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ORIGINAL ARTICLE

Strong genetic differentiation among east Atlantic populations of the sword razor shell (*Ensis siliqua*) assessed with mtDNA and RAPD markers

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Abstract The sword razor shell *Ensis siliqua* (Linnaeus, 1758) is a bivalve with a high commercial value being appreciated in fresh and processed markets. However, the genetic studies carried out in populations of *E. siliqua* are scarce. In this work, the genetic variability and differentiation of the sword razor shell was assessed using PCR–RFLPs of a fragment of the 16S rRNA mitochondrial gene and random amplified polymorphic loci (RAPD) in nine localities from Ireland, Spain, and Portugal. In the 314 individuals examined for the mitochondrial fragment, 12 composite haplotypes were observed; meanwhile, a unique phenotype was observed for each of the 242 individuals analyzed with 61 RAPD loci. Two of the mitochondrial composite haplotypes accounted for the majority of individuals (89.81%) and showed a remarkably disjoint distribution between Irish and Iberian samples, with the exception of Aveiro which exhibited as the most frequent haplotype the same found in Ireland. The level of variability observed for each sample was generally correlated with both types of markers and the results obtained suggest the existence of a strong population differentiation between Irish and Iberian localities, except for the Portuguese sample from Aveiro which is surprisingly closer to Irish individuals, although it is probably highly differentiated.

Keywords PCR–RFLPs 16S rRNA · RAPDs · Genetic variability · Population structure

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Introduction

The existence in many marine organisms with high dispersal potential, at least in part of its life cycle, together with the absence of clear barriers to dispersion over broad geographical ranges, would imply low or absence of genetic differentiation. This is the case of some studies carried out with marine bivalves, such as mussels of the genus *Mytilus* (Skibinski et al. 1983) or clams (Benzie and Williams 1992; Vadopalas et al. 2004). Nevertheless, there are increasing reports of genetically differentiated bivalve populations over large (Beaumont and Zouros 1991; Reeb and Avise 1990) or small (Buroker 1983; Ridgway 2001; Luttikhuizen et al. 2003) scales. The present genetic differentiation for each species would be the result of the interaction of historical events, hydrogeographic features and human intervention.

The sword razor shell *Ensis siliqua* (Linnaeus, 1758) belongs to the family Pharidae and inhabits fine sand, silt or sandy-mud bottoms at depths between 3 and 12 m, where they form extensive and dense beds (Gaspar et al. 1999). This species is found mainly from Norway to the Atlantic coast of the Iberian Peninsula. It is increasingly captured in Europe (mainly Ireland, the United Kingdom, the Netherlands, Spain and Portugal), being a highly appreciated seafood especially in Spain, which imports most of the production. Nevertheless, it is quite difficult to estimate the real landings of *E. siliqua*, since the official data do not

reflect exactly the extracted amounts, mainly due to illegal catches and to misidentification of different Solenidae species. The FAO statistics evidences the overexploitation status of *E. siliqua* stocks in different countries, with periods where most production came from a single country, followed by a fall in the catches to minimum values.

Therefore, in order to preserve the natural populations of this species, to promote the rational exploitation of the fishing resources and to evaluate the possibility of stock enhancement by transplanting individuals from other regions, it is essential to assess the genetic variation and population differentiation throughout the geographical range of *E. siliqua*. Furthermore, since different genetic methodologies used in the same species may depict different genetic structures (Karl and Avise 1992), it is important to employ several kinds of molecular markers. Among them, RAPDs (Welsh and McClelland 1990) allow a fast and convenient way to unravel the genetic diversity in species without a previous knowledge of the genome and have been extensively used to evaluate the population structure in molluscs (e.g. Huang et al. 2000; Holmes et al. 2003; Toro et al. 2004; Casu et al. 2005; Barreiro et al. 2006; Holmes and Miller 2006). Similarly, the analysis of mitochondrial DNA has been useful in determining the genetic population structure of molluscan species (e.g. Wilding et al. 1997; Heipel et al. 1999; Kong et al. 2003; Luttikhuizen et al. 2003; Nagashima et al. 2005; Mahidol et al. 2007).

Until now, the only genetic study of sword razor shell populations was carried out by Fernández-Tajes et al. (2007), employing 61 RAPD loci to examine samples from six collecting sites: one from Ireland, three from Spain and two from Portugal. This work revealed small differences between the localities from Spain and Portugal, and higher values between these localities in the Iberian Peninsula and Ireland. Furthermore, evidences of an isolation by distance process were observed. The aim of the present study was to assess the genetic variability and differentiation in *E. siliqua,* increasing the number of localities previously analyzed along the European coast (Fernández-Tajes et al. 2007) and employing an additional marker based on the mitochondrial DNA. The information gathered in the present study provides useful insights into the preservation of *E. siliqua* and for the sustainable management of the fishing resource.

Materials and methods

Sample collection and DNA extraction

Individuals of *E. siliqua* of commercial size (12–15 cm) were collected from Ireland, Spain and Portugal, three sites in each country, by dredging or by scuba diving (Fig. 1). Immediately after collection, the razor clams were brought alive to the laboratory, dissected and preserved in 95% ethanol. DNA was isolated through the method described by Fernández-Tajes et al. (2007).

