Genetics and Molecular Biology, 28, 2, 254-257 (2005)
Copyright by the Brazilian Society of Genetics. Printed in Brazil
www.sbg.org.br

Research Article

Genetic structure of the sea-bob shrimp (*Xiphopenaeus kroyeri* Heller, 1862; Decapoda, Penaeidae) along the Brazilian southeastern coast

Carolina Moreira Voloch and Antonio Mateo Solé-Cava

Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Genética, Laboratório de Biodiversidade Molecular, Rio de Janeiro, RJ, Brazil.

Abstract

The sea-bob shrimp, *Xiphopenaeus kroyeri*, is one of the most important economic marine resources along the entire Brazilian coast. Nevertheless, despite its economic importance, no studies have examined the population genetics of this species. In this paper, we used ten allozyme *loci* to study the pattern of genetic structuring in *X. kroyeri* along the southeastern Brazilian coast. Seven of the ten analyzed *loci* were polymorphic, yielding observed heterozygosity values higher than those reported for other penaeid shrimps. The population from São Paulo was significantly different from the other two populations (Rio de Janeiro and Espírito Santo), which, in turn, seem to form a single panmitic unit. Therefore, our results clearly indicate that conservation policies for this species should consider the São Paulo population as an independent stock from those of Rio de Janeiro and Espírito Santo.

Key words: population genetics, allozyme electrophoresis, fisheries stocks, Crustacea.

Received: December 22, 2004; Accepted: February 2, 2005.

Introduction

Xiphopenaeus kroyeri is a penaeid shrimp that occurs from North Carolina, USA to Rio Grande do Sul, Brazil (Boschi, 1963; Holthuis, 1980; Santos and Ivo, 2000), and is the only species of the genus *Xiphopenaeus* in the Western Atlantic (Pérez-Farfante and Kensley, 1997). In Brazil, this shrimp is one of the most important marine resources along the entire coast, particularly in the southeastern region of the country (IBAMA, 1993; Dias Neto, 1996).

The population genetics of many penaeid shrimps have been well studied, since the knowledge of the genetic structure of fisheries stocks is crucial to shape policies that will ensure sustainable stock viability (reviewed in Benzie, 2000). However, genetic studies of natural populations of Brazilian shrimps have been conducted only for the genera *Farfantepenaeus* and *Litopenaeus* (*e.g.*, Gusmão *et al.,* 2000; Maggioni *et al.,* 2001; Gusmão *et al.*, 2004). In addition, until now, despite its economic importance, the genetic structure of populations of *X. kroyeri* has never been studied.

Allozyme electrophoresis has been widely used to analyse the genetic population structure of many terrestrial and marine invertebrates (*e.g.*, David *et al.,* 2003; Nobrega *et al*., 2004). It has also been the most commonly used molecular marker for the study of penaeid populations (reviewed in Benzie, 2000). In this paper we use allozymes to analyse the population genetic structure of *X. kroyeri* along 700 km of the southeastern coast of Brazil.

Send correspondence to Antonio Mateo Solé-Cava. Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Genética, Laboratório de Biodiversidade Molecular, Avenida Pau Brasil 211, Bloco A, Sala A2-98, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brazil. E-mail: sole@biologia.ufrj.br.

Material and Methods

We collected 130 samples of *Xiphopenaeus kroyeri* between March and August 2001 from three locations on the Brazilian southeastern coast (Figure 1): Nova Almeida, in Espírito Santo (ES; 20°03' S 40°11' W); Cabo Frio, in Rio de Janeiro (RJ; 22°57' S 42°02' W); and Ubatuba, in São Paulo (SP; 23°26' S 45°04' W). Abdominal muscle tissue samples were retrieved in the field and stored immediately in liquid nitrogen until required for allozyme analysis.

Allozyme analyses

Allozyme electrophoreses was performed in 12.5% starch gels using the standard methodology (Murphy *et al*., 1996). Banding patterns were visualized using standard enzyme stain procedures (Manchenko, 1994). The two buffer systems selected after trial runs were TC7 (0.135 M Tris, 0.043 M citrate, pH 7.0; Shaw and Prasad, 1970) and TC8 (0.25 M Tris, 0.06 M citrate, pH 8.0; Ward and Beardmore, 1977). Out of the twenty enzyme systems essayed, we selected eight on the grounds of reproducibility and resolution: Alcohol dehydrogenase (*Adh*; Enzyme commission

