

## Letter to the Editor

### Evidence for Selection at Multiple Allozyme Loci Across a Mussel Hybrid Zone

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The frequency and strength with which selection shapes patterns of genetic variation is unknown. Whereas all loci should be roughly equally affected by demography and population history, selected loci may exhibit increased or decreased genetic differentiation relative to neutral loci (Cavalli-Sforza 1966). Thus, one way to test for selection on a specific category of loci is to compare geographic differentiation of this particular category relative to a category presumed to be neutrally evolving (McDonald 1994; McDonald, Verrelli, and Geyer 1996). A number of recent studies have followed this approach, in particular comparing patterns of genetic differentiation at allozyme to nonallozyme loci. Where discordances between allozymes and other nuclear markers have been reported, less genetic partitioning has been observed for allozymes, consistent with balancing selection reducing differences among geographical populations (e.g., Karl and Avise 1992; Pogson, Mesa, and Boutilier 1995; Latta and Mitton 1997). Here, we report the reverse situation; geographic partitioning is greater at allozymes relative to nonallozyme loci across a mussel hybrid zone.

Northern Hemisphere populations of the mussels *Mytilus trossulus* and *Mytilus edulis* are distinguished by nearly fixed allozyme allele frequency differences and some morphometric differences (McDonald, Seed, and Koehn 1991). *Mytilus edulis* is found throughout the northern Atlantic, whereas *M. trossulus* is found in three disjunct regions: the Pacific, Atlantic North America, and the Baltic Sea. A northern European hybrid zone between the Atlantic *M. edulis* and the Baltic Sea *M. trossulus* has been well described, based on allozyme surveys. A relatively sharp transition in mussel allozyme allele frequencies (over 100 km) occurs at the mouth of the Baltic with *edulis*-like alleles predominating in the Atlantic populations and *trossulus*-like alleles predominating in the Baltic. This pattern is particularly distinct for *Aap*, *Est-D*, *Gpi*, *Lap*, *Mpi*, and *Pgm* (Theison 1978; Bulnheim and Gosling 1988; Varvio, Koehn, and Väinölä 1988; Väinölä and Hvilson 1991), and these loci, with the exception of *Lap*, are diagnostic between *M. edulis* and *M. trossulus* throughout the Northern Hemisphere (Varvio, Koehn, and Väinölä 1988; McDonald, Seed, and Koehn 1991). Rapid clinal transitions in allele frequencies, in combination with significant linkage disequilibria (particularly involving *Gpi*, *Lap*, and *Pgm*), have supported the idea that this hybrid zone represents

a situation of secondary contact between pure populations of *M. edulis* and *M. trossulus* (Väinölä and Hvilson 1991). Recent mtDNA surveys, however, have found that all northern European *Mytilus*, including Baltic mussels, are fixed for *M. edulis*-type mtDNA such as might result from asymmetric mtDNA introgression into Baltic *M. trossulus* populations (Wenne and Skibinski 1995; Rawson and Hilbish 1998; Quesada, Wenne, and Skibinski 1999). Thus, mtDNA and allozymes portray conflicting information regarding the ancestry of Baltic mussels.

Here, we ask whether Baltic mussels represent a case of asymmetric (*M. edulis*) mtDNA introgression into an otherwise pure population of *M. trossulus*, or alternatively, whether the mtDNA introgression was accompanied by multiple *M. edulis* loci introgressing into the Baltic *M. trossulus* population. We employed four nuclear DNA markers that are diagnostic between *M. trossulus* and *M. edulis*: *Glu 5'* (Rawson, Joyner, and Hilbish 1996), *ITS* (Heath, Rawson, and Hilbish 1995), *MAL-I* (Rawson, Secor, and Hilbish 1996), and *PLIIa* (Heath, Rawson, and Hilbish 1995). *Glu 5'* and *PLIIa* primers target protein coding regions (*Glu 5'*: polyphenolic adhesive protein; *PLIIa*: protamine-like sperm packaging protein), whereas *ITS* primers amplify the *ITS-1*, *5.8S*, and *ITS-2* regions of rDNA, and *MAL-I* primers amplify the intron of a protein coding region of unknown function. *Glu 5'* primers produce species-specific-sized PCR products, whereas *ITS*, *MAL-I*, and *PLIIa* PCR products are digested with restriction enzymes to yield species-specific DNA fragments. These four markers were scored from 29 Baltic mussels collected from Håanko, Finland, a pure *M. trossulus* population based on allozymes (Väinölä and Hvilson 1991).

