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# Microgeographic Variation in *Caiman latirostris*

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*ABSTRACT* In theory, geographic scale is related to genetic variation at the population level, whereas microgeographic scale may reveal intra-population structure such as social groups and families. In the present work, both levels of genetic variation in the broad-snouted caiman (*Caiman latirostris*) were evaluated in small wetlands associated with the Piracicaba River and some of its tributaries in the state of São Paulo, Brazil. Genetic variation was determined using microsatellite DNA markers originally developed for the American alligator (*Alligator mississipiensis*) and previously tested in pedigreed captive broad-snouted caimans. Using these markers, we were able to detect variability among individuals from different sites, even those within a small geographic distance. Genetic results suggest that the groups sampled at each site are composed predominantly of related individuals. A possible combination of high mortality and low natality rates results in a low number of successfully dispersed individuals per generation. Future studies using a recently constructed *Caiman latirostris* microsatellite library (Zucoloto et al., 2002) might help us to understand metapopulation processes that may be occurring within this species. *J. Exp. Zool. (Mol. Dev. Evol.) 294:387–396, 2002.* 

## INTRODUCTION

The broad-snouted caiman is a medium-sized, endangered crocodilian (Groombridge, '87). Its remaining wild populations are widespread in the state of São Paulo, Brazil, throughout a network of small, more or less disconnected wetlands (Verdade, '98). This ecosystem has suffered considerable anthropogenic pressure due to pollution, drainage for agricultural purposes, and urbanization (Diegues, '90).

Contrary to the large and essentially continuous wetlands that comprise the Brazilian Pantanal, the wetlands of São Paulo are considered as a natural ''fragmented'' landscape, where a palustrine species such as the broad-snouted caiman presents a discontinuous distribution. In such circumstances, if patches are heterogeneous both in terms of area and resources, the balance between immigration and emigration can vary from negative in some patches to positive in others. This has been called a sink-source metapopulation pattern (Pulliam, '88). Patches with positive balance between immigration and emigration tend to export individuals (i.e., as a source) and patches with negative balance tend to import individuals by dispersal (i.e., as a sink). In such circumstances, if the source is lost, the entire metapopulation system can become extinct.

The state of São Paulo is centrally located within the geographical range of the broad-snouted caiman (Groombridge, '87). Local extinction of this species in São Paulo may result in isolation of more northern and southern populations, which could dramatically impact its conservation. However, this process would theoretically involve the network of small wetlands and occur at a local microgeographic level.

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In theory, geographic scale is related to processes at the populational level, whereas microgeographic scale may reveal intra-populational structure such as social groups and families whose identification would require individualization and relationship estimation among individuals (Pimm, '91). Geographic variation involving crocodilians is traditionally investigated by comparing the taxonomy of similar taxa (Mook, '21a; Freiberg and de Carvalho, '65; Iordansky, '73; Medem, '83; Hall, '89; Monteiro et al., '97; Monteiro and Soares, '97). However, some studies also relate to variations at a populational (Mook, '21b; Dodson, '75; Webb and Messel, '78; Montague, '84; Aquino, '94; Hall and Portier, '94), as well as at an individual level (Hall, '91; Monteiro, '97; Verdade, 2000).

General applications of genetic analyses in crocodilian conservation are reviewed by Forstner and Forstner (2002). Specifically analyses within Crocodylia include the analyses of relationships ranging from among genera (Densmore and Owen, '89; Densmore and White,'91) to subspecies complexes (Brazaitis et al., '97) and inter-population variation (Glenn et al., '98; Davis et al., 2001; Dever et al., 2002). Crocodilian isozyme studies have revealed low levels of interpopulational genetic variation, which is generally considered to be a likely historical bottleneck (e.g., O'Brien et al., '85). However, isozymes do not seem to be effective in measuring genetic variability of vertebrates (Avise, '94; Caughley '94).

Individualization analysis and estimation of relationship in crocodilians has been achieved by DNA fingerprinting with a Bkm-derived probe (Lang et al., '93). DNA fingerprinting utilizing multilocus and single locus probes can be used to identify individuals and determine paternity (Jeffreys et al., '85; Jeffrey et. al, '91). Microsatellites have been used to identify individual animals in *Crocodylus moreletii* (Dever et al., 2002). Assessment of genetic relationship (i.e., parentage) among individuals from a population may be an invaluable tool in the study of behavioral-ecological variables such as dispersal and mating system (Burke '89; Burke et al., '89, '91). Comprehension of these two components may be essential in the study of metapopulations (Hanski and Gilpin, '96; McCullough, '96).

