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Genetic study on two Adriatic lagoon populations of the clam *Ruditapes philippinarum*

Etude sur la génétique de deux populations lagunaires adriatiques de l'espèce Ruditapes philippinarum

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ABSTRACT

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Two populations of Ruditapes philippinarum, *reared in different brackish lagoons in the Po river delta (Caleri and Sacca di Scardovari), were studied to: 1) estimate levels of genetic variability, 2) test for differences between lagoons, 3) check for heterozygote deficiency and 4) investigate the relationships between heterozygosity for allozyme loci and morphometric traits. Allele frequencies differed in a large number of loci when samples from different lagoons were compared. Repeated sampling from the same lagoon showed a more homogeneous set of allele frequencies. Heterozygote deficiencies were frequently found (27 of 32 observations, for each polymorphic (0.05 level) and the four collected samples), even if only five of these were significant. The heterozygote deficiency index [(Ho-He) /He] ranged from - 0.077 to - 0.289 and was affected by season in the Caleri lagoon population only. Average heterozygosity was 0.34 - 0.37. Fourteen out of fifteen loci were polymorphic at the 5 % level in at least one sample, providing several possibilities for genetic tags. In order to detect the relationships between genetic variability and phenotype traits, the individuals were divided into two groups: the most homozygotic animals, with zero to two heterozygotic genes, and the most heterozygotic, with three or more heterozygotic loci. Means and variances of the measurements were calcutated for each group: significant differences between the means of the two groups were found for total shell weight and "density" in the Caleri lagoon. In these two cases the variances were significantly higher in the most homozygotic group. Length measurement variances were always higher, although not significantly, in the most homozygotic group.*

RÉSUMÉ

Fava G., E. Fonsatti, L. Meggiato, 1994 - [Etude sur la génétique de deux populations lagunaires adriatiques de l'espèce Ruditapes philippinarum]. Mar. Life, **4** (2) : 23-32.

Deux populations de Ruditapes philippinarum *provenant de différentes lagunes saumâtres, Caleri et Sacca di Scardovari, autour du delta du fleuve Pô, ont été étudiées dans le but de : 1) estimer le niveau de variabilité génétique, 2) vérifier si les populations diffèrent génétiquement entre lagunes, 3) vérifier s'il existe un déficit d'hétérozygotes et, 4) analyser les relations entre hétérozygosité pour les gènes codant des alloenzymes et les caractères morphologiques. Pour de nombreux gènes, les fréquences alléliques trouvées différaient dans les deux lagunes, une plus grande homogénéité existant entre les échantillons successifs provenant de la même lagune. La déficience en hétérozygotes (calculée pour chaque locus dans les quatre échantillons) a été observée pour 27 des 32 observations, bien que cinq seulement soient significatives. L'hétérozygosité moyenne était entre 0,34 et 0,37. La déficience en hétérozygotes a été calculée entre - 0,077 et - 0,289. Dans la lagune de Caleri ce paramètre a montré une variation saisonnière. Quatorze des quinze gènes testés étaient polymorphes*

(au seuil du 5 %), ce qui donne plusieurs possibilités d'obtenir des marqueurs génétiques. Pour établir une relation entre variabilité génétique et caractères morphométriques, les individus ont été répartis en deux groupes, selon le niveau d'hétérozygotie individuelle : 1) animaux avec zéro jusqu'à deux gènes hétérozygotes, et 2) animaux avec trois ou plus de gènes hétérozygotes. Les moyennes et les variances des caractères phénotypiques considérés ont été calculées pour chaque groupe.
Les moyennes pour le poids et la "densité" de la coquille, dans les deux groupes, différaient significativement chez les animaux de la lagune de Caleri, tandis que les variances étaient significativement plus élevées dans le groupe le plus homozygote, en tous les échantillons.

INTRODUCTION

In 1983, the Venetian Regional Fisheries Development Board introduced the Pacific clam *Ruditapes philippinarum* to the Adriatic sea, sowing 200,000 juveniles of 3 mm length in the Venetian lagoon (Pellizzato, 1990 a). The reason for this introduction was the decline in the productivity of the European *Ruditapes decussatus*. The production of the eastern clam was generally higher, and only in some areas was it too low for commercial exploitation (Mattei *et al.*, 1990), while the best performance has been obtained in the Po river delta area. Native clam production, fairly constant over the period considered (1985-1989), came almost entirely from the Venetian lagoon (Pellizzato 1990 a).

