Genetic relationships of Mytilus galloprovincialis Lmk. populations worldwide: evidence from nuclear -DNA markers

Claire Daguin, Philippe Borsa

Genetic relationships of *Mytilus galloprovincialis* Lmk. populations worldwide: evidence from nuclear-DNA markers

Claire Daguin and Philippe Borsa

Laboratoire Génome Populations Interactions and IRD, Station Méditerranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète, France

Corresponding author: P. Borsa (philippeborsa@yahoo.fr)
3724 words of text, 35 references, 2 tables, 3 figures
Abbreviated title: Geographical structure of *Mytilus galloprovincialis*

To be cited as:
Daguin C., Borsa P. 2000. – Genetic relationships of *Mytilus galloprovincialis* Lmk. populations worldwide: evidence from nuclear-DNA markers. *In* Crame A., Harper E., Taylor J. (eds.), *Bivalve Systematics and Evolution. Geological Society of London Special Publications* 177, 389-397.

Abstract: Allozyme surveys of genetic variation in *Mytilus galloprovincialis* Lmk. throughout the world have identified three groups within this species: a northeastern (NE) Atlantic group that also includes the *M. galloprovincialis* population of South Africa, a Mediterranean group that also includes the *M. galloprovincialis* populations from the eastern and the western coasts of the North Pacific, and an Australasian group. Hypotheses that have been proposed to account for the genetic differentiation patterns and disjunct, worldwide distribution of *M. galloprovincialis* include the recent introduction of this species into the Southern Hemisphere and the North Pacific through human agency, and an alternative hypothesis that each of the three groups is endemic. We used two nuclear-DNA markers (the polyphenolic adhesive protein gene *Glu-5'* and the first intron of the actin gene *mac-1*) to investigate in more depth the genetic relationships among *M. galloprovincialis* populations. Samples were taken between 1996 and 1999 from California, the NE Atlantic, the Mediterranean Sea, South Africa, Korea, Western Australia, Tasmania, and New Zealand. NE Atlantic *M. edulis* L. were used as an outgroup. While all *M. galloprovincialis* samples were fixed, or nearly so, for the diagnostic *G* allele at locus *Glu-5',* correspondence analysis of *mac-1* allele-frequency data highlighted the genetic distinctness of Australasian mussels relative to other *M. galloprovincialis* populations. The latter consisted of two differentiated groups (NE Atlantic and Mediterranean) as formerly reported at allozyme loci. Another sample, from Chile, was nearly identical to Mediterranean *M. galloprovincialis*. Nuclear-DNA data thus enforce the idea that *M. galloprovincialis* have probably been introduced from the Mediterranean to the North Pacific (and now Chile), and from the NE Atlantic to South Africa. We argue that Australasian mussels derive from a proto- *M. galloprovincialis* population introgressed by *M. edulis*-like genes, and should be considered as a regional subspecies of *M. galloprovincialis*.

Smooth-shelled *Mytilus* spp. mussels are distributed over the temperate and sub-polar regions of all oceans. Global surveys of allozyme variation in *Mytilus* spp. populations have focused on the clarification of the systematics of the genus (McDonald & Koehn 1988; Varvio *et al.* 1988; Koehn 1991; McDonald *et al.* 1991). These unambiguously showed that all smooth-shelled *Mytilus* populations in the Southern and the Northern Hemispheres could be ascribed to one of the three species *M. edulis* Linnaeus, 1758, *M. galloprovincialis* Lamarck, 1819, or *M. trossulus* Gould, 1850. Full species status is warranted by the fact that each of these entities maintains its genetic integrity over broad geographical areas in spite of hybridization in areas of overlap, and in the face of potential for long-distance dispersal by planktotrophic larvae (Koehn 1991). Although the majority of the genetic differences was accounted for by this systematic distinction, geographical differentiation was also detected within each species. For example, McDonald *et al.* (1991) recognized Australasian (Australia, Tasmania, New Zealand) *Mytilus* as *M. galloprovincialis*, but noted some differences in allele frequency between the latter, and their Northern Hemisphere conspecifics.

The present study is concerned with worldwide genetic variation in *Mytilus galloprovincialis*. Our main objective was to test with novel nuclear-DNA markers the biogeographical hypotheses arising from the analysis of allozyme loci.

Allozyme surveys of genetic variation in *Mytilus galloprovincialis* along the Iberian Peninsula have demonstrated clear-cut geographical isolation between the northeastern (NE) Atlantic and the Mediterranean populations of this species (Sanjuan *et al*. 1994; Quesada *et al*. 1995). Compilation and analysis of the dataset generated by the above allozyme studies has since clarified the worldwide picture of the geographic structure in *M. galloprovincialis* (Sanjuan *et al*. 1997). These authors examined the allele frequency data at five allozyme loci using multidimensional scaling analysis on the matrix of Nei's genetic distance estimates between populations. Unfortunately, they did not consider any outgroup, so their genetic networks were unrooted. They found evidence for three genetically differentiated groups of *M. galloprovincialis*: a NE Atlantic group that also included *M. galloprovincialis* from South Africa, a Mediterranean group that also included *M. galloprovincialis* from California and eastern Siberia, and a third group from Western Australia and Tasmania. New Zealand *Mytilus* appeared to be more closely related to North Pacific *M. galloprovincialis* than to the other Australasian populations.

