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Microgeographic allozyme differentiation in the hybrid zone of *Mytilus galloprovincialis* Lmk. and *M. edulis* L. on the continental European coast

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ABSTRACT: The European *Mytilus galloprovincialis* Lmk. and *M. edulis* L. coexist and hybridize in different proportions in extended areas of the British and Atlantic French coasts. *M. galloprovincialis* typical allozymes seem to predominate in wave exposed areas, at high levels of attachment and in larger mussels in the British hybrid zone. Mussel samples from exposed and sheltered areas, 200 m apart, and from high and low levels of attachment were collected from a location of the French hybrid zone in 1988–92. Pure *M. galloprovincialis* and *M. edulis* populations were also taken as controls. Diagnostic enzyme loci for both *Mytilus* (*EST-D**, *LAP-1**, *MPI**, *ODH**) and *AP-1**, *LAP-2** and *PGM** loci were studied. The frequencies of the *M. galloprovincialis* typical alleles were significantly greater in exposed populations than in sheltered samples (e.g. 0.729 to 0.803 vs 0.192 to 0.581 for *EST-D*90*), and at high level of attachment than at low level for the sheltered area (e.g. 0.581 vs 0.192 for *EST-D*90*). Putative *M. galloprovincialis* was more abundant on the exposed coast (0.591 and 0.702) than on the sheltered shore, where it predominated at the high shore but not at the low shore location (0.371 vs 0.045). Significantly positive correlations between shell length and typical *M. galloprovincialis* compound allele frequencies were found only for populations from exposed areas. Relationships between the *Mytilus* genetic differentiation and ecological factors are discussed.

INTRODUCTION

Mytilus galloprovincialis Lmk. and *M. edulis* L. are two European mussels which coexist and hybridize in varying proportions in some areas of the British and Continental or Atlantic French coast (Hoeh et al., 1991; Sanjuan et al., 1994; see Gardner, 1992, 1994; Gosling, 1992a, b; Seed, 1992). In the British Isles, typical alleles of *M. galloprovincialis* seem to predominate at wave-exposed sites whereas the typical alleles of *M. edulis* predominate in sheltered locations (Gosling & Wilkins, 1977, 1981; Skibinski et al., 1983). Nevertheless, no clear evidence of this feature was found in mixed populations from S. W. and N. E. England (Skibinski & Roderick, 1991). Moreover, in S. W. England, *M. galloprovincialis* seems to be the more abundant form at high levels of the shore in exposed areas (Skibinski, 1983), and probably in sheltered ones (Skibinski & Roderick, 1991). In W. Ireland, high *M. galloprovincialis* allele frequency was also found higher up the shore

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for the *ODH** locus but not for the *EST-D** locus, both diagnostic loci for *M. galloprovincialis* and *M. edulis* (Gosling & McGrath, 1990). Moreover, typical *M. galloprovincialis* alleles occur at higher frequency in large mussels than in small individuals from S.W. and N.E. English populations (Skibinski, 1983; Gardner & Skibinski, 1988; Skibinski & Roderick, 1991) but this correlation was not observed for W. Irish mussel populations (Gosling & McGrath, 1990). A selective mortality hypothesis in favour of *M. galloprovincialis* has been proposed to explain the different proportions of typical alleles of *M. galloprovincialis* and *M. edulis* according to ecological factors (salinity, wave exposure and height of attachment) and shell size (Skibinski, 1983; Gardner & Skibinski, 1988; Skibinski & Roderick, 1991; Gosling, 1992b; Gardner, 1994).

In the French hybrid zone, different proportions and degrees of hybridization of both *Mytilus* forms have been reported depending on the location (Coustau et al., 1991; McDonald et al., 1991; Sanjuan et al., 1994). Nevertheless, at present no detailed studies on the relationships of this genetic differentiation and ecological factors are available. Knowledge of the influence of ecological factors on the different proportions of *M. galloprovincialis* and *M. edulis* and their degree of hybridization outside the British hybrid zone may be important to help us understand the dynamics of the hybrid zone of both *Mytilus* taxa.

The main aim of this work was to study the microgeographic genetic differentiation of allozyme loci in mussel populations from the Continental or Atlantic French hybrid zone in relation to wave exposure and level of attachment. Samples from exposed and sheltered areas (only about 200 m apart) and from high and low levels of attachment were analysed for diagnostic loci for *Mytilus galloprovincialis* and *M. edulis* (*EST-D**, *LAP-1**, *MPI** and *ODH**) and for *AP-1**, *LAP-2** and *PGM** loci.

MATERIALS AND METHODS

Mussel populations were sampled in a location (Capbreton, C__) of the Continental or Atlantic French hybrid zone of *Mytilus galloprovincialis* Lmk. and *M. edulis* L. (Fig. 1; Table 1). Mussels were collected from a wave-exposed area (breakwater) and from a sheltered one (channel of Capbreton), about 200 m away, in April 1992 (except CFA which was sampled in March 1988). Samples were taken at random at high and low levels of attachment (Table 1; Fig. 1). Two pure *M. galloprovincialis* samples (GA1, GA2), collected in July and March 1992, and a pure *M. edulis* (ME), collected in March 1990, were also studied for comparison (Fig. 1, Table 1). The mussels were brought alive to the laboratory and frozen at –70 °C until required.

Horizontal starch-gel electrophoresis was carried out on 10 to 12 % gels at 4 °C using standard techniques (Harris & Hopkinson, 1976; Murphy et al., 1990). Portions of the digestive gland were homogenized in an equal volume of 0.01 M dithiothreitol solution and centrifuged at 8000 x g for 7 min. The supernatant was used as enzyme source for seven enzyme loci: *Aminopeptidase-1* (*AP-1**), *Esterase-D* (*EST-D**), *Leucine aminopeptidase-1* (*LAP-1**), *Leucine aminopeptidase-2* (*LAP-2**), *Mannose-6-phosphate isomerase* (*MPI**), *Octopine dehydrogenase* (*ODH**), *Phosphoglucomutase* (*PGM**). *EST-D**, *LAP-1**, *MPI** and *ODH** are partially diagnostic loci between *Mytilus galloprovincialis* and *M.edulis* (Ahmad & Beardmore, 1976; Skibinski et al., 1978, 1980, 1983; Grant & Cherry, 1985; Varvio et al., 1988; Sanjuan et al., 1990). Electrophoretic procedures and

