

INVITED REVIEW

Local adaptation and species segregation in two mussel (*Mytilus edulis* × *Mytilus trossulus*) hybrid zones

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Abstract

Few marine hybrid zones have been studied extensively, the major exception being the hybrid zone between the mussels *Mytilus edulis* and *Mytilus galloprovincialis* in southwestern Europe. Here, we focus on two less studied hybrid zones that also involve *Mytilus* spp.; *Mytilus edulis* and *Mytilus trossulus* are sympatric and hybridize on both western and eastern coasts of the Atlantic Ocean. We review the dynamics of hybridization in these two hybrid zones and evaluate the role of local adaptation for maintaining species boundaries. In Scandinavia, hybridization and gene introgression is so extensive that no individuals with pure *M. trossulus* genotypes have been found. However, *M. trossulus* alleles are maintained at high frequencies in the extremely low salinity Baltic Sea for some allozyme genes. A synthesis of reciprocal transplantation experiments between different salinity regimes shows that unlinked *Gpi* and *Pgm* alleles change frequency following transplantation, such that post-transplantation allelic composition resembles native populations found in the same salinity. These experiments provide strong evidence for salinity adaptation at *Gpi* and *Pgm* (or genes linked to them). In the Canadian Maritimes, pure *M. edulis* and *M. trossulus* individuals are abundant, and limited data suggest that *M. edulis* predominates in low salinity and sheltered conditions, whereas *M. trossulus* are more abundant on the wave-exposed open coasts. We suggest that these conflicting patterns of species segregation are, in part, caused by local adaptation of Scandinavian *M. trossulus* to the extremely low salinity Baltic Sea environment.

Keywords: allozyme, hybrid zone, *Lap*, mussel, selection, speciation

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Introduction

Although hybrid zones are most famous for the insights they can provide regarding the genetic architecture of reproductive isolation (e.g. Barton & Hewitt 1985; Harrison 1990), hybrid zones can also illuminate mechanisms for maintaining species boundaries. Frequently, parental species occupy different niches and segregate by habitat within a hybrid zone (Howard 1986; Rand & Harrison 1989; Vines *et al.* 2003). This raises the question of whether niche differentiation between species reflects ecological assortment of lineages that have already evolved niche specialization in allopatry, or whether adaptation to specific habitats has occurred locally via character displacement. In this review, we draw attention to hybridization between two mussel

species where the patterns of species segregation by habitat appear to differ in two replicated hybrid zones. In one of the hybrid zones there is strong evidence that some genes derived from one of the parental species are locally adapted.

Hybridization among marine taxa is probably fairly common, although few hybrid zones have been described in detail (Gardner 1997). The most intensely studied marine hybrid zones are those of mussels in the *Mytilus edulis* species complex (*M. edulis*, *Mytilus galloprovincialis*, and *Mytilus trossulus*), particularly the *M. edulis* × *M. galloprovincialis* hybrid zone in southwestern Europe (see Gosling 1992; Gardner 1994). Here, we review and synthesize the extensive literature pertaining to two less-studied *Mytilus* hybrid zones. *M. edulis* and *M. trossulus* have experienced secondary contact twice, yielding replicated hybrid zones on opposite sides of the Atlantic Ocean. Despite the involvement of the same parental species, these two hybrid zones are quite different in patterns of gene introgression and

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niches occupied by each species. We investigate whether ecological assortment of divergent lineages or local adaptation is the most likely explanation for the many differences between these two hybrid zones.

This review brings together a large body of literature and synthesizes results across studies, genetic loci, and geographical locations. Our aim here is not to provide definitive answers but to highlight the different characteristics of the two *M. edulis* × *M. trossulus* hybrid zones. We suggest several future avenues of research with the goal of understanding why these hybrid zones are so different.

Whether or not one considers the taxa under consideration to be valid species, *M. trossulus*, *M. edulis*, and *M. galloprovincialis* each maintain unique genetic cohesiveness throughout much of their ranges and have distinct evolutionary histories (e.g. Mallet 1995). Throughout this review, we will refer to these taxa as species for convenience, but recognize that they could also be considered as subspecies depending on ones' preferred definition of species boundaries.

Biogeography of *Mytilus*

Speciation among the three closely related *Mytilus* spp. was most likely allopatric. The first speciation was probably associated with the interchange of biotas that followed the opening of the Bering Strait approximately 3.5 million years ago (Ma) (Vermeij 1991; Dunton 1992; Cunningham & Collins 1994). Like many other molluscan genera previously restricted to the North Pacific, *Mytilus* fossils appear in North Atlantic, early Pliocene strata (Vermeij 1991). Speciation between Pacific *M. trossulus* and Atlantic mussels was presumably allopatric following this trans-Arctic dispersal. Speciation between *M. edulis* and *M. galloprovincialis* was probably vicariant (Barsotti & Meluzzi 1968), with a temporary barrier separating Atlantic *M. edulis* from Mediterranean *M. galloprovincialis* (although *M. galloprovincialis* has subsequently expanded its range beyond the Mediterranean). This biogeographical history of the three species is supported by a variety of genetic markers (e.g. mtDNA: Rawson & Hilbish 1995; Quesada *et al.* 1998; *ApaI* satellite DNA sequences: Martinez-Lage *et al.* 2002; *ITS* sequences: Riginos unpublished, M7 lysin: Riginos & McDonald 2003; allozymes: Varvio *et al.* 1988; Koehn 1991; McDonald *et al.* 1991).

More recently (in the Pleistocene or Holocene), Pacific mussels have again invaded the North Atlantic, founding populations of *M. trossulus* on both sides of the Atlantic (Fig. 1). (Tree building and other methods are detailed in the Appendix.) The descendants of these colonizing *M. trossulus* mussels now form two distinct hybrid zones with the Atlantic endemic *M. edulis*, one in the western Atlantic (Canadian Maritimes) and the second in the eastern Atlantic (Scandinavia).

These independent episodes of secondary contact have led to different outcomes on either side of the Atlantic

Ocean. Segregation of *M. edulis* and *M. trossulus* by habitat differs in both hybrid zones, as does the extent of gene introgression between species. On the basis of our re-analysis of genetic data from the literature, we conclude that there is compelling evidence for local adaptation of European *M. trossulus* at *Gpi* and *Pgm* to extremely low salinity environments, as typified by the Baltic Sea. In contrast, the limited data point to less tolerance of low salinity in Canadian *M. trossulus* relative to *M. edulis*.

Scandinavian hybrid zone

The Scandinavian hybrid zone encompasses the Baltic Sea, a region that was colonized by mussels recently. During the last glacial maximum, the Baltic Sea was completely covered with ice. Following deglaciation, it was an enclosed freshwater lake. A connection to the North Sea was established about 7500 years ago (Donner 1995), permitting mussels and other marine taxa to colonize the Baltic Sea. Subsequently, salinity has decreased as a result of extensive freshwater inputs (Donner 1995; Andr n *et al.* 2000). Current salinity in the Baltic ranges from 5%–10% to 10%–20% in the Kattegat, and 20%–30% in the Skagerrak (see map in Fig. 2).

The mussels inhabiting the Baltic Sea are distinct from mussel populations in the North Sea and Skagerrak. North Sea and Skagerrak mussels are morphologically indistinguishable from other *Mytilus edulis* populations, whereas Baltic mussels have comparatively thin shells and small sizes at maturity. The small size of Baltic mussels appears to be mostly a consequence of living in a low salinity environment, as the size of mussels transplanted from the Baltic to the high salinity North Sea approached that of native North Sea mussels after one year (Kautsky *et al.* 1990). The transplanted mussels, however, had thinner shells relative to the soft body and were more susceptible to sea star predation than the native mussels (Norberg & Tedengren 1995; sea stars are a common predator of mussels but are absent in the Baltic), pointing to some underlying genetic differences. Despite the small size and thin shells of Baltic mussels, multivariate morphometric analyses group Baltic mussels with *M. trossulus* from the Pacific and Canadian Maritimes (McDonald *et al.* 1991).

In concordance with morphology, allozyme surveys identify North Sea and Skagerrak populations as *M. edulis* and Baltic Sea populations as *M. trossulus* (McDonald & Koehn 1988; Varvio *et al.* 1988; McDonald *et al.* 1991; Gosling 1992) (and see Fig. 1). Kattegat populations have allele frequencies intermediate to the Baltic and North Seas, although changes in allozyme allele frequencies (notably involving the loci *Est-D*, *Gpi*, *Pgm*, *Lap*, and *Odh*) occur coincidentally and rapidly with a cline width of approximately 100 km through the islands of Denmark, the  resund, and the Belt Sea (Theisen 1978; Bulnheim & Gosling 1988; V in l  & Hvilson 1991).

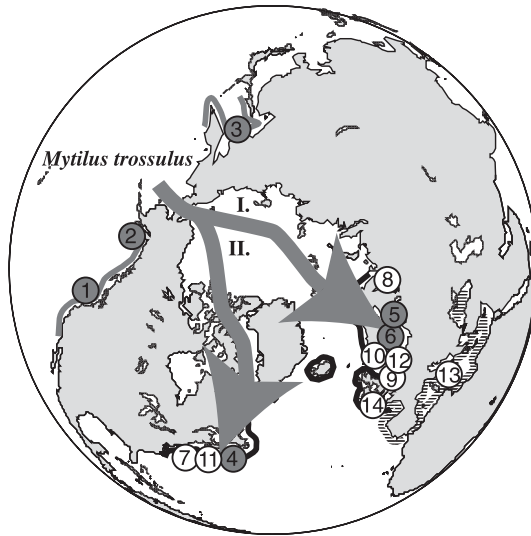
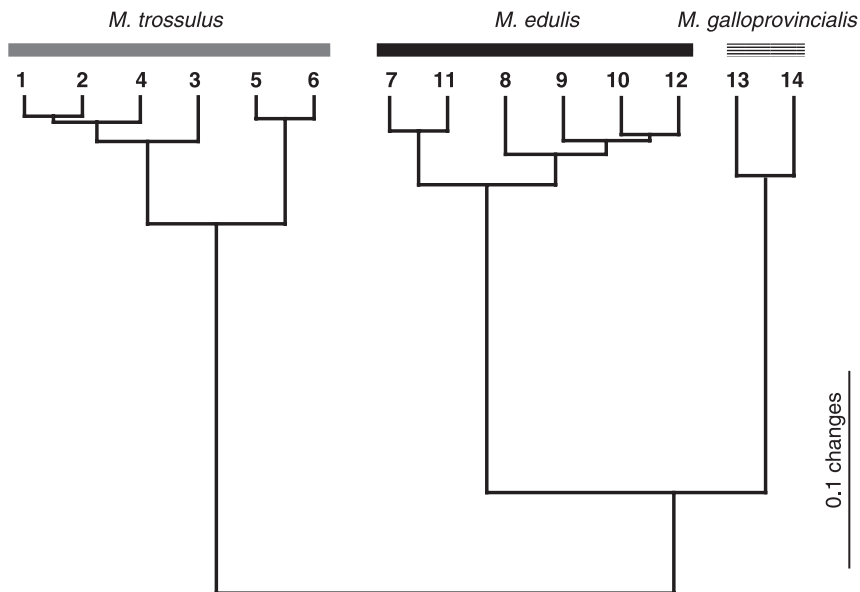


Fig. 1 Historical relationships among *Mytilus* species. UPGMA tree based on Cavalli-Sforza chord distances using allozyme loci *Ap*, *Gpi*, *Lap*, *Mpi*, and *Pgm*. Species distributions of are outlined following Gosling (1992). Populations are as follows: 1) Tillamook, 2) Petersburg, 3) Magadan, 4) Group III (*Mytilus trossulus* in the Canadian Maritimes), 5) Tvärminne, 6) Ystad, 7) Group I (*Mytilus edulis* in North America south of Cape Cod), 8) White Sea, 9) North Sea, 10) Galtö, 11) Group II (*M. edulis* in North America north of Cape Cod), 12) Århus, 13) Italy, and 14) Padstow. See Table 1 for allele frequencies. Arrows indicate the recent invasion(s) of Pacific *M. trossulus* to the North Atlantic.



