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Mytilus galloprovincialis and *M. edulis* on the coasts of the Iberian Peninsula

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ABSTRACT: *Mytilus edulis* L. and *M. galloprovincialis* Lmk. are 2 forms of mussels that live on the European coasts, and where they coexist, hybridize in varying proportions. While the Atlantic coast of the Iberian Peninsula is a key area for understanding the distribution of the genus *Mytilus* in Europe, the available evidence on the distribution from this area is scarce and contradictory. Five partially diagnostic allozyme loci (*aminopeptidase-1, esterase-D, leucine aminopeptidase-1, mannose phosphate isomerase* and *octopine dehydrogenase*) and the best diagnostic morphological characters (length of the anterior adductor muscle scar and internal radius of the hinge plate and shell length and height) between *M. edulis* and *M. galloprovincialis* were investigated in 37 and 39 samples respectively taken from the Iberian coasts. Concordance between morphological variation and enzyme polymorphisms is virtually complete and indicates that *M. galloprovincialis* occurs all along the Iberian Peninsula (Mediterranean, Lusitanian, Galician Rías and Cantabrian coasts). *M. galloprovincialis* follows, therefore, a continuous distribution all along the Atlantic coast of Europe, from the Mediterranean Sea to the English Channel and the British Isles. Moreover, *M. galloprovincialis* exhibits an abrupt discontinuity in allozyme frequencies of about 40% for Odh*100 and 25% for Ap-1*100 in the southeastern Mediterranean Iberian coasts. This strong genetic differentiation appears to be associated with an oceanographic current barrier (the Almeria-Oran front). On the other hand, evidence for the presence of *M. edulis* and hybrids of *M. edulis* and *M. galloprovincialis* was obtained only on the Atlantic coast near the Hispano-French frontier. Therefore, it seems that the meridional limit of the geographical distribution of *M. edulis* in Europe, and the concomitant limit of the hybrid zone, are between the mouths of the Bidasoa and Nervion rivers in Spain. These results clarify some evolutionary aspects of *M. edulis* and *M. galloprovincialis*.

KEY WORDS: Biogeography · Iberian Peninsula · *Mytilus* · Population genetics · Isoenzyme polymorphisms

INTRODUCTION

Three forms of mussels, *Mytilus edulis* L. (blue mussel), *M. galloprovincialis* Lmk. (Mediterranean mussel) and *M. trossulus* Gould, have been distinguished on the European coasts basically on grounds of morphology and allozymes (see Skibinski et al. 1983, Gosling 1984, 1992a, b, Koehn 1991, Gardner 1992, Seed 1992). *Mytilus edulis* has been recognized with confidence along the Atlantic coast of Europe, from the northern White Sea (McDonald et al. 1990) through the western European coast including Iceland and the British Isles (Seed 1976, Skibinski et al. 1983, Varvio et al 1988, McDonald et al. 1991) as far south as the Atlantic French coast (Seed 1972, 1976, Verduin 1979, Coustau et al. 1991, McDonald et al. 1991). *M. galloprovincialis* has been unambiguously identified on the Mediterranean coast, on the British Isles and on the Atlantic coast of France (Seed 1972, 1976, Verduin 1979, Skibinski et al. 1983, Coustau et al. 1991, McDonald et al. 1991; see Gosling 1984). A third form, *M. trossulus*, has been identified on the basis of electrophoretic data and, in Europe, seems confined to the Baltic Sea (Bulnheim & Gosling 1988, Varvio et al. 1988, Johan-

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nesson et al. 1990). *M. edulis* and *M. galloprovincialis* coexist in varying proportions in some areas of the British Isles and Atlantic coast of France, where they hybridize as shown by allozyme and mitochondrial DNA data (Ahmad & Beardmore 1976, Skibinski et al. 1978, 1983, Skibinski 1985, Edwards & Skibinski 1987, Beaumont et al. 1989, Coustau et al. 1991, Hoeh et al. 1991, McDonald et al. 1991; for reviews see Koehn 1991, Gardner 1992, Gosling 1992a, b, Seed 1992). The proportion of the 2 mussel forms and the degree of hybridization and introgression depend on the sample site and local conditions such as wave exposure and attachment height (see Gosling 1992b).

Current knowledge of the distribution of the different forms of mussels in the European coasts is incomplete, particularly with respect to the Iberian Peninsula coasts. The Atlantic coast of the Iberian Peninsula is a key area for understanding the distribution of the genus *Mytilus* on the Atlantic coasts of Europe for 2 important reasons. First, the occurrence of *Mytilus galloprovincialis* in the British Isles and on the Atlantic coasts of France can be understood differently according to whether the distribution of *M. galloprovincialis* is continuous or discontinuous from the Mediterranean Sea to those coasts. Second, study of the mussels on the Atlantic Iberian coast will establish the southern extent of the distribution of *M. edulis* in Europe and the range of the hybrid zone of *M. edulis* and *M. galloprovincialis* (Koehn 1991, Gardner 1992, Gosling 1992b, Seed 1992). In spite of this, the distribution of *Mytilus* spp. on the Atlantic coast of the Iberian Peninsula has not been investigated thoroughly and there are scarce and contradictory data. One opinion is that *M. edulis* extends over the whole of the Atlantic coast of Europe as far south as northern Africa; another opinion is that *M. galloprovincialis* is the predominant mussel on the Atlantic coasts of the Iberian Peninsula (Lubet 1973, 1976, Seed 1976, 1978, Suchanek 1985). To date, studies designed to extensively and reliably analyze mussel populations have been conducted only in northwestern Iberia, and they indicate the occurrence of *M. galloprovincialis* (Sanjuan et al. 1986, 1990). Two macrogeographical surveys with allozyme characters including other locations on the Iberian coasts indicate the occurrence of *M. galloprovincialis* (in Albufeira; Bulnheim & Gosling 1988) and hybrids of the 2 mussel forms (in Parede; McDonald et al. 1991). The other references about the distribution of *Mytilus* in the Atlantic Iberian coasts are based on partial data with limited numbers of samples or individuals or use unreliable discriminant characters (for reviews see Sanjuan et al. 1986, Sanjuan 1992, and references therein). To date, no clear conclusion has been reached with regard to the identity of the mussels living on these coasts. The main aim of this work is, therefore, to identify the form or forms of mussels that live on the coasts of Iberian Peninsula. For this purpose diagnostic allozyme loci and morphological characters have been studied in mussel populations from the Iberian Peninsula.

MATERIALS AND METHODS

Populations sampled. Mussel populations were sampled along the Atlantic and Mediterranean coasts of the Iberian Peninsula from 1985 to 1988 (Table 1, Fig. 1). Most samples were collected as close as possible to the low level of the mussel bed, but some came from commercial cultures and one came from submerged rocks at 9 m depth. For comparative purposes commercial and wild pure populations of *Mytilus edulis* from the northwestern Atlantic coasts of Europe were also studied (Table 1). Mussels were brought alive to the laboratory as quickly as possible, where they were dissected. The digestive gland was removed from each individual and frozen at −70°C until prepared for allozyme analysis.

Length of anterior adductor muscle scar and internal radius of the hinge plate, the best diagnostic conchological characters between *Mytilus edulis* and *M. galloprovincialis* (Lewis & Seed 1969, Seed 1974, 1978, Verduin 1979, Sanjuan et al. 1986, 1990, Beaumont et al. 1989, Sanjuan 1992), and length and height of the shells were examined in 39 mussel samples. The length of the anterior adductor muscle scar was measured in dry shells and the internal radius of the hinge

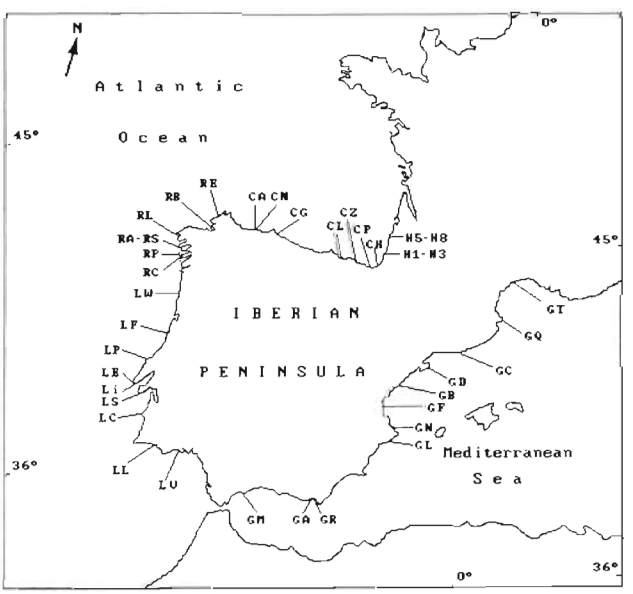

Fig. 1. Collection sites of *Mytilus* populations on the Iberian Peninsula coasts: Mediterranean (G_); Lusitanian (L_); Galician Rías (R_); Hispano-Cantabrian (C_) and French-Cantabrian (H_) populations. Population codes as in Table 1

