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Genomic reticulation indicates mixed ancestry in Southern-Hemisphere *Mytilus* spp. mussels

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Running title: Mixed ancestry in Southern-Hemisphere *Mytilus*

Previous surveys of allozyme variation in smooth-shell *Mytilus* spp. mussels have reported the presence in the Southern Hemisphere of both *M. edulis* and *M. galloprovincialis* mussels. Here, nuclear-DNA markers *mac-1* and *Glu-5'/Glu-3'*, both diagnostic for Northern-Hemisphere *M. edulis* and *M. galloprovincialis*, were used to further characterize the nuclear genomes of *M. edulis* from Kerguelen and *M. galloprovincialis* from Tasmania. Genomic reticulation was observed, with typical *M. edulis* allelomorphs fixed in both populations at locus *mac-1* while at locus *Glu-5'/Glu-3'*, allelomorphs characteristic of *M. galloprovincialis* were present in Kerguelen and nearly fixed in Tasmania. Kerguelen mussels had a genome of mixed *M. edulis* and *M. galloprovincialis* ancestry without evidence of barriers to merging as shown by Hardy-Weinberg and linkage equilibrium. Tasmanian mussels possessed a predominantly *M. galloprovincialis* genomic background introgressed by *M. edulis* allelomorphs at locus *mac-1*. Genetic drift superimposed on ancient hybridization and introgression may explain the genomic reticulation observed in both Kerguelen and Tasmanian mussels. There was no evidence of a recent introduction of Northern-Hemisphere *M. galloprovincialis* or *M. edulis* to Kerguelen or Tasmania.

ADDITIONAL KEYWORDS: intron-length polymorphism; nuclear DNA; hybridization; introgression; geographic structure; antitropical distribution.

INTRODUCTION

Gene trees do not necessarily parallel the phylogeny of populations and species (Hey, 2001; Nichols, 2001) and patterns of allopatric differentiation among populations may vary according to the genetic marker under scrutiny. This has several causes such as stochastic variance in coalescence time, within-genome differences in effective population sizes (e.g. mitochondrial vs. nuclear), horizontal transfer of genes across incompletely isolated evolutionary units (i.e. introgression) and selection (Hudson & Turelli, 2003). When secondary contact occurs with partial genetic isolation, additional genomic heterogeneity may emerge due to differential introgression (Barton & Hewitt, 1985; Harrison, 1990). The flow of genes directly involved in reproductive isolation is closed by selection, while the homogenization of neutral markers is slowed down in proportion to their linkage disequilibrium with the former (Barton 1979, 1986).

Variable rates of admixture and introgression characterize *Mytilus* spp. assemblages in the Northern Hemisphere, where various pre- and post-zygotic isolation mechanisms have been described: habitat specialization (Skibinski, Beardmore & Cross, 1983; Bierne *et al.*, 2002; 2003c), spawning asynchrony (Gardner & Skibinski, 1990; Secor, Day & Hilbish, 2001; Bierne *et al.*, 2003c), assortative fertilization (Bierne *et al.*, 2002) and hybrid depression (Beaumont, Abdul-Matin & Seed, 1993). Introgression seems to be variable across the genome as some loci are almost completely diagnostic whereas others do not allow discrimination between species (Skibinski, Beardmore & Cross, 1983; Rawson *et al.*, 1996; Bierne *et al.*, 2003a,b).

