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Genetic structure, population dynamics, and conservation of Black caiman (*Melanosuchus niger*)

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ABSTRACT

Microsatellite DNA polymorphisms were screened in seven populations of the largest Neotropical predator, the Black caiman *Melanosuchus niger* ($n = 169$), originating from Brazil, French Guiana and Ecuador. Eight loci were used, for a total of 62 alleles. The Ecuadorian population had the lowest number of alleles, heterozygosity and gene diversity; populations of the Guianas region exhibited intermediate diversities; highest values were recorded in the two populations of the Amazon and Rio Negro. During the last century *Melanosuchus* populations have been reduced to 1–10% of their initial levels because of hunting pressure, but no strong loss of genetic diversity was observed. Both the inter-locus *g*-test and the Pk distribution suggested no recent important recovery and/or expansion of current populations. On a global scale, the inter-population variation of alleles indicated strong differentiation ($F_{ST} = 0.137$).

Populations were significantly isolated from each other, with rather limited gene flow; however, these gene flow levels are sufficiently high for recolonization processes to effectively act at regional scales. In French Guiana, genetic structuring is observed between populations of two geographically close but ecologically distinct habitats, an estuary and a swamp. Similar divergence is observed in Brazil between geographically proximate "black water" and "white water" populations. As a consequence, the conservation strategy of the Black caiman should include adequate ecosystem management, with strong attention to preservation of habitat integrity. Distribution of genetic diversity suggests that current populations originated from the central Amazonian region. Dispersal of the species may thus have been deeply influenced by major climatic changes during the Holocene/Pleistocene period, when the Amazonian hydrographic networks were altered. Major ecological changes such as glaciations, marine transgressions and a hypothesized presence of an Amazonian Lake could have resulted in extension of Black caiman habitats followed by isolation.

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1. Introduction

The Black caiman *Melanosuchus niger* is the largest Neotropical predator. The species was formerly abundant in South America and present in the entire Amazon basin and in peripheral watersheds such as the eastern and western river drainages of the Guiana Shield region (Fig. 1). During the last century the Black caiman faced a strong hunting pressure for the leather industry (Smith, 1980; Plotkin et al., 1983), and a high rate of habitat loss. As a result, the total species population size may have decreased by 90% and concomitantly has experienced a high level of fragmentation (Ross, 1998). Remnant populations occur along the Amazon watershed, and in several edge locations, in French Guiana, North Brazil, southern Venezuela, Guyana, Peru and Bolivia. Although a significant fraction of populations benefit from protection programs, poaching still occurs in many areas. Black caimans, like other freshwater species, may also be threatened by mercury and lead contamination resulting from gold mining (Brazaitis et al., 1996; Uryu et al., 2001). Further, the Black caiman has life-history and ecological traits that are expected to limit its recovery. It is a sedentary diet and habitat specialist, found in slow-moving freshwater rivers, lakes, wetlands, black water swamps, and seasonally flooded areas of the Amazon. The species competes ecologically with the spectacled caiman *Caiman crocodilus*, a much more opportunistic species (Rebêlo and Magnusson, 1983; Herron, 1991; Herron, 1994; Thorbjarnarson, 1996) with population growth rate four fold higher than the Black caiman (Farias et al., 2004). To date, field surveys have been irregularly conducted all over the species' range, but available data reveal that the Black caiman is locally extinct in many Amazonian areas, and occurs in reduced densities in many others (Rebêlo and Lugli, 2001). On the other hand, field observations indicate that some populations may have recovered. The observations pertain mainly to Brazilian populations, since the complete protection of the Black caiman (Rebêlo and Magnusson, 1983; da Silveira and Thorbjarnarson, 1999), and possibly to Guyanan populations (Uruena, 1990).

Despite a recent change in the World Conservation Union directory which now considers the Black caiman "vulnerable" and no longer "endangered", its current conservation status remains precarious and highly dependant on conservation efforts at the population level (Hilton-Taylor, 2000).

The study of the patterns of genetic variation in natural populations is undoubtedly of major relevance for the conservation planning of threatened species (e.g., Hrbeck et al., 2005; Lawler et al., 2003; Mockford et al., 2005; Rivalan et al., 2006). Recent investigations of genetic diversity using the mitochondrial DNA cytochrome *b* gene confirmed a recovery potential of the species, and population expansion in some places (Farias et al., 2004). But mitochondrial DNA data also highlighted