Table 1. *Mytilus* spp. Iberian Peninsula mussel populations and typical *M. edulis* (E_) from northwestern Europe for comparison. Code is a population code; N: number of individuals scored; Morph.: samples analyzed for morphological characters; Loci: samples analyzed for allozyme loci

Populations	Code	Location	N	Morph.	Loci
Mediterranean coasts (*M. galloprovincialis*)					
Thau	GT	Basin de Thau (culture)	41		+
Cadaques	GQ	Cadaques	74		+
Cambrils	GC	Cambrils	65	+	+
Delta	GD	Delta Ebro (culture)	89	+	+
Benicasim	GB	Benicasim	100	+	
Cullera	GF	Faro de Cullera	74	+	
Denia	GN	Denia	50	+	
Altea	GL	Altea	67	+	
Almería-1	GR	Almería (east beach)	93	+	
Almería-2	GA	Almería (west beach)	82		+
Marbella	GM	Marbella (groynes)	78	+	+
Lusitanian coasts					
Vilamoura	LV	Vilamoura	105	+	+
Lagos	LL	Lagos	110	+	+
Cabo Sines	LC	Cabo Sines	65	+	+
Sezimbra	LS	Sezimbra	110	+	+
Lisboa	LI	Lisboa (harbour)	74	+	+
Estoril	LE	Estoril	110	+	+
Peniche	LP	Peniche (breakwater)	100		+
Figueira	LF	Figueira da Foz (harbour)	111		+
Varzim	LW	Povoa de Varzim	110		+
Galician Rías					
Pontevedra-5	RC	Aldán (culture)	137	+	+
Pontevedra-7	RP	Pontevedra	110	+	+
Arousa-3	RA	Carril	112	+	+
Arousa-4	RS	Illa da Rua (submerged)	107		+
Laxe-3	RL	Laxe (harbour)	100	+	+
Ares-5	RB	Sada	125	+	+
Cedeira	RE	Cedeira	100	+	+
Bay of Biscay					
Navia-1	CA	Navia	97	+	
Navia-2	CN	Navia	36	+	+
Gijón	CG	Gijón	110	+	+
La Arena	CL	La Arena (beach)	161		+
Zierbena	CZ	Zierbena (harbour)	121	+	+
Pasaia-B	CP	San Pedro de Pasaia	76		+
Hondarribia	CH	Hondarribia	120	+	+
Adour-DA	H1	Barre de L'Adour (breakwater)	120	+	+
Adour-DB	H2	Barre de L'Adour (breakwater)	30	+	+
Adour-CC	H3	Barre de L'Adour (channel)	109	+	+
Capbreton-CA	H5	Capbreton (channel)	110		+
Capbreton-CB	H6	Capbreton (channel)	226	+	+
Capbreton-FB	H7	Capbreton (groynes)	110	+	+
Capbreton-FA	H8	Capbreton (groynes)	23	+	
Northwestern Europe (*M. edulis*)					
France-R	ER	Riec-sur-Mer (culture)	70	+	+
France-D	ED	Dieppe	29	+	
Belgium	EO	Oostende	46	+	
Holland-1	EC	Holland (culture)	102	+	+
Holland-2	EN	Holland (culture)	57	+	+
Wales	EA	Anglesey	17	+	
Denmark-1	EH	Helsingør	43	+	
Denmark-2	EB	Blåvands	60	+	

plate was measured according to Verduin (1979). Because there are high correlations between some characters and shell length, the 3 original variables were transformed by dividing the measures by the shell length (Seed 1978, 1992, Beaumont et al. 1989).

Electrophoresis. Five partially diagnostic allozyme loci found to be informative for distinguishing between *Mytilus edulis* and *M. galloprovincialis* (see Ahmad & Beardmore 1976, Skibinski et al. 1978, 1980, 1983, Grant & Cherry 1985, Beaumont et al.

1989) were investigated by starch-gel electrophoresis in 37 samples. The digestive glands were homogenized in an equal volume of 0.01 M dithiothreitol solution. All the homogenized tissue samples were centrifuged at 9000 rpm (8000 × *g*) for 7 min and the supernatant was used as the enzyme source. Electrophoresis was carried out on 10 to 12% horizontal starch gels at 4 °C using standard techniques (Harris & Hopkinson 1976, Pasteur et al. 1987). Enzymatic systems were *aminopeptidase-1* (*Ap-1**), *esterase-D* (*Est-D**), *leucine aminopeptidase-1* (*Lap-1**), *octopine dehydrogenase* (*Odh**) and *mannose phosphate isomerase* (*Mpi**). Electrophoretic procedures and terminology were basically those described in Ahmad et al. (1977) and Skibinski et al. (1980) for *Ap-1**, *Est-D**, and *Lap-1**, Grant & Cherry (1985) for *Odh** and Sanjuan et al. (1990) for *Mpi**.

Data analysis. The genetic structure within and among populations was analyzed using Wright's *F*-statistics (F_{IS}, F_{ST} and F_{IT}). *F*-statistics were calculated by the method of Nei (1977, 1986; see also Nei & Chesser 1983). Estimation of F_{IS} index in each mussel population was carried out by a statistic developed by Robertson & Hill (1984), because it is unbiased for $F = 0$ and a significance test for $F = 0$ exists which has a higher statistical power than the usual chi-square test. The F_{IS} statistic was used to measure the deviation of genotype frequencies from Hardy-Weinberg proportions; positive values indicated a deficit of heterozygotes and negative values an excess. Estimation of Nei's genetic distance (Nei 1972) was carried out among pairs of populations.

Multivariate analyses were used to provide a low-dimensional representation of the data and the clearest possible description of the relations between the populations (Pimentel 1979, Reyment et al. 1984, James & McCulloch 1990). A hierarchical cluster analysis using the unweighted pair-group method with arithmetic averaging (UPGMA) was applied to the matrix of pairwise genetic distances (Sneath & Sokal 1973, Dunn & Everitt 1982). The cophenetic correlation was calculated and was used as a measure of the goodness of fit of the dendrogram to the original matrix of distances (Sneath & Sokal 1973). The samples were also ordinated with non-metric multidimensional scaling of the distance matrices (Dunn & Everitt 1982, Reyment et al. 1984). This method attempts to arrange the samples along few dimensions, so that similar samples are together and dissimilar samples are far apart. The stress, which measures the monoticity of the distances in the final configuration with the observed distances, was calculated. Low values of stress indicate a high degree of concordance of the rank orderings of new configuration and original distances. A minimum length spanning tree from the distance matrices was also computed and superimposed on the ordination diagram to graphically detect local distortions (Dunn & Everitt 1982, Rohlf 1990).

A composite genetic or hybrid index score for each individual based on its genotype at the 4 best diagnostic loci surveyed (*Est-D**, *Lap-1**, *Odh** and *Mpi**), was calculated. A score of −1 was assigned to the typical alleles of *Mytilus galloprovincialis* or diagnostic allozymes with a high frequency in *M. galloprovincialis* control populations ('G' compound allele), a score of +1 was assigned to the typical alleles of *M. edulis* ('E' compound allele), and a value of 0 was assigned to other alleles. The composite index score of an individual represented the sum of its scores over all 4 loci. So, the score of an individual could range from −8 (*M. galloprovincialis*) to +8 (*M. edulis*).

Multivariate analyses were carried out using the NTSYS-pc computer program (Rohlf 1990), and most genetic analyses were performed with GENET2 (Quesada et al. 1992). Conventional statistical calculations were carried out with the SPSS/PC package (Nie et al. 1975).