Allozymes separate Southern-Hemisphere *Mytilus* spp. populations into a group encompassing populations of mainland Australia, Tasmania and New Zealand (Australasia), that shows affinities with *M. galloprovincialis* from the western Mediterranean (the type locality for this species), and a South American group also including the Falkland and Kerguelen populations, which resembles typical *M. edulis* (McDonald, Seed & Koehn, 1991). The geographical distribution of the two female mitochondrial types that have been reported from the Southern Hemisphere (Hilbish *et al.*, 2000) leads to a partly different view: only the Kerguelen population harbours haplotypes of the *A* clade characteristic of *M. edulis*; all the

other *Mytilus* spp. populations in the Southern Hemisphere mostly or exclusively harbour a distinct, endemic subclade *D2* sister to subclade *D1* (the latter consisting of haplotypes unique to Mediterranean *M. galloprovincialis*). Through analysis of size-polymorphism of intron 1 of the actin gene *mac-1*, we (Daguin & Borsa, 2000) found that Australasian *M. galloprovincialis* had allelomorphs characteristic of northeastern Atlantic *M. edulis* at a total frequency of 80-100%, highlighting the genetic distinctness of Australasian mussels relative to Northern-Hemisphere *M. galloprovincialis* and meanwhile suggesting introgression.

Here we further characterized Southern-Hemisphere *Mytilus* spp. populations from Tasmania and the Kerguelen Islands using nuclear DNA markers together with previously published allozyme data, to address their genetic relationships to Northern-Hemisphere *M. edulis* and *M. galloprovincialis*.

MATERIALS AND METHODS

Southern-Hemisphere *Mytilus* spp. were collected in the Kerguelen Islands and in southeastern Tasmania (Table 1). The Tasmanian sample (TAS) consisted of six sub-samples that were chosen from a range of habitats varying from sheltered (CBL) to exposed (PAR), including a port city (HOB) where ballast water invasions are likely. This sampling strategy was designed to increase the likelihood that different forms (pure species, hybrids) be sampled in the case native mussels coexist, and eventually hybridize, with putative invaders. The mussels were either preserved frozen (Kerguelen sample) or in alcohol (all samples from Tasmania) prior to genotyping.

The DNA of each individual was extracted from muscle tissue using the Chelex 100 protocol (Walsh, Metzger & Higushi, 1991). DNA extracts were subjected to polymerase chain reaction (PCR)-amplification of a portion of intron 1 of the actin gene *mac-1* (Daguin & Borsa, 1999), using forward and reverse primers 5'-CGTCTAGCGTAGTACTTAAATTG-3' (there was a typing error in the original report) and 5'-CGAAAATTGTAGTCTAGTTTTGTG-3', respectively; the *Glu-5'* fragment at the 5'-end of the polyphenolic adhesive protein gene (Rawson *et al.*, 1996); and *Glu-3'* at the 3'-end of the same gene [Rawson *et al.* (1996), modified by labelling their *PR-8* primer with ^{33}P and setting the annealing temperature at 51°C for 35 PCR cycles, instead of 45°C for 30 PCR cycles]. The two *Glu* markers are separated by approximately 2 kb (Rawson *et al.*, 1996).

Size polymorphism of PCR-amplified fragments at locus *mac-1* was scored according to methods detailed in Daguin & Borsa (1999). The same protocol was used for distinguishing among the three size products at the *Glu-3'* locus [i.e. size-alleles (or allelomorphs) *214*, *220* (Rawson *et al.*, 1996) and *211*, a number which refers to this novel allelomorph's approximate length in base pairs as extrapolated from a comparison with allelomorphs *214* and *220* on a side-by-side run]. Characterization of *Glu-5'* allelomorphs was as in Borsa *et al.* (1999).

f and θ, Weir & Cockerham's (1984) equivalents of Wright's *Fis* and *Fst*, were estimated from genotype frequencies at each locus using the procedure FSTATS of GENETIX (Belkhir *et al.*, 1996). The significance of $\hat{f}$-estimates was assessed by a two-tailed permutation test (procedure PERMUTONS of GENETIX), with $p=(n+1)/(N+1)$, where n is the number of pseudo-values whose absolute value $=|$observed $\hat{f}$-value$|$ and N is the number of permutations (Sokal & Rohlf, 1995). The significance of $\hat{\theta}$-estimates was assessed similarly by a one-tailed permutation test. Linkage disequilibrium between loci was estimated using the LINKDIS algorithm (Black & Krafsur, 1985) implemented in GENETIX. The estimated value of R, the average allelomorph-pairwise correlation coefficient, was compared to zero using the χ^2 approximation of Weir (1979).

