Geographic Structure in the European Flat Oyster (*Ostrea edulis* L.) as Revealed by Microsatellite Polymorphism

S. Launey, C. Ledu, P. Boudry, F. Bonhomme, and Y. Naciri-Graven

Genetic differentiation of the flat oyster (*Ostrea edulis*) was studied along the European coast, from Norway to the Black Sea, by means of variation at five microsatellite loci. The results show a mild but significant isolation-by-distance profile, a noticeable between-sample variance in expected heterozygosity, and a tendency for Atlantic populations to be less variable than Mediterranean ones. This does not provide support for the existence of a single large panmictic population for this larvae-broadcasting species, but rather for the relative independence of local stocks. Comparison with data on allozyme variation from the literature confirms this view. It also leads us to suggest that the behavior of some sampled protein loci may depart from the average, so caution should be used when inferring neutral gene flow.

Marine species are classically described as showing less geographical differentiation than terrestrial species (Hauser and Ward 1998; Palumbi 1996; Ward et al. 1994). This is attributed to the absence of geographical barriers in the ocean, the large population sizes of marine species, and the frequent existence in their life cycle of a pelagic larval phase that ensures high dispersal, even in static species such as oysters. Others have observed, however, that some marine species show more differentiation than could be expected with this high dispersal capability (Burton and Lee 1994; Huvet et al. 2000b; Palumbi et al. 1997). Long-distance movements of larvae may in fact be impeded by the existence of hydrological or ecological barriers, such as currents, temperature, or salinity, whereas autorecruiting buckles may establish themselves wherever possible, favoring local differentiation (David et al. 1997). Genetic drift also occurs, since high fecundities are often associated with a very high variance in reproductive success (Hedgecock 1994; Li and Hedgecock 1998), which can greatly reduce effective population size.

All these factors are at play in most marine bivalves, where a sedentary adult phase is associated with a high reproductive effort through a planktonic larval phase. The European flat oyster (*Ostrea edulis* L.) is a marine bivalve whose natural geographical distribution ranges along the European Atlantic coast from Norway to Morocco and all along the Mediterranean as well as the Black Sea. It has also been introduced into many other parts of the world (e.g., United States, Canada, Japan) because of its aquacultural potential (Korringa 1976). Genetic studies of natural populations have focused on a limited number of populations (Blanc et al. 1986; Buroker 1982; Jaziri et al. 1987; Johannesson et al. 1989; Le Pennec et al. 1985; Saavedra et al. 1987; Wilkins and Mathers 1973) and showed low levels of intrapopulation polymorphism and interpopulation differentiation. More comprehensive studies of allozyme differentiation of the flat oyster over its natural range have been made by Jaziri (1990) and especially by Saavedra et al. (1993, 1995). They concluded that, although the overall differentiation was small, a significant divergence existed between Mediterranean and Atlantic populations. Jaziri (1990), working with a limited number of populations, observed a lower variability of the Atlantic stocks and suggested that during Pleistocene glaciations the Mediterranean Sea had been a refuge from which the Atlantic stock subsequently stemmed. Saavedra et al. (1993, 1995), analyzing 19 populations from Scandinavia to Greece with 14 enzymatic loci, observed clines in allelic frequencies

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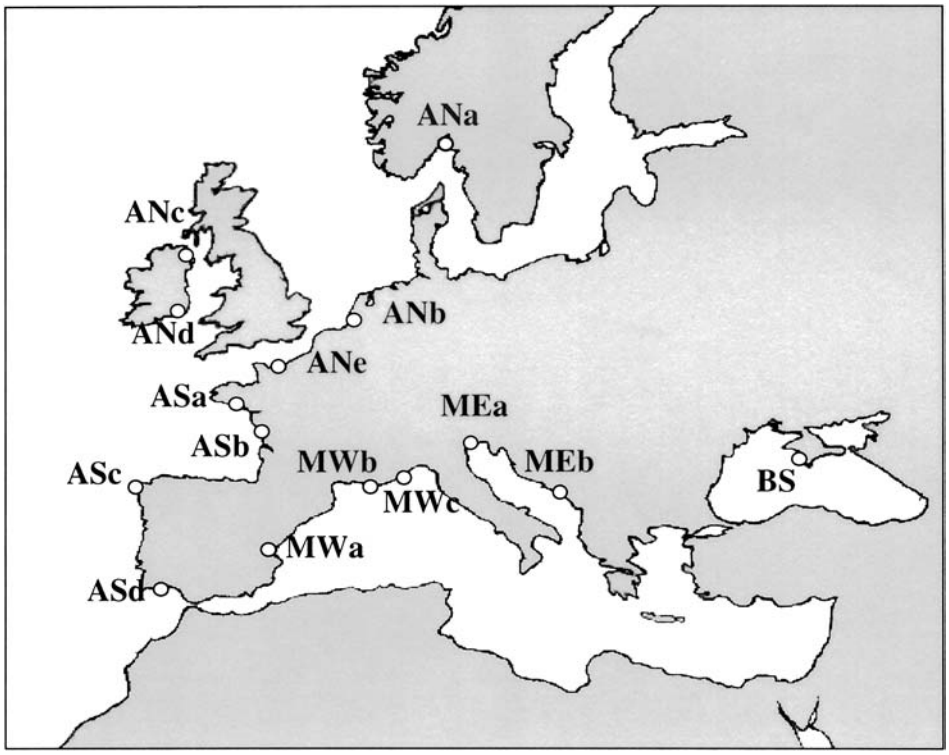

Figure 1. Geographical locations of the *O. edulis* populations sampled (sample size is given in parentheses). ANa: Oslofjorden, Norway (48); ANb: landlocked population of Grevelingen, Netherlands (27); ANc: Lough Foyle, Ireland (50); ANd: Cork, Ireland (50); ANe: Port en Bessin, France (34); ASa: Quiberon, France (49); ASb: La Rochelle, France (44); ASc: Vigo, Spain (35); ASd: Ria Formosa, Portugal (14); MWa: Murcia, Spain (45); MWb: Thau Lagoon, France (50); MWc: Port Saint Louis, France (50); MEa: Venice, Italy (23); MEb: Dubrovnik, Croatia (50); BS: Sevastopol, Black Sea, Ukraine (35).

for some loci on either side of the Straits of Gibraltar. They suggested that this pattern resulted from secondary contact of the two stocks (Atlantic and Mediterranean) at Gibraltar after separation during the last glaciation.

