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PRIMARY RESEARCH PAPER

Genetic structure of populations of the mangrove crab *Ucides cordatus* (Decapoda: Ocypodidae) at local and regional scales

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Abstract The crab *Ucides cordatus* (Decapoda: Ocypodidae) is a species of considerable economic and ecological importance in mangrove areas of the Western Atlantic coast. However, habitat loss, overfishing, and a new infectious disease are causing substantial reductions in local stocks of this species, leading to a pressing need to design efficient management strategies. A crucial step in this design in an understanding of how the genetic variability of *U. cordatus* is distributed among estuaries throughout its range. In this study we assess the degree of spatial structure in the pattern of genetic variation of *U. cordatus* over local (estuaries located within 100 km from each other) and geographical scales (estuaries located farther than 2700 km from each other). Ninety individuals were collected from nine estuaries and analyzed using PCR-RFLP and RAPD techniques. The percentage of polymorphic bands within populations ranged from 15% to 46% for RFLP markers and from 40% to 70% for RAPD markers. Our results failed to demonstrate significant geographical structure in the pattern of genetic variation, indicating that populations of *U. cordatus* are capable of extensive gene flow among estuaries. The implications of these results for the management of *U. cordatus* populations are discussed.

Keywords Phylogeography · RAPD · RFLP · AMOVA · Nested Clade analysis

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Introduction

Mangroves play essential roles in the dynamics of the estuarine systems where they are found, serving not only as nursery and feeding areas for a variety of fish and shellfish species, but also in the enrichment of coastal waters, in the stabilization of the shoreline, and in trapping silt and wastes from upland runoff (Marshall, 1994, Beck et al., 2001, Holguin et al., 2001). Despite the consensus among scientists regarding their importance, mangrove environments are being destroyed at an alarming rate in many parts of the

world, causing increasing concern with respect to the conservation and sustainable management of this ecosystem (Ong, 1995; Farnsworth & Ellison, 1997; Sathirathai & Barbier, 2001; Walters, 2004).

The crab *Ucides cordatus* (Decapoda: Ocypodidae) is considered a keystone species of West Atlantic mangroves, being responsible for the consumption and degradation of more than half of the leaf litter in these environments (Schories et al., 2003). Moreover, this species represents an important fishery resource for local communities along the Brazilian coast. For instance, in a survey of 21 fishermen communities located in the region of the Caeté estuary (State of Pará, Northern Brazil), *U. cordatus* is collected and sold by 42% of the households, and constitutes a main income source for 38% of them (Glaser, 2003). The fishery is usually done with extremely predatory techniques by the poorest communities on the coast of Brazil. Local populations of *U. cordatus* have declined in several regions along the Brazilian coast, not only due to overfishing, but also as a consequence of a newly discovered disease caused by a pathogenic black yeast (the Lethargic Crab Disease, Boeger et al., 2005). It has been reported that this disease caused a reduction of up to 84% in the fishing yield of crab catchers after mass mortality events in the estuary of the Rio Mamanguape, State of Paraíba (Nóbrega & Nishida, 2003). These factors underscore the need for the development of management strategies of natural populations of *U. cordatus* to preserve both this fishery resource and the integrity of the mangroves where they occur.

Adults of *Ucides cordatus* live in burrows in mangrove areas that are often quite distant from each other. These crabs are believed to be show little horizontal displacement and the genetic exchange between neighboring populations is done primarily through their larval forms, which are planktonic. There are two main demographic strategies for benthic estuarine macro-invertebrates that have a dispersive planktonic larval phase (Bilton et al., 2002): (1) retention of larval stages within the estuary; and (2) export of newly hatched stages from the estuary into shelf waters, with subsequent return migration to the same or other estuaries by a late larval or early post-larval stage. Both strategies have important consequences for the distribution of genetic variation, either through the differentiation of populations within and between estuaries or as the homogenization of genetic variation due to extensive gene flow, respectively.