Data at seven allozyme loci (*Ap*, *Est-D*, *Gpi*, *Lap*, *Mpi*, *Odh* and *Pgm*) were gathered from several studies reporting geographic variation in *Mytilus* spp. populations (Table 1). The Northern-Hemisphere *M. edulis* reference sample was 'Skagerrak' of Väinölä & Hvilsom (1991) for all loci except *Pgm*, for which it was 'SWE' of Varvio, Koehn & Väinölä (1988), to complete the data set. Correspondences between electromorphs from different studies (Table2) were established on the basis of the similarities in the frequencies of the most common electromorphs and in the relative mobilities of the other electromorphs in the pairwise comparisons of samples 'Shinnecock' of McDonald, Seed & Koehn (1991) with 'Skagerrak' of Väinölä & Hvilsom (1991); 'Venice' of McDonald, Seed & Koehn (1991) with 'Palavas' of Quesada, Zapata & Alvarez (1995) and 'Galicia' of Väinölä & Hvilsom (1991) with each 'Los Angeles' of McDonald & Koehn (1988) and 'Laxe' of Quesada, Zapata & Alvarez (1995). To complete the 'Laxe' data set we used the *Gpi* ('*Pgi*') data for sample 'Portosin' of Quesada, Zapata & Alvarez (1995). Where uncertainties remained, electromorphs were grouped as indicated in Table 2.

To compare nuclear DNA and allozyme data on a range of samples, we assumed that the samples of the present study and those designated as their homologues in Table 1 were drawn from the same populations. This assumption is based on the respective geographical proximities of these locations, and on the observation that *Mytilus* spp. populations generally are genetically homogeneous over large distances outside of hybrid zones and areas of recent introduction (McDonald, Seed & Koehn, 1991; Quesada *et al.*, 1995; Daguin & Borsa, 1999).

Absolute genetic distances (*Do*: Gregorius, 1984; Katz & Goux, 1986) between pairs of populations were estimated.

RESULTS

Allelomorph frequencies at loci *mac-1*, *Glu-5'* and *Glu-3'* in seven *Mytilus* spp. samples from the Southern Hemisphere (one from Kerguelen Islands, six from Tasmania) along with those of reference Northern-Hemisphere *M. edulis* and *M. galloprovincialis* are reported in Table 3. *mac-1* allelomorph *a3*, which was common in *M. edulis* and almost absent in *M. galloprovincialis*, was the most common in Kerguelen. The other *mac-1* allelomorphs found in the KER sample (*a2*, *a4*, and *c4*) were all characteristic of northeastern Atlantic *M. edulis*. Kerguelen mussels possessed at *Glu-5'* both allelomorphs characteristic of *M. edulis* (*E* and *E'*) and the allelomorph characteristic of *M. galloprovincialis* (*G*), and the same was observed at *Glu-3'* with allelomorphs *220* and *214*, respectively. *mac-1* allelomorph *a2* (otherwise known at substantial frequency from Northern-Hemisphere *M. edulis* only; Table 3) had a frequency >0.9 in all six samples from Tasmania. Three of the four other *mac-1* allelomorphs encountered in Tasmania (*a1*, *a3* and *a4*), all rare, were characteristic of Northern-Hemisphere *M. edulis*. Allelomorph *a0.5* was found only in sample ALO. Conversely, Tasmanian *Mytilus* samples possessed allelomorphs *Glu-5' G* and *Glu-3' 214*, which are characteristic of M. galloprovincialis, at high frequency. Allelomorphs *E* and *E'* at *Glu-5'*were absent from the Tasmanian samples.