In the present study we use microssatelite markers developed for *Alligator missisippiensis* to establish the relationship between microgeographic distance and genetic variation in wild broad-snouted caimans (*Caiman latirostris)*.

## MATERIALS AND METHODS

### *Study sites*

All field sites are located in the eastern-central region of the state of São Paulo, Brazil. Collection localities are all but one associated with Piracicaba River, one of the main northern tributaries of the Tietê River, the main river of the state (Fig. 1). *Volta Grande* (VG) and *Porto de Areia* (PA) are marginal wetlands of the Piracicaba River. *Pantanal* (PT) is a marginal wetland, and *Charqueada* (CH) is an artificial pond. Both are connected to Aracuá Creek, a tributary of the Piracicaba River. Distance between field sites is at most 15 km, with the exception of *Duraflora* (DF), located approximately 150 km from the others. The latter is a group of artificial ponds connected to Pederneiras Creek, a southern tributary of the Tietê River (Fig. 1, Table 1).

### *Capture techniques and blood collection*

Field studies were carried out from October 1995 to May 1996. Capture techniques consisted of approaching animals by boat at night with a spotlight. Juveniles ($<1.0$ m total length) were captured by hand, adapting the method described by Walsh ('87). Noosing, as described by Chabreck ('63), was unsuccessful for adults. Mature caimans were very wary and submerged before the noose was in place. Similar difficulties were described by Webb and Messel ('77) with *Crocodylus porosus* in Australia and by Hutton et al. ('87) with *C. niloticus* in Zimbabwe. Rope traps, adapted from Walsh ('87), were also unsuccessful for both juvenile and adult specimen. Therefore, only young individuals (230 to 2500g of body mass; 21.4 to 47.0 cm SVL) were sampled.

Animals were physically restrained during data collection without the use of tranquilizers. Body measurements were taken with a tape measure (1 mm precision). Body mass was taken with Pesola hanging scales ($300 \times 1$g, $1000 \times 2$g, $5000 \times 5$g, depending on individual body mass). Animals were sexed by manual probing of the cloaca (Chabreck, '63) and/or visual examination of genital morphology (Allstead and Lang, '95) with an appropriately sized speculum.

Blood was collected by puncturing the dorsal branch of the superior cava vein, which runs along the interior of the vertebral column of large reptiles (Olson et al., '75). After collection, blood was stored in lysis buffer: 100mM Tris-HCl,