In marked contrast to allozyme patterns, all of these markers show the majority of the Baltic alleles to be of *M. edulis* origin, with the estimated *M. edulis* allele frequencies ranging from 37%–75% (table 1). The high frequency of *M. edulis* alleles from Håanko refutes the idea that the Baltic mussels represent a pure *M. trossulus* population. Similarly, a survey of *Glu 5'* (one of the four markers employed in the present study) from a Gdansk, Poland population found high frequencies of *M. edulis* alleles (54%, Borsa et al. 1999). This observation of moderate to high frequencies of *M. edulis* nonallozyme loci points to extensive *M. edulis* introgression at nonallozyme nuclear loci into the Baltic mussel populations.

Multilocus genotypes also show extensive *M. edulis* introgression and are consistent with many generations of hybridization. Multilocus genotypes of individuals for our Baltic sample (excluding the dominant *PLIIa* locus) found two (out of 29 total) pure *M. edulis* individuals and no pure *M. trossulus* individuals. Several (14) individuals had F2-type genotypes and back-

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**Table 1**  
**Frequencies of *edulis*-type Alleles in Baltic Mussels for Loci Diagnostic Between *M. trossulus* and *M. edulis***

Marker	Locus	<i>n</i>	<i>M. edulis</i> Allele Frequency <sup>a</sup> (%)	Reference
Allozyme . . . . .	<i>Aap</i>	70	0	(Gosling 1992)
	<i>Est-D</i>	120	3	(Väinölä and Hvilson 1991)
	<i>Gpi</i>	315	2	(Väinölä and Hvilson 1991)
	<i>Mpi</i>	156	11	(Väinölä and Hvilson 1991)
	<i>Pgm</i>	160	3	(Väinölä and Hvilson 1991)
MtDNA . . . . .	<i>16S</i>	14	100	(Rawson and Hilbish 1998)
Nuclear. . . . .	<i>Glu 5'</i>	28	75	Present study
	<i>ITS</i>	27	70 <sup>b</sup>	Present study
	<i>MAL-I</i>	27	37	Present study
	<i>PLIIa</i>	29	72 <sup>c</sup>	Present study

<sup>a</sup> Allozyme loci have multiple alleles, and there are no complete fixed differences between allopatric *M. edulis* and *M. trossulus* populations. The allele frequencies presented here are the frequencies of the single allele that most typifies all Northern Hemisphere *M. edulis* populations. The low frequencies of *edulis*-like alleles among the Baltic mussels are similar to frequencies found in all pure *M. trossulus* populations. The total summed frequencies of all non-*M. trossulus*-type alleles (including alleles that are not fixed between any *M. edulis* and *M. trossulus* populations) are 8%, 21%, 10%, 23%, and 22%, respectively.

<sup>b</sup> *ITS* is a multicopy gene so that more than two alleles were undoubtedly PCR amplified from each individual. Although individual hybrid mussels have different proportions of *M. edulis*- and *M. trossulus*-type alleles, *ITS* allele frequencies were calculated as if each hybrid individual had equal proportions of the two allelic types.