The distribution of genotype frequencies did not depart from Hardy-Weinberg expectations as indicated by *f*-estimates, none of which was significant (Table 3). Slight genetic heterogeneity was possibly detected at locus *mac-1*, among samples from various habitats in southeastern Tasmania [Weir & Cockerham's (1984) $\hat{\theta}$=0.014; p<0.05]. No differences were evident at *Glu-5'* ($\hat{\theta}$=0.003; p>0.28) nor *Glu-3'* ($\hat{\theta}$=0.001; p>0.40). No linkage disequilibrium was found between *mac-1* and *Glu-5'* in the Kerguelen population [^R=0.121 (N=65); $\chi^2_{[6df]}$=7.23] nor *mac-1* and *Glu-3'* [^R=0.131 (N=56); $\chi^2_{[3df]}$=4.64]. As expected from their tight physical linkage, strong linkage disequilibrium was observed between *Glu-5'* and *Glu*-3' [^R=0.532 (N=61); $\chi^2_{[4df]}$=48.89: p<<0.001], although it did not

reach its maximal possible value. Recombinant *Glu-5'/Glu-3'* alleles were observed in both Kerguelen and Tasmanian populations, with frequencies of, respectively, 0.10 and 0.02, estimated from the ratio of recombinant di-locus genotype counts to the totals, after the double heterozygotes were excluded from the dataset.

All nuclear loci taken together, the two Southern-Hemisphere *Mytilus* spp. populations were clearly distinct from each other with an average $^{*}D_o$ (Table 4) of the same order as that between *M. edulis* and *M. galloprovincialis*. Tasmanian mussels were genetically closer to Northern-Hemisphere *M. galloprovincialis* than *M. edulis* while Kerguelen mussels were closer to Northern-Hemisphere *M. edulis* than *M. galloprovincialis* (Table 4). However, discrepancies among loci were evident in all the pairwise comparisons. Tasmanian mussels were genetically very close to *M. galloprovincialis* according to loci *Glu-5'* and *Mpi*, closer to *M. galloprovincialis* than to *M. edulis* at loci *Ap*, *Gpi* and *Odh*, closer to *M. edulis* at loci *Lap*, *Est-D* and *Pgm* and fixed for typical *M. edulis* allelomorphs at *mac-1*. Kerguelen mussels appeared to be close to Mediterranean *M. galloprovincialis* when considering allozyme locus *Odh*, while clearly different from this species at loci *Est-D*, *mac-1*, and *Mpi*, although close to *M. edulis* at loci *Est-D* and *Mpi* only (Table 4). In comparison, the genetic distance estimates between reference *M. edulis* and *M. galloprovincialis* were $^{*}D_o$=0.729±0.317 (GIL / SET), $^{*}D_o$=0.739±0.316 (GIL / CHI) and $^{*}D_o$=0.692±0.338 (GIL / STB), while those among *M. galloprovincialis* populations from the north-eastern Atlantic and the Mediterranean were $^{*}D_o$=0.136±0.124 (STB / SET) and $^{*}D_o$=0.167±0.147 (STB / CHI) and $^{*}D_o$=0.101±0.054 within the Mediterranean (SET / CHI).

DISCUSSION

The present results highlighted the distinct composition of Southern-Hemisphere *Mytilus* spp. populations relative to Northern-Hemisphere *M. edulis* and *M. galloprovincialis*. Allozyme-frequency differences between Tasmanian *M. galloprovincialis* and Kerguelen *M. edulis*, and their Northern-Hemisphere conspecifics (McDonald, Seed & Koehn, 1991; present results) indicate they are native rather than introduced. Both mitochondrial-DNA (Hilbish *et al.*, 2000) and *mac-1* data (present results) confirm that Tasmanian *M. galloprovincialis* are endemic. The same conclusion holds for Kerguelen *M. edulis* because of their unique, exclusive allelic composition at three nuclear-DNA loci.