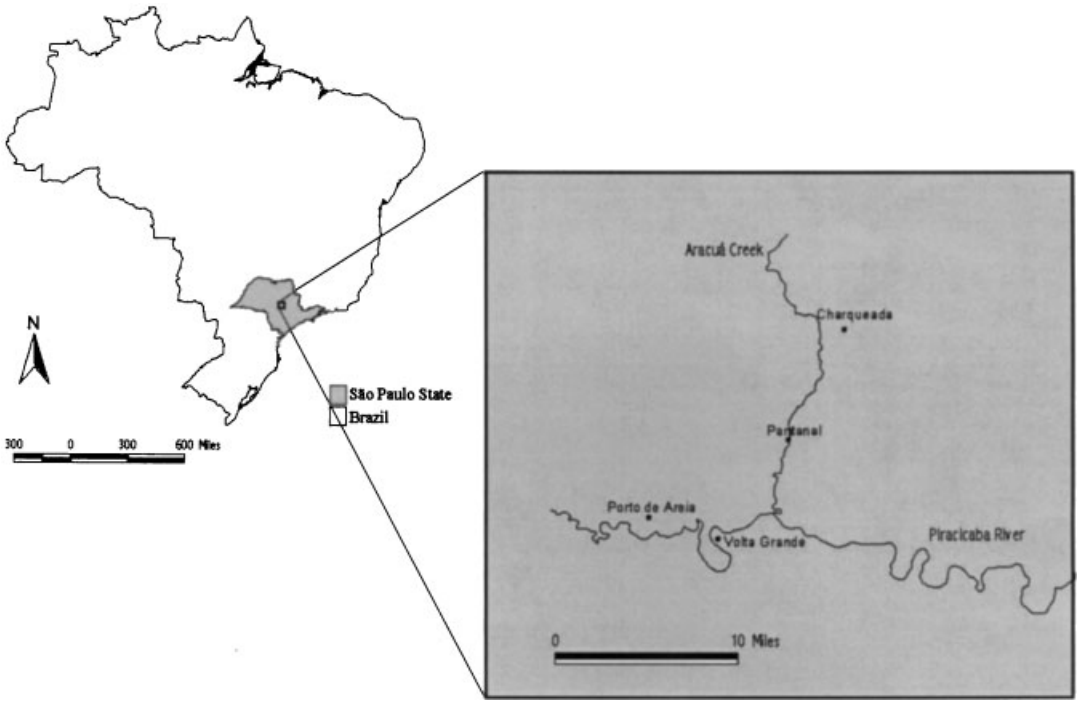


Fig. 1. Location of the study sites in São Paulo (*Duraflora* is westward and out of sight of the green exploded section).

pH 8.0; 100 mM EDTA, pH 8.0; 10 mM NaCl; 0.5% SDS (w/v) as in Hoelzel ('92).

### *Microsatellite analyses*

Blood stored in lysis buffer was digested with proteinase K to a final concentration of 0.5 μg/μl, proteins precipitated with 1.2 M NaCl and total DNA precipitated with ethanol (Hoelzel, '92; Olerup and Zetterquist, '92). Fourteen primer pairs originally developed for *Alligator mississipiensis* by Glenn et al. ('98) were tested in pedigreed captive broad-snouted caimans at the University of São Paulo (Zucoloto, '98). Conditions for an amplification reaction with a final volume of 25 μl, were: 1 × PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 μM of each primer pair, 0.02 U/μl Taq DNA polymerase and 100 ng of DNA. For markers *Amiμ11* and *Amiμ13*, bovine serum albumin (BSA) was added to a final concentration of 0.25 μg/μl. The amplification program was as follows: (1) 94 °C for 3 min, (2) 94 °C for 45 sec, (3) primer pair annealing temperature for 1 min (Table 2), (4) 73 °C for 1 min and 15 sec, (5) repeat steps 2, 3 and 4 n cycles according to Table 2, (6) 4 °C indefinitely.

Test phase amplifications were run in 3% agarose gels, stained with ethidium bromide and visualized in a UV transiluminator. Primers positive for amplification were FAM–labeled on the 5' end, and PCR products run on a 6% poliacrylamide gel in an ALF DNA sequencer. External and internal size standards were used to determine allele size.

Genepop Version 3.1d (Raymond and Rousset, '95) was used to test for linkage disequilibria among locus pairs, to calculate allele frequencies and estimate heterozygosities, to test allelic and genotypic differentiation across wild populations and to test Hardy-Weinberg equilibrium. Rst Calc (Goodman, '97) was used to estimate Rst and calculate the number of migrants per generation among pair wise wild populations assessing their significance; and NTSYS-pc 1.70 (Rohlf, '92) to make UPGMA phenograms and determine their significance.

## RESULTS AND DISCUSSION

### MICROSATELLITES

There was no significant linkage disequilibria deviation between pairs of loci studied across the populations sampled. This indicates that genotypes segregate independently in each locus

*TABLE 1. Study sites description*[1,2]

| | |
|---|---|
| A. Volta Grande | |
| Watercourse associated: | Piracicaba River |
| Latitude: | 22°40'41"S |
| Longitude: | 47°55'21"W |
| Area (ha): | ≈80 |
| Habitat type: | *várzea* (wetland) |
| Common aquatic plants: | Fam. Cyperaceae: *Cyperus* sp. Fam. Haloragaceae: *Myriophyllium brasiliensis* Fam. Pontediriaceae: *Pontederia* sp.1 Fam. Salviniaceae: *Salvinia rotundifolia* and S. *auriculata* |
| Apparent population size: | 9 |
| Sample size: | 2 |
| B. Porto de Areia | |
| Watercourse associated: | Piracicaba River |
| Latitude: | 22°39'02"S |
| Longitude: | 47°58'52"W |
| Area (ha): | ≈200 |
| Habitat type: | lagoon |
| Common aquatic plants: | Fam. Pontederiaceae: *Eichornia crassipes* and *E. azurea* Fam. Thyphaceae: *Typha angustifolia* Fam. Lentibulariaceae: *Utricularia* sp.1 Fam. Nymphaeaceae: *Nymphaea* sp.1 and sp.2 Fam. Hydrocharitaceae: *Elodea densa* Fam. Salviniaceae: *Salvinia auriculata* and S. *rotundifolia* |
| Apparent population size: | 21 |
| Sample size: | 9 |
| C. Pantanal | |
| Watercourse associated: | Aracua Creek |
| Latitude: | 22°35'58"S |
| Longitude: | 47°51'51"W |
| Area (ha): | ≈10 |
| Habitat type: | lagoon |
| Common aquatic plants: | Fam. Typhaceae: *Typha angustifolia* Fam. Haloragaceae: *Myriophyllium brasiliensis* Fam. Pontederiaceae: *Pontederia* sp.2 Fam. Nymphaeaceae: *Nymphaea* sp.3 |
| Apparent population size: | 22 |
| Sample size: | 3 |