In order to warrant adequate management of natural populations, it is imperative to understand in detail the biological characteristics of the managed species. In particular, it is important to understand the geographical distribution of the genetic variability among its sub-populations. In the present study, we use RAPD and PCR-RFLP markers to provide the first study describing the pattern of genetic variability and gene flow among populations of *U. cordatus* at local and regional scales by sampling individuals from two distant states in the Brazilian coast: Sergipe and Paraná. Our results failed to demonstrate significant geographical structure in the pattern of genetic variation, indicating that populations of *U. cordatus* are capable of extensive gene flow among estuaries, at least within the geographical limits of this study.

Materials and methods

Collection sites

Specimens of *U. cordatus* were collected from nine estuaries in Brazil, three in the State of Paraná (Guaratuba, Paranaguá, and Antonina) and six in the State of Sergipe (São Francisco, Sergipe, Japaratuba, Santa Maria—Vazabarris, Piauí, and Real) (Fig. 1). These states are distant more than 2,700 km from one another, whereas estuaries within each state are usually less than 70 km apart. The coast lines of the states of Paraná and Sergipe are 98 km and 178 km, respectively. Ten individuals were sampled in each estuary.

DNA extraction and amplification

Tissue samples, obtained from one of the pereiopods, were preserved in an EDTA–DMSO solution (Seutin et al., 1991) and maintained in a –20°C freezer, until being processed for DNA

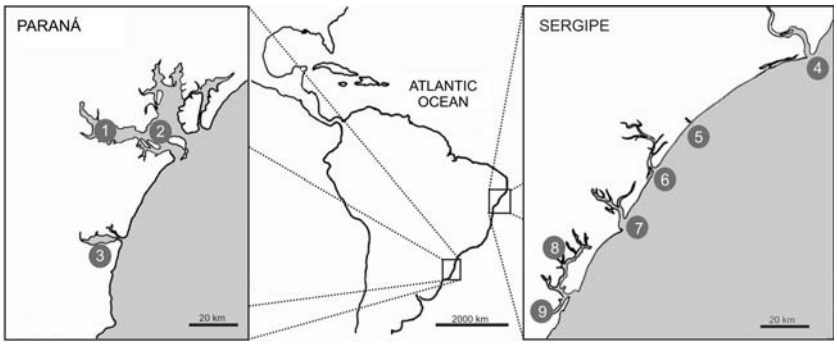

Fig. 1 Location of the estuaries where *Ucides cordatus* was collected. 1—Guaratuba; 2—Paranaguá; 3—Antonina; 4—São Francisco; 5—Japaratuba; 6—Sergipe; 7—Vazabarris; 8—Piauí; and 9—Real

extraction using the DNeasy kit (QIAGEN®). DNA concentration and purity were determined using a GeneQuant Pro spectrophotometer (Amersham Biosciences®). Two types of molecular methods were used to characterize the genetic structure of *U. cordatus*, namely Restriction Fragment Length Polymorphism (PCR-RFLP) of the control region of the mtDNA, and Random Amplification of Polymorphic DNA (RAPD). These methods are described in detail below.

The amplification of the control region of the mtDNA of *U. cordatus* was conducted using primers that were designed specifically for this study based on the flanking 12S rDNA and ILE–tRNA regions of the brachyuran mitochondrial genome (Table 1). The ≈1600 bp fragment was amplified in 25 μl reactions with the following final concentrations: 6 mM of $MgCl_2$, 1 mM of each dNTPs, 0.1 U/μl of Taq polymerase, 1× reaction buffer, 2 μM of each primer, and 1.2 ng/μl of template DNA. Thermocycler settings included an initial denaturation period of 2 min at 95°C, followed by 35 cycles of 20 s at 95°C, 30 s at 56°C, and 90 s at 72°C, and by a final extension period of 5 min at 72°C. Success in PCR amplification was checked by 1.5% agarose electrophoresis followed by ethidium bromide staining. PCR products were then digested using four restriction enzymes: *Mbo*I (BioLabs), *Alu*I (Invitrogen), *Dra*I (Invitrogen), and *Hinf*I (Jena Bioscience). Each 5 μl RFLP reaction included 2 μl of the PCR product, 0.5 μl (5U) of the restriction enzyme, and 2.5 μl of the reaction buffer. The solution was incubated for 4 h at 37°C and the results were visualized using a 2%-agarose-gel electrophoresis followed by staining by ethidium bromide.

