J. Mar. Biol. Ass. U.K. (2004), **84**, 235–238
Printed in the United Kingdom

Genetic variability of Tehuelche scallop, *Aequipecten tehuelchus*, populations from the Patagonian coasts (Argentina)

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Genetic variation was investigated in five subtidal locations from the north of San Matías Gulf (42°23′S) to the north of San Jorge Gulf (45°02′S). Fifteen loci were studied by means of vertical starch gel electrophoresis; ten loci were polymorphic. The percentages of polymorphic loci ($P_{0.99}$ and $P_{0.95}$ criteria) varied between 40% and 53.33% and between 33.33% and 46.67%, respectively. Expected mean heterozygosity per locus (He) ranged from 0.128 (Bajo Oliveira-El Sótano) to 0.160 (Puerto Madryn); this range is similar to those observed for other scallop species. All the loci were in Hardy–Weinberg equilibrium. The estimated genetic distances among populations were significantly lower than those reported for comparisons between populations of different pectinid species, suggesting that the five populations studied belong to the same species (*Aequipecten tehuelchus*). The absence of exclusive fixed alleles also supported that assumption. The results obtained suggest: (i) assignment of species or subspecies status to some morphological variants of the shells, like the '*madrynensis*' form is not supported; (ii) the commercially exploited Tehuelche scallop from San Matías and San José Gulfs should be managed as distinct stocks.

INTRODUCTION

The Tehuelche scallop, *Aequipecten (=Chlamys) tehuelchus* (d'Orbigny, 1835) is a warm-temperate bivalve that inhabits the shallow shelf bottoms from Brazil (23°S) to Argentina (45°S) (Figure 1A). In most of its geographical distribution (north of 42°30′S), this pectinid has typically 14 to 18 'squamous' and relatively rounded ribs; towards the southern end of its distribution (42°30′ to 45°S), the Tehuelche scallop presents 11 to 14 comparatively smoother and more marked ribs (in both cases rib number is not correlated with shell size). This geographic variant has been described as a separate species, *Chlamys madrynensis* (Lahille, 1906) or subspecies, *Chlamys tehuelchus madrynensis.* A correlation between morphological and genetic differentiation of the two phenotypes has not been explored.

The Tehuelche scallop of the San José Gulf is structured as a metapopulation whose components or populations (grounds) are interconnected by larval dispersion (Orensanz, 1986; Ciocco, 1992). The Tehuelche scallop of the San Matías Gulf probably has a similar ecological pattern. One relevant question for the management of this resource is whether San Matías and San José Gulfs's scallops are independent or inter-connected stocks, specially due to the collapse detected in recent years (Ciocco & Orensanz, 1997).

The purpose of this work was to ascertain the taxonomic status of the '*madrynensis*' form of the Tehuelche scallop and to explore the genetic structure of San Matías and San José Gulfs's stocks.

MATERIALS AND METHODS

Collection of samples

Samples were obtained from June 1999 to September 2000 manually by SCUBA diving at five locations of the upper infralitoral (15 to 25 m depth): (1) north of San Matías Gulf (Bajo Oliveira-El Sótano, 40°58′S to 65°06′W); (2) south of San Matías Gulf (Puerto Lobos, 42°01′S to 65°04′W); (3) San José Gulf (Punta Tehuelche, 42°23′S to 64°19′W); (4) Nuevo Gulf (Puerto Madryn, 42°45′S to 65°01′W); (5) San Jorge Gulf (Cabo Achaima, 45°02′S to 65°53′W) (Figure 1A). The individuals collected were transferred alive to the laboratory where they were dissected.

Shell morphometry and morphology

All the shells were carefully cleaned and dried off. The number of ribs was recorded as well as the shell's ornamentation. The number of ribs was compared among populations by single classification analysis of variance. The variable was normally distributed and the variance homogeneous. Tukey's test was applied to compare the means.

