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Genetic variation and population structure of two species of neo-tropical mud-mussels (*Mytella* spp)

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ABSTRACT. *Mytella guyanensis* Lamarck (1819) and *Mytella charruana* d'Orbigny (1846) are widespread euryhaline bivalves that have become commercially important in Brazil. Despite their importance, however, no genetic information that would be useful to orient governmental policies is available for these species. We analyzed, through allozyme electrophoresis, populations of *M. guyanensis* and *M. charruana* along 3,500 km of Brazilian coast. Pairwise comparisons among gene frequencies in *M. guyanensis* resulted in high levels of pairwise gene identity (I = 0.976 to 0.998). Conversely, significant levels of population structure were found in both *M. guyanensis* (F_{ST} = 0.089) and *M. charruana* (F_{ST} = 0.102). Heterozygosity levels for both species were high (H_e = 0.090 to 0.134 in *M. guyanensis* and H_e = 0.191 to 0.228 in *M. charruana*). The larger population size of *M. charruana* could explain, at least partially, the higher levels of genetic variability for this species. These levels of genetic variability yield an effective population size estimate of about 300,000 for *M. guyanensis*, and 540,000 for *M. charruana*, based on neutralist expectations. Remarkably, these numbers are much smaller than the estimated actual population sizes. This distortion might be ex-

plained by unstable population sizes and it suggests that long-term genetic variability studies are crucial to prevent artifactual viability analysis data for these commercially exploited species.

Key words: Mollusca, Allozymes, Brazil, Fisheries, Mytilidae

INTRODUCTION

The mud-mussel *Mytella guyanensis* Lamarck (1819) is a widespread euryhaline bivalve (Sibaja, 1986) that occurs half-buried in the intertidal zone of mangroves and estuaries from Baja California, Mexico to Santa Catarina, Brazil (Soot-Ryen, 1955; Klappenbach, 1965; Keen, 1971; Boffi, 1979). It is a gonocoric species (Cruz and Villalobos, 1993) with external fertilization and planktotrophic larvae. Because *M. guyanensis* grows very fast after settlement (0.333 mm/day on average for the first 30 days), it has become a very important fisheries resource, accounting for 60% of the whole net production of non-cultivated bivalves on the Brazilian coast (Ostini and Poli, 1990). Notwithstanding its commercial importance and large population size, nothing is known about the genetics or the population structure of *M. guyanensis*.

Mytella charruana d'Orbigny (1846) is also a euryhaline species (Leonel and Silva, 1988) that occurs in shallow lagoons and in mud-flats in bays, reaching large densities (Sibaja, 1985). The species range is known to be from Bahia de Petatlán, Mexico to San Antonio Cape, Argentina (Soot-Ryen, 1955; Keen, 1971). This species is also commercially important, being exploited by bank dwellers in large quantities. Genetic information about the populations of this species is also lacking, and there are no governmental policies dealing with extraction or cultivation for either of these species (Ostini and Poli, 1990).

We analyzed, through allozyme electrophoresis, populations of *M. guyanensis* and *M. charruana* along 3,500 km of the Brazilian coast. Knowledge about population structure is important both for the understanding of the biology of the species and for better management of their stocks (Carvalho and Pitcher, 1995; Thorpe et al., 2000).

MATERIAL AND METHODS

About 20 specimens or more (depending on the availability) of each species were collected from each population. *Mytella guyanensis* was collected manually from the intertidal zone from six localities along 3,500 km of the Brazilian coast and transported in liquid nitrogen to the laboratory (Figure 1). Horizontal gel electrophoresis was performed by standard methods using 12.5% starch gels (Solé-Cava and Thorpe, 1989; Murphy et al., 1990). The tissue used in all the experiments was the posterior adductor muscle. The gels were stained for 10 enzyme systems, being interpreted as the expression of 14 Mendelian loci for *M. guyanensis* and 11 loci for *M. charruana.* The buffers used were a discontinuous lithium hydroxide, pH 8.1 (Selander et al., 1971) and a continuous Tris-EDTA-maleate, pH 7.4 (Murphy et al., 1990).