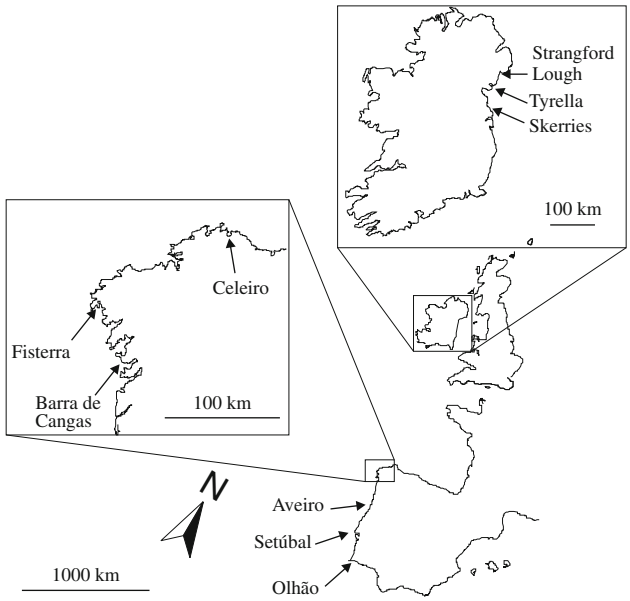

Fig. 1 Map showing the approximate location of *Ensis siliqua* localities analyzed in Ireland: Strandford Lough (Str), Tyrella (Tyr) and Skerries (Ske); Spain: Celeiro (Cel), Fisterra (Fis) and Barra de Cangas (Bar); and Portugal: Aveiro (Ave), Setúbal (Set) and Olhão (Olh)

PCR–RFLPs of 16S rRNA gene

Part of a mitochondrial gene fragment (16S rRNA) was amplified using the universal primers 16Sar and 16Sbr (Palumbi 1996). PCR was performed in 25 µl of a solution containing 1 ng/µl DNA, 10 mM Tris–HCl, 50 mM KCl pH 8.3, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 1 U of *Taq* DNA polymerase, and 1 µM of each primer. The PCR profile consisted of one initial denaturation cycle of 3 min at 94°C, followed by 35 cycles at 94°C for 20 s, 51°C for 20 s, and 72°C for 45 s. A final extension was carried out at 72°C for 5 min. Five µl of each PCR product were electrophoresed through a 1.0% agarose gel to check the amplification. Five µl of 16S rRNA PCR product were singly digested with *Acs* I, *Alu* I, *Cfo* I, *Dra* I, *Hae* III and *Sty* I, using the conditions recommended by the manufacturer (Roche). The digests were resolved after electrophoresis on 2.5% agarose gels in 1× TAE (40 mM Tris–acetate, 1 mM EDTA pH 8.0) and visualized after ethidium bromide staining.

RAPD amplification

The amplification and visualization of 61 RAPD loci obtained with five primers were carried out using the methodology described by Fernández-Tajes et al. (2007).

Data analysis

Restriction profiles from mtDNA gene were alphabetically coded, A being the commonest. Each razor clam was assigned a 6-letter code (that of 16S rRNA digested with *Acs* I, *Alu* I, *Cfo* I, *Dra* I, *Hae* III and *Sty* I) to describe its composite haplotype (Table 1). In the RAPD analyses, the presence or absence of band for each of the 61 RAPD loci was scored for the samples from Aveiro, Skerries and Tyrella, for the other *E. siliqua* localities, the data gathered in the previous study by Fernández-Tajes et al. (2007) was used.

Haplotypic data were analyzed using Arlequin version 3.0 (Excoffier et al. 2005) to estimate frequencies and gene diversity (Nei 1987) among samples**.** The percentage of polymorphic bands (PPB), number of effective alleles (n_e) and Nei's (1987) gene diversity (h) were obtained with the POPGENE software (Yeh et al. 1997) for RAPD data. In order to test if differences in sample size could be responsible for differences in the level of genetic diversity, data was bootstrapped. Ten thousand replicates, with a size equal to the minimum number of individuals analyzed for each type of marker (i.e. 19 or 22), were obtained, and the average expected heterozygosity was computed.

Haplotypic genetic differentiation was estimated with Arlequin by conventional pairwise F_{ST} computed from haplotype frequencies and an exact test of population differentiation (Raymond and Rousset 1995). The sequential Bonferroni correction (Rice 1989) was employed when multiple tests were performed. For RAPD data Nei's (1987) genetic distance, PhiPT (an analogous of F_{ST} fixation index), a Mantel test and a principal coordinate analysis (PCA) based on a matrix of Euclidean distances were calculated with GENALEX software (Peakall and Smouse 2006). A discriminant analysis was performed with R software (R Development Core Team 2009) on the resultant factors of RAPD data to assess the reliability with which individuals could be ascribed to their respective localities. An analysis of molecular variation (AMOVA) was also conducted with Arlequin and GENEALEX for haplotypic and RAPD data, respectively, to partition the total variance into variance components distributed among individuals, localities and regions.

