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GENETIC VARIATION IN VENEZUELAN MOLLUSCS. THE MUSSEL, PERNA PERNA (L.)

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RESUMEN: Por electroforesis en geles de almidón y poliacrilamida se estudió la variación genética en cuatro poblaciones del mejillón *Perna perna* (L), de las costas del nororiente de Venezuela. Se examinaron 23 sistemas proteicos que representan 32 loci génicos, de los cuales solamente 4 (12,50 %) resultaron polimórficos (LAP I, ES I, PGM y PGI). El número de alelos por locus varió entre 1 y 3 con un promedio de 1,56. Cada individuo fue heterocigoto en un 5,2 % de sus loci y el número de alelos efectivos promedio fue igual a 1,06. Se encontró una marcada similaridad genética entre las poblaciones estudiadas. Se analizó también los cambios en las frecuencias génicas de los loci polimórficos en el curso de seis años,.

ABSTRACT: Genetic variation in four populations of the mussel *Perna perna (L)*, from the shores of northeast Venezuela was studied using starch gel and polyacrylamide gelelectrophoresis. Twenty five protein systems were examined representing 23 loci, of which only 4 (12.50 %) showed polymorphism (LAP, ES, PGM, Y PGI). The number of alletes per locus varied from 1 to 3, with a mean 1.16. Each individual was heterozygous at 5.2 % of its loci and the number of effective alletes was 1.06. The populations analysed were closely similar. Changes in gene frequencies in the four polymorphic loci were also studied in the six year period of the study .

INTRODUCTION

Electrophoresis coupled with histochemical stains has been widely employed to determine the genetic variation of marine invertebrate populations (see reviews by NEVO, 1978; VALENTINE & AYALA, 1978; BURTON, 1983). However these techniques have been rarely applied to tropical molluscs and the knowledge of genetic variation in these organisms is scarce.

As one the of the initial stages of a project designed to improve the genetic condition of commercially important molluscs, we are determining the extent of genetic variation in populations of several species of tropical molluscs: *Arca zebra*, (PÉREZ, 1987). The mangrove oyster *Crassostrea rhizophorae*, and the American oyster *C. virginica*, (GUTIERREZ *et al.*, 1989), the scallops *Lyropecten nodosa* and *Pecten ziczac* (CORONADO *et al.* 1991). The present paper reports the genetic variation found in *Perna perna*, and how this variation changed through a six year period.

MATERIALS AND METHODS

Specimens of the mussel *Perna perna* were collected in Rio Caribe, Laguna Grande, Guatapanare and Turpialito, in northeast of Venezuela (Fig. 1) in 1982 and 1988 and transported live to the laboratory where the hepatopancreas and the adductor muscle were extracted, macerated and centrifuged. The supernatant was analyzed electroporeti-

cally using polyacrylamide gels (DAVIS, 1964) and starch gels (SMITHIES, 1955) in a Tris-Citrate-Borate system as described by PÉREZ (1974). For enzyme staining, the procedure described by SHAW & PRASSAD (1970) and HARRIS & HOPKINSON (1977) were followed.

Twenty-five enzymes were studied: acid phosphatase

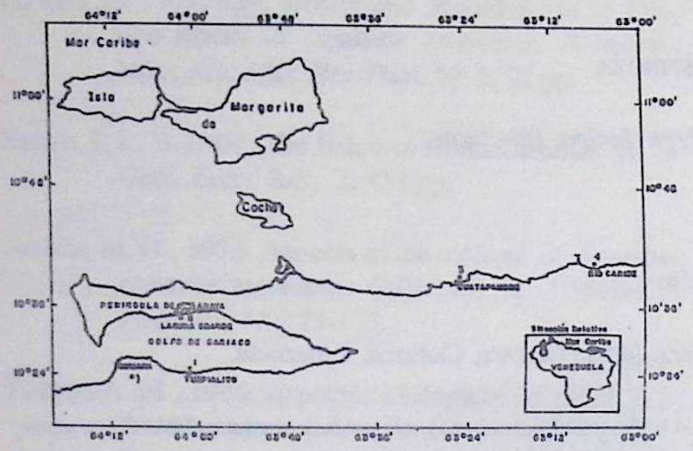


Fig. 1. Sampling localities.

(ACP), alkaline phosphatase (ALP), peptidases S, B, and D, glutamate pyruvate transaminase (GTP), leucine aminopeptidase (LAP), esterase (ES), xanthine oxidase (OX), tetrazolium oxidase (OT), malic enzyme (ME), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), hexokinase (HK), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), catalase (CAT), malate dehydrogenase (MDH), octopine dehydrogenase (ODH), glucosa 6 phosphate dehydrogenase (G-6PDH), pyruvate kinase (PK), arginine kinase (ARGK), glutamate oxaloacetate transaminase (GOT). A lettering system was use to differentiate between alleles, with the less anodal allele always being designates as "a". Where enzymes were coded by more than one locus, loci were numbered according to increasing mobility, with "I" representing the most anodal.