number (E.C.): 1.1.1.1); Adenylate kinase (*Ak*; E.C. 2.7.4.3); Isocitrate dehydrogenase (*Idh*; E.C. 1.1.1.42); Lactate dehydrogenase (*Ldh*; E.C. 1.1.1.27); Malate dehydrogenase (*Mdh*; E.C. 1.1.1.37); Peptidase (Leu-Gli-Gli; *Pep-1*; E.C. 3.4.1.1); Peptidase (Pro-Phe; *Pep-2*; E.C. 3.4.1.1); Phosphoglucose isomerase (*Pgi*; E.C. 5.3.1.9). The buffer TC7 was used for *Adh*, *Pgi* and *Idh*, and for the other enzyme systems we used the buffer TC8.

The BIOSYS-2 package (modified from Swofford and Selander, 1981) was used to estimate, from direct count genotype frequencies, the gene frequencies, observed and Hardy-Weinberg expected heterozygosities, unbiased genetic identities and distances (*I* and *D*, respectively: Nei, 1978).

Results and Discussion

The populations of *Xiphopenaeus kroyeri* along the southeastern coast of Brazil seem to be highly structured (F_{st} = 0.223), particularly due to the high differentiation between the population of São Paulo and those of Rio de Janeiro and Espírito Santo. Unbiased genetic distance (*D*) values found between the population from São Paulo and the other two are high ($D_{SP\text{-}RJ}$ = 0.17; $D_{SP\text{-}ES}$ = 0.19), whereas the populations from Rio de Janeiro and Espírito Santo are very similar to each other ($D_{RJ\text{-}ES}$ = 0.00).

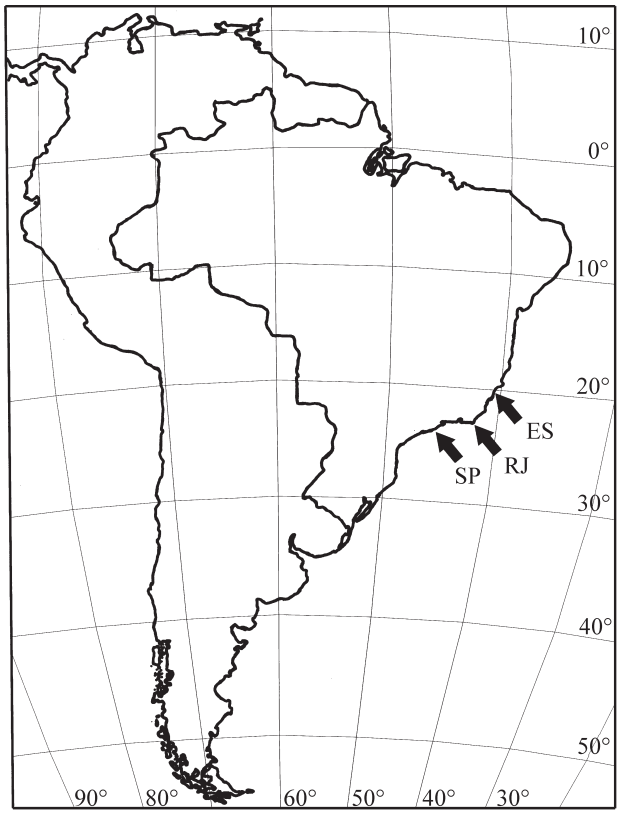

Figure 1 - Collecting sites: ES - Nova Almeida in Espírito Santo (20°03' S 40°11' W); RJ - Cabo Frio in Rio de Janeiro (22°57' S 42°02' W); SP - Ubatuba in São Paulo (23°26' S 45°04' W).

The eight enzymes studied coded for ten allozyme *loci* (Table 1). Two *loci, Pgi* and *Ldh2,* in the population from São Paulo had significant deviations from Hardy-Weinberg expectations (Fisher Exact Test; $p < 0.05$, corrected with Bonferroni adjustment; Lessios, 1992). Even though heterozygote deficiencies are often reported in marine invertebrates (Hare *et al.,* 1996), this phenomenon is not common in shrimp allozyme *loci* (Benzie, 2000). Explaining deviations from Hardy-Weinberg equilibrium is never an easy task, because of the multitude of factors that could result in heterozygote deficiencies. One such factor could be the difficulty in interpreting electrophoresis-banding patterns, due to possible bad resolution of the gels, which might lead to heterozygote individuals being left out of the analyses. However, this does not seem to be the explanation for the observed deficiencies, since, at the

Table 1 - Allele frequencies and sample sizes (N) at seven polymorphic *loci*. The other three *loci*: *Adh*, *Ak* and *Ldh1,* were monomorphic in all populations. H_o and H_e observed and expected heterozygosities respectively.

