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Nuclear-DNA evidence that northeastern Atlantic *Mytilus trossulus* mussels carry *M. edulis* genes

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The northeastern (NE) Atlantic region harbours three *Mytilus* L. species that have been identified on the basis of both morphological and allozyme data.^{1,2} The blue mussel, *M. edulis* L. ranges from the White Sea to the Bay of Biscay.^{2,3} It is reportedly absent from the Baltic Sea.^{4,5} *M. galloprovincialis* Lmk. is the only *Mytilus* species reported south of the French-Spanish border in Biscay (that is on the Atlantic coast of the Iberian peninsula, in northwestern (NW) Africa and in the Mediterranean^{2,3,6-9}). The range of *M. galloprovincialis* also extends northwards where it overlaps with that of *M. edulis*. The zone of overlap extends as far as the British Isles.^{1,10,11} *M. trossulus* Gould is apparently confined to the Baltic Sea where it has been identified on the basis of allozyme data.^{4,5,12}

While variable rates of admixture and introgression characterise *Mytilus* assemblages in the about 2000-km wide zone of contact between *M. edulis* and *M. galloprovincialis*,^{8,10,11} the Øresund region at the entrance of the Baltic Sea is the place of a sharp transition between *M. edulis* and *M. trossulus*, with a smooth North-to-South introgression gradient at allozyme loci over a geographic distance of about 100 km.⁵ Twelve of the 22 enzyme loci that were used to characterise North Sea (Skagerrak-Kattegat) *M. edulis* and Baltic Sea *M. trossulus* were strongly differentiated, 5 of which were nearly diagnostic between the two species.⁵

More recent, specific-polymerase chain reaction (PCR) investigations of genetic variability at a nuclear locus encoding the mussel polyphenolic adhesive protein, *Glu-5'*,^{13,14} showed that individuals of smooth-shell *Mytilus* species could be characterised by private alleles, one each for *M. galloprovincialis* from the Mediterranean and from California and for *M. trossulus* from the NE Pacific, and two for *M. edulis* from the NW Atlantic.¹⁴

Here we characterised *Glu-5'* allele polymorphism (Figure 1; Table 1) in *Mytilus* mussels from Gilleleje, Kattegat (56°08'N, 12°17'E); Sète, western Mediterranean (43°25'N, 03°44'E) and Gdansk, Baltic Sea (54°28'N, 18°34'E). Skagerrak or Kattegat samples have been used as

reference *M. edulis* in a number of allozyme^{5,12,15} and nuclear-DNA¹⁶ surveys of genetic variation; similarly, Sète samples have been used as reference *M. galloprovincialis*^{8,14,16,17}; samples from the Bay of Gdansk in the Baltic Sea have been used as reference *M. trossulus* in mitochondrial (mt) DNA¹⁸ and anonymous nuclear DNA¹⁹ surveys. We analysed additional mussel samples collected from two locations in northern California [Bodega Bay (38°20'N, 123°03'W) and Hog Island (38°17'N, 123°01'W)], a region where *M. trossulus* and introduced, *M. galloprovincialis* are known to co-occur and hybridise.²⁰⁻²²

Table 2 reports *Glu-5'* genotype frequencies in all 5 samples of the present study together with 3 reference samples from Ref. 14. Northeastern Atlantic *M. edulis* (sample Gilleleje) exhibited alleles characteristic of NW Atlantic *M. edulis* (sample Lewes, DE of Ref. 14) that is *E* at high frequency and *E'* at lower frequency. Mediterranean (Sète) *M. galloprovincialis* were characterised by allele *G*, but allele *E* was also detected. This suggests possible secondary contact, perhaps owing to the recent introduction to Sète of NE Atlantic *M. edulis* as spat attached to imported juvenile oysters, *Crassostrea gigas* Thunberg. One sample from California (Bodega Bay) exhibited mostly *M. galloprovincialis* alleles, but one individual was a *GT* heterozygote indicating that *M. galloprovincialis Glu-5'* genes have introgressed into *M. trossulus*. The other Californian sample (Hog Island), collected a few tens of km apart, consisted of a mixture of *GG* and *TT* homozygotes suggesting that pure *M. galloprovincialis* and pure *M. trossulus* were sampled on Hog Island, but not their hybrids. These results accord with previous reports on the geographic patchiness of the introgression of introduced *M. galloprovincialis* into native *M. trossulus* in the NE Pacific.²⁰⁻²² Allele *T* characteristic of NE Pacific *M. trossulus* (sample Newport, OR of Ref. 14) was common in the Baltic Sea sample but surprisingly, alleles *E* and *E'* characteristic of NE and NW Atlantic *M. edulis* were also present at high frequency. This was not expected on the view that smooth-shell *Mytilus* species are genetically homogeneous across vast distances and clearly differentiated from each other.^{1,2,12,15,20} The distribution of genotypes in sample Gdansk were in accordance with Hardy-Weinberg

expectations with an estimate of f (\equiv Wright's F_{is} ²³) = 0.010, not significantly different from 0 as indicated by permutation tests.²⁴ This result suggests strong introgression of *M. edulis* alleles into Gdansk *M. trossulus* at locus *Glu-5'*.