Phylogenetic inference is desirable for more in-depth understanding of the evolutionary processes including colonization and vicariance that shape the genetic composition of species. Fig. 1 is a neighbour-joining tree, rooted by *Mytilus edulis*, inferring the phylogeny of *M. galloprovincialis* populations from allele-frequency data at seven allozyme loci (McDonald *et al*. 1991; Väinölä & Hvilsom 1991; Quesada *et al*. 1995). The seven loci considered here included the five loci examined by Sanjuan *et al*. (1997). Jackknife resampling of loci, where each locus was omitted in turn from the dataset supported a principal separation between NE Atlantic / Mediterranean *M. galloprovincialis* (samples Sesimbra and Palavas) and Australasian *M. galloprovincialis* (samples Albany, Huon River, and Wellington). Fig. 1 also confirms that Californian *M. galloprovincialis* (Los Angeles) is genetically closer to the Mediterranean than the Atlantic population. The Wellington (New Zealand) sample occurred within an Australasian clade (that is, together with Albany and Huon River) 4/7 times, whereas in the remaining 3/7 pseudotrees it diverged slightly before the node separating the Australian (Albany + Huon River) clade from the NE Atlantic / Mediterranean clade.

Kenchington *et al*. (1995) obtained the sequence of 18S rDNA for several *Mytilus* spp. samples. These included *M. galloprovincialis* from an introduced and farmed population in Puget Sound in the eastern North Pacific, *M. edulis planulatus* Lamarck, 1819 from southeastern Tasmania [McDonald *et al*. (1991) had concluded that *M. edulis planulatus* were *M. galloprovincialis* according to both allozyme-frequency and morphometric data], and several *M. edulis* and *M. trossulus* samples. Kenchington and collaborators also analysed an "*M. galloprovincialis*" sample from Morgat (Britanny, France) but we disregarded this

identification because this location lies within the European hybrid zone between *M. edulis* and *M. galloprovincialis* (Coustau *et al.* 1991; Gosling 1992) where there is no evidence for the presence of non-introgressed *M. galloprovincialis* (Coustau *et al.* 1991; C. Daguin, F. Bonhomme, P. Borsa, unpublished data). The phylogeny inferred from the 18S rDNA sequences (Kenchington *et al.* 1995) strongly suggests an early separation of Northern Hemisphere *M. galloprovincialis* from the other smooth-shelled *Mytilus* spp., including *M. edulis*, *M. trossulus*, and Australasian *M. galloprovincialis*.

A number of hypotheses have been suggested to account for the differentiation patterns and disjunct worldwide distribution of *Mytilus galloprovincialis*.

(1) *Mytilus galloprovincialis* has been introduced to South Africa, the western North Pacific, the eastern North Pacific, and perhaps Australasia through human agency (Wilkins *et al.* 1983; Grant & Cherry 1985; McDonald & Koehn 1988; McDonald *et al.* 1991; Vermeij 1992). This hypothesis is based on the lack of evidence for *M. galloprovincialis*-like mussels in the fossil record and their absence from the literature or museum collections in South Africa and in the North Pacific until recently (Wilkins *et al.* 1983; Grant & Cherry 1985; McDonald & Koehn 1988, and references therein; Geller 1999). However, sub-fossil *Mytilus* are present in Aboriginal middens in Tasmania, southern Australia, and New Zealand (McDonald *et al.* 1991, and references therein). This leads to the following, modified hypothesis for the genetic affinities of Australasian *M. galloprovincialis*.

(2) Introduced *Mytilus galloprovincialis* have displaced native Australasian *Mytilus* sp. (suggested, although believed to be unlikely by McDonald *et al.* 1991) or introgressed with the latter (Seed 1992). Sanjuan *et al.* (1997) also write that "a human introduction of North Pacific mussels into Australia is [...] possible (Carlton 1987) [and] may explain the genetic heterogeneity of Australasian samples". Both displacement and introgression by presumably introduced *M. galloprovincialis* have affected native *M. trossulus* in California (McDonald & Koehn 1988; Geller 1999).

(3) North Pacific *Mytilus galloprovincialis* have been recently introduced from the South Pacific. Quoting Koehn (1991): "As *M. galloprovincialis* is probably native to large areas of the South Pacific, introductions into northern Pacific sites [...] may not have originated in Europe." However, this is contradicted by the quite large genetic dissimilarity between North Pacific and South Pacific *M. galloprovincialis*, which is at variance with the close genetic similarities between North Pacific and Mediterranean *M. galloprovincialis* (Gosling 1992).

(4) Australasian, North Pacific, and Mediterranean *Mytilus galloprovincialis* all derive from an ancestral Pacific stock (Sanjuan *et al.* 1997). While hypotheses (1) and (2) here above rely on the presumption that *M. galloprovincialis* is native to the NE Atlantic / Mediterranean, Sanjuan *et al.* (1997) consider the alternative hypothesis that either Australasia or the North Pacific be the geographic origin of *M. galloprovincialis*. According to this hypothesis, proto-*M. galloprovincialis* crossed the Equator in the Pacific Ocean; North

Pacific *M. galloprovincialis* subsequently crossed the Arctic to colonize the NE Atlantic and the Mediterranean. This amounts to propose that present-day Australasian, North Pacific, and Mediterranean populations are endemic forms of *M. galloprovincialis*. The data provided by Sanjuan *et al.* (their Table 4; 1997) however failed to substantiate part of this scenario, since the genetic distance estimate between the North Pacific and the Mediterranean [D = 0.029 (0.011-0.057)] was not significantly larger than that among sub-populations within the Mediterranean [D = 0.028 (0.007-0.054)].