*TABLE 1—Continued*

| | |
|---|---|
| D. Charqueada | |
| Watercourse associated: | Aracua Creek |
| Latitude: | 22°30'24"S |
| Longitude: | 47°49'29"W |
| Area (ha): | ≈2 |
| Habitat type: | *açude* (artificial pond) |
| Common aquatic plants: | Fam. Typhaceae: *Typha angustifolia* Fam. Cyperaceae: *Eleocharis* sp. Fam. *Poaceae*: sp.1 Fam. Nymphaeaceae: *Nymphaea* sp.4 |
| Apparent population size: | 6 |
| Sample size: | 3 |
| E. Duraflora | |
| Watercourse associated: | Pederneiras Creek |
| Latitude: | 22°26'32"S |
| Longitude: | 48°52'22"W |
| Area (ha): | ≈5 |
| Habitat type: | *açude* (artificial pond) |
| Common aquatic plants: | Fam. Haloragaceae: *Myriophyllium brasiliensis* Fam. Lentibulariaceae: *Utricularia* sp.2 Fam. Unknown: Algae Fam. Poaceae: sp.2 Fam. Cyperaceae: *Cyperus* sp. And *C. brevifolius* |
| Apparent population size: | 29 |
| Sample size: | 12 |

[1]Apparent population size: maximum number of animals seen during night-counts.
[2]Sample size: number of animals captured.

(Table 3). Under Markov chain parameters (9999 dememorization, 100 batches and 9999 iterations per batch), allelic differentiations among populations are significant ($P \leq 0.01$) in all loci analyzed except *Amiμ11* and highly significant considering the combination of loci across populations by Fisher's method ($\chi^2 \simeq \infty$, Df = 8, $P \leq 0.01$). Genotypic differentiation among populations was significant at the $P \leq 0.05$ level in *Amiμ08* and at $P \leq 0.01$, for *Amiμ13* and *Amiμ20 loci*, not significant in *Amiμ11*and significant considering the combination of loci across populations by Fisher's method ($\chi^2 \simeq \infty$, Df = 8, $P \leq 0.01$). With exception of the locus *Amiμ11* there is a large amount of differentiation among the populations studied, even in allelic and genotypic frequencies. As can be observed in Table 4, there are private alleles in some populations (e.g., the 260 bp allele from marker *Amiμ11* in Pantanal).

*TABLE 2. Primer pairs used to amplify Caiman latirostris DNA*

| *Locus* | Primer sequence | BSA | Annealing | Cycles | Size Range (bp) |
|---|---|---|---|---|---|
| *Ami*μ*8*a | CCTGGCCTAGATGTAACCTTC | No | 55°C | 30 | 115–117 |
| *Ami*μ*8*b | AGGAGGAGTGTGTTATTTCTG | | | | |
| *Ami*μ*11*a | AAGAGATGTGGGTGCTGCTG | Yes | 64°C | 35 | 229–237 |
| *Ami*μ*11*b | TCTCTGGGTCCTGGTAAAGTGT | | | | |
| *Ami*μ*13*a | CCATCCCCACCATGCCAAAGTC | Yes | 60°C | 35 | 240–270 |
| *Ami*μ*13*b | GTCCTGCTGCTGCCTGTCACTC | | | | |
| *Ami*μ*20*a | TTTTTCTTCTTTCTCCATTCTA | No | 55°C | 30 | 124–162 |
| *Ami*μ*20*b | GATCCAGGAAGCTTAAATACAT | | | | |

Two, five, eight, and eleven alleles, respectively, were observed across all populations for *Ami*μ*8*, *Ami*μ*11*, *Ami*μ*13* and *Ami*μ*20* markers (Table 4). The numbers and sizes of alleles observed in *Caiman latirostris* wild populations was similar to those observed by Glenn et al. ('98) in Louisiana and Florida for *Alligator mississippiensis* populations where markers *Ami*μ*8*, *Ami*μ*13* and *Ami*μ*20* presented ten, four, and eight alleles, respectively.

