JOURNAL OF EXPERIMENTAL ZOOLOGY 311A:258–269 (2009)

A Journal of Integrative Biology

Genetic and Morphometric Variability in *Caiman latirostris* (Broad-Snouted Caiman), Reptilia, Alligatoridae

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ABSTRACT *Caiman latirostris* (broad-snouted caiman) is a crocodilian species from Argentina subject of management plans. The goal of this study was estimating the distribution of genetic variability using RAPD markers and quantitative traits in wild populations of *C. latirostris* from Santa Fe province, Argentina. We sampled animals from four populations to obtain DNA and morphometric measurements. Eight RAPD primers were used and PCR products were analyzed on 4% polyacrylamide gels stained with silver nitrate. Eleven allometric measurements were obtained in animals within 48 hr after birth. We were able to reveal a relatively high number of variable markers in the studied populations. Our estimates of polymorphism and heterozygosity are higher than recorded values in other crocodilians using isozymes, the studied populations showed low levels of gene flow and some population subdivision. The study of quantitative traits conducted by nested analysis of variance and principal component analysis indicated higher levels of variance among nests within populations than among populations. We found that some head measurements have the highest contribution to morphological differences among populations; this fact could support the role of these traits in reproductive or feeding behavior. Estimated genetic differentiation value (F_{ST}) among populations was higher than quantitative trait differentiation value (Q_{ST}), suggesting a higher contribution of neutral than adaptive loci to the genetic differentiation among populations. Quantitative traits are probably more related with fitness and the differentiation among populations remained relatively lower. The high heritability estimated for some traits indicates great potential to improve them in management plans. *J. Exp. Zool. 311A:258–269, 2009.*

How to cite this article: Amavet P, Vilardi JC, Rosso E, Saidman B. 2009. Genetic and morphometric variability in *Caiman latirostris* (broad-snouted caiman), Reptilia, Alligatoridae. J. Exp. Zool. 311A:258–269.

Caiman latirostris (broad-snouted caiman) (Daudin, 1802) is a crocodilian species from Argentina belonging to the family Alligatoridae. It has a wide geographic distribution that embraces diverse aquatic environments of Parana River basin, which usually are shallow water bodies with dense vegetation (Larriera, '92). Considering their function as a carnivorous consumer, at the top of trophic levels, broad-snouted caiman could be considered as flag species in local ecosystems, whose viability guarantees the whole system viability.

C. latirostris wild populations in Santa Fe, Argentina, are subject of management plans carried out by the Proyecto Yacaré (Gobierno Santa Fe/MUPCN). The methodology applied is the ranching system that consists of harvesting wild eggs for rearing under captivity. The program intends to recover population densities, which were dramatically reduced through the last dec-

Grant sponsor: Universidad Nacional del Litoral; Grant number: CAI+D 2002 (P.I. 102/PACT 20); Grant sponsor: Proyecto Yacaré.

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Received 28 August 2008; Accepted 23 December 2008

Published online 4 February 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.523

ades, and to guarantee the conservation of the wetlands that this species shares with a lot of birds, mammalians, and other reptiles. The evident numeric recovery of broad-snouted caiman populations owing to this management system, made it possible that Convention on International Trade in Endangered Species of Wild Fauna and Flora in 1997 decided to change the population status of this species in Santa Fe province, authorizing their commercial use. This legal frame allows that a percentage of born animals from harvested eggs is derived to commercial fattening, producing economical yielding to people working on habitat conservation (Larriera, '98). Thanks to the commercial use, *C. latirostris* has turned into an important species at both national and international levels, owing to the high quality of their products.

Molecular analysis methods, especially in crocodilians, have provided valuable data (Forstner and Forstner, 2002) about reproductive mechanisms, gene flow, population effective size, geographic distribution, and genetic variability measurements, all of them fundamental information for the selection of appropriate management strategies.

Allozyme analysis (Gartside et al., '76; Lawson et al., '89; Flint et al., 2000) indicated that crocodilians have few polymorphic loci. There is scarce information about RAPD markers in this reptile order (Glenn et al., '98; Wu et al., 2002) and no previous RAPD data in *C. latirostris*. Recently, population studies on *C. latirostris* were conducted in Brazil (Verdade et al., 2002; Villela, 2004) by means of specific microsatellite primers developed for Zucoloto et al. (2002) that allowed detecting seven polymorphic loci in *C. latirostris* (Zucoloto et al., 2006).

RAPD technique is widely used (Dutra et al., 2008) because these markers usually display large numbers of polymorphic (di-allelic) loci. Tens to hundreds of polymorphic loci are commonly reported (Hardy, 2003), which offers opportunities to make inferences at much finer scales of resolution (Ritland, 2005). Furthermore, in comparison to co-dominant markers, dominant markers can be developed relatively easily even for species for which no prior genetic information is available and at a relatively low cost (Mueller and Wolfenbarger, '99). In spite of Dowling et al. ('96) criticisms, RAPD markers may represent excellent alternative tools to co-dominant markers to address questions requiring the estimation of pair-wise relatedness between individuals (Hardy, 2003) and to study the patterns of genetic variability (Li and Jin, 2006; Arruda and Morielle-Versute, 2008; Dutra et al., 2008; Lopera-Barrero et al., 2008; Torres et al., 2008).

