of Barrier Mechanisms

Once contact zones are established, genetic isolation can be maintained by prezygotic and/or postzygotic mechanisms. Prezygotic isolation typically involves loci under divergent ecological selection and/or loci involved in mate segregation (spatial, temporal, or behavioural) resulting in assortative mating. Postzygotic isolation can be maintained through intrinsic genetic incompatibilities or through extrinsic causes of selection against hybrids.

Atlantic cod (*Gadus morhua*) has a complex pattern of genetic differentiation along several environmental axes, including both genetic differentiation established prior to the Baltic Sea contact

Figure 1. Approximate positions of species contact zones are indicated along the salinity gradient. The majority of contact zones overlap with the steepest part of the salinity gradient (broken circle). These species are shown in the bottom panel; from top left: isopod (*Idotea baltica*), European plaice (*Pleuronectes platessa*), small sandeel (*Ammodytes tobianus*), common dab (*Limanda limanda*), blue mussel (*Mytilus edulis/trossulus*), Atlantic herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*), sand goby (*Pomatoschistus minutus*), Baltic clam [*Limecola (Macoma) balthica*], bladder wrack (*Fucus vesiculosus*), turbot (*Scophthalmus maximus*), three-spined stickleback (*Gasterosteus aculeatus*), sugar kelp (*Saccharina latissima*), Atlantic salmon (*Salmo salar*), diatom (*Skeletonema marinoi*), and greater sandeel (*Hyperoplus lanceolatus*). Corkwing wrasse (*Symphodus melops*), ballan wrasse (*Labrus bergyllta*), and common sole (*Solea solea*) contact zones are shifted 300–500 km outside the main zone. European flounder has two contact zones, one overlapping with the main zone and one shifted 300 km inside the Baltic Sea (the latter is the contact between the pelagic *Platichthys flesus* and the demersal *Platichthys solemdali*). A shallow-water and a deep-water population of the vase tunicate (*Ciona intestinalis*) form a vertical contact zone along the Swedish west coast (parallel lines). Finally, no contact zone is evident in the invasive bay barnacle (*Balanus improvisus*, top right) distributed over almost the complete salinity gradient. Photograph credits: Jan Drent, Ann-Margrethe Iseklint, Bo Johannesson, Lars-Ove Loo, Paul Naylor, Fredrik Plejelij, Anders Salesjö, Josefin Seftom, and Mark Thomas.

Table 1. Species with Available Genetic Data from Transect Samples Covering the North Sea–Baltic Sea Transition

Species	Common name	Dispersal potential ^a	Genetic data ^b	F_{ST} over zone	Outlier distribution	Selection agency	Fitted cline model ^c	Type of zone	Inferred from	Barrier strength; mechanism ^d	Refs
<i>Gadus morhua</i>	Atlantic cod	High	1.2 million	0.040	Three large inversions	Salinity, temperature	Too few data	Secondary	Phylogeny	Strong; separate spawning times, local adaptation	[42–48,69]
<i>Clupea harengus</i>	Atlantic herring	High	6 million	0.030	Haplotype blocks, one large inversion	Salinity, temperature	Stepped or segmented cline	Primary	Demography	Weak; local adaptation?	[38,56,90]
<i>Platichthys flesus</i>	European flounder	High	5472	0.005	Few and scattered	Salinity, temperature	Segmented cline	Secondary	Demography	Weak; local adaptation?	[39,73]
<i>P. flesus/Platichthys solendali</i>	Baltic flounder	Low	2051	0.025		Salinity	Too few data	Secondary	Demography	Strong; separate spawning habitats, local adaptation	[51,52]
<i>Scophthalmus maximus</i>	Turbot	High	3348	0.012	Many scattered	Salinity	Stepped cline	Primary	Demography	Moderate; unknown	[31]
<i>Pleuronectes platessa</i>	European plaice	High	6885	0.013	Two inversions	Salinity	Too few data	Primary	Demography	Weak; unknown	[39]
<i>Limanda limanda</i>	Common dab	High	3468	0.008	Clustered	Salinity	Stepped cline	Secondary	Demography	Weak; unknown	[39]
<i>Solea solea</i>	Common sole	High	3714	0.003	Very few		Too few data	Primary	Demography	Very weak; unknown	[40,41]
<i>Ammodytes tobianus</i>	Small sandeel	High	4039	0.041		Salinity, temperature	Segmented cline			Strong; separate spawning times?	[91,92]
<i>Hyperoplus lanceolatus</i>	Greater sandeel	High	4328	0.039		Salinity, temperature	Stepped cline			Strong; separate spawning times?	[92,93]
<i>Symphodus melops</i>	Corkwing wrasse	Low	50 130	0.120	Only two spurious outliers		Stepped or segmented cline	Secondary	Demography	Strong; density trough	[49]