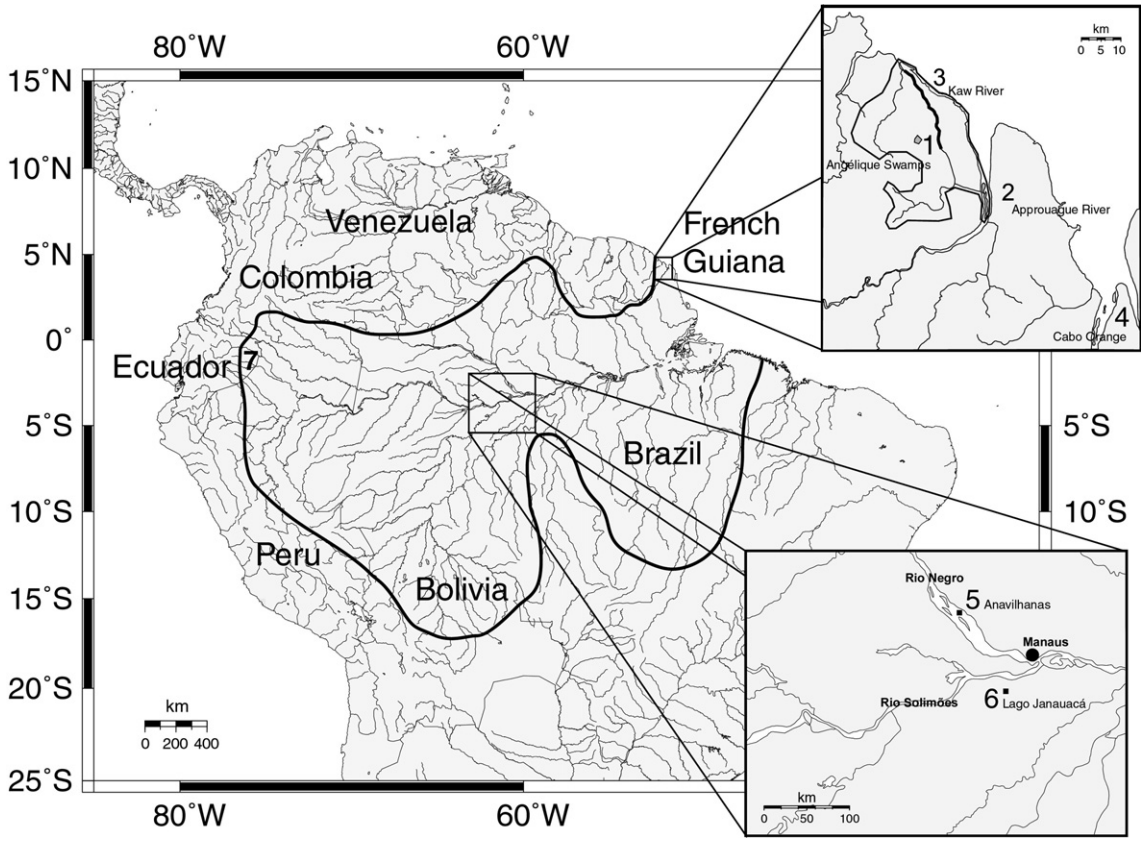

Fig. 1 – Distribution of the Black caiman *Melanosuchus niger* and locations of sampling sites (Bold line: distribution of the Black caiman. Numbers = locations of sampling sites. 1: Angélique swamps, French Guiana. 2: Approuague River, French Guiana. 3: Kaw River, French Guiana (in grey, Kaw Nature reserve). 4: Uaçá River, Brazil. 5: Rio Negro, Brazil. 6: Janauacá Lake, Brazil. 7: Cuyabeno Faunal Reserve, Ecuador).

that isolation-by-distance is a major characteristic of the species, and that some ecological constraints such as different water types of Amazônia (Sioli, 1984) could also play a role in population dynamics of this species (Farias et al., 2004).

In addition to mitochondrial DNA, evaluation of nuclear microsatellite DNA diversity is applied widely in conservation programs because of the high variability of these fragments, rendering them useful for the study of fine-scale population structure and contemporary evolutionary forces (Crandall et al., 2000). These tools have been sparsely used in crocodilians, with the notable exceptions of the American alligator *Alligator mississippiensis* (Davis et al., 2002), the broad-snouted caiman *Caiman latirostris* (Verdade et al., 2002) and the yacaré *Caiman yacare* (Godshalk, 2002). In order to contribute to a better knowledge of Black caiman ecology and of population dynamics in relation to conservation issues, we investigated microsatellite polymorphism in seven populations located in French Guiana, Brazil and Ecuador. Specific aims were (i) to use the allelic diversity and heterozygosity levels to assess the current status of populations (Frankham, 1996); (ii) to highlight gene flows and/or structuring of populations, helping for design and management of freshwater protected areas (Saunders et al., 2002); (iii) to assess recent population trends in relation to both major changes in habitat during the Pleistocene, and anthropogenic exploitation during the last century.

2. Material and methods

2.1. Study sites and sample collection

A total of 169 Black caimans was sampled from seven localities in French Guiana, Brazil, and Ecuador (Fig. 1). Three sites were located in the Kaw Swamp Nature Reserve (French Guiana): the Angélique swamps ($n = 35$ caimans) and the Kaw River ($n = 18$) are flooded swamps with black waters; the Approuague estuary ($n = 45$) is a mangrove coastal area with brackish waters. Three other sites were located in Brazil: the Uaçá River in the Cabo Orange National Park ($n = 14$) is a flooded herbaceous swamp with black waters; the Rio Negro ($n = 14$), in the gigantic Anavilhanas archipelago upstream of Manaus, where the habitat is an *igapó*, with floating meadows and acidic black waters; the Janauacá Lake ($n = 7$) is a confluence of fine rivers and creeks in connection with the Amazon River, the habitat is a *várzea*, i.e., seasonally flooded forest and meadows, with white type waters (Sioli, 1984). Animals of Ecuador ($n = 36$) were captured in the Cuyabeno Faunal Reserve, in the region of Lagartococha; this ecosystem is a seasonally flooded forest dominated by *Mauritia flexuosa* palms, with acid black waters.

Tissue samples were collected from the tail scutes obtained during the marking of animals and preserved in 95% ethanol.

2.2. Microsatellite genotyping

Scutes were dissolved overnight in a proteinase K/SDS solution and DNA was extracted with phenol/chloroform/isopropanol following standard procedures (Sambrook et al., 1989). Seven primer pairs originally developed for the broad-snouted caiman (Zucoloto et al., 2002): Claμ 2, Claμ 4, Claμ 5, Claμ 6, Claμ 7, Claμ 8, Claμ 9, and 28 primer pairs developed for the American alligator (Glenn et al., 1998; Davis et al., 2002): Amiμ 1, Amiμ 2, Amiμ 3, Amiμ 5, Amiμ 6, Amiμ 8 to Amiμ 20, Amiμ 101, Amiμ 102, Amiμ 202, Amiμ 203, Amiμ 204, Amiμ 208 Amiμ 210, Amiμ 212, Amiμ 213, were tested. Primer sequences of Amiμ 14 were modified as follows: sense 5′-ACA-ATTCCAGGTGGGGGGTG-3′; antisense 5′-CTTTCAGAGGGAG-CCAGGAACAAA-3′.

PCR amplifications were performed at a 15 μl final volume containing 1.5 mM $MgCl_2$, 1.2 mM dNTPs, ATGC-Biotechnologie Taq polymerase and 1 × Taq polymerase buffer, and ca. 15 ng genomic DNA. Amplifications were done as follows: denaturation 94 °C, 60 s; annealing at temperature recommended by authors, 60 s and latter optimized when necessary, extension 72 °C, 60 s for 30 cycles using one primer end-labelled with T4 polynucleotide kinase and [γ 33 P]-ATP. Products were separated on 8% polyacrylamide gels. For each locus, a Cytochrome *b* sequence was used as a size ladder to visualize the size of the amplified fragments from a first set of samples from several different animals; this yielded the actual size and the size range of the microsatellite. Subsequently, a sample of known size was loaded every six samples for use as standard for sizing microsatellite alleles. Amplified products were visualized after 24 h of exposure.