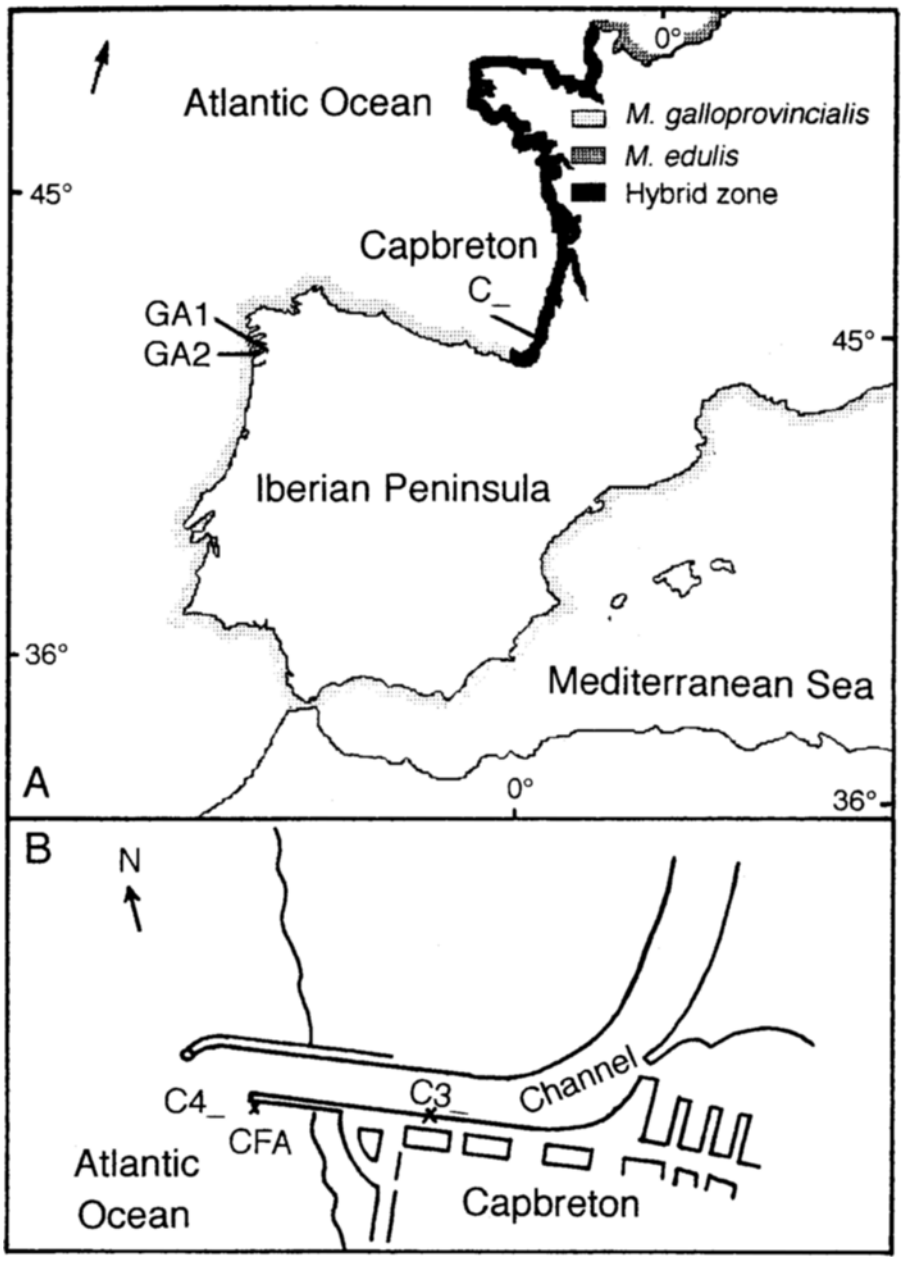

Fig. 1. A: geographic distribution of *Mytilus galloprovincialis* and *M. edulis* on the S.W. European coasts, pointing out the continental hybrid zone of both *Mytilus* (redrawn from Sanjuan, 1992) and pure *M. galloprovincialis* (GA1, GA2) and Capbreton (C_) sites. B: sample sites are shown in Capbreton (CFA and C4_: exposed samples; C3_ sheltered samples). Pure *M. edulis* were taken from The Netherlands (ME)

Table 1. Populations from the continental hybrid zone of *Mytilus galloprovincialis* and *M. edulis* (CFA, C4_, C3_) with pure *M. galloprovincialis* (GA_) and pure *M. edulis* (ME) as control populations. Degree of wave exposure and level of attachment are given for each population. N is the sample size and Code is a population code

Populations	Code	N	Degree of exposure and level of attachment
Rande B(Ría de Vigo)	GA1	133	sheltered, low
Patos B(Ría de Vigo)	GA2	126	semi-exposed, low
Capbreton FA breakwater	CFA	133	exposed, high
Capbreton 4A breakwater	C4A	123	exposed, high
Capbreton 4B breakwater	C4B	133	exposed, low
Capbreton 3A channel	C3A	124	sheltered, high
Capbreton 3B channel	C3B	132	sheltered, low
The Netherlands, culture	ME	34	

terminology were basically those described in Ahmad et al. (1977) and Skibinski et al. (1980) for *AP-1**, *EST-D**, *LAP-1**, *LAP-2** and *PGM**; Grant & Cherry (1985) for *ODH**, and Sanjuan et al. (1990) for *MPI**. Notation for allozymes were based on recommendations of Shaklee et al. (1990).

Estimation of *F* in each population was carried out by a statistic developed by Robertson & Hill (1984). This statistic is an unbiased estimate of *F*, and a significance test exists which has a higher statistical power than the usual chi-square test (Robertson & Hill, 1984; Sanjuan et al., 1990). The *F* statistic was used to measure the deviation of genotype frequencies from Hardy-Weinberg expected proportions; positive values indicated a deficit of heterozygotes, and negative values an excess. Comparisons among populations were made by homogeneity chi-square test of allele frequencies. Estimates of the null hypothesis probability were made by Monte Carlo simulation because of the expected low values (Roff & Bentzen, 1990).

Estimation of Nei's genetic distance (Nei, 1972) from the allele frequencies at the seven loci was carried out among all pairs of populations. 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 coefficient 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 a non-metric multidimensional scaling upon the distance matrices. The stress, which is a measure of the goodness of fit of the distances in the final configuration with the observed distances, was also calculated. A minimum spanning tree was superimposed on the non-metric multidimensional scaling plot to graphically detect local distortions (Dunn & Everitt, 1982; Reyment et al., 1984; Rohlf, 1990).

The frequencies of alleles at each diagnostic locus (*EST-D**, *LAP-1**, *MPI** and *ODH**) were pooled to form three synthetic alleles (**g*, **e*, **o*). The **g* synthetic allele included those alleles at high frequencies in *Mytilus galloprovincialis* (*EST-D*90*, *MPI*100*, *LAP-1*104* and *LAP-1*108* pooled and *ODH*100* and *ODH*129* pooled); the **e* alleles included those alleles at high frequencies in *M. edulis* (*EST-D*100*, *MPI*200*, *LAP-1*96* and *LAP-1*100* pooled and *ODH*115*); the **o* alleles included other alleles.

The *g and *e synthetic alleles for each locus were averaged over the four diagnostic loci to calculate mean *G and *E compound alleles according to the method of Skibinski (1983). A hybrid index or composite genetic index using the 4 diagnostic loci was also applied to identify the mussel forms according to Sanjuan et al. (1994). The hybrid index score for each individual could range from −8 to +8. Individuals with values between −8 and −5 were regarded as putative *M. galloprovincialis*, between +5 and +8 as putative *M. edulis*, and individuals with 0 value and tetraheterozygotes **eg* as hybrids. Corrected normalized values were used for individuals where one locus was unscored.

The shell length of the mussels from 4 mixed populations (C4A, C4B, C3A, C3B) was measured to study the relationship between the frequency of *Mytilus galloprovincialis* typical alleles and shell length. Length categories of 0.5 cm interval were considered. For this particular analysis, a larger number of small and large individuals were chosen from each population to increase the sample size of smallest and largest length categories. The frequencies of *G and *E compound alleles were calculated for each length category. Linear regression between frequency of *G allele arcsin transformed and the mid-point of each length category was made (see Gardner & Skibinski, 1988).

Most genetic analyses were performed with GENET-2 (Quesada et al., 1992) and Zaykin & Pudovkin (1993) computer programs. Multivariate analyses were carried out using the NTSYS-pc computer program (Rohlf, 1990) and conventional statistical calculations with SPSS/PC package (Nie et al., 1975).

RESULTS

Allele frequencies at diagnostic (*EST-D**, *LAP-1**, *MPI**, *ODH**) and non-diagnostic loci (*AP-1**, *LAP-2**, *PGM**) for analysed samples are shown in Table 2. Allele frequencies for diagnostic loci for pure *Mytilus galloprovincialis* (GA_) and pure *M. edulis* (ME) populations agreed with those previously reported (see Gardner, 1992; Gosling, 1992b; Sanjuan et al., 1994). Populations from the hybrid zone (C__) showed most abundant alleles for both diagnostic and non-diagnostic loci in intermediate frequencies between those for pure control populations (GA_ and ME). For example, for *EST-D** locus, the *EST-D*90* allele had a frequency of about 0.900 for the *M. galloprovincialis* populations (GA_), 0.074 for the *M. edulis* control population (ME), and ranged from 0.803 to 0.192 for populations from the hybrid zone (C__) (Table 2).