RESULTS

Allozyme frequencies at the 5 loci *Ap-1**, *Est-D**, *Lap-1**, *Mpi** and *Odh** for the 37 samples are shown in Tables 2 to 6. These allozyme loci are partially diagnostic for *Mytilus edulis* and *M. galloprovincialis* (see Skibinski et al. 1983, McDonald et al. 1991, Gardner 1992, Gosling 1992a, b, Seed 1992). The allele frequency distributions at the 5 loci in mussel populations from northwestern Europe coast (E_) and from the Mediterranean coast (G_), were used as control samples of *M. edulis* and *M. galloprovincialis* respectively and agreed with allele frequencies observed by other authors for both mussel forms (see Gardner 1992, Gosling 1992b). *Est-D** and *Mpi** loci presented a single allozyme (Est-D*90 and Mpi*100), and *Lap-1** and *Odh** a pair of pooled allozymes (Lap-1*104 and Lap-1*108, and Odh*100 and Odh*129) at high frequency in *M. galloprovincialis* collections (about 70 to 97%) and at low frequency in *M. edulis* (1 to 13%). In contrast, other allozymes at each locus, such as Est-D*100, Mpi*200, Odh*115, and Lap-1*96 and Lap-1*100 pooled, were at low frequency (1 to 29%) in *M. galloprovincialis* and at high frequency (87 to 97%) in *M. edulis*. *Ap-1** had a weaker discriminatory power.

Data of the allozyme frequencies for all populations are summarized by cluster and ordination analyses based on Nei's (1972) genetic distance. The UPGMA dendrogram (Fig. 2) shows that the deepest dichotomy (distance = 1.08) occurred between a small group, which includes the control *Mytilus edulis* (E_) and

Table 2. *Mytilus* spp. Allele frequencies at the *Ap-1** locus for Iberian Peninsula mussel populations and for typical *M. edulis*

Population	86	93	100	108	114	122	128
Mediterranean coasts (*M. galloprovincialis*)							
GT	0	0.012	0.195	0.280	0.378	0.122	0.012
GQ	0	0.007	0.164	0.452	0.233	0.137	0.007
GC	0	0.008	0.162	0.400	0.285	0.123	0.023
GD	0	0.007	0.184	0.390	0.250	0.162	0.007
GA	0	0.024	0.421	0.341	0.177	0.037	0
GM	0	0.045	0.344	0.390	0.175	0.026	0.019
Lusitanian coasts							
LV	0	0.013	0.475	0.275	0.206	0.031	0
LL	0	0.014	0.423	0.355	0.150	0.050	0.009
LC	0	0	0.400	0.369	0.177	0.054	0
LS	0	0.009	0.436	0.327	0.191	0.036	0
Li	0	0.007	0.493	0.314	0.150	0.036	0
LE	0	0.023	0.393	0.369	0.192	0.023	0
LP	0	0.010	0.400	0.355	0.185	0.050	0
LF	0	0.009	0.450	0.351	0.144	0.041	0.005
LW	0	0.014	0.418	0.327	0.209	0.032	0
Galician Rías							
RC	0.011	0.011	0.407	0.332	0.200	0.032	0.007
RP	0.006	0	0.392	0.380	0.169	0.054	0
RA	0	0.005	0.429	0.344	0.160	0.057	0.005
RS	0	0.005	0.446	0.322	0.188	0.040	0
RL	0.005	0.014	0.389	0.361	0.144	0.083	0.005
RB	0.004	0.012	0.455	0.361	0.127	0.041	0
RE	0	0.010	0.485	0.285	0.165	0.050	0.005
Bay of Biscay							
CN	0	0.014	0.361	0.306	0.250	0.069	0
CG	0	0.005	0.464	0.318	0.191	0.023	0
CL	0.003	0.006	0.440	0.326	0.168	0.047	0.009
CZ	0.004	0.004	0.425	0.325	0.196	0.038	0.008
CP	0	0.007	0.375	0.342	0.197	0.079	0
CH	0	0.004	0.432	0.297	0.212	0.051	0.004
H1	0	0.025	0.500	0.303	0.143	0.025	0.004
H2	0	0	0.433	0.283	0.267	0.017	0
H3	0	0	0.532	0.255	0.167	0.046	0
H5	0.004	0.017	0.558	0.262	0.146	0.008	0.004
H6	0.004	0.004	0.681	0.201	0.073	0.029	0.007
H7	0	0.014	0.436	0.332	0.182	0.032	0.005
Northwestern Europe (*M. edulis*)							
ER	0.007	0.007	0.799	0.157	0.030	0	0
EC	0	0.021	0.819	0.111	0.042	0.007	0
EN	0	0	0.850	0.140	0.010	0	0

some French-Cantabrian populations (H5, H6), and a large group, which includes the control Mediterranean *M. galloprovincialis* populations (G_) and the remaining populations (Atlantic Iberian populations and the other French-Cantabrian samples H1, H2, H3 and H7). For the *M. galloprovincialis* cluster, another dichotomy occurred at a distance of 0.08 with a small group of 4 Mediterranean samples from the northeastern Iberian coast (GT, GQ, GC, and GD) and a larger group, including 2 Mediterranean populations from southern Iberian coasts (GA, GM) and the Atlantic samples (L_, R_, C_, and H1, H2, H3, H7). Some samples from Capbreton (H5 and H6) were grouped with pure *M. edulis* populations and the other (H7) with the *M. galloprovincialis* cluster. The plots of 2-dimensional projections resulting from a non-metric multidimensional analysis based on Nei's (1972) genetic distance upon the 5 loci and each individual loci clarified the relationship between the populations (Fig. 3). The overall pattern based upon the 5 loci shows the extreme separation of the 3 *M. edulis* control samples (E_) and the Mediterranean *M. galloprovincialis* control samples (G_). Moreover, the 4 Mediterranean samples from the northeastern Iberian coast (GT, GQ, GC, and GD) were separated from the large group, including the 2 Mediterranean populations from the south Iberian littoral (GA, GM) and most of the Atlantic Iberian populations (Lusitanian L_, Galician R_ and Hispano-Cantabrian

Table 3. *Mytilus* spp. Allele frequencies at the *Est-D** locus for Iberian Peninsula mussel populations and for typical *M. edulis*

Population	76	82	87	90	93	100	103	107	110
Mediterranean coasts (*M. galloprovincialis*)									
GT	0.049	0.012	0	0.878	0	0.061	0	0	0
GQ	0.034	0.007	0	0.939	0	0.020	0	0	0
GC	0.031	0.038	0	0.900	0	0.031	0	0	0
GD	0.007	0	0	0.971	0	0.022	0	0	0
GA	0	0.037	0	0.939	0.012	0.012	0	0	0
GM	0.006	0.058	0	0.909	0	0.013	0.013	0	0
Lusitanian coasts									
LV	0	0.031	0	0.931	0.006	0.031	0	0	0
LL	0	0.051	0	0.866	0.032	0.046	0.005	0	0
LC	0	0.023	0	0.883	0.008	0.086	0	0	0
LS	0	0.055	0	0.864	0.014	0.068	0	0	0
Li	0.007	0.058	0	0.870	0	0.065	0	0	0
LE	0.005	0.037	0	0.911	0.009	0.037	0	0	0
LP	0.015	0.030	0.010	0.890	0	0.055	0	0	0
LF	0	0.059	0	0.891	0.009	0.041	0	0	0
LW	0.005	0.048	0	0.936	0.011	0	0	0	0
Galician Rías									
RC	0.004	0.040	0	0.885	0	0.072	0	0	0
RP	0	0.026	0	0.953	0.005	0.016	0	0	0
RA	0.005	0.029	0	0.880	0.005	0.063	0.005	0.010	0.005
RS	0.009	0.014	0	0.907	0.019	0.051	0	0	0
RL	0	0.037	0	0.903	0.005	0.056	0	0	0
RB	0	0.039	0	0.917	0.020	0.020	0	0.005	0
RE	0	0.040	0	0.935	0	0.025	0	0	0
Bay of Biscay									
CN	0.014	0.014	0	0.917	0	0.056	0	0	0
CG	0.005	0.050	0	0.901	0.005	0.036	0.005	0	0
CL	0.003	0.034	0	0.910	0.003	0.047	0.003	0	0
CZ	0.004	0.033	0	0.896	0	0.063	0.004	0	0
CP	0	0.052	0	0.870	0	0.078	0	0	0
CH	0	0.029	0	0.917	0	0.050	0	0.004	0
H1	0	0.038	0	0.811	0	0.139	0.008	0.004	0
H2	0	0.033	0	0.800	0	0.150	0.017	0	0
H3	0.009	0.018	0	0.786	0	0.182	0.005	0	0
H5	0.004	0.016	0	0.423	0	0.537	0.004	0.016	0
H6	0.002	0.013	0	0.271	0.002	0.687	0	0.013	0.011
H7	0	0.036	0	0.655	0.018	0.286	0	0.005	0
Northwestern Europe (*M. edulis*)									
ER	0	0	0	0.014	0	0.943	0	0.007	0.036
EC	0	0	0	0.022	0	0.941	0	0.007	0.029
EN	0	0	0	0.050	0	0.910	0	0.020	0.020