There were fixed alleles in VG and DF populations. In VG this pattern could be explained by the small sample size (two individuals). We could not assume Hardy-Weinberg equilibrium in PA and DF populations, where significant deviations occurred (Table 5). A more dramatic deviation occurred in DF at loci *Ami*μ*11*, *Ami*μ*13* and *Ami*μ*20* were the deviations were highly significant ($P \leq 0.01$). An excess of homozygozity at locus *Ami*μ*13* and *Ami*μ*20* and an allele fixed at *locus Ami*μ*8* were observed in this population.

The Rst estimator Rho (ρ) presented high values across populations at *loci Ami*μ*08*, *Ami*μ*13* and *Ami*μ*20* (Table 6), corroborating the observations of differentiation in allelic and genotypic frequencies among populations. These populations appear to have no differences at locus *Ami*μ*11*, where the larger variability resides within rather than between populations. The mean value of ρ for these loci over variance components was 0.186 ($P \leq 0.01$), suggesting at least moderate gene flow between populations. However, the estimated number of migrants per generation (Nm), calculated as in Goodman ('97) was rather low, 1.1. Actually, this would be better called ''successfully dispersed individuals'' (i.e., the ones who successfully reach suitable patches and reproduce).

*TABLE 3. P-value to test linkage disequilibria for each locus pair across all populations by Fisher's method*

| *Locus* pair | $\chi^2$ | df | P-value |
|---|---|---|---|
| *Ami*μ*08* × *Ami*μ*11* | 3.593 | 6 | 0.73157 |
| *Ami*μ*08* × *Ami*μ*13* | 1.694 | 2 | 0.42880 |
| *Ami*μ*11* × *Ami*μ*13* | 1.591 | 4 | 0.81039 |
| *Ami*μ*08* × *Ami*μ*20* | 0.366 | 2 | 0.83263 |
| *Ami*μ*11* × *Ami*μ*20* | 2.338 | 4 | 0.67385 |
| *Ami*μ*13* × *Ami*μ*20* | 0.950 | 4 | 0.91730 |

*TABLE 4. Allele frequencies in wild populations*

| *Ami*μ*8* | Pop | PA (9) | VG (2) | PT (3) | CH (4) | DF (12) |
|---|---|---|---|---|---|---|
| *Alleles (pb)* | 15 | 0.778 | 1.000 | 0.333 | 0.625 | 1.000 |
| | 117 | 0.222 | | 0.667 | 0.375 | |
| *Ami*μ*11* | Pop | PA (9) | VG (2) | PT (3) | CH (4) | DF (12) |
| Alleles (pb) | 227 | 0.222 | 0.250 | | 0.250 | 0.208 |
| | 229 | 0.278 | 0.500 | 0.667 | | 0.250 |
| | 231 | | | | | 0.042 |
| | 235 | 0.500 | 0.250 | 0.333 | 0.625 | 0.250 |
| | 237 | | | | 0.125 | 0.250 |
| *Ami*μ*13* | Pop | Pa (8) | VG (2) | PT (3) | CH (4) | DF (12) |
| Alleles (pb) | 254 | | 0.250 | 0.333 | | |
| | 260 | | | 0.500 | | |
| | 262 | | | | | 0.042 |
| | 264 | 0.250 | | 0.167 | 0.500 | 0.083 |
| | 266 | | | | | 0.083 |
| | 268 | | | | | 0.792 |
| | 270 | | | | 0.125 | |
| | 272 | 0.750 | 0.750 | | 0.375 | |
| *Ami*μ*20* | Pop | PA (9) | VG (2) | PT (3) | CH (4) | DF (11) |
| Alleles (pb) | 116 | 0.056 | 0.500 | | | |
| | 124 | 0.444 | 0.250 | | | 0.273 |
| | 126 | 0.167 | 0.250 | 0.167 | 0.375 | 0.455 |
| | 128 | | | | 0.250 | 0.136 |
| | 130 | 0.111 | | | | |
| | 144 | 0.167 | | 0.333 | 0.125 | |
| | 152 | | | | | 0.045 |
| | 154 | | | | | 0.045 |
| | 156 | | | 0.500 | 0.125 | |
| | 158 | 0.056 | | | 0.125 | |
| | 164 | | | | | 0.045 |