The Robertson & Hill (1984) estimate of *F* per locus in each population is shown in Table 3. Mixed populations from the sheltered area (C3_) showed significant positive *F* values for at least three of the four diagnostic loci but it was not detected for non-diagnostic loci (*AP-1**, *LAP-2**, *PGM**). These results and their intermediate frequencies between pure *Mytilus galloprovincialis* (GA_) and *M. edulis* (ME)samples, may be related with a Wahlund effect, because larger differences in the allele frequencies for diagnostic loci than for non-diagnostic loci exist for pure *M. galloprovincialis* and *M. edulis* (Table 2). Two mixed populations from the exposed area (C4_) and a control *M. galloprovincialis* (GA2) showed significant positive *F* values for at least two diagnostic loci. On the other hand, *LAP-1** and *ODH** loci showed significant positive *F* values for five of a total of eight samples including pure *M. galloprovincialis* and *M. edulis* populations.

The UPGMA dendrogram (Fig. 2A) showed the deepest dichotomy (genetic distance = 0.58) between a small group, which included the control *Mytilus edulis* (ME) and a

Table 2. Allele frequencies at diagnostic loci for *Mytilus galloprovincialis* and *M. edulis* (*EST-D**, *LAP-1**, *MPI**, *ODH**) and at *AP-1**, *LAP-2** and *PGM** loci for mussel populations from the hybrid zone of *M. galloprovincialis* and *M. edulis* (C_). Pure *M. galloprovincialis* (GA_) and *M. edulis* (ME) samples are given as control populations. N is the sample size. Population codes as in Table 1

	Populations							
Locus	GA1	GA2	CFA	C4A	C4B	C3A	C3B	ME
*EST-D**								
**76*	0.000	0.012	0.000	0.000	0.000	0.008	0.004	0.000
**82*	0.056	0.047	0.027	0.029	0.012	0.024	0.011	0.000
**87*	0.004	0.000	0.000	0.008	0.000	0.000	0.000	0.000
**90*	0.902	0.894	0.803	0.781	0.729	0.581	0.192	0.074
**93*	0.004	0.004	0.004	0.008	0.012	0.004	0.000	0.000
**100*	0.034	0.043	0.159	0.174	0.244	0.378	0.771	0.897
**107*	0.000	0.000	0.008	0.000	0.000	0.004	0.015	0.015
**110*	0.000	0.000	0.000	0.000	0.004	0.000	0.008	0.015
N	133	126	132	121	129	123	133	34
*LAP-1**								
**93*	0.000	0.008	0.000	0.009	0.000	0.000	0.000	0.015
**96*	0.024	0.000	0.011	0.017	0.043	0.052	0.118	0.162
**100*	0.077	0.086	0.226	0.132	0.177	0.326	0.630	0.794
**102*	0.008	0.000	0.000	0.004	0.012	0.004	0.000	0.000
**104*	0.350	0.500	0.344	0.423	0.350	0.304	0.160	0.015
**108*	0.516	0.391	0.419	0.397	0.409	0.304	0.092	0.015
**110*	0.024	0.016	0.000	0.017	0.008	0.009	0.000	0.000
N	123	64	93	117	127	115	119	34
*MPI**								
**25*	0.013	0.012	0.000	0.000	0.000	0.000	0.000	0.015
**100*	0.970	0.938	0.975	0.858	0.792	0.679	0.252	0.044
**200*	0.017	0.049	0.025	0.138	0.208	0.321	0.744	0.941
**300*	0.000	0.000	0.000	0.004	0.000	0.000	0.004	0.000
N	117	81	20	123	120	120	129	34
*ODH**								
**80*	0.004	0.011	0.006	0.004	0.008	0.012	0.000	0.031
**95*	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
**100*	0.428	0.447	0.500	0.491	0.432	0.354	0.176	0.000
**102*	0.004	0.000	0.000	0.000	0.004	0.008	0.004	0.000
**112*	0.000	0.000	0.000	0.009	0.004	0.000	0.000	0.000
**115*	0.174	0.117	0.256	0.222	0.252	0.439	0.748	0.938
**120*	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000
**129*	0.390	0.404	0.237	0.256	0.282	0.183	0.069	0.031
**140*	0.000	0.021	0.000	0.004	0.019	0.004	0.000	0.000
N	132	94	78	117	133	123	131	32
*AP-1**								
**86*	0.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000
**93*	0.015	0.004	0.027	0.004	0.011	0.024	0.008	0.000
**100*	0.389	0.419	0.454	0.451	0.489	0.463	0.682	0.735
**108*	0.374	0.335	0.313	0.325	0.299	0.297	0.205	0.221
**114*	0.187	0.185	0.149	0.167	0.163	0.167	0.072	0.015
**122*	0.031	0.052	0.053	0.045	0.038	0.049	0.034	0.029
**128*	0.004	0.004	0.004	0.004	0.000	0.000	0.000	0.000
N	131	124	131	123	132	123	132	34

Table 2 (continued)

Locus	Populations							
	GA1	GA2	CFA	C4A	C4B	C3A	C3B	ME
*LAP-2**								
**90*	0.000	0.004	0.000	0.012	0.000	0.008	0.013	0.015
**95*	0.044	0.044	0.071	0.041	0.064	0.065	0.126	0.076
**100*	0.492	0.504	0.457	0.516	0.557	0.569	0.559	0.606
**102*	0.000	0.004	0.000	0.012	0.004	0.008	0.000	0.000
**105*	0.440	0.425	0.429	0.382	0.341	0.319	0.286	0.258
**107*	0.000	0.004	0.000	0.004	0.000	0.000	0.000	0.000
**110*	0.024	0.013	0.043	0.033	0.034	0.032	0.017	0.045
N	125	114	127	123	132	124	119	33
*PGM**								
**88*	0.004	0.000	0.000	0.000	0.000	0.000	0.004	0.000
**92*	0.008	0.022	0.008	0.020	0.011	0.016	0.023	0.000
**96*	0.149	0.163	0.111	0.139	0.158	0.133	0.106	0.188
**100*	0.617	0.511	0.603	0.598	0.602	0.565	0.617	0.646
**102*	0.000	0.022	0.024	0.012	0.008	0.000	0.011	0.021
**104*	0.210	0.253	0.246	0.217	0.199	0.250	0.223	0.146
**107*	0.012	0.017	0.000	0.012	0.015	0.032	0.011	0.000
**110*	0.000	0.011	0.008	0.000	0.008	0.004	0.004	0.000
N	124	89	63	122	133	124	132	24

mixed population from the sheltered area (C3B), and another group, which included the *M. galloprovincialis* control populations (GA_) and the remaining mixed populations (CFA, C4A, C4B, C3A). In this latter group, the population from the sheltered area and with high level of attachment (C3A) was separated from most samples. The 2-dimensional projection plot of the non-metric multidimensional analysis and the superimposed minimum spanning tree showed that the populations from the hybrid zone were situated in an intermediate position between the extreme *M. galloprovincialis* (GA_) and *M. edulis* (ME) control populations (Fig. 2B). The population from the sheltered area and with low level of attachment (C3B) was near the ME control population. The populations on the exposed shore (CFA, C4_) were close to the GA_ control populations, whereas the population from the sheltered area and high level of attachment (C3A) had an intermediate position between the sheltered population with low level of attachment (C3B) and the samples from exposed area (CFA, C4_). The intermediate position of the C3A sample could be a consequence of the intermediate allele frequencies for diagnostic loci (*EST-D**, *LAP-1**, *MPI**, *ODH**) between those samples from exposed area (CFA, C4_) and those from the sheltered area and low level of attachment (C3B) (see Table 2). The same topology and ordination of samples was obtained when the genetic distances were calculated using only the diagnostic loci (data not shown). These data revealed the distinct *Mytilus* composition of each sample from the hybrid zone.