Originally introduced as hatchery juveniles, *Ruditapes philippinarum* seems to be able to reproduce in several areas of the northern Adriatic : wild beds have been recorded in the Venetian lagoon (Pellizzato, 1990 b), in the Sacca di Scardovari (Milia, 1990) and in the Sacca di Goro with a possible spread to the beach in front of Rimini (Paesanti, 1990).

Therefore, this clam is of increasing importance, both from the economic and ecological points of view: its uncontrolled spread and rapid growth might deeply affect coastal ecosystems, reducing their productivity because of the depletion of slowly renewable resources.

The fact that *Ruditapes philippinarum* is able to reproduce in Adriatic lagoons provides a good opportunity for studying the evolution of the genetic structure during the process of colonisation, to throw some light on the relationships between the genetic characteristics of a species and its capability as competitor and successful coloniser. Obviously, a comparison with *Ruditapes decussatus,* apparently a poorer and slower growing coloniser, will be of crucial importance.

Moreover, in order to improve the management of natural resources, it is useful to analyse the genetic constitution of the exploited strains. The search for genotypes particularly adapted to reproduction and growth, as found in *Mulinia lateralis* (Kohen *et al.*, 1988) and *Mercenaria mercenaria* (Adamkewicz *et al.*, 1984), and testing for the existence of differences between lagoons might help to increase farming efficiency and prevent an uncontrolled spread to the nearby marine environment.

The present research was undertaken to obtain preliminary indications on the genetic characteristics of the strains reared in two different brackish lagoons in the Po river delta: the Caleri lagoon and the Sacca di Scardovari. The study aimed to: 1) estimate levels of genetic variability, 2) test for differences between lagoons, 3) check for heterozygote deficiencies, an apparently common phenomenon in bivalves, and 4) investigate the relationships between heterozygosity at allozyme loci and morphometric traits.

Morphometric traits and growth have been considered in a few studies made on clams with reference to the effect of ecological variables (Gérard, 1978; Mann, 1979; Maître-Allain, 1983; Goulletquer *et al.*, 1988; Bacher and Goulletquer, 1989). However, in the present investigation the interest is rather focused on the effect of heterozygosity: Lerner (1954) suggested that highly heterozygotic individuals have better developmental homeostasys that leads to a decrease in phenotypic variance and asymmetry, as recently found also in rainbow trout (Leary *et al.*, 1983). A further effect of heterozygosity is enhanced growth, as observed in some bivalve species (Garton *et al.*, 1984; Koehn and Gaffney, 1984).

MATERIAL AND METHOD

Seasonal temperature variations in the Sacca di Scardovari are typical for northern Adriatic lagoons: minimum values, around 5°C, have been recorded in winter, and maxima slightly over 27°C in July-August. A salinity range between 11 and 30 °/oo has been observed (Fava and Volkmann, 1977). In 1991, several observations were made in 5 stations on the Caleri lagoon: temperature changes were normal, from 3°C (January) to 29°C (June), while salinity variations were unpredictable, within the range 21-36 °/oo (Andreoli, personal communication). Both lagoons are, thus, harsh environments for the populations they harbour: salinity variations and environmental stress were proven to affect the genetic constitution of some bivalve molluscs (Koehn *et al.*, 1976; Bulnheim and Gosling, 1988; Scott and Koehn, 1990).

Animals were provided by fish farmers from Caleri and Scardovari: two samples per lagoon, collected in April and September, were tested. Clams were kept in a deep-freezer (-70°C) for a maximum of two months.

Electrophoretic runs were made in Tris-Citric pH 8.0 buffer (Tris 30.25 g/l, Citric acid 11.97 g/l, at 100 V and about 45 mA, for five hours; gels were done with 26 g Sigma Starch in 250 ml buffer (Tris 2.06 g/l, Citric acid 0.483 g/l, pH 8.0). Staining methods were those suggested by Borsa and Thiriot-Quiévreux (1990) and Pasteur *et al.*, 1988. Eight enzyme systems, giving a total of 15 loci, were considered. Loci and alleles nomenclature is that suggested by Allendorf and Utter (1979): the most common allele is labelled 100, the others with a number which represents their mobility relative to that of the most frequent allele.

Shell measurements, shown in Figure 1, are some of those suggested by Jarne *et al.*, 1988 in *Ruditapes decussatus,* to estimate individual size and shape. Height of the right and left valves provided an evaluation of individual asymmetry. All the measurements were made on the right valve. The lengths in mm, were measured with a 0.1 mm precision caliper.