<sup>c</sup> *PLIIa* is a dominant marker for the *M. trossulus* allele; to estimate the frequency of recessive *M. edulis* alleles, a Hardy-Weinberg equilibrium was assumed.

cross individuals were predominantly *edulis*-like (10 *edulis*-like, 3 *trossulus*-like). Thus, from the four loci examined here, many Baltic mussels carry both *M. edulis* and *M. trossulus* alleles, and *edulis*-like genotypes are at a higher proportion than *trossulus*-like genotypes. We tested for deviations from Hardy-Weinberg expectations for *Glu 5'* and *MAL-I*, the two codominant single locus markers, and found a significant deficit of heterozygotes for *Glu 5'* (tested following Guo and Thompson 1992,  $P < 0.001$ ) and no deviations from Hardy-Weinberg for *MAL-I*. The low frequencies of *Glu 5'* hybrids may indicate selection against hybrids at *Glu 5'* or a linked locus, although no deviations from Hardy-Weinberg were detected for *Glu 5'* among mussels from Gdansk (Borsa et al. 1999).

In addition to frequency-based estimates of *M. edulis* introgression at nuclear loci, we used a genealogical approach to verify that individual Baltic mussels carry both *M. edulis*- and *M. trossulus*-derived alleles. The *ITS* regions of rDNA were PCR amplified using the *ITS* primers described above (Heath, Rawson, and Hilbish 1995) with high fidelity polymerase (Expand Hi-Fidelity Polymerase, Roche). We sampled from a Baltic population (Hänko, Finland,  $n = 5$ ), two pure *M. edulis* populations (Trondheim, Norway,  $n = 5$ ; Wood's Hole, Mass.,  $n = 2$ ), a pure population of *M. trossulus* (Coveville, Wash.,  $n = 5$ ), and four individuals from an *M. trossulus*-*M. edulis* hybrid zone in Canada identified by our markers as pure *M. trossulus* individuals (Wolfville, Nova Scotia, Canada,  $n = 3$ ; North Harbor, Newfoundland, Canada,  $n = 1$ ). PCR products were TA cloned (Invitrogen), and an average of 10 clones per individual was sequenced to give 449 basepairs of sequence, including *ITS-1* and 5.8S regions. Because rDNA has multiple copies, much of the within-individual variation is likely caused by imperfect gene conversion and is larger than expected from simple polymerase error. A subset of representative sequences was aligned with ClustalX (Thompson et al. 1997), and the remainder was manually aligned to the Clustal alignment. Sequences have

been deposited in GenBank (accession numbers AF440869–AF441078).

Because there was considerable ambiguity in the alignment, phylogenies were estimated both including (fig. 1) and excluding (not shown) gapped regions. Kimura's (1980) model of substitution with a gamma rate distribution was identified by MODELTEST (Posada and Crandall 1998) as the most appropriate model for our *ITS* sequences. The ratio of transitions to transversions and the gamma parameter were estimated from a parsimony tree and used as the input values for a maximum likelihood search (under a Kimura 1980 with gamma model). A heuristic search with the tree-bisection-reconnection method in PAUP\* (Swofford 1998) found 27 equally likely trees when gapped regions were included and one likely tree when gaps were excluded. All trees, both including and excluding gapped regions, found reciprocally monophyletic lineages for *M. edulis* and *M. trossulus*, with Baltic alleles falling unambiguously in both the *M. edulis* and *M. trossulus* clades. Thus, the *ITS* gene tree points to hybrid origins of the Baltic mussel population. Three Baltic individuals had one *M. trossulus* allele each, whereas all five had multiple *M. edulis* alleles (fig. 1). Hence in this sample of *ITS* from five Baltic individuals, there were two pure *M. edulis* individuals, three hybrids, and no pure *M. trossulus*.