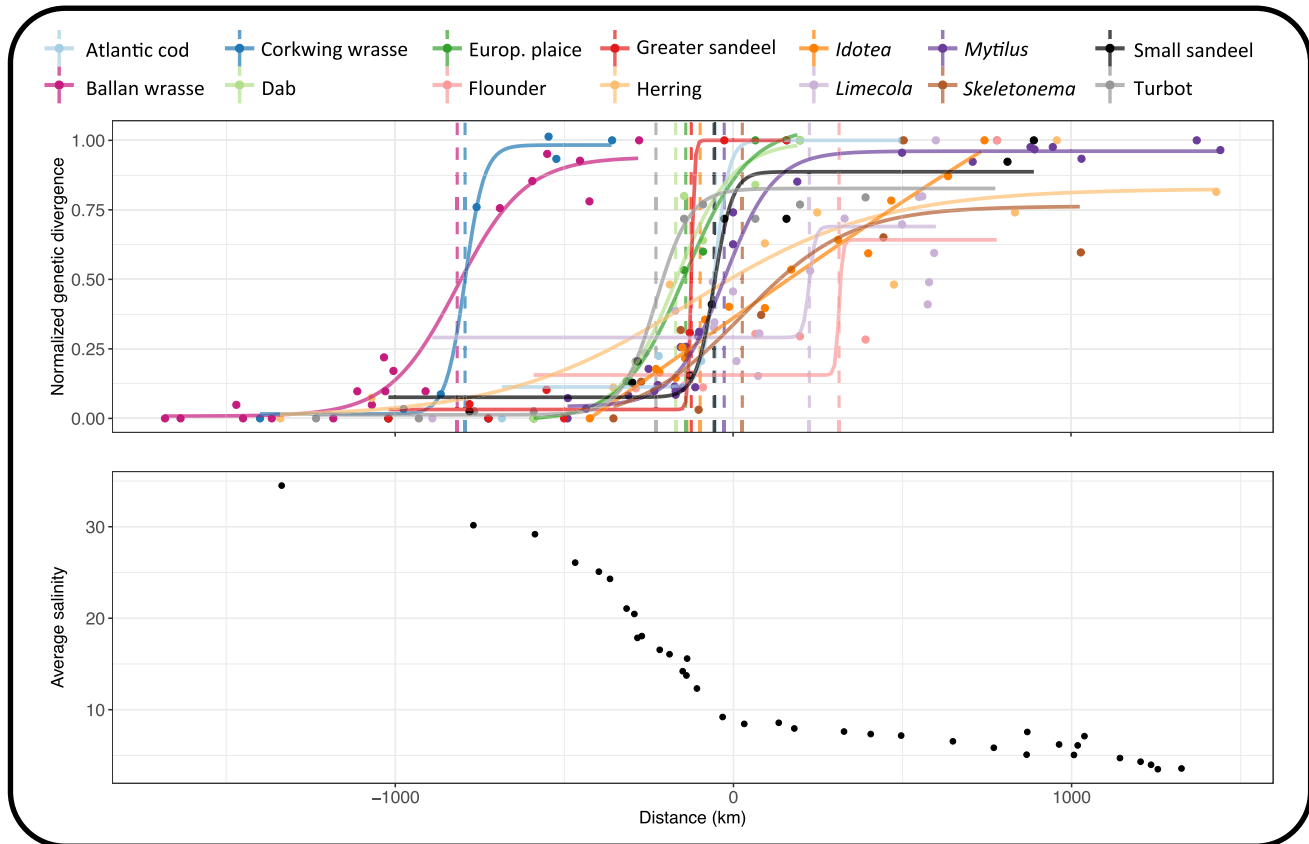
<i>Labrus bergyllia</i>	Balkan wrasse	Low	82	0.027				Stepped cline				Moderate; density trough	[50]
<i>Pomatoschistus minutus</i>	Sandgoby	Low	22 190	0.020	Distributed	Salinity, turbidity		Too few data	Secondary	Demography		Moderate; local adaptation	[94,95]
<i>Gasterosteus aculeatus</i>	Three-spined stickleback	Low	30 000	0.015	Enriched in regions	Salinity, temperature		Too few data	Primary	Demography		Moderate; local adaptation	[67,96]
<i>Salmo salar</i>	Atlantic salmon	High	5034	0.132				Too few data	Secondary	Demography		Strong; separate spawning areas	[8,97]
<i>Ciona intestinalis</i>	Vase tunicate	Low	1653	0.180		Salinity		Vertical cline (no test)	Secondary	Demography		Strong; partly separate spawning times, local adaptation	[53,55]
<i>Idotea baltica</i>	'Isopod'	Low	33 774	0.024		Salinity		Linear cline				Weak; unknown	[98]
<i>Balanus improvisus</i>	Bay barnacle	High	mtDNA, four microsatellites	0.011	No outliers			No cline				No barriers	[25,99]
<i>Limecola (Macoma) balthica</i>	Baltic clam	High	84 AFLPs	0.100		Salinity		Linear cline (but see [75])	Secondary	Phylogeny		Weak; unknown	[100]
<i>Mytilus edulis/trossulus</i>	Blue mussel	High	60	0.344		Salinity		Stepped cline	Secondary	Phylogeny		Weak; local adaptation	[66,101]
<i>Fucus vesiculosus</i>	Bladder wrack	Low	Five microsatellites	0.115		Salinity		Too few data				Weak; local adaptation	[22,26, 102–104]
<i>Saccharina latissima</i>	Sugar kelp	Low	Twelve microsatellites	0.120		Salinity		Too few data				Unknown	[27]
<i>Skeletonema marinoi</i>	'Diatom'	High	Eight microsatellites	0.080		Salinity		Segmented cline				Medium; local adaptation	[29]