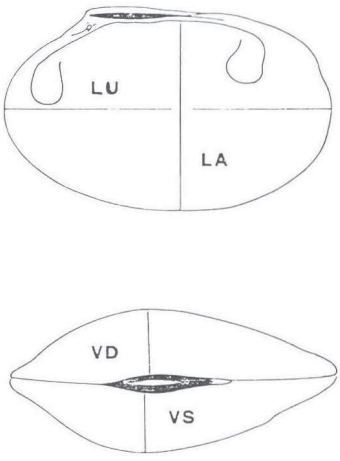

Figure 1 - Morphometric measurements on the shell. Above: internal view, right valve: LU = length, LA = width. Below: upper view, VD = height of the right valve, VS = height of the left valve.
Mesures morphométriques de la coquille. Au-dessus : vue interne, valve droite : LU = longueur, LA = largeur. Au-dessous : vue supérieure : VD = hauteur de la valve droite, VS = hauteur de la valve gauche.

The first and second samples from Scardovari will be referred to as S1 and S2; those from Caleri as C1 and C2.

The significance of differences between frequencies was tested with the G test (Sokal and Rohlf, 1981). For the morphological measurements (which were normally distributed) the means between the two groups, differing by heterozygosity levels, were compared by the t-test. The heterozygote deficiency indexes were compared by the non-parametric Mann-Whitney U test (Sokal and Rohlf, 1981).

The PGI was stained as follows:

4 mg NADP, 20 mg Fructose-6-phosphate, 1 mg PMS, 5 mg MTT, 10U, G6PDH, 40 mg $MgCl_2$ were dissolved in 10 ml Tris-HCl 0.1M pH 8.0.

500 mg agar, in 40 ml Tris-HCl 0.1M pH 8.0, were heated to boiling point: when the suspension cooled to 40°C about the two solutions were mixed and poured over the starch gel slice.

PGI-1 stained strongly and rapidly than PGI-2 thus, assuming the dimeric structure of the enzyme, the interpretation of the zymograms was rather easy.

An intermediated area of light activity was assumed to be due to hybrid molecules between the two loci.

Gels were made with 26 g of Sigma Starch In 250 ml of buffer (gel buffer: Tris 2.06 g/l; Citric acid 0.483 g/l; pH 8.0) and run (running buffer: Tris 30.25 g/l; Citric acid 11.97 g/l; pH 8.0) at 100 V and 45 mA about, for five hours.

Average migratory distances were:

PGI-1						
allele	175	150	125	100	75	50
mm	28	24	20	16	12	8
PGI-2						
allele	110	100	90			
mm	42	38	34			

As stated above, the number which identifies an allele is its relative mobility, that is the ratio between its average mobility and that of the most common allele, multiplied by 100 (thus PGI-1, 150 = (24/16) x 100, and so on).

RESULTS

Expected and observed allelic frequencies and heterozygosities are reported in Table I : according to the 5 % criterion to define a locus as polymorphic, *Mdh-1* was always monomorphic, *Pgi-2* appeared polymorphic in two samples and *Sod-1* in one sample.

A further index of variability was calculated by counting, for each individual, the number of loci in a heterozygotic state from the following eight genes: *Aat-1, Pgm-1, Glo, Idh-1, Idh-2, Lap-1* and *Sod-2*. This restriction was due to the fact that the remaining loci permitted a reliable scoring for a limited number of individuals or samples. The locus *Idh-2* was not considered in C2 as it had a poor resolution. The statistical parameters for each population are summarised in Table II.

In order to verify the degree of heterogeneity between lagoons and samples collected in different seasons, the following variables were compared: 1) allelic frequencies for all loci where more than one allele was found, 2) observed and expected heterozygosity for each gene, so that an estimate of heterozygote deficiencies was obtained, and 3) the number of heterozygotic loci per individual.