Among the 35 microsatellite loci tested, eight were successfully amplified and polymorphic in the Black caiman: Claμ 6, optimal annealing temperature $T_{opt} = 61$ °C; Claμ 8, $T_{opt} = 60$ °C; Amiμ 8, $T_{opt} = 57$ °C; Amiμ 16 and Amiμ 20, $T_{opt} = 56$ °C; Amiμ 11, $T_{opt} = 55$ °C; and Amiμ 13, $T_{opt} = 55$ °C, with 4.5 mM $MgCl_2$ instead of 1.5 mM; Amiμ 14 $T_{opt} = 55$ °C. Other primers did not amplify, or did not amplify optimally.

2.3. Genetic analysis

Population genetic analyses were performed using GENEPOP version 3.4 (Raymond and Rousset, 1995). Linkage disequilibrium was tested following a strict Bonferroni correction, using an exact contingency test for each pair of loci in each population. A global Fisher test of differentiation was obtained by combining the probabilities for each population. Genetic polymorphism for each population was measured as observed number of alleles (A), observed heterozygosity (H_o) and the heterozygosity expected under Hardy–Weinberg proportions (H_e). Gene diversity was estimated per locus and per population using an unbiased estimator (Nei, 1987) with the program FSTAT version 2.9.3.2. (Goudet, 1999). Deviation from Hardy–Weinberg equilibrium was tested using Fisher's exact test for fit of genotype proportion (Guo and Thompson, 1992) with the alternative hypothesis H_1 = heterozygote deficiency. The genetic structure of populations was examined by use of F_{IS}, the inbreeding coefficient of an individual relative to its subpopulation, and F_{ST}, a measure of the amount of differentiation among subpopulations (Weir and Cockerham, 1984).

The significance of F_{ST} was determined by a log-likelihood G-based test (Goudet et al., 1996). Isolation by distance was investigated by examining relationship between genetic and geographic distances, using the Mantel test (Mantel, 1967) implemented in the GENEPOP program. For this purpose, $F_{ST}/1\text{-}F_{ST}$ was used to construct the genetic matrix (Rousset,

1997). The strength of this relationship was further investigated with IBD software (Bohonak, 2002; Jensen et al., 2005) using a RMA regression with log-transformed genetic and geographic distances (Slatkin, 1993). Values of N_m, the number of migrants per subpopulation per generation, and the Nei's unbiased genetic distance (Nei, 1978) calculated for all pair-wise comparison of populations, were calculated with GENETIX v. 4.05.02 (Belkhir et al., 2001). Nei's distances were used to construct the genetic distance tree with Neighbor-Joining distances, and bootstrap values were calculated on individuals with 1000 replicates, using POPULATION 1.2.28 (http://www.cnrs-gif.fr/pge/bioinfo/populations/). Population structuring was further investigated with a Bayesian model-based clustering algorithm with STRUCTURE v.2 (Pritchard et al., 2000). The admixture model was used to determine the K number of ancestral clusters, by comparing log-likelihood ratios in multiple runs (50,000 run burn-in period, 1,000,000 iterations per run) for values of K ranging from 1 to 7. Evidence of bottlenecks was investigated using the program BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996), looking for a significant excess of heterozygosity . We implemented the two-phase model of microsatellite evolution (TPM) allowing 90% of mutations to follow the step-wise mutation model (SMM), and 10% of mutations to follow the infinite allele mutation model (IAM). Statistical significance was determined with a Wilcoxon signed rank test. In BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996) we also explored a mode shift in the distribution of allele frequencies which reflects a loss of low frequency alleles following a bottleneck. This test is more qualitative but allows detecting more recent bottlenecks, i.e., within the last dozen generations (Luikart and Cornuet, 1998), which in the case of the Black caiman would be approximately 100–150 years (Thorbjarnarson, 1996).

The Pk distribution method was used to evaluate the inference of recent population expansion; this method is based on the shape of the distribution of pair-wise differences in repeat number among all alleles at each locus, averaged across loci (Shriver et al., 1997). The inter-locus g-test for population expansion was also used (Reich and Goldstein, 1998). The variance of the variance of allele lengths is expected to be larger in constant-sized populations than in growing populations, because the variance of an allele length distribution depends mainly of the age of most recent bifurcations. A low value of the ratio $g = \mathrm{var}[Vj]/(4/3\ V^2 + 1/6V)$, where V = the average variance across the j loci, is interpreted as a sign of expansion. Cut-off values depend of sample sizes and number of loci: for 8 loci and 20–40 samples, one would not expect to see g-score below 0.15 unless a population expansion had occurred (Reich et al., 1999).

3. Results

3.1. Genetic diversity

No statistically significant linkage was observed among the eight loci ($p > 0.05$ for each pair of loci across all samples). The number of alleles per locus ranged from three for Amiμ 8 and Amiμ 14–13 for Amiμ 16, for a total of 62 alleles. The population in Ecuador had the lowest total number of alleles, as well as the lowest heterozygosity and gene diversity (Table 1). In contrast, high values were recorded in the two populations of the central Amazon area of Janauacá and Rio Negro. Due to small sample sizes, these values for these two populations may have been underestimated, and may thus reinforce the high genetic diversity in these areas. Intermediate heterozygosities and diversities were observed in populations of the Guianas region, French Guiana and Uaçá (Table 1). Among the seven populations, only caimans of Uaçá and Janauacá Lake were at Hardy–Weinberg equilibrium ($F_{IS} = -0.007$, $p = 0.3$; and $F_{IS} = -0.08$, $p = 0.6$, respectively). Other populations showed significant disequilibria with an overall heterozygote excess ($p = 10^{-4}$): Angélique $F_{IS} = -0.099$ ($p = 0.0001$); Approuague: $F_{IS} = -0.203$ ($p = 10^{-5}$); Kaw: $F_{IS} = -0.085$ ($p = 0.004$); Ecuador: $F_{IS} = -0.137$ ($p = 10^{-5}$); and Rio Negro: $F_{IS} = -0.136$ ($p = 0.01$).