The allozyme-based phylogeny proposed in Fig. 1 suggests that Australasian *Mytilus galloprovincialis* differentiated early from all the other *M. galloprovincialis*, supporting the hypothesis that Australasian *M. galloprovincialis* are endemic (Koehn 1991; McDonald *et al.* 1991). Fig. 1 further shows that the divergence between NE Atlantic and Mediterranean *M. galloprovincialis* is more recent than the separation between the Australasian and the NE Atlantic / Mediterranean groups. The close genetic relationship of Californian with Mediterranean *M. galloprovincialis* (see above; Fig. 1) reflects an even more recent common origin, which is indeed compatible with the hypothesis that North Pacific *M. galloprovincialis* were introduced from the Mediterranean through human agency. Sanjuan *et al.* (1997) instead propose that "a genetic mixing between [*M. galloprovincialis* and *M. edulis*], perhaps combined with selective pressure, may explain the larger allozyme divergence between *M. galloprovincialis* of Mediterranean vs Atlantic populations, than between Mediterranean vs North Pacific populations". If this hypothesis were true, allele frequencies in NE Atlantic *M. galloprovincialis* would be intermediate between those of *M. edulis* and Mediterranean *M. galloprovincialis*. This appears to be the case at only one (*Ap*), perhaps two (adding *Gpi*) out of the six loci (*Ap*, *Est-D*, *Gpi*, *Lap*, *Mpi*, *Odh*) at which the most significant differences were observed between NE Atlantic and Mediterranean *M. galloprovincialis* (Quesada *et al.* 1995) and which were also scored in *M. edulis* (Skibinski *et al.* 1980; Coustau *et al.* 1991; Sanjuan *et al.* 1994). All these loci were taken into account for computing the neighbour-joining tree of Fig. 1. The distinctness of the NE Atlantic vs Mediterranean *M. galloprovincialis* thus far appears to reflect vicariance more than differential introgression by *M. edulis* allozyme genes.

To what extent do nuclear-DNA markers enforce and refine the conclusions drawn from allozyme data?

Methods

Mytilus galloprovincialis populations were sampled in California (sample BOD of Fig. 2), the NE Atlantic (STB), the Mediterranean Sea (SET), South Africa (SAF), eastern Asia (KOR), Western Australia (AUS), Tasmania (TAS), and New Zealand (NZL) between 1996 and 1999. Reference *M. edulis* L. samples were collected in the North Sea (GFP), the Skagerrak (FLO), and the Kattegat (GIL) in 1996 and 1997. A sample of uncertain taxonomic status was collected in southern central Chile (CHL) in 1998. All mussel shells in this sample were *M.*

galloprovincialis according to morphology. *Mytilus* samples from Chile examined so far at allozyme loci and using morphometrics were *M. edulis*-like (McDonald *et al.* 1991; Fig. 1), but a sample from southern Chile recently analysed at nuclear-DNA and mitochondrial-DNA loci exhibited alleles that are identical to those of New Zealand *M. galloprovincialis* (Toro 1998) raising the possibility that this species might also be present in southern Chile.

The total genomic DNA was extracted from each individual and used as template for polymerase-chain reactions (PCR) using primer pairs specific of a fragment of the polyphenolic adhesive protein gene *Glu-5'*, and a fragment of intron 1 of the actin gene *mac-1*. The *Glu-5'* marker was developed by Inoue & Odo (1994) and Rawson *et al.* (1996). The *mac-1* marker was developed by Ohresser *et al.* (1997) and Daguin & Borsa (1999). Protocols for DNA extraction, PCR, gel electrophoresis, and allele nomenclature at locus *Glu-5'* have been reported in Borsa *et al.* (1999). Those for locus *mac-1* have been detailed in Daguin & Borsa (1999). *mac-1* is the only non-coding locus out of the ten nuclear loci (also including 7 allozyme loci, 18S rDNA, and *Glu-5'*) considered in this paper.

Correspondence analysis (FCA: Benzécri 1982) was performed using the AFC procedure implemented in BIOMECO (Lebreton *et al.* 1990) on the matrix of allelic frequencies per sample. This method, previously used by Coustau *et al.* (1991) on allozyme data, is well-adapted to express the genetic differences present in a data set because the eigenvalues of each FCA's axis are analogue to S. Wright's *Fst* (Guinand 1996).

Results and discussion

Table 1 gives the allele frequencies at locus *Glu-5'*. Allele *G* was fixed or nearly fixed in all *Mytilus galloprovincialis* samples [thereby extending the preliminary findings of Rawson *et al.* (1996) and Borsa *et al.* (1999)] and in the *Mytilus* sp. sample from Chile. The *Glu-5'* marker, being quasi-monomorphic, is therefore of little help to analyse the genetic relationships among *M. galloprovincialis* populations.

In contrast, preliminary surveys of genetic variation at the *mac-1* locus have demonstrated considerable polymorphism, with up to 18 size-alleles in *Mytilus galloprovincialis* samples from the NE Atlantic and the Mediterranean (Daguin & Borsa 1999). Here, allele-frequency data at locus *mac-1* suggested closer genetic affinities of Australasian *Mytilus* with *M. edulis* rather than *M. galloprovincialis* (Table 2): all three Australasian samples possessed *mac-1* allele *a*2 at high frequency (this allele is found at moderate frequency in NE Atlantic *M. edulis*, but at significantly lower frequency in *M. galloprovincialis*: Daguin & Borsa 1999; Table 2) and the Tasmanian and New Zealand samples did not possess any of the alleles (*b*2, *c*2) inferred to be characteristic of Mediterranean and NE Atlantic *M. galloprovincialis* (Daguin & Borsa 1999; Table 2) although these were present at moderate frequency in Western Australia. The three-dimensional projection of the FCA of *M. galloprovincialis* samples collected worldwide (Fig. 3) illustrates