Electrophoretic analysis

Sections around 0.5 g of digestive gland (DG) and adductor muscle (AM) from each individual (30 to 32 from each location) were dissected, labelled and stored in

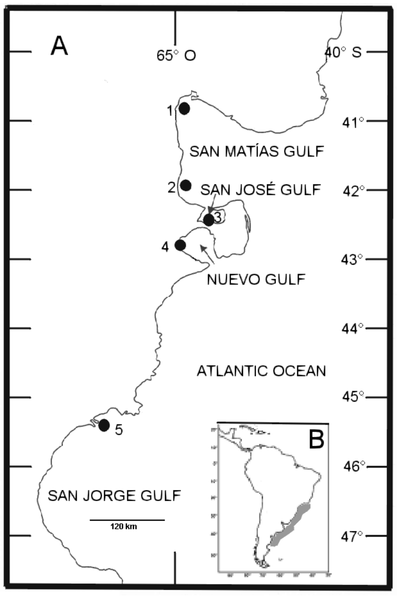

Figure 1. (A) Location of sampling sites: 1, San Matías Gulf (north); 2, San Matías Gulf (south); 3, San José Gulf; 4, Nuevo Gulf; 5, San Jorge Gulf; (B) geographical distribution of the Tehuelche scallop.

liquid nitrogen. The tissue samples (DG and AM) were individually homogenized in distilled water using refrigerated glass mortars and then centrifuged at 10,000 rpm for 10 min. The supernatants of each sample were electrophoresed in vertical starch gel (10%; 4°C). Eleven enzymes (*Gpi*, *Pgm*, *Aat*, *Mdh*, *Idh*, *Sdh*, *Fac-1*, *Fac-2*, *Est-1*, *Est-2*, *Lap-1*, *Lap-2*, *Hxdh*, *Sod-1* and *Sod-2*) were studied and two buffer systems were used: Tris–boric–EDTA and Tris–citric. The gel was cut in slices and the cut surfaces were exposed to mixtures of specific staining to reveal the activity of each enzyme by standard procedures. After staining, the gels were photographed.

Nomenclature

The loci were designed with the abbreviation of the corresponding enzyme, with the first letter in upper case. In the case of isozymes controlled for two or more loci, the fastest anodally migrating protein was designated ***1*** and slower migrating loci were numbered consecutively. Enzymes originated by multiple alleles in the same *locus* (allozymes) were designated with consecutive lower case letters, ***a*** corresponding to that with the fastest anodal migration.

The genotypes were designated using the symbol of each locus followed by the two lower letters corresponding to the alleles present in each individual.

Statistical analysis

The genotypes were inferred from the electrophoretic patterns according to those described in other species in which the genetic control is clearly known. The expected genotypes distribution according to the Hardy–Weinberg equilibrium was calculated using the Levene's correction (1949) for small samples. The probability of deviation between observed and expected genotypes frequencies was estimated by the Chi-square test. Intra- and inter-population allozymic variation was estimated by the proportion of polymorphic loci ($P_{0.95}$ and $P_{0.99}$ criteria), mean observed (Ho) and expected heterozygosity (He) and the average number of alleles per locus (A). Genetic distance between populations was estimated according to Nei (1975) and Rogers modified by Wright (1978). Population structure was analysed using Wright's F statistics (1965) modified by Nei & Chesser (1983). The index F_{ST} was used to estimate the genetic divergence between populations and the index F_{IS} was used to estimate the deviation of the expected and observed heterozygosity within populations. The significance of F_{IS} and F_{ST} values were analysed using a permutation test according to the FSTAT program (Goudet, 1999). Relationships between populations based on Rogers distance modified by Wright (1978) were summarized as a UPGMA (Sneath & Sokal, 1973). Calculations were performed using BIOSYS-2 (Black, 1997) and FSTAT (Goudet, 1999) programs.

RESULTS

Shell morphometry and morphology

According to the number and the clearly discontinuous form of the ribs, the shells from San Matías (north and south locations) and San José Gulfs would belong to the '*tehuelchus*' form. In contrast, the shells from Nuevo and San Jorge Gulfs correspond to the '*madrynensis*' form. Significant differences were detected in the number of ribs when the five populations were compared ($P < 0.001$). Those of Cabo Achaima (12.50 ±0.89 ribs; San Jorge Gulf) and Puerto Madryn, (12.50 ±0.83; Nuevo Gulf) did not differ significantly ($P > 0.9$), while the remaining ones (Bajo Oliveira-El Sótano: 15.9 ±0.79; Puerto Lobos: 16.2 ±0.77; Tehuelche: 15.6 ±0.85) were similar to each other and they differed significantly from the two first ones ($P < 0.001$).

Allozyme variation

The zymograms of the 11 analysed enzymes offered information for 15 loci; *Mdh*, *Sdh*, *Idh*, *Fac*-1 and *Lap*-2 were monomorphic for the same allele in the five populations.

The electrophoretic patterns were not well defined for catalase and hexoquinase as to asign genotypes unequivocally. However, both loci were polymorphic.