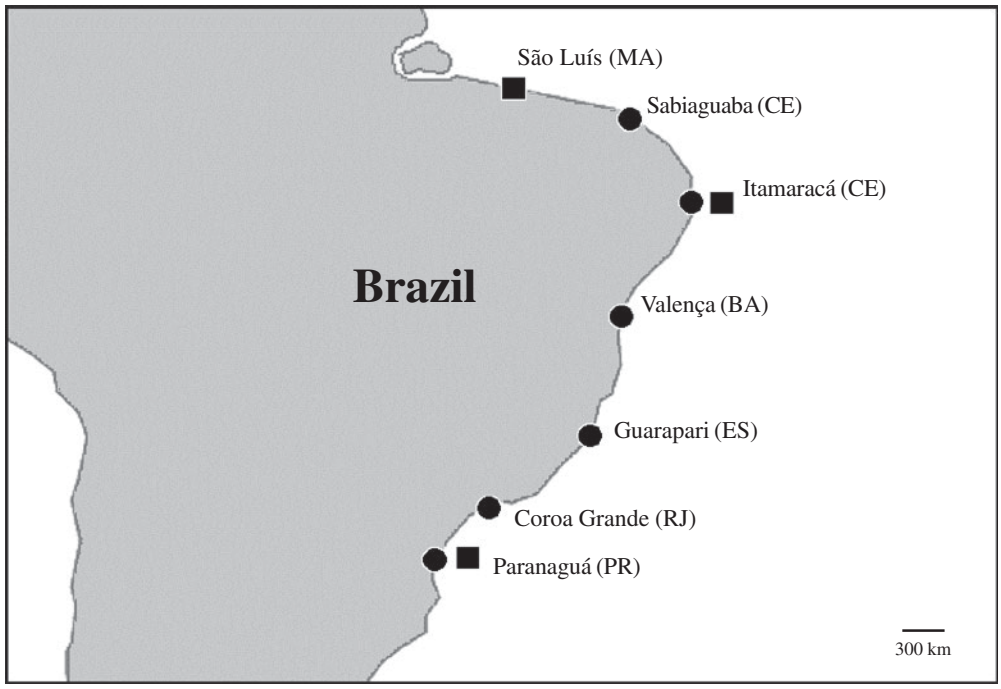

Figure 1. Collection points of *Mytella guyanensis* (circles) and *M. charruana* (squares).

The estimation of the levels of gene variation was accomplished by a direct count of observed heterozygotes (H_o) and by an estimation of the mean Hardy-Weinberg expected number of heterozygotes (H_e) per locus (Nei, 1978). The proportion of polymorphic loci was obtained by direct count. Conformance to Hardy-Weinberg equilibrium (HWE) was tested with Fisher's exact test, with pooling of rare alleles (Swofford and Selander, 1981). The significance of the results of the HWE tests was adjusted by the use of a Bonferroni correction for multiple tests (Lessios, 1992). Population structure was measured using Wright's fixation indexes (Wright, 1950) corrected for sampling size (Nei, 1987). Genetic relatedness between the populations was also estimated through the use of unbiased genetic identities (Nei, 1978) and cluster UPGMA analyses (Sneath and Sokal, 1973). Gene frequencies, observed and expected heterozygosities, departures from HWE, genetic identities and fixation indices were calculated using the BIOSYS 1.7 program (Swofford and Selander, 1981). To test for a possible relationship between geographic distances and gene distances (Nei, 1978) between pairs of *Mytella* populations, we used the Mantel test, with 1,000 replicates (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

The genetic variability in both species was high (H_e = 0.090 to 0.134 in *M. guyanensis* and H_e = 0.191 to 0.228 in *M. charruana*; data not shown). Significant departures from Hardy-Weinberg expectations ($P < 0.05$ after Bonferroni correction) were detected only for the *Pgm-1* locus in the Itamaracá population of *M. guyanensis* and for the *Pgm* locus in the Paranaguá population of *M. charruana*.

Table 1. Unbiased gene identities (Nei, 1978; above the diagonal) and distances (below the diagonal) between populations of *Mytella guyanensis*.

Population	Paranaguá	Coroa Grande	Guarapari	Valença	Itamaracá	Sabiaguaba
Paranaguá - PR	-	0.998	0.976	0.989	0.980	0.995
Coroa Grande - RJ	0.002	-	0.977	0.988	0.980	0.997
Guarapari - ES	0.025	0.023	-	0.983	0.989	0.988
Valença - BA	0.011	0.012	0.017	-	0.989	0.988
Itamaracá - PE	0.020	0.020	0.011	0.011	-	0.983
Sabiaguaba - CE	0.005	0.003	0.012	0.012	0.017	-

Table 2. Unbiased gene identities (Nei, 1978; above the diagonal) and distances (below the diagonal) between populations of *Mytella charruana*.

Population	Paranaguá	Itamaracá	São Luís
Paranaguá - PR	-	0.989	0.968
Itamaracá - PE	0.011	-	0.925
São Luís - MA	0.033	0.078	-

The six populations of *M. guyanensis* had high levels of pairwise gene identity (I = 0.976 to 0.998; Table 1). Nevertheless, their populations were genetically structured (mean F_{ST} = 0.089; H_o = panmixis; $P < 0.05$) in the area studied. Similar levels of differentiation (F_{ST} = 0.102; $P < 0.05$) were found among the three populations of *M. charruana* (Table 2). The differentiation was not correlated with geographic distance in either of the species (Mantel test, $P > 0.30$). This indicates that they do not follow an isolation-by-distance model of population structuring.