The electrophoretic analysis, particularly for

Table I - Allele frequencies for the variable loci. Alleles are indicated with numbers giving their relative mobility. Sam = sample; N = sample size (individuals). He = expected and Ho = observed heterozygosity. n = number of heterozygotic individuals, f = frequency of heterozygotes. / *Fréquences alléliques pour les gènes variables. Les allèles sont indiqués avec des nombres qui donnent la mobilité relative. Sam = échantillon ; N = nombre d'individus. He = hétérozygosité attendue et Ho = observée. n = nombre des individus hétérozygotes, f = fréquences des hétérozygotes.*

			Alleles				He		Ho	
Locus	Sam	N	180	100		20	n	f	n	f
Aat-1	S1	100	0.135	0.855		0.010	25.1	0.251	23	0.230
	C1	87	0.103	0.874		0.023	19.6	0.225	18	0.207
	S2	80	0.144	0.837		0.019	22.2	0.278	22	0.275
	C2	45	0.055	0.856		0.089	11.5	0.256	7	0.156
			180	140	100	60	n	f	n	f
Aat-2	S1	73	0.062	0.164	0.767	0.007	27.8	0.381	27	0.370
	C1	86	0.029	0.134	0.837	—	24,2	0.281	20	0.233
	S2	55	0.109	0.082	0.809	—	18.0	0.327	15	0.273
	C2	33	—	0.045	0.758	0.197	12.7	0.385	6	0.182
			125	100	75	50	n	f	n	f
Pam-1	S1	93	0.054	0.414	0.532	—	50.5	0.543	39	0.419
	C1	88	0.085	0.619	0.296	—	45.9	0.522	38	0.432
	S2	94	0.085	0.564	0.346	0.005	52.2	0.555	44	0.468
	C2	56	0.080	0.581	0.339	—	30.3	0.541	19	0.339
			15	100		85	n	f	n	f
Pam-2	C1	71	0.063	0.894		0.043	13.8	0.195	12	0.169
	S2	59	0.110	0.729		0.161	25.4	0.431	15	0.254
			175	150	125					
Pgi-1	S1	100	0.080	0.100	0.120					
	C1	89	0.067	0.062	0.135					
	S2	98	0.015	0.092	0.128					
	C2	60	0.050	0.083	0.133					
			100	75		50	n	f	n	f
	S1		0.575	0.105		0.020	62.7	0.627	57	0.570
	C1		0.455	0.264		0.017	61.9	0.696	66	0.742
	S2		0.566	0.199		—	60.3	0.615	64	0.653
	C2		0.450	0.200		0.083	43.4	0.724	39	0.650

diagnostic loci *Mdh, Aat, Idh, Lap* and *Sod,* revealed that in the samples from the Caleri lagoon both *Ruditapes philippinarum* and *Ruditapes decussatus* were present. This was particularly the case for C2, where only 61 of 100 individuals belonged to the asiatic species (93 of 100 for C1).

The most representative comparisons were those between samples from the same lagoon collected in different periods (S1 vs S2; C1 vs C2), and those between animals sampled in different lagoons but in the same season. (S1 vs C1; S2 vs C2).

Allelic frequencies

The significance of the G test values are summarised in Table III: allele frequencies differed in an higher number of loci when samples from different lagoons were compared. Repeated sampling from the same lagoon showed a more homogeneous set of allele frequencies.

Observed and expected heterozygosity

The expected and observed values calculated for each variable locus, and all samples, are reported in Table I. Table IV shows, for each locus and the four samples, the heterozygote deficiency index [defined as D = (Ho - He) /He] (Zouros and Foltz, 1984; Volckaert and Zouros, 1989). The G test was used to compare the observed number of heterozygotes to those expected from Hardy-Weinberg equilibrium. A deficiency was frequently found (27 out of 32 observations), even if only five of these were significant. For *Aat-1, Aat-2, Pgm-1, Lap-1,* and