All of these studies relied on allozyme data. In theory, genetic drift and gene flow affects all loci similarly; however, selection, and to a lesser extent mutation, may influence the geographical structuring of polymorphism at some loci. This can lead to a heterogeneity among loci, with high levels of genetic differentiation at some loci and little or none at others. Actually, bivalves display some of the exemplary cases where selection has been shown at certain allozyme loci (e.g., Hilbish and Koehn 1985; Moraga and Tanguy 2000; Pogson 1991; Sarver et al. 1992). Here we analyzed geographical genetic structuring in *O. edulis* as revealed by polymorphic nuclear markers (microsatellites), which are supposedly neutral, and compared our results to previously published allozyme data with a comparable sampling.

Materials and Methods

Sampling

Fifteen populations of *O. edulis* were sampled along the European Atlantic and Mediterranean coasts and in the Black Sea (14–50 individuals per sample). Populations were chosen that either had not been commercially exploited in the recent past or were harvested from local stocks. When possible, we tried to avoid populations with a documented history of imports from foreign stocks (MacKenzie et al. 1997). We used the same coding convention as Saavedra et al. (1995): populations were named according to their broad geographical origin (AN: Atlantic, northern part of the study area; AS: Atlantic, southern part of the study area; MW: Mediterranean, west; ME: Mediterranean, east; and BS, Black Sea sample, which was not in Saavedra et al.'s sampling scheme) (Figure 1).

Development of Microsatellite Markers

A genomic library was constructed after extraction of genomic DNA from a whole adult flat oyster (*O. edulis*). Genomic DNA was digested to completion with *Alu*I, *Rsa*I, and *Hae*III. Fragments between 400 and 800 bp were size selected and eluted from an agarose gel and ligated into the *Sma*I-linearized pBluescript II KS plasmid (Stratagene). Recombinants were then transformed into competent DH5α *Escherichia coli* cells. Recombinant clones were screened for microsatellites with [α-^{33}P]-dATP-labeled $d(AC)_n$ and $d(AG)_n$ probes (Pharmacia). After identification of positive clones, extraction of plasmid DNA by alkaline-lysis miniprep, and sequencing using both forward and reverse primer with the Pharmacia T7 sequencing kit, 29 clones containing at least one microsatellite repeat were identified. Primers were designed for five loci using PRIMER software (Whitehead Institute for Biomedical Research, Cambridge, MA) (Table 1).

DNA Extraction and Amplification

DNA was extracted with a rapid procedure using Chelex (Biorad), adapted from Estoup et al. (1996). A small piece of gill tissue was heated at 55°C in 150 μl 5% Chelex, 15 μl TE (Tris EDTA), and 10 μl proteinase K (Eurogentec, 10 mg/ml). Samples were then boiled for 10 min and centrifuged for 5 min (4000 rd/min), and the supernatant was collected and kept frozen (−20°C) before use. Polymerase chain reactions (PCRs) were performed in a 10 μl reaction mix containing 2 μl template DNA, 1.5 μM $MgCl_2$, 75 μM each dNTP, 0.25 μM α-^{33}P-labeled forward primer, 0.4 μM reverse primer, 0.35 units of Goldstar Licensed Polymerase (Eurogentec), and 1× polymerase buffer (supplied by the manufacturer). Amplifications were processed as follows: predenaturation (94°C, 2 min) followed by 30 cycles of denaturation/annealing/polymerization (94°C, 1 min; T_a, 1 min; 72°C, 1 min 15 s) and a final elongation step (72°C, 5 min). T_a is the optimal annealing temperature for each pair of primers (Table 1). Amplification products were analyzed on 7 M urea, 6% polyacrylamide gel using individuals of known genotype as size markers.

Genetic Analysis

For each population and locus we calculated the number of alleles (N_a), the observed heterozygosity (H_o), and the expected heterozygosity (H_e) according to Nei's unbiased estimate (Nei 1978). One-tailed Mann–Whitney tests (Sokal and Rohlf 1995) were used to determine whether the monolocus genic diversities

Table 1. Characteristics of the five microsatellite loci used in this study

Locus	Repeat motif	Size range of PCR product	T_a (°C)	Primer sequences[a]	
*Oedu*H15	$(ATCT)_n$[b]	165–225	50°C	H15-R	TAA TGA TTT CGT TCG TTG AC
				H15-F	TTT TGA CTC TGT GAT ATC GAC
*Oedu*J12	$(GT)_{14}$	216–272	50°C	J12-R	TCG TCA CCT CCC TCT CAG AG
				J12-F	GCT GTA TTT CCA TCA ATT CGA G
*Oedu*O9	$(GA)_{36}$	140–176	53°C	O9-R	ACT TCA ATG TCT GTT CTA ATG G
				O9-F	ATT CAA TTG ATT TTA GGT TGG
*Oedu*U2	$(AC)_{21}(AG)_7$	146–206	50°C	U2-R	GAA AGA AAT GGA GGC AAT AAC
				U2-F	ACC AAT GAA CAC AGA TCA CC
*Oedu*T5	$(CA)_{15}$	106–166	55°C	T5-R	TAG TGA ATG GTC TTG CAT TCC
				T5-F2	CTT CGT TCT TGT ACG TAA GCG

T_a = optimal annealing temperature.

[a] GenBank accession number AF310009–AF310015.

[b] Full sequence of the repeat: $(ATCT)_4ATGT(ATCT)_2ATGTATCTATATATCTATGT(ATCT)_5A(TACC)_4AATTTTTC-T(ATCT)_3$.

are higher for the Mediterranean populations than for the Atlantic ones, as suggested by Jaziri (1990).

Mean F_{IS} (F_{ST}) were computed over loci and/or populations according to Weir and Cockerham's estimators, using Genetix 4.0 software (Belkhir et al. 1996–2001). Significance levels were assessed by permutation of the alleles (multilocus genotypes) within populations (across populations). Reynolds's genetic distance (Reynolds et al. 1983) was calculated using the PHYLIP 3.57 software package (Felsenstein 1989). The distance matrix was visualized as a neighbor-joining tree (Saitou and Nei 1997, using PHYLIP). The robustness of the nodes of the unrooted tree was assessed by bootstrapping over loci.