C_ samples). The French-Cantabrian populations (H_), except H2, were intermediately situated between the *M. edulis* cluster and the *M. galloprovincialis* group as shown by the minimum spanning tree diagram superimposed on the ordination analysis (Fig. 3; 5 loci). Some samples from the same locality of Capbreton as H5 and H6 (Capbreton channel) were close to the *M. edulis* cluster whereas H7 (Capbreton groynes) was near to the *M. galloprovincialis* group. Individual loci contributed differentially to the overall pattern of genetic divergence. *Est-D**, *Lap-1**, and *Mpi** loci produced non-metric multidimensional patterns with 2 main groups corresponding to *M. edulis* and *M. galloprovincialis* populations, whereas *Ap-1** and *Odh** loci gave the above 3 groups (see Fig. 3 for each locus). In all cases mussel populations from the French-Cantabrian coasts fell between the *M. edulis* and *M. galloprovincialis* extreme clusters: H5 and H6 (Capbreton channel) were closer to the *M. edulis* cluster in all cases, whereas H1, H2, and H3 (from Barre l'Adour) and H7 (from Capbreton groynes) were nearest to the *M. galloprovincialis* cluster with a varying arrangement.

In addition, a multivariate analysis of 3 diagnostic conchological characters between *Mytilus edulis* and *M. galloprovincialis* was carried out on the mussel populations. The raw data from each population are in Sanjuan (1992). The representation of non-metric

Table 4. *Mytilus* spp. Allele frequencies at the *Lap-1** locus for Iberian Peninsula mussel populations and for typical *M. edulis*

Population	93	96	100	102	104	108	110
Mediterranean coasts (*M. galloprovincialis*)							
GT	0	0	0.049	0.122	0.390	0.427	0.012
GQ	0.007	0.020	0.027	0.027	0.372	0.534	0.014
GC	0	0	0.048	0.056	0.476	0.373	0.048
GD	0	0.044	0.074	0.059	0.346	0.456	0.022
GA	0	0	0.056	0.031	0.395	0.494	0.025
GM	0	0	0.027	0.009	0.420	0.518	0.027
Lusitanian coasts							
LV	0	0.006	0.038	0.025	0.438	0.456	0.038
LL	0	0	0.052	0.033	0.392	0.500	0.024
LC	0	0.008	0.016	0.016	0.430	0.438	0.094
LS	0.009	0.009	0.027	0.009	0.432	0.477	0.036
Li	0	0	0.029	0.014	0.464	0.478	0.014
LE	0	0.025	0.025	0.010	0.445	0.480	0.015
LP	0	0	0.051	0.025	0.460	0.444	0.020
LF	0	0.009	0.064	0.018	0.390	0.482	0.037
LW	0	0.009	0.050	0.027	0.450	0.427	0.036
Galician Rías							
RC	0	0.011	0.051	0.011	0.394	0.504	0.029
RP	0	0	0.063	0.049	0.438	0.438	0.014
RA	0	0.010	0.029	0.025	0.436	0.495	0.005
RS	0	0.009	0.047	0.023	0.360	0.537	0.023
RL	0	0	0.034	0.053	0.403	0.471	0.039
RB	0	0.020	0.059	0.010	0.455	0.421	0.035
RE	0	0.005	0.047	0.021	0.326	0.547	0.053
Bay of Biscay							
CN	0	0.028	0.056	0	0.514	0.389	0.014
CG	0	0.005	0.046	0.005	0.361	0.546	0.037
CL	0	0.006	0.075	0.013	0.381	0.481	0.044
CZ	0	0.004	0.034	0.017	0.381	0.513	0.051
CP	0.007	0.013	0.033	0.053	0.380	0.507	0.007
CH	0.004	0.008	0.067	0.021	0.395	0.445	0.059
H1	0.004	0.025	0.182	0.025	0.352	0.403	0.008
H2	0	0.056	0.074	0.019	0.444	0.333	0.074
H3	0	0.046	0.116	0.042	0.315	0.477	0.005
H5	0.009	0.112	0.461	0.026	0.168	0.216	0.009
H6	0.018	0.151	0.539	0.026	0.138	0.123	0.004
H7	0	0.047	0.217	0.042	0.292	0.377	0.024
Northwestern Europe (*M. edulis*)							
ER	0.007	0.257	0.693	0	0.043	0	0
EC	0.025	0.108	0.825	0.025	0.017	0	0
EN	0.021	0.167	0.792	0	0.021	0	0

multidimensional scaling analysis based upon Euclidean distances of the sample means of the 3 ratios, previously standardized, is shown in Fig. 4. The control samples of *M. edulis* (E_) were strongly separated from most mussel populations. Some French-Cantabrian samples (H2, H6, H7 and H8) had intermediate positions between the 2 mussel forms. There were clear-cut morphological differences between *M. edulis* control samples and most of the Iberian mussel populations (*M. galloprovincialis*), except some French-Cantabrian mussel populations. On the other hand, there was no evidence of morphological differentiation within *M. galloprovincialis* populations as found in the allozyme data. Thus, the allozyme and morphological data showed a clear-cut difference between Mediterranean *M. galloprovincialis* and northwestern European *M. edulis*. Also, the samples from the Lusitanian, Galician and Hispano-Cantabrian coasts presented morphological and allozyme characters similar to *M. galloprovincialis*, and some mussel samples from the French-Cantabrian coast exhibited an intermediate position.

An analysis of the genetic structure of the Iberian mussel populations by mean of *F*-statistics is shown in Table 7. Two aspects are interesting: there were consistent and significant deficiencies of heterozygotes in some cases which are mainly associated with the locus *Odh**. Second, only samples from the French Atlantic

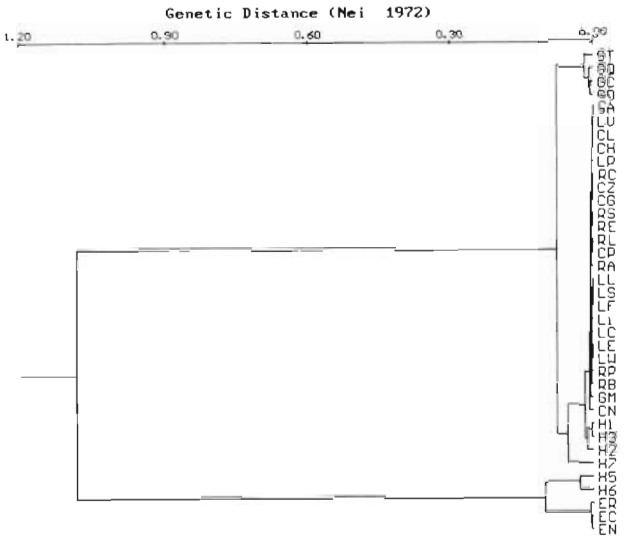

Fig. 2. *Mytilus* spp. Dendrogram showing genetic relationships among sampled populations (Mediterranean: G_; Lusitanian: L_; Galician Rías: R_; Hispano-Cantabrian: C_; French-Cantabrian: H_; control *M. edulis*: E_). Nei's genetic distance from allozyme frequencies at 5 loci were clustered using unweighted pair-group method of analysis (UPGMA). Cophenetic correlation of dendrogram is r = 0.876. Population codes as in Table 1

coast (H1 to H6) presented significant deficiencies of heterozygotes for at least 3 of the 4 best diagnostic loci (*Est-D**, *Lap-1**, *Mpi** and *Odh**). This can be explained by a mixture of 2 populations with different allozyme frequencies (Wahlund effect).