*TABLE 5. Heterozigozity, fixation index and exact test for Hardy-Weinberg equilibrium*

| | PA | VG | PT | CH | DF |
|---|---|---|---|---|---|
| *Amiμ8* | | | | | |
| He | 0.366 | | 0.533 | 0.536 | |
| Ho | 0.000 | | 0.000 | 0.750 | |
| f | 1.000* | Fixed | 1.000 | −0.500 | Fixed |
| *Amiμ11* | | | | | |
| He | 0.400 | 0.833 | 0.533 | 0.607 | 0.801 |
| Ho | 1.000 | 0.500 | 0.000 | 0.500 | 1.000 |
| f | −0.565* | 0.500 | 1.00 | 0.200 | −0.263** |
| *Amiμ13* | | | | | |
| He | 0.400 | 0.500 | 0.733 | 0.679 | 0.373 |
| Ho | 0.500 | 0.500 | 0.333 | 0.500 | 0.083 |
| f | −0.273 | Not done | 0.600 | 0.294 | 0.784** |
| *Amiμ20* | | | | | |
| He | 0.771 | 0.833 | 0.733 | 0.857 | 0.727 |
| Ho | 0.667 | 1.000 | 0.667 | 0.750 | 0.364 |
| f | 0.143 | −0.333 | 0.111 | 0.143 | 0.512** |

*$p \leq 0.05$; **$p \leq 0.01$

Considering the age at sexual maturity of 10 years for the species (Verdade and Sarkis, '98) and a relative generation time, the number of approximately one individual successfully dispersed per generation seems rather low. Since the wetlands are all connected by rivers and creeks, we can conclude there is no physical barrier to dispersal. Therefore, the relatively low number of successfully dispersed individuals can be due to a possible combination of high mortality rates and low natality rates.

The pairwise ρ comparisons between populations discriminate only between the most divergent pairs of populations (Table 7). There was a significant divergence between PA and DF, CH and DF ($P \leq 0.05$); and between PA and PT, PT and DF ($P \leq 0.01$). These patterns are compatible with the geographic distance between DF and the other sites. The negative values of estimated ρ should be interpreted carefully since they were not significant. However, they *could* indicate that PA and VG, and CH and DF are panmictic, which would be logical for the former but wrong for the latter based on geographic distances. Nm values should be analyzed only if the divergence between populations compared were highly significant (Goodman, '97). When this analysis was possible, the results indicated that the more divergent the population pairs were, the smaller the number of migrants per generation was between them (Table 7). The $(\delta\mu)^2$ distances in Table 7 were used to cluster populations using UGMA phenogram (Fig. 2). Even the geographically closest populations showed high genetic distances (Table 7).

*TABLE 6. RHO values over all populations*

| Locus | SA (Across) | SW (Within) | RHO (Among) |
|---|---|---|---|
| *Amiμ08* | 0.29053 | 0.94251 | 0.23562 |
| *Amiμ11* | −0.05044 | 0.94279 | −0.05653 |
| *Amiμ13* | 0.38170 | 1.16663 | 0.24652 |
| *Amiμ20* | 0.25360 | 0.76966 | 0.24783 |
| Overall results | | | |
| RHO (averaging variance components) =0.18637 | | | |
| Nm=1.09140 | | | |
| P=0.00640 | | | |
| Number of permutations=10000 | | | |

*TABLE 7. RHO values averaging over variance components, estimated Nm and $(\delta\mu)^2$ distances, under 10000 permutations*

| PoPS | (VAR COMP) RHO | Nm | P | Distance $(\delta\mu)^2$ |
|---|---|---|---|---|
| PA × VG | −0.00484 | −51.9381 | 0.38600 | 0.33272 |
| PA × PT | 0.52328 | 0.2278 | 0.00960** | 2.26298 |
| PA × CH | 0.00897 | 27.6288 | 0.27550 | 0.21893 |
| PA × DF | 0.06904 | 3.3709 | 0.03500* | 0.17354 |
| VG × PT | 0.51260 | 0.2377 | 0.10700 | 2.75850 |
| VG × CH | 0.13817 | 1.5594 | 0.08140 | 0.82849 |
| VG × DF | −0.05655 | −4.6706 | 0.30440 | 0.22553 |
| PT × CH | 0.31528 | 0.5430 | 0.05710 | 1.34225 |
| PT × DF | 0.54935 | 0.2051 | 0.00330** | 2.05812 |
| CH × DF | 0.07944 | 2.8972 | 0.03070* | 0.32409 |

*$p \leq 0.05$; **$p \leq 0.01$

Parentage between pairs of individuals from natural populations can be assessed with molecular markers without having previous pedigree information (Ritland, '96; Lynch and Ritland, '99). Microsatellite markers may be extremely useful for this purpose. However, several informative alleles are normally needed to estimate high level parentage between two individuals chosen randomly (Ritland, '96; Lynch and Ritland, '99).