the clear distinctness of Australasian *Mytilus*, with the first axis of FCA separating *M. edulis* and Australasian mussels from all the other samples. The second axis then clearly distinguished Australasian mussels from *M. edulis*. The third axis yielded evidence of comparatively slighter, further distinction between NE Atlantic (STB) and Mediterranean (SET) *M. galloprovincialis*. The homogeneity of *mac-1* allele frequencies within each of the two latter regions has been reported elsewhere (Daguin & Borsa 1999; unpublished). South African mussels clustered together with NE Atlantic *M. galloprovincialis* while mussel samples from California, Chile and Korea clustered together with Mediterranean *M. galloprovincialis*. The estimate of *q*, the equivalent of Wright's *Fst* (Weir & Cockerham 1984), was $\hat{q} = 0.027$ between these two groups. This value compares with the value reported at locus *mac-1* between NE Atlantic (northwestern African) and Western Mediterranean *M. galloprovincialis* ($\hat{q} = 0.016$; $p = 0.02$; Daguin & Borsa 1999) and with the mean *Gst* value reported for 13 allozyme loci between samples from the western and the eastern coasts of the Iberian Peninsula (*Gst* = 0.029; $p < 0.001$; Quesada *et al.* 1995).

Thus, *mac-1* data (Daguin & Borsa 1999; present results) are in accordance with the results of former allozyme-based surveys in (1) allowing a clear distinction between NE Atlantic and Mediterranean *Mytilus galloprovincialis* populations (Sanjuan *et al.* 1994; Quesada *et al.* 1995; Sanjuan *et al.* 1997); (2) demonstrating the genetic affinities of South African *M. galloprovincialis* with NE Atlantic *M. galloprovincialis* (Sanjuan *et al.* 1997); (3) demonstrating the genetic affinities of *M. galloprovincialis* from both the eastern and the western coasts of the North Pacific with Mediterranean *M. galloprovincialis* (Sanjuan *et al.* 1997; Fig. 1); (4) arguing against the possibility that Australasian *M. galloprovincialis* were transported to the North Pacific (Gosling 1992; Fig. 1).

Australasian *Mytilus* are *M. galloprovincialis* when considering allozyme data (McDonald *et al.* 1991), even though substantial differences with Northern Hemisphere *M. galloprovincialis* were detected using multidimensional scaling (Sanjuan *et al.* 1997) and neighbour-joining phylogenetic inference (Fig. 1). Australasian *Mytilus* remained grouped with *M. galloprovincialis* when considering *Glu-5'* data (Table 1). However, *mac-1* data (Table 2; Fig. 3) revealed strong differences between Australasian *Mytilus* and Northern Hemisphere *M. galloprovincialis*. The summing up of all data available regarding the genetic characterization of Australasian *Mytilus* (McDonald *et al.* 1991; Kenchington *et al.* 1995; this study) shows noticeable discrepancies among loci. For instance, let us examine allele frequency patterns at the four loci (allozyme loci *Est-D* and *Mpi*; nuclear-DNA loci *Glu-5'* and *mac-1*) that can be considered as diagnostic between *M. galloprovincialis* and *M. edulis* (Skibinski *et al.* 1983; Rawson *et al.* 1996; Daguin & Borsa 1999). Australasian *Mytilus* possess fixed or nearly fixed *M. galloprovincialis* alleles at *Mpi* and *Glu-5'*, while at *Est-D* the frequency of *M. edulis* alleles was zero in sample Wellington, ≈0.25 in sample Albany, and ≈0.5 in sample Huon River (McDonald *et al.* 1991); at locus *mac-1*, Australasian *Mytilus*

possess at high frequency a size-allele (*a*2) which is more characteristic of *M. edulis* than *M. galloprovincialis* (Table 2). At other allozyme loci, Australasian mussels can be considered as either closer to *M. galloprovincialis* than *M. edulis* (*Aap*, *Ap*, *Gpi*, and *Lap*), or the contrary (*Pgm*), or quite different from both (*Odh*) (McDonald *et al*. 1991). Finally, 18S rDNAs of Tasmanian *Mytilus* showed higher sequence similarity with *M. edulis* (and *M. trossulus*) than with *M. galloprovincialis* (Kenchington *et al*. 1995).

The present results provided new insight into the biogeography of *Mytilus galloprovincialis*.

First, Australasian *Mytilus* now appear to be clearly distinct from all *M. galloprovincialis* populations elsewhere in the world. We therefore reject the hypothesis that these mussels were recently introduced by man (from, e.g. the North Pacific), or that they may have undergone recent, substantial introgression by alien *M. galloprovincialis* (e.g. Seed 1992). Instead, Australasian *M. galloprovincialis* have a patchy genetic architecture, with high frequency of *M. edulis*-like alleles at some loci and of *M. galloprovincialis*-like alleles at other loci. Assuming that the choice of *M. edulis* as an outgroup of *M. galloprovincialis* holds valid, this suggests that Australasian *Mytilus* originate from a proto-*M. galloprovincialis* population that underwent introgression by proto-*M. edulis*. In other words, introgression by *M. edulis*-like alleles was detected in Australasian mussels at loci *mac-1* and *Est-D* (and, perhaps, *Pgm*) but this may be ancient since at locus *mac-1* these alleles are now fixed or quasi-fixed. It is also possible that the *M. edulis*-like genes of Australasian mussels do not derive from a proto-*M. edulis* form, but originate from more modern Southern Hemisphere *M. edulis* (South America, Falklands, Kerguelen; McDonald *et al*. 1991). We expect that sequencing analysis will allow us to tell whether the *mac-1* alleles present in Australasian mussels are phylogenetically closer to those from Northern Hemisphere, or Southern Hemisphere *M. edulis*. On the basis of the close morphological and allozymic resemblance borne by Australasian mussels to *M. galloprovincialis* (McDonald *et al*. 1991), we propose that these be attributed a subspecific rank within *M. galloprovincialis*.