The interpretation of these coincident clines has differed. Väinölä & Hvilson (1991) have argued that this situation results from secondary contact between Skagerrak *M. edulis* and Baltic Sea *M. trossulus*, with limited hybridization in the Kattegat (perhaps conforming to a tension zone model, where hybrid individuals are inherently less fit). Because the Kattegat is a region of rapidly changing salinity, Bulnheim & Gosling (1988) and Johannesson *et al.* (1990) have argued that primary differentiation of *M. edulis* (associated with adaptation to the low salinity conditions of the Baltic Sea; 5%–10%) was the best explanation for the observed pattern. This explanation, however, does not explain why alleles characteristic of Pacific and western Atlantic *M. trossulus* are found at high frequencies in the Baltic Sea. Moreover, *ITS* sequences confirm the presence of some *M. trossulus* alleles in the Baltic Sea, falling in the same clade

as *ITS* alleles from the Pacific and western Atlantic (Riginos *et al.* 2002), so that the hypothesis of *M. edulis* primary differentiation, in the absence of any hybridization, can be rejected.

Whereas allozyme loci show differentiation across the Scandinavian hybrid zone, other genetic loci indicate extensive gene flow. The most extreme example is mtDNA. There has been complete asymmetric introgression of *M. edulis* female mtDNA into Baltic Sea populations, such that no (native) *M. trossulus* F-mtDNA has been detected (Rawson & Hilbish 1998; Quesada *et al.* 1999). Furthermore, standard male *M. trossulus* mtDNA (Box 1) is also not detected. In heteroplasmic individuals, all mtDNA are closely related to female *M. edulis*, which suggests that some *M. edulis* female mtDNA has acquired male mtDNA functions (Wenne & Skibinski 1995; Quesada *et al.* 1999;

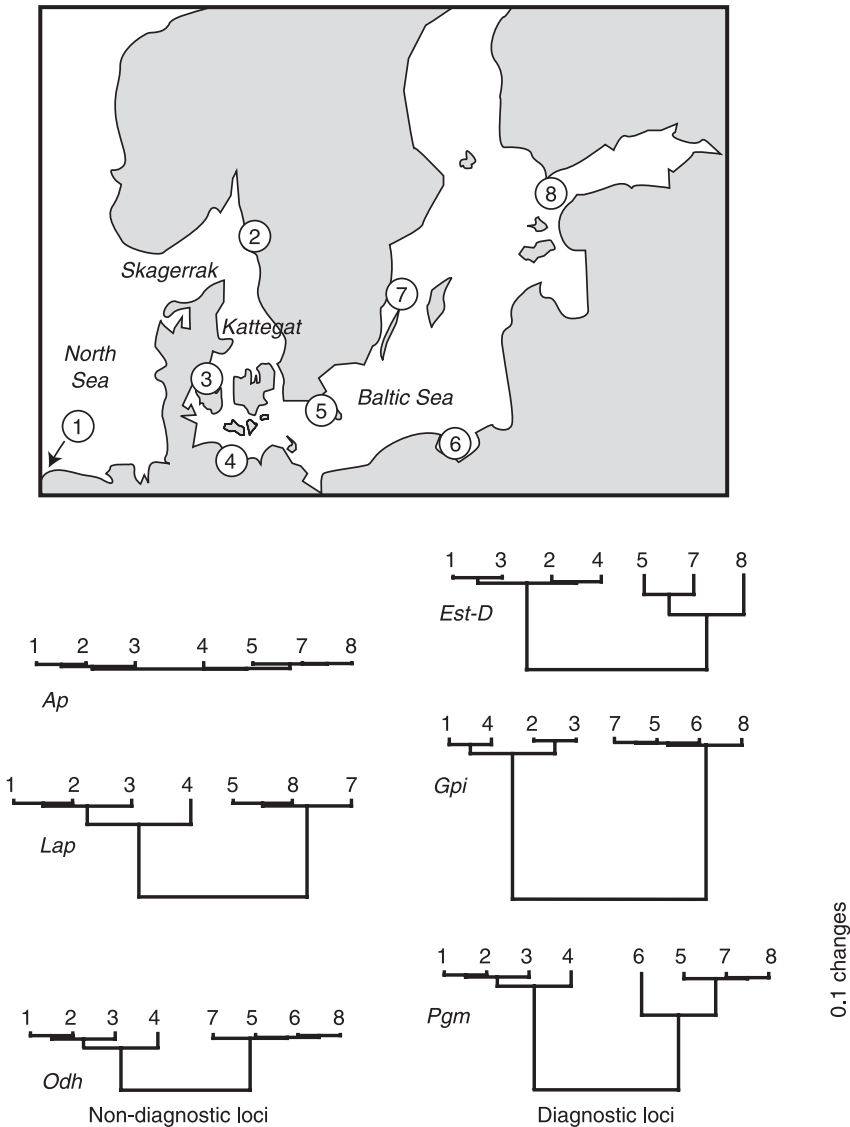


Fig. 2 Population relationships across the Baltic hybrid zone shown by UPGMA trees based on Cavalli-Sforza chord distances. There is strong concordance among loci regarding population relationships, with *Mytilus edulis* alleles predominating among non-Baltic populations (1–3) and *Mytilus trossulus* alleles predominating among Baltic Sea proper populations (6–8). Branches for nondiagnostic loci are shorter than for the diagnostic loci, but reflect the same pattern. Populations are as follows: 1) Netherlands, 2) Galtö, 3) Vejle, 4) Niendorf, 5) Kaseberga, 6) Gdansk, 7) Vastervik, and 8) Tvärminne. Allele sources are Bulnheim & Gosling (1988) for populations 1, 3–5, and 7; Väinölä & Hvilso (1991) for population 2; and Hummel *et al.* (2001) for population 6. *Aap* and *Mpi* are omitted because they were not sampled by Bulnheim & Gosling (1988).

Box 1 MtDNA inheritance and sex determination

Mussels have an unusual system of mtDNA transmission with separate maternally and paternally inherited mitotypes (via egg and sperm, respectively). In general, female mussels only have female mtDNA (F-mtDNA), whereas male mussels are heteroplasmic, having both male (M-) and female (F-) types of mtDNA (Skibinski *et al.* 1994; Zouros *et al.* 1994a) (See Zouros 2000 for a complete review). This system of inheritance has been described as double uniparental inheritance (DUI; Zouros *et al.* 1994a) and is clearly associated with sex determination (Saavedra *et al.* 1997; Kenchington *et al.* 2002b).

The most common type of male (M-) mtDNA is paralogous to female (F-) mtDNA, and the evolutionary

dynamics of these two mitotypes are quite different. In general, M-mtDNA evolves at a significantly faster rate, with more substitutions within and between species (Stewart *et al.* 1995; Stewart *et al.* 1996; Quesada *et al.* 1998; Quesada *et al.* 1999), probably because it is heteroplasmic with F-mtDNA in male tissues except in sperm and testes (Garrido-Ramos *et al.* 1998). Sometimes, however, the second mitotype of heteroplasmic males is more closely related to F-mtDNA than to the standard male type, and has probably assumed male mtDNA functions. This phenomenon, called masculinization, may occur more readily in hybrid zones as do other deviations from DUI (Rawson *et al.* 1996b; Hoeh *et al.* 1997; Saavedra *et al.* 1997; Quesada *et al.* 1999; Ladoukakis & Zouros 2001).

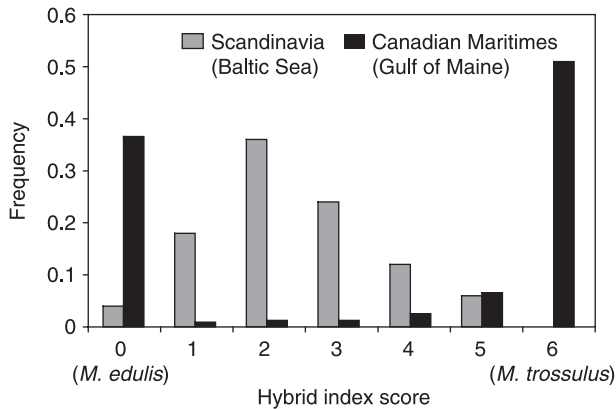


Fig. 3 Frequency of hybrid genotypes. Mussels were given a score of zero to six based on their total number of *Mytilus trossulus* alleles for the loci *Glu 5'*, *ITS*, and *MAL-I*. A score of zero is consistent with a pure *Mytilus edulis* genotype, whereas a score of six is consistent with a pure *M. trossulus* genotype. Hybrids are rare in the Gulf of Maine but comprise the majority of individuals in the Baltic Sea. Data from the Gulf of Maine are from Rawson *et al.* (2001). Data from the Baltic Sea are from Riginos *et al.* (2002) and the present study.

Quesada *et al.* 2003; Zbawicka *et al.* 2003a; Zbawicka *et al.* 2003b). Although less dramatically than mtDNA, nuclear DNA markers (nDNA; in this case, where alleles are determined by size differences or restriction digests of PCR products) also show considerable introgression, particularly of *M. edulis* alleles into Baltic Sea populations (Borsa *et al.* 1999; Riginos *et al.* 2002; Wood *et al.* 2003) (Table 2). There is also some export of Baltic alleles to the Skagerrak, the most extreme example being the T0 allele of *EFbis*, found at 60% in Flødevigen, in southern Norway (Baltic frequency = 95%) (Bierne *et al.* 2003b).

