Journal of Herpetology, Vol. 25, No. 3, pp. 357–361, 1991

Genetic Variation in Populations of the American Crocodile

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The 11 extant species of the genus *Crocodylus* appear to be minimally divergent biochemically, despite their wide, circumtropical distribution. In his studies of the biochemical and immunological systematics of Crocodilia, Densmore (1981, 1983) found marked biochemical similarities among species of crocodiles. Specifically, starch gel electrophoresis of proteins coded by

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TABLE 1. Gene frequencies of three populations of *Crocodylus acutus*. Abbreviations: JM = Jamaica; FL = Florida; DR = Dominican Republic; alb = plasma albumin; alpha and beta globulins = those plasma protein zones; per = peroxidase; pep = peptidases; est = esterases; ald = aldolase; np = nucleoside phosphorylase; hk = hexokinase; mpi = mannose phosphate isomerase; g6pd = glucose-6-phosphate dehydrogenase; pgm = phosphoglucomutase; pgi = phosphoglucose isomerase; ca = carbonic anhydrase; ldh = lactate dehydrogenase; 6pgd = 6-phosphogluconate dehydrogenase; mdh = malate dehydrogenase; fh = fumarase; cat = catalase; sod = super oxide dismutase. Buffers are listed in the order of best results and preference. See methods and materials for details.

Locus / Population	Buffer	Genotype frequency: ranked from most to least anodal 1/1	1/2	2/2	2/3
alb	E				
JM		20			
FL		9			
DR		28			
alpha-1g	E				
JM		11	5	4	
FL		6	1	2	
DR		11	13	4	
alpha-2g	E				
JM		18	2		
FL		7	2		
DR		21	4	3	
alpha-3g	E				
JM		17	3		
FL		5	3	1	
DR		14	12	2	
beta g	E				
JM		20			
FL		9			
DR		28			
per 1	A, E				
JM				17	
FL		1		8	
DR				26	
per 2	A, E				
JM		12	5	4	
FL		5	3	2	
DR		24	5		
pep d	E				
JM		17			
FL		8			
DR		25			
pep a	E				
JM		10			
FL		20			
DR		24			
est 1	E, A				
JM		20			
FL		11			
DR		29			
est 2	E, A				
JM		13	1	2	
FL		8			
DR		18	3		
est 3	E, A				
JM			6	9	
FL		1	5	5	
DR		2	14	13	
ald 1	B, E				
JM			1	16	
FL			5	18	
DR			5	26	
ald 2	B, E				
JM		17			
FL		19			
DR		30			
np	B, E				
JM		2	1	15	
FL		1	4	18	1
DR		7	9	14	
hk 1	B				
JM		18			
FL		18			
DR		23			
hk 2	B				
JM		18			
FL		10			
DR		20			
mpi	B				
JM				9	
FL			1	9	
DR				14	
g6pd 1	B				
JM			3	8	
FL			3	11	
DR			17	8	
g6pd 2	B				
JM		12			
FL		12	2		
DR		19	7		
pgm	B, D, A				
JM				15	1
FL			7	11	3
DR			6	21	1
pgi	C, B				
JM		21			
FL		22	2		
DR		34			
ca	D, B, A				
JM			1	17	
FL		4	10	11	
DR			25	10	
ldh 1	A				
JM				18	1
FL			4	17	2
DR			2	26	
ldh 2	A				
JM		15	2		
FL		19	4		
DR		21	7	1	
6pgd	B, C, D				
JM				15	
FL			1	15	
DR			5	20	

TABLE 1. Continued.

Locus / Population	Buffer	Genotype frequency: ranked from most to least anodal 1/1	1/2	2/2	2/3
mdh 1	D				
JM				16	
FL		3	3	12	
DR			1	28	
mdh 2	D				
JM		16			
FL		15	2	1	
DR		26	4		
fh	D				
JM		13			
FL		11			
DR		16	2		
cat	E				
JM		10			
FL		13			
DR		20			
sod 1	E				
JM		11			
FL		16			
DR		21			
sod 2	E				
JM		11			
FL		18			
DR		18	2		

21 presumed gene loci failed to differentiate clearly more than two species of *Crocodylus*, including species from four different zoogeographic realms. Such protein conservatism may be characteristic of the Crocodilia in that three studies of *Alligator mississippiensis* found a low degree of heterozygosity (Gartside et al., 1977; Menzies et al., 1979; Adams et al., 1980). Three disjunct populations were distinguishable by the presence of distinctive allele frequencies at only three loci. Given the emerging pattern of genetic conservatism among the Crocodilia, it is of interest to consider further the question of allozymic variation within other species of this group.

We report a study of genetic variation in three populations of the American crocodile (*Crocodylus acutus*). Animals were sampled from three disjunct populations in Florida, Jamaica, and the Dominican Republic. Genotype frequency data were obtained from 32 presumptive protein loci in plasma, red blood cells and, in some cases, muscle tissue. We tested the hypothesis that these populations are not electrophoretically distinguishable. The results are relevant to an understanding of crocodilian genetics, gene flow among insular populations, and crocodilian conservation.