^a'High' denotes several week-long pelagic larvae and/or highly mobile adults giving the species a potential for high gene flow. 'Low' denotes a short pelagic larval (or zygote/spore) stage and sessile or less mobile adults.

^bFigure denotes number of SNPs if not specified.

^cThe change in genetic divergence over the salinity gradient was analysed for each taxon separately using three different models, which were then compared with the Akaike information criterion (AIC): a linear model, a stepped cline model [84], and a regression model with segmented relationships (R package 'segmented' [105]), compatible with isolation by distance, stepped clines shaped by one contact, and complex clines shaped by more than one contact, respectively.

^dSuggested mechanisms that are not experimentally confirmed are followed by '?'. Note that other mechanisms, not yet investigated, might add to barriers.



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Figure 2. Position and Shape of Genetic Clines in 14 Species. Top panel: Dots represent normalised pairwise F_{ST} estimates between the outermost population and all other populations of the same species, plotted against waterway distance from the entrance of the Baltic Sea. To illustrate cline shapes and positions, clines [88] are fitted to the data (unbroken lines), and cline centres are also indicated (broken vertical lines); see Table 1 for tests of cline fits. We omitted species without any contact (*Balanus improvisus*), species with vertical contact (*Ciona intestinalis*), the second contact of European flounder (*Platichthys flesus/solemdali*), five species with poorly resolved geographic data [*Fucus vesiculosus*, *Saccharina latissima*, common sole (*Solea solea*), sandgoby (*Pomatoschistus minutus*), and three-spined stickleback (*Gasterosteus aculeatus*)], and Atlantic salmon (*Salmo salar*), where we lacked F_{ST} data. Bottom panel: Sea-surface (yearly average) salinity plotted over the same geographic interval.

and differentiation evolved *in situ* [5,42,43]. A major genetic divide is between the eastern Baltic Sea population and all other populations, with a genetic shift of allele frequencies overlapping with the steepest part of the salinity gradient (Figure 2). Today, gene flow over this contact zone seems very restricted, as first-generation hybrids are rarely found [23,44]. Critical traits contributing to barriers, such as spawning season and egg buoyancy, differ between populations on either side of the contact zone due to divergent selection [45]. Additional genetic divergences are established among stationary fjord populations outside the Baltic Sea, migratory North Sea cod populations, and stationary eastern North Sea populations, with behavioural barriers contributing to isolation [46,47]. As discussed further below, at least three large chromosomal inversions that are under selection play various and important roles in this divergence [41,46,48].

Current data suggest that barriers to gene flow involve prezygotic mechanisms, such as spatial or temporal segregation (six of the 22 contact zones), and local adaptation resulting from divergent ecological selection (ten of the 22 zones) (Table 1). However, in several species there is no information on the mechanisms involved. In addition, it is most likely that gene flow is impeded by

more than one isolation mechanism in many of the contact zones. The lack of evidence for postzygotic mechanisms is most likely due to the absence of studies targeting such mechanisms.

While the majority of the Baltic Sea contact zones are associated with the centre of the transition, a few displaced zones shed further light on the details of the barrier mechanisms involved. For example, corkwing wrasse (*Symphodus melops*), a small fish confined to coastal seaweed habitats, forms a sharp genetic cline that is strongly shifted outside the major contact (Figure 2). The divergence is genome wide but only few loci are outliers and under divergent selection [49]. Demographic analysis shows that bottlenecks and divergence prior to contact 5500 years ago shaped this contact zone, which is today trapped in a hostile habitat (a 60-km sandy-beach area) at the margin of the transition (Figure 1). This example clearly illustrates the importance of ancestral divergence and the trapping of a secondary contact zone at a density trough. Notably, population-genetic data show the presence of a contact zone in a taxonomically close and ecologically very similar species, ballan wrasse (*Labrus bergylta*), overlapping with the corkwing wrasse contact [50] (Figure 2).

In European flounder, a second contact zone appears inside the Baltic Sea (Figure 1) separating pelagic spawning and benthic spawning populations [51,52]. Demographic analysis suggests that divergence started during a very recent period of isolation after the formation of the Baltic Sea, forming a stronger barrier to gene flow than over the contact at the steep salinity shift (Table 1). The inner contact is associated with low salinity where pelagic eggs are no longer buoyant [52]. This is an example where selection on a critical trait (egg buoyancy) strongly impacts the strength and position of the contact zone.

The vase tunicate (*Ciona intestinalis*) appears with two genetically divergent populations separated in their vertical distributions over the transition; one is confined to the high-salinity water below 20 m outside the entrance of the Baltic Sea and the other is present in the brackish surface water flowing out from the Baltic Sea [53,54]. **Demographic modelling** and heterozygote deficits suggest secondary contact with limited gene flow [55]. Still, populations are interfertile in laboratory crosses (J. Hudson, personal communication) and show high larval plasticity in different salinities following **reciprocal transplants** [21]. This contact zone supports the importance of the salinity gradient, as the genetic divergence in this species coincide with the halocline (the salinity change). Both the physical barrier to dispersal of larvae over the halocline and divergent selection between shallow and deep water might contribute to impede gene flow. Furthermore, the reproductive periods of the two populations only partly overlap [53].