Table I. - Cont

			110	100	90	n	f	n	f
Pgi-2	S1	100	0.015	0.955#	0.030	8.8	0.088	8	0.080
	C1	87	0.057	0.897	0.046	16.5	0.190	8	0.207
	S2	20	0.025	0.870	0.100	4.5	0.224	2	0.100
			135	100	65	n	f	n	f
Glo	S1	89	0.039	0.865	0.096	21.5	0.241	24	0.270
	C1	84	0.203	0.708	0.089	37.8	0.450	37	0.440
	S2	98	0.071	0.796	0.133	33.7	0.344	36	0.367
	C2	61	0.016	0.820	0.164	18.3	0.300	16	0.262
			120	100	80 60	n	f	n	f
Idh-1	S1	89	0.174	0.775	0.039 0.011	32.7	0.368	29	0.326
	C1	92	0.245	0.701	0.054 —	41.0	0.446	41	0.446
	S2	98	0.235	0.724	0.041 —	41.1	0.419	36	0.367
	C2	45	0.233	0.744	0.022 —	17.6	0.392	13	0.289
			112	100	88	n	f	n	f
Idh-2	S1	94	0.016	0.926	0.058	13.1	0.139	9	0.096
	C1	93	0.075	0.860	0.065	23.3	0.251	22	0.237
	S2	78	0.064	0.885	0.051	16.4	0.210	11	0.141
			100	90	80	n	f	n	f
Lap-1	S1	92	0.571	0.418	0.011	45.9	0.499	33	0.359
	C1	86	0.773	0.221	0.006	30.4	0.354	23	0.267
	S2	96	0.766	0.219	0.015	35.0	0.365	27	0.281
	C2	61	0.762	0.238	—	22.1	0.363	11	0.180
			120	110	100	n	f	n	f
Lap-2	C1	67	0.015	0.373	0.612	32.6	0.514	26	0.388
	S2	76	0.125	0.434	0.441	45.7	0.398	42	0.553
			140	100	60	n	f	n	f
Mdh-1	S1	80	0.006	0.962#	0.032	5.6	0.070	6	0.075
	C1	71	0.028	0.972#	—	3.8	0.054	4	0.056
	S2	100	0.005	0.995#	—	1.0	0.010	1	0.010
	C2	61	0.033	0.967#	—	3.9	0.064	4	0.066
			175	100	25	n	f	n	f
Sod-1	C1	52	0.058	0.933	0.009	6.6	0.126	7	0.135
	S2	100	0.035	0.965#	—	6.8	0.068	7	0.070
	C2	56	0.027	0.973#	—	3.0	0.053	3	0.055
			130	100	70	n	f	n	f
Sod-2	S1	80	0.075	0.906	0.019	13.8	0.173	11	0.137
	C1	86	0.070	0.872	0.058	19.9	0.231	18	0.209
	S2	100	0.040	0.915	0.045	15.9	0.159	15	0.150
	C2	55	0.118	0.827	0.055	16.4	0.299	16	0.291

#, not polymorphic at 5 % level

Sod-2 a heterozygote deficiency was always present. The average heterozygote deficiency index found in sample C2 was lower (Mann-Whitney U test, $P < 0.05$) than in C1 (same locality, different season) and in S2 (different localities, same season).

Number of heterozygotic loci per individual

The values reported in Table II were used to test if the populations had the same level of average individual genetic variability. The results of the Student's *t* test were:

Comparison	S1 vs S2	S1 vs C1	C1 vs C2	S2 vs C2
t	0.504	3.809	2.831	0.657
P	n.s.	< 0.001	< 0.01	n.s.

For the reasons given above, the number of genes considered were eight for the first two comparisons and seven for the two last.

Morphological traits

In addition to the traits shown in Figure 1, valve weight (in g) was measured in both samples

Table II - Average number of heterozygotic loci per individual and the relative standard deviation. Values within brackets are calculated without Idh-2./ *Nombre moyen de gènes hétérozygotes par individus et écart type relatif. Les valeurs entre parenthèses ont été calculées sans Idh-2.*

Samp.	Mean	St-Dev.	N
S1	2.37	1.118	68
C1	3.14	1.310	80
	(2.92)	(1.329)	
S2	2.47	1.221	72
	(2.32)	(1.208)	
C2	(2.15)	(1.278)	33

Table III - Allelic frequencies: significance of the G test comparisons. ns = P > 0.05./ *Fréquences alléliques : significativité des comparaisons avec le test G. ns = P> 0,05.*

	Comparison			
Locus	S1 - S2	S1 - C1	C1 - C2	S2 - C2
Aat-1	ns	ns	P < 0.05	P < 0.005
Aat-2	ns	ns	ns	ns
Pgm-1	P < 0.005	P < 0.005	ns	ns
Pgi-1	P < 0.005	P < 0.005	ns	P < 0.005
Pgi-2	ns	P < 0.05	—	—
Glo	ns	P < 0.005	P < 0.005	ns
Idh-1	ns	ns	ns	ns
Idh-2	ns	P < 0.05	—	—
Lap-1	P < 0.005	P < 0.005	ns	ns
Mdh-1 -	P < 0.05	ns	ns	ns
Sod-2	ns	ns	ns	P < 0.05

Table IV - Comparisons between observed and expected numbers of heterozygotes for polymorphic loci (5 % level): G test. ns = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.005. The values give the relative difference D = (Ho - HE) / He./ *Comparaison entre le nombre d'hétérozygotes attendus et observés pour les gènes polymorphes (niveau : 5 %) : test G. ns = P> 0,05 ; * P< 0,05 ; ** P< 0,01 ; *** P < 0,005. Les nombres donnent la différence relative D = (Ho - HE) / He.*