It is notable that even in the three hybrid individuals, we found many distinct *M. edulis* alleles (beyond what is expected from polymerase error) but only found one *M. trossulus* allele each in three hybrid individuals. Similarly, *M. trossulus* bands, for the majority of individuals scored as hybrids, were substantially less intense on ethidium bromide-stained agarose gels than *M. edulis* bands. Although the high proportion of *M. edulis* alleles could be explained by the preferential amplification of *M. edulis* alleles from the tandem arrays, it may truly reflect a small proportion of *M. trossulus* alleles in hybrid genomes. If Baltic mussels do contain fewer *M. trossulus* *ITS* copies relative to *M. edulis* cop-

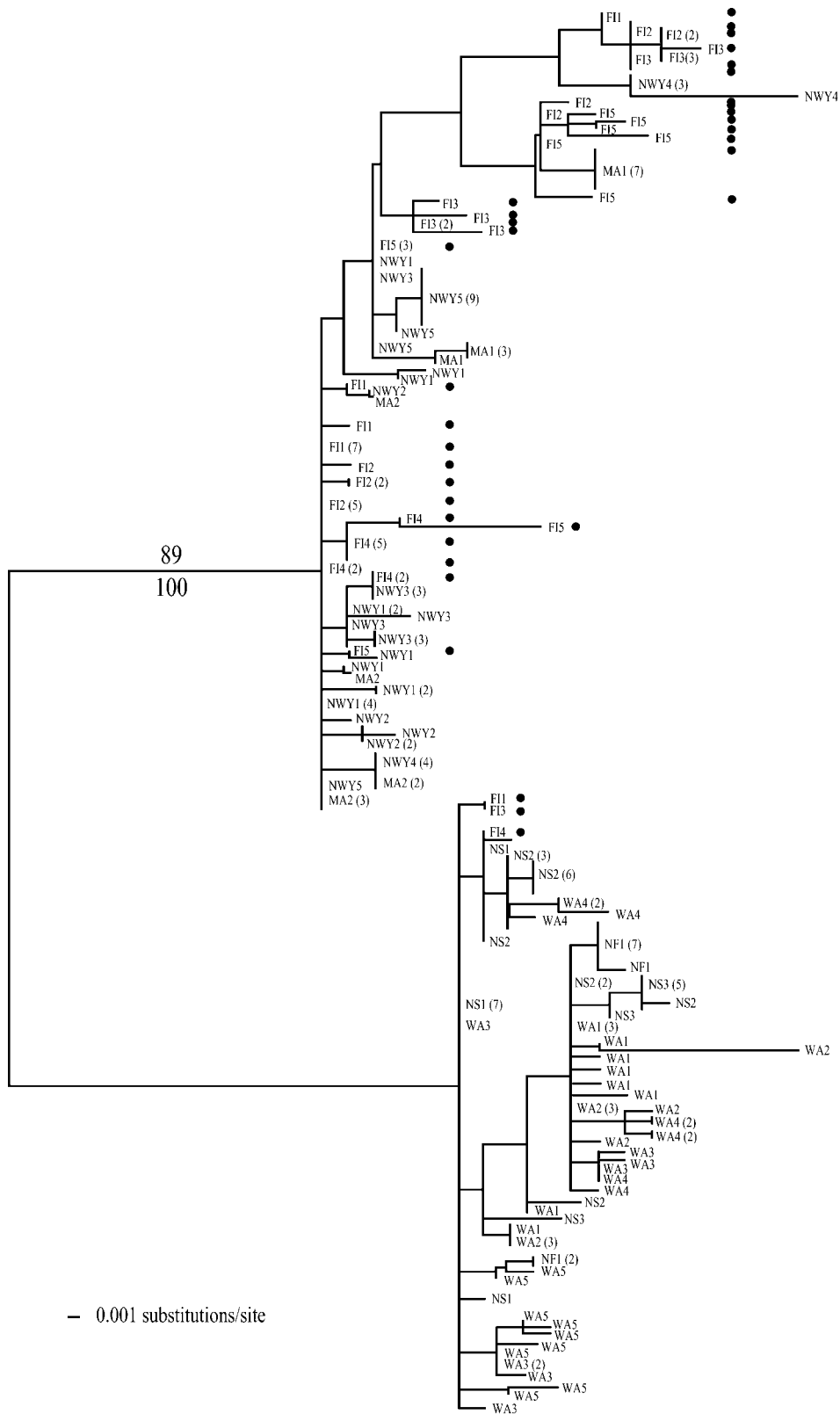


FIG. 1.—Maximum likelihood tree from *ITS* clones. This is one of 27 equally likely trees under a Kimura (1980) model of substitution with a gamma rate distribution and gapped sites included. Clones from each mussel are labeled by geographic origin of the mussel (FI: Finland; MA: Massachusetts; NF: Newfoundland; NS: Nova Scotia; NWY: Norway; and WA: Washington State) and also assigned a unique number for that location. Where multiple identical clones are included from the same mussel, the number of identical clones is given in parentheses. For example, MA1 (7) refers to seven identical clones from MA individual one. Mussels from MA and NWY are pure *Mytilus edulis*, and mussels from NF, NS, and WA are pure *M. trossulus*. The two major clades corresponding to *M. edulis* and *M. trossulus* alleles are well supported with 89% bootstrap support using parsimony (above branch; 1,000 replicates) and 100% consistency (below branch) among all 27 maximum likelihood trees. Filled circles indicate alleles from Finnish mussels.

ies, this would be further evidence of long-term introgression by *M. edulis* alleles.

In summary, mtDNA and all nonallozyme nuclear markers examined in the Baltic mussels show that there has been extensive introgression of the *M. edulis* genome into the Baltic population of *M. trossulus*. In contrast, there has been no apparent evidence for extensive *M. edulis* introgression among previously published allozyme surveys (e.g., Theison 1978; Bulnheim and Gosling 1988; Varvio, Koehn, and Väinölä 1988; Väinölä and Hvilson 1991; Wenne and Skibinski 1995) where mussels were collected from 1976 (Theison 1978) to 1991 (Wenne and Skibinski 1995). There is no reason to suspect that allozyme frequencies at Hånko or any