In relation to the phenotypic variability estimated through allometric measurements, Monteiro et al. ('97) analyzed ontogenetic changes in the cranial shape among *C. latirostris* and other two caiman species and concluded that the differences in ontogenetic processes probably determine dietary differences among cited species. Verdade (2000) analyzed in *C. latirostris* 18 quantitative body-size traits and ten relative measurements (proportions between absolute measurements) at different ages. The author concluded that all variables are age-dependent and, consequently associated with growth rate. Moreover, Verdade (2000) found sexual dimorphism in the cranial allometric growth. More recently, the same author (Verdade, 2001) and Larriera et al. (2004) recorded a significant association between female and their offspring allometric measurements.

The goal of this study was estimating the distribution of genetic variability using RAPD markers and analyzing genetic variation of quantitative traits in wild population of *C. latirostris* from Argentina. The results obtained for these population parameters can be useful to evaluate and, perhaps, modify, the current management programs.

MATERIALS AND METHODS

Biological material (sampled populations)

As national laws prevent working with wild populations outside the rules of management programs, we have limited our sampling to animals from the facilities of Proyecto Yacaré of Santa Fe city. We sampled specimens from four populations of Santa Fe province in Argentina: ''Estancia El Estero'' (EEE)—Departamento San Javier (30° 29′S 59° 59′W), ''Costa del Salado'' (CSA)—Departamento San Cristóbal (29° 58′S 60° 50′W), ''Estero del Paraje 114'' (EDP)—Departamento San Javier (30° 43′S 60° 17′W), and ''Arroyo El Espín'' (AES)—Departamento Vera (29° 58′S 60° 04′W) (Fig. 1).

All animals were part of the stock of captivity specimens of Proyecto Yacaré of Santa Fe City. The animals were captured by hand and measured, weighed and sexed. The capture site of each specimen was identified by means of labels consisting of cuts in their tail scales that symbolize

the birth year and the nest number. The animals were labeled within 24 hr after birth by staff members of Proyecto Yacaré. For molecular markers analysis we used ten individuals coming from different nests of each studied population, making a total of 40 animals. For the morphometric study we randomly sampled four nests per population, and picked at random ten newborns from each selected nest, making a total of 160 individuals (four populations × four nests × ten individuals).

RAPD markers analysis

We obtained whole blood samples from specimens of *C. latirostris* by puncture to the post occipital supra-vertebral sinus at the level to the cervical vertebra (Tourn et al., '93). EDTA or lysis buffer (Longmire et al., '88) for long-term blood storage at room temperature, according to White and Densmore ('92), was used as anticoagulant.

For DNA isolation we used the method described in Murray and Thompson ('80). It consists of a treatment of whole blood with a lysis solution (buffer TE: 10 mM Tris-HCl pH 7.5, 10 mM Na_2EDTA 2 H_2) at room temperature making three washes, and then with an extraction solution (2% (w/-v) CTAB, 1.4 M NaCl, 0.2% (w/-v) 2-mercaptoetanol, 20 mM EDTA, 200 mM Tris HCl pH 7.5) at 60°C for 3 hr. The pellet was washed in chloroform, precipitated with pro-analysis isopropyl alcohol and hydrated in 1 mL sterile double distilled water.

We quantified all DNA samples before amplification assays on 2% agarose gels. DNA concentration was estimated by comparison with bands provided by a K562 marker (Promega, Madison) at 10 ng/μL.

We conducted amplification reactions in a final volume of 25 μL of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% Gelatin (Promega), 200 pM dATP, dTTP, dGTP, and dCTP, 5 pM of 10-base primer, 1.25 unit *Taq* DNA polymerase (Promega), and 25 ng genomic DNA. A negative control containing all reagents but without genomic DNA was included in each reaction. DNA amplification was performed in a thermal cycler (PTC-100 Peltier Thermal Cycler, MJ Research, Watertown) with a program of 45 cycles of 2 min at 94°C, 1 min at 36°C, and 2 min at 72°C, with a final extension at 72°C for 10 min.

Initially, we analyzed a set of 20 random 10-mer primers from Promega (B050-10 and B051-10) and we selected eight of them that showed the best resolution and reproducible bands: A01 (CCC AAG GTC C), A02 (GGT GCG GGA A), A03 (AAG ACC CCT C), A05 (CAC CAG GTG A), A06 (GAG TCT CAG G), B04 (TGC CAT CAG T), B05 (GCG CTC ACG C) and B07 (AGA TCG AGC C). We analyzed