By displaying all pairwise individuals by parentage relationships (Fig. 3), it was possible to demonstrate that some individuals appear to be more closely related to animals from sites different from the site of capture. Such individuals might possibly be migrants. For example, PA07 grouped closer to DF03 and DF06 than with its fellow-neighbors. This observation may indicate a possible dispersal pattern of relatively large distances (150 km).

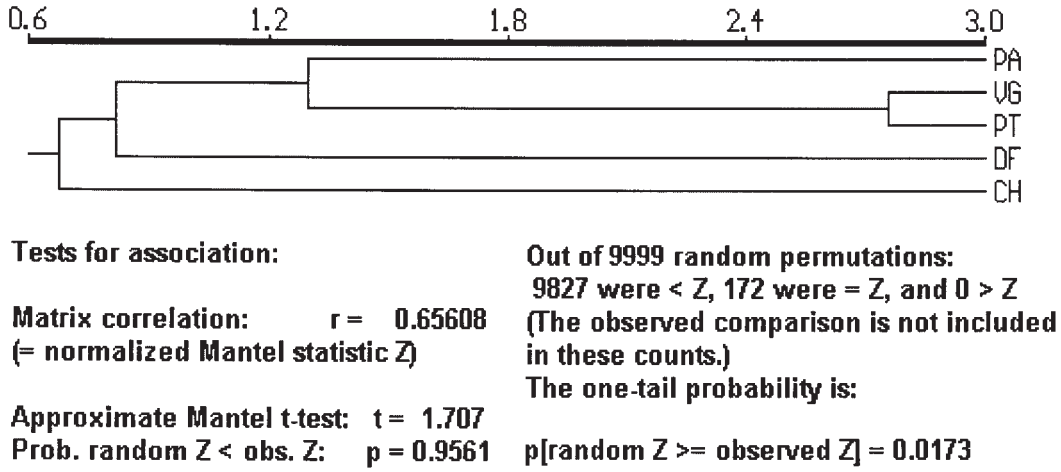


Fig. 2. $(\delta\mu)^2$ distance among populations by UPGMA method.