Deviations from expected values were tested by Chi-square analysis and the deficiency or excess of heterozygotes was analyzed by the D statistic (SELANDER, 1970). In this study, genetic variation is described by three indices, namely the proportion of polymorphic loci, calculated from loci that clearly exhibited polymorphism, divided by the total number of clearly resolved loci. The observed heterozygosity at a locus (Ho) was taken as the observed proportion of individual heterozygotes at that locus. The effective number of alletes at the locus (Ne), (which was the reciprocal of the sum of squares of the allele frequencies at a locus), was used as a measure of genetic variability of alleles, because it takes into account the reduced contribution to variability of the rare alleles (BEAUMONT & BEVERIDGE, 1984). Genetic similarity was calculated according to NEI (1972).

RESULTS AND DISCUSSION

Twenty five protein systems were studied, apparently determined by 32 loci: ACP, ALP, ME, LDH, HK, IDH, CAT, MDH, ODH, XO, G6PDH, PK, GOT. PEP-D, PEP-SI, PEP-SII, PEP-BI, PEP-BII, GPT-I, GPT-II, SOD-I, SOD-II, ARGK-I, ARGK-II, ARGK-III, ARGK-IV, LAP-II, and ES-II were monomorphic. On the other hand, PGM, PGI, LAP I and ES-I were polymorphic.

Results for PGM and PGI loci are given in Table 1, for samples collected in 1988, while Table 2 shows the results for LAP I and ES-I for samples collected in 1982 and 1988.

The proportion of polymorphic loci was 12.50 %; the mean number of alleles was 1.16, while the mean effective number of alleles per locus(Ne) was 1.06 and the observed mean heterozygosity per locus (Ho) varied between 0.048 (Turpialito) to 0.057 (Guatapanare). Genetic variability was low in the mussell *Perna perna,* compared with the blue mussell *Mytilus edulis*, where KOEHN *et al* (1976) studying 22 loci. found 0.130 for Ho and 31,0 % of polymorphism in a population collected in the Atlantic coast of USA. AHMED *et al* (1977) studying 34 loci in European populations found 0.095 for Ho, 38 % of polymorphisms and 2.38 alleles per loci. - Low genetic variability found in *P. perna* and other tropical molluscs, two species of scallops (CORONADO *et al,* 1990), two species of oysters (GUTIERREZ *et al.* 1989) and in the arcid *Arca zebra* (PÉREZ, 1987) seems to contradict the VALENTINE & AYALA (1976) hypothesis that genetic variation should be high in trophically stable environments such as exist in the tropics. Our results seem to be explained best by the hypothesis that relate high genetic variation with changes in the environments (LEVINS, 1968), and suggested that genetic variation could be and adaptive strategy to increase adaptability in organisms living in heterogeneous environments, then we would expect low variability in more stable environments such as the tropics. MITTON & LEWIS (1989)

have recently found supports for this hypothesis in bony fishes.

In relation to the polymorphisms, PGM presented three phenotypes, the heterozygotes showing two bands as all monomeric enzymes, (the same situation was observed for LAP-I and ES-I) and in general a slight excess of heterozygotes was observed in all populations with the exception of the population of Guatapanare that showed a rather high proportion of heterozygotes being also the only population that was not in HARDY WEINBERG equilibrium for this locus. PGM have been repeatedely reported as polymorphic in several populations of *M. edulis* (AHMED *et al.* 1977; BEAUMONT & BEVERIDGE, 1983; GOSLING & WILKINS, 1981).

PGI on the other hand showed three bands in the heterozygote, indicating a dimeric constitution. PGI was also studied in samples collected in 1982, but on that occassion the enzyme appeared to be monomorphic, a second allele has now been detected in all populations but is being expressed only in the heterozygotes, due to the very low frecuency of this new allele (Table 1). Gene flow needs to be very high between the populations studied, to explain how the new allele came to be present in all of them in similar frequencies. In fact values of genetic similarity ranged from 0.955 (the lowest value) between Guatapanare and Turpialito and 0.993 (the highest value) between Laguna Grande and Rio Caribe.