Population structuring in both *Mytella* species, along 3,500 km of the Brazilian coast, was moderate to high, indicating that gene flow is limited along their Brazilian range. For benthic species, such as *Mytella* spp, the larval phase is the main source of gene flow between populations (Hedgecock, 1986). Unfortunately, no detailed studies have been performed to determine the parameters of larval dispersal and settlement of *M. guyanensis*. Laboratory experiments with *M. charruana*, however, showed that larvae can remain from 10 to 15 days in the water column before settling (Paranaguá, 1972). This is much less than the reported larval duration of *Mytilus edulis* (six months prior to settlement, due to their capacity to delay growth and metamorphosis (Lane et al., 1985) in the absence of a suitable substrate for fixation). Indeed, levels of population structuring in *M. edulis* and the Brazilian venerid, *Anomalocardia brasiliana* are, likewise, smaller than those of the two mytilids studied here (Silva and Solé-Cava, 1994).

The expected heterozygosity levels for *M. guyanensis* were high (mean H_e = 0.126) and the percentage of polymorphic loci varied from 42.9 to 57.1. In *M. charruana*, heterozygosity levels were even higher (mean H_e = 0.213), and there was a larger proportion of polymorphic loci (from 72.7 to 81.8). According to neutralist predictions, the larger population size of *M. charruana* can explain, at least partially, the higher levels of variability. *Mytella charruana*

forms dense aggregations, so their populations can reach very high densities. For example, in Lepanto Beach, Costa Rica, densities of up to 5,400 *M. charruana* individuals/m^2 were recorded, leading to an impressive estimation of 61 million mussels within a single population (Sibaja, 1985). *Mytella guyanensis* does not form such aggregations. They form clumps of two to seven individuals, half-buried in the mud in mangroves, and their densities reach only 5.2 mussels/m^2 (Nishida and Leonel, 1995).

Neutralist expectations for the relationship between effective population size and heterozygosity are, for neutrally evolving genes, $H = 4N_e\mu / (1 + 4N_e\mu)$, where N_e is the effective population size, and μ is the mutation rate (Kimura and Crow, 1964; Solé-Cava and Thorpe, 1991). The mean mutation rate is estimated at around 10^{-7} for allozymes (Kimura, 1983). This gives estimated effective population sizes of about 300,000 for *M. guyanensis*, and 540,000 for *M. charruana*. These numbers are much smaller than the estimated population sizes, at least for *M. charruana* in Costa Rica. One possible explanation is that population size in that species is unstable. Great rates of mortality have been recorded for *M. charruana* populations, generally after uncommonly rainy seasons (Paranaguá, 1972; Oliveira and Kjerfve, 1993). Recolonization occurs after each episode of massive mortality (Paranaguá, 1972), suggesting that there may be a great capacity of recolonization in this species. The frequent events of population bottlenecks, in a continuous process of expansion and contraction, characterize a metapopulation with a corresponding decrease in effective population size (Hastings and Harrison, 1994). Alternatively, the lower-than-expected levels of gene variation might be due to purifying selection (if the allozymes that are observed are not selectively equivalent), or to large variances in reproductive input at each generation.

In any case, the genetic variability supported by *Mytella* populations is much less than that expected with the population size registered. This is an important result that reinforces the need for long-term genetic variability studies that appear to be crucial in order to avoid artifactual viability analysis data for these commercially exploited species.

The levels of population structure found in the two species also indicate that attempts to extensively cultivate this species (Nanni, 2002) should try to work with seeds from local populations, in order to profit from possible local adaptations.

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REFERENCES

Boffi, A.V. (1979). *Moluscos Brasileiros de Interesse Médico e Econômico*. Hucitec, São Paulo, SP, Brasil.

Carvalho, G.R. and **Pitcher, T.J.** (1995). *Molecular Genetics in Fisheries*. Chapman and Hall, London, England.

Cruz, R.A. and **Villalobos, C.R.** (1993). Shell length at sexual maturity and spawning cycle of *Mytella guyanensis* (Bivalvia: Mytilidae) in Costa Rica. *Rev. Biol. Trop. 41*: 93-96.

Hastings, A. and **Harrison, S.** (1994). Metapopulation dynamics and genetics. *Ann. Rev. Ecol. Syst. 25*: 167-188.

Hedgecock, D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bull. Mar. Sci. 39*: 550-564.

Keen, A.M. (1971). *Sea Shells of Tropical West America. Marine Molluscs from Baja California to Peru.* Stanford University Press, Palo Alto, CA, USA.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge, England.

Kimura, M. and **Crow, J.F.** (1964). The number of alleles that can be maintained in a finite population. *Genetics 49*: 725-738.