Table 1 List of primers used in the present study and their respective sequences

Primer	Sequence
OPA–01	5′-CAGGCCCTTC-3′
OPA–07	5′-GAAACGGGTG-3′
OPA–08	5′-GTGACGTAGG-3′
OPA–09	5′-GGGTAACGCC-3′
OPA–11	5′-CAATCGCCGT-3′
OPA–17	5′-GACCGCTTGT-3′
12SUCAF3	5′-CCAGTANRCCTACTA TGTTACGACTTAT-3′
ILEUCAR3	5′-GCTAYCCTTTTAAAT CAGGCAC-3′

Twenty RAPD primers were initially screened for amplification quality and degree of polymorphism (Series I, Operon Technologies), of which six were selected for the present study: OPA–1, OPA–7, OPA–8, OPA–9, OPA–11, and OPA–17 (Table 1). Each 15 μl reaction had the following final concentrations: 0.25 mM of each dNTP, 1× reaction buffer, 4 mM of $MgCl_2$, 0.1 U/μl of Taq polymerase, 0.4 μM of primer, and 0.4 ng/μl of template DNA. Thermocycler settings included an initial denaturation period of 3 min at 95°C, followed by 35 cycles of 15 s at 94°C, 30 s at 35°C, and 60 s at 72°C, and by a final extension period of 4 min at 72°C. Results were visualized using 2%-agarose electrophoresis followed by ethidium bromide staining. The entire process was repeated at least once for each primer and each individual.

Data analysis

The existence of statistically significant geographical structure in the pattern of genetic variability among populations of *U. cordatus* was assessed using the Analysis of Molecular Variance (AMOVA, Excoffier et al., 1992), as implemented in the program AMOVA (Dyer, in prep). RAPD and RFLP data were analyzed separately. Band sizes were determined by comparison with a 100-bp ladder and recorded in a binary matrix (0/1) of a particular band for each individual. Faint bands were excluded from the datasets prior to the analyses.

We also used a nested clade analysis (NCA, Templeton et al., 1995; Templeton, 1998) to separate patterns of population history from current gene flow. This method incorporates the evolutionary relationships of haplotypes and their current geographic distributions, unlike traditional F_{ST} approaches that are based on current haplotype distributions. Subdivision is detected by comparing the geographic center of all members of a clade to the geographic centers of each haplotype or subclade. An inference key (Templeton et al., 1995) is then used to determine the patterns of subdivision resulting from history or gene flow. We used a minimum spanning tree as implemented in Arlequin (Schneider et al., 2000) to construct the haplotype network and GeoDis 2.0 (Posada et al., 2000) to perform the NCA. Nested clades were determined following the guidelines of Templeton & Sing (1993) and Templeton et al. (1995). Haplotype nesting is determined by the number of mutational steps and position (tip or interior) in the haplotype network.

Results

There was considerable genetic variation in the sampled populations of *U. cordatus* (Fig. 2). A total of 21 PCR-RFLP haplotypes were identified across all estuaries (Table 2). All of the used restriction enzymes resulted in the digestion of the control region mtDNA fragment, with *Dra*I generating five distinct digestion profiles, followed by *Alu*I (three profiles), *Hinf*I (four profiles), and *Mbo*I (two profiles). Thirteen of the 21 haplotypes were found only once (in single individuals), whereas the most common haplotype was present in 41% of the individuals. Only 3.3% of the haplotypes were not shared among estuaries from Paraná and Sergipe.