Second, we reject the hypothesis, implicit in Sanjuan *et al*. (1997), that North Pacific and Mediterranean mussels are endemic forms of *M. galloprovincialis* because there was no evidence of genetic differentiation between populations of these two regions. The close genetic similarity at all loci including *mac-1*, of populations separated by such geographical distances and by geographical barriers (continents) is conform to the hypothesis of recent introduction by man. Since fossil *M. galloprovincialis* have been found in the Mediterranean (Mars 1956) and have not been reported from the North Pacific (see Introduction), it is legitimate to presume that North Pacific *M. galloprovincialis* were introduced from the Mediterranean and not the contrary. Rejecting the present existence of endemic North Pacific *M. galloprovincialis* does not necessarily imply that *M. galloprovincialis* is a Mediterranean offshoot of North Atlantic *M. edulis* as frequently assumed (Barsotti & Meluzzi 1968; Skibinski *et al*. 1983; Gosling 1984, 1992; Grant & Cherry 1985; Seed 1992; Rawson &

Hilbish 1998). As emphasized by Vermeij (1992), "It is possible that the original Tethyan *Mytilus* persisted in the Mediterranean regions throughout the Neogene, and that the Pleistocene and Recent *M. galloprovincialis* is derived from this purported stock rather than from the Pacific-derived *trossulus-edulis* group", although fossil evidence for this scenario remains to be found.

Third, *M. galloprovincialis* was here identified for the first time in Chile. Its high genetic similarity, at locus *mac-1*, with Mediterranean and North Pacific *M. galloprovincialis* suggests it has been recently introduced to Chile from either of these regions by maritime transport or perhaps by unreported, intentional transplantation. The population from which our Chilean sample originates thus appears to be different from any of the *M. edulis*-like Chilean populations sampled by McDonald *et al.* (1991), but it is perhaps the same as the one sampled by Toro (1998). In this case, the taxonomic status proposed by Toro for these mussels (a subspecies of *M. edulis*) should be disregarded.

Note added in proof: A recent mitochondrial-DNA survey aimed at elucidating the origin of the antitropical distribution pattern of *Mytilus* spp. [Hilbish *et al.*, *Mar. Biol.* 136, 69-77 (2000)] yielded results that confirm the main conclusions of the present study. Most Australasian *Mytilus* spp. female mitochondrial lineages clustered into a single clade (*D2*) whose closest relative was the *D* clade found in northern *M. galloprovincialis* (*D1*), demonstrating both the originality of Australasian *Mytilus* sp. and their close relatedness to *M. galloprovincialis*. A few Australasian mussels however possessed mitochondria of the *A* type characteristic of *M. edulis*: this is consistent with our hypothesis that these Australasian mussels derive from a proto-*M. galloprovincialis* population introgressed by *M. edulis*–like genes. Mitochondrial-DNA data were also consistent with the hypothesis that North Pacific *M. galloprovincialis* originate from the Mediterranean through human agency (all mussels from San Diego, California possessing mitochondria of type *D1* or *A* as do Mediterranean mussels), but Hilbish *et al.* (2000) failed to detect *D1* haplotypes in their Chilean samples.

We are grateful to N. Bierne, F. Bonhomme, B. Delay, M. Raymond and R.D. Ward for discussions; to J. Taylor, R.D. Ward, and an anonymous referee for comments on an earlier manuscript; to J. Beesley, W. Borgeson, P. Boudry, P. Fréon, A. Leitao, C. Lemaire, D. Moraga, M. Ohresser, J. Panfili, C. Perrin, M. Raymond and C. Riquelme for providing samples; to V. Rolland for carefully examining the Chilean *Mytilus galloprovincialis* shells; to S. Ramos Caetano for assistance in the laboratory; to IFREMER URM 16 for research funds; to MENRT and IRD for our salaries.

References

BARSOTTI, G. & MELUZZI, C. 1968. Osservazioni su *Mytilus edulis* L. e *Mytilus galloprovincialis* Lamarck. *Conchiglie*, **4**, 50-58.

BENZÉCRI, J.-P. 1982. *L'Analyse des Données. 2. L'Analyse des Correspondances*. Dunod, Paris.

BORSA, P., DAGUIN, C., RAMOS CAETANO, S. & BONHOMME, F. 1999. Nuclear-DNA evidence that northeastern Atlantic *Mytilus trossulus* mussels carry *M. edulis* genes. *Journal of Molluscan Studies*, **65**, 524-527.

CARLTON, J.T. 1987. Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science*, **41**, 452-465.

COUSTAU, C., RENAUD, F. & DELAY, B. 1991. Genetic characterization of the hybridization between *Mytilus edulis* and *M. galloprovincialis* on the Atlantic coast of France. *Marine Biology*, **111**, 87-93.

DAGUIN, C. & BORSA, P. 1999. Genetic characterisation of *Mytilus galloprovincialis* Lmk. in North West Africa using nuclear DNA markers. *Journal of Experimental Marine Biology and Ecology*, **235**, 55-65.

FELSENSTEIN, J. 1993. *PHYLIP 3.5 (Phylogeny Inference Package)*. University of Washington, Seattle, Washington, USA.

GELLER, J. B. 1999. Decline of a native mussel masked by sibling species invasion. *Conservation Biology*, **13**, 661-664.