Correlation between geographical and genetic distances was estimated by a Mantel test (Mantel 1967, using Genetix). Geographical distances were measured along the coast lines; when different routes were possible, we opted for the most likely, according to the principal current flow in the area. $F_{ST}/(1 - F_{ST})$ was used as a measure of genetic distance between each pair of populations, as suggested by Rousset (1997). For allozyme data, pairwise F_{ST} values were recalculated from pairwise Reynolds's genetic distance provided in the initial publication (Saavedra et al. 1993; Saavedra C, personal communication).

Results

Intrapopulation Variability

The number of alleles and expected and observed heterozygosity per locus and per population are given in Table 2. As expected for bivalves, the flat oyster populations show high levels of polymorphism (mean number of allele/locus/population = 18.5 ± 4.5, mean H_e = 0.914 ± 0.018). Mediterranean populations (including the Black Sea sample) were more polymorphic than Atlantic populations (mean ± standard error = 20.6 ± 4.2 versus 17.2 ± 4.2 alleles/locus/population, $P < .001$).

The one-tailed Mann–Whitney tests were highly significant (and remained significant after Bonferroni correction) for *Oedu*U2 and *Oedu*O9 ($\alpha = 0.0025$), indicating that the genic diversities are higher for the Mediterranean populations when compared to the Atlantic ones for these loci. The one-tailed tests were marginally significant for *Oedu*J12 and *Oedu*T5 ($0.05 < \alpha < 0.10$) and nonsignificant for *Oedu*H15 ($\alpha > 0.10$).

Thirteen of the 15 samples showed an overall heterozygote deficiency, as indicated by significantly positive values of the multilocus F_{IS} (see Table 2). Heterozygote deficiencies were especially high for the *Oedu*H15 locus, since heterozygote deficiencies remained significant in only four samples when *Oedu*H15 was discarded from the analysis (Table 2).

Interpopulation Differentiation

The global multilocus estimate for F_{ST} was 0.019 ± 0.003. This estimate is significantly different from zero ($P < .001$), indicating a heterogeneous distribution of the genetic variability of the species over the whole sample collection. The different loci did not contribute equally to the interpopulation differentiation, with monolocus F_{ST}s ranging from 0.009 ($P < .001$, *Oedu*U2) to 0.030 ($P < .001$, *Oedu*H15). If we omit *Oedu*H15, the multilocus estimate for F_{ST} remains significantly different from zero (F_{ST} = 0.017, $P < .001$).

The overall pattern of genetic differentiation is summarized by the neighbor-joining tree (Figure 2A), while the allozymic tree obtained from Saavedra et al.'s 1995 data is given in Figure 2B. For microsatellite data, bootstrap values appear quite low. The populations are clustered according to their geographical origin, with an Atlantic cluster, a western Mediterranean cluster (including the ASd population from Portugal), and an eastern Mediterranean cluster (including Black Sea). This clustering is supported by weak bootstrap values (only values higher than 50 are shown in Figure 2). However, bootstrap values for allozyme data are not higher (Figure 2B). Another approach to genetic distance significance is given by the matrix in Table 2, which shows, after Bonferroni correction, the significance of pairwise F_{ST} coefficients. They range from 0 (between French Atlantic samples) to 0.058 (between ANa/Oslo and MEa/Venice samples). Apart from the northernmost population, Atlantic populations are not significantly differentiated from each other. The populations from the Adriatic and Black seas, however, are divergent from every other

Table 2. Intrapopulation genetic diversity at five microsatellite loci for 15 *O. edulis* populations

Population	Sample size	N_a	H_o	H_e	F_{IS}	F_{IS} without *Oedu*H15
ANa	47	16.6	0.861	0.791	**0.083**	−0.042
ANb	27	16.8	0.913	0.814	**0.110**	**0.077**
ANc	48	17.6	0.913	0.785	**0.141**	**0.085**
ANd	32	16.6	0.905	0.834	**0.079**	0.045
ANe	39	19.2	0.925	0.873	**0.057**	0.050
ASa	49	19.8	0.914	0.840	**0.082**	0.051
ASb	44	19.2	0.912	0.840	**0.081**	**0.077**
ASc	35	15.6	0.890	0.828	**0.070**	−0.002
ASd	14	13	0.931	0.92	0.014	−0.019
MWa	45	20.8	0.935	0.881	**0.059**	0.054
MWb	49	21.8	0.924	0.862	**0.068**	0.029
MWc	50	21.0	0.914	0.885	0.032	−0.009
MEa	23	18.4	0.924	0.812	**0.124**	0.062
MEb	50	22.8	0.930	0.863	**0.072**	0.033
BS	35	18.8	0.917	0.751.	**0.183**	**0.121**

N_a = mean number of alleles per locus; H_o = mean observed heterozygosity; H_e = mean expected heterozygosity. Values in bold are significantly different from zero after sequential Bonferroni correction.