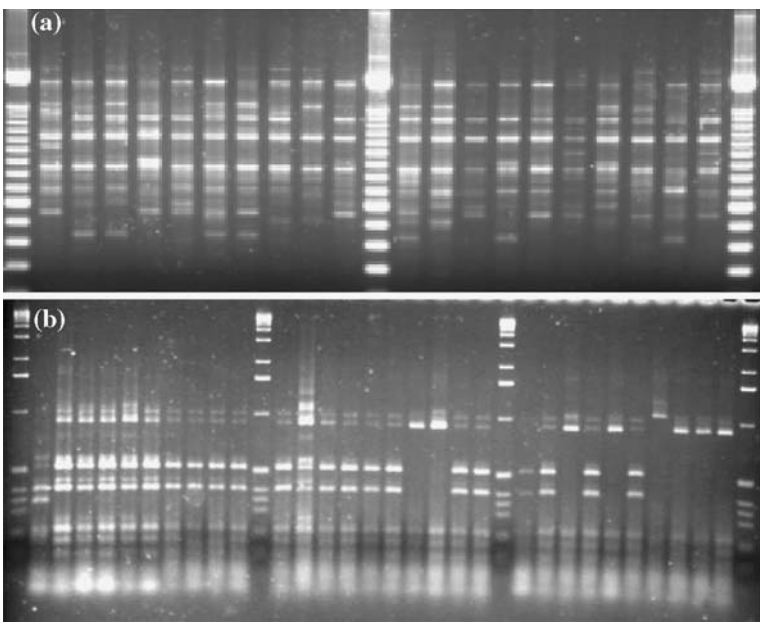

Fig. 2 Representative RAPD (**a**) and PCR–RFLP (**b**) gels used in the present study

Table 2 PCR-RFLP haplotypes (H) identified in the present study and their relative frequency in the different estuaries of the States of Paraná and Sergipe

	Sergipe						Paraná		
	SF	JA	SE	VB	PI	RE	P	G	A
H1	1	0	0	0	0	0	0	0	0
H2	1	0	0	0	0	0	0	0	0
H3	0	0	0	0	0	0	0	0	1
H4	7	4	4	3	5	5	4	3	3
H5	0	0	0	0	1	1	0	0	0
H6	0	0	0	1	0	0	0	0	0
H7	1	3	0	2	2	0	3	0	4
H8	0	0	0	0	0	2	0	0	1
H9	0	0	1	1	0	0	0	2	1
H10	0	0	0	0	0	0	0	1	0
H11	0	0	0	0	0	0	1	0	0
H12	0	0	0	0	0	1	0	0	0
H13	0	0	0	0	0	0	1	0	0
H14	0	0	1	0	0	0	0	0	0
H15	0	0	0	0	0	1	0	0	0
H16	0	2	3	1	2	1	2	3	2
H17	0	0	0	2	0	0	0	1	0
H18	0	0	1	0	0	0	0	0	0
H19	0	0	0	0	0	0	1	0	0
H20	0	0	0	0	0	0	0	1	0
H21	0	1	0	0	0	1	0	1	0
Total	**10**	**10**	**10**	**10**	**10**	**10**	**12**	**12**	**12**

S—Rio Sergipe, J—Rio Japaratuba, R—Rio Real, P—Rio Piauí, SF—Rio São Francisco, V—Rio Vazabarris, G—Baía de Guaratuba, P—Baía de Paranaguá, A—Baía de Antonina

RAPD bands used in the present study ranged between 100 and 1900 bp in size. A total of 40 loci could be safely identified, of which 29 were polymorphic, and none were exclusive to a single estuary. The percentage of polymorphic bands within populations ranged from 15% to 46% in the case of PCR-RFLP and from 40% to 70% in RAPD markers. Nei's unbiased gene diversity *He* (Nei, 1973) was higher for RAPD than for PCR-RFLP, with average within population gene diversities of 0.16 and 0.03, respectively.

Analyses of molecular variance of either dataset did not detect any evidence of geographical structuring in the genetic variability among populations of *U. cordatus*, with Φ_{st} values very close to zero (Table 3). Also, a comparison between pairwise Φ_{st} values between different estuaries and their respective geographical distances failed to demonstrate an effect of isolation by distance (Fig. 3). Finally, although the minimum spanning tree of the RFLP data indicated two main lineages (Fig. 4), the permutation tests of the NCA failed to show any statistically significant association between haplotypes and geography. It is important to note that some of the sampled locations are almost 3,000 km apart, suggesting that there is considerable gene flow among populations from different estuaries. Finally, matrices of the genetic distances among populations based on either marker show modest interpopulation divergences, as one would expect if there is strong gene flow among populations (Table 4).