Genetic variability

The genetic variability of the five populations was calculated starting from the allelic frequencies in all the loci. The $P_{0.99}$ values oscillated between 40% and 53.33%; $P_{0.95}$ values ranged between 33.33% and 46.53% and He varied from 0.128 (Bajo Oliveira-El Sótano) to 0.160 (Puerto Madryn) (Table 1).

In all the polymorphic loci, observed genotype frequencies were in agreement with those expected under Hardy–Weinberg equilibrium. F_{IS} values were also not significant (Table 2).

The F_{ST} values varied between 0.013 (*Hxdh*) and 0.167 (*Est*-2) (average value: 0.032; $P < 0.01$), indicating a

Table 1. *Estimations of genetic variability in five populations of* Aequipecten tehuelchus *(standard errors in parentheses).*

	Populations				
	San Jorge Gulf	San Matías Gulf (south)	San Matías Gulf (north)	Nuevo Gulf	San José Gulf
Percentage of polymorphic loci ($P_{0.95}$)	33.33	40	46.67	40	40
Percentage of polymorphic loci ($P_{0.99}$)	40	53.33	53.33	46.67	53.33
Mean number of alleles per locus (A)	1.9 (0.3)	1.9 (0.3)	2.0 (0.3)	2.1 (0.4)	2.0 (0.3)
Mean observed heterozygosis (Ho)	0.138 (0.057)	0.148 (0.054)	0.121 (0.050)	0.162 (0.060)	0.158 (0.054)
Mean expected heterozygosis (He)	0.140 (0.061)	0.155 (0.058)	0.128 (0.054)	0.160 (0.061)	0.157 (0.056)

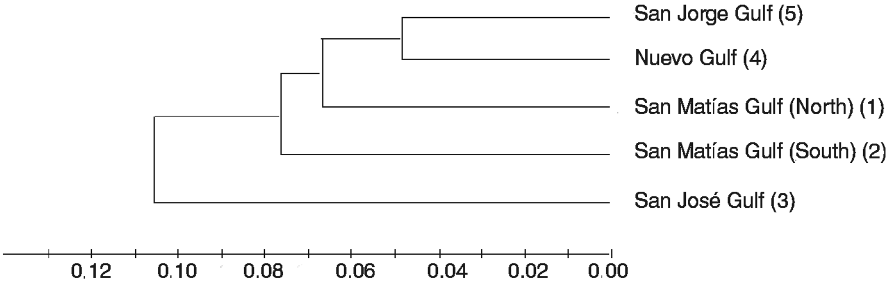

Figure 2. UPGMA dendogram based on Rogers modified genetic distance (Wright, 1978) between populations.

Table 2. *F statistics in population samples of* Aequipecten tehuelchus.

Locus	F_{IS}	F_{ST}
Gpi	0.011	0.02*
Aat	−0.033	0.015
Pgm	−0.048	0.018
Fac-2	−0.059	0.045**
Est-1	−0.075	0.056**
Est-2	0.149	0.167**
Lap-1	0.116	0.049**
Hxdh	−0.117	0.013
Sod-1	−0.028	0.018
Sod-2	−0.14	0.021
Average	0.019	0.032**

*, $P<0.05$; **, $P<0.01$.

significant level of differentiation among populations. This significance was mainly contributed by the *Gpi*, *Est*-1, *Est*-2, *Lap*-1 and *Fac*-2 loci (Table 2).

The values of genetic distance calculated according to Nei (1975) and to Rogers modified by Wright (1978) oscillated from 0.003 to 0.015 and from 0.048 to 0.111, respectively. Figure 2 shows the Rogers' index obtained. Genetic distance analysis showed that Cabo Achaima (San Jorge Gulf), Puerto Madryn (Nuevo Gulf) and Bajo Oliveira-El Sótano (north of San Matías Gulf) were similar, while the Tehuelche (San José Gulf) population was the most different population. Puerto Lobos (south of San Matías Gulf) population showed an intermediate position among the first three populations and Tehuelche (Figure 2).

DISCUSSION

This is the first study on the genetic structure of pectinid populations from the south-west Atlantic Ocean. The percentage of polymorphic loci ($P_{0.99}$) (40 to 53.3%) was similar to that observed in other scallop species (Beaumont & Zouros, 1991). The expected values of mean heterozygosity per locus (parameter less influenced by the number of studied loci and for the sampling size than P) estimated in this study (0.128 to 0.160) were within the range reported for pectinids (0.094 to 0.321; Beaumont & Beveridge, 1984; Beaumont & Zouros, 1991; Beaumont et al., 1993; Rios et al., 1999).