other Baltic location have changed in the last decade, although we cannot currently exclude this possibility. If the allozyme allele frequencies given in table 1 are representative of current allele frequencies at Hånko, then the probability that all four nuclear DNA loci have higher *M. edulis* allele frequencies than the five diagnostic allozyme loci is less than 0.008 (based on 126 possible combinations of 4 items from 9 total loci).

Taken at face value, the strong discordance between allozyme and nonallozyme markers can only be explained by selection acting on some loci. Although *M. edulis* mtDNA and other (nonallozyme) nuclear loci could, in principle, have some selective advantage over native *M. trossulus* loci, it is more likely that multiple *M. trossulus* allozyme loci, which are involved in metabolic functions, have been selectively maintained among the Baltic mussels. Cohesion of the *M. trossulus* allozyme phenotype may be caused by either the coadaptation of *M. trossulus* genes to each other or by independent local adaptation. Salinity in the Baltic is very low, and strong allozyme differentiation between Atlantic and Baltic populations has been observed for several osmoconformers (such as *Mytilus*) but not for osmoregulators (reviewed by Bulnheim and Gosling 1988; Väinölä and Hvilson 1991). Thus, it seems probable that the Baltic environmental conditions affect the performance of mussel allozyme alleles and may act to keep *M. trossulus*-like alleles in high frequencies among the Baltic mussel populations.

Whatever the cause of selection on allozyme loci, the discordant patterns of allozyme and nonallozyme introgression across the Baltic mussel hybrid zone presents a qualitatively different pattern than other examples of discordance between allozymes and other nuclear markers. In American oysters (Karl and Avise 1992), Atlantic cod (Pogson, Mesa, and Boutilier 1995), and limber pine (Latta and Mitton 1997), allozymes show less differentiation among geographically distant populations than nonallozyme markers, consistent with balancing selection maintaining similar allozyme allele compositions among populations. Here, allozymes are more differentiated between the Baltic *M. trossulus* and Atlantic *M. edulis* populations than nonallozyme loci. Although the patterns are qualitatively different, these studies point to the same conclusion: selection may frequently shape patterns of genetic variation at multiple allozyme loci.

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