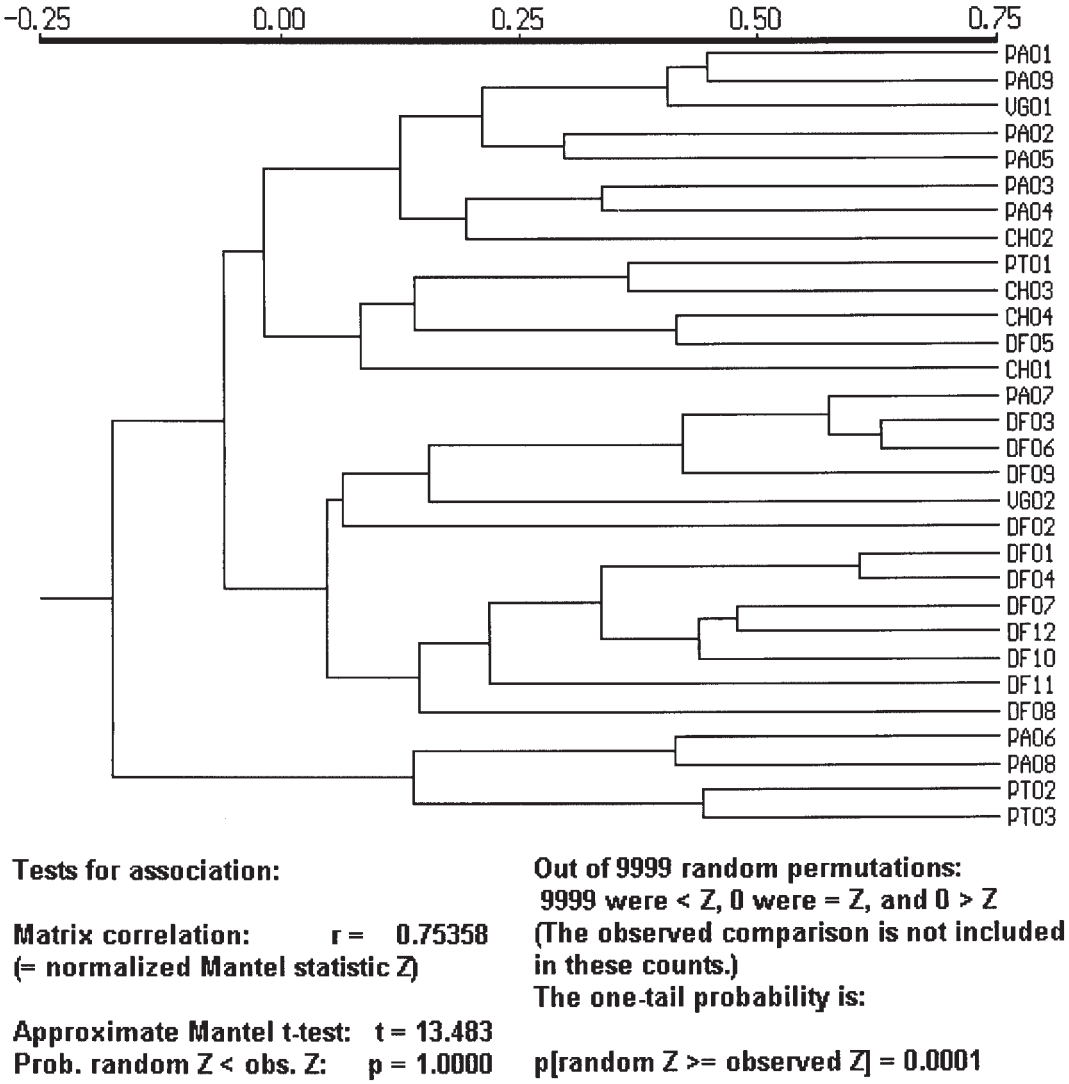


Fig. 3. UPGMA phenogram among wild individuals by information method for calculate parentage (Coelho, 2001).

### *Ecological interpretation*

There was a significant variation at the microgeographic scale among populations of broad-snouted caiman. This indicates that there is some degree of isolation among groups of animals from different sites. The pattern of genetic distance among groups roughly follows their geographic distance (i.e., DF significantly differs from PA, PT, and CH). The only exception is the relationship between PA and PT, which are geographically close but genetically distinct (Table 7).

Considering that gene flow is closely related to dispersal, we can assume that the colonization process of small wetlands is not as random as it would be if the populations were spread over a continuous landscape. This reinforces the hypothesis that this species probably presents a metapopulation structure. Larger wetlands, closer to the rivers, may serve as source populations because besides having more food resources, they seldom get completely dry and hunting pressure is possibly lower due to difficult access. On the other hand, smaller wetlands, associated with creeks, besides possibly having less food resources, may become completely drained in some dry years and may also suffer higher hunting pressure.

By definition, the source-populations may theoretically present birth rates higher than mortality rates, likely exporting individuals by dispersal and at the same time not likely suffering local extinction. On the other hand, the sink populations may in theory present mortality rates higher than birth rates, and therefore likely suffering local extinction and recolonization from time to time.

In such a situation, elevation of the water level, as suggested by the state government for the improvement of fluvial transportation (Oliveira and Caixeta-Filho, '97), may cause source populations to disappear, as this species is a palustrine not a riverine crocodilian. Consequently, the extinction of source populations might result in the extinction of the metapopulation as a whole. Assessing dispersal and mating system (as suggested by McCullough, '96) using molecular markers might help testing this hypothesis as well produce data that could aid in avoiding its occurrence.

## CONCLUSIONS

- Populations of *Caiman latirostris* differ microgeographically both in terms of genetic diversity and heterozygosity
- Assessment of individuals' sites of origin in microgeographic terms as well as parentage among individuals, using molecular techniques might be possible in the near future, although the precision of the method is still low to medium
- The relationship between geographic distance and genetic distance in the broad-snouted caiman, although substantial is not completely clear
- Genetic diversity of this species seems similar to that reported in the American alligator
- A possible combination of high mortality and low natality rates appears to result in a low number of successfully dispersed individuals per generation
- Genetic analyses might be used complementarily in behavioral ecological studies of wild caimans

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