A multilocus analysis can also be informative about the genetic composition of the mussel populations. Associations by the chi-square test for different 2-locus genotype combinations were calculated. 'E' and 'G' composite alleles were considered. Allele 'E' by locus was Ap-1*100, Est-D*100, Lap-1*96 and Lap-1*100, Mpi*200, and Odh*115, and allele 'G' was Ap-1*108, Est-D*90, Lap-1*104 and Lap-1*108, Mpi*100, and Odh*100 and Odh*129. A low percentage of the chi-square tests were significant in the *Mytilus galloprovincialis* (11/57 = 19.3%) and *M. edulis* (2/20 = 10.0%) control populations, and in the Lusitanian (6/86 = 7.0%), Galician (9/70 = 12.9%) and Hispano-French (5/59 = 8.5%) populations, whereas a large number of association chi-square values (40/50 = 80.0%) were highly significant in the French-Cantabrian populations. Moreover, only French-Cantabrian populations (H_) showed a highly significant chi-square association tests ($p < 0.001$) for the combinations of the 4 best diagnostic loci. These results imply either a significant excess of double homozygote genotypes for 2 loci (E/E E/E or G/G G/G) and/or a deficit of double heterozygotes (E/G E/G) and suggest, therefore, a mixture of *M. edulis* and *M. galloprovincialis* populations. Other samples from the Iberian coast showed varying significant association values which depended of the absence or the low values of 'E/E' or 'E/G' double genotypes.

Table 5. *Mytilus* spp. Allele frequencies at the *Mpi** locus for Iberian Peninsula mussel populations and for typical *M. edulis*

Population	25	100	200	300
Mediterranean coasts (*M. galloprovincialis*)				
GT	0.012	0.927	0.061	0
GQ	0	0.973	0.027	0
GC	0	0.962	0.038	0
GD	0	0.941	0.059	0
GA	0.006	0.945	0.049	0
GM	0.013	0.941	0.046	0
Lusitanian coasts				
LV	0	0.956	0.044	0
LL	0.014	0.950	0.037	0
LC	0.023	0.931	0.046	0
LS	0.018	0.936	0.045	0
Li	0.007	0.936	0.050	0.007
LE	0.014	0.972	0.014	0
LP	0	0.935	0.065	0
LF	0.005	0.937	0.059	0
LW	0.009	0.973	0.018	0
Galician Rías				
RC	0.007	0.929	0.064	0
RP	0.008	0.944	0.040	0.008
RA	0.022	0.888	0.084	0.006
RS	0.009	0.939	0.051	0
RL	0	0.919	0.081	0
RB	0.010	0.933	0.053	0.005
RE	0.005	0.940	0.055	0
Bay of Biscay				
CN	0	0.971	0.029	0
CG	0.005	0.936	0.059	0
CL	0.006	0.944	0.050	0
CZ	0	0.962	0.038	0
CP	0	0.933	0.067	0
CH	0.004	0.925	0.063	0.008
H1	0	0.849	0.151	0
H2	0	0.833	0.167	0
H3	0.009	0.809	0.182	0
H5	0	0.439	0.553	0.009
H6	0.004	0.314	0.672	0.009
H7	0.009	0.705	0.282	0.005
Northwestern Europe (*M. edulis*)				
ER	0	0.059	0.941	0
EC	0.007	0.049	0.875	0.069
EN	0.010	0.020	0.970	0

The elaboration of a hybrid index or composite genetic index allowed an analysis of the populations at the individual level (Fig. 5). The hybrid index values of *Mytilus edulis* control samples (EN, ER) exceeded 0 (1 to 8) and the Mediterranean samples of *M. galloprovincialis* ranged from 0 to -8 (Fig. 5a). The Lusitanian (L_), Galician (R_) and Hispano-Cantabrian (C_) samples (except Hondarribia, CH) showed exclusively hybrid index values corresponding to the *M. gallo-*

Table 6. *Mytilus* spp. Allele frequencies at the *Odh** locus for Iberian Peninsula mussel populations and for typical *M. edulis*

Population	80	95	100	102	112	115	120	129	140
Mediterranean coasts (*M. galloprovincialis*)									
GT	0	0	0.211	0	0	0.316	0	0.474	0
GQ	0.007	0	0.103	0	0	0.288	0	0.603	0
GC	0.015	0	0.146	0	0	0.192	0	0.646	0
GD	0.007	0	0.162	0	0	0.140	0	0.691	0
GA	0	0	0.677	0	0	0.104	0	0.213	0.006
GM	0.007	0	0.651	0	0	0.066	0	0.270	0.007
Lusitanian coasts									
LV	0.019	0	0.625	0	0	0.112	0	0.244	0
LL	0	0	0.527	0.005	0.023	0.082	0	0.359	0.005
LC	0.016	0	0.508	0	0	0.090	0	0.377	0.008
LS	0.005	0	0.523	0	0.014	0.130	0	0.329	0
Li	0	0	0.500	0	0	0.100	0.014	0.379	0.007
LE	0.005	0	0.542	0	0	0.126	0	0.308	0.019
LP	0.010	0	0.638	0.005	0	0.117	0	0.230	0
LF	0.009	0	0.523	0	0	0.149	0.005	0.306	0.009
LW	0.009	0	0.564	0	0.005	0.095	0.005	0.323	0
Galician Rías									
RC	0.004	0.004	0.568	0.004	0.004	0.129	0	0.289	0
RP	0	0	0.500	0	0.005	0.149	0	0.346	0
RA	0.005	0	0.518	0	0	0.191	0	0.264	0.023
RS	0.005	0	0.528	0	0	0.159	0	0.308	0
RL	0	0	0.578	0.005	0.009	0.128	0	0.280	0
RB	0.020	0	0.574	0	0.004	0.098	0.008	0.283	0.012
RE	0	0	0.535	0	0	0.131	0.005	0.328	0
Bay of Biscay									
CN	0	0	0.500	0	0.014	0.114	0	0.329	0.043
CG	0.014	0	0.586	0	0	0.109	0	0.273	0.018
CL	0.003	0	0.615	0	0.003	0.127	0	0.252	0
CZ	0	0	0.593	0	0.004	0.102	0	0.301	0
CP	0	0	0.588	0	0	0.095	0	0.311	0.007
CH	0	0	0.621	0	0.004	0.162	0	0.208	0.004
H1	0.004	0	0.471	0	0.008	0.225	0.004	0.287	0
H2	0.017	0	0.567	0	0	0.150	0	0.267	0
H3	0	0	0.505	0	0.005	0.220	0	0.266	0.005
H5	0.017	0	0.291	0	0	0.543	0	0.141	0.009
H6	0.007	0	0.223	0.004	0.004	0.677	0	0.080	0.004
H7	0.005	0	0.468	0	0.005	0.286	0	0.232	0.005
Northwestern Europe (*M. edulis*)									
ER	0	0	0.088	0	0	0.868	0	0.044	0
EC	0	0	0.040	0	0	0.960	0	0	0
EN	0	0	0.021	0	0	0.947	0	0.021	0.011

provincialis range. The samples from Hondarribia (CH) and the French-Cantabrian coast (H_, Barre de L'Adour and Capbreton) exhibited values in the range of *M. edulis* and *M. galloprovincialis* as well as individuals with 0 value in a varying proportion (Fig. 5b, c, d). Most individuals with 0 value from Hondarribia and French-Cantabrian coast, where there are *M. edulis* and *M. galloprovincialis* individuals, were 'E/G' tetraheterozygotes and therefore presumably hybrids of both mussel forms. In contrast, individuals with a hybrid index of 0 from other Atlantic populations and from Mediterranean samples were never 'E/G' tetraheterozygotes.