Our analyses of Baltic Sea populations using three such nuclear genes (*Glu 5'*, *ITS*, and *MAL-I*, Riginos *et al.* 2002 and present study) find that the majority of mussels are hybrids (96%). The distribution of Baltic genotypes follows a unimodal distribution, where complex hybrids (hybrid scores of 2–4) are most common and parental species (hybrid scores 0 and 6) are virtually absent (Fig. 3). Although individuals with equal proportions of *M. edulis* and *M. trossulus* alleles are common (hybrid score of 3), we have not found any mussels with F1 genotypes (i.e. heterozygous at every locus). These conclusions, which are based on allele frequencies, are corroborated by *ITS* sequences from Riginos *et al.* (2002), who found examples of individual Baltic mussels with higher proportions of *M. edulis* alleles than *M. trossulus* alleles (*ITS* forms multiple arrays of tandem repeats so that more than two alleles are expected per individual). Thus, based on these nDNA markers, Baltic mussel populations are best described as a hybrid swarm. Clearly, no strong reproductive barriers are currently acting to maintain a cohesive *M. trossulus* genome, and, in fact, we have found no individuals with a nuclear genotype con-

sistent with being a pure *M. trossulus* individual. Although absence of F1 type individuals is often taken as evidence for reproductive barriers between parental species, in this case it seems more likely to be a consequence of the low (or even negligible) frequency of *M. trossulus* parental types.

Contrasting patterns of gene flow for allozyme vs. nDNA loci in Scandinavia

Despite the extensive introgression of *Mytilus edulis* nDNA alleles into Baltic populations (as described above), allozymes indicate the overall genetic integrity of two species (e.g. Skagerrak *M. edulis* and Baltic Sea *Mytilus trossulus*). This striking contrast among genetic markers led both Borsa *et al.* (1999) and Riginos *et al.* (2002) to conclude that selection was likely to be affecting at least some allozyme loci. Recently, Bierne *et al.* (2003b) have challenged this conclusion by arguing that genetic differentiation across the hybrid zone varies greatly among allozyme loci and that some allozyme loci show minimal population structure, similar to nDNA loci. This argument, however, fails to take into account the fact that some allozyme loci also have minimal genetic differentiation between allopatric populations of *M. edulis* and *M. trossulus*. Such loci that show little differentiation between nonhybrid *M. edulis* and *M. trossulus* (likely the result of incomplete lineage sorting of ancestral polymorphism) are also expected to show little differentiation across a hybrid zone, whether or not there has been recent gene flow (see Box 2 for an extended discussion).

Here, we compare all allozyme and nDNA loci for which we have information regarding differentiation between allopatric populations. It is not necessary to restrict comparisons to loci generally considered diagnostic (see Gosling 1992) between *M. edulis* and *M. trossulus* in order to demonstrate that gene flow between Skagerrak *M. edulis* and Baltic Sea *M. trossulus* is restricted for allozymes relative to nDNA.

The most extensive survey of allozyme variation across the Scandinavian hybrid zone was that conducted by Väinölä & Hvilsum (1991) who examined 22 loci. Whereas there is a range of geographical partitioning among these loci, for many there is no information regarding how much divergence is found in populations of *M. edulis* and *M. trossulus* outside of the hybrid zone. We re-examine all loci that have been scored both in the Scandinavian hybrid zone and in allopatric *M. edulis* and *M. trossulus* populations. The analyses that follow include the loci that best discriminate between *M. edulis* and *M. trossulus* (e.g. *Aap*, *Est-D*, *Gpi*, *Mpi*, and *Pgm*, see Gosling 1992) and *Ap*, *Lap*, and *Odh*, which are generally not used to discriminate between these two species (see also Table 1).

There is strong concordance among allozyme loci regarding population relationships across the Scandinavian hybrid

Table 1 Allozyme frequencies among *Mytilus* populations

Species allopatric/ sympatric with other <i>Mytilus</i> spp.	<i>M. trossulus</i>						<i>M. edulis</i>						<i>M. galloprovincialis</i>		
	A			S			A			S			A	S	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Locus, allele	Tillamook*	Petersburg*	Magadant†	Group III‡	Tvärminne*	Ystad§	Group I¶	White Sea**	North Sea††	Galtö§	Group II‡	Århus†	Italy§§	Padstow¶¶	
<i>Aap</i>															
120–125	0	0	0	0	0	—	0	0	0	—	0	0	0.06	0.04	
110–115	0.02	0	0	0	0	—	0	0.02	0.03	—	0	0	0.78	0.83	
100–105	0.06	0	0.02	0.95	0	—	0.88	0.80	0.79	—	0.85	0.86	0.14	0.07	
95	0.48	0.63	0.34	0.01	0.92	—	0.08	0.12	0.16	—	0.05	0.12	0.02	0.06	
90	0.36	0.06	0.34	0.04	0.08	—	0.04	0.06	0.02	—	0.10	0.02	0	0	
85	0.06	0.31	0.26	0	0	—	0	0	0	—	0	0	0	0	
80	0.02	0	0.04	0	0	—	0	0	0	—	0	0	0	0	
<i>Ap</i>															
108–125	0.06	0.02	0	0.02	0	0	0.02	0	0	0.03	0.03	0	0.40	0.15	
105	0.22	0.22	0.16	0.34	0.06	0.16	0.43	0.24	0.13	0.21	0.45	0.24	0.42	0.24	
100	0.62	0.54	0.76	0.44	0.84	0.74	0.30	0.72	0.82	0.69	0.32	0.68	0.18	0.56	
95	0.08	0.21	0.08	0.06	0	0.01	0.01	0	0.04	0.01	0.02	0.04	0	0.04	
90	0.02	0.01	0	0.15	0.10	0.09	0.24	0.04	0.01	0.06	0.18	0.04	0	0.01	
<i>Est-D</i>															
110	0	0.02	0	0	0	0	0.04	0	0.05	0	0.03	0.02	0	—	
100	0	0.01	0	0	0	0.03	0.92	0.98	0.91	0.90	0.96	0.94	0	—	
95	0.06	0	0	0.03	0.22	0.18	0	0	0	0.02	0.01	0	0	—	
90	0.90	0.95	0.98	0.96	0.78	0.79	0.02	0.02	0.04	0.08	0	0.04	1	—	
80	0.04	0.02	0.02	0.02	0	0	0.02	0	0	0	0.01	0	0	—	
<i>Gpi</i>															
107–110	0	0	0	0.04	0	0.02	0.24	0.66	0.52	0.55	0.26	0.54	0.02	0.11	
105	0.02	0	0.02	0	0	0	0	0	0.12	0.08	0	0	0.06	0.22	
100–102	0.12	0.06	0.08	0.12	0.02	0.01	0.55	0.20	0.33	0.18	0.36	0.22	0.92	0.50	
98	0.56	0.70	0.32	0.57	0.94	0.90	0	0	0.01	0.17	0.02	0.04	0	0.05	
86–96	0.30	0.24	0.58	0.27	0.04	0.07	0.21	0.14	0.02	0.02	0.36	0.20	0	0.12	
<i>Lap</i>															
100	0.04	0.01	0.06	0.01	0.62	0.49	0.01	0	0.02	0.02	0.02	0	0.12	0.01	
98	0.12	0.06	0	0.12	0.02	0.01	0.27	0.38	0.18	0.25	0.55	0.22	0.42	0.47	
96	0.28	0.34	0.26	0.47	0.16	0.32	0.20	0.52	0.55	0.64	0.35	0.70	0.46	0.49	
92–94	0.56	0.59	0.68	0.41	0.20	0.18	0.52	0.10	0.25	0.09	0.09	0.08	0	0.03	

Table 1 Continued

Species allopatric/ sympatric with other <i>Mytilus</i> spp.	<i>M. trossulus</i>						<i>M. edulis</i>						<i>M. galloprovincialis</i>	
	A			S			A			S			A	S
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Locus, allele	Tillamook*	Petersburg*	Magadant†	Group III‡	Tvärminne*	Ystad§	Group I¶	White Sea**	North Sea††	Galtö§	Group II‡	Århus†	Italy§§	Padstow¶¶
<i>Mpi</i>														
104–110	0.02	0.02	0.02	0.01	0	0	0.02	0.08	0	0	0	0	0	0
100	0	0	0	0	0	0.11	0.88	0.90	0.94	0.89	0.84	0.94	0	0.06
94	0.92	0.94	0.98	0.96	0.90	0.77	0	0	0	0.04	0	0	0	0
92	0	0	0	0	0	0	0	0.02	0.04	0	0	0	1	0.93
84–90	0.06	0.04	0	0.03	0.10	0.12	0.10	0	0.02	0.07	0.16	0.06	0	0.01
<i>Odh***</i>														
108–110	–	–	–	–	–	0.07	–	0.03	0.05	0.09	–	–	0.58	0.20
105	–	–	–	–	–	0.61	–	0.01	0.01	0.04	–	–	0.22	0
100	–	–	–	–	–	0.29	–	0.92	0.91	0.85	–	–	0.18	0.06
94	–	–	–	–	–	0.02	–	0.01	0.01	0	–	–	0.01	0.01
90	–	–	–	–	–	0.01	–	0.03	0.02	0.02	–	–	0.21	0.73
<i>Pgm</i>														
114	0.06	0.10	0.06	0.09	0.08	0.11	0	0	0	0	0	0	0	0
111	0.52	0.39	0.52	0.49	0.86	0.80	0.02	0	0.05	0.04	0.04	0	0.02	0.13
106	0.32	0.36	0.32	0.22	0.02	0.04	0.12	0.05	0.27	0.19	0.11	0.10	0.28	0.15
93–100	0.10	0.15	0.10	0.21	0.04	0.05	0.84	0.59	0.53	0.70	0.85	0.82	0.64	0.56
89	0	0	0	0	0	0	0.03	0.36	0.15	0.07	0	0.08	0.06	0.16

*McDonald & Koehn (1988); †McDonald *et al.* 1990 for all loci except *Pgm*, which was taken from Gosling (1992); ‡Koehn *et al.* (1984) except *Aap*, which is from Varvio *et al.* (1988), and *Est-D* and *Mpi* from Comesaña *et al.* (1999); average *M. edulis* frequencies for Group II and average *M. trossulus* frequencies for Group III; §Väinölä & Hvilsom (1991); ¶Koehn *et al.* (1984) except *Aap*, which is Shinnecock, NY from McDonald & Koehn 1988; **Kandalaksha from McDonald *et al.* 1990 for all loci except *Odh* and *Pgm*, which are the average of White Sea frequencies from Hummel *et al.* (2001); ††North Sea (Helgoland) from Bulnheim & Gosling 1988 for all loci except *Aap*, which is the Netherlands from Comesaña & Sanjuan 1997; §§Venice from McDonald & Koehn 1989 except for *Odh*, which is La Spezia from Hummel *et al.* (2001); ¶¶Varvio *et al.* (1988); ***The *M. edulis* and *M. trossulus* characteristic alleles of *Odh*¹⁰⁰ and *Odh*¹⁰⁵ do not resolve at a pH 8.0 buffer employed by many studies. For this locus, the allelic notation of Väinölä & Hvilsom (1991) is used, whereas the rest of the loci use the allele names of Gosling (1992).