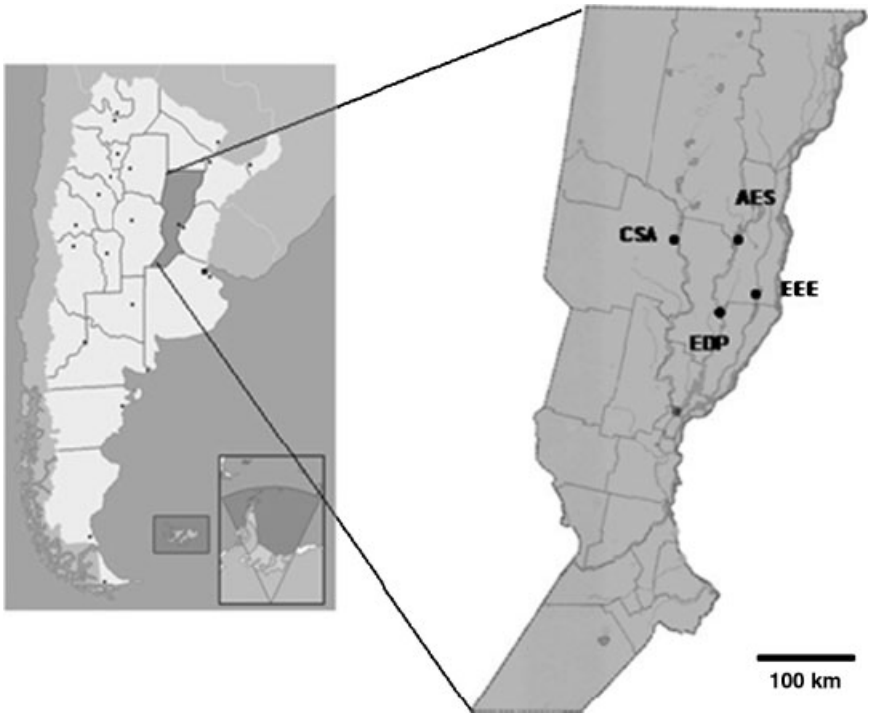

Fig. 1. Range map including the four sampled populations.

PCR products by electrophoresis on 4% polyacrylamide gels of 33 cm × 39 cm, to 2200 V and 75 W in TBE buffer, stained with silver nitrate solution (Bassam et al., '91). We used pGem ladder as a marker of molecular weight. Stained gels were photographed with a digital camera (Kodak C330; Eastman Kodak Company, Rochester, NY) using the macro mode.

We performed routinely re-amplifications to ensure reproducibility of banding patterns. The usual cautions needed to prevent contamination of PCR experiments with previously amplified fragments were observed. In particular, pre- and postamplification procedures were separated and fresh aliquots of reagents were used for each experiment wherever possible. To test the reliability of PCR products, we used routinely several controls, one without primer, a second maintaining no *Taq* DNA polymerase, and the third with no genomic DNA. No amplification occurred in any of these controls.

Molecular markers data analysis

Binary matrices (''1'' and ''0'' for presence and absence of bands, respectively) including all obtained bands were analyzed using the TFPGA program (ver. 1.3) (Miller, '98) to estimate descriptive statistics. Binary matrices consisting of only polymorphic bands were transformed into allelic frequencies by a Bayesian method with nonuniform prior distribution (Zhivotovsky, '99) using the program AFLP-SURV 1.0 (Vekemans, 2002). We quantified genetic variability following the approach of Lynch and Milligan ('94) by means of the following parameters: Average number of alleles per locus (A), Percentage of polymorphic loci (P, 5% criterion), and unbiased expected heterozygosity (H_e, Nei, '78). We analyzed population structure by means of nonhierarchical F_{ST} (Wright, '51), and following the approach of Lynch and Milligan ('94), we estimated within population (H_w) and among population (H_b) variability components. Confidence intervals for the F_{ST} estimated were obtained by 1,000 random permutations of individuals among populations. F_{ST} statistics is equivalent to a nonhierarchical analysis of molecular variance (Excoffier, 2003) that can be applied to dominant markers and is widely used in population structure analysis (Frankham et al., 2002). Indirect estimations of gene flow (Nm) were obtained from the differentiation among populations (F_{ST}) according to the relationship $Nm = (1 - F_{ST})/4F_{ST}$ (Nei, '78).

Using the same software, we estimated pair-wise genetic distances between populations by two methods, unbiased Nei's ('78) distance and pair-wise F_{ST} coefficients. From the genetic distance matrices we obtained phenograms representing relative phenetic relationships by the unweighted pair group method with arithmetic means (Sneath and Sokal, '73) using the program R (ver. 2.7.1) (R Development Core Team, 2008).

The structure of genetic and geographic distance matrices were compared by Mantel test using the ape package (Paradis et al., 2006) of the R program.

Morphometric analysis

We measured specimens within 48 hr after birth. For each animal, the date of birth, the nest number, and the population of origin were recorded. The sex in newborns of *C. latirostris* cannot be detected without sacrificing the animals, which is not permitted by national legislations. For this reason, we did not record sex effects in this study. Eleven allometric measurements (Verdade, 2001) were obtained in all animals (Table 1, Fig. 2). The traits total length (TTL) and snout–vent length (SVL) were measured at the nearest millimeter. Head traits were measured at the nearest 0.01 mm using a digital electronic Vernier caliper. Body mass (BM) was recorded with an OHaus CS 200 balance with a precision of 0.1 g.

The analysis of distribution of quantitative trait variation followed a hierarchical (nests within populations) design. We conducted analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) analysis with the R program and estimated variance components at different hierarchical levels using the package *lme4*. Nests and populations were considered random factors. Following this model, the ANOVA F value among populations was calculated as MS_P/MS_N (where MS_P and MS_N represent respectively among population and among nest mean squares). The same software was used to conduct a principal component analysis (PCA) to identify the most important variables for differentiating populations and nests.