The hidden population genetic structure was examined employing a model-based clustering method implemented in STRUCTURE 2.3 (Pritchard et al. 2000). In order to estimate the number of populations, the distribution of the estimated likelihood of K was examined and the ΔK summary statistic of Evanno et al. (2005) was computed. Similarity among runs was calculated according to Rosenberg et al. (2002) using the R-script Structure-sum (Ehrich et al. 2007) with minor modifications. The program was run under the admixture and non admixture model, considering correlated and independent allele frequencies and employing or not sampling locations as prior information to assist the clustering, for K values between 1 and 9. For each K value, 10 runs with a 2×10^4 burnin followed by a 2×10^4 steps (different runs lengths ranging 10^4–10^6 were tested). Furthermore, since Evanno et al. (2005) showed that this ΔK method detects the uppermost level of population structure

Table 1 Relative composite haplotypes frequencies in *Ensis siliqua* from digestion of part of the 16S rRNA gene in 314 individuals; order of restriction enzymes: *Acs* I, *Alu* I, *Cfo* I, *Dra* I, *Hae* III and *Sty* I

Code	Haplotype	Str	Tyr	Ske	Cel	Fis	Bar	Ave	Set	Olh
H1	AAAAAA				0.962	0.940	0.887		1.000	0.923
H2	ADAAAA				0.038*					
H3	AEAAAA						0.019*			
H4	BAAAAA							0.037*		
H5	BBAAAA	0.771	1.000	1.000				0.667		
H6	BBAAAB	0.125								
H7	BBAABB	0.104								
H8	CAAAAA					0.020*				
H9	CBAAAA							0.296		
H10	DAAAAA					0.040	0.057			
H11	ECAAAA						0.038			
H12	FEABAA									0.077
	N	48	22	38	26	50	53	27	24	26
	Haplotype diversity	0.387	0.000	0.000	0.077	0.117	0.213	0.484	0.000	0.148

* Composite haplotypes carried by single individuals. *N*: sample size. Locality abbreviations according to Fig. 1

when several hierarchical levels exist, the analyses were repeated on each of the groups inferred.

Results

In all individuals, one fragment of 510 bp of the 16S rRNA was obtained. After digestion, in some individuals (7.96%) the sum of fragments was higher than the size of the PCR product and these patterns might be attributed to the presence of heteroplasmy in *E. siliqua*. The fact that they were consistently reproducible allows for their use as genetic markers (González Ittig and Gardenal 2002).

In the 314 individuals examined, 12 composite haplotypes were observed, with four of these being present in only one individual (Table 1). Gene diversity (Nei 1987) oscillated from zero in the localities with one composite haplotype to 0.484 in Aveiro (mean = 0.158). No composite haplotype was shared among all localities, with H1 and H5 accounting for the majority of individuals (89.81%) and being present in five and four samples. It is remarkable that the most frequent haplotype in Aveiro (H5) was the same that in the Irish localities, instead of H1 that was present in geographically nearer sites.

Table 2 Genetic variation statistics of the localities of *Ensis siliqua* examined with 61 RAPD loci

Locality	*N*	PPB (%)	n_e	I	*h*
Str	32	98.361	1.544	0.490	0.324
Tyr	22	85.246	1.449	0.415	0.272
Ske	28	75.410	1.389	0.358	0.235
Cel	24	91.803	1.548	0.479	0.320
Fis	35	98.361	1.625	0.535	0.361
Bar	33	98.361	1.623	0.527	0.356
Ave	19	86.885	1.365	0.358	0.228
Set	26	93.443	1.524	0.463	0.308
Olh	23	96.721	1.521	0.465	0.307

For each site the sample size (*N*), percentage of polymorphic bands (*PPB*) effective number of alleles (n_e), Shannon index (*I*) and expected heterozygosity (*h*) are indicated

Locality abbreviations according to Fig. 1

The 242 sword razor shells scored for 61 RAPD loci obtained with five primers showed a unique phenotype. The proportion of polymorphic loci observed in these nine collecting sites ranged from 75.41% for Skerries to 98.36% for Fisterra, Barra, and Strangford Lough. The effective number of alleles per locus, the Shannon Index and the Nei's gene diversity (Table 2) were 1.365 to 1.625, 0.358 to 0.535 and 0.228 to 0.361, respectively, with Aveiro, Tyrella and Skerries presenting the lower values.

The mean expected heterozygosity values obtained after bootstrapping were remarkably similar to those obtained with the original data (the maximum discrepancy between values was 0.020 and 0.018 for PCR–RFLPs and RAPDs, respectively, data not shown), suggesting that differences in sample sizes could not explain differences between methods or localities.

The pairwise F_{ST} values and differentiation tests computed from haplotype frequencies showed similar results (Table 3). After sequential Bonferroni correction 23 and 24 values were significant for F_{ST} values and differentiation tests, respectively. Based on these results, the Spanish and Portuguese localities (except Aveiro) formed a homogeneous group. On the other hand, the Irish localities showed significant differences, with Strangford Lough differing from Skerries, but not from Tyrella. Finally, the pairwise F_{ST} value, but not the differentiation test, was significant between Aveiro and Strangford Lough.