	Sample			
Locus	S1	C1	S2	C2
Aat-1	- 0.084 ns	- 0.080 ns	- 0.011 ns	- 0.391 ns
Aat-2	- 0.029 ns	- 0.171 ns	- 0.165 ns	- 0.527 **
Pgm-1	- 0.228 *	- 0.172 ns	- 0.157 ns	- 0.373 ***
Pgi-1	- 0.091 ns	+ 0.066 ns	+ 0.062 ns	- 0.102 ns
Glo	+ 0.120 ns	- 0.022 ns	+ 0.067 ns	- 0.127 ns
Idh-1	- 0.114 ns	0.000	- 0.124 ns	- 0.263 ns
Lap-1	- 0.281 *	- 0.246 ns	- 0.230 ns	- 0.504 ***
Sod-2	- 0.208 ns	- 0.095 ns	- 0.057 ns	- 0.027 ns
$\bar{x}$	- 0.114	- 0.090	- 0.077	- 0.289
s	0.127	0.103	0.110	0.189

from Caleri and in the second from Scardovari. Total weight (PT) and a value (PSPA) referred to as 'specific gravity' (obtained by dividing PT by the surface (LT x LA) of the rectangle circumscribed to the shell, which might be less size-dependent) are reported. All the measurements are given in Tables 5 and 6. The *t* test values were calculated for S1 vs. C1, and C1 vs. C2, excluding sample S2, as it was composed of more homogeneous individuals with a "commercial size".

Total length (LT), width (LA) and shell height (VD), and the asymmetry (AS) calculated as the absolute difference between right and left height (I VD - VS I), never gave significant values. Weight comparisons were only made for the Calery lagoon. Both PT and PSPA were found to be higher in the spring sample ($t = 2.27$ / $P < 0.05$ and $t = 3.59$ / $P < 0.001$, respectively).

As shown in Table VI, the individuals of the asiatic species were those with the greater average values. PSPA was also higher in *Rudipates philippinarum,* whose shell appears heavier.

Genetic variability and morphological traits

In order to detect the relationships between genetic variability and phenotype traits, the individuals were divided into two groups. The first contained the most homozygotic animals, that is those with zero to two heterozygotic genes; the second, those with three or more heterozygotic loci.

Means and variances of the measurements were calculated for each group (Table V). The second sample from Caleri did not permit this kind of comparison, because only a small number of individuals had good electrophoretic resolution for all the enzymes. S1 and C1 samples included individuals of any size and were representative of the whole population. Thus they made it possible to verify whether the heterozygote deficiency, and the relationship between heterozygosity and metric traits, were age-dependant as reported for other bivalve species.

Significant differences were found for: i) total weight ($P < 0.05$) in C1, where the most homozygotic animals were those with the highest mean; ii) PSPA in C1 ($P < 0.05$) still with the higher average for the most homozygotic group. In these two cases the variances were higher in the most homozygotic group (F test, respectively, $F = 2.05$ and $F = 1.99$; $P < 0.05$).

For the remaining comparisons, the variances of the two groups never differed significantly. However, considering only the homogeneous measures, it can be seen (Table V) that the length (LT, LA, VD) variances were always higher in the most homozygotic group: that is nine outcomes out of nine. If the variance is independant of the degree of heterozygo-

Table V - Relationship between number of heterozygotic loci per individual and metric traits. In groups ≤ 2 and ≥ 3, the animals have two or less, or three and more heterozygotic loci. In the groupe "all", mean and variance of all measured individuals are reported. The t test, to compare the averages of the groups ≤ 2 and ≥ 3, was only significant for PT and PSPA in C1 ($P < 0.05$). / *Relation entre le nombre d'hétérozygotes pour chaque individu et caractères morphométriques. Les animaux dans les groupes ≤ 2 et ≥ 3 ont deux ou moins, ou trois ou plus gènes hétérozygotes. Dans le groupe "all", sont données les moyennes et les variances de tous les individus mesurés. Le test t, pour comparer les moyennes des groupes ≤ 2 et ≥ 3 était significatif, seulement pour PT et PSPA dans l'échantillon C1 (P< 0,05).*