The coupling between the variation in the environment and the distribution of the contact zones can help us understand the nature of the genetic barriers. In the North Sea–Baltic Sea transition, it is obvious that salinity is a strong forcing factor, causing both horizontal and vertical genetic clines. However, as illustrated by the two species of wrasse, a few contacts appear less affected by divergent ecological selection related to the salinity gradient while being instead trapped by density troughs in environments that are hostile for these specific species.

Genomic Landscapes of Divergence

Recent progress in understanding the evolution of diverged lineages comes from mapping the distribution of divergent genomic regions to positions on the genome. Although this is as yet limited to a handful of fish species in the North Sea–Baltic Sea transition, a diversity of genomic landscapes emerges, from divergent regions distributed across the entire genome (e.g., Baltic populations of cod, turbot, herring, and demersal flounder) to divergence restricted to a few genomic regions or loci (e.g., dab, plaice, pelagic flounder) [39,41,42,51,56]. Thus, multispecies contact zones like in

the Baltic Sea cover a wide range of stages of divergence along the speciation continuum in a wide range of taxa, and studies of these zones can provide us with a broader understanding of the genetic mechanisms involved in barrier formation and speciation. In the few examples from fish species here, these differences do not appear to be linked to major differences in dispersal (a key life-history trait) as all of these fish species have highly mobile adult stages and have long-lived pelagic larvae. More likely, these variable patterns are, at least in part, explained by different demographic histories and by the presence of chromosomal rearrangements.

Chromosomal inversions that reduce recombination and establish large ‘supergenes’ might contribute important barriers in contact zones in general. Inversions play a pivotal role in divergence in Atlantic cod, European plaice, and Atlantic herring and possibly also in demersal flounder (*P. Momigliano*, personal communication). In cod, an inversion on chromosome 2 includes loci important in osmoregulation and one of the two **chromosomal arrangements** is overrepresented in the Baltic Sea [42,47]. In plaice, most of the Baltic Sea differentiation is clustered on two putative inversions, which appear to be significantly enriched in immune-related genes [57]. In herring, most of the differences are concentrated in regions of reduced recombination, including **haplotype blocks** and in four large inversions [38,56,58].