Locus		Population		
		Nova Almeida (ES)	Cabo Frio (RJ)	Ubatuba (SP)
Pgi	(N)	39	35	36
	A	0.000	0.000	0.708
	B	0.885	0.857	0.292
	C	0.115	0.143	0.000
Mdh1	(N)	50	39	40
	A	0.010	0.013	0.000
	B	0.930	0.974	0.988
	C	0.060	0.013	0.012
Mdh2	(N)	39	30	32
	A	0.462	0.350	0.031
	B	0.192	0.233	0.109
	C	0.346	0.417	0.859
Idh	(N)	45	34	31
	A	1.000	0.985	1.000
	B	0.000	0.015	0.000
Ldh2	(N)	49	34	36
	A	1.000	0.971	0.806
	B	0.000	0.029	0.194
Pep1	(N)	22	13	9
	A	0.000	0.000	0.167
	B	0.864	0.885	0.278
	C	0.136	0.115	0.556
Pep2	(N)	50	40	40
	A	0.960	1.000	0.975
	B	0.040	0.000	0.025
H_o		0.111	0.116	0.085
H_e		0.130	0.126	0.169

Pgi and *Ldh2* loci, almost all individuals were typed. Furthermore, even if the few unscored individuals were assumed to be heterozygotes, the deficiencies would still remain.

The Wahlund effect, which is the artifactual deficiency of heterozygotes caused by the analysis of a mixture of different populations (Wahlund, 1928), could be another factor responsible for the observed phenomenon. The complete absence of heterozygotes at the *Ldh2* locus, in the population from São Paulo, is particularly puzzling, and warrants further investigation.

To better understand the patterns of structuring, we have performed pairwise F_{ST} and heterogeneity analyses between the populations studied (Table 2). The results of the contingency table analysis once again indicate that a barrier to gene flow exists between the São Paulo and Rio de Janeiro populations, whereas no significant differences exist between Rio de Janeiro and Espírito Santo populations.

The sampling area covered in this study is a very small fraction of the entire geographical distribution of the sea-bob shrimp. Nova Almeida (ES) is 380 km north of Cabo Frio (RJ), which is 310 km north of Ubatuba (SP) and hence the observed structuring may not be explained by isolation by the distance model, since Cabo Frio is closer to Ubatuba than to Nova Almeida. Two aspects that might influence the population dynamics of *X. kroyeri* are their distribution exclusively in shallow waters (D'Incao, 1995) and their independence from estuaries to complete their life-cycle (Valentini *et al.,* 1991). This indicates that other environmental factors could be the cause of the observed structuring. For instance, D'Incao (1995) attributes the limits of distribution of many penaeids to temperature, since the vast majority of them occur between the 20 °C isotherms of both hemispheres. In Cabo Frio, an upwelling phenomenon is responsible for lowering water temperature from 23 °C to 12 °C during some times of the year. This temperature variation may be influencing the population dynamics of *X. kroyeri*. The influence of upwelling on distribution boundaries has already been described for other Penaeoidea species, like *Artemesia longinaris* and *Pleoticus muelleri*, which have the northern limits of their distribution in the Cabo Frio region (D'Incao, 1995).

It would be interesting to study sites between Cabo Frio and Ubatuba (*e.g.*: Angra dos Reis-RJ) to better locate the population discontinuity. In any case, an immediate application of this work is that *X. kroyeri* from Ubatuba and Nova Almeida/Cabo Frio must be treated as independent stock units for management purposes.

Table 2 - Contingency table results, and pairwise estimation of F_{ST} between the populations of sea-bob from Ubatuba (SP), Cabo Frio (RJ) and Nova Almeida (ES).

	χ^2	g.l.	p	F_{st}
Total	252.6	22	$< 10^{-40}$	0.223
ES *vs.* RJ	12.2	9	> 0.20	≈ 0
RJ *vs.* SP	139.8	11	$< 10^{-23}$	0.239
ES *vs.* SP	174.4	10	$< 10^{-31}$	0.251

Acknowledgments

We thank Renata Schamma for help in the collection of samples and in electrophoretic work. We are also very thankful to Claudia A.M. Russo for helping with the data analysis, and to Carla Zilberberg for suggestions on an early version of the manuscript. This work is part of the thesis by Carolina Voloch and was supported by grants from CNPq, FAPERJ, PADCT and FUJB.