All the populations studied were in Hardy–Weinberg equilibrium. This is supported by the non-significance of F_{IS} values in all polymorphic loci. In bivalves, including several pectinids, reported heterozygote deficiency has been attributed to selective mortality, autofertilization or severely reduced genetic flow among subpopulations. Our results do not show heterozygote deficiency.

Genetic distances (Rogers' index modified by Wright, 1978) estimated in this study were smaller than 0.11 and higher than those recorded for conspecifics populations of *Pecten jacobeus* (Rios et al., 1999) and *P. maximus* (Huelvan, 1985; Rios et al., 1999). Regarding interspecific comparisons, the genetic distances calculated in this study (Nei's index range: 0.003 to 0.015) were generally lower than those reported for *Pecten jacobeus* and *P. maximus* populations from the Mediterranean and Atlantic coasts of Europe, respectively (Huelvan, 1985: 0.09 units; Ríos et al., 1999: 0.148 units).

The F_{ST} values between population pairs showed significant levels of differentiation in five out of ten polymorphic loci, suggesting that the Tehuelche scallops of the Patagonian coast does not constitute an unique panmictic population.

Beaumont & Zouros (1991) reported that *P. magellanicus* from the north-western Atlantic is structured as a geographically dispersed population with enough genic flow as to prevent the accumulation of significant differences in the allelic frequencies. These authors estimated interpopulational distances (Nei's index) between 0.02 at 0.13. Similar levels of interpopulational heterogeneity have been reported in *Pecten maximus* and in *Chlamys islandica* from the North Atlantic. The interpopulational genetic distances estimated in this study were significantly lower than the interspecific distances reported for other pectinids, suggesting that the five studied populations belong to the same species. Absence of exclusive fixed alleles in the '*tehuelchus*' and the '*madrynensis*' forms contradicts the hypothesis of two different species.

Our results show no correlation between genetic variation and morphological differences which led some authors to propose a subspecific status for the '*madrynensis*' form. Several examples of geographical variations (morphological phenotypes, reproductive patterns, growth rates and survival) previously reported are not always consistent with genotypes; in some cases they are related to environmental conditions (Orensanz et al., 1991). Differences in colour, form, relative weight and shine or growth rings among conspecific individuals from different grounds can often be discerned at first sight. The Tehuelche scallops from San José Gulf are, on average, higher than those from San Matías Gulf (Orensanz, 1986). It has been suggested that a 'sedentary' outline like this (proportionally higher shells, more asymmetric auricles) might be favoured by selective pressures because it could improve scallop attachment to the bottom. Individuals attached more firmly could have a lower chance of dying due to beach stranding, a significant component of natural mortality in San José Gulf, but not in San Matías (Orensanz, 1986; Orensanz et al., 1991).

The pelagic larval period in pectinids varies between 6 and 70 days. There is no precise information for *Aequipecten tehuelchus* in the natural environment, but the time elapsed since massive spawning and settlement peaks on artificial collectors suggests three to six weeks on average. In laboratory experiments the Tehuelche scallop's larvae live about 30 days. Passive accumulation of larvae seems to be one of the most important mechanisms generating the distribution patterns in pectinids. Hydrodynamic conditions, in turn, determine the dispersion and retention of pelagic stage. However, important questions about larval retention and dispersion are not answered yet as, for example, the behaviour of larvae in the water column (Orensanz et al., 1991). In the case of the north Patagonian Gulfs, the pattern of water exchange between the San Matías and the San José Gulfs is not well known. It is likely that platform water enters from the south of San Matías Gulf, flows along the western coast of the Gulf, and flows out of the Gulf in the north, implying a water residence time in San Matías Gulf of about 300 days (Rivas & Beier, 1990). This period is much longer than the estimated life time of *A. tehuelchus* larvae (3–6 weeks), and could explain the genetic differences between the northern and southern populations from the San Matías Gulf. In the same sense, the existence of a cyclonal eddy in the north of the San Matías Gulf would interfere with the exchange of larvae with the rest of the gulf. Similarly, circulation studies in the San José Gulf suggest a scenario for larval retention to the east. Local natural selection, as mentioned above (sedentary phenotype favoured in the San José Gulf), would also contribute to promote genetic divergence between scallops from both gulfs.

We are grateful to Dr Maite Narvarte, Luis de Francesco and José Ascorti for providing some of the samples. We also express our gratitude to the Population Genetics and Evolution Laboratory staff (F.C.E.F. y N., University of Cordoba) and to Norberto De Garin and Dr Pedro Barón (CENPAT-CONICET).

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Submitted 11 February 2002. Accepted 18 November 2003.