In the population structure analysis the Q_{ST} value (Spitze, '93) of quantitative traits is equivalent to F_{ST} for molecular data (Pressoir and Berthaud, 2004). We estimated variance components by REML (restricted maximum likelihood). We considered the total phenotypical variance as:

TABLE 1. Description of allometric measurements obtained (Adapted from Verdade, 2001)

Acronym	Description
TTL	Total length: anterior tip of snout to posterior tip of tail
SVL	Snout–vent length
DCL	Dorsal cranial length: Anterior tip of snout to posterior surface of occipital condyle
SL	Snout length: Anterior tip of snout to anterior orbital border, measured diagonally
LCR	Length of the postorbital cranial roof: Distance from the posterior orbital border to the posterolateral margin of the squamosal
CW	Cranial width: Distance between the lateral surfaces of the mandibular condyles of the quadrates
SW	Basal snout width: Width across anterior orbital borders
WN	Maximal width of external nares
IOW	Minimal interorbital width
ML	Mandible length: Anterior tip of dentary to the posterior tip of the retroarticular process
BM	Body mass

$\sigma^2_T = \sigma^2_{Pop} + \sigma^2_{nest} + \sigma^2_{resid}$, where $\sigma^2_{Pop} = \sigma^2_{ST}$ and we estimated Q_{ST} as:

$$Q_{ST} = \frac{\sigma^2_{ST}(1 + F_{IS})}{2\bar{\sigma}^2_{IS} + \sigma^2_{ST}(1 + F_{IS})}$$

Ignoring epistatic effects, the covariance among full sibs (= variance among nests) is composed of $1/2V_A + 1/4V_D + V_{EC}$ (where V_A, V_D, and V_{EC} are, respectively, variance components attributed to additive effects, dominant deviance, common environment factors) (Falconer and Mackay, '96). Then, σ^2_{nest} is the maximum expected value of V_A. As it is not possible to remove experimentally V_D and V_{EC}, we estimated the maximum expected σ^2_{IS} value as $2\sigma^2_{nest}$. Considering random mating in these populations we assumed $F_{IS} = 0$. Then, we calculated the minimum expected Q_{ST} value as:

$$Q_{ST} = \frac{\sigma^2_{ST}}{2\bar{\sigma}^2_{IS} + \sigma^2_{ST}}$$

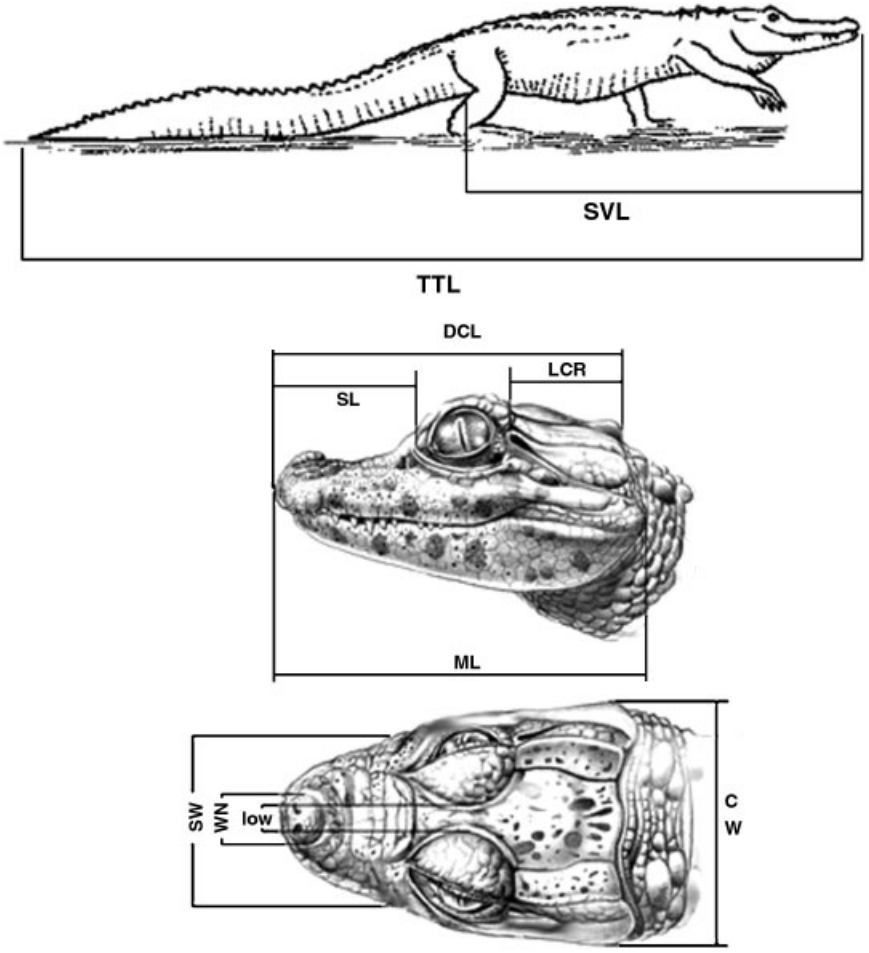

Fig. 2. Allometric measurements obtained.

To estimate heritability values (h^2) we calculated variance components in each population by one-way ANOVA. We obtained heritability estimates as:

$$h^2 = \sigma^2_{IS}/\sigma^2_{TOT}$$

RESULTS

RAPD analysis

Polyacrylamide gels revealed between 20 and 40 bands per primer (Fig. 3) with sizes ranging from 350 to 2645 bp. Two hundred and thirty-three RAPD markers were analyzed, of which only 32 were polymorphic. Twenty of the polymorphic loci were produced by a single primer, B05.