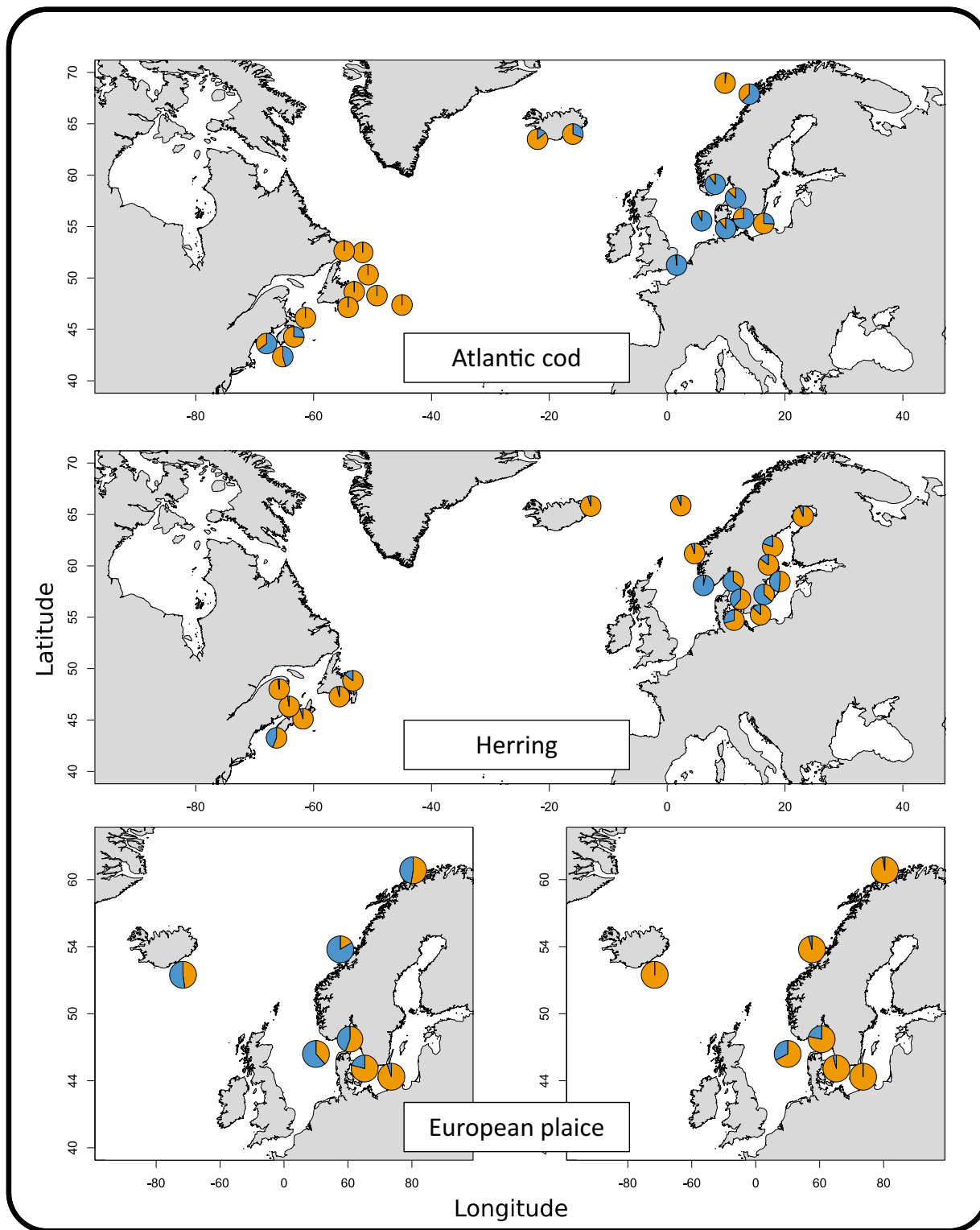
From these few examples, it seems as if large chromosomal rearrangements, such as inversions, are important components of divergence over contact zones. The coupling of loci that constitute barriers to gene flow provided by the inversion is an efficient way of reducing gene flow between populations in contact [9,59–61]. Multispecies contact zones allow quantitative assessment of the role of inversions and other large chromosomal rearrangements in barrier formation and of the relationship between recombination and the pattern of differentiation along the genome.

Contribution of Ancestral Variation

Inversions that contribute to species divergence and local adaptation tend to be old [62]. In three of the fish clines over the North Sea–Baltic Sea transition, the chromosomal arrangements now present in the Baltic Sea are older than the Baltic Sea [47,56,57] and also present outside the Baltic Sea (Figure 3). As inversions are typically polymorphic, they constitute important parts of the standing genetic variation of populations [9,62]. Consequently, arrangements containing alleles that promote local adaptation to a specific environment can rapidly increase in frequency and promote the formation of local genetic clines over similar environmental transitions, as observed in Atlantic herring and Atlantic cod [47,56,58].

The cases of secondary divergence also provide obvious evidence for the contribution of ancestral variation. For instance, the Baltic Sea populations of the blue mussel and the Baltic clam with recent origins in the Pacific were already separated from the east Atlantic coast populations millions of years ago [63–65]. However, the blue mussel adaptation to brackish water conditions in the Baltic Sea by *M. trossulus* seems to be recent and by primary divergence, since in eastern Canada it is the other lineage (*M. edulis*) that has established in brackish water conditions [66].