Klappenbach, M.A. (1965). Sobre las fechas de las especies de Mytilidae que describiera a d'Orbigny. *Neotropica 36*: 118-120.

Lane, D.J.W., Beaumont, A.R. and **Hunter Jr.** (1985). Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Mar. Biol. 84*: 301-308.

Leonel, R.M.V. and **Silva, I.N.** (1988). Estudo da sobrevivência e da capacidade de isolamento de *Mytella guyanensis* (Mollusca: Bivalvia). em diferentes salinidades. *Rev. Nordestina Biol. 6*: 35-41.

Lessios, H.A. (1992). Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Mar. Biol. 112*: 517-523.

Murphy, R.W., Sites, J.W., Buth, D.G. and **Haufler, C.H.** (1990). Proteins I. Isozyme electrophoresis. In: *Molecular Systematics* (Moritz, C., ed.). Sinauer Associates, Sunderland, MA, USA, pp. 45-126.

Nanni, S. (2002). Cultivo de mexilhão do mangue: pioneirismo na Ilha Comprida. *Regional Agrícola online* 10, March 2002. [http://www.rgt.matrix.com.br/jornaljr/agricola/agricola15.html]. Accessed March 10, 2005.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*: 583-590.

Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY, USA.

Nishida, A.K. and **Leonel, R.M.V.** (1995). Occurrence, population dynamics and habitat characterization of *Mytella guyanensis* (Lamarck, 1819) (Mollusca, Bivalvia) in the Paraiba do Norte River estuary. *Bol. Inst. Oceanogr. São Paulo 43*: 41-49.

Oliveira, A.M. and **Kjerfve, B.** (1993). Environmental responses of a tropical coastal lagoon system to hydrological variability: Mundau-Manguaba, Brazil. *Estuar. Coast. Shelf Sci. 37*: 575-591.

Ostini, S. and **Poli, C.R.** (1990). A situação do cultivo de moluscos no Brasil. In: *Reun. Grupo de Trabajo Tecnico, Ancud* (Hernandez, R.A., ed.). Red de Entidades y Centros de Acuicultura de America Latina, Chiloe, Chile, pp. 137-170.

Paranaguá, M.N. (1972). Distribuição, ecologia e desenvolvimento de *Mytella falcata* (d'Orbigny, 1846) nos estados do Rio de Janeiro e Guanabara. Ph.D. thesis, Universidade de São Paulo, São Paulo, SP, Brasil.

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E. and **Gentry, J.B.** (1971). *Biochemical Polymorphism and Systematics in the Genus Peromyscus*. I. *Variation in the Old-Field Mouse* (*Peromyscus polionotus*). *Studies in Genetics*. University of Texas Publication, Austin, TX, USA, pp. 49-90.

Sibaja, W.G. (1985). Shell parameters of *Mytella strigata* in Pacific Costa-Rica. *Rev. Biol. Trop. 33*: 59-60.

Sibaja, W.G. (1986). Madurez sexual en el mejillón chora *Mytella guyanensis* Lamarck, 1819 (Bivalvia: Mytilidae) del manglar en Jicaral, Puntarenas, Costa Rica. *Rev. Biol. Trop. 34*: 151-155.

Silva, E.P. and **Solé-Cava, A.M.** (1994). Genetic variation and population structure in the tropical marine bivalve *Anomalocardia brasiliana* (Gmelin) (Veneridae). In: *Genetics and Evolution of Aquatic Organisms* (Beaumont, A.R., ed.). Chapman & Hall, London, England, pp. 159-168.

Sneath, P.H.A. and **Sokal, R.R.** (1973). *Numerical Taxonomy*. W.H. Freeman and Co., San Francisco, CA, USA.

Sokal, R.R. and **Rohlf, F.J.** (1995). *Biometry: The Principles and Practice of Statistics in Biological Research.* W.H. Freeman and Co., San Francisco, CA, USA.

Solé-Cava, A.M. and **Thorpe, J.P.** (1989). Biochemical correlates of genetic variation in marine lower invertebrates. *Biochem. Genet. 27*: 303-312.

Solé-Cava, A.M. and **Thorpe, J.P.** (1991). High levels of genetic variation in natural populations of marine lower invertebrates. *Biol. J. Linn. Soc. 44*: 65-80.

Soot-Ryen, T. (1955). A report on the family Mytilidae. *Allan Hancock Pacific Exped. 20*: 1-175.

Swofford, D.L. and **Selander, R.B.** (1981). BIOSYS-1, a FORTRAN programme for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered. 72*: 281-283.

Thorpe, J.P., Solé-Cava, A.M. and **Watts, P.C.** (2000). Exploited marine invertebrates: genetics and fisheries. *Hydrobiologia 420*: 165-184.

Wright, S. (1950). The genetical structure of populations. *Ann. Eugenics 15*: C323-C354.