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Transferrin polymorphism in Amazon turtle (*Podocnemis expansa*) stocks

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ABSTRACT

The transferrin gene locus (Tf) was investigated in five populations of the Amazon turtle (*Podocnemis expansa*) sampled from five geographical areas in the Amazon region. This locus was polymorphic, showing three genotypes (Tf^aTf^a, Tf^aTf^b and Tf^bTf^b), presumably encoded by two co-dominant alleles, Tf^a and Tf^b. All populations showed good genetic balance according to Hardy-Weinberg expectations, and may sustain the hypothesis of a single stock in the area investigated. The data are consistent with free flow of genes among the population samples examined.

INTRODUCTION

The Amazon turtle (*Podocnemis expansa*) is considered the biggest freshwater Chelonia in South America. With a wide distribution in the Amazon region, this species populates diverse water bodies (rivers, natural canals, and lakes). Among all the Amazon Chelonia, it is the most appreciated by man both as food and sub-products, such as cosmetics. Popular local dishes include *sarapatel* (a stewed mixture of viscera, fat and blood), eggs, etc.

Conservation and management programs of Amazon turtle have been carried out for about three decades by the former Instituto Brasileiro de Desenvolvimento Florestal (IBDF), now known as Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA).

Several studies have been made on biological and reproductive aspects (Alho and Pádua, 1982a,b; Alho *et al.*, 1984, 1985; Danni and Alho, 1985). Frair *et al.* (1978) studied blood proteins to examine phylogenetic relationships in the genus *Podocnemis*, including *P. expansa*. However, very little is known about the population structure of this species. As for any other organism, Amazon Chelonia management, specifically of the Amazon turtle, depends on whether this species forms a single stock or different stocks, genetically adapted to their respective distributions. In the former case the animals could be transported without questioning their origin. In the latter case, it would be advisable to respect and conserve the independent genetic character of each stock unit.

Several genetic markers (proteins and specific enzymes) have been analyzed by a series of genetic-biochemical methods (e.g., starch gel electrophoresis). These have been used for identifying and delimiting commercial fish stocks. These methods provide protein gene data which have helped in the conservation and practical management of the main marine teleost species (Jamieson and Turner, 1978; Smith and Robertson, 1981; Jamieson and Birley, 1989).

Among the various genetic markers so far examined, the transferrin locus, which shows a high level of polymorphism in many vertebrate species, appears to be very efficient for distinguishing fish stocks of commercial importance in the North Atlantic Ocean (Jamieson and Turner, 1978; Jamieson and Smith,

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1987; Jamieson and Birley, 1989). The transferrin locus, separated by electrophoresis, has also demonstrated promising results in genetic studies performed at the Instituto Nacional de Pesquisas da Amazônia (INPA) involving commercial fish species in the Amazon (Teixeira and Jamieson, 1985; Teixeira *et al.*, 1990).

The Amazon turtle was chosen in the present study because: 1) it represents an important item of the Amazon populations' diet, 2) there is a lack of information on the variability of this species, and 3) this study could help IBAMA to establish the conservation and management policy of the species.

MATERIAL AND METHODS

A total of 205 specimens of the Amazon turtle (*P. expansa*) were caught from five geographical areas in the Amazon region (Figure 1), separated from each other by an average distance of 860 km. Blood specimens were drawn from the femoral artery into 5-ml BD vacutainer tubes, containing 0.5 ml of 3.8% sodium citrate as anticoagulant. Blood plasma specimens were separated by centrifugation at 3,000 rpm for 20 min and stored at -25°C, until electrophoresis was run. A rivanol precipitation method was applied for isolating plasma transferrins prior to electrophoresis (Jamieson and Turner, 1978). The blood plasma specimens were treated with rivanol solution (2%) at a ratio of 1:1. Hatchlings, juveniles and adults were sampled at one locality.