DISCUSSION

Enzyme polymorphisms and morphological variation have revealed the ubiquitous presence of *Mytilus galloprovincialis* on the Iberian coast. The allozyme frequencies for the best diagnostic loci (*Est-D**, *Lap-1**, *Mpi** and *Odh**; Tables 3 to 6) and the UPGMA dendrogram and nonmetric multidimensional scaling from genetic and morphological distances (Figs. 2, 3 & 4) showed the clustering of most of Iberian mussel populations with pure *M. galloprovincialis* populations from the Mediterranean coast. The concordance between both morphological variation and enzyme polymor-

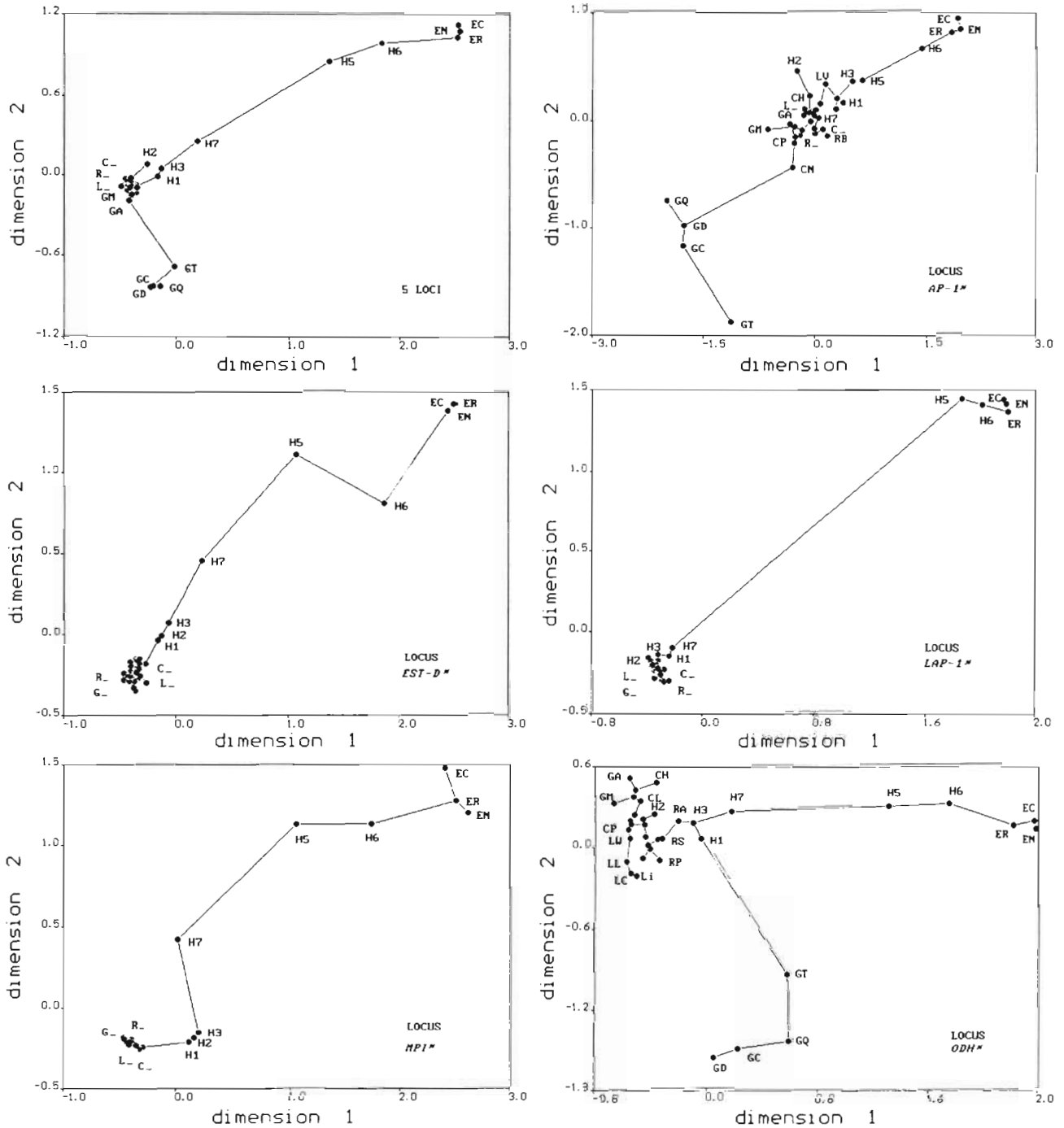

Fig. 3. *Mytilus* spp. Ordination of sampled populations in 2 dimensions by non-metric multidimensional scaling (MDS) of Nei's genetic distances calculated among all pairs of populations for the 5 loci, as well as for each locus separately (*Ap-1**, *Est-D**, *Lap-1**, *Mpi** and *Odh**). Stress of each MDS plot is 0.020, 0.076, 0.006, 0.007, 0.004, and 0.009 respectively. Minimum spanning tree is superimposed. Population codes as in Table 1

phisms in the present work is virtually complete and indicates that *M. galloprovincialis* commonly occurs along the coasts of the Iberian Peninsula: the Mediterranean, Lusitanian, Galician and Bay of Biscay coasts. These results from the Atlantic Iberian coast permit a fuller understanding of the data of Sanjuan et al. (1986, 1990), which show the presence of *M. galloprovincialis* in the NW Iberian area, and some partial data in other scattered locations (Verduin 1979, Bulnheim & Gosling 1988, McDonald et al. 1991). The present investigation

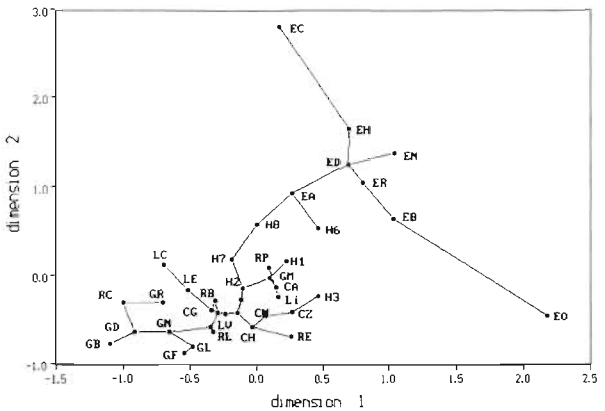

Fig. 4. *Mytilus* spp. Ordination of sampled populations in 2 dimensions by non-metric multidimensional scaling (MDS) of Euclidean distances calculated among all pairs of populations for the 3 conchological ratios. Stress is 0.099, which is a good goodness of fit. Minimum spanning tree is superimposed. Population codes as in Table 1

shows a continuous distribution of the *M. galloprovincialis* form from the Mediterranean Sea to the English Channel and British Isles. This continuous distribution of *M. galloprovincialis* seems to eliminate the possibility that British *M. galloprovincialis* populations evolved from an *M. edulis*-like ancestral population (Skibinski et al. 1978, 1983) and it suggests instead a migration and northward extension from the Mediterranean populations as suggested by Barsotti & Meluzzi (1968).

On the other hand, there was no evidence of *Mytilus edulis*, or hybrids, in mussel populations from the Mediterranean, Lusitanian, Galician and Hispano-Cantabrian (except in Hondarribia, CH) coasts, and the presence of *M. edulis* was only detected in samples near the Hispano-French frontier. In general, the UPGMA dendrogram and the nonmetric multidimensional scaling from genetic distances (Figs. 2 & 3 respectively) show that mussel populations of that

