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GENETIC VARIATION IN VENEZUELAN MOLLUSCS. II CRASSOSTREA RHIZOPHORAE AND C. VIRGINICA (OSTREIDAE)

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ABSTRACT: Genetic variation in some populations of the two edible oysters, *Crassostrea rhizophorae* and *C. virginica*, was studied using starch and polyacrylamide gel electrophoresis, coupled with histochemical stains. 19 enzymatic systems were analized, codified apparently by 30 loci in both species. The proportion of polymorphic loci was 16.7% in *C. rhizophorae* and 3.3% in *C. virginica*. The observed mean heterozygosity per locus and the mean effective number of alleles was 0.059 and 1.17 for *C. rhizophorae* and 0.021 and 1.03 for *C. virginica*.

RESUMEN: La variación genética en poblaciones de las ostras *Crassostrea rhizophorae* y *C. virginica*, se estudió por electroforesis en geles de almidón y de poliacrilamida, unido a tinciones histoquímicas. Se analizaron 19 sistemas enzimáticos en las dos especies, codificados aparementemente, por 30 loci. La proporción de loci polimórficos fue de 16,7% en *C. rhizophorae* y 3,3% para *C. virginica*. El promedio de heterocigosidad individual y el número de alelos efectivos por locus fue de 0,059 y 1,17 para *C. rhizophorae* y de 0,021 y 1,03 para *C. virginica*.

INTRODUCTION

As the second paper in this series, (see PEREZ, 1987), we present here the results of our observations on two commercially important oyster species (*Crassostrea virginica* and *C. rhizophorae*), which occur commonly in the coastal waters of eastern Venezuela. Of the two species, *C. virginica* has a very wide latitudinal distribution in the western Atlanctic, being recorded from as far north as the Gulf of St. Lawrence, Canada, to the Gulf of Mexico and the West Indies, while *C. rhizophorae* has a limited distribution range, being recorded so far only in the Caribbean sea and Brasil (ABBOTT, 1974).

MATERIALS AND METHODS

Specimens of *Crassostrea rhizophorae* were collected from La Restinga and Laguna Grande del Obispo, while those of *C. virginica* were collected from Caño Ajíes, Guariquén, in eastern Venezuela (Fig. 1). Animals were transported live to the laboratory, where the hepatopancreas and the adductor muscle were extrated, macerated and centrifuged. The supernatant was analyzed electrophoretically using polyacrylamide gels (DAVIS, 1964) and starch gels in a Tris-Citrate-Borate system as described by PEREZ (1974).

For enzyme staining the procedure described by SHAW & PRASSAD (1970) and HARRIS & HOPKINSON (1976) were followed. Twenty-three enzymes were studied: acid phosphatase (ACP), alkaline phosphatase (ALP), superoxide dismutase (SOD), malic enzyme (ME), lactate dehydrogenase (LDH), peptidase-S (PEP-S), peptidase-D (PEP-D), hexokinase (HK), isocitrate dehydrogenase (IDH), glutamate pyruvate transaminase (GPT), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), catalase (CAT), leucylamino peptidase (LAP), malate dehydrogenase (MDH), octopine dehydrogenase (ODH), esterase (ES), xanthine oxidase (XO), glucose-6-phosphate dehydrogenase (GPT), phosp-