Extreme inter-locus variance in allelic composition was also reported, which we interpret as evidence of past introgression as explained in the following.

In Tasmanian mussels, the quasi-fixation of *M. edulis* allelomorphs at some nuclear loci is at first view hardly compatible with the presence of typical *M. galloprovincialis* allelomorphs at several other loci, as well as with mitochondrial-DNA data. Such a pattern of genomic reticulation in Tasmanian mussels indicates secondary contact with *M. edulis* either before or after the foundation of the Tasmanian population. When secondary contact occurs, gene flow can be restrained by partial genetic isolation, whose effect is to accentuate heterogeneity among neutral loci because of differential introgression rates (Barton & Hewitt, 1985; Barton, 1986; Harrison, 1990). The action of a semi-permeable barrier to gene flow on the inter-locus variance in introgression rate was described in Baltic Sea *M. trossulus* (Väinölä & Hvilsom, 1991; Bierne *et al.*, 2003b). A proposed mechanism for the fixation of *M. edulis mac-1* allelomorph *a*2 in Tasmanian *M. galloprovincialis* is genetic drift, perhaps due to the small size of the founding population or to subsequent reduction in effective population size, enhanced by the geographical isolation of Tasmania. Genetic drift likewise may explain the fixation or quasi-fixation of *M. galloprovincialis*-like allelomorphs at loci *Gpi*, *Mpi*, and *Glu-5'* in Tasmania. An alternative possibility is that *mac-1* allelomorph *a2* has been introgressing a *M. galloprovincialis* background by selection at a nearby locus (pseudo-selection). Under this scenario, a favourable gene previously fixed in a *M. edulis*-like population that came into

contact with the ancestral Tasmanian population would have easily crossed the inter-specific genetic barrier (Barton, 1979; Pialek & Barton, 1997) and neutral alleles at linked loci may have hitchhiked with it. Explaining that only one *M. edulis* allele, *a2*, has hitchhiked through the barrier would however require strong directional selection and / or tight linkage.

Kerguelen mussels were characterized by *M. edulis* allelomorphs at locus *mac-1*, and by a mixture of *M. edulis*- and *M. galloprovincialis*-like allelomorphs at *Glu-5'* and *Glu-3'*. There was no evidence that this mixture was either recent or maintained by reproductive isolation, because of the absence of significant heterozygote deficiencies at *mac-1* and *Glu-5'* or *Glu-3'* (all polymorphic in the Kerguelen population) and the absence of significant linkage disequilibrium between *mac-1* and either *Glu-5'* or *Glu-3'*. Taken together, allozyme and nuclear-DNA data thus demonstrated the genetic originality of the Kerguelen population, even though it simultaneously possesses at several loci allelomorphs that are characteristic of either *M. edulis* or *M. galloprovincialis*. The co-occurrence of the latter in the Kerguelen population supports the hypothesis that its evolutionary history has been affected by hybridization between ancestors of these two species. This hybridization event is not likely to be recent because of the occurrence of recombinant *Glu-5'/Glu-3'* allelomorphs at a substantial frequency. For reasons similar to those raised hereabove to explain the genetic architecture of Tasmanian *Mytilus*, hybridization followed by genetic drift and pseudo-selection may explain that at some loci Kerguelen mussels exhibit affinities with Northern-Hemisphere *M. galloprovincialis* while other loci are fixed for *M. edulis*-like allelomorphs. It is worth noting that locus *Glu-5'* which up to now was considered to be fully diagnostic between *M. edulis* and *M. galloprovincialis* (Rawson *et al.*, 1996; Borsa *et al.*, 1999; Bierne *et al.*, 2003a) has now been found polymorphic for hetero-specific alleles and at Hardy-Weinberg equilibrium in another genetic context than the *M. edulis / M. galloprovincialis* hybrid zone in the Northern Hemisphere. This result suggests that reproductive isolation between *M. edulis*- and *M. galloprovincialis*-like genomes may have been weaker in the Kerguelen Islands as it appears to be nowadays in the Northern Hemisphere. We speculate that the reproductive isolation genes putatively responsible for the strong barrier to gene flow at locus *Glu-5'* in the Northern Hemisphere (Bierne *et al.*, 2003a) either had not yet evolved at the time when the contact occurred in Kerguelen, or did not produce a sufficient barrier under the demographic and ecological conditions met in Kerguelen. Alternatively, the *Glu-5'/Glu-3'* polymorphisms may be old and pre-date the speciation events. However, this hypothesis seems less likely because it would imply that differential fixation had occurred in the Northern Hemisphere where distribution ranges are nonetheless much greater than in the small and isolated Kerguelen Islands.