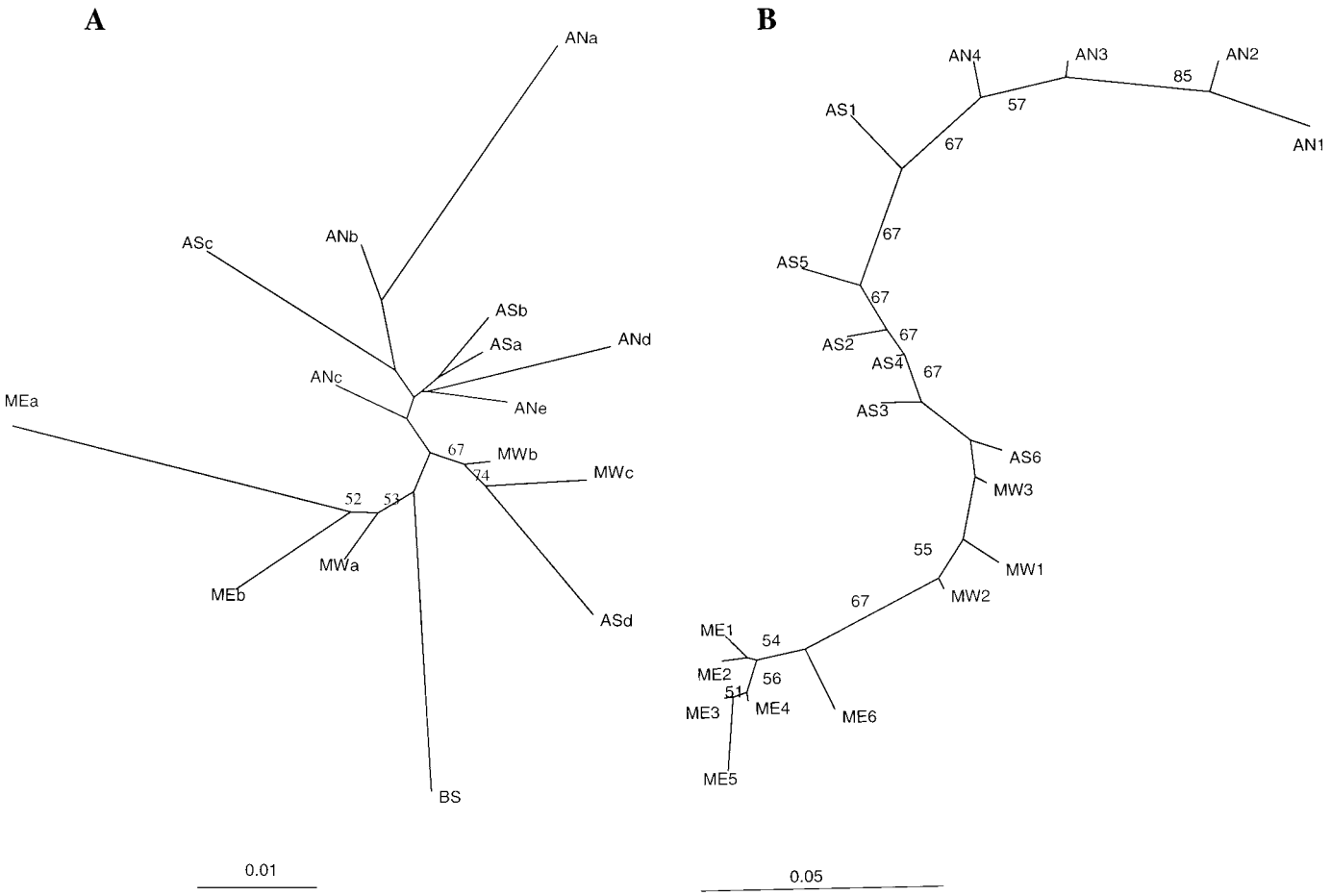

Figure 2. Neighbor-joining tree based on Reynolds's genetic distances between flat oyster populations, calculated from **(A)** microsatellite data and **(B)** allozyme data.

population. Note the intermediate position (between the Atlantic and Mediterranean populations) of the samples from Thau (ASb in this study; similar to Marseille/AS6 in Saavedra et al. 1995), which can be linked to transplantation of Atlantic farmed stocks known to have occurred there (Goulletquer and Héral 1996).

Regression of $F_{ST}/(1 - F_{ST})$ over coastal distances in kilometers showed a positive correlation between genetic and geographical distances (Figure 3). A Mantel test on the two matrices showed that this correlation was significant ($r = .58$, $P \leq .003$). The correlation remained significant when the three eastern Mediterranean populations were omitted from the sample ($r = .39$, $P \leq .018$), indicating that the correlation between geographical and genetic distances is not an artifact caused solely by the strong differentiation of the eastern Mediterranean samples. Similarly the correlation remains significant when we omit the locus that shows the higher interpopulation differentiation, *Oedu*H15 ($r = .362$, $P < .05$).

Discussion

We compared the geographical structure of genetic diversity in *O. edulis*, as revealed by microsatellite markers, with the results already published using allozyme markers (Jaziri 1990; Saavedra et al. 1993, 1995). As developed hereafter, results obtained by both types of markers are highly congruent, although their overall interpretation may differ from what has been proposed previously.

Several interacting mechanisms could account for the distribution of the genetic variability in *O. edulis*, revealed by both allozyme and microsatellite markers.

Life Cycle Traits and Intrapopulation Differentiation

As expected, microsatellites reveal a much higher level of intrapopulation genetic variability than allozymes, both in terms of the number of alleles and gene diversity. All five microsatellite loci are highly polymorphic, with a mean of 18.5 alleles per locus and per population, in contrast to 1.8–2.8 alleles per allozyme locus (Jaziri et al. 1987; Saavedra et al. 1995). Gene diversity for microsatellite loci is very high ($H_e = 0.930$) versus 0.176 $\pm$ 0.052 for allozyme loci (Saavedra et al. 1995). These results confirm the high levels of polymorphism already observed for the flat oyster with other microsatellite markers ($H_e = 0.664–0.910$; Naciri et al. 1995) and for another marine bivalve, the Pacific oyster (*Crassostrea gigas*), with gene diversity higher than 0.8 (Huvet et al. 2000a,b; Magoulas et al. 1998). Similar levels of microsatellite instability have been found for microsatellite results in other marine organisms. DeWoody and Avise (2000) compared microsatellite variation in 78 species of freshwater and marine fishes and found that the latter have higher heterozygosities and number of alleles

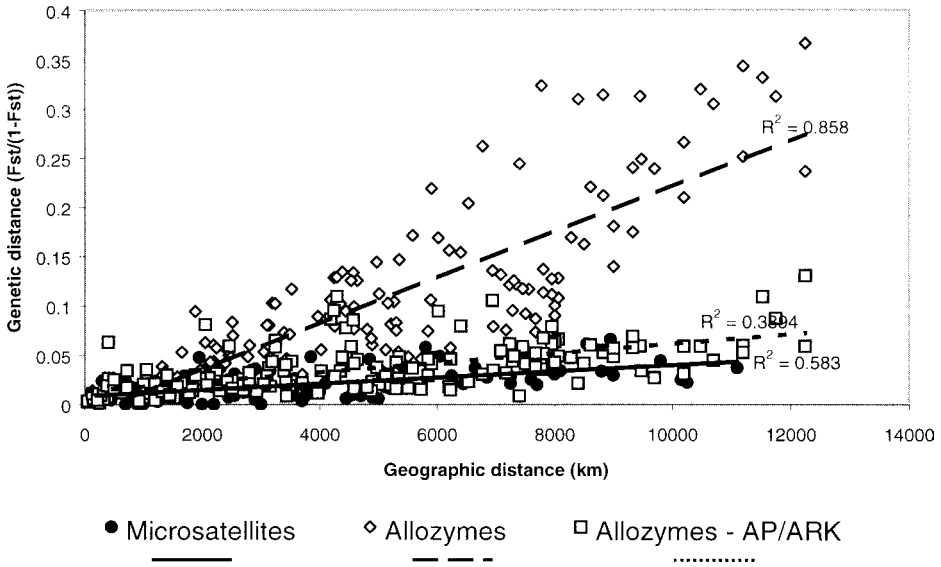

Figure 3. Regression of genetic distance (computed as $F_{ST}/(1 - F_{ST})$) versus geographic distance in flat oyster populations for microsatellite data (filled symbols and dotted line) and allozyme data (open symbols and full lines).

(H_e = 0.78 and N_a = 20.6 for marine species versus H_e = 0.58 and N_a = 7.1 for freshwater ones). They attribute this greater genetic variation to larger effective population sizes of marine species, in turn related to the larger and more continuous nature of the marine environment.

Heterozygote deficiencies relative to Hardy–Weinberg expectations seem to be a common observation in marine bivalve populations as shown by allozyme loci (see for instance, Fairbrother and Beaumont 1993; Zouros and Foltz 1984), but also microsatellite loci (Huvet et al. 2000b). Our microsatellite data also show a general heterozygote deficiency in all but two samples (ASb/Ria Formosa and MWc/Port Saint Louis), as shown by the high positive F_{IS} values. Different explanations have been put forward, ranging from population causes (Wahlund effect, mating system), to selection effects, to technical artifacts (null alleles). In our case, heterozygote deficiencies seem to be largely due to locus *Oedu*H15 (mean F_{IS} = 0.253). If we omit this locus, heterozygote deficiencies are greatly reduced in all samples (average F_{IS} = 0.041, $P < .001$) and multilocus F_{IS} values remain significant in only four samples. A Wahlund effect can probably be ruled out since the observed F_{ST} values (F_{ST} = 0.019) are considerably lower than the mean F_{IS} values including all five loci (0.082), and more than 10 times lower than the F_{IS} at *Oedu*H15. Similarly, if inbreeding had occurred, it would increase the observed homozygosity at every locus in the same manner, which is not the case. Null alleles (i.e., nonamplified alleles due to mutation in the primer sites; Pemberton et al. 1995) could be a likely cause for heterozygote deficiencies at *Oedu*H15. Similarly, null alleles have been reported at several microsatellite loci in *C. gigas* (McGoldrick et al. 2000), where they are also likely to explain the observed heterozygote deficiencies in this species (Huvet et al. 2000a). According to the method of Brookfield (1996), we estimated that the frequency of null alleles that would explain the observed deficiency at *Oedu*H15 would be, on average, 19%. If we omit this locus, F_{IS} values for most populations are not significantly different from zero, as shown in Table 2, indicating that, on average, flat oyster populations are either at or close to panmixia.

Locus *Oedu*H15 was retained, however, for the interpopulation differentiation study. Indeed, the F_{ST} value for this locus was in the same range as the other four (Table 3). Moreover, a null allele is expected to be randomly associated with any other "visible" alleles so that multiallelic F_{ST}, a parameter based on a weighted mean of the contribution to the overall variance of each allele considered separately (Weir and Cockerham 1984) will not likely be biased by an unseen allele. We have also seen that when we omit this locus, the overall pattern of distribution of genetic variability is not affected, and the differentiation between populations remains significant (although the magnitude of the divergence is lower without this locus).

Interpopulation Differentiation

The genetic structure of *O. edulis* populations has probably been influenced by both long-term evolutionary history and present and past human activities, which are not always easy to separate (for a review see MacKenzie et al. 1997). Nevertheless, our data are consistent with a model of isolation by distance, which is apparent in both distance trees, where the populations are roughly ordered according to their geographic origin. As already shown by Borsa et al. (1997), previously published allozyme data (Saavedra et al. 1993, 1995) also show a positive correlation with geographical distance (Mantel test: r^2 = .859, $P < .001$). Both data sets are plotted simultaneously on Figure 3. The slope of the regression line for allozymes is higher than for microsatellites, as expected from the overall F_{ST} values, which are higher for allozymes (F_{ST} = 0.082) than for microsatellites (F_{ST} = 0.019). A priori, this discrepancy in the intensity of the

Table 3. Pairwise F_{ST} values between 15 *O. edulis* populations

	ANb	ANc	ANd	ANe	ASa	ASb	ASc	ASd	MWa	MWb	MWc	MEa	MEb	BS
ANa	0.015	0.029	0.044	0.035	0.025	0.034	0.032	0.044	0.042	0.030	0.045	0.055	0.058	0.035
ANb		0.009	0.009	0.004	0.004	0.007	0.017	0.005	0.016	0.007	0.017	0.024	0.029	0.024
ANc			0.011	0.003	0.004	0.006	0.017	−0.003	0.013	0.006	0.016	0.020	0.032	0.021
ANd				0.007	0.011	0.008	0.020	0.009	0.016	0.009	0.017	0.026	0.037	0.040
ANe					0.000	0.003	0.015	0.002	0.012	0.008	0.015	0.020	0.026	0.028
ASa						0.000	0.013	0.007	0.017	0.006	0.018	0.030	0.035	0.026
ASb							0.016	0.007	0.015	0.009	0.018	0.027	0.034	0.031
ASc								0.022	0.027	0.021	0.034	0.036	0.052	0.040
ASd									0.000	−0.007	−0.004	0.009	0.029	0.020
MWa										0.001	0.004	0.004	0.021	0.022
MWb											0.001	0.015	0.029	0.023
MWc												0.022	0.034	0.031
MEa													0.023	0.027
MEb														0.033

Values in bold are significantly different from zero ($P < .05$) after sequential Bonferroni corrections.

signal between the different types of markers can be attributed to two causes: (1) a higher mutation rate at microsatellite loci generating homoplasy, which reduces large-scale genetic variation, or (2) differential selection on some allozyme loci. In our case, however, monolocus F_{ST} values are highly variable among allozyme loci; F_{ST} is greater than 0.1 for two loci only, *ARK* and *AP* (F_{ST} = 0.29 and 0.11, respectively), while the other loci reveal levels of differentiation comparable to what is observed with microsatellites (F_{ST} = 0.008–0.054) (Figure 4). If we exclude *ARK* and *AP* from the calculation, then the level of differentiation of *O. edulis* populations revealed by allozyme markers (F_{ST} = 0.032) is comparable to that revealed by microsatellites, a fact that indicates the low impact of homoplasy on these marker loci when migration is prominent over mutation. Similarly, when *AP* and *ARK* are excluded, the correlation between geographical and genetic distance is still significant (Mantel test: $r^2 = .624$, $P < .0001$), but the slope of the regression line becomes comparable to that of microsatellite loci (see Figure 3).

If one implements Lewontin and Krakauer's (1973) test, one finds a quite high value for the coefficient k [var(F_{ST}) across loci = $k/n - 1^*(E(F_{ST}))^2$] of 28.19. This value drops to 9.47 when *ARK* is removed, and to 3.99 when both *ARK* and *AP* are removed. Despite the fact that this test has been severely criticized, recently it has been empirically revisited by Baer (1999), who proposed from a review of a large number of real data sets that 7.4 could be considered a threshold value above which a departure from neutrality should be suspected. We are well above this value in our case, and we believe that the positions of *ARK* and *AP* exemplified in Figure 4 are clearly those of outliers. It could further be debated whether this corresponds to "quirks" of a chaotic population history (this seems unlikely to us) or to other forces acting upon these two loci. These two loci (*ARK* and *AP*) have been shown to display highly significant clines in allelic frequencies from the North Atlantic to the Eastern Mediterranean (Saavedra et al. 1995). The question of the existence of eventual selective forces acting on them, as compared to the other enzymes and microsatellite loci which would represent neutrality, is thus left open.

Comparison of allozyme and microsatellite data sets is becoming more common in the literature and helps establish a neutral baseline. In marine organisms, it is not uncommon to find situations where some enzyme loci are clear outliers for which the action of selection is suspected (see, for instance, Lemaire et al. 2000 and cases reported therein for sea bass). At any rate, it seems statistically more sound to exclude them from the estimation of neutral gene flow.

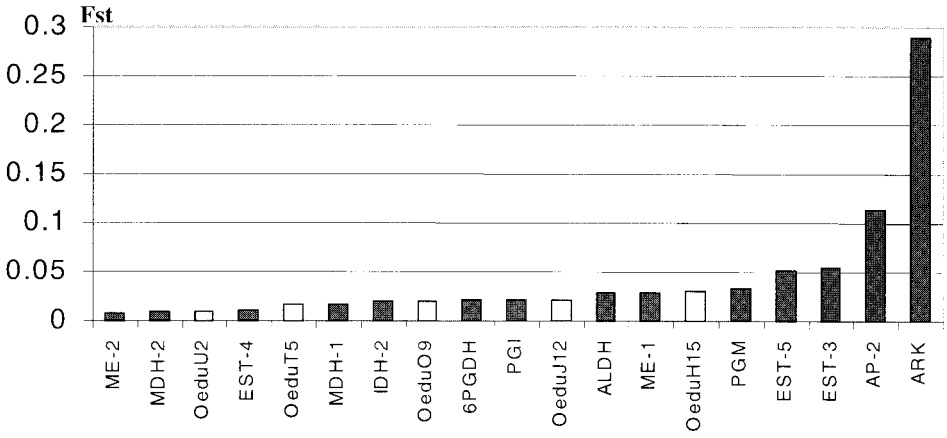

Figure 4. Monolocus F_{ST} values for *O. edulis* populations. Data for microsatellite loci (white histograms) are based on 15 populations (this study); data for allozyme loci (black histograms) are based on 19 populations (Saavedra et al. 1993, 1995) spanning the same geographical range. Loci are ordered according to ascendant F_{ST} values.

Unlike the conclusions drawn by Saavedra et al. (1995), our results failed to show an obvious genetic discontinuity between Atlantic and Mediterranean flat oyster populations. In fact, a closer look at their data (Figure 2B) shows that, while the populations are ordered in a "geographical" fashion, the transition between both marine basins is neither marked nor supported by a significant bootstrap value. Therefore it does not seem that Saavedra et al.'s conclusion, according to which the actual structuring of genetic diversity could be explained by a secondary contact between two ancient Atlantic and Mediterranean stocks, has very strong support based on our analysis, neither from the standpoint of allozymes nor microsatellites. At the very least, if the initial situation was indeed conforming to Saavedra et al.'s hypothesis (which we cannot exclude), our data show that clear traces of the initial separation in two stocks would have vanished by subsequent distance-limited but rather homogeneous gene flow. The situation for *O. edulis* may thus be quite different from that of another widespread bivalve, *Mytilus galloprovincalis*, which appears to show no isolation by distance in each basin, accompanied by a small, but clear genetic divergence of the two entities from the standpoint of both allozymes (Quesada et al. 1995) and nuclear DNA markers (Daguin and Borsa 2000). Similarly in the sea bass, another marine species analyzed with the same kind of data set, a clear discontinuity exists east of Gibraltar, with two rather homogeneous ensembles on each side (Naciri et al. 1999). At the other extreme, the palourde clam (*Ruditapes decussatus*) seems to show no differentiation at all across Gibraltar (Borsa et al. 1994). Thus the question remains open as to why different species react apparently differently to the same historical contingencies.