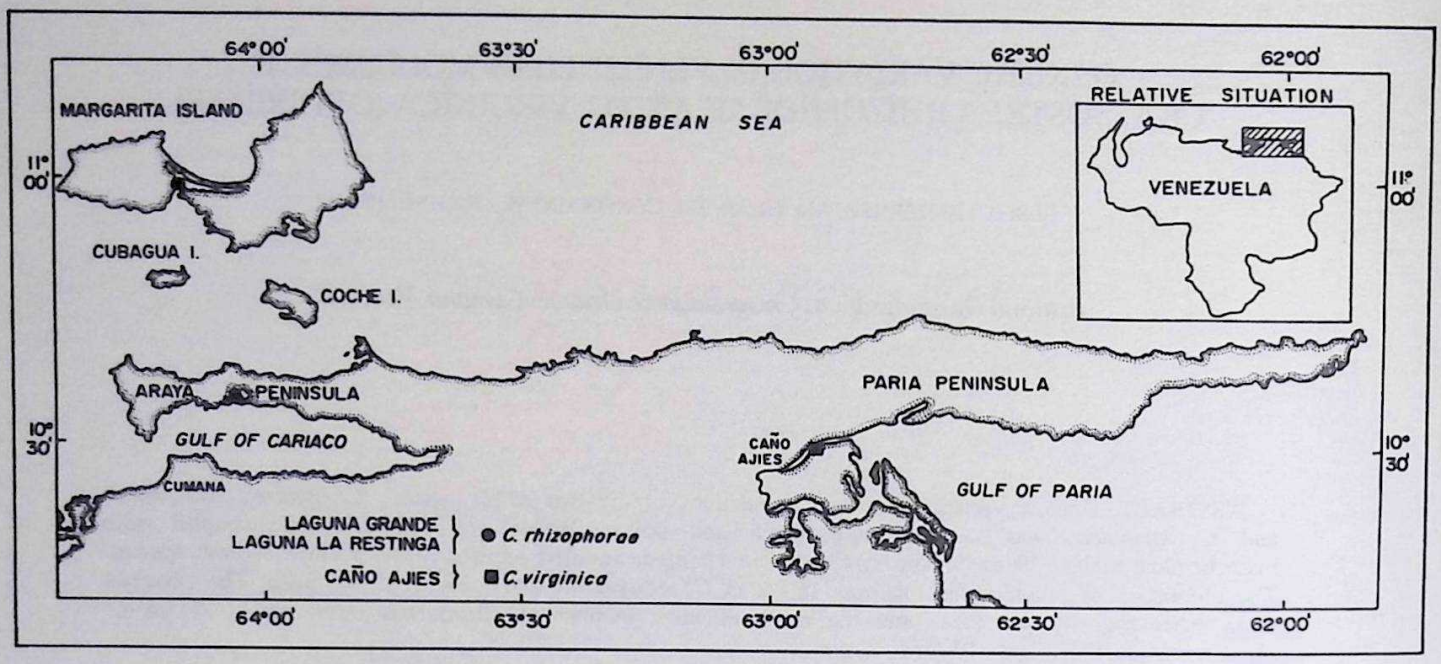


Fig. 1.- Map of northeastern Venezuela, showing the collection sites.

hoglucomutase (PGM), phosphoglucose isomerase (PGI), catalase (CAT), leucylamino peptidase (LAP), malate dehydrogenase (MDH), octopine dehydrogenase (DDH), esterase (ES), zanthine oxidase (XO), glucose-6-phosphate dehydrogenase (G6PDH), glucose dehydrogenase (GDH), pyruvate kinase (PK), arginine kinase (ARK) and glutamate oxalacetate transaminase (GOT).

A lettering was used to differentiate between alleles, with the most anodal allele always being designated as "a". Where enzymes were coded by more than one locus, loci were numbered according to increasing mobility, "I" being the most anodal.

Deviations from expected genotype numbers were tested by chi-square analysis and the deficiency or excess of heterozygotes was analyzed by the D statistics (SELANDER, 1970). In this study, genetic variation is described by there indices. The proportion of polymorphic loci, is calculated from loci that clearly exhibited polymorphisms, divided by the total number of clearly, resolved loci. The observed heterozygosity at a locus (Ho), is the observed proportions of individual heterozygotes at that locus. The effective number alleles at of locus, Ne (the reciprocal of the sum of squares of the allele frecuencies at a locus), is used as a measure of genetic variabilily rather than the actual number of alleles, because it takes into account the reduced contributions to variability of the rare alleles (BEAUMONT & BEVERIDGE, 1984).

RESULTS

Nineteen protein systems were detected in both *Crassostrea rhizophorae* and *C. virginica*, which were apparently determined by 30 loci (LDH, GDH, PK and ODH were not distinguished clearly).