Nei's genetic distance and PhiPT values for RAPD data (Table 4) depicted a similar situation to that observed with mtDNA. The lowest values were observed between Spanish

Table 3 Genetic differentiation between samples of *Ensis siliqua* based on PCR–RFLPs of part of the 16S rRNA gene

	Str	Ske	Tyr	Cel	Fis	Bar	Ave	Set	Olh
Str		0.003**	0.058	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
Ske	0.137**		NC	0.000**	0.000**	0.000**	0.001**	0.000**	0.000**
Tyr	0.103*	0.000		0.000**	0.000**	0.000**	0.004**	0.000**	0.000**
Cel	0.732**	0.969**	0.958**		0.411	0.356	0.000**	1.000	0.476
Fis	0.750**	0.934**	0.920**	−0.010		0.684	0.000**	1.000	0.227
Bar	0.703**	0.878**	0.855**	0.007	−0.004		0.000**	0.729	0.170
Ave	0.108*	0.319**	0.250**	0.716**	0.742**	0.683**		0.000**	0.000**
Set	0.755**	1**	1**	−0.003	0.004	0.029	0.746**		0.492
Olh	0.706**	0.940**	0.920**	0.001	0.003	0.003	0.682**	0.036	

Pairwise F_{ST} values below the diagonal and *P*-values of the differentiation test above the diagonal

NC not calculable. Locality abbreviations according to Fig. 1

* Significant at $P < 0.05$. ** Significant after sequential Bonferroni Correction.

Table 4 Pairwise Nei's genetic distance (below the diagonal) and PhiPT values (above the diagonal) between nine *Ensis siliqua* localities obtained with 61 RAPD loci

	Str	Tyr	Ske	Cel	Fis	Bar	Ave	Set	Olh
Str		0.142	0.252	0.209	0.184	0.225	0.147	0.241	0.202
Tyr	0.086		0.352	0.327	0.292	0.309	0.246	0.363	0.316
Ske	0.141	0.179		0.360	0.316	0.357	0.295	0.392	0.327
Cel	0.130	0.208	0.226		0.052	0.051	0.331	0.126	0.196
Fis	0.087	0.172	0.182	0.040		0.069	0.291	0.160	0.178
Bar	0.128	0.189	0.227	0.043	0.046		0.319	0.149	0.137
Ave	0.060	0.101	0.131	0.190	0.135	0.169		0.330	0.300
Set	0.114	0.223	0.237	0.068	0.070	0.067	0.161		0.190
Olh	0.103	0.204	0.195	0.117	0.092	0.070	0.152	0.091	

Locality abbreviations according to Fig. 1

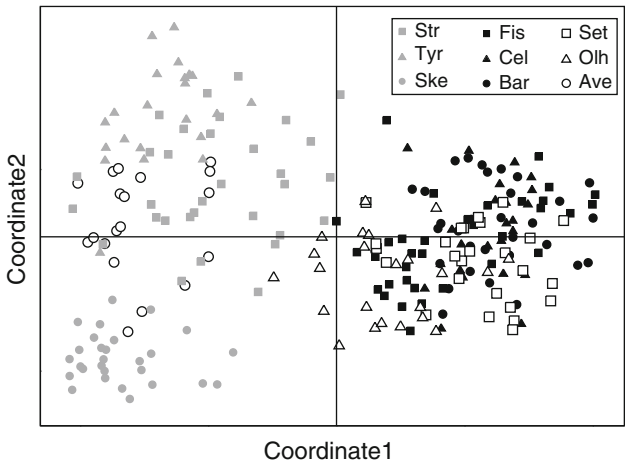

Fig. 2 Principal coordinate analysis of nine *Ensis siliqua* localities based on 61 random amplified polymorphic DNA loci. Locality abbreviations according to Fig. 1

samples and the highest between Irish sampling sites and the remaining localities, except Aveiro that showed a lower genetic distance/PhiPT with Irish populations. The Mantel test revealed a low but significant correlation ($R^2 = 0.153$, $P < 0.050$) between genetic and geographic distances. When the locality of Aveiro was removed from the analysis, the coefficient of determination increased its value to 0.381 and was highly significant ($P < 0.001$).

Genetic similarities among the 242 *E. siliqua* individuals for RAPD data were also examined by principal coordinate analysis (PCA). The first three coordinates explained 67.33% of the total variation and the plot of the first two factors supported the previous results, revealing two groupings, one composed by the localities from the Iberian Peninsula (except Aveiro) and another containing the Irish populations together with Aveiro (Fig. 2). Furthermore, this analysis also pointed to the existence of differentiation between Skerries and the rest of Irish localities. The discriminant analysis indicated that 57.44 and 98.76% of individuals could be assigned to their original localities and the two groups previously described, respectively.

Analysis of molecular variance based on the groups inferred from previous analyses (i.e. one group composed by the Iberian samples except Aveiro and other comprising the rest of localities) revealed that 80.39 and 14.08% of the total variance could be attributed to among groups differences for mtDNA and RAPDs, respectively (Table 5). Any attempt to form other groupings resulted in a decrease in the among groups variance.