3.2. Gene flow and structuring of populations

On a global scale, the inter-population variation of alleles indicated an overall differentiation ($F_{ST} = 0.137$), with g-like tests indicating significant population structure ($p < 10^{-5}$) at all loci (Table 2). Although all populations except Angélique and Kaw River were significantly isolated from each other, Fst values were rather low, ranging from 0.03 to 0.25. At a

Table 1 – Observed (H_o) and (H_e) expected Hardy–Weinberg heterozygosity, number of alleles (A), and gene diversity (GD) for the seven populations of *Melanosuchus niger*

Loci (total number of alleles)	Angélique ($n = 35$) (FG) $H_o/H_e/A/GD$	Approuague ($n = 45$) (FG) $H_o/H_e/A/GD$	Kaw ($n = 18$) (FG) $H_o/H_e/A/GD$	Ecuador ($n = 36$) $H_o/H_e/A/GD$	Uaçá ($n = 14$) (Br) $H_o/H_e/A/GD$	Janauacá ($n = 7$) (Br) $H_o/H_e/A/GD$	Rio Negro ($n = 14$) (Br) $H_o/H_e/A/GD$
Claμ 6 (10)	0.85/0.78/6/0.80	0.73/0.66/7/0.66	0.75/0.68/5/0.70	0.33/0.41/4/0.42	0.50/0.63/5/0.66	0.88/0.80/7/0.65	0.71/0.66/5/0.69
Claμ 8 (11)	0.86/0.78/6/0.79	0.86/0.78/6/0.73	0.89/0.78/ 6/0.79	1.00/0.55/5/0.59	0.92/0.80/6/0.83	1.00/0.80/7/0.85	1.00/0.80/7/0.83
Amiμ 8 (3)	0.06/0.20/2/0.21	0.22/0.36/3/0.37	0.11/0.35/2/0.36	0.00/0.00/1/0.00	0.00/0.00/1/0.00	0.00/0.00/1/0.00	0.64/0.50/2/0.51
Amiμ 16 (13)	0.71/0.68/4/0.69	0.69/0.57/3/0.57	0.61/0.58/4/0.59	0.56/0.57/4/0.58	0.53/0.45/4/0.47	0.63/0.75/7/0.81	0.86/0.86/10/0.90
Amiμ 14 (6)	0.46/0.43/3/0.44	0.71/0.51/4/0.51	0.47/0.36/2/0.39	0.53/0.50/4/0.51	0.33/0.48/4/0.50	0.63/0.49/4/0.52	0.64/0.54/4/0.56
Amiμ 13 (5)	0.57/0.47/2/0.47	0.42/0.45/2/0.46	0.39/0.48/2/0.50	0.22/0.24/2/0.24	0.36/0.38/2/0.39	0.38/0.49/2/0.54	0.57/0.50/5/0.52
Amiμ 11 (3)	0.80/0.48/2/0.48	0.98/0.50/2/0.50	0.88/0.49/2/0.50	0.53/0.39/2/0.39	0.67/0.48/2/0.49	0.88/0.55/3/0.57	0.64/0.43/2/0.45
Amiμ 20 (11)	0.74/0.70/4/0.72	0.82/0.69/4/0.69	0.89/0.77/6/0.79	0.56/0.57/6/0.58	0.73/0.66/5/0.68	1.00/0.80/5/0.84	0.57/0.50/3/0.52
All loci	0.63/0.57/29/0.58	0.67/0.56/31/0.56	0.62/0.56/29/0.58	0.47/0.40/28/0.41	0.51/0.49/29/0.50	0.67/0.59/36/0.6	0.70/0.60/38/0.62

n = Sample size; FG, French Guiana; Br, Brazil.

Table 2 – Fixation indexes (F_{ST}) between populations and associated probability (above); number of migrants (below)

	Angélique (n = 35)	Approuague (n = 45)	Kaw river (n = 18)	Ecuador (n = 36)	Uaçá (n = 14)	Janauacá (n = 7)	Rio Negro (n = 14)
Angélique	–	0.05 ($p < 10^{-5}$)	0.01 ($p = 0.2$)	0.19 ($p < 10^{-5}$)	0.09 ($p < 10^{-5}$)	0.07 ($p < 10^{-5}$)	0.13 ($p < 10^{-5}$)
Approuague	4.5	–	0.03 ($p = 3 \times 10^{-4}$)	0.25 ($p < 10^{-5}$)	0.09 ($p < 10^{-5}$)	0.10 ($p < 10^{-5}$)	0.19 ($p < 10^{-5}$)
Kaw river	29.4	7.8	–	0.24 ($p < 10^{-5}$)	0.05 ($p = 3 \times 10^{-3}$)	0.08 ($p < 10^{-5}$)	0.14 ($p < 10^{-5}$)
Ecuador	1.1	0.8	0.8	–	0.22 ($p < 10^{-5}$)	0.16 ($p < 10^{-5}$)	0.17 ($p < 10^{-5}$)
Uaçá	2.6	2.4	4.8	0.9	–	0.09 ($p < 10^{-5}$)	0.18 ($p < 10^{-5}$)
Janauacá	3.4	2.1	2.9	1.4	2.6	–	0.08 ($p < 10^{-5}$)
Rio Negro	1.6	1.1	1.5	1.3	1.1	2.8	–