In *C. rhizophorae*, the 52 specimens from La Restinga Lagoon and the 52 specimens from Laguna Grande del Obispo, showed monomorphism in the following enzymatic systems: ARK I, ARK II, ARK III, PEP-S I, PEP-S II, PEP-S III, G6PDH I, PEP-D I, PEP-D II, HK I, HK II, IDH I, IDH II, MDH I, MDH II, OT I, OT II, XO I, XO II, CAT I, EM I, PAC I, GOT I, PGI I. Esterases showed five zones of activity, but only one (ES-V) was clearly resolved, the leucylamino peptidase system presented three activity zones, but one of them LAP I was poorly resolved. GPT I, ES V. LAP II, LAP III were polymorphic in both populations, while PAL I was monomorphic in Laguna Grande del Obispo and polymorphic in La Restinga. However, the situation for PGM I was reverse, i.e. polymorphic for Laguna Grande del Obispo and monomorphic for La Restinga lagoon populations.

TABLE 1. POLYMORPHIC LOCI INDICATING THE NUMBER OF SPECIMENS (N), ALLELE FREQUENCY, EFFECTIVE NUMBER OF ALLELES (Ne), FRECUENCY OF OBSERVED AND EXPECTED HETEROZYGOTES, OBSERVED DEVIATION AMONG HETEROZYGOTES AND VALUES OF X^2 IN SAMPLES OF *C. VIRGINICA* FROM POPULATIONS OF CAÑO AJIES AND *C. RHIZOPHORAE* FROM POPULATIONS OF LA RESTINGA (LR) AND LAGUNA GRANDE DEL OBISPO (LG).

LOCI	N	ALLELIC FREQUENCIES	Ne	OBSERVED	EXPECTED	D	X^2
C. virginica							
LAP II	50	0.470 - 0.530	1.993	0.620 (31)	0.498 (24.9)	0.20	3.2
C. rhizophorae							
GPT (LR)	52	0.317 - 0.683	1.764	0.212 (11)	0.442 (22.6)	- 1.09	13.5
GPT (LG)	52	0.240 - 0.760	1.574	0.135 (7)	0.365 (19.0)	- 1.70	20.7
ESV (LR)	52	0.135 - 0.865	1.305	0.192 (10)	0.231 (11.8)	- 0.20	1.7
ESV (LG)	52	0.231 - 0.769	1.551	0.346 (18)	0.346 (18.4)	0.00	0.0
PGM (LR)	52	0.000 - 1.000	1.000	----------	----------	-------	-----
PGM (LG)	52	0.269 - 0.731	1.648	0.192 (10)	0.346 (20.5)	- 1.05	13.6
PAL (LR)	48	0.594 - 0.406	1.932	0.354 (17)	0.484 (23.2)	- 0.37	3.4
PAL (LG)	48	0.000 - 1.000	1.000	----------	----------	-------	-----
LAP II (LR)	70	0.300 - 0.600 - 0.100	2.174	0.571 (40)	0.543 (37.8)	0.05	0.8
LAP II (LG)	52	0.346 - 0.346 - 0.308	2.991	0.731 (38)	0.673 (34.6)	0.08	4.9
LAP III (LR)	66	0.114 - 0.469 - 0.311- 0.106	2.933	0.394 (26)	0.652 (43.5)	- 0.66	47.4
LAP III (LG)	52	0.144 - 0.635 - 0.221	2.115	0.423 (22)	0.523 (27.2)	- 0.24	6.7

The proportion of polymorphic loci was 16,7% for each population and 1.17 as the effective number (Ne) for the species, being the observed mean heterozygosity per locus (Ho) equal to 0.059. Table 1 shows the observed and expected phenotyps according to Hardy-Weinberg distribution, values of X^2 and D for the polymorphic loci.