As illustrated with the Baltic Sea data, individual phylogenetic and demographic analyses can be used to address the origin of contact zone divergences, and studies of multiple overlapping contacts will support the identification of extrinsic mechanisms potentially affecting whole communities of species.



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The Role of Selection

Multiple contact zones trapped by the same environmental gradient show that divergent selection strengthens barriers in many of the contacts, including those formed by secondary contact. Over the North Sea–Baltic Sea transition, there is also evidence from reciprocal transplant experiments and field studies that migrants crossing a contact zone perform worse than native individuals [22,27,47,66–68]. In addition, experiments suggest that the salinity gradient, sometimes in combination with a parallel temperature gradient, is a major selective force (Table 1 and references therein). Atlantic cod was one of the first species where a sharp genetic cline in haemoglobin was observed over the transition zone [23] and this was later attributed to detailed selection mechanisms at the molecular level ([69,70] and see [71]). On the analysis of candidate genes, other molecular functions have been suggested to be associated with local adaptation in cod, such as heat-shock proteins [72]. Proteins from the same family have also been found as candidates for selection along the North Sea–Baltic Sea transition in other fish species [57,73,74]. Similarly, the same single-base mutation in the rhodopsin gene is found repeatedly in populations of several fish species with vision adapted to red-shifted waters as, for example, in the Baltic Sea [75]. Such convergent evolution lends strong support to selection and provides general knowledge about the role of a specific biological function in a given environment. Furthermore, a multispecies contact zone appears to be an ideal framework to study convergent evolution by providing replicates of evolution under shared environmental conditions. For instance, several species converge to increased asexual reproduction inside the Baltic Sea [12], possibly resulting from selection favouring uniparental reproduction during colonisation of a new habitat [76]. Moving forwards in our understanding of the role of natural selection, the integration of whole-genome sequencing into comparative frameworks in multispecies contact zones would allow us to characterise convergent evolution occurring at levels from SNPs to biological functions.

A Combinatorial View on Divergence

Hybridisation followed by **introgression** can be central to the evolution of diverging populations. Although hybridisation and introgression mostly contribute genetic homogenisation, or break up locally adapted gene clusters [77], rare introgression between diverging lineages can provide new genetic material for the evolution of local adaptation [3,78]. There is evidence from demographic modelling that historic introgression has played a key role in the evolution of some of the Baltic Sea lineages; for example, *Mytilus* and *Limecola* [37,79,80]. These introgressions have increased the standing genetic variation of both species and probably promoted local adaptation to the marginal Baltic Sea environment [81]. It is also suggested that introgression between divergent lineages has supported the formation of the brackish water population of *C. intestinalis* [55]. In addition, introgression from the demersal flounder formed inside the Baltic Sea possibly supported the adaptation of the pelagic flounder to brackish-water conditions [52].

Introducing new genetic variation by hybridisation and introgression over contact zones is probably a much more efficient way to promote rapid adaptation than waiting for new mutations when there is a shortage of available genetic variation [78]. Such a combinatorial view provides an alternative to the classical primary versus secondary divergence dichotomy.

Dispersal Potential and Width of Selected Clines

Dispersal can be critical in shaping genetic clines. At equilibrium, the width of allele frequency clines over a contact zone with gene flow is expected to be related to the balance between

Figure 3. Distribution of Inversions in Atlantic Cod, Atlantic Herring, and European Plaice. Pie charts indicate the frequencies of the two chromosomal arrangements of the inversion on chromosome 2 in Atlantic cod (*Gadus morhua*) [47,89], the inversion on chromosome 12 in Atlantic herring (*Clupea harengus*) [56], and the two inversions on chromosomes 19 and 21 in European plaice (*Pleuronectes platessa*) [57]. There are no data for plaice on the American side.