Table 3 Measures of population differentiation of *Ucides cordatus* based on analyses of molecular variance, both within and between states

	Comparison	Φst	*p*
RAPD	Within Sergipe	–0.00926	0.73
	Within Paraná	0.02597	0.09
	Sergipe vs. Paraná	–0.00036	0.55
RFLP	Within Sergipe	–0.00511	0.61
	Within Paraná	0.03072	0.12
	Sergipe vs Paraná	–0.00077	0.58

Discussion

The results of the present study are consistent with the preponderance of the larval export strategy throughout the evolutionary history of *U. cordatus*, as revealed by the very modest degree of differentiation over a wide geographical area. Indeed,

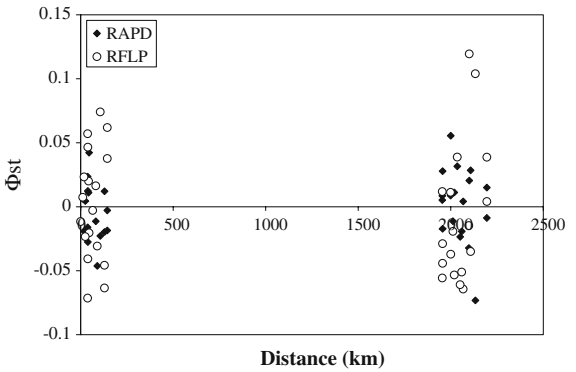

Fig. 3 Correlation between pairwise Φ_{st} values between different estuaries and their respective geographical distances. $R = 0.11$, $p = 0.50$ and $R = -0.08$, $p = 0.65$ for RAPD and RFLP data, respectively

field collections of larvae of *U. cordatus* in the Furo Grande Region, State of Pará, observed synchronized massive releases of larvae that were exported up to 200 km off the coast (Diele, 2000). Nevertheless, the observed pattern is remarkable given the increasing evidence that a variety of physical oceanographic factors, including temperature gradients, oceanic currents and wind patterns, can restrict larval dispersal, leading to population structure in marine species with pelagic larvae (e.g., Reeb & Avise, 1990; McCartney et al., 2000). Moreover, there are decapod species that show significant geographical differentiation along the Brazilian coast over much smaller geographical distances (Weber & Levy, 2000). It is important to note that samples from very large populations tend to underestimate population differentiation, causing our results to be conservative (Waples, 1998). However, a caveat is in order. Although RAPD markers are known to provide unreliable results under certain conditions, the similarity between replicate reactions and the congruence with the PCR-RFLP dataset indicate that the RAPD markers used in the present study are informative. Another limitation of our dataset is the use of only 10 individuals in each location. Future studies using sequence data will be instrumental to corroborate the patterns observed in the present study (Table 5).

The lack of significant genetic differentiation among estuaries has important implications for the management of natural populations of *U. cordatus*. First, the considerable gene flow among populations from different estuaries indicates that local populations are demographically interdependent, with substantial exchange