In *C. virginica*, the 50 specimens studied showed ARK I, ARK II, ARK III, G6PDH I, PEP-D I, PEP-D II, PEP-D III, PGM I, PGM II, HK I, HK II, IDH I, IDH II, MDH I, MDH II, OT I, ES I, ES II, GPT I, GOT I, PEP-S I, PEP-S II, XO I, CAT I, EM I, PAC I, PAL I, PGI I, LAP II to be monomorphic. The only polymorphic locus was LAP I.

Polymorphism for *C. virginica* is 3.3% and the effective number of alleles is 1.03 and Ho equals 0.021. Table 1 shows the observed and expected phenotype, values of X^2 and D for locus LAP II.

DISCUSSION

BUROKER *et al* (1979) found polymorphisms in a population of *C. rhizophorae* from the Salt River estuary, the U.S. Virgin Islands, 30% of polymorphic loci and 0,100 of individual heterozygosity. However in two populations of *C. virginica*, one from Nova Scotia, Canada and the other from West Florida, USA; these values were shown to be 46.9; 47.1; 0.191, and 0.166 respectively. As shown in the present paper, the genetic variability in the same 2 species but occurring in eastern Venezuela, is lower than the one found by BUROKER *et al* (1979).

Low genetic variability has been attributed in salmonids (ALTUKHOV & SALMENKOVA, 1987) due to their tetraploid origin and the high degree of duplication. A similar explanation was also given by CORONADO *et al* (1991), for the two scallops *Pecten ziczac* and *Lyropecten nodosa*, occurring in eastern Venezuela. From the data provided by BUROKER *et al* (1979), we calculated a ratio of 1.4 loci per protein in *C. rhizophorae*, while *C. virginica* (from Nova Scotia) showed 1.5 and 1.6 (West Florida). Our calculations showed 1.6 loci per protein for *C. rhizophorae* (both populations) and for *C. virginica*.

Gene duplication could be at least one possible explanation for the low genetic variation found in these two species. Gene duplications that occur in the h eterozygote will allow two alleles to segregate as a unit. If the heterozygote is superior to either homozygotes, the duplication should increase in frequency in the population (MCINTYRE, 1976) and this would be the ideal situation for the advantage of the heterozygote: all the members of the population being "heterozygous". Although duplication could increase fitness in the "heterozygous", genetic variation will however be reduced, i.e., one polymorphic locus being converted to two monomorphic loci. However, the ratio of 1.6 for both species, similar to the ratios calculated for the populations studied by BUROKER *et al.* (1979), indicated that the adaptive strategy of gene duplications apparently used by the pectinid species *Pecten ziczac* and *Lyropecten nodosa* (Coronado *et al.* 1991) were not followed in these species of oysters.

One explanation for the low degree of variability in *C. virginica* could be the high degree of inbreading due to small size of the population.

The low genetic variation observed in *C. virginica*, and *C. rhizophorae*, is markedly different with that observed in other benthic invertebrates (see VALENTINE & AYALA, 1978). Also, the hypotesis proposed by Campbell *et al* (1975) to explain the high genetic variability found in trophically stable environments, such as the tropics, is not consistent with the observed low genetic variation in these molluscs. On the other hand, it is well known that the northeastern zone of Venezuela, where the specimens were collected, is an intense zone of upwelling (MARGALEFF, 1965), and in terms of productivity is comparable to temperate region. However the genetic variation in the two species studied, is lower than species from temperate zones.

This hypothesis predicts a negative correlation between the amount of temporal variation in the food resources of a species and the amount of genetic variation. However the result of BUROKER (1980) in several *Crassostrea* and *Saccostrea* species, did not show the expected trend of increasing levels of genetic variation for decreasing latitudinal species range, and provided some support of a corollary of the theory, which states that within a species, the higher latitudinal population should be more genetically variable than those from lower latitudes. The result of BUROKER (1980) and our results, are in agreement with this last hypothesis that predicts less variation, as we approach lower latitudes. BUROKER (1980) collected *C. virginica*, at 45°N. Lat.- 30°N Lat. and *C. rhizophorae* at 18°N. Lat.

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