Results found for RAPDs data using STRUCTURE under different models were congruent and confirmed the clear genetic differentiation observed in the previous analysis. On the contrary, the assignments obtained with mtDNA PCR–RFLPs were roughly symmetric to all populations, suggesting no population structure, except when sampling locations were employed as prior information to assist the clustering. This is not an unexpected result bearing in mind that only one marker was employed. Therefore, to allow the comparison between results obtained with both type of markers the admixture model, recommended as a starting point for most analyses in the STRUCTURE documentation, with correlated allele frequencies and using sampling location as prior information was employed (Fig. 3). MtDNA PCR–RFLPs and RAPDs showed a high value for Evanno et al's (2005) ΔK at $K = 2$, the other values being close to zero (Online Resource 1). Similarly, mtDNA PCR–RFLPs showed the higher likelihood at this value of K. On the contrary, for RAPDs, the likelihood continued to increase after $K = 2$, but this value seemed to capture the major structure of the data and runs with higher K producing less consistent results. The average similarity for $K = 2$ between runs for each type of marker and between markers was higher than 0.97 and 0.80, respectively. According to Rosenberg et al. (2002), these values correspond to large and moderate similarity coefficients, respectively. In order to detect lower level structure, as suggested by Evanno

Table 5 AMOVA analysis, based on (a) PCR–RFLPs of part of the 16S rRNA gene and (b) RAPD data for *Ensis siliqua*

Source of variation	*d f*	Sum of squares	Variance components	Percentage of variation	*P*-value
(a)					
Among groups	1	61.725	0.398	80.39	<0.001
Among localities within groups	7	2.953	0.010	2.00	<0.001
Within localities	305	26.596	0.087	17.61	0.008
Total	313	91.274	0.495		
(b)					
Among groups	1	315.555	2.083	14.08	0.001
Among localities within groups	7	480.992	2.182	14.75	0.001
Within localities	233	2453.065	10.528	71.17	0.001
Total	241	3249.612	14.793		

Two groups were considered, one composed by the Iberian localities but Aveiro and other by the remaining samples

(a) mtDNA, $F_{SC} = 0.102$, $F_{ST} = 0.824$, $F_{CT} = 0.804$ (b) RAPDs $F_{SC} = 0.141$, $F_{ST} = 0.172$, $F_{CT} = 0.288$

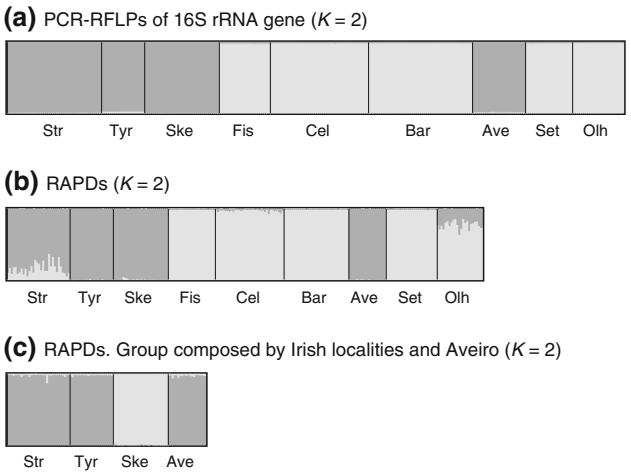

Fig. 3 Most likely population structure, obtained with STRUCTURE under the admixture model, independent frequencies allele frequencies and using sampling locations as prior information (see text for details)

et al. (2005), the analysis was repeated within the groups. In each analysis, *K* was set to vary between 1 and the number of localities in each group. For mtDNA, PCR–RFLPs data results suggested absence of population structure for both groups. Runs for *K* different from one displayed a very high variance and no individuals were strongly assigned. For the group composed by the Iberian samples except Aveiro, the RAPDs results showed for $K = 4$ the higher likelihood and the maximum ΔK. Nevertheless, the absence of clear break among the likelihoods of the different *K* resulted in a very low ΔK, indicating absence of genetic differentiation. On the other hand, the maximum value for the estimated likelihood of *K* was found at $K = 4$ for the group comprising the Irish localities and Aveiro. Nevertheless, for *K* values higher than 2, the likelihood showed only a slight increase and ΔK displayed a high peak at $K = 2$, suggesting the presence of two differentiated populations, separating Skerries from the rest of localities (Online Resource 1).

Discussion

The analysis of *E. siliqua* samples with a fragment of the 16S rRNA gene digested with six enzymes and five RAPD primers allowed identifying 12 composite haplotypes and examining 61 RAPD loci, respectively. Two composite haplotypes, H1 and H5, accounted for most of the individuals and showed a remarkable disjoint distribution. On the contrary, each individual characterized with the RAPD loci displayed a unique pattern, which is not unexpected taking into account the high genetic variation usually detected using RAPD primers and the number of loci examined. The high frequency of H1 and H5, together with their presence in several localities, suggests their older origin and the existence of gene flow, past or present, between the localities sharing one of the haplotypes. In contrast, the other haplotypes (except H10), were limited to a single locality. This indicates a more recent origin of these haplotypes and in case of isolation could result in private haplotypes characteristic of a specific locality.