Box 2 Distinguishing between incomplete lineage sorting and recent gene flow

Some gene polymorphisms can persist for long periods of time and can even be retained among closely related taxa. Allelic variants that predate speciation present a particular challenge in a hybrid zone situation where one wishes to make inferences about recent gene flow between parental species. In such cases, a lack of population structure across a hybrid zone could be the result of incomplete lineage sorting of ancestral polymorphism regardless of whether there has been recent gene flow.

One solution to this issue is to only employ markers that are highly diagnostic between nonhybrid populations of the parental species. In the most extreme case, different alleles are fixed in each species so that when an allele from one species is found in the other species' genetic background, gene introgression can be safely concluded. For such diagnostic loci, reduced differentiation across a hybrid zone can be safely assumed to have resulted from recent gene flow. Of course, in some situa-

tions it may not be possible to identify loci that are absolutely diagnostic between species, because some alleles are shared between species.

In the case of *Mytilus* spp., several nDNA based markers (length variants or restriction digests of PCR products) appear to be completely diagnostic (100% fixed) between species (Heath *et al.* 1995; Rawson *et al.* 1996a; Rawson *et al.* 1996b). However, the classical markers for identifying mussel species are allozymes (particularly *Aap*, *Est-D*, *Gpi*, *Mpi*, and *Pgm*, Gosling 1992). Although many allozymes are strongly differentiated among species, they do not have completely fixed differences (see Table 1). Therefore, in a hybrid zone situation, some parental species mussels are likely to be scored as hybrids when their genotypes include a rare (for their species) allele. Markers that are not completely fixed between species (including all allozymes in *Mytilus*) are, thus, predisposed to find more hybrid individuals (see Comesaña *et al.* 1999 for an empirical example) and more gene flow across a hybrid zone than markers that are fixed between species.

zone. *M. edulis* alleles predominate among non-Baltic Sea populations (Fig. 2: populations 1–3), whereas *M. trossulus* alleles predominate among Baltic Sea populations (populations 5–8). (No study sampled *Aap* or *Mpi* through the Kattegat and Baltic well enough to include in this analysis.) The Niendorf population (population 4) clusters with North Sea populations for all loci except *Ap*, where it is intermediate between North Sea and Baltic Sea, and this is consistent with its position in the middle of the hybrid zone. Other populations show strong identity to parental types across all loci. Note that branches for 'nondiagnostic' loci are shorter than for the 'diagnostic' loci, but reflect the same essential division. This observation argues against any inherent bias of estimates based on diagnostic loci vs. nondiagnostic loci, and simply demonstrates that there is a greater rate of divergence for diagnostic loci.

Consistency among 'diagnostic' and 'nondiagnostic' allozyme loci is also evident in Fig. 4, which shows divergence across the Skagerrak–Baltic Sea hybrid zone scaled by divergence between nonhybrid populations of *M. edulis* and *M. trossulus*. Like all measures of genetic distance, Δp (Barton 2000) ranges from zero to one, where zero is no difference in allele frequencies between two populations and one is when no alleles are shared across populations. In the absence of any gene flow across the hybrid zone, the difference in allele frequencies, Δp , for each locus should be approximately the same between allopatric populations (x -axis) and across the hybrid zone (y -axis) and fall near the one-to-one line. Gene flow across the hybrid zone

would cause allele frequencies to become more similar and would reduce Δp on the y -axis such that it would fall below the one-to-one line. This graphical depiction allows one to distinguish between loci that share alleles as a result of incomplete lineage sorting (small genetic distance between parental species; *Ap* and *Lap*) and those that have experienced recent gene flow across the hybrid zone (circled).

Most allozyme loci have similar amounts of genetic differentiation across the hybrid zone relative to differentiation between North Sea *M. edulis* and Pacific *M. trossulus* populations and fall close to the one-to-one line. *Gpi*, *Lap* and *Pgm*, however, have greater differentiation across the hybrid zone than between parental species. Note that *Lap* is not usually diagnostic between *M. edulis* and *M. trossulus* (Table 1), but has a high Δp (= 0.56) across the hybrid zone (Δp = 0.33 between parental species). The elevated genetic distances of *Gpi*, *Lap* and *Pgm* across the hybrid zone could be a consequence of genetic drift resulting from a small effective population size within the Baltic Sea, or could result from directional selection on these or linked loci (we examine the latter possibility in the following section).

One locus that is notable for falling below the one-to-one line is *Ap*. Varvio *et al.* (1988) noted that there is greater within species (particularly within *M. edulis*) genetic differentiation than between species at *Ap*, and suggested that this locus may be more permeable to gene flow between species than other (allozyme) loci. However, in the context of this hybrid zone, several other loci (*Est-D*, *Mpi* and *Odh*) have a similar pattern.

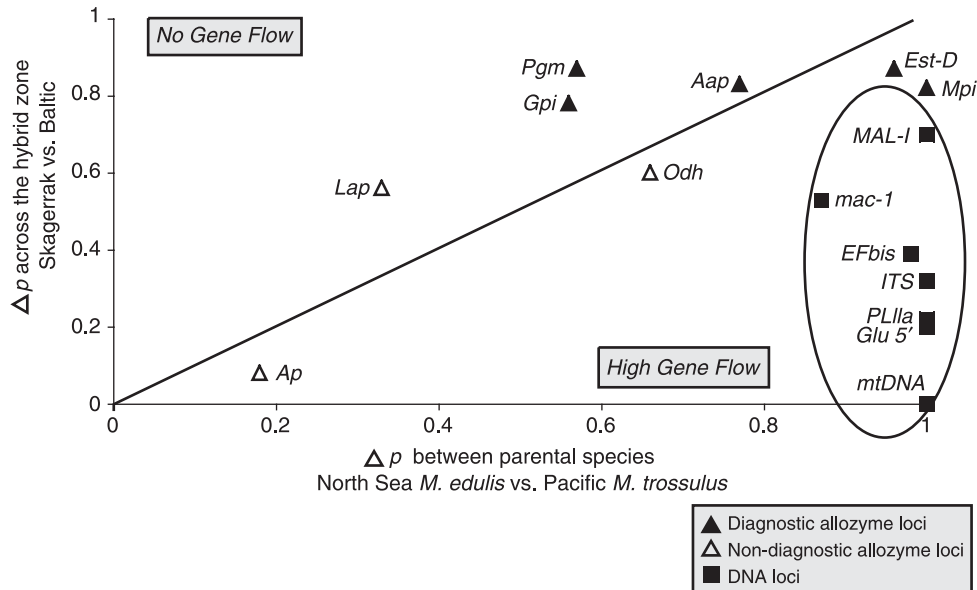


Fig. 4 Contrasting genetic divergence between allozyme and DNA loci across the Scandinavian hybrid zone relative to species divergence. The graph uses Δp values taken directly from Table 2 of Bierne *et al.* (2003b) for allozymes and the DNA loci *EFbis* and *mac-1*. For the remaining nDNA markers Δp 's are given in our Table 2. Similar results are obtained using other sources of allele frequencies or using metrics such as \hat{F}_{ST} and $\hat{F}_{ST(w)}$.

All DNA loci (mt and nDNA) have high Δp scores between parental species. For nDNA, this is probably an artefact of their being chosen for analysis because they are diagnostic between species (but less so for *mac-1* and *EFbis*, which do not have fixed differences between species). But, all nDNA loci show significantly less divergence across the hybrid zone as compared to allozyme loci when scaled by divergence between parental species (Mann–Whitney $U = 46$, $P < 0.003$ between nDNA loci and allozymes).

The relative contributions of *M. edulis* and *M. trossulus* alleles to hybrid Baltic populations were also estimated in a coalescent framework with Bayesian inference (Chikhi *et al.* 2001) for allozymes and nDNA (see Appendix for further details). The posterior probabilities of the 95% credibility intervals were not overlapping (for the proportion of *M. edulis* alleles, mode of $p^{\text{alloz}} = 0.06$, CI = 0–0.21; mode of $p^{\text{nDNA}} = 0.54$, CI = 0.36–0.87). We also tested for a difference between posterior probability distributions of p^{alloz} and p^{nDNA} by randomizing the order of values obtained from steps in the Markov Chain and by taking the difference,

$$\delta = p_i^{\text{nDNA}} - p_i^{\text{alloz}}$$

(where i is a random step in the chain; Holsinger & Wallace 2004). The distribution of δ was significantly greater than zero (CI = 0.26–0.71), indicating that *M. edulis* has contributed significantly more nDNA alleles than allozyme alleles to Baltic Sea populations. This coalescent method assumes that admixture was a one-time event and not

recurrent, while in reality the introgression of *M. edulis* alleles into the Baltic is probably ongoing. Nonetheless, these results bolster our conclusion Baltic Sea populations are more permeable to nDNA than to allozyme introgression.

The observed discordance between nDNA and allozyme loci is difficult to explain without invoking some form of selection on one type of genetic marker. Either the allozymes are more resistant to gene flow or nDNA are more permeable to gene flow. In general, it seems more reasonable to expect there to be greater fitness consequences for changes in protein amino acid sequences (e.g. allozymes), than changes in length and/or restriction sites for noncoding DNA (e.g. most of the nDNA loci used here). Moreover, in the following section, we provide direct evidence for adaptation of some allozyme loci to different salinity regimes and, thus, argue that selection on allozyme loci is most likely to be the causal agent for the previously described pattern. It is not our contention that allozymes are universally subject to selection. In the case of mussels, however, some of the most frequently employed allozyme loci behave in a manner inconsistent with nDNA. Of course, to determine which specific loci deviate from the overall genomic level of gene flow (e.g. Beaumont & Nichols 1996) it would be useful to consider many more loci. For example, it would be informative to examine the remaining allozyme loci surveyed by Väinölä & Hvilsom (1991) across the hybrid zone. But, without information about divergence between *M. edulis* and *M. trossulus* allopatric populations, it is not possible to discriminate between similarity across the hybrid zone resulting from incomplete lineage sorting or recent gene flow.