The frequencies of the synthetic alleles for each locus (**g*, **e*, **o*) are shown in Table 4. Populations from the hybrid zone (C__) showed intermediate allele frequencies between those of pure *Mytilus galloprovincialis* and *M. edulis*. Populations from the exposed shores (CFA, C4_) showed **g* allele frequencies (0.714 to 0.975) near those of

Table 3. Estimates of the *F* statistics for each locus after Robertson & Hill (1984) with standard error (SE) for $F = 0$ for populations from the continental hybrid zone of *Mytilus galloprovincialis* and *M. edulis* (CFA, C4_, C3_), and pure *M. galloprovincialis* (GA_) and pure *M. edulis* (ME). Population codes as in Table 1

	Populations							
Locus	GA1	GA2	CFA	C4A	C4B	C3A	C3B	ME
*EST-D**								
F	−0.047	−0.048	−0.004	0.140	0.423***	0.277**	0.234**	−0.071
SE	0.061	0.063	0.087	0.091	0.088	0.090	0.087	0.172
*LAP-1**								
F	0.194**	0.512***	0.159*	0.051	0.019	0.123*	0.243***	0.242
SE	0.064	0.088	0.073	0.065	0.051	0.054	0.053	0.172
*MPI**								
F	0.328***	−0.048	0.000	0.185*	−0.006	0.144	0.200*	−0.001
SE	0.065	0.111	0.224	0.090	0.091	0.091	0.088	0.172
*ODH**								
F	0.107	0.091	0.153	0.178**	0.213***	0.142*	0.245***	0.507***
SE	0.062	0.073	0.080	0.065	0.061	0.064	0.062	0.125
*AP-1**								
F	−0.056	0.025	0.045	−0.016	0.004	0.010	−0.028	0.047
SE	0.050	0.052	0.050	0.052	0.050	0.052	0.050	0.172
*LAP-2**								
F	0.160*	−0.050	0.066	0.063	0.185***	0.030	−0.086	−0.052
SE	0.063	0.066	0.051	0.052	0.050	0.052	0.065	0.101
*PGM**								
F	−0.060	0.134	0.064	0.126*	0.090	0.031	0.041	0.030
SE	0.064	0.075	0.089	0.064	0.061	0.052	0.062	0.144

*$P < 0.05$ **$P < 0.01$ ***$P < 0.001$ (significance levels for X^2 test)

M. galloprovincialis control populations (GA_; 0.818 to 0.970) and greater than those from the sheltered area (C3_; 0.192 to 0.679). In the sheltered area, the population from low level of attachment (C3B) showed **g* allele frequencies about 0.192 to 0.252, near those of *M. edulis* control population (ME; 0.030 to 0.074), whereas the population from high level (C3A) showed intermediate **g* allele frequencies (0.537 to 0.679) between C3B sample (0.192 to 0.252) and those from the exposed area (CFA, C4_; 0.714 to 0.975).

The distributions of the hybrid index for each sample are shown in Figure 3. The CFA sample was not considered in this and subsequent analyses because it was collected at a different date (March 1988). The histogram for *Mytilus galloprovincialis* (GA1) and *M. edulis* (*ME*) control populations showed a unimodal distribution with a mode at −8 value (51 % of individuals) and +8 value (74 % of individuals), respectively (Fig. 3A). One individual had 0 value, but it was not an **eg* tetraheterozygote. The mixed populations (C__) showed bimodal or trimodal distributions (Fig. 3B). The modes with the highest frequencies were at −8 value for C4A, C4B and C3A with 46 %, 33 % and 22 % of sampled

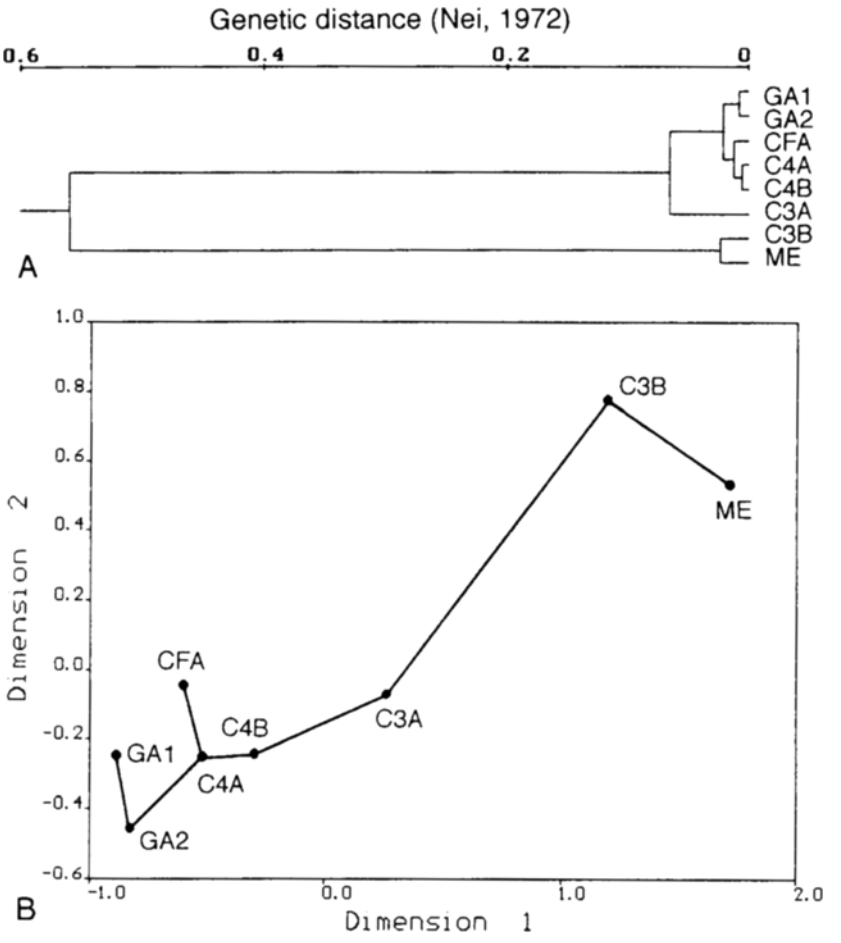

Fig. 2. A: UPGMA dendrogram; B: non-metric multidimensional scaling plot of mussel populations from the continental hybrid zone (C__) and pure *Mytilus galloprovincialis* (GA_) and *M. edulis* (ME) upon genetic distances (Nei, 1972) using alleles frequencies at seven enzyme loci (*AP-1**, *EST-D**, *LAP-1**, *LAP-2**, *MPI**, *ODH** and *PGM**). The cophenetic correlation coefficient for the UPGMA dendrogram was $r = 0.829$. The final stress for the non-metric multidimensional scaling analysis was $S = 0.007$, which means an excellent adjustment. Minimum spanning tree was superimposed on the non-metric multidimensional scaling plot. Population codes as in Table 1

individuals respectively, and at +8 value for C3B with 30 %. The other modes were at 0 value for C4A (9 %) and C3A (20 %), at 2 value for C4B (8 %) and at –2 and –4 value for C3B (5 %) populations. C4A and C3B populations seem to show also a weak third mode at 8 (2 %) and –8 (4 %) values, respectively. These results clearly suggested that each mixed sample comprises a mixture of *M. galloprovincialis*, *M. edulis*, hybrid and introgressed forms in varying proportions.