The values of haplotype diversity per locality (0 to 0.484) were similar to those reported for other bivalves for the 16S rRNA mitochondrial gene (Wilding et al. 1997; Saavedra and Pena 2004; Fernández-Moreno et al. 2008). However, haplotype diversity is a rough measure of variability that is highly dependent on the number or restriction enzymes employed, the technique chosen and the rate of mutation of the mitochondrial region used. On the other hand, the genetic variability observed for the RAPD markers within samples was higher than that obtained from other

marine species (e.g. De Wolf et al. 1998; Ma et al. 2000; Aranishi and Okimoto 2004).

The level of variability observed for each sample was generally correlated in both types of markers. Nevertheless, some striking differences were detected, such as Aveiro showing the highest and the lowest values of haplotype and RAPD diversity, respectively, and Fisterra with an intermediate and maximum mitochondrial and RAPD diversity, respectively. The discrepancy between mtDNA and RAPD diversity could be the result of the different nature of these markers. First, RAPDs are less likely to be subjected to selection than mtDNA, since most of the regions targeted probably correspond to nonexpressed DNA. Second, the high mutation rate in RAPDs would result in rapid recovery of RAPD variation from population bottlenecks. Third, the mitochondrial genome acts as a single locus, providing a single estimate, and has an effective size that is half of nuclear markers being more sensitive to genetic drift.

The differences observed for Fisterra might be related to historical fluctuations in the abundance of *E. siliqua*. This locality suffered an interruption in extractive activity from 1978 to 1983 (due to low cost-effectiveness) and an important decrease in the production in the last years of the 20th century (Guerra Díaz and Gabín Sánchez 2008). In the case of Aveiro, a nonexploited locality, one or more of the factors stated before could have contributed to the observed discrepancy.

The existence of genetic differentiation among the samples analyzed in this study is supported by both mitochondrial PCR–RFLPs and RAPD markers. The different analyses suggested absence or low genetic differentiation among Spanish and Portuguese localities (except Aveiro), but heterogeneity with the remaining samples. Therefore, these localities might be acting as a single interbreeding population with high levels of gene flow among them. The Mantel test was significant even when Aveiro locality was included, although the low coefficients of determination seemed to not support an isolation by distance process, contrasting with the results observed by Fernández-Tajes et al. (2007), this discrepancy being probably caused by the inclusion of a single Irish locality in that work.

The comparison among Irish localities usually showed higher values of differentiation and Aveiro was surprisingly close to those sites. The analyses globally suggested the existence of homogeneity of the Strangford Lough sample with Tyrella but not with Skerries. Strangford Lough is a long, narrow, enclosed sea basin, generally less than 10 m depth, with a narrow tidal outlet to the Irish Sea in its southern end and with extremely strong currents up to 4 m/s. The structure of this lough and an estimated water residence time of 4–28 days (Ferreira et al. 2008) suggest that a certain larval retention might occur. Therefore, even if Skerries and Strangford Lough are relatively near (less than 50 km apart), some restriction to gene flow might exist.

In the case of Aveiro, only the pairwise F_{ST} values for mitochondrial PCR–RFLPs and PhiPT for RAPD data indicated homogeneity with another locality, Strangford Lough. However, these localities presented a considerable frequency of locality-specific mitochondrial haplotypes, suggesting that these samples are in fact highly differentiated. The lack of similarity between Aveiro and the remaining samples suggests that this locality may be predominantly self-recruiting.

Several works have suggested the existence of some mechanism that might affect the larval dispersion in Aveiro (Santos et al. 2004, 2007; Peliz et al. 2007). Santos et al. (2007) found that the upwelling events could significantly change the distribution of early larval stages of *Sardina pilchardus*, and Peliz et al. (2007) working with a dispersal model for larvae crab obtained a good fit between the release of floats from Aveiro and a large part of the floats retained in the shelf zone near the emission points. In addition, Shanks and Brink (2005) observed that the larvae of the congeneric species *E. directus* were below the thermocline, being swept onshore during upwelling events. In the Atlantic coast of the Iberian Peninsula, southward winds during summer generate coastal upwelling, and this period overlaps with the spawning season of *E. siliqua* in Portugal (Gaspar and Monteiro 1998). Therefore, larval retention might explain the results obtained in the present study, although species-specific studies are needed and considerable interannual variation might exist.

To explain the fact that the dominant haplotype is the same in Ireland and Aveiro, and the low genetic differentiation obtained with RAPD markers for these localities, some hypotheses can be proposed. Although possible, an independent origin of the genetic composition is unlikely to have occurred. Since nucleotide mutations in mtDNA are usually rare, individuals that share a particular mutation are generally assumed to descend from a common ancestor (Hartl and Clark 1989) and it is highly improbable that the frequencies for the 61 RADP loci reached similar values merely by random changes.

It is possible that the genetic variability observed in this study was already present in ancestral populations. The commonest haplotypes, H1 and H5, might be an antique polymorphism, one of them being removed (for example due to genetic drift) or maintained (if the haplotypes had a selective advantage to particular habitats) in the individuals that originated the populations analysed in this work. The origin and distribution of molecular variation of these populations is expected to have been shaped by the major climatic changes that occurred during the Pleistocene, and specifically by the last glacial maximum (approximately 20,000 years ago). The subsequent expansion after the glacial

events would have created opportunities for population subdivision, and the succession of multiple founder effects along the colonizing route from southern Europe could have lead to loss of haplotypes and changes in the frequency of RAPD loci. But again the RAPD markers, given their high mutation rate, should have accumulated more changes in their allelic frequencies.