Ideally, the sample size of nDNA markers should also be increased to get a better estimate of neutral differentiation (along with variance among neutral loci; see Bierne *et al.* 2003b) across the hybrid zone. In addition, the best markers would be single locus (not *ITS*) and codominant (not *PLIIa*) (see Riginos *et al.* 2002).

The most serious caveat to the argument that selection may affect the distribution of alleles at multiple allozyme loci (and not nDNA) is that some loci might be physically linked (see Varvio *et al.* 1988 for additional discussion). If there were strong selection on one gene in a linkage group, genetic hitch-hiking would create the appearance of selection at multiple loci. Some of the genes under consideration might even be contained within a chromosomal rearrangement, so that gene flow between the *M. edulis* and *M. trossulus* alleles would be blocked within this region but would be uninhibited for nonrearranged genomic regions (as in Noor *et al.* 2001; Rieseberg 2001). In fact, there is some evidence of linkage among some of the genes in question. For example, Hilbish *et al.* (1994) found that *Est-D* and *Odh* alleles cosegregate in the *M. edulis* × *Mytilus galloprovincialis* hybrid zone, while Beaumont (1994) found that *Est-D*, *Lap*, *Mpi*, *Odh* and possibly *Gpi* were linked in *M. edulis* × *M. galloprovincialis* crosses. Hvilsom & Theisen (1984), however, found no support for linkage among the same set of loci in crosses of Danish mussels collected from the Kattegat and Öresund (thus, probably involving either pure *M. edulis* or *M. edulis* × *M. trossulus* hybrids). Therefore, the data on linkage are conflicting, but this matter is clearly relevant and important to all studies of *Mytilus* genetics employing this particular group of loci.

In the case of mussels in Scandinavia, if *Est-D*, *Gpi*, *Lap*, *Mpi*, and *Odh* are physically linked, then it seems likely that a target of selection lies somewhere in this linkage group. Moreover, there is no evidence that *Pgm* belongs to this putative linkage group (Hvilsom & Theisen 1984; Beaumont 1994) and, as the results below show, the evidence for selection on *Pgm* is as strong or stronger than for other genes in the possible linkage group. Thus, it seems that there is evidence for selection acting on at least two independently segregating genomic regions.

Salinity-induced selection on Scandinavian allozyme alleles

Gpi, *Pgm* and *Lap* exhibit the greatest differentiation across the hybrid zone (relative to parental species; Fig. 4) and reciprocal transplantation experiments demonstrate that many individual alleles of these loci respond in a correlated manner to salinity change. We compared the direction of allele frequency changes among local mussel populations across the Skagerrak/Kattegat/Baltic Sea salinity gradient to allele frequency changes in transplantation experiments carried out by Johannesson *et al.* (1990) and Theisen (1978).

Johannesson *et al.* transplanted mussels from the Skagerrak (Tjärnö: 19‰–30‰) to the Baltic Sea (Askö, 5‰–7‰) and vice versa. Similarly, Theisen performed a transplant from low (8‰–10‰) to high salinity (14‰–17‰) within the Baltic Sea and Kattegat regions.

In addition to these transplantation experiments, we include the survey of Ridgway (2001). Ridgway sampled mussels in the vicinity of Bergen on the western coast of Norway, approximately 700 km from the hybrid zone, in areas presumably only populated by *M. edulis*. His transect compared allele frequencies of mussels from an oceanic site (Espeland: 27‰–32‰) to allele frequencies in brackish fjord waters (Bergen and Hylkje: 5‰–30‰), approximately 40 km distant from the coast.

Remarkably, individual *Pgm* and *Gpi* alleles responded to salinity change in the same way across transplantation experiments and on the west coast of Norway (Fig. 5). The alleles that typify *M. edulis*, *Pgm*^{93–100} and *Gpi*^{107–110}, increased in frequency at high salinity, whereas the alleles that typify *M. trossulus* (outside of the Baltic), *Pgm*¹¹¹ and *Gpi*⁹⁸, increased in frequency in low salinity conditions. Frequency changes for other alleles were concordant but less dramatic. The single exception, *Pgm*¹⁰⁶, is generally the second most frequent allele in both *M. trossulus* and *M. edulis* populations, so that it is not at all diagnostic between species. Although mussels that survived Baltic to Skagerrak transplantation (from Johannesson *et al.* 1990) had alleles characteristic of native Skagerrak populations, they maintained physiological differences better suited for low salinity, such as a greater rate of nitrogen excretion and a negative energy balance (Tedengren *et al.* 1990).

In Norway, the difference between coastal and fjord *Pgm* and *Gpi* allele frequencies was striking and clearly allies the brackish water populations of Bergen and Hylkje with *M. trossulus* (Fig. 6). But, these alleles that are typically characteristic of *M. trossulus* are also found in low frequencies in all *M. edulis* populations. Thus, at present, it is not possible to determine whether *Pgm*¹¹¹ and *Gpi*⁹⁸ are inherently better suited to low salinity (and are *M. edulis*-derived in Bergen and Hylkje) or whether they are *M. trossulus*-derived alleles, which have escaped from the Baltic into northern European mussel populations via adaptive introgression.

Despite the a priori expectation for *Lap* to be under selection associated with salinity (Box 3), individual *Lap* alleles did not behave as expected (see Väinölä & Hvilsom 1991 for an extended discussion). Given that *Lap*⁹⁴ decreases in low salinity conditions in New York *M. edulis* (Koehn *et al.* 1976), it is somewhat surprising that *Lap*⁹⁴ is found at higher frequencies within Baltic populations (18%) than Skagerrak populations (9%). The most common allele in the Baltic is *Lap*¹⁰⁰, which is in very low frequencies in all other northern hemisphere mussel populations. One possible explanation is that *Lap*¹⁰⁰ is linked to another allele that is the real target of selection in the Baltic Sea.

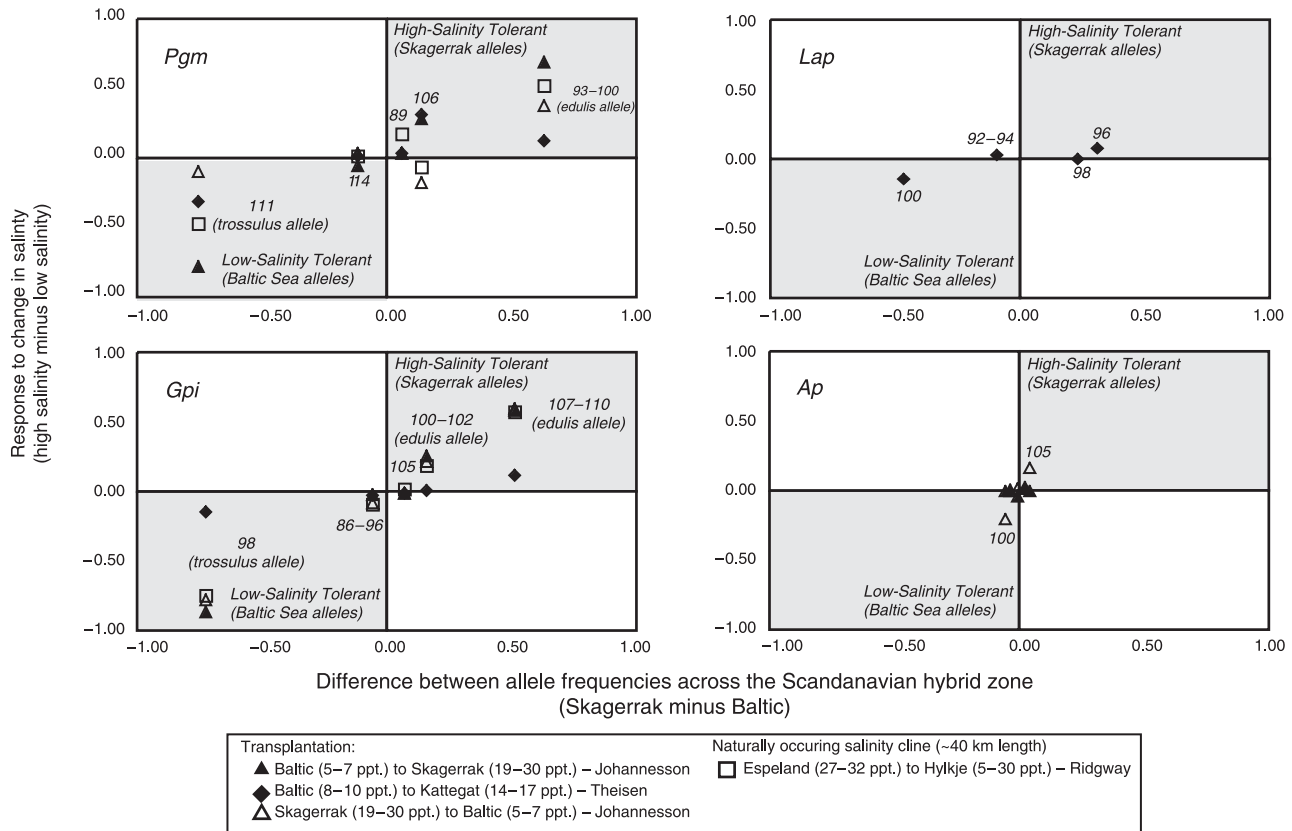


Fig. 5 Response of individual alleles to salinity change. Most alleles respond in the same manner across the hybrid zone (x -axis) as when transplanted to a different salinity regime (y -axis). Values are the difference between high salinity and low salinity conditions (x -axis: Galtö — Ystad; Väinölä & Hvilson 1991 across the hybrid zone, y -axis: 1) AS86* — AS83, Johannesson *et al.* 1990, 2) Tjärnö average — TJ84*; Johannesson *et al.* 1990, 3) station 6 — Transplant; Theisen 1978, and 4) Espeland — Hylkje; Ridgway 2001). For example, *Pgm*¹¹¹ is more common in the Baltic than Skagerrak and also increases in frequency following transplantation of mussels from high salinity to low salinity. Conversely, *Pgm*⁹³⁻¹⁰⁰ is more common in the Skagerrak than Baltic and also increases in frequency following transplantation of mussels from low salinity to high salinity.

Box 3 Salinity induced selection on *Lap*

One of the best-studied examples of environmentally induced selection is the effect of salinity on *Lap* alleles in *Mytilus edulis*. Mussels are osmoconformers and regulate their cell volumes by altering their concentrations of free amino acids. The protein product of the *Lap* locus, aminopeptidase-I, catabolizes proteins into free amino acids. In high salinities, mussels adjust their osmotic pressure by increasing the amount of free amino acids in their cells. Thus, the overall activity of *Lap* is higher in mussels inhabiting open ocean sites than in estuaries (Koehn *et al.* 1980a). Oceanic populations of *M. edulis* on the east coast of North America have high proportions (~55%) of *Lap*⁹⁴; the *Lap*⁹⁴ allele confers 20% greater catalytic efficiency relative to other *Lap* alleles (Koehn & Siebenaller 1981) and is therefore well suited for high salinity conditions. The greater catalytic efficiency of *Lap*⁹⁴, however, is a liability for mussels in low salinity

conditions where an excess of amines are excreted and *Lap*⁹⁴ mussels waste energy reserves on unnecessary catabolism (Hilbish *et al.* 1982).