GOSLING, E. M. 1984. The systematic status of *Mytilus galloprovincialis* in western Europe: a review. *Malacologia*, **25,** 551-568.

GOSLING, E. M. 1992. Systematics and geographic distribution of *Mytilus*. *In*: Gosling E. (ed) *The Mussel* Mytilus*: Ecology, Physiology, Genetics and Culture*. Elsevier, Amsterdam, Developments in Aquaculture and Fisheries Science, **25**, 1-20.

GRANT, W. S. & CHERRY, M. I. 1985. *Mytilus galloprovincialis* in South Africa. *Journal of Experimental Marine Biology and Ecology*, **90,** 179-191.

GREGORIUS, H. R. 1984. An unique genetic distance. *Biometrical Journal*, **26**, 13-18.

GUINAND, B. 1996. Use of a multivariate model using allele frequency distributions to analyse patterns of genetic differentiation among populations. *Biological Journal of the Linnean Society*, **58**, 173-195.

INOUE, K. & ODO, S. 1994. The adhesive protein cDNA of *Mytilus galloprovincialis* encodes decapeptide repeats but no hexapeptide motif. *Biological Bulletin*, **186**, 349-355.

KENCHINGTON, E., LANDRY, B. & BIRD, C. J. 1995. Comparison of taxa of the mussel *Mytilus* (Bivalvia) by analysis of the nuclear small-subunit rDNA gene sequence. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 2613-2620.

KOEHN, R. K. 1991. The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture*, **94**, 125-145.

LEBRETON, J.-D., ROUX, M., BANCO, G. & BACOU, A.-M. 1990. *BIOMECO (Biométrie-écologie), Logiciel d'Ecologie Statistique pour PC et Compatibles. Version 3.9*. Centre National de la Recherche Scientifique, Montpellier, France.

MARS, P. 1956. Faunes malacologiques du Pliocène et du Quaternaire de Milazzo (Sicile). *Bulletin du Muséum d'Histoire Naturelle de Marseille*, **16**, 33-52.

MCDONALD, J. H. & KOEHN, R. K. 1988. The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. *Marine Biology*, **99**, 111-118.

MCDONALD, J. H., SEED, R. & KOEHN, R. K. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the northern and southern hemispheres. *Marine Biology*, **111**, 323-333.

OHRESSER, M., BORSA, P. & DELSERT, C. 1997. Intron-length polymorphism at the actin gene locus *mac-1*: a genetic marker for population studies in the marine mussels *Mytilus galloprovincialis* Lmk and *M. edulis* L. *Molecular Marine Biology and Biotechnology*, **6**, 123-130.

QUESADA, H., ZAPATA, C. & ALVAREZ, G. 1995. A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Marine Ecology-Progress Series*, **116**, 99-115.

RAWSON, P. D. & HILBISH, T. J. 1998. Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* spp.). *Evolution*, **52**, 100-108.

RAWSON, P. D., JOYNER, K. L., MEETZE, K. & HILBISH, T. J. 1996. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. *Heredity*, **77**, 599-607.

SAITOU, N. & NEI, M. 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4,** 406-425.

SANJUAN, A., ZAPATA, C. & ALVAREZ, G. 1994. *Mytilus galloprovincialis* and *M. edulis* on the coasts of the Iberian Peninsula. *Marine Ecology-Progress Series*, **113**, 131-146.

SANJUAN, A., ZAPATA, C. & ALVAREZ, G. 1997. Genetic differentiation in *Mytilus galloprovincialis* Lmk. throughout the world. *Ophelia*, **47**, 13-31.

SEED, R., 1992. Systematics, evolution and distribution of mussels belonging to the genus *Mytilus*: an overview. *American Malacological Bulletin*, **9**, 123-137.

SKIBINSKI, D. O. F., BEARDMORE, J. A. & CROSS, T. F. 1983. Aspects of the population genetics of *Mytilus* (Mytilidae; mollusca) in the British Isles. *Biological Journal of the Linnean Society*, **19**, 137-183.

SKIBINSKI, D. O. F., CROSS, T. F. & AHMAD, M. 1980. Electrophoretic investigation of systematic relationships in the marine mussels *Modiolus modiolus* L., *Mytilus edulis* L., and *Mytilus galloprovincialis* Lmk. (Mytilidae; Mollusca). *Biological Journal of the Linnean Society*, **13**, 65-73.

TORO, J. E. 1998. PCR-based nuclear and mtDNA markers and shell morphology as an approach to study the taxonomic status of the Chilean blue mussel, *Mytilus chilensis* (Bivalvia). *Aquatic Living Resources*, **11**, 347-353.

VÄINÖLÄ, R. & HVILSOM, M. M. 1991. Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations. *Biological Journal of the Linnean Society*, **43,** 127-148.

VARVIO, S.-L., KOEHN, R. K. & VÄINÖLÄ, R. 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. *Marine Biology*, **98**, 51-60.

VERMEIJ, G. J. 1992. Trans-equatorial connections between biotas in the temperate eastern Atlantic. *Marine Biology*, **112**, 343-348.

WILKINS N.P., FUJINO, K. & GOSLING, E.M. 1983. The Mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. *Biological Journal of the Linnean Society*, **20**, 365-374.