The distributions of putative *Mytilus galloprovincialis* (Mg), putative *M. edulis* (Me) and their hybrids (H) for mixed populations (C__) are compared in Table 5. Putative

Table 4. Frequencies of the synthetic alleles *g (typical of *Mytilus galloprovincialis*), *e (typical of *M. edulis*) and *o (others) for populations from the hybrid zone of *M. galloprovincialis* and *M. edulis* (CFA, C4A, C4B, C3A, C3B) and for pure *M. galloprovincialis* (GA1, GA2) and *M. edulis* (ME) populations as control. N is the sample size. Population codes as in Table 1

	Populations							
Locus	GA1	GA2	CFA	C4A	C4B	C3A	C3B	ME
*EST-D**								
*g	0.902	0.894	0.803	0.781	0.729	0.581	0.192	0.074
*e	0.034	0.043	0.159	0.174	0.244	0.378	0.771	0.897
*o	0.064	0.063	0.038	0.045	0.027	0.041	0.038	0.030
N	133	127	132	121	129	123	133	34
*LAP-1**								
*g	0.866	0.891	0.763	0.820	0.760	0.609	0.252	0.030
*e	0.102	0.086	0.237	0.150	0.220	0.378	0.748	0.956
*o	0.033	0.023	0.000	0.030	0.020	0.013	0.000	0.015
N	123	64	93	117	127	115	119	34
*MPI**								
*g	0.970	0.938	0.975	0.858	0.792	0.679	0.252	0.044
*e	0.017	0.049	0.025	0.138	0.208	0.321	0.744	0.941
*o	0.013	0.012	0.000	0.004	0.000	0.000	0.004	0.015
N	117	81	20	123	120	120	129	34
*ODH**								
*g	0.818	0.851	0.737	0.748	0.714	0.537	0.244	0.031
*e	0.174	0.117	0.256	0.222	0.252	0.439	0.748	0.938
*o	0.008	0.032	0.006	0.030	0.034	0.024	0.008	0.031
N	132	94	78	117	133	123	131	32

M. galloprovincialis was clearly the most abundant form in exposed populations (C4_) (70.2 % and 59.1 %), whereas it was at lower frequencies in sheltered samples (C3_)(37.1 % and 4.5 %). Significant differences in the distribution of putative *M. galloprovincialis* (Mg), *M. edulis* (Me) and hybrids (H) were found between exposed and sheltered samples for high and low level of attachment (Table 5). Also significant differences in the allele frequencies of diagnostic loci were detected between exposed and sheltered populations on high level shore (*EST-D** $X^2 = 16.2$, $P < 0.001$; *LAP-1** $X^2 = 17.8$, $P < 0.001$; *MPI** $X^2 = 12.7$, $P < 0.01$; *ODH** $X^2 = 16.5$, $P < 0.01$) as well as at low level of attachment (*EST-D** $X^2 = 80.8$, $P < 0.001$; *LAP-1** $X^2 = 70.7$, $P < 0.001$; *MPI** $X^2 = 72.4$, $P < 0.001$; *ODH** $X^2 = 69.3$, $P < 0.001$). For non-diagnostic loci, significant differences were detected only for *AP-1** locus ($X^2 = 10.8$, $P < 0.05$) at low level of attachment. In relation to the level of attachment, putative *M. galloprovincialis* was more abundant at high level of attachment both at exposed (70.2 % vs 59.1 %) and sheltered (37.9 % vs 4.5 %) sites, but significant differences were found only among sheltered populations (C3A vs C3B). Significant differences in allele frequencies at diagnostic loci for homogeneity chi-square test were detected among sheltered populations (C3A vs C3B) (*EST-D** $X^2 = 44.7$, $P < 0.001$; *LAP-1** $X^2 = 34.5$, $P < 0.001$; *MPI** $X^2 = 45.7$, $P < 0.001$; *ODH** $X^2 = 29.3$, $P < 0.001$) and also for the non-diagnostic *AP-1** locus ($X^2 = 14.0$, $P < 0.05$). Hybrid individuals exhibited the

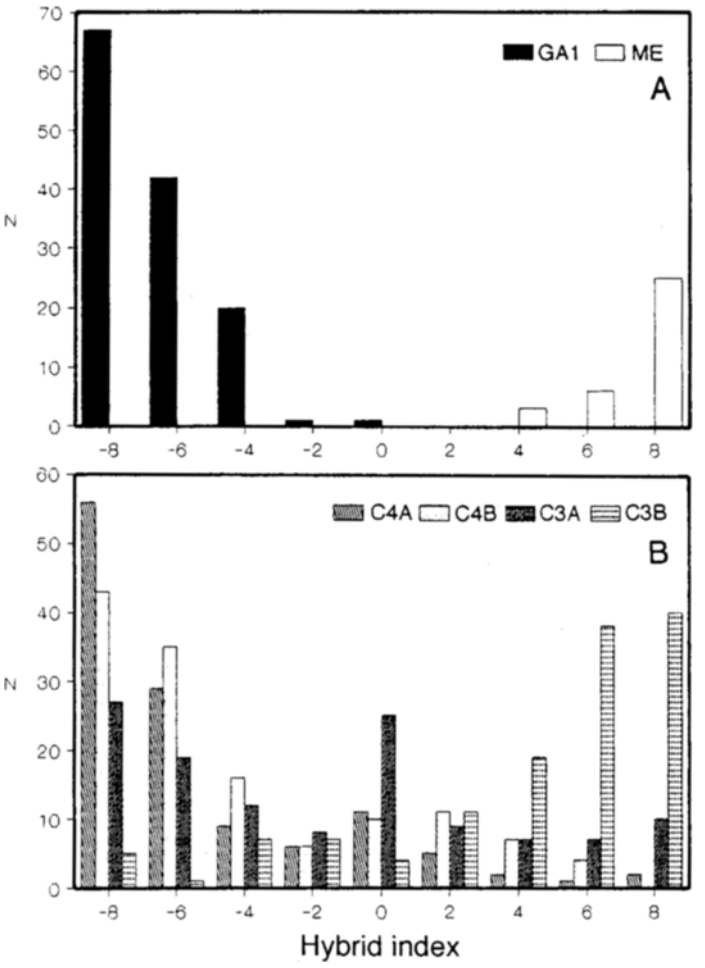

Fig. 3. Histograms showing the distributions of the hybrid index for (A) pure *Mytilus galloprovincialis* (GA1) and *M. edulis* (ME) control populations and for (B) four populations from the hybrid zone (C_). N is the number of individuals. Population codes as in Table 1

highest frequency (12.9 %) in the C3A sample (high level of attachment of the sheltered population).

The relationship between the frequency of the compound allele of *Mytilus galloprovincialis* (**G*) and the shell length for populations from the hybrid zone is shown in Figure 4. An increase of **G* allele frequency with length may be observed for exposed samples (C4_), but it was not clear for sheltered samples (C3_). Linear regression analysis between mean compound **G* allele frequency arcsin transformed and mid-point of each shell length category showed significant positive correlation coefficients only for the exposed populations (Table 6), where the *M. galloprovincialis* form is the most abundant (see Table 5).

Table 5. Distribution and percentage of putative *Mytilus galloprovincialis* (Mg), putative *M. edulis* (Me) and its putative hybrids (H) for four populations of the hybrid zone (C4A, C4B, C3A, C3B), according to the degree of wave exposure and the level of attachment to the shore. An individual was considered putative *M. galloprovincialis* (Mg) when the hybrid index was between −5 to −8, putative *M. edulis* (Me) between +5 to +8, and hybrids (H) when it was zero and tetraheterozygote. The percentage of each taxon in respect of the sample size is given in parentheses. The homogeneity chi-square test values among pairs of populations are also given (X^2). N is the sample size. Population codes as in Table 1

Degree of Exposure	Level of attachment: High level	Low level	X^2
Exposed:	C4A population	C4B population	
Mg	85 (70.2 %)	78 (59.1 %)	
H	8 (6.6 %)	6 (4.5 %)	0.4
Me	3 (2.5 %)	4 (3.0 %)	
N	121	132	
Sheltered:	C3A population	C3B population	
Mg	46 (37.1 %)	6 (4.5 %)	
H	16 (12.9 %)	3 (2.3 %)	78.6***
Me	17 (13.7 %)	78 (59.1 %)	
N	124	132	
X^2	22.6***	129.5***	

*$P < 0.05$ **$P < 0.01$ ***$P < 0.001$ (significance levels for X^2 test)

Table 6. Linear regression of the arcsin transformed of the *G compound allele frequency against mid-point of shell length category for four populations from the hybrid zone (C4A, C4B, C3A, C3B). Population codes as in Table 1

Population	Intercept	Slope	Coefficient(r)	Significance
C4A	0.7044	0.1431	0.9354	**
C4B	0.7816	0.1071	0.8475	**
C3A	0.6539	0.0662	0.4421	ns
C3B	0.3027	0.0919	0.5708	ns

** $P < 0.01$ (significance level for F test; ns = no significant level)

DISCUSSION

Clear genetic microdifferentiation depending on ecological factors and shell size was found in populations from a location of the continental European hybrid zone of *Mytilus galloprovincialis* and *M. edulis* (see Fig. 1). The UPGMA dendrogram and the multidimensional scaling plot showed that populations from an exposed shore (CFA, C4A, C4B) were grouped with those of *M. galloprovincialis* control populations (GA1, GA2) (Fig. 2A, B); one population from a sheltered area and with a low level of attachment (C3B) was grouped with the *M. edulis* control population (ME); and the other sheltered population with a high level of attachment (C3A) had an intermediate position (Fig. 2B). The relationship among samples seems to depend on the proportions of the typical *M. gallopro-*

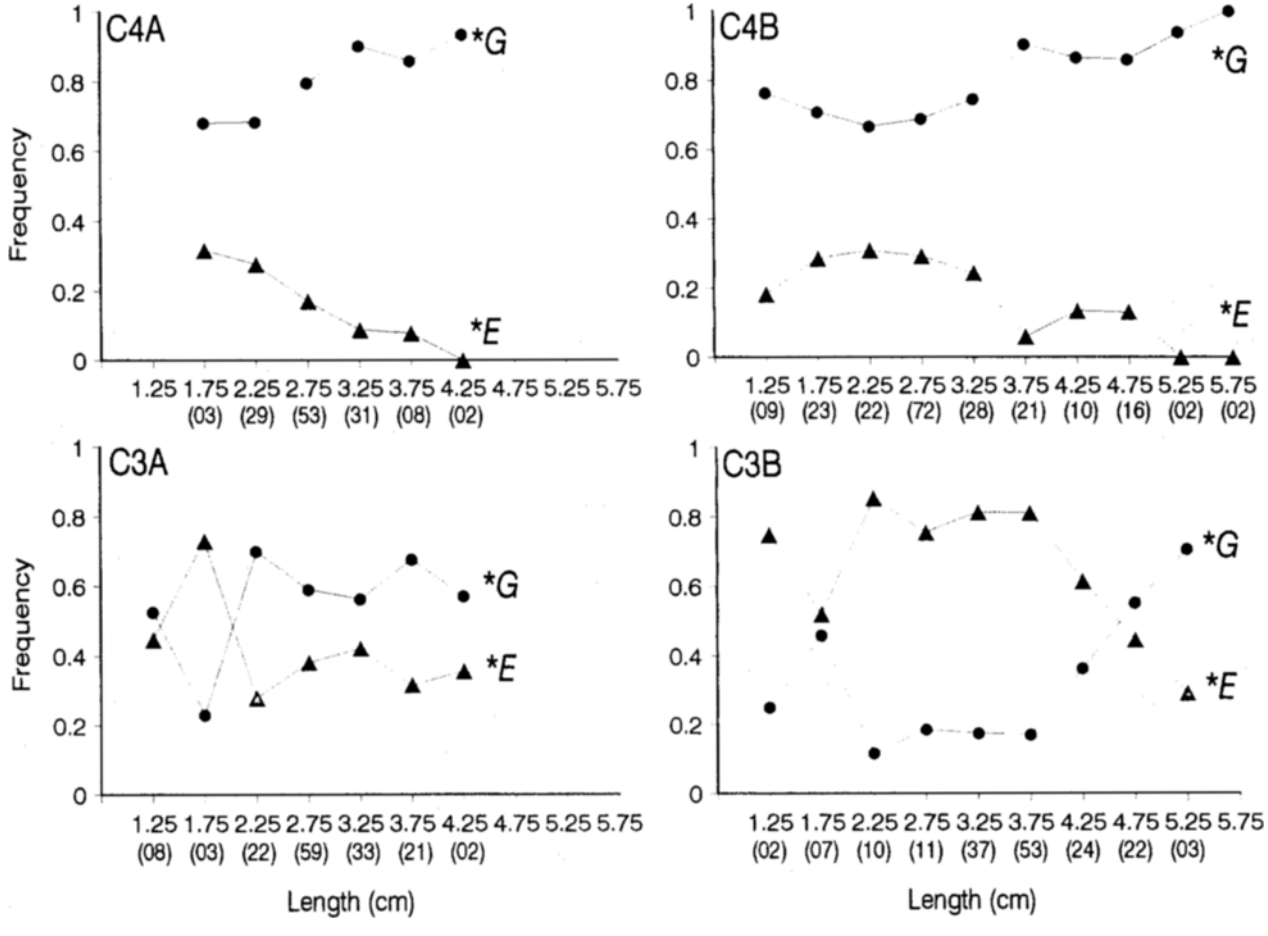

Fig. 4. Frequencies of compound alleles *G (*Mytilus galloprovincialis*: circles) and *E (*M. edulis*: triangles), averaged over the four diagnostic loci (*EST-D**, *LAP-1**, *MPI**, *ODH**), plotted against mid-point of shell length category for four populations from the hybrid zone (C4A, C4B, C3A, C3B). Population codes as in Table 1. The number of individuals analysed for each length category is given in parentheses

vincialis and *M. edulis* alleles at the diagnostic enzyme loci, and consequently on the proportions of both *Mytilus* forms. Typical *M. galloprovincialis* alleles for diagnostic loci showed significantly higher frequencies for exposed samples (CFA, C4_) than for sheltered populations (C3_) (Table 2, 4). The present results from continental mixed *Mytilus* populations are in accordance with those found in Irish and English hybrid zones for diagnostic loci (Gosling & Wilkins, 1977, 1981; Skibinski et al., 1983; but see Skibinski & Roderick, 1991). On the other hand, non-diagnostic loci (*AP-1**, *LAP-2**, *PGM**) did not show significant differences between exposed and sheltered populations, except *AP-1** for samples with a low level of attachment. These latter results contrast with those genetic differences found between exposed and sheltered Irish populations at *LAP-2** and *PGM** loci (Gosling & Wilkins, 1981). The proportion of putative *M. galloprovincialis* individuals was significantly higher in exposed populations than in sheltered samples separated by only 200 m (see Table 5). These results are in accordance with the ratios of *M. edulis* and *M. galloprovincialis*, as identified on morphological bases, varying according to the wave exposure in the French hybrid zone (Seed, 1972). Consequently, it seems that the predominance of *M. galloprovincialis* on wave-exposed coasts is a general phenomenon in the European hybrid zone of *M. galloprovincialis* and *M. edulis* (cf. Gosling, 1992b; Gardner, 1994). It has been suggested that the *M. galloprovincialis* form has an advantage in exposed regions and differential viability is thought to be the most important cause (Skibinski, 1983; cf. Gosling, 1992b; Gardner, 1994). The strength of attachment to the substrate is greater in *M. galloprovincialis* than in *M. edulis* (Gardner & Skibinski, 1991; Willis & Skibinski, 1992). In addition, the shell shape of *M. galloprovincialis* may be particularly well suited for exposed locations (cf. Gosling, 1992b; Gardner, 1994). If the wave action acts as a selective agent in exposed areas, the greater strength of attachment to the substrate gives *M. galloprovincialis* an adaptive advantage, and it can explain the present results. Salinity variation could explain differences in the genetic composition between exposed and sheltered shore mussels (see Gardner, 1994), but the sheltered shore investigated in this work is very close to the exposed shore studied (200 m; Fig. 1B), and the possible freshwater input does not seem to strongly affect the salinity conditions because a marine lake joins the freshwater stream before it flows into the Capbreton channel. Nevertheless, the factors which result in the reduced presence of *M. galloprovincialis* at most of the sheltered sites of the hybrid zone are still unknown (Gosling, 1992b; Gardner, 1994).