Crocodiles (25 individuals) from Florida were collected from the wild in Florida Bay from 1979–1982. Animals of Jamaican origin (N = 21) were obtained from the living collection at Gatorama in Palmdale, Florida, in April 1979. Nine were from animals collected in Jamaica, and twelve from offspring, most known to be from different parents than those sampled. Samples from Dominican Republic animals were collected at the National Zoological Park (Zoodom) in Santo Domingo. Blood was obtained from 35 unrelated animals, as documented by Zoo records, ranging from three months of age to adults. Most of the animals originated in Lago Enriquillo, Dominican Republic.

Blood samples were obtained by vein puncture (Gorzula et al., 1976). Sample volumes ranged from 0.3 ml from hatchlings to 10 ml from adults. In some cases, entire hatchlings which did not survive hatching were frozen intact. Muscle tissue was sometimes obtained from adults by biopsy from the inner surface of the hind leg (Menzies et al., 1983). A small incision (about 2 cm) was made through the skin to the surface of the muscle, a small piece was removed, and the wound painted with gentian violet.

Collection and transport conditions for samples collected from Jamaican and Dominican Republic animals were similar; red cells and plasma were separated when collected and frozen separately, and muscle tissue was also frozen; all samples were transported to the laboratory on dry ice. Samples from Florida animals were collected in the field over several years. Blood and tissue were kept on ice until centrifugation of blood and freezing could be done on shore. Specimens were transported to the laboratory frozen, as above.

For electrophoresis, plasma was analyzed directly or diluted ten fold with a solution of 10 mM sodium phosphate, 1 mM EDTA, 1 mM Mg^{++}, and 1 mM beta mercaptoethanol, pH 7.5. Red cell pellets and muscle tissue were homogenized in two to four volumes of the same solution. The homogenate was centrifuged at 10,000 × g for 20 min and the supernatant saved for electrophoretic analysis.

Electrophoretic procedures used have been described earlier (Harris and Hopkinson, 1976; Menzies and Kerrigan, 1979; Menzies, 1981). In most cases, vertical starch gel electrophoresis was used. A mixture of 11% Electrostarch (Electrostarch Corp., Madison, Wisconsin) and 2% Connaught Starch (Fisher Scientific, Inc.) was used with a variety of buffers (see Table 1). (A) Bridge; 0.1 M Tris base, 0.025 M citrate, pH 7.5; Gel; 1:10 dilution, pH 7.5. (B) Bridge; 0.1 M Tris base, 0.1 M Maleate, 0.01 M EDTA, 0.01 M $MgCl_2$, pH 7.4; Gel; 1:10 dilution, pH 7.4. (C) Same as B but no $MgCl_2$ or EDTA. (D) Bridge; 0.1 M sodium phosphate, 0.0001 M NADP in cathode buffer, pH 7.0; Gel; 1:40 dilution with NADP added after degassing. (E) Bridge; 0.3 M borate, pH 8.6; Gel; 0.005 M Tris citrate, 0.01 M $MgCl_2$, pH 8.6. Staining procedures for enzyme and proteins were as described by Harris and Hopkinson (1976). Gels were scored with the gel reader not knowing the origin of coded samples. One or more samples whose genotype was known was placed on each gel to minimize ambiguity in scoring.

The standard deviation of allele frequency was calculated as $s = (pq/n)^{0.5}$, where p and q are the allele frequencies of the most and least common allele, respectively, and n is the number of alleles observed (Kempthorne, 1957). When more than two alleles were present, q was taken as the composite frequency of the group of least common alleles. Tests of significance by t, G and χ^2 procedures were done as described in Sokal and Rohlf (1969), and Nei (1987). Observed average heterozygosity (H_o) was obtained by averaging heterozygote frequencies over all loci.

TABLE 2. Genetic variation in three populations of *Crocodylus acutus*.

Variation[1] parameters	Population		
	Jamaica	Florida	Dominican Republic
% polymorphism[2]	40.6	59.4	59.4
H_o	0.055	0.117	0.158
Variance (H_o)	0.009	0.017	0.039
H_e	0.0813	0.1600	0.1492
Variance (H_e)	0.0005	0.0009	0.0009

[1] Calculations of H_o, H_e and their variances are described in the text.

[2] Locus considered polymorphic if frequency of most common allele ≤ 0.99.

TABLE 3. Genetic relatedness of three populations of *Crocodylus acutus*.

Population comparisons	D*	Var D*	Allele frequency differences[1]
Jamaica vs. Florida	0.0098	0.00001657	est-2, pgm, ca, ldh-1, mdh-1, mdh-2
Jamaica vs. Dom. Rep.	0.0131	0.00002203	alpha 2G, alpha 3G, per-2, np, g6pd-2, pgm, ca, 6pgd, mdh-2
Florida vs. Dom. Rep.	0.0104	0.00001653	np, g6pd-1, mdh-1

[1] Loci showing significant allele frequency differences based on t-test analysis and by Chi square according to Nei (1987).