PATARNELLO *et al.* have found in the amphipod *Gammarus insensibilis* that heterozygotes exhibited higher survival at 27 °C that homozygotes and also higher activity at three different temperatures, showing that probably PGI locus is subject to selection and the heterozygous genotypes possess superior fitness. In *Mytilus edulis* it has been also found that temperature plays an important role as a selective agent (HALL, 1985). Thus, it may be that natural selection is acting at the PGI locus in *P. perna*, and favoring the rare allele.

In LAP I three alleles were present in both 1982 and 1988 samples, but as it can be observed in Table 2, there were several changes in the frequencies of the alleles. In all populations (in 1982 and 1988) the frequency of the fast moving allele was very low, but is increasing in frecuency, especially in Laguna Grande, where allele LAP-I-a lowered their frecuency; increasing LAP-I-b has also happened in Turpialito and Río Caribe; and only in Guatapanare has the opposite situation ocuurred.

Table 1.-Polymorphic loci in samples of *Perna perna*. D= observed deviation among heterozygotes, P= populations (T = Turpialito, LG = Laguna Grande, G = Guatapanare, RC = Río Caribe, C = Cubagua). All samples were of 52 specimens, collected in 1988.

Loci	P	Allele Frequencies a	b	Frecuency of heterozygotes obs.	exp.	D	X^2
PMG	T	0.644	0.356	0.519	0.459	+0.13	0.94
	LG	0.548	0.452	0.558	0.495	+0.13	0.80
	G	0.615	0.385	0.692	0.474	+0.46	11.15
	RC	0.462	0.538	0.577	0.497	+0.16	1.37
PGI	T	0.952	0.048	0.096	0.091	+0.05	0.10
	LG	0.933	0.067	0.135	0.125	+0.08	0.24
	G	0.942	0.058	0.115	0.109	+0.06	0.22
	RC	0.942	0.058	0.077	0.109	-0.29	3.73

It has been shown that this enzyme plays an important role in the osmotic regulation in molluscs, and that the polymorphism found in *M. edulis* is salinity dependant (see review of KOEHN & HILBISH, 1987). A rather similar situation has been found in the oyster *Crassotrea rhizophorae* colleted in Margarita Island (NIRCHIO *et al.* 1987, 1991). So it seems possible that the LAP I polymorphism in *P. perna* could also be salinity de–pendant.

With the exception of samples colleted in 1988 in Laguna Grande and Guatapanare (the two populations where the changes in frequencies were higher) the other samples were in HARDY-WEINBERG equilibria (Table 2).

The other polymorphic locus was ES I; this enzyme has been found to be polymorphic in several populations of *M. edulis* (AHMED *et al.* 1977, BEAUMONT & BEVERIDGE, 1983;).

In P. perna, ESI was the polymorphic fraction, with three genotypes, being coded by two alleles; whose relative frequency changed over the six years period of this study. In the populations outside the Cariaco Gulf, allele ES-I-a

Tabla 2. Polymorphic loci in samples of *Perna perna*, populations (P) were colleted in 1982 (*) and in 1988. N = Sample size.

Loci	P	N	Allele Frequencies a	b	c	Frecuency of heterozygotes obs.	exp.	D	X^2
ESI	T*	40	0.587	0.413		0.375	0.485	-0.23	2.06
	T	52	0.567	0.433		0.327	0.491	-0.33	5.84
	LG*	40	0.575	0.425		0.300	0.489	-0.39	5.79
	LG	52	0.577	0.423		0.308	0.488	-0.37	7.13
	G*	50	0.310	0.690		0.420	0.427	-0.02	0.02
	G	52	0.625	0.375		0.404	0.469	-0.14	1.01
	RC*	60	0.508	0.492		0.383	0.499	-0.23	3.26
	RC	52	0.538	0.462		0.385	0.497	-0.23	2.62
			a	b	c				
LAP I	T*	40	0.475	0.500	0.025		0.550	0.458	+0.10
	T	52	0.404	0.577	0.019	0.615	0.503	+0.22	6.04
	LG*	40	0.637	0.363	0.000	0.275	0.462	-0.40	6.55
	LG	42	0.488	0.440	0.072	0.333	0.563	-0.41	23.99
	G*	50	0.450	0.530	0.020	0.420	0.516	-0.18	4.38
	G	36	0.611	0.319	0.069	0.306	0.518	-0.41	18.77
	RC*	58	0.466	0.517	0.017	0.448	0.515	-0.13	2.73
	RC	52	0.404	0.529	0.067	0.481	0.552	-0.13	7.43

(the slow moving allele), increased, while in Laguna Grande there was no change, and in Turpialito the frequency of allele ES-I-b increased (Tabla 2). Population from Laguna Grande (samples collected in 1982) and also Turpialito (sample of 1988) were not in Hardy-Weinberg equilibrium.

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