regional scale, animals living in the black waters of Kaw swamp (Angélique swamp and Kaw River) in French Guiana were differentiated from those of the estuarine area of the Approuague (F_{ST} = 0.03, $p = 3 \times 10^{-4}$). Similarly, animals living in the Brazilian black waters of the lower Rio Negro were differentiated from the geographically proximal white water animals of Janauacá Lake (F_{ST} = 0.08, $p = <10^{-5}$) (Table 2). Gene flow levels ranged 0.8–29, and are below the critical threshold of one animal per generation only between the Ecuadorian population *vs.* other populations. According to the Mantel test, geographic distance significantly drives genetic isolation of populations (p = 0.005); RMA regression confirmed that geographic and genetic distances were correlated, despite a low correlation coefficient (r^2 = 0.56), possibly resulting from population structuring at small geographic levels. An unrooted tree of Nei's genetic distances (Fig. 2) graphically demonstrates the relationships and genetic divergence of animals of the studied populations. Low bootstrap values are concordant with the low level of structuring among the three groups of French Guiana. Bayesian population analysis assignment tests revealed a much higher probability of five ancestral clusters (p = 0.999; *vs.* $P < 0.001$ for 1, 2, 3, 4 and 7 clusters, and p = 0.001 for six clusters) (Fig. 3): one for Ecuadorian animals, two for the two central Amazon populations, one for the Angélique swamp, and one for animals of Kaw River, Approuague estuary and Uaçá River.

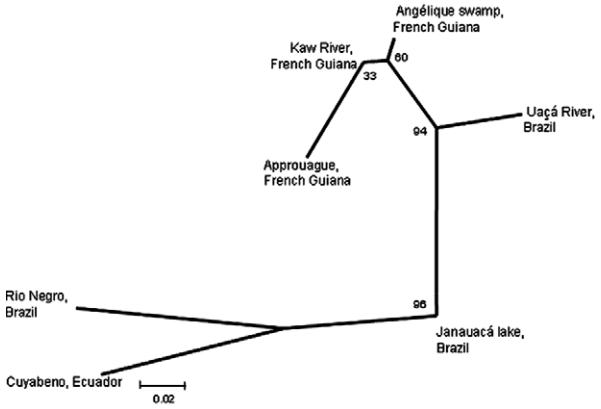

Fig. 2 – Nei's (1978) nuclear DNA distances between seven populations of Black caiman *Melanosuchus niger*, with associated bootstrap values.

3.3. *Population trends*

Evidence of a recent bottleneck was strong in the Angélique population, with both a significant Wilcoxon signed rank test (p = 0.01) and a shift in distribution of allele frequencies. On the Kaw River, only the Wilcoxon signed rank test was significant (p = 0.01), with no shift in distribution of allele frequencies; on Uaçá River, only the latter test was significant: for these two populations, bottlenecks were inferred to be less intense. Values obtained with the inter-locus g-test ranged from 1.1 and 1.5, far higher than expected cut-off thresholds. Also, the Pk distribution showed strong P_0 peaks for all populations: these two tests concordantly suggested no recent important recovery and/or expansion of populations.

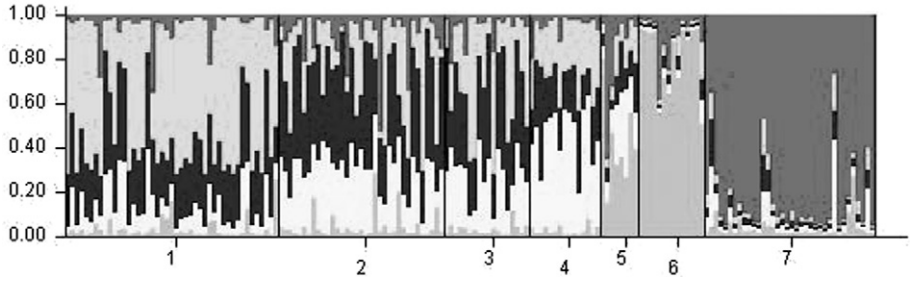

Fig. 3 – A Bayesian population assignment test based on eight microsatellite loci showing five distinct ancestral clusters in seven populations of Black caimans: 1 = Angélique Swamp, French Guiana, Guiana; 2 = Approuague River, French Guiana; 3 = Kaw River, French Guiana; 4 = Uaçá River, Brazil; 5 = Janauacá Lake, Brazil. 6 = Rio Negro, Brazil. 7 = Cuyabeno Faunal Reserve, Ecuador.

4. Discussion

The Black caiman was historically widely distributed in Neotropical freshwaters, but during the last century faced dramatic harvest pressure throughout its entire range: millions of *C. crocodilus* and *M. niger* skins were exported from Brazilian Amazônia over a few decades (Brazaitis et al., 1996). Black caiman populations have been reduced to 1–10% of their initial levels, and are now greatly fragmented (Groombridge and Wright, 1982; Ross, 1998). Such small populations are more prone to experience lower levels of genetic variability (Frankham, 1996), and isolation is expected to decrease both number of alleles and heterozygosity (Slatkin, 1985). But as we already reported for mitochondrial DNA haplotypes (Farias et al., 2004), nuclear DNA diversities are high in all populations, with genetic diversities ranging from 0.4 to 0.9 (Table 1). Heterozygosities in Black caimans are comparable to those recorded in large populations of the alligator *A. mississippiensis* (Davis et al., 2002). First, loss of genetic diversity subsequent to extensive but recent threats (i.e., some 5–10 generations ago), such as due to habitat fragmentation or over-harvesting, may be difficult to identify (Zhivotovsky et al., 2000). For instance, recent population isolation neither resulted in significantly lower genetic diversities in the American alligator (Ryberg et al., 2002) nor in the Australasian skink (Sumner et al., 2004). Similarly to Brazil, the distribution of Black caiman is now reduced in French Guiana compared to historical records (de Thoisy, 2004). Historical data on species abundances are scarce, but surveys conducted in 1993 showed mean kilometric index on several French Guiana rivers to be 10–15-fold higher than the current index calculated during the 1999–2003 period (de Thoisy, 2004). These last surveys also revealed that size structure of the Kaw River population is similar to that previously reported from depleted areas, with an absence of both hatchlings and large breeders (Rebêlo and Magnusson, 1983). In contrast, high numbers of animals, favorable size distributions, and presence of breeding areas were recorded in the Angélique swamp (de Thoisy, 2004), although no significant difference in genetic diversity between these two locations is observed. Thus, field monitoring remains a necessary complement to molecular approaches in conservation programs, mainly when focusing on species with low effective population sizes and low dispersal rates (Sumner et al., 2004).