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Table 1. *Mytilus* spp. List of samples analysed for the present survey, and homologous samples (i.e. taken from presumably the same population, at the same or a nearby location) from previous allozyme surveys

Sample (present survey)				Sample (previous allozyme survey)	
Abbreviation	Location	Coordinates	Date	Name	Reference
Southern-Hemisphere *Mytilus* spp.					
KER	Mayes Island, Kerguelen Islands	49°28'S, 69°58'E	July 1997	'Kerguelen Islands'	McDonald, Seed & Koehn (1991)
ADB (TAS)	Adventure Bay, Bruny Island	43°21'S, 147°22'E	Mar.1997	–	–
ALO (TAS)	Alonnah wharf, d'Entrecasteaux Channel	43°18'S, 147°15'E	Mar. 1997	–	–
CBL (TAS)	Cloudy Bay Lagoon, Bruny Island	43°26'S, 147°12'E	Feb. 1997	–	–
HOB (TAS)	CSIRO marine labs waterfront, western shore of River Derwent, Hobart	42°53'S, 147°20'E	Feb. 1997	–	–
PAR (TAS)	Rocky point southwest of Partridge Narrows	43°25'S, 147°06'E	Mar. 1997	–	–
SIM (TAS)	Simpson's or Isthmus Bay, Bruny Island	43°17'S, 147°20'E	Mar. 1997	'Huon River Estuary, Tasmania'	McDonald, Seed & Koehn (1991)
Reference *M. edulis*					
GIL	Gilleleje, Kattegat	56°07'N, 12°19'E	Sep. 1996	'Skagerrak' 'SWE'	Väinölä & Hvilsom (1991) Varvio, Koehn & Väinölä (1988)
Reference *M. galloprovincialis*					
STB	Setubal, Portugal	38°29'N, 8°56'E	Sep. 1997	'Sesimbra'	Quesada, Zapata & Alvarez (1995)
SET	Sète, Western Mediterranean	43°24'N, 3°41'E	May 1996	'Palavas'	Quesada, Zapata & Alvarez (1995)
CHI	Chioggia, Adriatic Sea	45°13'N, 12°18'E	June 1997	'Venice'	McDonald, Seed & Koehn (1991)

Table 2. Denomination, correspondence across studies, and groupings of electromorphs at 7 allozyme loci in *Mytilus* spp. Data obtained by cross comparisons of electromorph frequencies in McDonald & Koehn (1988) (MK88), McDonald, Seed & Koehn (1991) (MD91), Väinölä & Hvilsom (1991) (VH91) and Quesada, Zapata & Alvarez (1995) (HQ95). Locus *Lap* of MK88, MD91 and VH91 is *Lap-2* of HQ95

	Locus																	
	Ap									*Gpi*								
MK88	–	–	*100*	*103*	*105*	*108*	*117*	*120*	*125*	–	–	–	*100*	–	*105*	*107*	–	
MD91	*90*	*95*	*100*	–	*105*	*108*	*117*	*120*	*125*	*93*	*96*	*98*	*100*	*102*	*105*	*107*	*110*	
VH91	*90*	–	*100*	–	*105*	*108*	*117*	–	–	*93*	*96*	*98*	*100*	*102*	*105*	*107*	–	
HQ95	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	–	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	