We assumed that geographic variation within *M. trossulus* is weak, but is this assumption correct? Actually, heterogeneity in allozyme frequencies and also significant differences in gene diversity were detected when making comparisons across *M. trossulus* samples from the Baltic Sea, the NW Atlantic and the NW Pacific, while allozyme frequencies in a sample from Gdansk Bay¹⁸ were very close to other Baltic Sea samples⁵ (Table 3). Nevertheless, the important point here is that across-ocean genetic variation (as estimated using Nei's G_{st} ²⁵) at locus *Glu-5'* by far exceeded that of allozymes (Table 3). Two additional observations support the view that in contrast to other *M. trossulus* populations, Baltic Sea *Mytilus* possess *M. edulis* characters: PCR using specific primers for the actin gene *mac-1* intron 1^{16,17} yielded DNA fragments whose size is characteristic of *M. edulis*, in the Gdansk sample but not in the Hog Island sample (our unpublished data); Gdansk Bay and other Baltic Sea *M. trossulus* also possess mtDNA that is much more similar to NE Atlantic *M. edulis* mtDNA than to the mtDNA of NW Atlantic or NE Pacific *M. trossulus*.^{18,26,27}

Thus, nuclear-DNA and possibly mtDNA markers reveal the presence of *M. edulis* genes in Gdansk Bay *Mytilus*, a result that was not apparent from earlier allozyme studies.^{5,18,20} We can offer the following tentative explanations for this finding. (1) We haphazardly sampled the progeny of *M. edulis* x *M. trossulus* crosses in Gdansk Bay. This supposes that *M. edulis* has been introduced to the Gdansk region where it has survived until reproduction and has introgressed *M. trossulus*. Such successful cases of accidental or on-purpose introduction of smooth-shell *Mytilus* spp. have been reported elsewhere.^{20-22,28,29} We have not independently tested this hypothesis by using allozyme markers. However, this is challenged by the following observations: while hybridisation of *M. trossulus* with introduced *M. galloprovincialis* has been documented in California (Ref. 20-22,28; present study) and in Japan,²⁹ it is not clear whether

M. trossulus x *M. edulis* hybridisation naturally occurs in the NW Atlantic^{30,31} or in the NE Pacific²⁸; also, the narrowness of the Øresund hybrid zone between *M. edulis* and *M. trossulus*⁵ may well be explained by strong selection against hybrid genotypes; samples of individuals with *M. edulis* allozyme genotypes experimentally transferred from the Skagerrak to the Baltic Sea underwent catastrophic mortality^{32,33} showing that the extreme environment of the Baltic Sea is lethal to NE Atlantic *M. edulis*. (2) A second hypothesis is that the *M. edulis* / *M. trossulus* hybrid zone is more permeable to *Glu-5'*, *mac-1* or mtDNA than to allozyme, genes. As a corollary to this, the maintenance of a narrow hybrid zone at allozyme loci would be due to strong selection against the latter while other genes including *Glu-5'* and mtDNA would spread across the hybrid zone because they would be weakly counter-selected or not counter-selected. Selection against allozyme loci as a group, if it occurs, might well be enhanced by linkage since two (*Est-D*, *Mpi*), possibly three (adding *Gpi*) of the 5 allozyme loci that show clearcut differences between Skagerrak *M. edulis* and Baltic Sea *M. trossulus*⁵ belong to the same linkage group in NE Atlantic *M. edulis*.³⁴ Differential introgression, which has been documented at nuclear loci in *Mytilus* spp. (Ref. 28; our unpublished data) points towards differential selection across loci. Assuming that the introgression in the Baltic Sea of *M. trossulus* by *M. edulis* genes is recent, this situation offers a unique opportunity to study the dynamics of selection in natural populations of *Mytilus* mussels.

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Table 1. Approximate sizes in base pairs (bp) of the 5 *Glu-5'* allelomorphs in *Mytilus* spp. samples of the present study, and identification to alleles reported by Rawson *et al.*¹⁴ on the basis of the close correspondence between *Approximate size*^a and *Size*^b.