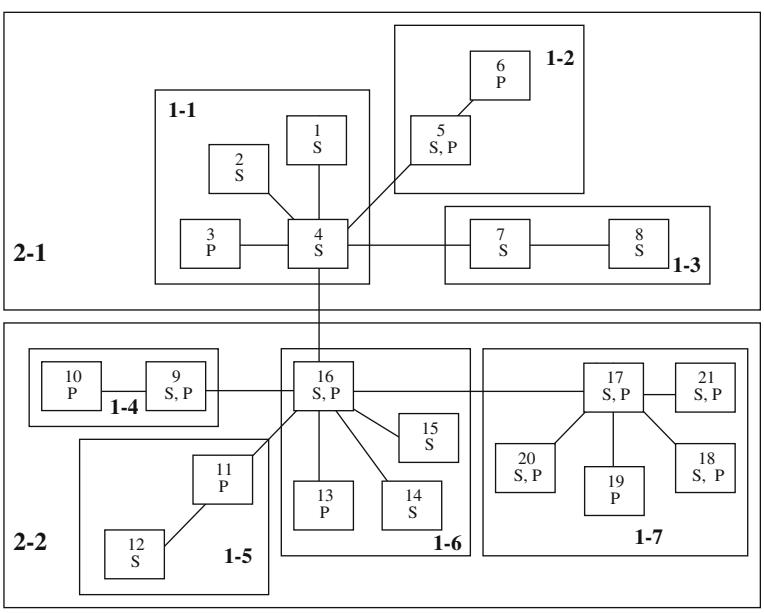

Fig. 4 The nesting design inferred from the cladogram estimation of 21 haplotypes detected for *Ucides cordatus*. Each line in the network represents one mutational change. The number inside each square represents haplotypes. ''P''(State of Paraná) or ''S''(State of Sergipe) indicates the locality where the haplotype was found

Table 4 Nei's genetic distances among populations from different estuaries based on PCR–RFLP (upper diagonal) and RAPD (lower diagonal), as well as the percentage of polymorphic loci and Nei's genetic diversity within each estuary

	VA	PI	RE	SF	JA	SE	P	G	A
VA	–	0.003	0.006	0.015	–	0.003	0.003	0.004	0.001
PI	0.019	–	0.009	0.003	–	0.007	0.001	0.006	0.001
RE	0.027	0.010	–	0.023	0.015	0.002	0.015	0.005	0.005
SF	0.01	0.006	0.001	–	0.005	0.020	0.003	0.016	0.014
JA	0.004	0.007	0.011	0.004	–	0.008	–	0.003	0.002
SE	0.018	0.003	0.015	0.007	0.009	–	0.009	0.001	0.002
P	0.015	0.002	0.012	0.001	0.004	0.004	–	0.005	0.004
G	0.020	0.006	0.014	0.003	0.019	0.005	0.004	–	0.003
A	0.023	0.009	0.016	0.011	0.001	0.010	0.005	0.018	–
% polymorphic RAPD loci	50.0	60.0	60.0	52.5	52.5	52.5	50	52.5	47.5
He (RFLP)	0.12	0.06	0.09	0.03	0.07	0.10	0.07	0.10	0.09
He (RAPD)	0.16	0.17	0.17	0.17	0.16	0.16	0.17	0.16	0.14

VA. Vaza–barris; P. Piauí; RE. Real; SF. São Francisco; JA. Japaratuba; SE. Sergipe; P. Paranaguá; G. Guaratuba; A. Antonina

Table 5 Nested clade distance analysis of mtDNA control region haplotypes observed in *Ucides cordatus*

Haplotypes			1-step clades			2-step clades		
	Dc	*Dn*	Clade	*Dc*	*Dn*	Clade	*Dc*	*Dn*
1	0	605						
2	0	605	1–1	1262	1164			
3	0	1650						
4	1253	1219						
I-T	1253	269						
5	1530	1500	1–2	0	720	2–1	1344	1222
6	0	1173						
I-T	1530	326						
7			1–3	1530	1189			
8			I-T	–26	49			
9	1650	1466						
10	0	880	1–4	1530	1192			
I-T	1650	586						
11	0	2200						
12	0	2200	1–5	2200	1131			
I-T	0	0						
13	0	1344						
14	0	977						
15	0	977	1–6	1494	1281	2–2	1491	1204
16	1514	1419						
I-T	1514	319						
17	1760	1243						
18	1760	1243	1–7	1571	1184			
19	0	1375						
20	0	1375						
21	0	1100						
I-T	880	-19,9	I-T	–135	100			

Brackets reflect the nesting structure. *Dc* and *Dn* are clade and nesting clade distances, respectively. The average difference between interior vs. tip clades for both distance measures is given in the row labeled I-T

of immature forms among adjacent estuaries. This property might facilitate the design of management strategies, such that well-preserved estuaries can work as sources of larvae to disturbed areas and allow for their recolonization. However, despite the strong gene flow among populations, it is still unknown if larger estuaries contribute disproportionately more larvae to the regional larval pool than smaller estuaries. Future studies using more sensitive markers such as microsatellites will be necessary to address this issue.

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