	Locus																
	Est-D				*Lap*					*Mpi*							
MK88	*80*	*90*	–	–	–	–	*96*	*98*	*100*	–	–	*92*	–	–	–		
MD91	*80*	*90*	*95*	*100*	*92*	*94*	*96*	*98*	*100*	*84*	*90*	*92*	*94*	*96*	*100*		
VH91	–	*90*	–	*99*	–	*94*	*96*	*98*	–	*84*	*90*	*92*	–	–	*100*		
HQ95	*1,2*	*4*	*5*	*6*	*1*	*2*	*3*	*5*	*7*	*1*	–	*2*	–	–	*3*		

	Locus														
	Odh						*Pgm*								
MK88	–	*90*	–	*100*	*110*	–	*86*	*89*	*93*	*100*	*106*	*111*	–		
MD91	*80*	*90*	*98*	*100*	*110*	*120*	*86*	*89*	*93*	*100*	*106*	*111*	*114*		
VH91	–	*90*	*98*	*100*	*108,110*	–	–	*88*	*100*	*103*	–	*111*	*114*		
HQ95	*1*	*3*	*4,5*	*6*	*8*	*9*	*1*	*2*	*3*	*4*	*6*	*8*	–		

Table 3. Allelomorph frequencies at loci *mac-1*, *Glu-5'* and *Glu-3'* in *Mytilus* spp. from the Southern Hemisphere. *^f*, Weir & Cockerham's (1984) estimate of *Fis*; *ns*, not significant according to permutation test (Belkhir *et al.*, 1996). Size homologies between allelomorphs from different samples were ascertained by side-by-side electrophoretic runs. *mac-1* allelomorph nomenclature follows Daguin & Borsa (1999); *Glu-5'* allelomorphs *G*, *E* and *E'* (Borsa *et al.*, 1999) are allelomorphs *300*, *350* and *380*, respectively, in Rawson *et al.* (1996); nomenclature for *Glu-3'* allelomorphs accords with Rawson *et al.* (1996). Primary data at loci *mac-1* and *Glu-5'* for reference samples (GIL, STB, SET, CHI) from Borsa *et al.* (1999), Daguin & Borsa (2000), and Daguin *et al.* (2001)