Finally, the similarity among Aveiro and the Irish localities may be caused by the migration of individuals, with the haplotype H5 and the RAPD frequency composition being originated in one of those regions. The main surface and intermediate currents over the North Atlantic (see Beaugrand et al. 2001 and references therein) suggest that the most probable route of transport of larvae is from the Iberian Peninsula to the east coast of Ireland. Nevertheless, if a continuous genetic exchange existed between Aveiro and Ireland it would probably have also influenced the haplotypic and RAPD composition of the neighbor localities, which was not observed in this study. Alternatively, an anthropogenic intervention, for example through the transport of larvae in the ballast water of ships is a feasible process. This was how a closely related species of the same genus, the American jack-knife clam *E. directus*, was involuntary introduced in Europe (Von Cosel et al. 1982). Therefore, and taking into account that the Aveiro harbor has a remarkable activity, the anthropogenic transport of larvae is the most plausible explanation to explain the similarity between Aveiro and the Irish localities.

The present results obtained employing mitochondrial and RAPD markers suggest the existence of a strong population differentiation between Irish and Iberian localities (except Aveiro) and absence of gene flow. This corroborates the growing evidence that significant population structure may exist in marine species, especially those with low or null mobility as adults. Therefore, management plans that take into account the differences among localities identified in this work are needed to carry out a rational exploitation of these fishing resources. The unexpected genetic composition of the sword razor shell from Aveiro, which was more similar to the Irish populations than to the other Iberian localities (but that is probably highly differentiated), indicates that this population may be predominantly self-recruiting and that the genetic variability and population differentiation in *E. siliqua* probably has been shaped by human intervention.

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References

Aranishi F, Okimoto T (2004) Genetic relationship between cultured populations of Pacific oyster revealed by RAPD analysis. J Appl Genet 45:435–443

Barreiro R, Couceiro L, Quintela M, Ruiz JM (2006) Population genetic structure of the prosobranch *Nassarius reticulatus* (L.) in a ria seascape (NW Iberian Peninsula) as revealed by RAPD analysis. Mar Biol 148:1051–1060

Beaugrand G, Ibanez F, Lindley JA (2001) Geographical distribution and seasonal and diel changes in the diversity of calanoid copepods in the North Atlantic and North Sea. Mar Ecol Prog Ser 219:189–203

Beaumont AR, Zouros E (1991) Genetics of scallops. In: Shumway SE (ed) Scallops: biology, ecology and aquaculture. Elsevier, Amsterdam, pp 585–617

Benzie JAH, Williams ST (1992) Genetic-structure of giant clam (*Tridacna maxima*) populations from reefs in the Western Coral Sea. Coral Reefs 11:135–141

Buroker N (1983) Genetic differentiation and population structure of the American oyster *Crassostrea virginica* (Gmelin) in Chesapeake Bay. J Shellfish Res 3:153–167

Casu M, Maltagliati F, Cossu P, Lai T, Galletti MC, Castelli A, Commito JA (2005) Fine-grained spatial genetic structure in the bivalve *Gemma gemma* from Maine and Virginia (USA), as revealed by inter-simple sequence repeat markers. J Exp Mar Biol Ecol 325:46–54

De Wolf H, Backeljau T, Verhagen R (1998) Congruence between allozyme and RAPD data in assessing macrogeographical genetic variation in the periwinkle *Littorina striata* (Mollusca, Gastropoda). Heredity 81:486–492

Ehrich D, Gaudeul M, Assefa A, Koch MA, Mummenhoff K, Nemomissa S, Consortium I, Brochmann C (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. Mol Ecol 16:2542–2559

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:50

Fernández-Moreno M, Arias-Pérez A, Freire R, Méndez J (2008) Genetic analysis of *Aequipecten opercularis* and *Mimachlamys varia* (Bivalvia: Pectinidae) in several Atlantic and Mediterranean localities, revealed by mitochondrial PCR-RFLPs: a preliminary study. Aquac Res 39:474–481

Fernández-Tajes J, Gaspar M, Martinez-Patino D, McDonough N, Roberts D, Gonzalez-Tizon A, Martinez-Lage A, Mendez J (2007) Genetic variation of the razor clam *Ensis siliqua* (Jeffreys, 1875) along the European coast based on random amplified polymorphic DNA markers. Aquac Res 38:1205–1212

Ferreira JG, Hawkins AJS, Monteiro P, Moore H, Service M, Pascoe PL, Ramos L, Sequeira A (2008) Integrated assessment of ecosystem-scale carrying capacity in shellfish growing areas. Aquaculture 275:138–151

Gaspar MB, Monteiro CC (1998) Reproductive cycles of the razor clam *Ensis siliqua* and the clam *Venus striatula* off Vilamoura, southern Portugal. J Mar Biol Assoc U K 78:1247–1258