We have been able to reveal a relatively high number of variable markers in *C. latirostris* (Table 2), being "Arroyo El Espín" the most variable population. The analysis of gene diversity revealed a total diversity $H_T = 0.23$, most part of it (73%) was explained by diversity within populations ($H_W = 0.16 \pm 0.03$), whereas the diversity among populations was 27% ($H_B = 0.06 \pm 0.003$).

The F_{ST} value obtained (0.27 ± 0.08) was relatively high for conspecific populations, and highly significant ($P < 10^{-4}$ based on 1,000 permutations). Consequently, the estimated Nm (0.3) was lower than unity.

The phenogram based on unbiased Nei's ('78) genetic distances showed that population AES is highly differentiated from the rest (Fig. 4). However, the bootstrap support (based on 1,000 resamplings) for nodes were relatively low (<50%). The phenogram obtained from pair-wise F_{ST} values between populations yielded similar results (not shown).

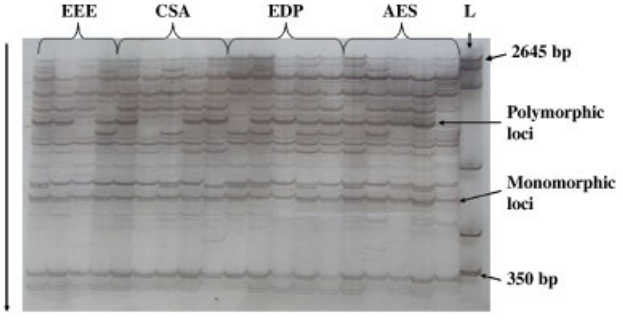

Fig. 3. RAPD bands visualized on a 4% polyacrylamide gel stained with silver nitrate solution. EEE, CSA, EDP, and AES: samples of individuals from each population. The vertical arrow on the left shows the direction of electrophoresis. L: Ladder pGem.

The Mantel test based on 100 permutations indicated that geographic and genetic distance matrices were not significantly correlated ($Z = 30.31$, $P = 0.61$ for Nei's distances and $Z = 106.23$, $P = 0.69$ for pair-wise F_{ST} estimates).

Morphometric analysis

Descriptive statistics for each trait in each nest are summarized in Table 3. Univariate analyses of variance, considering nests and populations as random factors, indicated that the differences among populations (F_{POP}) are not significant whereas, differences among nests within populations (F_{NEST}) are in all cases highly significant.

TABLE 2. Genetic variability at 32 loci in all populations

Population	*N*	*A*	*P*	*H*	Var *I*%	Var *L*%
EEE	10	1.51 (0.51)	51.10 (7.45)	0.15 (0.03)	34.6	65.4
CSA	10	1.42 (0.50)	42.20 (7.36)	0.13 (0.03)	22.1	77.9
EDP	10	1.40 (0.50)	40.00 (7.30)	0.14 (0.03)	20.2	79.8
AES	10	1.64 (0.48)	64.4 (7.14)	0.24 (0.03)	26.4	73.6

N: sample size; *A*: average number of alleles per locus; *P*: percentage of polymorphic loci (5% criterion); *H*: expected heterozygosity; Var *I*%: percent of total variance explained by variance among individuals; Var *L*%: percent of total variance explained by variance among loci (standard error in parentheses).

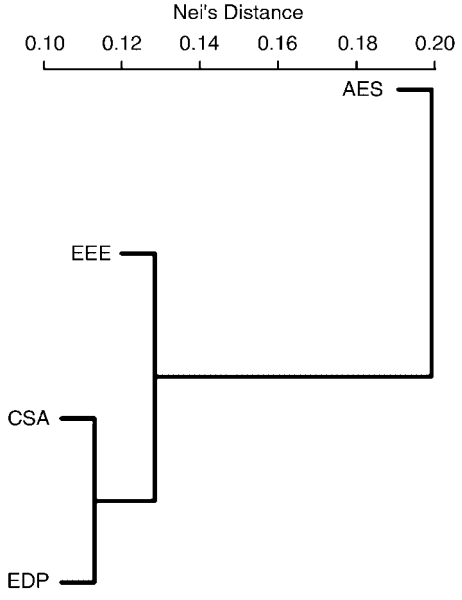

Fig. 4. Nei's ('78) genetic distances among populations.

TABLE 3. Mean, standard deviation (in parentheses) of morphometric variables in each nest and ANOVA statistics for the differentiation at population (F_{POP}) and nest (F_{NEST}) levels

Pop	EEE				CSA			EDP				AES						
Nest	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	FPOP	FNEST
TTL	24.790	24.140	23.880	24.660	24.550	24.410	23.360	24.430	24.210	24.070	24.880	23.650	24.540	24.490	23.270	23.800	0.28	8.02**
(cm)	(0.425)	(0.732)	(0.492)	(0.519)	(0.591)	(0.532)	(0.980)	(0.386)	(0.949)	(0.682)	(0.278)	(0.350)	(0.386)	(0.468)	(0.416)	(0.716)		
SVL	12.210	11.730	11.540	11.900	11.780	11.760	11.430	12.260	11.890	11.940	12.180	11.740	12.210	11.920	11.220	11.510	0.32	12.34**
(cm)	(0.247)	(0.452)	(0.324)	(0.298)	(0.181)	(0.217)	(0.397)	(0.117)	(0.482)	(0.217)	(0.114)	(0.284)	(0.260)	(0.343)	(0.257)	(0.185)		
DCL	34.038	33.379	33.317	33.457	33.545	33.166	32.244	34.142	32.999	33.053	33.817	32.734	33.963	33.630	32.976	33.589	0.55	8.40**
(mm)	(0.414)	(0.891)	(0.540)	(0.611)	(0.454)	(0.613)	(0.708)	(0.423)	(0.800)	(0.711)	(0.482)	(0.531)	(0.470)	(0.447)	(0.340)	(0.539)		
SL	11.579	11.784	11.696	11.964	11.588	11.422	11.070	12.144	11.406	11.687	12.070	11.680	11.443	11.451	11.187	11.722	0.91	5.22**
(mm)	(0.541)	(0.619)	(0.292)	(0.388)	(0.540)	(0.220)	(0.476)	(0.328)	(0.372)	(0.417)	(0.454)	(0.329)	(0.385)	(0.383)	(0.279)	(0.298)		
LCR	12.215	12.095	11.965	12.018	11.994	11.653	11.743	12.347	11.796	11.864	12.143	12.067	12.597	12.455	11.875	12.048	1.24	4.50**
(mm)	(0.360)	(0.154)	(0.370)	(0.431)	(0.323)	(0.300)	(0.256)	(0.488)	(0.366	(0.387)	(0.180)	(0.385)	(0.517)	(0.481)	(0.454)	(0.281)		
CW	20.582	21.061	21.544	21.627	20.636	20.759	20.454	21.933	20.970	21.057	21.734	20.760	21.16	20.892	20.775	21.264	0.23	7.74**
(mm)	(1.003)	(0.495)	(0.523)	(0.456)	(0.388)	(0.388)	(0.755)	(0.529)	(0.605)	(0.780)	(0.383)	(0.467)	(0.302)	(0.477)	(0.304)	(0.279)		
SW	15.022	14.869	14.643	14.914	14.383	14.421	14.061	15.059	14.469	14.168	15.038	13.926	15.078	14.439	14.444	14.475	1.23	4.65**
(mm)	(0.633)	(0.519)	(0.347)	(0.387)	(0.494)	(0.512)	(0.362)	(0.555)	(0.471)	(0.743)	(0.531)	(0.729)	(0.480)	(0.717)	(0.446)	(0.353)		
WN	5.344	5.082	4.953	5.087	5.137	5.153	4.667	4.899	4.767	4.776	4.549	5.153	4.852	4.913	5.072	5.051	1.62	4.28**
(mm)	(0.394)	(0.199)	(0.239)	(0.217)	(0.317)	(0.371)	(0.481)	(0.254)	(0.346)	(0.253)	(0.288)	(0.263)	(0.371)	(0.246)	(0.192)	(0.183)		
IOW	2.038	2.116	2.123	2.534	1.836	2.151	2.346	2.098	2.498	2.227	2.163	2.349	1.652	1.984	2.044	2.012	2.88	7.36**
(mm)	(0.175)	(0.155)	(0.113)	(0.166)	(0.104)	(0.283)	(0.345)	(0.134)	(0.206)	(0.314)	(0.236)	(0.252)	(0.337)	(0.153)	(0.247)	(0.174)		
ML	35.407	35.528	35.615	36.122	35.410	36.073	33.489	36.312	35.371	34.866	35.630	34.457	36.344	35.578	35.207	36.202	0.80	8.21**
(mm)	(0.903)	(1.090)	(0.884)	(0.915)	(0.845)	(1.048)	(0.937)	(0.544)	(0.999)	(1.148)	(0.507)	(0.486)	(0.546)	(0.741)	(0.637)	(0.710)		
BM	50.040	42.98	45.400	54.510	47.880	45.18	52.500	50.050	44.210	46.350	46.600	43.300	50.740	49.330	44.300	47.660	0.98	31.15**
(gr)	(1.255)	(1.641)	(1.363)	(2.126)	(1.644)	(2.007)	(2.403)	(2.127)	(2.784)	(2.614)	(1.465)	(1.491)	(1.691)	(1.821)	(1.817)	(1.768)		

**$P < 0.01$.

However, the multivariate global test (MANOVA) showed highly significant differences among populations (Wilks = 0.328, $P < 10^{-15}$).

In the principal components analysis, all standardized traits were used to obtain three noncorrelated principal axes (Table 4). Although the first three axes explained only 64.2% of total variance, further axes were not considered because their eigenvalues were lower than 1. The PC1 depends on almost the same proportion on all traits. PC2 was mainly determined by CW, WN, and IOW, and PC3 depends essentially on WN, SVL, and BM.

Nested ANOVA test using the three principal components, applying a random model, showed borderline significant differences among populations for PC2. Pair-wise comparisons between populations through TukeyHSD tests yielded nonsignificant results for PC1 whereas, most of the comparisons for PC2 were significant. For PC3, only one comparison was significant (Table 5).

In the analysis of population structure, estimated Q_{ST} values were 0 for seven traits (Table 6). Four head measurements (LCR, SW, WN, and IOW) showed Q_{ST} values different from 0.

Estimated heritability values were significant in most cases (Table 7). Notably, the EEE population showed heritability values of 0 in four traits. The trait with the highest heritability estimate in all populations was BM.

TABLE 4. Loadings of each trait on the first three principal component (PC), eigenvalues and proportion of total variance explained by each PC according to the principal component analysis

	PC1	PC2	PC3
TTL	–0.373	0.060	–0.265
SVL	–0.333	0.046	–0.400
DCL	–0.413	0.146	0.056
SL	–0.320	–0.333	0.244
LR	–0.281	0.109	0.080
CW	–0.324	–0.404	0.109
SW	–0.333	–0.040	0.250
WN	–0.072	0.482	0.607
IOW	0.057	–0.660	0.152
ML	–0.354	0.073	0.156
BM	–0.219	0.111	–0.459
SD	2.178	1.127	1.022
Eigenvalue	4.744	1.270	1.045
Prop of variance	0.431	0.115	0.095
Cumulative prop	0.431	0.547	0.642

DISCUSSION

Management plans of profitable natural resources should be applied taking into account fundamental biological aspects, including information proceeding from both neutral markers and quantitative traits because wild populations whose genetic variability has been eroded might have reduced ecological

TABLE 5. Pair-wise comparisons between populations by TukeyHSD Test. P values are given in parentheses

Pairs	PC1	PC2	PC3
CSA–AES	0.379 (0.860)	–0.540 (0.076)	–0.344 (0.418)
EDP–AES	0.532 (0.690)	–1.524 (0.000)	–0.144 (0.918)
EEE–AES	–0.537 (0.682)	–0.740 (0.006)	0.341 (0.427)
EDP–CSA	0.152 (0.990)	–0.984 (0.000)	0.200 (0.807)
EEE–CSA	–0.915 (0.233)	–0.200 (0.804)	0.685 (0.014)
EEE–EDP	–1.068 (0.124)	0.784 (0.003)	0.484 (0.138)

TABLE 6. Variance components and Q_{ST} values for each trait

Pop	Name	Variance	St. dev.	QST
	Nest	0.205	0.453	
TTL	Pop	0.000	0.000	0.000
	Residual	0.349	0.591	
	Nest	0.083	0.288	
SVL	Pop	0.000	0.000	0.000
	Residual	0.086	0.293	
	Nest	0.224	0.473	
DCL	Pop	0.000	0.000	0.000
	Residual	0.336	0.580	
	Nest	0.069	0.262	
SL	Pop	0.000	0.000	0.000
	Residual	0.167	0.409	
	Nest	0.049	0.220	
LCR	Pop	0.004	0.062	0.019
	Residual	0.139	0.373	
	Nest	0.164	0.404	
CW	Pop	0.000	0.000	0.000
	Residual	0.295	0.543	
	Nest	0.104	0.322	
SW	Pop	0.008	0.088	0.018
	Residual	0.284	0.533	
	Nest	0.030	0.172	
WN	Pop	0.006	0.077	0.047
	Residual	0.090	0.300	
	Nest	0.032	0.180	
IOW	Pop	0.018	0.132	0.119
	Residual	0.051	0.225	
	Nest	0.481	0.693	
ML	Pop	0.000	0.000	0.000
	Residual	0.698	0.836	
	Nest	11.100	3.330	
BM	Pop	0.000	0.000	0.000
	Residual	3.700	1.920	

TABLE 7. Heritability estimates of each trait in each sampled population

Population	TTL	SVL	DCL	SL	LCR	CW	SW	WN	IOW	ML	BM
EEE	0.67**	0.75**	0.29+	0	0	0.61**	0	0.41*	1.34**	0	1.81**
CSA	0.76**	1.27**	1.31**	1.04**	0.81**	1.19**	0.78**	0.46*	0.81**	1.35**	1.37**
EDP	0.72**	0.41*	0.59**	0.55**	0.23+	0.60**	0.65**	0.79**	0.38*	0.45*	0.62**
AES	1.13**	1.44**	0.84**	0.48*	0.65**	0.49*	0.42*	0.13	0.65**	0.71**	1.40**

+$0.05 \leq P < 0.1$.
*$0.01 \leq P < 0.05$.
**$P < 0.01$.

stability. Comparative studies of population differentiation in marker genes and genes coding quantitative traits have been performed in recent years (Merilä and Crnokrak, 2001; McKay and Latta, 2002), but no antecedent of these analyses is recorded in crocodilians.

In this study, we were able to identify a higher number of variable markers than other similar studies in crocodilians. Our estimates of P and H_e are higher than recorded values in crocodilians using isozymes (Gartside et al., '76; Menzies et al., '79; Lawson et al., '89; Flint et al., 2000). This may be attributed to the different ability of DNA and biochemical markers to detect polymorphisms. Using RAPD markers on agarose gels, Wu et al. (2002) found 3.11 and 10.88% polymorphism in two subpopulations of *Alligator sinensis*. The genetic distance between the two subpopulations was only of 0.0009. Our values of genetic distance were much higher. This difference may be partially explained by technique differences because we used polyacrylamide gels that typically detect more bands than agarose gels (Stift et al., 2003). However, the higher variability and among population differentiation may be a reflection of a particular population structure of *C. latirostris*. The studied populations showed low levels of gene flow and some population subdivision. Genetic distances were lower among EEE, CSA, and EDP populations, probably owing to the fact that all of them share stable environments where water levels are constant. In contrast, the AES population has a transitional environment that receives water and gene flow from two water basins (Parana and Salado Rivers), and may be subject to higher levels of disturbance that may influence their variability (Nevo, 2001). Also, other environmental aspects probably can be related to genetic variation, such as nesting habitat and sanitary status of the egg-laying females. Unfortunately these aspects could not be analyzed owing to limitations in the sampling design.

Variance components obtained from the RAPD analysis showed that although the sampling size was relatively small, we succeeded in capturing 73% of the total genetic variance within samples. The most part of H_e variance (ranging from 65 to 80%) was explained by variation among loci, suggesting that with the number of individuals sampled the probability of losing alleles is low.

The hierarchical analysis of variance of quantitative traits indicated that most differences occur among nests within populations. Individual ANOVAs failed to show significant differences among populations, but the MANOVA did yield highly significant differences. These results appear to suggest that considering trait by trait, the differences among populations are very low, but considering all traits as a global phenotype, the low differences would accumulate yielding significant results.

According to the PCA most of traits have similar contribution to PC1. This fact suggests that this component reflects body size differences. In PC2, traits CW, WN, and IOW have important contribution with different signs, suggesting that this axis is associated to body shape differences. In the same way, in PC 3 WN and SVL contributed with opposing signs reflecting body shape differences.

Variance components estimated by REML for the three principal components also showed higher levels of variance among nests than among populations. Although differentiation among populations is relatively low, pair-wise specific comparison revealed significant differences.

Sex can affect head morphometrics even in *C. latirostris* hatchlings, (Piña et al., 2007). Because of this, a considerable part of the variance found among clutches of the same population might be attributed to different sex ratio. Unfortunately, in this study, we could not determine sex because the diagnosis implies sacrificing the hatchlings, which was not possible because the

animals were part of stock of captivity specimens of Proyecto Yacaré. Currently, the group of Proyecto Yacaré is trying to diagnose sex in hatchlings using ultrasound (Piña, personal communication).

As only PC2 and PC3 showed significant differences in pair-wise comparisons we may conclude that the phenotypic differentiation is related with shape rather than size differences. Wu et al. (2006) in agreement with Verdade ('97) established that morphological variation was low in captive population of *A. sinensis* and *C. latirostris*. In this study we found high variation among nests in allometric measurements, perhaps because the animals proceed from wild populations.

Verdade (2000) observed sexual dimorphism in the upper region of the cranium of juveniles and adults of *C. latirostris*, and Piña et al. (2007) obtained similar results comparing newborns 24 hr after hatching. These authors suggested that this fact may be evolutionarily related to the visual recognition of sex when individuals exhibit only the top of their heads above the surface of the water. The fact that we found that head measurements as WN and IOW have important influence on morphological variability, seems to support the role of these traits in reproductive or, perhaps, feeding behavior. In order to study in depth the morphometrics in *C. latirostris* populations, we consider important, in further studies, to apply geometric morphometrics to compare their potential for discriminating populations in relation with traditional morphometrics (Maderbacher et al., 2008).

Comparative studies of population differentiation at both genetic and morphological levels are based on Wright's ('69) assumptions that the degree of quantitative trait differentiation among populations, as measured by the Q_{ST} index is comparable to that of the F_{ST} index, estimated from neutral marker genes. The relative magnitudes of these two indices are therefore informative about the role of natural selection and genetic drift as a cause of the observed degree of population differentiation (O'Hara and Merilä, 2005). Estimated F_{ST} values in this study were higher than Q_{ST} estimates, suggesting a higher contribution of neutral than adaptive loci to the genetic differentiation among populations. Quantitative traits are probably more related with fitness and the differentiation among populations remained relatively low. In spite of past events of overexploitation of *C. latirostris*, resulting in a reduction in population size, the genetic variability remained relatively high.

Estimated heritability values may be upward biased**,** because we did not take into account dominance, epistasis, maternal effects, and common environment, which increase covariance among full sibs (Lynch et al., '99), then our estimated values may be a valid upper limit for real heritability values. However, as most of differences among nests are highly significant, we suggest that an important genetic variability exist within populations for most traits in most populations. Besides, the high heritability estimates for some traits (TTL, SVL, CW, and BM) indicate great potential to improve them in management plans. In particular, TTL (total length), SVL (snout-vent length), and BM (body mass) are traits taken into account in the growing of animals in nurseries for commercial use in ranching programs. Owing to their high heritability values their response to directional selection is expectedly high.

The results so far obtained suggest that despite the reduction in population sizes underwent before 1990 (year of beginning of management program in Santa Fe, Argentina) owing to overexploitation, natural populations of *C. latirostris* have substantial genetic variation for both molecular markers and quantitative traits. Although more extensive samplings are needed, the present results encourage the development of breeding programs to genetically improve profitable traits.

ACKNOWLEDGMENT

This study was supported by Proyecto CAI+D 2002 (P.I. 102/PACT 20) from Universidad Nacional del Litoral, and Proyecto Yacaré (Gobierno Santa Fe/MUPCN), Santa Fe, Argentina. We thank to all members of Proyecto Yacaré, especially Pablo Siroski and Gisela Poletta for their assistance in providing samples. B. O. S. and J. C. V. are members of Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

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