Estimated average heterozygosity per locus (H_e) and variance were calculated according to Nei (1975, 1987). Genetic distance (D) was calculated as defined by Nei (1975, 1987), but modified by Hillis (1984), and Tomiuk and Graur (1988) (as D*). Variance of D* was calculated according to Tomiuk and Graur (1988).

Thirty-two presumed protein loci were detectable in most of the specimens. Table 1 lists the numbers of animals in each genotype group per locus, per population. Table 2 summarizes several indices of genetic variability. Percent polymorphism ranged from 40.6% for the Jamaican population to 59.4% for both the Florida and Dominican Republic populations (using an allele frequency of ≤0.99 as the cut-off criterion). Observed frequencies of heterozygotes per locus (H_o) were 0.055, 0.117, and 0.158 for Jamaica, Florida, and the Dominican Republic, respectively; estimated heterozygosities per locus (H_e) were 0.081, 0.16, and 0.149 for those populations.

Genetic Distance (D*) was of the order 0.01, indicating very few codon differences between population pairs (Table 3). By this criterion, little genetic differentiation has occurred. However, in each case the D* calculated over all loci was significantly different from zero. Allele frequencies were also compared for population pairs at each locus. According to Nei (1987), if a significant difference exists at a locus between population pairs, then the genetic distance is greater than zero. Table 3 lists those loci that had significant allelle frequency differences as tested by both t-test, using the variance described by Kempthorne (1957), and χ^2, as described by Nei (1987). Therefore, these loci are the basis of the non-zero genetic distance, D*, over all loci.

The electrophoretic analyses indicate that more genetic variation exists within populations of the American crocodile than in most other species of vertebrates (Nevo, 1978), especially the American alligator, the only other crocodilian studied on a populational basis (Gartside et al., 1977; Menzies et al., 1979; Adams et al., 1980). Because H_o values for the Florida and Dominican Republic populations were similar as were those of H_e, we do not believe the high heterozygosity was a sampling artifact. The lower heterozygosity (0.081) of the Jamaican sample may reflect some genetic relatedness among the captive individuals sampled. The high heterozygosity was not a function of certain protein groups often associated with high polymorphism. Removing data of protein groups such as hydrolases and plasma proteins, caused a decrement of the order of .01 to .03 in H_o and H_e.

The high genetic variation we found was also unexpected because of the results of Densmore's (1981, 1983) studies. He found *C. acutus* to be biochemically indistinguishable from other congenerics on the basis of qualitative genetic data derived from single or few samples per species. It now seems that species of *Crocodylus* (at least *C. acutus*) might be distinguishable from each other on the quantitative basis of gene frequencies, rather than on fixed allelic differences. This was also the case in each of the alligator studies cited.

Nonetheless, our results are consistent with Densmore's (1983) principal conclusion that the species of *Crocodylus* are closely related. We calculate genetic distances, D*, between populations on the order of 0.01, indicating little divergence has occurred. Because the land masses where these populations reside have been separated for 40–80 million years (Hedges, 1982), it is remarkable that more differentiation or even speciation has not taken place if the populations were separated at the time of land mass separation. Thus, Densmore's (1983) suggestion is plausible, that the genus radiated post-Pliocene, and the various Caribbean populations arose by colonization rather than by vicariance. The same mechanism is likely responsible for periodic gene exchange which retarded genetic differentiation. Occasional gene influx could also serve to maintain high heterozygosity. Periods of greater or lesser gene flow might be related to changes in sea levels and currents in the Caribbean and Florida Straits over Pliocene and Pleistocene periods (Riggs, 1984).

Occasional movement of crocodiles between various populations seems possible under certain conditions (Densmore, 1981; Dunson, 1982). In recent years, American crocodiles in Florida have been observed at sea and are known to travel more than 100 km (Kushlan and Mazzoti, 1989). Distances between Florida, Cuba, Yucatan, Jamaica and Hispanola are on the same order of magnitude. If aided by currents, especially wind-driven as in the case of hurricanes, adults of this species should be able to survive such a trip.

These results have implications for the conservation of crocodile populations. American crocodile populations differ in their quantitative genetics and some of this variation may be of adaptational value. From the standpoint of conserving natural gene pools of crocodiles, restocking approaches to conservation should be considered only as a last resort. The conservation of crocodilians should be undertaken on a populational rather than species basis by approaches such as suggested by Kushlan (1988).

Acknowledgments.—We especially thank the curators who permitted us access to their collections. C. Clemons, of Gatorama, Palmdale, Florida, graciously allowed us to obtain samples from his valuable collection of Jamaican crocodiles. The staff of Zoodom in the Dominican Republic was extraordinarily helpful. We thank especially Dr. A. Ferreira, J. A. Ottenwalder, and C. Sanlley (Dominican Republic) for their support and assistance. We wish to thank S. Raney, E. Menzies and Lyle Kochinski (NOVA University Oceanographic Center), Frank Mazzotti, and Terri Jacobsen for their assistance in this study. This study was supported in part by the National Park Service, Nova University Oceanographic Center, Academy of Marine Sciences (Miami), Institution for Medical and Marine Research, and NSF grant DAR 8009353.

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Accepted: 6 March 1991.