MEASURE		S1			C1			S2		
	GROUP	n	mean	var	n	mean	var	n	mean	var
LT	≤ 2	38	35.53	34.87	23	35.51	50.14	39	36.98	19.93
	≥ 3	30	35.81	23.44	57	33.78	39.93	33	37.54	15.14
	all	99	35.29	30.61	89	33.94	42.33	100	37.44	17.68
LA	≤ 2	38	23.66	14.43	23	24.59	22.66	39	24.16	8.77
	≥ 3	30	23.59	11.89	57	23.35	16.44	33	25.01	7.23
	all	99	23.33	13.52	89	23.42	17.92	100	24.73	8.53
VD	≤ 2	38	8.57	2.74	23	8.85	4.36	39	9.00	1.76
	≥ 3	30	8.59	2.21	57	8.25	2.97	33	9.31	1.20
	all	99	8.50	2.56	89	8.30	3.28	100	9.23	1.60
AS	≤ 2	38	0.133	0.011	20	0.150	0.061	39	0.123	
0.015										
	≥ 3	30	0.132	0.020	53	0.196	0.043	33	0.103	
0.008										
	all	99	0.143	0.164	80	0.177	0.045	100	0.129	
0.015										
PT	≤ 2	—	—	—	23	6.29	12.36	39	6.38	10.01
	≥ 3	—	—	—	57	4.83	6.04	33	6.67	8.11
	all	—	—	—	89	5.07	7.88	100	6.72	9.09
PSPA^≤ 2		—	—	—	23	6.36	3.67	39	6.78	2.21

Table VI - Measurements on 119 individuals for the two clam species in second Caleri sample (C2). Test t : * P < 0.05 ; ** P < 0.001. / *Mesures de 119 individus des deux espèces de palourde dans le deuxième échantillon de Caleri (C2). Test t : * P < 0,05 ; ** P < 0,01.*

	R. philippinarum n = 77		*R. decussatus* n = 42		
Measure	$\bar{x}$	s^2	$\bar{x}$	s^2	P
LT	33.51	17.99	30.44	27.84	***
LA	22.89	10.30	20.24	10.39	***
VD	8.09	1.95	6.93	1.68	***
PT	4.10	7.02	2.62	4.67	**
PSPA^	4.9	1.90	3.8	1.03	***

^ mean x 10E-3 variance x 10E-6

sity, the probability of obtaining the above result is 0.0039 (sign test) (Sokal and Rohlf, 1981).

DISCUSSION

The comparison of the allelic frequencies indicated the existence of some degree of differentiation between lagoon populations, which appeared seasonally variable. In the spring samples, a significant difference was found in six out of eleven genes, and the genetic variability (mean number of heterozygotic loci per individual) was higher in the Caleri lagoon (Table III). In the samples collected at the end of summer, only three out of nine genes had different allelic frequencies and the genetic variability was similar in the two lagoons.

This outcome might reflect the existence of some differences between the juveniles introduced in the two lagoons. However, the two seasonal samples were collected a few months apart, and they can be considered to belong to the same cohort: thus the differences between the comparisons S1-C1 and S2-C2 are probably due to a different effect of seasonal variations on different genotypes. An alternative hypothesis is that a different regime of selection pressures in the two lagoons ends in a genetic diversification between originally identical populations. Further studies are required to determine which hypothesis is true.

A previous comparative study of allele frequencies in three lagoon areas in the Po delta was performed by Mattoccia (1991), who found substantial genetic homogeneity between naturalised populations which differed, however, from the hatchery animals in the Goro lagoon. The most interesting result reported by Mattoccia (1991) was the great differentiation observed between the animals raised in Goro and those grown in the Sabaudia lake.

After considering that: 1) both strains were subsamples from a stock of juveniles sharing, at that time, the same gene pool, and that 2) chemical and physical characteristics of the two lagoons were quite different, Mattoccia suggested that these findings were due to the different selective pressures existing in Sabaudia lake and Goro lagoon (Mattoccia, 1991).

If this is the case, it would appear that there is a possibility of selecting a number of strains that are particularly suitable for each lagoon.

A further aspect which could be relevant for practical purposes is the high level of genetic variability that seems to characterise *Ruditapes philippinarum*: the present populations have an average heterozygosity between 0.34 and 0.37, not too far from the value 0.343 reported by Borsa and Thiriot-Quiévreux (1990), but slightly higher than those found by other workers (0.24 to 0.29, Mattoccia (1991); 0.263, Moraga (1986). The percentage of variable loci was also rather high. This provides a wide reservoir for selection, and good possibilities of finding some efficient genetic tags, both for wild or commercial strains. In any case, the commercially interesting venerid species studied so far all appear rather variable: this was the case for *Ruditapes decussatus* (Worms and Pasteur, 1982 ; Moraga and Lucas, 1983; Borsa *et al.*, 1991) and *Chamelea (Venus) gallina* (Stella and Rodino, 1986).