In any case, an important result lies in the fact that significant F_{ST} values could be found, even at a rather small scale (among the 105 pairs of populations tested, 73 were significantly differentiated). At the same scale, gene diversities were also quite variable, showing that populations with different diversities may coexist in close proximity (e.g., ANc/Lough Foyle versus ANd/Cork; ASc/Vigo versus ASd/Ria Formosa). This points toward the fact that, despite the possibility of larval dispersal, local stocks may be quite independent dynamically and harbor varied instantaneous effective sizes likely to shape the gene diversity they contain. Genetic variability (especially in terms of the number of alleles) is lower in Atlantic populations than in populations from the Mediterranean Sea (especially the Adriatic and Black seas). Previous allozyme studies

have also shown a lower genetic variability in Atlantic populations than in Mediterranean populations: $0.08 < H_o < 0.12$ versus $0.12 < H_o < 0.15$, 1.8–2.1 alleles/locus versus 2.2–2.8 (Jaziri 1990; Johannesson et al. 1989; Magennis et al. 1983; Saavedra et al. 1987, 1993, 1995; Wilkins and Mathers 1973).

This result and the pattern of isolation by distance, supported by both types of markers, could be explained by a globally smaller evolutionary effective size for Atlantic populations compared to Mediterranean populations. Two main explanations can be put forward for such a difference. First, it is noticeable that the favorable period for reproduction is sometimes very short in the Atlantic (especially for the northernmost populations), and shorter than that in the Mediterranean. This allows for a high variance in effective sizes in the Atlantic (and therefore a reduced variance N_e), and for a lower variance in effective sizes in the Mediterranean (and therefore a higher variance N_e). For instance, northern populations are genetically different from the other populations, as shown by the high F_{ST} values between AN populations and all the other geographical zones for both type of markers. These populations also show a lower level of intrapopulation variability, as already observed with allozymes (Johannesson et al. 1989). These populations are at the northernmost limit of the species' range, and reproduction might not occur every year. Such conditions may result in natural and recurrent bottlenecks that reduce both the effective size and the number of alleles while increasing the effect of genetic drift.

The second explanation deals with oyster parasites (*Marteilia refringens* and *Bonamia ostreae*), which have had a more critical effect on *O. edulis* stocks in the Atlantic than in the Mediterranean. For instance, complete disappearance of flat oyster stocks following an outbreak of diseases in the late 1960s and 1970s has already been documented in the Wadden Sea (McKenzie et al. 1997), and dramatic stock decreases have been documented on the Atlantic coast in French Brittany, the Netherlands, Spain, Denmark, Ireland, and England (Goulletquer and Héral 1996; Naciri-Graven et al. 1998).

Finally, the impact of human activities exemplified by overfishing and stock transfer can also be invoked to explain lower effective sizes in harvested areas and the intermediate positions of some populations, like Thau and Marseille, which are suspected to have benefited from transplantation of farmed stocks from Atlantic populations.

References

Baer C, 1999 Among-locus variation F_{st}: fish, allozymes and the Lewontin-Krakauer test revisited. Genetics 152:653–659.

Belkhir K, Borsa P, Chikhi L, Raufaste N, and Bonhomme F, 1996–2001. GENETIX 4.02, logiciel sous Windows TM pour la génétique des populations. Montpellier, France: Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II.

Blanc F, Jaziri H, and Durand P, 1986. Isolement génétique et taxonomie des huîtres plates dans une lagune du sud de la Méditerranée occidentale. C R Acad Sci Paris III 303:207–210.

Borsa P, Jarne P, Belkhir K, and Bonhomme F, 1994. Genetic structure of the palourde *Ruditapes decussatus* in the Mediterranean. In: Genetics and evolution of aquatic organisms (Beaumont AR, ed). London: Chapman & Hall; 122–134.

Borsa P, Naciri M, Bahri L, Chikhi L, García de León FJ, Kotoulas G, and Bonhomme F, 1997. Zoogéographie infra-spécifique de la mer Méditerrannée. Vie Milieu 47:295–305.

Brookfield JFY, 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453–455.

Buroker NE, 1982. Allozyme variation in three nonsibling *Ostrea* species. J Shellfish Res 2:157–163.

Burton RS and Lee BN, 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californianus.* Proc Natl Acad Sci USA 91:5197–5201.

Daguin C and Borsa P, 2000. Genetic relationships of *Mytilus galloprovincialis* Lmk. populations worldwide: evidence from nuclear-DNA markers. In: The evolutionary biology of the bivalvia (Harper EM, Taylor JD, and Crame JA, eds). Special publication no. 177. London: Geological Society; 389–397.

David P, Berthou P, Noel P, and Jarne P, 1997. Patchy recruitment patterns in marine invertebrates: a spatial test of the density-dependent hypothesis in the bivalve *Spisula ovalis.* Oecologia 111:331–340.

DeWoody JA and Avise JC, 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J Fish Biol 56:461–473.

Estoup A, Largiader CD, Perrot E, and Chourrout D, 1996. Rapid one-tube extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol Mar Biol Biotechnol 5:295–298.

Fairbrother JF and Beaumont AR, 1993. Heterozygote deficiencies in a cohort of newly settled *Mytilus edulis* spat. J Mar Biol Assoc UK 73:647–653.

Felsenstein J, 1989. PHYLIP-phylogeny inference package (version 3.2). Cladistics 5:164–166.

Goulletquer P and Héral M, 1996. Marine molluscan production trends in France: from fisheries to aquaculture. In: History, present conditions and future of the molluscan fisheries of North America and Europe. NOAA Technical Report 129. Washington, DC: National Marine Fisheries Service; 137–164.

Hauser L and Ward RD, 1998. Population identification in pelagic fish: the limit of molecular markers. In: Advances in molecular ecology (Carvalho G, ed). Amsterdam: IOS Press; 191–224.

Hedgecock D, 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Genetics and evolution of aquatic organisms (Beaumont AR, ed). London: Chapman & Hall; 122–134.

Hilbish TJ and Koehn RK, 1985. Dominance in physiological phenotypes and fitness at an enzyme locus. Science 229:52–54.

Huvet A, Boudry P, Ohresser M, Delsert C, and Bonhomme F, 2000a. Variable microsatellites in the Pacific cupped oyster *Crassostrea gigas* and other cupped oyster species. Anim Genet 3:71–72.