Table 1. *Allele frequencies at locus* Glu-5' *in samples of* Mytilus galloprovincialis *from the Northern and the Southern Hemispheres, and in two* M. edulis *reference samples*

Allele*	Sample†										
	BOD	CHL	STB	SET	SAF	KOR	AUS	TAS	NZL	FLO	GIL
E	–	–	–	0.01	0.05	–	–	–	–	0.89	0.50
E'	–	–	–	–	–	–	–	–	–	0.11	0.47
E''	–	–	–	–	–	–	–	–	–	–	0.03
G	0.98	1.00	1.00	0.99	0.95	1.00	1.00	1.00	1.00	–	–
T	0.02	–	–	–	–	–	–	–	–	–	–
(*N*)‡	(23)	(48)	(19)	(56)	(65)	(19)	(46)	(25)	(77)	(35)	(16)

* nomenclature of Borsa *et al.* (1999)

† abbreviations for samples as in legend to Fig. 2; data for GIL, BOD, and SET from Borsa *et al.* (1999); additional data for SET from Rawson *et al.* (1996)

‡ sample size

Table 2. *Allele frequencies at locus* mac-1 *in samples of* Mytilus galloprovincialis *from the Northern and the Southern Hemispheres, and in three* M. edulis *reference samples*

Allele*	Sample†											
	BOD	CHL	STB	SET	SAF	KOR	AUS	TAS	NZL	FLO	GIL	GFP
*f*1	–	–	0.02	–	0.02	–	–	–	–	–	–	–
*f*2	–	–	–	–	0.01	–	–	–	–	–	–	–
*f*3	–	0.01	–	–	0.01	–	–	–	–	–	–	–
*b*0	–	–	–	–	0.01	–	–	–	–	–	–	–
*b*05	–	–	–	–	–	–	–	–	0.02	–	–	–
*b*2	0.04	0.05	0.04	0.05	0.04	0.02	–	–	–	–	–	–
*b*1	0.32	0.32	0.15	0.21	0.09	0.42	0.04	–	–	–	–	0.01
*b*3	–	0.01	–	–	–	0.02	–	–	–	–	–	–
*b*4	–	–	0.02	–	–	–	–	–	–	–	–	–
*b*5	–	–	0.02	–	–	–	–	–	–	–	–	–
*c*1	0.04	0.08	0.10	0.07	0.10	0.05	–	–	–	–	–	0.01
*c*12	–	0.01	–	–	–	–	–	–	–	–	–	–
*c*15	–	–	–	–	0.01	–	–	–	–	–	–	–
*c*2	0.41	0.39	0.50	0.54	0.53	0.43	0.16	–	–	–	–	–
*c*3	–	0.01	0.02	–	0.04	–	–	–	–	–	–	–
*c*4	–	–	–	–	–	–	–	–	–	0.05	–	0.02
*c*6	0.01	–	–	0.01	0.01	–	–	–	–	–	–	–
*a*0	–	–	–	–	–	–	–	–	–	0.02	–	–
*a*05	–	–	–	–	–	–	–	–	0.04	–	–	–
*a*1	–	–	0.02	–	–	–	–	–	–	0.06	0.02	0.01
*a*15	–	–	–	–	–	–	0.01	–	0.01	–	–	–
*a*2	–	0.03	0.02	–	0.02	0.02	0.62	0.99	0.92	0.10	0.15	0.20
*a*3	0.03	–	0.04	0.01	0.03	–	0.16	0.01	0.02	0.29	0.31	0.24
*a*4	–	0.01	–	–	–	–	–	–	–	0.07	0.17	0.18
*a*5	0.10	0.01	–	–	0.02	–	–	–	–	0.38	0.27	0.29
*a*6	–	0.01	–	0.01	0.02	–	0.01	–	–	–	0.08	0.04
*a*7	0.01	0.03	0.02	0.04	0.04	0.03	–	–	–	–	–	–
*a*8	0.01	0.05	0.04	0.06	0.01	0.02	–	–	–	–	–	–
*a*9	–	–	–	–	–	–	–	–	–	0.01	–	–
d	–	–	–	–	–	–	–	–	–	0.01	–	–
(*N*)‡	(34)	(76)	(26)	(68)	(62)	(30)	(38)	(40)	(79)	(47)	(26)	(42)

* nomenclature of Daguin & Borsa (1999); alleles are presented from the slower migrating (*f*1) to the faster migrating (*d*)

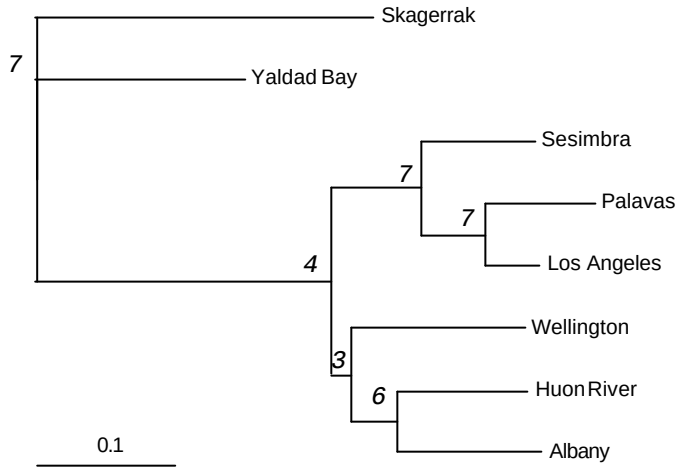
† abbreviations for samples as in legend to Fig. 2; data for GIL and SET from Daguin & Borsa (1999)