The frequencies of the *Mytilus galloprovincialis* typical alleles were greater in mussels with a high level of attachment than in those with a low level, in both exposed and sheltered areas; but significant differences were found only in the sheltered populations (Table 2, 4). This may be a consequence of the significantly higher proportion of putative *M. galloprovincialis* at high shore (37.9 %) than at low shore locations (4.5 %) for sheltered populations (Table 5). A greater frequency of *M. galloprovincialis* typical alleles at several diagnostic loci were also found for mussels higher up the shore than those lower down on English exposed and sheltered coasts (Skibinski, 1983; Gardner & Skibinski, 1988; Skibinski & Roderick, 1991), but amongst Irish exposed populations, genetic differences were only observed at the *ODH** locus but not at the *EST-D** locus (Gosling & McGrath, 1990). The degree of exposition to wave action in the investigated exposed shore may be different from that of the English and Irish reported sites, and this, together with the fact that mussels at the high shore were not the upper mussels, could be a cause of

these distinct results. Moreover, at some exposed sites, wave action may affect in a similar and homogeneous way both high and low level areas and it could be hiding the effect of other factors which could make those areas different environments. In sheltered sites, such as enclosed bays or estuaries, other ecological factors such as salinity fluctuations, aerial emersion, or temperature could overlap the wave action effect as a selective force (cf. Gosling, 1992b; Gardner, 1994). Genetic differences related to shore height have been suggested to derive from recruits composed of genetically distinct cohorts which settle preferentially at different levels on the shore or from recruits genetically homogenous but once settled diverge genetically over time due to different selective pressures such as air exposure (Gosling & Wilkins, 1985; Gosling & McGrath, 1990; Gosling, 1992b; Gardner, 1994). At present, it is clearly not possible to disregard one of these hypotheses. However, *M. galloprovincialis* maintains a higher feeding rate and a net energy balance, compared with *M. edulis*, under temperature conditions between 20°C and 25°C, and these are clearly important characteristics that determine the capacity of this form to thrive in warm-temperate waters (Hilbish et al., 1994). In addition, *M. galloprovincialis* may be better able to withstand longer periods of emersion than *M. edulis* (Gosling, 1992b). These abilities of *M. galloprovincialis* could be useful for life higher up on the shore in sheltered areas, where there are periods of time in which mussels are exposed to the air, and the temperature of the water inside the valves increases. Consequently, wave action may not be the main selective agent related to shore height on sheltered coasts, and other factors such as air exposure and temperature may play a role in furthering the different survival of both forms of mussels. Again, different viability may be involved in the high level of attachment on sheltered shore to the advantage of *M. galloprovincialis*. However, no clear factors explain the predominance of *M. edulis* at low shore locations on sheltered coasts (cf. Gosling, 1992b; Gardner, 1994).

A positive correlation between the frequency of the typical compound allele of *Mytilus galloprovincialis* (G^*) and shell length was found, but only for exposed populations (Table 6, Fig. 4). These results are in line with those found for English populations (Skibinski, 1983; Gardner & Skibinski, 1988; Skibinski & Roderick, 1991; Gardner et al., 1993). No such correlation was found for Irish exposed populations (Gosling & McGrath, 1990), and it may be explained because only a low percentage of mussels greater than 6 mm in length were considered (Gardner, 1994). The mussels studied in this investigation ranged from 10 to 60 mm, and the correlation was found for exposed populations. Local environmental conditions, such as different degrees of wave exposure, or different degrees of hybridization could explain these different results. A higher viability coefficient in *M. galloprovincialis* than in *M. edulis* regarding the strength of attachment of the byssus has been suggested to explain the correlation phenomenon (cf. Gosling, 1992b; Gardner, 1994). This hypothesis could explain why that correlation is found in exposed environments and not in sheltered ones, as a consequence of the strong effect of wave action as a selective force in exposed areas but not in sheltered ones.

With regard to the stability of the hybrid zone in time, homogeneity chi-square test for allele frequencies at diagnostic and non-diagnostic loci showed no significant differences between populations from the same place sampled in 1988 (CFA) and 1992 (C4A) (data not shown; see Table 2). This can be taken as evidence that both samples exhibit short-term temporal genetic stability and supports the idea that *Mytilus* hybrid zones are stable in structure (Gardner & Skibinski, 1988; Beaumont et al., 1989; Gardner

et al., 1993). Nevertheless, Viard et al. (1994) report strong genetic changes in time in their samples in the French hybrid zone after 3 years, but ecological data are not reported. Considering the microhabitat differences found for *Mytilus* mixed populations, care must be taken to choose the same sample area in different years, and it could explain this latter discordant result.

The environmental factors which may cause the genetic differences between exposed/sheltered and high/low shores can interact making it difficult to foresee the genetic composition of the *Mytilus* mixed populations. Coustau et al. (1991) note that the distribution of nine populations along the French hybrid zone does not correspond with a geographical gradient. No details of physical conditions at each site are given, and no conclusions concerning the relationship of ecological factors and genetic differentiation can be reached from this study. Moreover, these above described results contrast with the gradient observed in the *M. galloprovincialis* typical alleles for mixed populations from the southern limit of *M. edulis* (South French hybrid zone) in the same environment (Sanjuan et al., 1994). Results of this latter report and present data agreed with the *Mytilus* hybrid zone being considered a mosaic hybrid zone, where its structure is strongly influenced by the underlying mosaic of environmental variation (see Gardner, 1994 and references therein). More detailed genetic studies covering different sites of the continental hybrid zone and considering ecological factors may clarify the micro- and macrogeographic differentiation of the French hybrid zone of *M. galloprovincialis* and *M. edulis*.

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LITERATURE CITED

Ahmad, M. & Beardmore, J. A., 1976. Genetic evidence that the "Padstow mussel" is *Mytilus galloprovincialis*. – Mar. Biol. *35*, 139–147.

Ahmad, M., Skibinski, D. O. F. & Beardmore, J. A., 1977. An estimate of the amount of genetic variation in the common mussel *Mytilus edulis*. – Biochem. Genet. *15*, 833–846.

Beaumont, A. R., Seed, R. & Garcia-Martinez, P., 1989. Electrophoretic and morphometric criteria for the identification of the mussels *Mytilus edulis* and *M. galloprovincialis*. In: Reproduction, genetics and distributions of marine organisms. Ed. by J. S. Ryland & P. A. Tyler. Olsen & Olsen, Fredensborg, 251–258.

Coustau, C., Renaud, F. & Delay, B., 1991. Genetic characterization of the hybridization between *Mytilus edulis* and *M. galloprovincialis* on the Atlantic coast of France. – Mar. Biol. *111*, 87–93.

Dunn, G. & Everitt, D. S., 1982. An introduction to mathematical taxonomy. Cambridge University Press, Cambridge, 151 pp.

Gardner, J. P. A., 1992. *Mytilus galloprovincialis* (Lmk.)(Bivalvia, Mollusca): the taxonomic status of the Mediterranean mussels. – Ophelia *35*, 219–243.

Gardner, J. P. A., 1994. The structure and dynamics of naturally occurring hybrid *Mytilus edulis* Linneus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia: Mollusca) populations: review and interpretation. – Arch. Hydrobiol.(Suppl.) *99*, 37–71.