Huvet A, Lapègue S, Magoulas A, and Boudry P, 2000b Mitochondrial and nuclear DNA phylogeography of *Crassostrea angulata*, the Portuguese oyster endangered in Europe. Conserv Genet 1:251–262.

Jaziri H, 1990. Variations génétiques et structuration biogéographique chez un bivalve marin: l'huître plate *Ostrea edulis* (L.) (PhD dissertation). Montpellier: Université Montpellier II—Sciences et Techniques du Languedoc.

Jaziri H, Durand P, and Blanc F, 1987. Genetic diversity between and within population of the European oyster, *Ostrea edulis.* In: World Symposium on Selection, Hybridization, and Genetic Engineering in Aquaculture, Bordeaux, May 27–30, 1986 (Tiews K, ed). Berlin: Heenemann Verlagsellschaft MBH; 177–187.

Johannesson K, Rodstrom EM, and Aase H, 1989. Low genetic variation in Scandinavian populations of *Ostrea edulis* L.—possible causes and implications. J Exp Mar Biol Ecol 128:177–190.

Korringa P, 1976. Farming the flat oysters of the genus *Ostrea*—a multidisciplinary treatise. Developments in aquaculture and fisheries science, vol. 3. Amsterdam: Elsevier.

Lemaire C, Allegrucci G, Naciri M, Bahri-Sfar L, Kara H, and Bonhomme F, 2000. Do discrepancies between microsatellite and allozyme variation reveal differential selection between sea and lagoon in the sea bass (*Dicentrarchus labrax*)? Mol Ecol 9:57–467.

Le Pennec M, Moraga D, Blanc F, Pichot P, and Thiriot-Quievreux C, 1985. Recherches de différences morphogénétiques, biochimiques et cytogénétiques entre *Ostrea edulis* sensu stricto et *Ostrea edulis* "pied de cheval." Vie Marine 7:29–39.

Lewontin RC and Krakauer J, 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics 74:175–195.

Li G and Hedgecock D, 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. Can J Fish Aquat Sci 55:1025–1033.

MacKenzie CL, Burrell VG, Rosefield A, and Hobart WL, 1997. The history, present condition, and future of the molluscan fisheries of north and central America and Europe. Vol. 3, Europe. NOAA Technical Report 129. Washington, DC: National Marine Fisheries Service.

Magennis BA, Gosling E, and Wilkins NP, 1983. Irish oyster populations: a historical and genetic study. Proc R Irish Acad 83B:291–299.

Magoulas A, Gjetvag B, Terzoglou V, and Zouros E, 1998. Three polymorphic microsatellites in the Japanese oyster, *Crassostrea gigas* (Thurnberg). Anim Genet 29:69–70.

Mantel N, 1967. The detection of disease clustering and generalized regression approach. Cancer Res 27:209–220.

McGoldrick DJ, Hedgecock D, English LJ, Baoprasertkul P, and Ward RD, 2000. The transmission of microsatellite alleles in Australian and north American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. J Shellfish Res 19:779–788

Moraga D and Tanguy A, 2000. Genetic indicators of herbicide stress in the Pacific oyster *Crassostrea*

gigas under experimental conditions. Environ Toxicol Chem 19:706–711.

Naciri M, Lemaire C, Borsa P, and Bonhomme F, 1999. Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). J Hered 90:591–596.

Naciri Y, Vigouroux Y, Dallas J, Desmarais E, Delsert C, and Bonhomme F, 1995. Identification and inheritance of (GA/TC)n and (AC/GT)n repeats in the European flat oyster *Ostrea edulis* (L.). Mol Mar Biol Biotechnol 4:83–87.

Naciri-Graven Y, Martin AG, Baud JP, Renault T, and Gérard A, 1998. Selecting flat oyster *Ostrea edulis* for survival when infected by the parasite *Bonamia ostreae.* J Exp Mar Biol Ecol 224:91–107.

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Palumbi SR, 1996. Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. In: Molecular zoology: advances, strategies, and protocols (Ferraris JD and Palumbi SR, eds). New York: Wiley Liss; 101–117.

Palumbi SR, Grabowski G, Duda T, Geyer L, and Tachino N, 1997. Speciation and population genetic structure in tropical pacific sea urchins. Evolution 51:1506–1517.

Pemberton JM, Slate J, Bancroft DR, and Barrett JA, 1995. Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol Ecol 4:249–252.

Pogson GH, 1991. Expression of overdominant for specific activity at the phosphoglucomutase-2 locus in the Pacific oyster, *Crassostrea gigas.* Genetics 128:133–141.

Quesada H, Zapata C, and Alvarez G, 1995. A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. Mar Ecol Prog Ser 116:99–115.

Reynolds J, Weir BS, and Cockerham CC, 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105:767–779.

Rousset F, 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228.

Saavedra C, Zapata C, and Alvarez G, 1995. Geographical patterns of variability at allozyme loci in the European oyster *Ostrea edulis.* Mar Biol 122:95–104.

Saavedra C, Zapata C, Guerra A, and Alvarez G, 1987. Genetic structure of populations of flat oyster (*Ostrea edulis* [Linneo, 1758]) from the NW of the Iberian Peninsula. Invest Pesq 51:225–241.

Saavedra C, Zapata C, Guerra A, and Alvarez G, 1993. Allozyme variation in European populations of the oyster *Ostrea edulis.* Mar Biol 115:85–95.

Saitou N and Nei M, 1997. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Sarver SK, Katoh M, and Foltz DW, 1992. Apparent overdominance of enzyme specific activity in two marine bivalves. Genetica 85:231–239.

Sokal RR and Rohlf FJ, 1995. Biometry: the principles and practice of statistics in biological research, 3rd ed. New York: W. H. Freeman.

Ward RD, Woodward M, and Skibinski DOF, 1994. A comparison of genetic diversity levels in marine, freshwaters and anadromous fishes. J Fish Biol 44:213–232.

Weir BS and Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

Wilkins NP and Mathers NF, 1973. Enzyme polymorphisms in the European oyster, *Ostrea edulis* L. Anim Blood Groups Biochem Genet 4:41–47.

Zouros E and Foltz DW, 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25:583–591.

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