dispersal and the strength of divergent selection following $w \propto \sqrt{(\sigma^2/s)}$, where w is the cline width defined as $1/\text{maximum slope}$, σ is the per-generation dispersal, and s is the strength of selection [1,82,83]. Marine organisms typically have either high or low dispersal potential. Accordingly, under the bold assumption that the magnitude of divergent selection is roughly similar among species, we expect wider genetic clines in species with high dispersal potential than in those with very limited dispersal (Table 1). However, this is not what we observe. For example, some species with high dispersal potential (herring and the diatom) have wide clines, while others (blue mussel, cod, plaice, dab, and the greater and small sandeel) have narrow clines (Figure 2). What seems to be an uncoupling between cline shape and dispersal can have various explanations, such as differences in the strength of divergent selection, the demographic history, or the genomic architecture [7]. In addition, if clines have not reached equilibrium, we expect that clines formed from secondary contact initially have steep slopes that will successively erode under gene flow until they reach a steady state [7]. Primary clines, by contrast, build successively, with alleles with large effects establishing narrow clines faster than alleles with smaller effects [7,84].

Marine multispecies contact zones have a strong potential for comparisons of the effects of dispersal potential on barrier formation, but we need many more data, including data on barrier strength, hybrid formation, and hybrid fitness. Furthermore, comparative studies including species that vary in other life-history traits will offer possibilities to relate the shape of genetic barriers to variation in traits such as generation time and reproductive potential.

Concluding Remarks and Future Perspectives

To understand the nature of barriers to gene flow between diverging taxa, we need studies of contact zones. Using multispecies contact zones, we can address new questions and derive more general answers to old ones, such as: how is divergence affected by the type and origin of the barriers; and what are the roles of ancestral genetic variation, the genomic architecture, and life-history traits? While young multispecies contact zones contain clear footprints of demographic and divergence histories prior to contact, older contacts converge with respect to history but more clearly express patterns of selection, introgression, and gene flow. For both, we need the power of the new genetic tools to compare the barrier effects of primary and secondary divergence and their interactions. Furthermore, we need to compare the effects of different chromosomal architectures, demographic histories, and life histories among species. Over the North Sea–Baltic Sea transition, divergence in many of the species is shaped by a combination of processes strongly linked to evolution at different temporal scales [85], leading to the conclusion that the primary–secondary divergence dichotomy might be less relevant. To fully explore the complexity of this and other multispecies contact zones (see Outstanding Questions), high genomic coverage and geographic reference outside the contact zone are needed. We also need data that are less taxonomically biased and include more information on dispersal, locally adapted traits (not least associated with reproduction), and hybrid fitness. Thus, experimental ecological approaches are necessary and complementary to the current boom of genomic studies. Marine contact zones seem ideal for broad taxonomic comparisons combined with oceanographic modelling of dispersal patterns [86]. Finally, the incorporation of empirical data into theoretical models that explore the mechanisms of the evolution of reproductive barriers will further increase our understanding of divergence and speciation.

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Outstanding Questions

What are the barriers to gene flow over contact zones? Are barriers the result of the coupling of a large number of traits or of divergence in a few large-effect loci? The poor link between general divergence (e.g., F_{ST}) and the strength of barriers (e.g., the absence or presence of hybrids) over the North Sea–Baltic Sea transition is puzzling.

What is the role of dispersal potential in contact zone evolution? Our expectation that more long-range dispersal would result in wider genetic clines was not observed over the North Sea–Baltic Sea transition.

How are contact zone mechanisms related to life-history traits (other than dispersal)? For example, extending the comparisons to include species with much shorter generation times (such as microalgae) and with different reproductive strategies (sexual vs asexual) would be desirable.

How do primary and secondary processes interact in population divergence over contact zones and is the relative importance of the two processes linked to species traits, to the environmental and historical context, or to a combination of the two?

How important are chromosomal inversions for the evolution of barriers over contact zones? Old inversion polymorphisms seem to contribute key elements of divergence in some species over the North Sea–Baltic Sea transition zone. However, the general importance of inversion polymorphisms in the formation of barriers over contact zones cannot be judged from a few examples; we need data from additional species.

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