The relatively high genetic diversities of individual populations could be maintained by migrants. Estimated migration rates in most cases have remained above the threshold of one migrant per generation (Mills and Allendorf, 1996; Wang, 2003). These gene flow levels may have helped to maintain current populations by preventing local extinction, and will probably be of importance for future population recovery. Structuring of populations nevertheless occurs, a result not unexpected given that the Amazon region is composed of several independent drainage systems, and has at least three chemically and limnologically distinct water types. Additionally, populations may become differentiated from each other via genetic drift as census sizes decrease (Frankham et al., 2002), for example as a result of over-harvesting. Our nuclear DNA results are indeed concordant with structuring revealed with mitochondrial DNA markers between populations occupying white waters *vs.* black waters (Farias et al., 2004). They also are concordant with a phylogeographic pattern revealing significant differentiation between Amazon basin and coastal Atlantic drainage populations of the related crocodilian *C. crocodilus* (Vasconcelos et al., 2006). Microsatellite markers even suggest population structuring at a very fine geographic scale, e.g., in French Guiana, between populations of two geographically close but ecologically distinct habitats: the Approuague River estuary and the Kaw-Angélique swamp located only 30 km away. Comparably strong differentiation is also observed between *M. niger* of the Anavilhanas Archipelago on the Rio Negro and the Januacá Lake which are separated by approximately 150 km. The observed genetic divergence between geographically proximate populations inhabiting different water types may be the result of selection. If selection is strong enough, even gene flow levels greater than one effective migrant per generation will not be able to homogenize the two populations (Endler, 1986; Frankham et al., 2002). Ecological processes structuring crocodile populations at small geographic scales have been reported in *Caiman latirostris* (Verdade et al., 2002), *C. yacare* (Godshalk, 2002) and *A. mississippiensis* (Ryberg et al., 2002). However, in contrast to the patterns of genetic structuring among populations of the Black caiman occurring in nutrient-rich white waters *vs.* the nutrient-poor and acid black waters, water type appears to have no observed consequences on gene flow among *C. crocodylus* populations (Vasconcelos et al., 2006). These ecological constrains on the Black caiman will require further investigation, as they will need to be considered for protected areas design, maintenance of source–sink systems and/or design of corridors (Mech and Hallet, 2001).

Further data on biology and ecology of the Black caiman also have to be gathered. Heterozygote excess recorded in most of populations could be also related to a gender-biased dispersal strategy of breeders (Lawler et al., 2003). In the American alligator, the mating system is based on multiple paternity and movements of females prior to copulation (Davis et al., 2002). Different microhabitat use between males and females has been suggested in Black caiman (da Silveira and Thorbjarnarson, 1999) and may indicate that suitable areas for nesting may not be equally distributed and may induce large movements of breeding females. As no data on gender dispersion are available for any Neotropical crocodilian species, further studies should be performed to understand male and female movements during the mating period.

Together with ecological data, the spatial distribution of microsatellite alleles may also be useful for understanding recent population history. Distribution of genetic diversity (Farias et al., 2004; this study: Table 1) suggests that current populations should have originated from the central Amazonian region. Greatest genetic diversity and phylogenetically oldest alleles are expected to be found in the center of a species distribution (Castelloe and Templeton, 1994). Dispersal of the species may thus have been deeply influenced by major climatic changes during the Holocene/Pleistocene period, when the Amazonian hydrographic networks faced alternations of low and high water levels. Expansion of freshwater ecosystems may have occurred during the "Lago Amazonica" hypothesized by Frailey et al. (1988) and Marroig and Cerqueira (1997) which would have resulted in an 50–150 m water

level increase above the current level of the Amazon watercourse, from 35,000 to 5000 BC. The lake would have influenced the distribution of numerous animal species (Marroig and Cerqueira, 1997). The central and western part of the distribution of the Black caiman (e.g., the Ecuadorian population) may also be related to this event. Nevertheless the lake did not connect the water drainages of Amapá such as the Uaçá River and eastern French Guiana with the Amazon, and thus may not explain the northern distribution of this species. Connectivity between these drainages probably occurred later. First, crocodiles may have colonized northern swamps areas during the last glacial periods, since salt water is supposed to be a barrier to dispersal for modern aligatorids (Brochu, 2001); low sea water level may thus have allowed expansion towards northern habitats. Later, a rise in sea level of 15–50 m at the Amazon mouth occurred ca. 5000 years ago (Vuilleumier, 1971). This rising did not cause a transgression, but rather backed up the Amazon River and resulted in freshwater flooding in the lower Amazon region, and the creation of "varzea-like" habitats which are the preferred habitat of *Melanosuchus*. This would have created a conduit for dispersal from the central Amazonian region to the swamps of Amapá and French Guiana. Finding of genetically differentiated populations with lower genetic diversity resulting from subsampling, genetic drift and bottleneck at the western-most (Faunal Reserve of Cuyabeno, Ecuador) and eastern-most (Angélique swamp and Kaw River, French Guiana; Uaçá Indigenous reserve, Brazil) populations further supports our hypothesis of geographic expansion from the central Amazon region. This expansion was followed by some isolation and fragmentation due to a combination of natural and anthropogenic processes. Indeed, considering a generation time of ca. 15 years for *M. niger* (Thorbjarnarson, 1996), these demographic events would have occurred 800–1200 generations ago, a time scale easily detectable with DNA microsatellite variations (Zhivotovsky et al., 2000).

As for the American alligator (Ryberg et al., 2002), the study of microsatellite polymorphisms suggests that the conservation strategy of the Black caiman should be focused at the regional level, including both protection of key sites (e.g., nesting areas), implementation and management of sustainable habitat use, and maintenance of strict habitat integrity and ecological functioning of the entire area, providing opportunities for the maintenance of gene flow. Although we were unable to highlight any population expansion, recolonization could be expected from pristine to depleted areas (Pulliam, 1988). Nevertheless, current pressures on habitats, including unmanaged tourism, together with persistence of poaching, may hinder these weak recolonization process, further accentuating the lower recovery rates of crocodiles in black waters due to the low productivity of these habitat types (Rebêlo and Lugli, 2001).

Although rare, some protected areas were established specifically to protect freshwater species and their habitats (Saunders et al., 2002). In South America, the Kaw Swamp Nature Reserve in French Guiana was initially created for the protection of the Black caiman. Similarly, *Arapaima gigas*, another neotropical freshwater predator, has been used as a focal species for the design of the Pacaya-Samiria National Reserve in Peru and the Mamirauá Sustainable Developmental Reserve in Brazil. Molecular investigations could be of major interest for the design or optimized management of such controlled areas. A recent analysis of mitochondrial DNA haplotypes of *A. gigas* suggest that *Arapaima* forms one large panmictic population that may best be managed within a source–sink metapopulational model (Hrbeck et al., 2005). Although genetically depleted, *A. gigas* appears not to be fragmented, and several areas of high genetic diversity were observed (Hrbeck et al., 2005). Furthermore, the natural distribution of *Arapaima* is almost entirely within Brazil, facilitating the implementation of conservation and management policies. In contrast, the conservation of Black caiman is more challenging. While the species is physically fragmented into geographically isolated populations, strong gene flow exists between the animals of the Kaw area (French Guiana) and Cabo Orange/Uaçá river (Brazil); these two regions should be managed as a single conservation unit. But so far these two protected areas are neither physically nor politically connected. Strong attention to the entire landscape quality and conservation, and a better knowledge of movement rates of animals (Fahrig, 2001) are critical points to be considered for an efficient regional and trans-border action plan for the most northern Black Caiman population.

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REFERENCES

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2001. GENETIX Version 4.02. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000. Université de Montpellier II, France.

Bohonak, A.J., 2002. IBD (Isolation By Distance): a program for analyses of isolation by distance. Journal of Heredity 93, 153–154.

Brazaitis, P., Rebêlo, G.H., Yamashita, C., Odierna, E.A., Watanabe, M.E., 1996. Threats to Brazilian crocodilian populations. Oryx 30, 275–284.

Brochu, C.A., 2001. Congruence between physiology, phylogenetics, and the fossil record on crocodilian historical biogeography. In: Grigg, G., Seebacher, F., Franklin, C.E. (Eds.), Crocodilian Biology and Evolution. Surrey Beatty and Sons, Sydney, Australia, pp. 9–28.

Castelloe, J., Templeton, A.R., 1994. Root probabilities for intraspecific gene trees under neutral coalescent theory. Molecular Phylogenetic and Evolution 3, 102–113.

Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., Wayne, R.K., 2000. Considering evolutionary processes in conservation biology. TREE 15, 290–291.

da Silveira, R., Thorbjarnarson, J.B., 1999. Conservation implications of commercial hunting of black and spectacled caimans in the Mamiraua sustainable development reserve, Brazil. Biological Conservation 88, 103–109.

Davis, L.M., Glenn, T.C., Strickland, D.C., Guillette, L.J., Elsey, R.M., Rhodes, W.E., Dessauer, H.C., Sawyer, R.H., 2002. Microsatellite DNA analysis support an East–West phylogeographic split of American Alligator populations. Journal of Experimental Zoology 294, 352–372.

de Thoisy B., 2004. French Guiana regional report. IUCN/SSC/ Crocodile Specialist Group Newsletter 24, 4–5.

Endler, J.A. (Ed.), 1986. Natural Selection in the Wild. Princeton University Press, Princeton, NJ.

Fahrig, L., 2001. How much habitat is enough? Biological Conservation 100, 65–74.

Farias, I.P., da Silveira, R., de Thoisy, B., Monjeló, L.A., Thorbjarnarson, J., Hrbek, T., 2004. Genetic diversity and population structure of Amazonian crocodilians. Animal Conservation 7, 1–8.

Frailey, C.D., Lavina, E.L., Rancy, A., de Souza, J.P., 1988. A proposed Pleiostene/Holocene lake in the Amazon basin and its significance to Amazonian geology and biogeography. Acta Amazonica 18, 119–143.

Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10, 1500–1508.

Frankham, R., Ballou, J.R., Briscoe, D.A. (Eds.), 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Glenn, T.C., Dessauer, H.C., Braun, M.J., 1998. Characterization of microsatellite DNA loci in American alligators. Copeia 3, 591–601.

Godshalk, R., 2002. Conservation genetics for *Caiman yacare* in Bolivia: potential forensic applications. In: Proceedings of the 16th Working Meeting of the Crocodile Specialists Group. IUCN, Gland, Switzerland, p. 219.

Goudet, J., 1999. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.8.

Goudet, J., Raymond, M., De Meeüs, T., Rousset, F., 1996. Testing differentiation in diploid populations. Genetics 144, 933–1940.

Groombridge, B., Wright, L. (Eds.), 1982. Amphibia–Reptilia Red Data Book. Part 1: Testudines, Crocodylia, Rhynocephalia. IUCN, Gland, Switzerland.

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48, 361–372.

Herron, J.C., 1991. Growth rates of Black caiman *Melanosuchus niger* and spectacled caiman *Caiman crocodylus*, and the recruitment of breeders in hunted caiman populations. Biological Conservation 55, 103–113.

Herron, J.C., 1994. Body size, spatial distribution, and microhabitat use in the caimans, *Melanosuchus niger* and *Caiman crocodilus*, in a Peruvian lake. Journal of Herpetology 28, 508–513.

Hilton-Taylor, C. (Ed.), 2000. 2000 IUCN Red List of Threatened Species. IUCN, Gland, Switzerland.

Hrbeck, T., Farias, I.P., Crossa, M., Sampaio, I., Porto, J.I.R., Meyer, A., 2005. Population genetic analysis of *Arapaima gigas*, one of the largest freshwater fishes of the Amazon basin: implications for Ist conservation. Animal Conservation 8, 297–308.

Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genetics 6, 13. Available from: <http:// phage.sdsu.edu/~jensen/>.

Lawler, R.R., Richard, A.F., Riley, M.A., 2003. Genetic population structure of the white sifaka (*Propithecus verraeauxi*) at Beza Mahafaly Special Reserve, southwest Madagascar (1992–2001). Molecular Ecology 12, 2307–2317.

Luikart, G., Cornuet, J.M., 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12, 228–237.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27, 209–220.

Marroig, G., Cerqueira, R., 1997. Plio-Peistocene South American history and the Amazon Lagoon Hypothesis: a piece in the puzzle of Amazonian diversification. Journal of Comparative Biology 2, 103–119.

Mech, S.G., Hallet, J.G., 2001. Evaluating the effectiveness of corridors: a genetic approach. Conservation Biology 15, 467–474.

Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per generation rule in conservation and management. Conservation Biology 10, 1509–1518.

Mockford, S.W., McEachern, L., Herman, T.B., Snyder, M., Wright, 2005. Population genetic structure of a disjunct population of Blanding's turtle (*Emydoidea blandingii*) in Nova Scotia, Canada. Biological Conservation 123, 373–380.

Nei, M., 1978. Estimates of average heterozygosity and genetic distance from a small number of individuals between populations. Genetics 89, 583–590.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Plotkin, M.J., Medem, F., Mittermeier, R.A., Constable, I.A., 1983. Distribution and conservation of the black caiman (*Melanosuchus niger*). In: Rhodin, A., Mitaya, K. (Eds.), Advances in Herpetology and Evolutionary Biology. Museum of Comparative Zoology, Cambridge, MA, pp. 695–705.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Pulliam, H.R., 1988. Sources, sinks, and population regulation. American Naturalist 132, 652–661.

Raymond, M., Rousset, F., 1995. GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity 86, 248–249.

Rebêlo, G.H., Lugli, L., 2001. Distribution and abundance of four caiman species (Crocodilia: Alligatoridae) in Jau National Park, Amazonas, Brazil. Revista de Biologia Tropical 49, 1095–1109.

Rebêlo, G.H., Magnusson, W.E., 1983. An analysis of the effect of hunting on *Caiman crocodilus* and *Melanosuchus niger* based on the sizes of confiscated skins. Biological Conservation 26, 95–104.

Reich, D.E., Goldstein, D.B., 1998. Genetic evidence for a Paleolithic human population expansion in Africa. Proceedings of the National Academy of Sciences 95, 8119–8123.

Reich, D.E., Feldman, M.W., Goldstein, D.B., 1999. Statistical properties of two test that use microsatellite data sets to detect population expansions. Molecular Biology and Evolution 16, 453–466.

Rivalan, P., Dutton, P.H., Baudrya, E., Roden, S.E., Girondot, M., 2006. Demographic scenario inferred from genetic data in Leatherback turtles nesting in French Guiana and Suriname. Biological Conservation 130, 1–9.

Ross, P. (Ed.), 1998. Crocodiles: An Action Plan for their Conservation. IUCN, Gland, Switzerland.

Rousset, F., 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145, 1219–1228.

Ryberg, W.A., Fitzgerald, L.A., Honeycutt, R.L., Cathey, J.C., 2002. Genetic relationships of American alligator populations distributed across different ecological and geographic scales. Journal of Experimental Zoology 294, 325–333.

Sambrook, J.E., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning a Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, New York.

Saunders, D.L., Meeuwig, J.J., Vincent, A.C.J., 2002. Freshwater protected areas: strategies for conservation. Conservation Biology 16, 30–41.

Shriver, M.D., Jin, L., Ferrel, R.E., Deka, R., 1997. Microsatellite data support an early population expansion in Africa. Genome Research 7, 586–591.

Sioli, H., 1984. The Amazon and its main affluents: hydrography, morphology of the river courses and river types. In: Sioli, H. (Ed.), The Amazon. Limnology and Landscape Ecology of a Mighty Tropical River and its Basin. Dr. W. Junk Publishers, Dordrecht, The Netherlands, pp. 127–165.

Slatkin, M., 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics 16, 393–430.

Slatkin, M., 1993. Isolation by distance at equilibrium and non-equilibrium populations. Evolution 47, 264–279.

Smith, N.J.H., 1980. Caimans, capybaras, otters, manatees and man in Amazônia. Biological Conservation 19, 177–187.

Sumner, J., Jessop, T., Paetkau, D., Moritz, C., 2004. Limited effect of anthropogenic habitat fragmentation on molecular diversity in a rain forest skink, *Gnypetoscincus queenslandiae*. Molecular Ecology 13, 259–269.

Thorbjarnarson, J.B., 1996. Reproductive characteristics of the order Crocodylia. Herpetologica 52, 8–28.

Uruena, E., 1990. Black caiman in Guyana. Crocodile Specialist Group Newsletter 9, 24.

Uryu, Y., Malm, O., Thornton, I., Paynes, I., Cleary, D., 2001. Mercury contamination of fish and its implications for other wildlife of the Tapajós basin, Brazilian Amazon. Conservation Biology 15, 438–446.

Vasconcelos, W.R., Hrbek, T., Da Silveira, R., de Thoisy, B., Marioni, B., Farias, I.P., 2006. Population genetic analysis of *Caiman crocodilus* (Linnaeus, 1758) from South America. Genetics and Molecular Biology 29, 220–230.

Verdade, L.M., Zucoloto, R.B., Vilela, P.M.S., Coutinho, L.L., 2002. Genetics of *Caiman latirostris*. In: Proceedings of the 16th Working Meeting of the Crocodile Specialists Group. IUCN, Gland, Switzerland, p. 212.

Vuilleumier, B.S., 1971. Pleistocene changes in the fauna and flora of South America. Science, 771–779.

Wang, J., 2003. Application of the one-migrant per generation rule to conservation and management. Conservation Biology 18, 332–334.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Zhivotovsky, L.A., Bennett, L., Bowcock, A.M., Feldman, M.W., 2000. Human population expansion and microsatellite variation. Molecular Biology and Evolution 17, 757–767.

Zucoloto, R.B., Verdade, L.M., Coutinho, L.L., 2002. Microsatellite DNA library for the *Caiman latirostris*. Journal of Experimental Zoology 294, 346–351.