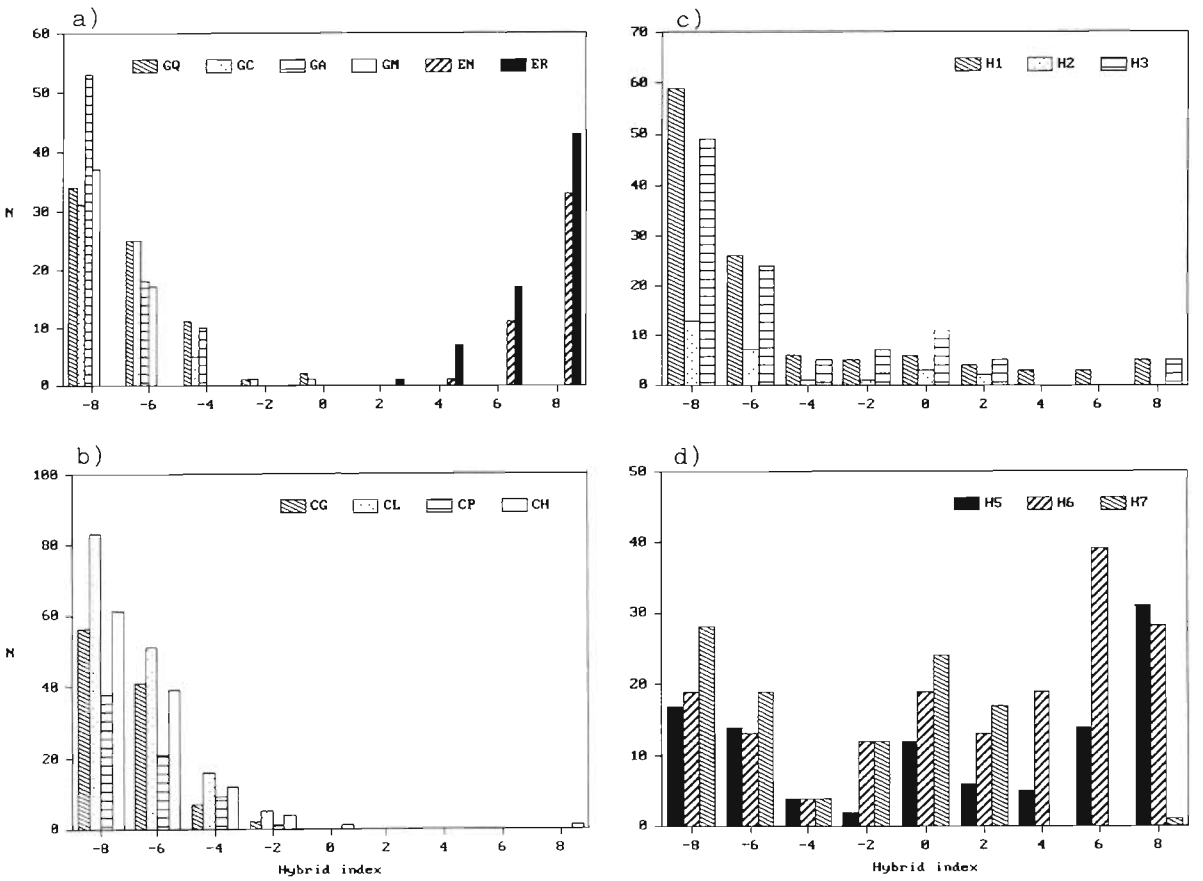

Fig. 5. *Mytilus* spp. Histograms showing distributions of the composite genetic or hybrid index in: (a) Mediterranean *M. galloprovincialis* (G_) and Atlantic *M. edulis* (E_) control populations; (b) Hispano-Cantabrian populations (C_); (c) 3 samples from Barre de L'Adour (H1, H2 and H3); and (d) 3 samples from Capbreton (H5, H6 and H7). To obtain the hybrid index, +1 was assigned to each typical allele of *M. edulis*, −1 to each typical allele of *M. galloprovincialis*, and 0 to others. The score for each individual is the sum of values across the 4 diagnostic loci (*Est-D**, *Lap-1**, *Mpi** and *Odh**). Each class is constituted by individuals with 2 consecutive hybrid index values (e.g. class −8 groups individuals with −8 and −7 scores), except class 0 which only contain individuals with 0 score for hybrid index. N: number of individuals scored

Table 7. *Mytilus* spp. Estimates of the *F*-statistics for the mussel populations from the Iberian Peninsula. F_{ST}' and F_{IT}' are Wright's fixation indices estimated after Nei & Chesser (1983), and *F* in each sample is estimated after Robertson & Hill (1984) with standard error. *M. edulis* (E_) and hybrid zone (H1 to H7) populations were not considered in calculating F_{IS}, F_{ST}', F_{IT}' and homogeneity χ^2 tests, $^{\bullet}p < 0.05$; $^{\bullet\bullet}p < 0.01$; $^{\bullet\bullet\bullet}p < 0.001$

Population	*Ap-1*• *F* ± SE	*Est-D*• *F* ± SE	*Lap-1*• *F* ± SE	*Mpi*• *F* ± SE	*Odh*• *F* ± SE
Mediterranean coasts (*M. galloprovincialis*)					
GT	0.288 ± 0.090••	0.165 ± 0.110	−0.098 ± 0.090	−0.055 ± 0.156	0.528 ± 0.115•••
GQ	−0.003 ± 0.068	−0.031 ± 0.116	−0.090 ± 0.116	−0.021 ± 0.116	0.271 ± 0.083••
GC	−0.019 ± 0.072	−0.030 ± 0.072	0.055 ± 0.063	−0.033 ± 0.124	0.075 ± 0.088
GD	0.044 ± 0.070	−0.016 ± 0.121	−0.023 ± 0.061	0.212 ± 0.121	−0.082 ± 0.086
GA	0.012 ± 0.064	−0.035 ± 0.110	0.092 ± 0.064	−0.046 ± 0.110	0.160 ± 0.078•
GM	0.106 ± 0.066	−0.061 ± 0.114	−0.020 ± 0.134	−0.043 ± 0.115	−0.007 ± 0.081
Lusitanian coasts					
LV	−0.124 ± 0.065	−0.028 ± 0.079	0.141 ± 0.065•	−0.040 ± 0.112	0.091 ± 0.079
LL	0.012 ± 0.055	0.025 ± 0.056	0.101 ± 0.056	0.226 ± 0.096•	0.252 ± 0.067•••
LC	0.066 ± 0.071	−0.094 ± 0.125	0.062 ± 0.088	−0.044 ± 0.124	0.121 ± 0.091
LS	−0.007 ± 0.055	0.176 ± 0.067••	−0.048 ± 0.067	−0.045 ± 0.095	0.120 ± 0.068
Li	0.013 ± 0.069	−0.065 ± 0.085	0.112 ± 0.120	−0.048 ± 0.120	0.026 ± 0.085
LE	−0.079 ± 0.068	−0.037 ± 0.068	0.202 ± 0.100•	−0.024 ± 0.097	0.151 ± 0.068•
LP	0.118 ± 0.058•	−0.044 ± 0.071	0.132 ± 0.071	0.266 ± 0.100••	0.224 ± 0.074••
LF	0.050 ± 0.055	0.070 ± 0.067	0.172 ± 0.055••	0.115 ± 0.095	0.205 ± 0.067••
LW	−0.036 ± 0.055	−0.047 ± 0.103	0.105 ± 0.055	−0.024 ± 0.095	0.052 ± 0.067
Galician Rías					
RC	0.040 ± 0.049	0.049 ± 0.060	−0.023 ± 0.060	−0.066 ± 0.085	0.110 ± 0.060
RP	0.105 ± 0.063	−0.023 ± 0.103	−0.089 ± 0.068	−0.035 ± 0.126	0.241 ± 0.073•••
RA	−0.018 ± 0.056	0.091 ± 0.098	0.058 ± 0.099	−0.080 ± 0.106	0.154 ± 0.067•
RS	−0.013 ± 0.057	−0.046 ± 0.097	0.041 ± 0.068	0.142 ± 0.097	0.254 ± 0.068•••
RL	0.048 ± 0.056	0.084 ± 0.068	0.202 ± 0.049•••	0.174 ± 0.098	0.186 ± 0.068••
RB	0.161 ± 0.052	−0.042 ± 0.099	−0.010 ± 0.057	−0.053 ± 0.098	0.106 ± 0.064
RE	−0.010 ± 0.058	−0.040 ± 0.100	−0.078 ± 0.059	0.139 ± 0.100	−0.040 ± 0.071
Bay of Biscay					
CN	0.035 ± 0.096	0.519 ± 0.167••	0.465 ± 0.118•••	−0.015 ± 0.169	−0.170 ± 0.098
CG	0.101 ± 0.067	−0.044 ± 0.067	−0.065 ± 0.056	−0.059 ± 0.095	0.130 ± 0.067
CL	−0.048 ± 0.046	0.029 ± 0.056	−0.017 ± 0.046	0.082 ± 0.079	0.224 ± 0.056•••
CZ	−0.002 ± 0.053	−0.045 ± 0.065	0.066 ± 0.053	0.197 ± 0.091•	0.136 ± 0.065•
CP	−0.022 ± 0.066	0.065 ± 0.081	0.073 ± 0.067	−0.065 ± 0.116	0.110 ± 0.082
CH	−0.037 ± 0.053	−0.044 ± 0.091	0.043 ± 0.053	0.078 ± 0.091	0.071 ± 0.065
H1	0.092 ± 0.065	0.225 ± 0.065•••	0.251 ± 0.065•••	0.417 ± 0.092•••	0.229 ± 0.065•••
H3	0.035 ± 0.056	0.197 ± 0.095•	0.103 ± 0.048•	0.084 ± 0.095	0.163 ± 0.068•
H5	0.100 ± 0.065	0.443 ± 0.090•••	0.244 ± 0.054•••	0.585 ± 0.093•••	0.258 ± 0.065•••
H6	0.021 ± 0.047	0.308 ± 0.066•••	0.116 ± 0.038••	0.419 ± 0.066•••	0.175 ± 0.047•••
H7	0.098 ± 0.055	−0.098 ± 0.067	−0.023 ± 0.049	−0.254 ± 0.095••	0.111 ± 0.067
Northwestern Europe (*M. edulis*)					
ER	0.181 ± 0.122	−0.032 ± 0.120	0.140 ± 0.085	0.212 ± 0.121	0.116 ± 0.086
EC	0.062 ± 0.083	−0.026 ± 0.121	0.038 ± 0.129	0.091 ± 0.083	0.386 ± 0.127••
EN	0.178 ± 0.141	−0.049 ± 0.141	0.579 ± 0.144•••	– –	0.337 ± 0.084•••
F_{IS}	0.028	−0.001	0.065	0.022	0.127
F_{IT}'	0.048	0.009	0.073	0.028	0.187
F_{ST}'	0.021	0.010	0.009	0.006	0.069
χ^2	230.8•••	40.7•	75.3•	30.1	419.3•••