Gaspar MB, Castro M, Monteiro CC (1999) Effect of tooth spacing and mesh size on the catch of the Portuguese clam and razor clam dredge. ICES J Mar Sci 56:103–110

González Ittig RE, Gardenal CN (2002) Haplotype diversity of the mitochondrial DNA D-loop region in *Calomys musculinus* (Rodentia, Muridae) detected by PCR-RFLP. Biochem Genet 40:293–302

Guerra Díaz A, Gabín Sánchez C (2008) Capítulo 11: Navajas y longueirones comerciales en España. In: Guerra Díaz A, Lodeiros Seijo C (eds) Navajas y longueirones: biología, pesquerías y cultivo, 1st edn. Xunta de Galicia, Consellería de Pesca e Asuntos Marítimos, pp 273–293

Hartl DL, Clark AG (1989) Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts

Heipel DA, Bishop JDD, Brand AR (1999) Mitochondrial DNA variation among open-sea and enclosed populations of the scallop *Pecten maximus* in western Britain. J Mar Biol Assoc U K 79:687–695

Holmes SP, Miller N (2006) Aspects of the ecology and population genetics of the bivalve *Corbula gibba*. Mar Ecol Prog Ser 315:129–140

Holmes SP, Witbaard R, van der Meer J (2003) Phenotypic and genotypic population differentiation in the bivalve mollusc *Arctica islandica*: results from RAPD analysis. Mar Ecol Prog Ser 254:163–176

Huang BX, Peakall R, Hanna PJ (2000) Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar Biol 136:207–216

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters–implications from nuclear RFLPs. Science 256:100–102

Kong X, Yu Z, Liu Y, Chen L (2003) Intraspecific genetic variation in mitochondrial 16S ribosomal gene of Zhikong scallop *Chlamys farreri*. J Shellfish Res 22:655–660

Luttikhuizen PC, Drent J, van Delden W, Piersma T (2003) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. J Evol Biol 16:260–272

Ma XL, Cowles DL, Carter RL (2000) Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Mar Environ Res 50:559–563

Mahidol C, Na-Nakorn U, Sukmanomon S, Taniguchi N, Nguyen TTT (2007) Mitochondrial DNA diversity of the Asian moon scallop, *Amusium pleuronectes* (pectinidae), in Thailand. Mar Biotechnol 9:352–359

Nagashima K, Sato M, Kawamata K, Nakamura A, Ohta T (2005) Genetic structure of Japanese scallop population in Hokkaido, analyzed by mitochondrial haplotype distribution. Mar Biotechnol 7:1–10

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics, 2nd edn. Sinauer Associates, Sunderland, Mass, pp 205–247

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peliz A, Marchesiello P, Dubert J, Marta-Almelda M, Roy C, Queiroga H (2007) A study of crab larvae dispersal on the Western Iberian Shelf: physical processes. J Mar Syst 68:215–236

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Reeb CA, Avise JC (1990) A genetic discontinuity in a continuously distributed species: mitochondrial-DNA in the American oyster, *Crassostrea virginica*. Genetics 124:397–406

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Ridgway G (2001) Interpopulation variation in blue mussels. *Mytilus edulis* L., over short distances. Sarsia 86:157–161

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW (2002) Genetic structure of human populations. Science 298:2381–2385

Saavedra C, Pena JB (2004) Phylogenetic relationships of commercial European and Australasian king scallops (*Pecten* spp.) based on partial 16S ribosomal RNA gene sequences. Aquaculture 235:153–166

Santos AMP, Peliz A, Dubert J, Oliveira PB, Angelico MM, Re P (2004) Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. Cont Shelf Res 24:149–165

Santos AMP, Chicharo A, Dos Santos A, Moita T, Oliveira PB, Peliz A, Re P (2007) Physical-biological interactions in the life history of small pelagic fish in the Western Iberia upwelling ecosystem. Prog Oceanogr 74:192–209

Shanks AL, Brink L (2005) Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. Mar Ecol Prog Ser 302:1–12

Skibinski DOF, Beardmore JA, Cross TF (1983) Aspects of the population-genetics of *Mytilus* (Mytilidae, Mollusca) in the British-Isles. Biol J Linn Soc 19:137–183

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Toro JE, Ojeda JA, Vergara AM (2004) The genetic structure of *Mytilus chilensis* (Hupe 1854) populations along the Chilean coast based on RAPDs analysis. Aquacult Res 35:1466–1471

Vadopalas B, Leclair LL, Bentzen P (2004) Microsatellite and allozyme analyses reveal few genetic differences among spatially distinct aggregations of geoduck clams (*Panopea abrupta*, Conrad 1849). J Shellfish Res 23:693–706

Von Cosel R, Dörjes J, Mühlenhardt-Siegel U (1982) Die amerikanische Schwertmuschel *Ensis directus* (Conrad) in der Deutschen Bucht. I. Zoogeographie und Taxonomie im Vergleich mit den einheimischen Schwertmuschel-Arten. Senckenber Marit 14:147–173

Welsh J, Mcclelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Wilding CS, Beaumont AR, Latchford JW (1997) Mitochondrial DNA variation in the scallop *Pecten maximus* (L) assessed by a PCR-RFLP method. Heredity 79:178–189

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada