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LOSS OF GENETIC VARIATION IN A STRONGLY ISOLATED AZOREAN POPULATION OF THE EDIBLE CLAM, *TAPES DECUSSATUS*

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ABSTRACT We used allozyme electrophoresis to compare the genetic variation of an introduced and strongly isolated population of the edible clam *Tapes decussatus* in the Azores (Lagoa de Santo Cristo, São Jorge) with populations from the main range of the species (Ria and Thau). Observed and expected heterozygosity values, number of polymorphic loci, and mean number of alleles per locus in the main-range populations fall within the limits reported for *T. decussatus* and other Venerid clams. In contrast to previous studies on Venerid clams, we observed no heterozygote deficiencies. In the introduced Lagoa population, we observed a strong reduction of allelic diversity and expected heterozygosities and an effective population size of only 5.30. The Lagoa population is only slightly differentiated from populations from the species' main range and may thus be of low "biological value." Exploitation of *T. decussatus* could therefore be allowed to continue but must follow strict collection guidelines, especially given that only 15% of the area is suitable for exploitation. Otherwise, a unique component of the Azorean fauna that also serves as a fishery resource may be lost.

KEY WORDS: Azores, allozymes, founder effect, *Tapes decussatus,* population genetics, conservation

INTRODUCTION

Small or isolated populations can contribute substantially to biodiversity, and the conservation of such populations must be an important part of any effective Biodiversity Action Plan (Usher 1997). The genetic effects in small populations are manifold (Harris 1984, Usher 1987). Small effective population sizes (N_e) often show a loss of genetic variability (i.e., founder effects, bottlenecks) caused by genetic drift (Lacy 1987). Apart from losing (rare) alleles, small populations often lose common alleles by chance (Nei et al. 1975, Simberloff 1988) and may show elevated inbreeding, which may impair reproductive fitness. In addition, the loss of genetic variability may limit the ability of a population to adapt to changing environments (Frankel and Soulé 1981, Thorpe et al. 1995). Over the long term, these effects may enhance the risk of extinction (Soulé 1987). Effective conservation or management plans require a thorough knowledge of the genetic population structure before adequate measures can be taken. In this study, we estimated the effective population size and investigated whether genetic variation is reduced in an introduced, isolated population of the commercial edible clam *Tapes decussatus* (Linnaeus, 1758).

The main range of *T. decussatus* extends from Great Britain in the north to Senegal in the south, along the Iberian peninsula, and into the Mediterranean to the east (Tebble 1966). Outside its main range, the species has been introduced in the Lagoa de Santo Cristo, a small and isolated lagoon situated at the north coast of the island of São Jorge in the Azores, approximately 1,400 km from the African/European coasts, where it was discovered for the first time in 1967 (Morton 1967). This lagoon has a total area of 0.86 km^2 (length, 500 m; width, 250 m; and maximum depth, 6 m) and harbors a unique fauna (Morton 1967, Santos 1985, Santos and Martins 1986, Morton and Tristão da Cunha 1993, Morton et al. 1998). The lagoon was classified as a Natural Partial Reserve in 1984 on the basis of its unique origin, geology, and the presence of the edible clam *T. decussatus.* In 1989, it was also declared a Special Ecological Area, to safeguard the unique breeding population of *T. decussatus* and to maintain the ecological equilibrium

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of the area. Although there is no written record, *T. decussatus* was probably introduced in the lagoon by humans, especially since the species occurs nowhere else in the Azores (Morton 1967, Morton and Tristão da Cunha 1993). Moreover, the planktonic stage of the larvae lasts approximately 10 days, during which larvae are transported by sea currents over a distance of 10–100 km (Borsa et al. 1991). Larval transport from the main range to the Azores by sea currents seems therefore unlikely.

At this moment, *T. decussatus* is the main commercially exploited species of the lagoon (Fonseca et al. 1995). Santos and Martins (1987), Santos et al. (1989), and Gonçalves and Martins (1991) showed that the population of *T. decussatus* in the Lagoa de Santo Cristo was declining through overexploitation, especially in the intertidal parts of the lagoon, where clam collection is easy. The intense fishery resulted in smaller individuals in the intertidal area. These potential detrimental impacts on the clams and other species of the lagoon have obliged the Azorean government to establish a management program for the Lagoa de Santo Cristo. Therefore, the clam fishery at the lagoon is nowadays closed during a period that largely coincides with the breeding season of the species (May 15 to August 15; Santos and Martins 1987, 1991). The present research was performed to provide genetic data that may be relevant for further substantial management of the clam population.

MATERIALS AND METHODS

Four samples of *T. decussatus* were collected from three sites: Lagoa de Santo Cristo (SC; July 1992 and June 1993), Étang de Thau (Thau; French Mediterranean coast; August 1993), and Playa do Testal (Ria; Ria de Muros y Noya, Galicia, Spain; December 1993). Specimens were immediately frozen in liquid nitrogen for transport to the laboratory, where they were stored at −80 °C.

Forty specimens of each sample were surveyed for allozyme variation with vertical polyacrylamide gel electrophoresis (PAGE). Individual tissue homogenates were prepared by dissecting specimens in ice-cold distilled water and removing the digestive gland, the gills, the foot muscle, and the adductor muscles. Each of the tissues was separately weighted and homogenized in a 20% (w/v) aqueous sucrose solution (5 μL sucrose solution per mg tissue). Crude homogenates were centrifuged for 45 min at ±27,000 *g* at 5 °C to obtain clear supernatants for electrophoresis.

PAGE was performed as described by Backeljau (1987, 1989). Two electrophoretic buffer systems were used: (1) Tris/glycine pH 9.0 in the tray and Tris/HCl pH 9.0 in the gels and (2) Tris/citric acid pH 8.0 in both the tray and the gels. Enzyme staining recipes were adapted from Harris and Hopkinson (1976).

Twenty-six enzyme systems were screened in the four tissues (see Backeljau et al. 1994). Seven of these enzymes yielded interpretable genetic polymorphisms and were retained for further analysis (Table 1).

Alleles were designated alphabetically according to decreasing electrophoretic mobilities (A = most anodal = fastest-migrating allele). Previously typed specimens were included with each run to compare different gels. The BIOSYS-1 version 1.7 package (Swofford and Selander 1981) was used for estimating allele frequencies, mean numbers of alleles per locus (MNA), observed heterozygosities (H_o, direct count) and Nei's (1978) unbiased expected heterozygosities (H_e). Numbers of polymorphic loci (P) were simply counted. Weir and Cockerham's (1984) fixation indices (F_{is}) were estimated with GENEPOP version 3.0 (Raymond and Rousset 1995), and genotype frequencies were evaluated for departures from Hardy-Weinberg (HW) equilibrium expectations with the probability test implemented by the same program. The significance of F_{is} values was tested with FSTAT version 1.2 (Goudet 1995). Linkage disequilibria (LD) between loci were tested with the exact probability test in GENEPOP version 3.0. Whenever needed, testing procedures were corrected for multiple testing with the sequential Bonferroni method (Rice 1989). Nei's (1978) unbiased genetic distance between populations was calculated with BIOSYS-1 version 1.7.

The effective population size (N_e) of the population from the Lagoa was estimated in two different ways. One method estimates N_e from the changes in expected heterozygosity. In a population of size N_e, the initial heterozygosity (H_o) will decrease to H_t after t generations. The relationship between H_o and H_t is given by the equation $H_t = H_o (1 - 1/2N_e)^t$ (Crow and Kimura 1970). A second method (i.e., the temporal method) estimates N_e from temporal changes of gene frequencies as described by Waples (1989) and Hedgecock et al. (1992). Although a few *T. decussatus* individuals may spawn in their first year (Vilela 1950), the vast majority of individuals reach their sexual maturity at the beginning of their second year (Gallois 1977). Therefore, we used a generation time of 1 y for *T. decussatus*. An assumption of both methods is that the allozyme polymorphisms studied are selectively neutral. To test this, we performed the Ewens-Watterson test using the algorithm given in Manly (1985) and implemented by the program POPGENE version 1.31 (updated version of POPGENE version 1.2 of Yeh and Boyle [1997]).

Because many bivalves show a positive correlation between

TABLE 1.

Enzymes studied, E.C. numbers, enzyme codes, the tissue from which the enzyme was extracted, and the buffer system (TC = Tris/citric acid; TG = Tris/glycine) used to examine genetic variation in four *T. decussatus* populations.

Enzyme	EC Number	Code	Tissue	Buffer
Malate dehydrogenase	1.1.1.37	*Mdh*	Adductor muscle	TC
D-Octopine dehydrogenase	1.5.1.11	*Opdh*	Adductor muscle	TC
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*Idhp*	Digestive gland	TC
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	Digestive gland	TC
3-Hydroxybutyrate dehydrogenase	1.1.1.30	*Hbdh*	Digestive gland	TG
Leucylalanine peptidase	3.4.13.11	*Pep*	Gills	TG
Phosphoglucomutase	5.4.2.2	*Pgm*	Adductor muscle	TG

shell size and individual heterozygosity (e.g., Zouros and Foltz 1984), we checked for such a relationship to avoid the possibility that discrepancies in H_o values would merely reflect size differences between populations. Therefore, Pearson's product-moment correlation was calculated between shell length and numbers of heterozygous loci, as outlined by Diehl and Koehn (1985) and Fevolden (1992).

RESULTS

Pep revealed two independent banding zones, the cathodal of which was clearly polymorphic in the Thau and Ria populations, but monomorphic in the Lagoa population. Yet, because the bands in this zone were often confused, they were not used for genotypic analysis. The six remaining enzymes yielded information for seven putative loci (Table 1), the population genetic data of which are provided in Tables 2 and 3. Out of 18 HW tests, only 2 were significant (*Pgm* in Thau and *Idhp* in Ria; Table 2), but this was no longer so after sequential Bonferroni correction. Not surprisingly, F_{is} values taken over all loci in all populations were not significantly different from 0 ($0.193 < P < 0.27$). However, compared with the Lagoa population, the Thau and Ria populations had higher heterozygosity levels and nearly twice as many polymorphic loci and mean numbers of alleles per locus (Table 2). Only two of the 31 LD tests were significant (data not shown), but both cases were no longer significant after sequential Bonferroni correction. Nei's (1978) unbiased genetic distance between the samples ranged from 0.036 (between two samples from the Azores) to 0.23 (between Thau and two samples from the Azores) (Table 3).

The estimate of N_e with the temporal method was infinity. This result is probably an artifact caused by the small number of loci analyzed ($n = 3$) (Table 2). It simply indicates that the change in allozyme frequencies observed between the 2 years was not large enough to be distinguished from sampling error. The estimate of N_e obtained from the reduction of heterozygosity was 5.30. The test for neutrality gave nonsignificant results.

We found no significant correlation between individual heterozygosity and shell length (Thau, $r = 0.173$, $P = 0.733$; Ria, $r = 0.36$, $P = 0.556$; and Lagoa (pooled samples), $r = 0.48$, $P = 0.409$).

DISCUSSION

Observed and expected heterozygosity values, number of polymorphic loci, and mean number of alleles per locus in the Ria and Thau populations fall within the limits reported for *T. decussatus* and the palourde *Ruditapes philippinarum* (Table 4). As in many other bivalve species, heterozygote deficiencies have often been reported in *T. decussatus* and *R. philippinarum* (see references in Table 4), but at present the causes of this remain unclear (Zouros et al. 1988). Yet, in our study, we observed no heterozygote deficiencies. Nevertheless, our population genetic data of the Thau population are very similar to the results obtained by Jarne et al. (1988), Borsa and Thiriot-Quiévreux (1990), and Borsa et al. (1994) for the same population and for the nearby population of Étang du Prévost (Worms and Pasteur 1982). Moreover, genetic distances between our populations are similar to those reported by Jarne et al. (1988) (compare our Table 3 with their Table 4).

However, in the Lagoa population of *T. decussatus* in the Azores, we observed a strong reduction of allelic diversity and expected heterozygosities, but not heterozygote deficiencies, compared with main-range populations. Substantial losses of genetic diversity have also been observed in bivalves for which hatchery stocks have been established from only a few individuals (e.g., the oysters *Crassostrea gigas* [Gosling 1982, Hedgecock and Sly 1990] and *C. virginica* [Vrijenhoek et al. 1990, Gaffney et al. 1992]). This may have important implications when management and exploitation practices are developed. Many hatchery stocks or introduced populations have a low N_e value despite densities that can be very high (e.g., Saavedra 1997 and references therein). In the Lagoa, population densities of *T. decussatus* may reach 400 individuals/m^2 (Gonçalves and Martins 1991). Yet we estimated an effective population size of only 5.30 individuals. Founder effects, genetic drift, intentional selection, and inadvertent selection during culture are likely to reduce the genetic diversity of the Lagoa population further. The introduction of a small number of individuals a few decades ago probably resulted in the loss of genetic variation via founder effects. The strong isolation of this population probably does not allow transport of larvae from nearby populations (see Introduction), and genetic drift and inbreeding may further reduce genetic variability. These effects are probably reinforced by human activities such as selection during harvesting (e.g., the collection of only large adults). Indeed, the exploitation of *T. decussatus* in the Lagoa follows a classic "fishery" picture with old (i.e., large) shells lacking among empty shells in the lagoon because they were collected for consumption when alive (Morton and Tristão da Cunha 1993). It is unclear whether such selective harvesting affects the genetic structure of the population, because there was no association between individual heterozygosity and size. Yet this topic deserves further study, as Borsa et al. (1994) and Passamonti et al. (1997) found a high level of intrapopulation structuring, probably related to year-cohort heterogeneities, that perhaps indicate short-term selection or genetic drift (Borsa et al. 1994). Thus, harvesting a single age cohort (i.e., oldest and largest individuals) could affect the genetic population structure.

In none of the populations did we observe a significant correlation between shell size and individual heterozygosity. Some other studies also failed to show a relationship between individual heterozygosity and morphological traits such as size and growth (Adamkewicz et al. 1984, Volckaert and Zouros 1989, Gaffney 1990, Slattery et al. 1991), but others report negative (Wilkins 1978) or positive (Garton et al. 1984, Koehn and Gaffney 1984, Zouros and Foltz 1984, Gaffney 1990) associations, although associations may differ among populations (Gaffney 1990).

A positive relation between heterozygosity, body size, and survival was found in a population of *T. decussatus* that survived natural anoxic stress (Borsa et al. 1992). However, in other populations of the same species, Jarne et al. (1988) observed no association between asymmetry of left and right valves (as a measure of fitness, i.e., the more asymmetric the less fit) and heterozygosity, and an increased variance for morphological traits in the classes with low heterozygosity. This also appears to be the case for some of the *R. philippinarum* populations in the Po river lagoon in Italy (Fava et al. 1994). In that study, individual heterozygosity and phenotypic variability appeared to be negatively correlated, but the relationship was heterogeneous between populations (Fava et al. 1994).

TABLE 2.

Allozyme variation in four populations of *T. decussatus* (for full population names we refer to the text).

	Thau (n = 40)	Ria (n = 40)	SC92 (n = 40)	SC93 (n = 40)
Mdh				
A	0.837	1.000	1.000	1.000
B	0.163			
H_e	0.272			
H_o	0.325			
F_{is}	−0.182			
P_{exact}	0.564			
Omdh				
A	0.625	0.538	0.488	0.600
B	0.213	0.225	0.262	0.212
C	0.162	0.237	0.250	0.188
H_e	0.538	0.604	0.631	0.569
H_o	0.575	0.575	0.675	0.575
F_{is}	−0.067	0.061	−0.057	−0.015
P_{exact}	0.500	0.801	0.526	0.458
Idhp				
A	0.113	0.038		
B	0.887	0.962	1.000	1.000
H_e	0.200	0.072		
H_o	0.125	0.025		
F_{is}	0.385	0.661		
P_{exact}	0.057	0.038*		
Pgdh				
A	0.138			
B	0.200	0.225	0.462	0.375
C	0.349			
D	0.175	0.613	0.338	0.400
E	0.138	0.162	0.200	0.225
H_e	0.769	0.548	0.632	0.649
H_o	0.500	0.525	0.650	0.650
F_{is}	0.102	0.054	−0.036	0.011
P_{exact}	0.384	0.881	0.973	0.576
Hbdh-1				
A	0.250	0.225		
B	0.724	0.762	1.000	1.000
C	0.013	0.013		
D	0.013	0.013		
H_e	0.412	0.368		
H_o	0.400	0.375		
F_{is}	0.041	0.264		
P_{exact}	0.832	0.144		
Hbdh-2				
A	0.987	1.000	1.000	1.000
B	0.013			
H_e	0.025			
H_o	0.025			
F_{is}	−0.013			
P_{exact}	1.000			
Pgm				
A	0.400	0.586	0.887	0.937
B	0.537	0.363	0.113	0.063
C	0.062	0.038		
D		0.013		
H_e	0.547	0.522	0.200	0.117
H_o	0.675	0.500	0.175	0.125
F_{is}	−0.222	0.055	0.136	−0.054
P_{exact}	0.011*	0.192	0.896	1.000

TABLE 2.

Continued

	Thau (n = 40)	Ria (n = 40)	SC92 (n = 40)	SC93 (n = 40)
Overall				
H_e	0.400	0.306	0.212	0.192
(SE)	(0.096)	(0.104)	(0.114)	(0.119)
H_o	0.404	0.271	0.214	0.193
(SE)	(0.100)	(0.100)	(0.116)	(0.110)
MNA	3.0	2.4	1.7	1.7
P	7/7	5/7	3/7	3/7
$P_{1\%pop}$	3/8	6/8	3/8	3/8

H_e, expected heterozygosity; H_o, observed heterozygosity; F_{is}, fixation index; P_{exact}, exact P-values (*$P < 0.05$); MNA, mean number of alleles per locus; P, proportion of polymorphic loci; SE, standard error.

Our allozyme data indicate that the Lagoa population from the Azores is genetically depauperate and only slightly differentiated from populations from the main range and may thus be of low "biological value" (i.e., in terms of biodiversity). Gathering of *T. decussatus* could therefore be allowed to continue. Nevertheless, given the lower genetic diversity of *T. decussatus* in the Lagoa, the low effective population size, and the depauperate intertidal region (Santos et al. 1985, Santos and Martins 1987), exploitation of this species must follow strict collection guidelines (see also Santos 1989), especially given that only 15% of the area is suitable for exploitation (Morton and Tristão da Cunha 1993). Otherwise, a unique component of the Azorean fauna that also serves as a small fishery resource may be lost. In addition, there is much to compare between Ilhéu de Vila Franca on the island of São Miguel in the Azores and the Lagoa de Santo Cristo. The faunistic and scientific value of Ilhéu de Vila Franca is strongly reduced because of tourism. Thus, opening up the Lagoa for tourism could be disastrous for the fauna too. Therefore, in view of the unique origin, geology, fauna, and flora, the place should be declared a "Site of Special Scientific Interest" (Morton and Tristão da Cunha 1993).

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TABLE 3.

Nei's (1978) unbiased genetic distance between the four populations of *T. decussatus* (for population names we refer to the text).

	Thau	Ria	SC92	SC93
Thau				
Ria	0.352			
SC92	0.239	0.129		
SC93	0.230	0.129	0.036	

TABLE 4.

Allozyme variation reported in other studies of *T. decussatus* and *R. philippinarum*.

Species	H_o	H_e	MNA	P	Reference
T. decussatus					
		0.28	2.75	0.83	Worms and Pasteur (1982)
		0.23–0.28	2.18–2.73	0.64–0.73	Jarne et al. (1988)
	0.22	0.26	2.33	0.78	Borsa and Thiriot-Quiévreux (1990)
	0.18–0.24	0.23–0.33	1.54–1.99	0.54–0.66	Passamonti et al. (1997)
	0.19–0.40	0.19–0.40	1.71–3.00	0.43–1.00	This study (all populations)
R. philippinarum					
	0.26	0.26	3.18	0.73	Moraga (1986)
	0.16–0.20	0.18–0.22	2.67–3.44	0.22–0.33	Kijima et al. (1987)
	0.17–0.25	0.20–0.27	2.6–3.6	0.43–0.57	Oniwa et al. (1988)
	0.33	0.34	2.89	0.89	Borsa and Thiriot-Quiévreux (1990)
	0.34–0.37		2.80–3.80	0.80–0.93	Fava et al. (1994)
	0.19–0.22	0.20–0.27	1.57–1.63	0.54–0.75	Passamonti et al. (1997)
	0.27	0.27	3.15–3.35	0.75–0.85	Yokogawa (1998)

H_o, observed heterozygosity; H_e, expected heterozygosity; MNA, mean number of alleles per locus; P, percentage of polymorphic loci.

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