Locus,	Sample										
Allelomorph	KER	TAS						GIL	STB	SET	CHI
		ADB	ALO	CBL	HOB	PAR	SIM				
mac-1											
f1	–	–	–	–	–	–	–	–	0.02	–	–
b2	–	–	–	–	–	–	–	–	0.04	0.05	–
b1	–	–	–	–	–	–	–	–	0.15	0.21	0.28
b4	–	–	–	–	–	–	–	–	0.02	–	0.01
b5	–	–	–	–	–	–	–	–	0.02	–	–
c1	–	–	–	–	–	–	–	–	0.10	0.07	0.06
c2	–	–	–	–	–	–	–	–	0.50	0.54	0.57
c3	–	–	–	–	–	–	–	–	0.02	–	0.01
c4	0.08	–	–	–	–	–	–	–	–	–	–
c6	–	–	–	–	–	–	–	–	–	0.01	–
a0.5	–	–	0.01	–	–	–	–	–	–	–	–
a1	–	–	–	0.03	–	–	–	0.02	0.02	–	–
a2	0.20	0.95	0.99	0.92	0.98	1.00	0.99	0.15	0.02	–	–
a3	0.70	0.04	–	0.03	–	–	0.01	0.31	0.04	0.01	–
a4	0.01	–	–	0.02	0.02	–	–	0.17	–	–	–
a5	–	–	–	–	–	–	–	0.27	–	–	–
a6	–	–	–	–	–	–	–	0.08	–	0.01	–
a7	–	–	–	–	–	–	–	–	0.02	0.04	0.03
a8	–	–	–	–	–	–	–	–	0.04	0.06	0.03
(N)	(83)	(28)	(59)	(32)	(31)	(30)	(40)	(26)	(26)	(68)	(47)
^f	0.106^{ns}	-0.019^{ns}	0.000^{ns}	-0.040^{ns}	0.000^{ns}	–	0.000^{ns}	0.173^{ns}	-0.010^{ns}	0.123^{ns}	0.032^{ns}
Glu-5'											
E'' (~550 bp)	–	–	–	–	–	–	–	0.03	–	–	–
E' (~410 bp)	0.03	–	–	–	–	–	–	0.47	–	–	–
E (~390 bp)	0.32	–	–	–	–	–	–	0.50	–	0.06	–
i (~350 bp)	–	0.02	–	0.03	0.02	–	–	–	–	–	–
G (~320 bp)	0.65	0.96	1.00	0.97	0.98	1.00	1.00	–	1.00	0.94	1.00
ii (~290 bp)	–	0.02	–	–	–	–	–	–	–	–	–
(N)	(79)	(26)	(25)	(29)	(26)	(25)	(38)	(16)	(19)	(39)	(18)
^f	0.064^{ns}	-0.010^{ns}	–	-0.018^{ns}	0.000^{ns}	–	–	0.088^{ns}	–	-0.027^{ns}	–
Glu-3'											
223	–	–	–	–	–	–	–	0.03	–	–	–
220	0.48	0.03	–	0.02	0.03	–	0.03	0.97	–	–	–
214	0.51	0.94	1.00	0.98	0.94	0.97	0.92	–	1.00	1.00	1.00
211	0.01	0.03	–	–	0.03	0.03	0.05	–	–	–	–
(N)	(65)	(17)	(28)	(30)	(29)	(17)	(30)	(20)	(24)	(17)	(18)
^f	0.046^{ns}	-0.016^{ns}	–	0.000^{ns}	-0.024^{ns}	-0.016^{ns}	-0.051^{ns}	0.000^{ns}	–	–	–

Table 4. Single-locus genetic-distance (*do*: Gregorius, 1984) estimates between *Mytilus* spp. populations of the Southern Hemisphere (KER, TAS) and reference Mediterranean *M. galloprovincialis* (CHI, SET), Atlantic *M. galloprovincialis* (STB) and northeastern Atlantic *M. edulis* (GIL). *^Do*, across-locus average *^do*; *SD*, estimate of standard deviation

Locus	KER *vs.*	KER *vs.*				TAS *vs.*			
	TAS	CHI	SET	STB	GIL	CHI	SET	STB	GIL
Ap	0.670	0.680	0.685	0.435	0.170	0.350	0.415	0.375	0.570
Est-D	0.500	0.975	0.990	0.945	0.100	0.475	0.490	0.445	0.420
Gpi	0.515	0.620	0.575	0.560	0.825	0.125	0.190	0.345	0.970
Lap	0.105	0.540	0.505	0.560	0.270	0.455	0.420	0.475	0.185
Mpi	0.940	0.980	0.950	0.930	0.110	0.040	0.010	0.030	0.960
Odh	0.495	0.100	0.075	0.375	0.635	0.595	0.510	0.150	0.890
Pgm	0.290	0.400	0.335	0.265	0.200	0.340	0.295	0.245	0.200
mac-1	0.788	0.996	0.973	0.929	0.523	0.996	0.996	0.965	0.839
Glu-5'	0.350	0.350	0.286	0.350	0.651	0.000	0.064	0.000	1.000
Glu-3'	0.447	0.490	0.490	0.490	0.520	0.083	0.083	0.083	0.967
^Do	0.510	0.613	0.586	0.584	0.400	0.346	0.347	0.311	0.700
SD	0.243	0.301	0.314	0.259	0.261	0.306	0.292	0.286	0.328