References

Benzie JAH (2000) Population genetic structure in penaeid prawns. Aquacult Res 31:95-119.

Boschi EE (1963) Los camarones comerciales de la familia Peneidae de la costa Atlántica de América del Sur. Clave para el reconocimiento de las especies y datos bioecológicos. Bol Instit Biol Mar 3:1-39.

David JP, Huber K, Failloux AB, Rey D and Meyran JC (2003) The role of environment in shaping the genetic diversity of the subalpine mosquito, *Aedes rusticus* (Diptera, Culicidae). Mol Ecol 12:1951-1961.

D'Incao F (1995) Taxonomia, padrões distribucionais e ecológicos dos Dendrobranchiata (Crustacea, Decapoda) do Brasil e Atlântico Ocidental. PhD Thesis, Universidade Federal do Paraná, Paraná.

Dias Neto J (1996) Diagnóstico da Pesca Marítima do Brasil. IBAMA, Brasília, 165 pp.

Gusmão J, Lazoski C and Solé-Cava AM (2000) A new species of penaeus (Crustacea, Penaeidae) revealed by allozyme and cytochrome oxidase I analises. Mar Biol 137:435-446.

Gusmão J, Lazoski C and Solé-Cava AM (2004) Population genetic structure in Brazilian shrimp species (*Farfantepenaeus* spp. and *Litopenaeus schmitti* Decapoda, Penaeidae). Gen Mol Biol (in press).

Hare MP, Karl SA and Avise JC (1996) Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. Mol Biol Evol 13:334-345.

Holthuis LB (1980) Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO species catalogue, v. 1, FAO fisheries Synopsis, FAO, Roma, 271 pp.

IBAMA (1993) Camarões do Sudeste e Sul: Relatório da IX reunião do Grupo Permanente de Estudos (GPE) de Camarões, Centro de Pesquisa e Extensão Pesqueira das Regiões Sudeste e Sul (CEPSUL), Itajaí, SC. Coleção Meio Ambiente. Série estudos, pesca; n. 5. IBAMA, Brasília, 63 pp.

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

Maggioni R, Rogers AD, Maclean N and D'Incao F (2001) Molecular phylogeny of western Atlantic *Farfantepenaeus* and *Litopenaeus* shrimp based on mitochondrial 16S partial sequences. Mol Phylog Evol 18:66-73.

Manchenko GP (1994) Handbook of detection of enzymes on electrophoretic gels. CRC Press Inc., Boca Raton, 341 pp.

Murphy RW, Sites JW, Buth DG and Haufler CH (1996) Proteins: Isozyme electrophoresis. In: Hillis DM, Moritz C and Mable BK (eds) Molecular Systematics. 2nd edition. Sinauer Associates, Sunderland, pp 51-120.

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

Nobrega R, Solé-Cava AM and Russo CA (2004) High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic Coast of the Americas. J Exp Mar Biol Ecol 303:173-181.

Pérez-Farfante I and Kensley B (1997) Penaeoid and Sergestoid Shrimps and Prawns of the World. Editions du Muséum National d'Histoire Naturelle, Paris, 233 pp.

Santos MCF and Ivo CTC (2000) Pesca, Biologia e Dinâmica Populacional do Camarão *Xiphopenaeus kroyeri* (Heller, 1862) (Crustacea, Decapoda, Penaeidae) Capturado em Frente ao Município de Caravelas (Bahia-Brasil). Boletim Técnico CEPENE 8:131-164.

Shaw CR and Prasad R (1970) Starch gel electrophoresis of enzymes - A compilation of recipes. Biochem Genet 4:297-320.

Swofford DL and Selander RB (1981) Biosys-1: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72:281-283.

Valentini H, D'Incao F, Rodriquez LF, Neto JER and Domit LG (1991) Análise da pesca do camarão Sete-Barbas (*Xiphopenaeus kroyeri*) nas regiões sudeste e sul do Brasil. Atlântica 13:171-177.

Wahlund S (1928) Zusammensetzung von Populationen und Korelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65-105.

Ward RD and Beardmore JA (1977) Protein variation in the plaice (*Pleuronectes platessa*). Genet Res 30:45-62.

Editor: Fábio de Melo Sene