This study		Rawson <i>et al.</i> ¹⁴	
Allele name	Approximate size ^a	Allele name	Size ^b
<i>E''</i>	550 bp	-	-
<i>E'</i>	410 bp	380	427 bp
<i>E</i>	390 bp	350	397 bp
<i>G</i>	320 bp	300	322 bp
<i>T</i>	240 bp	240	244 bp

^a Estimate from visual comparison with 100-bp ladder

^b Nucleotide count from sequences presented in Fig. 2 of Ref.14

Table 2. Inferred genotype frequencies at locus *Glu-5'* in smooth-shell *Mytilus* spp. samples from the northern hemisphere. *N*, sample size.

Sample	(N)	Inferred genotype										
		<i>E''E</i>	<i>E'E'</i>	<i>E'E</i>	<i>EE</i>	<i>EG</i>	<i>E''T</i>	<i>E'T</i>	<i>ET</i>	<i>GG</i>	<i>GT</i>	<i>TT</i>
Gilleleje, Skagerrak	(16)	0.06	0.25	0.44	0.25	-	-	-	-	-	-	-
Sète, Mediterranean	(39)	-	-	-	-	0.03	-	-	-	0.97	-	-
Gdansk, Baltic Sea	(30)	-	-	0.03	0.20	-	0.03	0.17	0.37	-	-	0.20
Bodega Bay, CA	(23)	-	-	-	-	-	-	-	-	0.96	0.04	-
Hog island, CA	(36)	-	-	-	-	-	-	-	-	0.17	-	0.83
Lewes, DE ^a	(25)	-	0.08	0.16	0.76	-	-	-	-	-	-	-
Sète, Mediterranean ^b	(17)	-	-	-	-	-	-	-	-	1.00	-	-
Newport, OR ^c	(42)	-	-	-	-	-	-	-	-	-	-	1.00

^a, ^b, ^c Respectively, reference *M. edulis*, *M. galloprovincialis* and *M. trossulus* samples in Ref. 14

Table 3. *Mytilus trossulus*. Estimates of genetic diversity and genetic differentiation (Gst^{25}) at different geographic scales. Hs , within-subpopulation estimate of genetic diversity; SD , across-locus jackknife estimate of standard deviation.

Geographic scale, Sample	Source	$Hs \pm SD$	$Gst \pm SD$

Inter-oceanic (allozymes) ^{a,b,c}			0.060 ± 0.028
NE Pacific: Tillamook	Ref. 20	0.544 ± 0.202	
NE Atlantic: Tvärminne	Ref. 20	0.295 ± 0.164	
NW Atlantic: Group III	Ref. 12	0.531 ± 0.120	
Inter-oceanic (<i>Glu-5'</i>)			0.266
NE Pacific: Newport	Ref. 14	0.000	
NE Atlantic: Gdansk	present study	0.606	
Baltic Sea (allozymes) ^{a,d}			0.005 ± 0.001
Ystad	Ref. 5	0.385 ± 0.050	
Gdansk	Ref. 18	0.359 ± 0.051	

^a Five allozyme loci (*Est-D*, *Gpi*, *Lap-2*, *Odh* and *Pgm*) were taken into account because these were scored in all 4 source studies

^b *Est-D* was not scored in sample Group III¹²

^c To allow comparisons across studies, some poolings (+) of electromorphs were necessary. These were the following. *Gpi*: electromorphs 86 + 89 of Ref. 20 to compare with 91 of Ref. 12; *Pgm*: electromorphs 86 + 89 + 93 of Ref. 20 to compare with <96 of Ref. 12, and 108 + 111 of ref. 12 to compare with 111 of Ref. 20.

^d *Est-D*: electromorphs 90 + 97; *Pgm*: 100 + 103 and 120 + 122

Figure 1. Allelomorph polymorphism at locus *Glu-5'* in *Mytilus* spp. of the NE Atlantic and inferred genotypes. Mussel DNA extracts were subjected to PCR in a Robocycler thermocycler (Stratagene, San Diego) according to protocols that were identical to those of Ref. 14 except that the annealing temperature here was set at 54°C instead of 53°C. The PCR products were electrophoresed at 100 V for 2 hrs on ethidium-bromide stained 2.5% agarose gels and photographed under ultraviolet light. The sizes of the PCR products were estimated approximately by visual comparison with the 100-bp ladder (L) of Pharmacia Biotech (Uppsala). Banding patterns were the same as formerly described (Figure 1a of Ref. 14). *Glu-5'* alleles were denominated as *E*, *E'*, *E''*, *G* and *T*. We chose this new nomenclature to avoid confusion between allele name, approximate allele size and nucleotide count, when comparing the present study with Ref. 14 (see Table 1).

L GG E"G ET E'E E'E' TT E'G E'T GG L