Comparisons between observed and expected frequencies of heterozygotes revealed a general lack of heterozygotes, in agreement with the findings of Mattoccia (1991) both for *Ruditapes philippinarum* and *Ruditapes decussatus*. The heterozygote deficiency index was strongly affected by season in the Caleri lagoon population, where a high value was found in the autumn sample. Many factors could have been responsible for this phenomenon (see Gaffney *et al.*, 1990 for a review) but specific causes are quite hard to recognize. At the present state of the art, it seeems reasonable to assert that *".. the causes of heterozygotes deficiency, a common phenomenon in populations of marine bivalves, remain obscure"* (Zouros *et al.*, 1988).

Considering the phenotypic traits, it was seen that length measurements were homogeneous between lagoons and seasons. On the other hand, both PT and PSPA weight estimates were affected by seasons: during the summer the shell tended to lighten, probably because metabolism increased with higher water temperatures.

It is worth while to note that the average PSPA was higher in *Ruditapes philippinarum* than in *Ruditapes decussatus*.

No relationship was found between size and multi-locus heterozygosity (Table V). The lack of difference between mean morphometric measurements of the groups with the number of heterozygotic loci ≤ 2, or ≥ 3, suggested that heterozygosity was substantially independent of age. In fact, under the hypothesis of dependence, younger (smaller) and older (larger) individuals are expected to distribute preferentially in one of the two groups, giving rise to different averages.

In contrast, the variance of length measures was always higher in the group with lower heterozygosity. This suggests that, at least in the lagoons

considered, the most heterozygotic individuals were those with the lower phenotypic variability, possibly because they shared an increased buffering capacity against developmental perturbations produced by the wide fluctuations of chemical and phycical variables of these environments, according to Lerner's hypothesis on genetic homeostasis (1954).

Valve weight also appeared to be related to multi-loci heterozygosity: the most homozygotic animals were in the group with the highest mean and variance. However, this relationship was only significant in the Caleri population (C1). Thus, this population also differed from that of Scardovari in this respect.

Heterogeneous results between populations were also found by Mattoccia (1991).

The existence of different relationship models between genetic variability and phenotype in molluscs is a documented, although not yet understood, phenomenon. Wilkins, for example, in a single gene *(Pgi)* study found that the larger individuals of *Pecten maximus* were less heterozygotic (1978). In: *Mercenaria mercenaria,* two loci *(Lap* and *Pgm-3)* affect shell length, but a specific effect of heterozygosity *per se* (Adamkewicz *et al.,* 1984; Slattery *et al.,* 1991) was not present. Volckaert and Zouros (1989) were unable to detect any correlation between multi-locus heterozygosity and growth in *Placopecten magellanicus.* Absence of association between multi-loci heterozygosity and several phenotypic means and variances have been reported for the terrestrial gastropod *Cerion bendalli* (Booth *et al.,* 1990).

Moreover, in *Mytilus edulis* the relationship between size and genetic variability can be positive, negative or not significantly different from zero depending on the samples, and Gaffney (1990) explained these findings, by suggesting that selection acted on factors which are only temporarily associated with allozymic variability.

With reference to the species in the genus *Ruditapes,* a positive relation between heterozygosity, body size and survival was found in a population of *Ruditapes decussatus* that survived natural anoxic stress (Borsa *et al.,* 1992). Borsa *et al.,* 1991 reported that in *Ruditapes decussatus* the heterozygotic deficiency was, on average, lower in juveniles (the smaller individuals) than in adults. However in different populations of the same species, Jarne *et al.,* 1988 observed no association between asymmetry and heterozygosity and an increased variance for morphological traits in the classes with low heterozygosity.

As stated above, this also appears to be the case for some of the *Ruditapes philippinarum* populations in the Po river lagoons.

The present preliminary results can be summarised as follows: 1) The populations introduced in the Adriatic Sea retain the levels of genetic variability of the species, providing a wide reservoir for selection and good possibilities of finding some efficient genetic tags for wild and commercial strains. 2) Strains from the two lagoons show some degree of genetic differentiation. 3) A generalised deficit of heterozygotes was present. 4) Individual heterozygosity and phenotypic varability appears to be negatively related.

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