As would be expected from the biochemical and metabolic properties of the *Lap*⁹⁴ gene product, frequencies of *Lap*⁹⁴ are much lower in estuaries along the east coast of North America as compared to oceanic populations (Koehn *et al.* 1976). Summer larval recruits entering Long Island Sound (and presumably other brackish waters) have similar allelic proportions to open coastal populations, but as autumn progresses and newly settled mussels are exposed to salinity fluctuations in the Sound, the frequency of *Lap*⁹⁴ drops over time (to ~15%) (Koehn *et al.* 1980b). In Pacific *Mytilus trossulus*, *Lap*⁹⁴ is also found at lower frequencies in estuarine populations, as compared to open ocean populations, although the change in average frequency is much less than in Atlantic *M. edulis* (*M. trossulus* ocean populations: 54%, estuarine populations: 47%, McDonald & Sieten 1988).

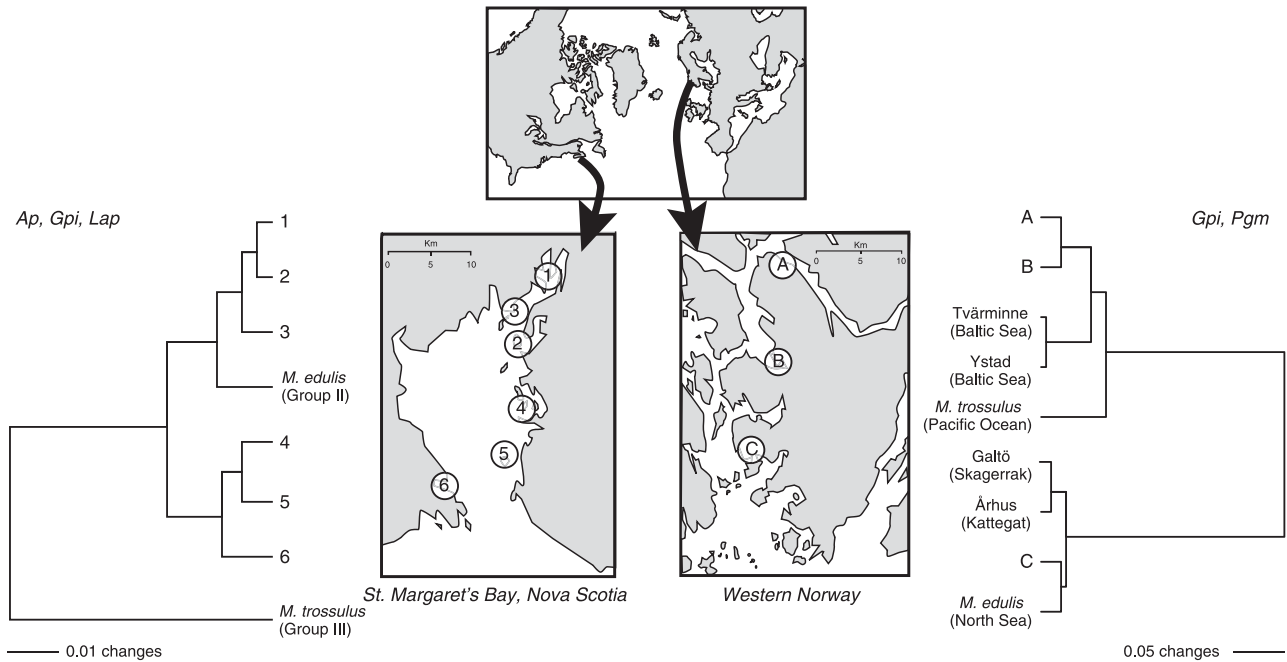


Fig. 6 Distributions of *Mytilus edulis* and *Mytilus trossulus* alleles in western and eastern Atlantic inlets. The UPGMA tree from the western Atlantic groups populations from the head of St. Margaret's Bay where salinity fluctuates (periodically exposing native mussels to low salinity conditions) with *M. edulis*. In contrast, the UPGMA tree from the eastern Atlantic shows low salinity populations grouping with *M. trossulus* and a high salinity populations grouping with *M. edulis*. Data are from Gartner-Kepkay *et al.* (1983), Ridgway (2001), and Table 1 (alignments in supplemental material). Although Gartner-Kepkay *et al.* (1983) scored *Aap* and *Mpi*, their allele frequencies could not be reasonably aligned with other Atlantic populations and therefore had to be omitted from our reanalysis.

The alleles for *Ap* (a nondiagnostic locus) responded as predicted in one transplant direction (high to low salinity) but the response was negligible in the reverse direction. Given that there is not much of a difference in *Ap* allele frequencies across the hybrid zone, it would be difficult to detect any response.

The parallel responses among *Gpi*, *Pgm* and *Lap* alleles are consistent with physical linkage, and indeed Väinölä & Hvilson (1991) found significant linkage disequilibria among these three loci. Because none of the salinity experiments report actual genotypes, however, we cannot test for linkage. As discussed previously, *Pgm* does not seem to be physically linked to other allozyme loci.

One note of caution is that the validity of the transplantation experiments conducted by Johannesson *et al.* (1990) have been challenged by Väinölä (1990) on statistical grounds. Väinölä argued that there would have been insufficient genetic variation in source populations to permit the observed changes in allele frequencies, given the extremely high mortality of transplanted mussels. He concluded that local mussel recruits must have contaminated the transplanted populations. In addition, mussels from the Baltic to Skagerrak transplantation survived for 16 months before large mortalities were observed (Johannesson *et al.* 1990); this mass mortality may have been caused by a

disease, rather than an inherent ability to acclimatize to ambient environmental conditions (Väinölä 1990).

However, the identical results (for *Pgm* and *Gpi*) of Theisen (who marked each transplanted mussel) and Ridgway point to a shared phenomenon and not contamination of the transplanted population by local mussels. The results of Ridgway are particularly compelling, as the salinity changes of the Norwegian fjord mimic the environmental gradient of the Kattegat, but over an extremely small geographical distance (*c.* 40 km), much less than the likely dispersal distance of mussels whose larvae are planktonic. Moreover, because Ridgway sampled natural populations, contamination is not a concern.

Alleles typical of Pacific *M. trossulus* are at high frequencies in the extremely low salinity Baltic Sea (notably *Gpi*⁹⁸ and *Pgm*¹¹¹, but also see Table 1 for other loci). Pacific *M. trossulus* viability appears unaffected by moderate estuarine conditions (*c.* 20‰ Sarver & Foltz 1993; Matson *et al.* 2003), so *M. trossulus* might have been preadapted for low salinity. However, *M. edulis* also tolerates low salinity both in North America (salinity in Long Island Sound = 26‰, Koehn *et al.* 1980b; see also Box 3) and in Europe (Gardner 1994), so it is difficult to argue that either species is inherently better suited to the extreme conditions of the Baltic Sea (5%–10%).

Although we have presented salinity as the most likely environmental factor causing the segregation of specific alleles, in principle, other correlated environmental differences could also play a role. For example, the Baltic Sea has virtually no tide and no major predators (particularly sea stars, which target mussels, Norberg & Tedengren 1995) or competitors (Kautsky 1982). In addition, much of the surface water freezes during the winter. Any one of these environmental factors could similarly affect gene distributions in the Baltic Sea and Norwegian fjords.

Canadian Maritime hybrid zone

In contrast to the regional structure of the Scandinavian hybrid zone, in the western Atlantic there is a large geographical area of sympatry between *M. trossulus* and *M. edulis*. This range overlap extends at least as far north as Hudson Bay, Canada (Koehn *et al.* 1984) and south as far as Little Machias Bay, Maine (Rawson *et al.* 2001). (Given that the zone of sympatry is primarily in Canada, we will refer to this region as the Canadian Maritime hybrid zone). This hybrid zone is best described as a mosaic, where *M. trossulus*, *M. edulis*, and hybrid individuals can be found throughout the region (Bates & Innes 1995; Mallet & Carver 1999; Penney & Hart 1999). The frequency distribution of genotypes follows a bimodal distribution, as shown in Fig. 2, for the Gulf of Maine. Individuals with parental genotypes (i.e. scores of zero or six) are most prevalent (74%–87%; Comesaña *et al.* 1999; Rawson *et al.* 2001), followed by individuals with mostly *M. trossulus* or *M. edulis* alleles (i.e. scores of one and five; backcross hybrids). Among these backcross individuals, *M. trossulus*-backcrosses are more common than *M. edulis*-backcrosses. Mussels with F1-consistent genotypes (heterozygous for both parental alleles at all nuclear loci) are rare (0%–2.5%, Saavedra *et al.* 1996; Rawson *et al.* 2001).

This bimodal distribution of genotypes has been documented throughout the hybrid zone for adult populations (Maine: Rawson *et al.* 2001; Nova Scotia: Saavedra *et al.* 1996; Comesaña *et al.* 1999; Newfoundland: Bates & Innes 1995) and for Newfoundland larval populations (Toro *et al.* 2004). This pattern is also consistent among markers used for species identification, although allozymes (which are not completely diagnostic between species – see Box 2) provide less resolution, particularly between parental and backcross hybrids (see Comesaña *et al.* 1999). MtDNA introgression is also infrequent, as mtDNA (both female and male type) almost always matches the majority nuclear background (Saavedra *et al.* 1996; Comesaña *et al.* 1999). The two species also maintain subtle but distinct morphological differences throughout the region (McDonald *et al.* 1991; Mallet & Carver 1995; Innes & Bates 1999). The rarity of F1 hybrids suggests that there are reproductive barriers between species, nonetheless, given the presence of many

backcross hybrids some interspecific matings must occur and produce fertile offspring.

Jiggins & Mallet (2000) have suggested that bimodal hybrid zones represent situations where speciation is nearly complete, either as a result of selection for prezygotic isolation (reinforcement) or because of prezygotic barriers that arose before secondary contact. In either case, prezygotic isolation is expected. Although Canadian Maritime *M. edulis* and *M. trossulus* have overlapping spawning periods (Toro *et al.* 2002; Maloy *et al.* 2003), interspecific fertilization between *M. edulis* and *M. trossulus* from areas of sympatry is highly inefficient as compared with intraspecific fertilization, implicating some barrier to cross-species fertilization (Rawson *et al.* 2003). Whether or not allopatric *M. edulis* and *M. trossulus* are reproductively isolated is unknown.