area (CH, Hondarribia, and H_, Barre de L'Adour and Capbreton) exhibited intermediate positions between pure populations of *M. edulis* and Atlantic *M. galloprovincialis*. Intermediate allozyme frequencies for the diagnostic loci between those characteristic of pure *M. edulis* and *M. galloprovincialis* (Tables 2 to 6), consistent and significant deficits of heterozygotes for 3 of the 4 best diagnostic loci (*Est-D*•, *Lap-1*•, *Mpi*• and *Odh*•; Table 7), and highly significant associations between the 4 best diagnostic loci are all evidence for the mixture of 2 genetic pools, i.e. the coexistence of *M. edulis* and *M. galloprovincialis* in varying proportions. Moreover the hybrid index showed typical *M. edulis* and *M. galloprovincialis* individuals as well

as hybrids (E/G tetraheterozygotes) of *M. edulis* and *M. galloprovincialis* in the samples from this same area (CH, Hondarribia, and H_, Barre l'Adour and Capbreton; Fig. 5). The coexistence of these *Mytilus* forms on the Atlantic coast of France is in accordance with other morphological (Lubet 1959, 1973, Seed 1972, Verduin 1979, Lubet et al. 1984) and allozyme data (Coustau et al. 1991, McDonald et al. 1991). Consequently, the meridional limit of the distribution of European *M. edulis*, and the concomitant limit of the hybrid zone between *M. edulis* and *M. galloprovincialis*, can be accurately established between the mouths of the Bidasoa and Nervion rivers in Spain. This finding contradicts the previously held and widespread opinion that European *M. edulis* ranges from the Arctic to North Africa (see Seed 1976, 1978, 1992, Gosling 1984, Suchanek 1985, Gardner 1992). Only McDonald et al. (1991) cited allozyme evidence of hybrids (and *M. galloprovincialis*) in a southern location (Parede), but their study did not provide strong evidence for the extension of the hybrid zone because no evidence of *M. edulis* was found and these must be present in order to maintain a hybrid zone (an accidental or artificial introduction could explain those data).

The frequency of characteristic allozymes of *Mytilus edulis* and *M. galloprovincialis* forms in the hybrid zone of the Bay of Biscay (Tables 2 to 6) and the pattern of the hybrid index (Fig. 5) seem to be dependent on the location (CH, Hondarribia, or H_, Barre de L'Adour or Capbreton) as well as on environmental factors such as the wave exposure (channel or groynes) or the attachment height of the mussel zone, as is found in other areas of the hybrid zone (Gosling & Wilkins 1981, Skibinski 1983, Skibinski et al. 1983, Gardner & Skibinski 1988, 1991, Willis & Skibinski 1992; see Gosling 1992b for a review). A more detailed analysis of this hybrid area will be published elsewhere.

Another feature worthy of mention is that *Mytilus galloprovincialis* from the southern Mediterranean exhibits an abrupt discontinuity in allozyme frequencies of the loci *Ap-1** and *Odh**. The mean frequencies (±SE) for some common allozymes at the *Odh** and *Ap-1** loci (in the 4 samples from the northeastern Iberian coasts and in the 24 samples from southern and Atlantic Iberian coasts respectively) were as follows: Odh*100, 0.566 ± 0.011 and 0.156 ± 0.022; Odh*129, 0.296 ± 0.010 and 0.604 ± 0.047; Ap-1*100, 0.423 ± 0.008 and 0.176 ± 0.008. The frequency of these allozymes followed a stepped cline pattern with mean variations of about 40, 30 and 25% for Odh*100, Odh*129, and Ap-1*100, respectively (Tables 2 & 6). A highly significant genetic differentiation among *M. galloprovincialis* was detected at the *Ap-1** and *Odh** loci (F_{ST} was 0.021 and 0.069 respectively; Table 7). The 4 samples from the northeastern Iberian coast (GT, GQ, GC, GD) constituted a small cluster clearly separated from most *M. galloprovincialis* as shown by the UPGMA dendrogram and the non-metric multidimensional plots (Figs. 2 & 3). This strong genetic differentation within *M. galloprovincialis* over a small geographic distance calls into question the fact that *M. galloprovincialis* can be an homogeneous genetic pool as has been supposed in recent reports (Koehn 1991, McDonald et al. 1991; but see Gosling 1992b). Moreover, it contributes to the explanation of the 'intriguing pattern of variation' of *Ap** locus (and others) in mussel populations from the European coast (Varvio et al. 1988) and can also help to explain the 'large differences in mtDNA genotype' between British and Mediterranean populations (Ward 1989, Karakousis & Skibinski 1992).

The area of the Western Mediterrenean Sea where *Mytilus galloprovincialis* exhibits abrupt discontinuity in allozyme frequencies appears to be associated with an oceanographic current front. The Almería-Oran front, a strong large-scale ocean front between Almería (SE Spain) and Oran (Algeria) (Tintore et al. 1988, La Violette 1989), could be an important barrier to the migration of the mussel larvae between the 2 sides of this front. These 2 adjacent areas have different biotic and abiotic conditions: the Mediterranean Sea has characteristically warm-temperate floral and faunal assemblages (Rodriguez 1982, Ben-Tuvia 1985, Ketchum 1985, Pérès 1985, Ros et al. 1985, Sara 1985), and the Alboran Sea represents a transition zone between the Atlantic water mass and the inner Mediterranean Sea (Pérès 1967, Estrada et al. 1985, Conde 1990). In addition, no mussels were seen along the intertidal coastal level between Almería and northern Altea (Alicante) during 3 scientific trips to collect mussel samples (Sanjuan 1992), perhaps because the physical (substrate, oceanographic factors) or biological (phytoplankton concentration) conditions of the area are unsuitable for growing mussels. This lack of the appropiate conditions could cause an interruption or reduction of abundance of the mussels. These facts suggest and make plausible a discrete subpopulation model, i.e. European *M. galloprovincialis* seems subdivided at least into 2 relatively separate genetic pools. An ancient isolation of Mediterranean from Atlantic *M. galloprovincialis* populations with genetic divergence, followed by secondary contact with a restricted gene flow could explain this stepped cline pattern of allozyme variation (Endler 1977). A similar mechanism has been suggested to account genetic differentiation of *Limulus polyphemus* (Selander et al. 1970, Saunders et al. 1986) and other estuarine species in southeastern North America (see Avise 1992), and in *Platichtys flessus* (Galleguillos & Ward 1982) and *Ostrea edulis* (Saavedra et al. 1993) between Mediterranean and

Atlantic populations. The large climatic-eustatic oscillations of the Mediterranean Basin during geological periods, which have been associated with changes in the Mediterranean organism composition (Rodriguez 1982, Maldonado 1985, Pérès 1985, Raffi et al. 1985, Sara 1985, Vermeij 1989), can support this historical hypothesis.

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