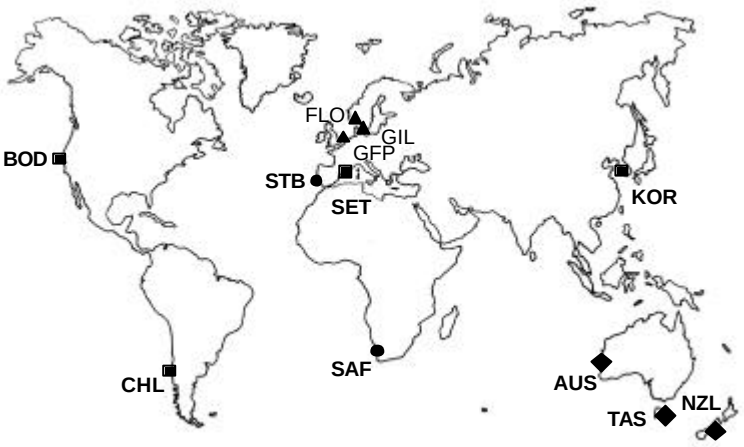
‡ sample size

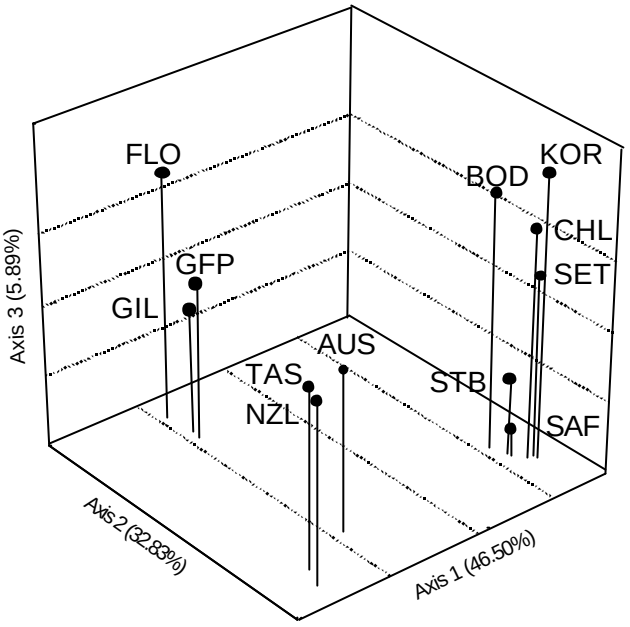
Fig. 1. *Mytilus galloprovincialis*. Neighbour-joining tree (Saitou & Nei 1987; NEIGHBOR procedure of PHYLIP: Felsenstein 1993) constructed from the matrix of absolute genetic distance estimates (Gregorius 1984), based on electromorph frequency data at 7 allozyme loci (*Ap*, *Est-D*, *Gpi*, *Lap*, *Mpi*, *Odh*, *Pgm*) in samples 'Los Angeles, California' (McDonald *et al*. 1991), 'Palavas' (Mediterranean) and 'Sesimbra' (NE Atlantic) (Quesada *et al*. 1995), 'Yaldad Bay, Chile', 'Albany, Western Australia', 'Huon River Estuary, Tasmania' and 'Wellington, New Zealand' (McDonald *et al*. 1991). Numbers at a node are scores of across-locus jacknife resampling. Electromorph identities were deduced from cross-comparisons of electromorph frequencies in McDonald & Koehn (1988), McDonald *et al*. (1991), Väinölä & Hvilsom (1991) and Quesada *et al*. (1995). *M. edulis* sample 'Skagerrak' of Väinölä & Hvilsom (1991) which is also 'SWE' of Varvio *et al*. (1988) was used as outgroup to root the tree. These samples were chosen because of their geographical proximity (=) with samples BOD (= 'Los Angeles'), CHL (= 'Yaldad Bay'), STB (= 'Sesimbra'), SET (= 'Palavas'), AUS (= 'Albany'), TAS (= 'Huon River'), NZL (= 'Wellington'), and FLO (= 'Skagerrak') of the present study (see Fig. 2). Rooting the entire tree with *M. trossulus* sample 'Tillamook, Oregon' of McDonald *et al*. (1991) did not change its topology (data not shown), confirming that the choice of *M. edulis* as outgroup for all *M. galloprovincialis* was appropriate. Scale bar = 0.1 unit absolute genetic distance

Fig. 2. Sampling sites for *Mytilus galloprovincialis*. Abbreviations for samples: *BOD* Bodega Bay, California; *CHL* Dichato, southern Central Chile; *STB* Setubal, Portugal; *SET* Sète, France [= sample SETE of Daguin & Borsa (1999)]; *SAF* Bloubergstrand, South Africa; *KOR* western coast of South Korea; *AUS* Nedlands, Western Australia; *TAS* d'Entrecasteaux Channel, Tasmania; *NZL* Dunedin, New Zealand. Symbols refer to the present nuclear-DNA characterization of samples as "Mediterranean" *M. galloprovincialis* (squares), "Atlantic" *M. galloprovincialis* (circles), and "Australasian" *M. galloprovincialis* (diamonds). Triangles symbolize *M. edulis* reference samples: *FLO* Flodevigen, Skagerrak; *GFP* Grand Fort Philippe, North France; *GIL* Gilleleje, Kattegat [= sample GILL of Daguin & Borsa (1999)]

Fig. 3. *Mytilus galloprovincalis* and *M. edulis*. Three-dimensional representation of the outcome of correspondence analysis (Benzécri 1982) on the matrix of *mac-1* allelic frequencies per sample. Abbreviation for samples as in legend to Fig. 2


Skagerrak
7
Yaldad Bay
Sesimbra
7
Palavas
7
Los Angeles
4
Wellington
3
Huon River
6
Albany
0.1


FLO
GIL
GFP
BOD
STB
SET
KOR
SAF
CHL
AUS
NZL
TAS


FLO
KOR
BOD
CHL
SET
GFP
GIL
AUS
TAS
STB
NZL
SAF
Axis 3 (5.89%)
Axis 2 (32.83%)
Axis 1 (46.50%)