Post-zygotic isolation in the hybrid zone has also not been extensively investigated. In the single study where *M. edulis* and *M. trossulus* were experimentally crossed, some hybrids showed deviations from the usual association of male mtDNA with male individuals (Zouros *et al.* 1994b) (see Box 1), but fitness of these and other hybrid individuals was not assayed. Crossing experiments in mussels are difficult given the one-year minimum generation time and the necessity of rearing larvae through their planktonic stage. Nevertheless, the fitness of naturally occurring hybrids could be experimentally tested.

Species segregation by habitat in the Canadian Maritimes

Whereas salinity (or a correlated environmental factor) affects some species-specific gene distributions in Scandinavia, associations between salinity and species distributions have not been explicitly examined in the Canadian Maritime hybrid zone, although the study of Gartner-Kepkay *et al.* (1983) is often cited. They sampled mussels in St. Margaret's Bay, Nova Scotia where populations at the head of the Bay were occasionally subjected to low salinity (no exact values were reported, also see Kenchington *et al.* (2002a) for a description of St. Margaret's Bay), and found substantial genetic differentiation between the head of the Bay populations and oceanic populations (particularly at *Lap*). This paper, however, predated the discovery and description of two separate mussel species in this region and is, thus, difficult to interpret (Koehn *et al.* 1984; Varvio *et al.* 1988). Here, we re-examine the data of Gartner-Kepkay *et al.* with reference to the two species.

In St. Margaret's Bay, there is some evidence for species segregation. In this case, mussel populations at the head of the Bay (sometimes exposed to low salinity) have a greater affinity with *Mytilus edulis* (Fig. 6), the opposite association with that found in Scandinavia. This result is partially caused by *Lap*. Although *Lap*⁹⁴ is at a high frequency in oceanic populations south of Cape Cod (*M. edulis*, Group I, Koehn *et al.*

1984), the frequency of *Lap*⁹⁴ in all estuarine western Atlantic mussel populations is low (see Box 3), including St. Margaret's Bay and for Canadian *M. edulis* in general (Koehn *et al.* 1984; Varvio *et al.* 1988; Comesaña *et al.* 1999). *Gpi* allele frequencies, which are much more useful for species diagnosis, are also *M. edulis*-like at the head of the Bay and *M. trossulus*-like at the mouth, providing greater support for the partitioning of species by habitat. (As with most allozyme studies, Gartner-Kepkay *et al.* only reported overall allele frequencies and not genotypes. Therefore, we were not able to extrapolate the actual proportion of hybrids and parental species.)

Salinity, in St. Margaret's Bay and many other Canadian Maritime locations, is likely to be correlated with wave exposure. Oceanic sites have both high salinity and high wave action, whereas many sheltered sites are also likely to have freshwater input. Wave exposure is also a determinant of species distributions in the European *M. edulis* × *M. galloprovincialis* hybrid zone (where *M. galloprovincialis* are found at high frequencies at wave exposed sites: Gosling & Wilkins 1981; Skibinski *et al.* 1983; Bierne *et al.* 2003a).

Although few studies have directly examined the role of wave exposure, an emerging pattern is that *M. trossulus* are more frequent at locations subject to high wave action (exposed and intertidal), whereas *M. edulis* are more frequent in sheltered (and possibly subtidal) sites. For example, Bates & Innes (1995) surveyed mussel populations from a variety of habitats in Newfoundland, and typed them as *M. edulis*- and *M. trossulus*-like (they used the loci *Ap*, *Est-D*, *Lap* and *Pgm*, which could not discriminate between backcross hybrids and parental species). Significantly more *M. trossulus*-like mussels were found at exposed intertidal sites than sheltered intertidal sites (77% and 34%, respectively, $\chi^2 = 18.5$, d.f. = 1, $P < 0.001$; data taken from Bates & Innes Table 3 and normalized by sample size). The only subtidal site (less wave action than intertidal) had a very low frequency of *M. trossulus*-like mussels (8%). Similarly, the three (natural) subtidal sites sampled by Penney & Hart (1999) also had high proportions of *M. edulis*, especially at Shag Rocks (100% *M. edulis*; *Gpi*, *Mpi*, *Lap* and *Pgm* were used for determining species identity).

Comesaña *et al.* (1999) re-examined exposed and sheltered populations from Chance Cove and Bellevue sites of Bates & Innes (1995) and were able to distinguish between parental and hybrid mussels (using *Est-D*, *Mpi*, *Glu 5'*, *ITS*, and mtDNA). They also found that the frequency of pure *M. trossulus* increased with wave exposure (significantly so for Chance Cove), even though all their sampling sites were subtidal. In Maine, Rawson *et al.* (2001) found higher frequencies of *M. edulis* (55%) at the upriver site of Machias Bay as compared to 37% *M. edulis* in intertidal coastal populations (based on the nuclear DNA markers *Glu 5'*, *MAL-I*, and *ITS*).

Field (Kenchington *et al.* 2002a) and laboratory (Freeman *et al.* 2002) experiments of larval settlement have also been

consistent with the adult distribution patterns described above. At depths greater than 5 m (subtidal), *M. edulis* recruits predominated, whereas, at shallow depths (analogous to intertidal), proportions of the two species were equal (laboratory) or with a majority of *M. trossulus* (field). Freeman & MacQuarrie (1999) also found that presettlement *M. trossulus* larvae maintain higher positions, on average, in laboratory water columns.

In natural populations of larvae and newly settled mussels, *M. trossulus* genotypes are the overall most frequent (Pederson *et al.* 2000; Toro *et al.* 2004). The postsettlement mortality rates of both species, however, may be affected by habitat. Pederson *et al.* (2000) found that there was increased mortality of *M. edulis*-like settlers (relative to *M. trossulus*-like settlers, typed by *Lap* and *Pgm*) at an exposed intertidal site. At two protected subtidal sites, (Toro *et al.* 2004) described a decrease in *M. trossulus* genotypes and an increase in *M. edulis* genotypes (in the 2–15 mm shell length size classes). These patterns of differential post-settlement mortalities are consistent with *M. trossulus* adaptation to exposed sites and *M. edulis* adaptation to sheltered sites. In groups of mussels held subtidally (in conditions used for mussel mariculture), Penney & Hart (2002), however, reported a significant increase in *M. trossulus* *Mpi* alleles at one experimental site. These results are somewhat ambiguous, as no significant changes in allele frequencies were found at the other two experimental sites, and subtidal conditions in mesh culturing bags are quite different from natural habitats.

Although adult *M. trossulus* have the greatest absolute abundance in most natural populations, *M. trossulus* become less abundant in the larger adult size classes (20+ mm shell length) (Comesaña *et al.* 1999; Toro *et al.* 2004). This could be the result of a greater rate of mortality (see discussion in Toro *et al.* 2004) or because of a slower growth rate compared to *M. edulis* (Mallet & Carver 1995). In either case, the outcome is that the frequency of *M. edulis* increases with cohort age.

Many of these studies point to some assortment of *M. edulis* and *M. trossulus* by habitat, but the relative effects of salinity and wave exposure are not clear. Although we have argued that sheltered sites may be periodically exposed to low salinity, it is also true that salinity (and other environmental factors) is more variable in intertidal relative to subtidal locations. To further complicate this matter, experiments that have exposed adult and larval mussels to different salinity regimes have given conflicting results. Gardner & Thompson (2001) showed that *M. trossulus* (typed by *Pgm* and *Est-D*) had lower fitness than *M. edulis* when exposed to low salinity conditions, matching the habitat associations described here. However, Qiu *et al.* (2002) found that early ontogenetic stages of *M. edulis* had lower survivorship in low salinity than *M. trossulus* (typed by *Glu 5'*), a finding that is inconsistent with observed adult distributions.

Moreover, there are some deviations from the overall pattern of species segregation by habitat. For example, Traytown, Newfoundland is a type location for *M. trossulus* (Group III) (Koehn *et al.* 1984; Varvio *et al.* 1988) but is sometimes described as an estuarine site (Gardner & Thompson 2001). Water conditions around Traytown vary and are affected by freshwater runoff (from melting snow and ice). Without knowing exactly where mussels have been collected and their ambient conditions, it is difficult to draw any firm conclusions about species segregation at this and other sites. Similarly, the estuarine (20%–30%) Bras D'Or Lake in Nova Scotia is reputed to have pure *M. trossulus* populations (based on *Mpi*: Mallet & Carver 1999; *Gpi*, *Mpi*, *Lap*, and *Pgm*: Penney & Hart 1999; *Glu 5'*: Qiu *et al.* 2002), however, a recent collection at this site found a high proportion of individuals with *M. edulis* alleles (*Glu 5'*, *ITS*, and *MAL-I*: Yund & Slaughter, pers. comm.). These conflicting results highlight the importance of describing habitat conditions at collecting sites. If *M. edulis* and *M. trossulus* are adapted to different habitats, then species segregation by habitat may help explain how they maintain genetic distinctiveness in sympatry. Also, identifying locations with pure species is of considerable interest to mussel farmers, given that commercially cultured *M. edulis* yield a superior product because of their larger size and faster rate of growth (Mallet & Carver 1995; Penney & Hart 1999; Penney *et al.* 2002).

Whether and how habitat affects species distributions in the Canadian Maritimes remains to be determined. More studies are needed to explicitly test the possible effects of wave exposure and salinity. In addition, markers that have the capacity to discriminate between parental and hybrid individuals should be utilized to provide greater resolution. It is clear, however, that both environmental factors and species distributions vary over microgeographical scales typical of mosaic hybrid zones. While species transitions between the Skagerrak and Baltic Sea encompass a larger geographical area (> 100 km), so do the relevant environmental conditions. In both hybrid zones, habitat features appear to influence species and species-specific gene distributions.

Why are the two hybrid zones so dissimilar?

Patterns of hybridization between *Mytilus edulis* and *Mytilus trossulus* are quite different in the western and eastern Atlantic. In the Canadian Maritimes, the two species largely maintain their independent genetic integrities. But in Scandinavia, gene introgression has been so extensive that there are no remaining pure *M. trossulus* mussels, just remnants of their original genome in Baltic Sea populations. The different patterns of introgression in these two hybrid zones could represent alternative possible outcomes (possibly resulting from local adaptation) or simply result from difference in age of secondary contact.

At present, it is not possible to precisely date the appearance of *M. trossulus* in the Canadian Maritimes or Scandinavia (and date the subsequent secondary contact with *M. edulis*). Both female and male mtDNA surveys suggest that *M. trossulus* has been in the western Atlantic since before the last glacial maximum (Rawson unpublished; Riginos unpublished). Because eastern Atlantic *M. trossulus* have lost their native mtDNA, it is not possible to use mtDNA to infer the history of the *M. trossulus* component of Baltic mussel genomes.