Gardner, J. P. A. & Skibinski, D. O. F., 1988. Historical and size-dependent genetic variation in hybrid mussel populations. – Heredity *61*, 93–105.

Gardner, J. P. A. & Skibinski, D. O. F., 1991. Biological and physical factors influencing genotype-dependent mortality in hybrid mussel populations. – Mar. Ecol. Prog. Ser. *71*, 235–243.

Gardner, J. P. A., Skibinski, D. O. F. & Bajdik, C.D., 1993. Shell growth and viability differences between the marine mussels *Mytilus edulis* (L.), *Mytilus galloprovincialis* (Lmk.) and their hybrids

from two sympatric populations in S.W. England. – Biol. Bull. mar. biol. Lab., Woods Hole *185*, 405–416.

Gosling, E.M., 1992a. Systematics and geographic distribution of *Mytilus*. In: The mussel *Mytilus*: ecology, physiology, genetics and culture. Ed. by E. M. Gosling. Elsevier, Amsterdam, 1–20.

Gosling, E. M., 1992b. Genetics of *Mytilus*. In: The mussel *Mytilus*: ecology, physiology, genetics and culture. Ed. by E. M. Gosling. Elsevier, Amsterdam, 309–382.

Gosling, E. M. & Wilkins, N. P., 1977. Phosphoglucoisomerase allele frequency data in *Mytilus edulis* from Irish coastal sites: its ecological significance. In: Biology of benthic organisms. Ed. by B.F. Keegan, P. O'Ceidigh & P. J. S. Boaden. Pergamon Press, London, 297–309.

Gosling, E. M. & Wilkins, N. P., 1981. Ecological genetics of the mussels *Mytilus edulis* and *M. galloprovincialis* on Irish coasts. – Mar. Ecol. Prog. Ser. *4*, 221–227.

Gosling, E. M. & Wilkins, N. P., 1985. Genetics of settling cohorts of *Mytilus edulis* (L): preliminary observations. – Aquaculture *44*, 115–123.

Gosling, E. M. & McGrath, D., 1990. Genetic variability in exposed-shore mussels, *Mytilus* spp., along an environmental gradient. – Mar. Biol. *104*, 413–418.

Grant, W. S. & Cherry, M. I., 1985. *Mytilus galloprovincialis* Lmk. in Southern Africa. – J. exp. mar. Biol. Ecol. *90*, 179–191.

Harris, H. & Hopkinson, D.A., 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publ., Amsterdam, Loose-leaf publ.

Hilbish, T. J., Bayne, B.L. & Day, A., 1994. Genetics of physiological differentiation within marine mussel genus *Mytilus*. – Evolution *48*, 267–286.

Hoeh, W. R., Blakley, K. H. & Brown, W. M., 1991. Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. – Science, N.Y. *251*, 1488–1490.

McDonald, J. H., Seed, R. & Koehn, R. K., 1991. Allozymes and morphometric characters of three species of *Mytilus*, in the northern and southern hemispheres. – Mar. Biol. *111*, 323–333.

Murphy, R. W., Sites, J. W., Buth, D. G. & Haufler, C. H., 1990. Proteins I: Isozyme electrophoresis. In: Molecular systematics. Ed. by D. M. Hillis & C. Moritz. Sinauer Ass., Sunderland, Mass., 45–126.

Nei, M., 1972. Genetic distance between populations. – Am. Nat. *106*, 283–292.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H., 1975. SPSS: statistical package for the social sciences. McGraw Hill, New York, 675 pp.

Quesada, H., Sanjuan, J. & Sanjuan, A. 1992. GENET2: a BASIC program for deviations from Hardy-Weinberg proportions. – J. Hered. *83*, 460–461.

Reyment, R. A., Blackith, R. E. & Campbell, N. A., 1984. Multivariate morphometrics. Acad. Press, London, 233 pp.

Robertson, A. & Hill, W. G., 1984. Deviations from Hardy-Weinberg proportions, sampling variances and use in estimation of inbreeding coefficients. – Genetics *107*, 703–718.

Roff, D. A. & Bentzen, P., 1990. The statistical analysis of mitochondrial DNA polymorphisms: X^2 and the problem with small samples. – Mol. Biol. Evol. *6*, 539–545.

Rohlf, F. J., 1990. NTSYS-PC. Numerical taxonomy and multivariate analysis system, ver. 1.50. Exeter Software, New York, Loose-leaf publ.

Sanjuan, A., 1992. La identificación del mejillón de la Península Ibérica: variabilidad morfológica y polimorfismos alozímicos. Tesis Doctoral, Univ. Santiago de Compostela, 186 pp.

Sanjuan, A., Quesada, H., Zapata, C. & Alvarez, G., 1990. On the occurrence of *Mytilus galloprovincialis* Lmk. on the N.W. coast of the Iberian Peninsula. – J. exp. mar. Biol. Ecol. *143*, 1–14.

Sanjuan, A., Zapata, C. & Alvarez, G., 1994. *Mytilus galloprovincialis* and *M. edulis* on the coasts of the Iberian Peninsula. – Mar. Ecol. Prog. Ser. *113*, 131–146.

Seed, R. A., 1972. Morphological variations in *Mytilus* from the French coasts in relation to the occurrence and distribution of *Mytilus galloprovincialis* (Lmk). – Cah. Biol. mar., *13*, 357–384.

Seed, R. A., 1992. Systematics, evolution and distribution of mussels belonging to the genus *Mytilus*: an overview. – Am. malac. Bull. *9*, 123–137.

Shaklee, J. B., Allendorf, F.W., Morizot, D. C. & Whitt, G. S., 1990. Gene nomenclature for protein-coding loci in fish. – Trans. Am. Fish. Soc. *119*, 2–15.

Skibinski, D. O. F., 1983. Natural selection in hybrid mussel populations. In: Protein polymorphism: adaptive and taxonomic significance. Ed. by G. S. Oxford & D. Rollinson. Acad. Press, London, 283–298.

Skibinski, D. O. F, Ahmad, M. & Beardmore, J. A., 1978. Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis*. – Evolution *32*, 354–364.

Skibinski, D. O. F., Cross, T. D. & Ahmad, M., 1980. Electrophoretic investigation of systematic relationships in the marine mussels *Modiolus modiolus* L., *Mytilus edulis* L. and *Mytilus galloprovincialis* Lmk (Mytilidae; Mollusca). – Biol. J. Linn. Soc. *13*, 65–73.

Skibinski, D. O. F., Beardmore, J. A. & Cross, T. F., 1983. Aspects of the population genetics of *Mytilus* (Mytilidae; Mollusca) in the British Isles. – Biol. J. Linn. Soc. *19*, 137–183.

Skibinski, D. O. F. & Roderick, E. E., 1991. Evidence of selective mortality in favour of the *Mytilus galloprovincialis* Lmk phenotype in British mussel populations. – Biol. J. Linn. Soc. *42*, 351–366.

Sneath, P. H. A. & Sokal, R. R., 1973. Numerical taxonomy. Freeman, San Francisco, 573 pp.

Varvio, S.-L., Koehn, R. K. & Väinölä, R., 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. – Mar. Biol. *98*, 51–60.

Viard, F., Delay, B., Coustau, C. & Renaud, F., 1994. Evolution of the genetic structure of the bivalve cohorts at hybridization sites of the *Mytilus edulis-M. galloprovincialis* complex. – Mar. Biol. *119*, 535–539.

Willis, G. L. & Skibinski, D. O. F., 1992. Variation in strength of attachment to the substrate explains differential mortality in hybrid mussel (*Mytilus edulis* and *Mytilus galloprovincialis*) populations. – Mar. Biol. *112*, 403–408.

Zaykin, D. V. & Pudovkin, A. I., 1993. Two programs to estimate chi-square values using pseudo-probability test. – J. Hered. *84*, 152.