Allozymes show Baltic Sea populations (Fig. 1: populations 5 and 6) to be more diverged from Pacific populations (populations 1–3) than Canadian Maritime populations (population 4). At face value, this greater divergence would imply an older age of colonization from the Pacific and is consistent with the Baltic Sea population representing an Atlantic relict of a formerly circum-Arctic *M. trossulus* distribution (Koehn 1991; Väinölä & Hvilson 1991). But, introgression of *M. edulis* alleles or directional selection would also lengthen the branch connecting the Baltic Sea population to other *M. trossulus* populations. Indeed, if many of the allozyme loci used to build the tree shown in Fig. 1 have been affected by selection and genetic hitch-hiking, rather than drift, then this tree may inaccurately represent histories among several taxa (but relationships among species are confirmed by other loci – see Introduction). Sequences of nuclear genes or rapidly evolving microsatellite markers may be helpful for reconstructing the biogeographical history of *M. trossulus* in Scandinavia.

If the Scandinavian hybrid zone is older, then the extensive gene introgression could simply be a consequence of the prolonged period of secondary contact. If this is the case, recurrent gene flow has eroded the reproductive isolating mechanisms that appear to still operate in the Canadian Maritimes. Alternatively, if the Canadian Maritime hybrid zone is older, maybe there have been more opportunities for prezygotic isolation to evolve (*sensu* Jiggins & Mallet 2000). Resolving the relative ages of the hybrid zones and genetic barriers to introgression will be informative for generally understanding the evolution of reproductive isolation between mussel taxa.

Whether or not the two hybrid zones differ in age, evidence points to local adaptation of Scandinavian *M. trossulus* to brackish conditions. In this scenario, the first mussels to colonize the Baltic, following establishment of the marine connection through the Kattegat ~7500 years ago (Donner 1995), were probably *M. trossulus* (Varvio *et al.* 1988; Väinölä & Hvilson 1991). As salinity gradually decreased, these mussels became adapted to the ambient brackish waters. Although secondary contact between *M. trossulus* and *M. edulis* could have followed Baltic colonization, it is also possible that the hybrid zone is older and has shifted position following the range expansion of *M. trossulus* into the Baltic Sea. In either case, we propose that the allozyme loci maintaining the *M. trossulus* characteristic alleles either

were directly the targets of selection or linked to genes for low salinity adaptation. In the western Atlantic, *M. trossulus* seem not as well adapted to low salinity as the native *M. edulis*, although species segregation by salinity (and other environmental factors) needs further investigation before clear conclusions can be drawn.

Both the apparent conflicting patterns of species segregation in the two hybrid zones and the fact that both *M. trossulus* and *M. edulis* can withstand brackish waters outside the hybrid zones (Koehn *et al.* 1980b; Sarver & Foltz 1993; Gardner 1994; Matson *et al.* 2003) argue against ecological assortment of species resulting from traits that predate secondary contacts. Again, knowing the age and history of *M. trossulus* in the Atlantic will further our understanding of adaptation to local habitats.

Conclusions

Regardless of relative ages, the contrast between the two *Mytilus edulis* × *Mytilus trossulus* hybrid zones should be useful for studying the dynamics of gene introgression, genome integrity, and mechanisms of mtDNA inheritance. For example, what is the source and strength of reproductive isolation in the Canadian Maritimes and is there any reproductive isolation between *M. edulis* and Baltic mussels? Are some portions of the species' genomes porous to introgression in both hybrid zones? Do breakdowns in DUI of mtDNA inhibit hybridization in the Canadian Maritimes? Is the complete introgression of *M. edulis* mtDNA into the Baltic connected with rampant nuclear gene introgression? These and many other issues await examination.

Many of our conclusions regarding the exact patterns and dynamics of hybridization depend on the markers used for identifying species (or the species origin of genomic segments). Neutrality of allozymes may be sometimes suspect, given that many of the most popular loci (such as *Gpi*, *Mpi*, *Pgm*) have been chosen because of their polymorphism among diverse taxa. In the case of mussels, these loci have been frequently used precisely because different frequencies of alleles are found between species. In addition, the possibility of linkage among many diagnostic allozyme loci (*Est-D*, *Gpi*, *Lap*, *Mpi*, and *Odh*) means that many studies could be looking only at a few linkage groups rather than assessing genome-wide patterns. This should be especially worrisome given that *Est-D* and *Odh* alleles covary and are strongly correlated with growth and feeding rate (in the European *M. edulis* × *Mytilus galloprovincialis* hybrid zone; Hilbish *et al.* 1994) and *Lap* allele frequencies are affected by salinity (Koehn *et al.* 1980b; Hilbish *et al.* 1982). Thus, some members of a possible linkage group are candidate targets of selection. In addition, many of the other allozymes used in *Mytilus* genetics (not just *Lap*) are genes involved in protein catabolism and glycolysis (e.g. *Aap*, *Ap*, *Gpi*, *Mpi*, and *Pgm*) and thus are likely to experience different selective

pressures, especially under different salinity regimes. Experimental crosses to determine recombination rates among loci (nDNA as well as allozyme) are needed to resolve this issue.

On the other hand, if mussel allozyme loci are targets of selection, then mussels provide a wonderful system in which to determine how genes respond to the environment and hybridization (see Hilbish *et al.* 1994). In the case of *M. edulis* × *M. trossulus* hybridization, some allozyme loci segregate by species group in response to salinity (or correlated factors). The evidence for this is strongest for *Gpi* and *Pgm* in Scandinavia, but there is sufficient evidence for species segregation by salinity and wave exposure in the Canadian Maritimes to merit further investigation. Clearly, much work is needed to irrefutably establish the action of salinity-induced selection at specific loci. Nevertheless, in the two *M. edulis* × *M. trossulus* hybrid zones we have the rare opportunity to study the effects of selection on multiple genes and to elucidate two different solutions to the challenges of living in brackish water.

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Supplementary material

Supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2379/MEC2379sm.htm>

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Cynthia Riginos studies how historical events and natural selection contribute to population and species differences in marine organisms. One of her ongoing projects is to determine how Baltic Sea mussels have become adapted to an extremely low salinity environment. Cliff Cunningham is generally interested in the evolution and biogeography of marine invertebrates, particularly those residing in the North Atlantic.

Appendix

A common framework for synthetic analyses and specific methods

In this paper, we present analyses using data taken from multiple studies, most of which surveyed allozyme electrophoretic variation. Differences in electrophoretic conditions (buffers, running duration, etc.) can affect resolution among allozyme alleles, leading to slight differences in allele numbers described by different studies. Also, investigators often assign different names to the same alleles. In order to create a unified dataset, we compared studies that had sampled from similar locations outside of hybrid zones. From these locations, it was relatively straightforward to determine which alleles were the same across studies, although usually it was necessary to resolve minor ambiguities by binning some alleles. The original data are given in the supplemental material, which also show how alleles were binned on a per-locus basis. Table 1 presents the resultant core dataset employed in most analyses.

In addition, we gathered new data for the DNA loci *Glu 5'*, *ITS*, *MAL-I*, and *PLIIa* (Table 2) as described in Riginos *et al.* (2002). Mussels were sampled from representative *M. trossulus* (Penn Cove, WA; Pacific Coast of the USA) and *M. edulis* (Woods Hole, MA; Atlantic Coast of the USA) populations and from either side of the Scandinavian hybrid zone; Tjärnö, Sweden in the Skagerrak and Askö, Sweden in the Baltic Sea. We also include the loci *EFbis* and *mac-1* from Bierne *et al.* (2003b) with Hog Island (Pacific Ocean) and Helgoland (North Sea) as representative *Mytilus trossulus* and *Mytilus edulis* populations and Flødevigen (Skagerrak) and Gdansk (Baltic) from the hybrid zone.

All allozyme trees were constructed with the Cavalli-Sforza chord measure, implemented in the GENDIST program in PHYLIP 3.573 (Felsenstein 1993). This measure is most appropriate where the major differences between populations are frequency differences and not presence or

absence of alleles. The outfile was analysed with NEIGHBOUR to create neighbour-joining and UPGMA phenograms, which had the same topology in all cases (UPGMA phenograms are shown in figures).

Differences in allozyme frequencies were measured with the metric Δp of Barton (2000), following Bierne *et al.* (2003b). The measure has the advantage of giving the same value under different binning strategies, as long as allelic classes that are binned behave in a similar manner. For example, if two alleles are both more frequent in species 1 than species 2, then Δp is the same whether or not the two alleles are binned or treated as two separate alleles. In addition, we measured genetic distances using Nei's F_{ST} (1986), both by reducing multiallelic data to two alleles, \hat{F}_{ST} , and by taking an unweighted average of F_{ST} for each locus, $\hat{F}_{ST(u)}$. These measures are appropriate ways to compare multiallelic data such as allozymes to biallelic data, such as several of the nDNA markers used in this paper (McDonald 1994). All three genetic distance measures yielded similar results to that shown in Fig. 4.

The coalescent estimations of admixture proportions were obtained using the program LEA (likelihood-based estimation of admixture, Chikhi *et al.* 2001). LEA uses Markov Chain Monte Carlo sampling to give Bayesian posterior probabilities of allele proportions at the time of admixture. The prior distribution of allelic frequencies in parental populations and parental contributions to the admixed population cover all possible histories under a simple model of one-time admixture. For each analysis, a chain of 200 000 steps was run, following a burn-in of 50 000 steps. Tillamook, North Sea, and Tvärminne were used as representative populations of *M. trossulus*, *M. edulis*, and the Baltic Sea, respectively, with data from our Tables 1 and 2 and from Table 1 of Bierne *et al.* (2003b). Because McDonald *et al.* (1991), the source for Tillamook frequencies, did not resolve *Odh*¹⁰⁰ and *Odh*¹⁰⁵, we assumed allele frequencies of 50% for *Odh*¹⁰⁵ and 0% for *Odh*¹⁰⁰ for Tillamook. Analyses were repeated completely excluding *Odh*, with no effect on the results.

Table 2 Frequencies of *Mytilus edulis*-type alleles for DNA loci^a

Locus	<i>M. edulis</i> Atlantic USA (MA)	<i>M. trossulus</i> Pacific USA (WA)	Tjärnö Skagerrak	Askö Baltic Sea	Δp Skagerrak-Baltic
<i>Glu 5'</i>	1	0	0.95	0.75	0.20
<i>ITS</i>	1	0	1	0.68	0.32
<i>MAL-I</i>	1	0	1	0.30	0.70
<i>PLIIa</i>	1	0	0.95	0.77	0.22

^aTwenty mussels were sampled from each population..