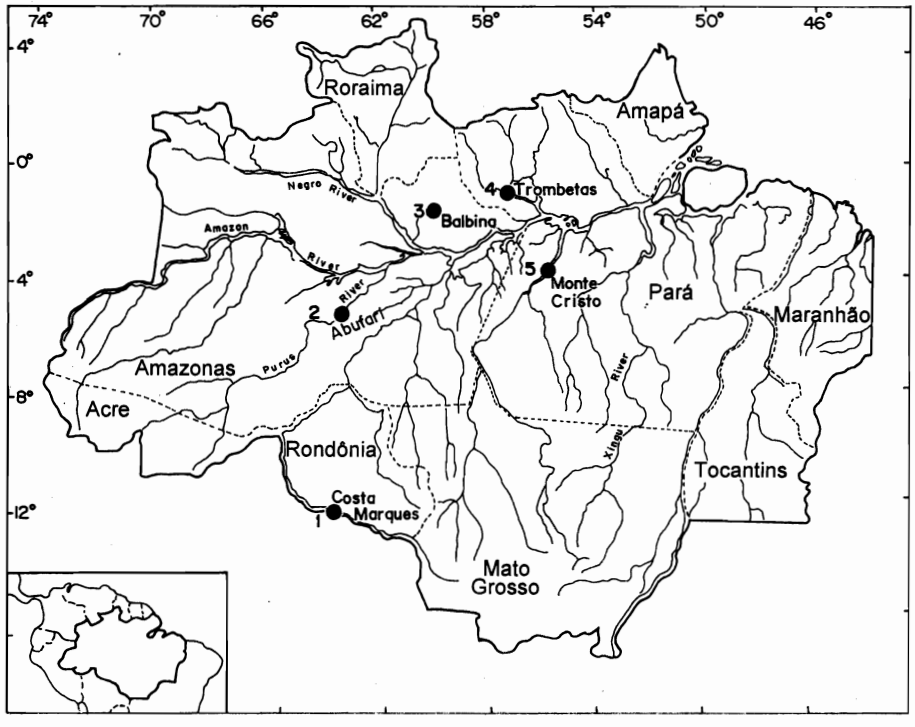

Figure 1 - The sampling locations of the Amazon turtle (*Podocnemis expansa*).

The electrophoresis procedures for the preparation of gel-electrode buffer systems (Tris-citrate; lithium hydroxide-boric acid), preparation of starch gel medium, sample application and gel staining (1% amido Black for 5 min) followed Jamieson and Turner (1978). In the absence of an electrophoretic destaining apparatus, the gel slices were immersed several times in a vessel containing a solution of 5:5:1 water, methanol and acetic acid, respectively, for about 15 h.

The two putative transferrin alleles were classified as **a** and **b**.

Statistical tests for genetic balance assuming Hardy-Weinberg equilibrium were applied to compare the observed and expected numbers of transferrin genotypes within and between the Amazon turtle population samples examined. Contingency tests on the overall transferrin allele distributions were applied by calculating chi-square (χ^2) and log-likelihood ratio chi-square (G^2) values in the population samples.

RESULTS

Transferrin genotypes in the Amazon turtle

The genotypes designated Tf^aTf^a, Tf^aTf^b, and Tf^bTf^b were observed in the Amazon turtle (*P. expansa*) and were assumed to be encoded by two co-dominant alleles (Tf^a and Tf^b) segregating at the Tf gene locus (Figure 2). The electrophoretic mobility of allele **a** toward the anode exceeded that of allele **b**. Homozygotes showed a single transferrin band, whereas heterozygotes presented two bands, suggesting a monomeric structure for transferrin molecules and a co-dominant gene locus, as reported in other vertebrates. The absence of the Tf^bTf^b genotypes was noticed in five out of the seven population samples examined: Costa Marques, Balbina and Trombetas (a), (b) and (c) (Table I).

Transferrin allele frequency and genotype distribution in the population samples

All population samples showed a good fit to Hardy-Weinberg equilibrium. The population totals and the heterogeneity chi-square tests indicated no significant statistical difference

between transferrin genotypes and population sample examined (Table I). Contingency tests on the overall transferrin allele frequency distributions among the population samples found no statistical difference, showing similar results when the observed and expected allele frequencies were assessed by either a simple chi-square (χ^2) or a log-likelihood ratio chi-square (G^2) test (Table II). When the above tests were applied to the 21 pair-wise comparisons, which represent all possible pair-wise combinations among the seven population samples examined, one pair comparison (Abufari versus Balbina) was significant at the 5% level, one such difference is expected, indicating that this is a statistical artifact (Table III).

The present data are consistent with a free flow of genes among the population samples examined.

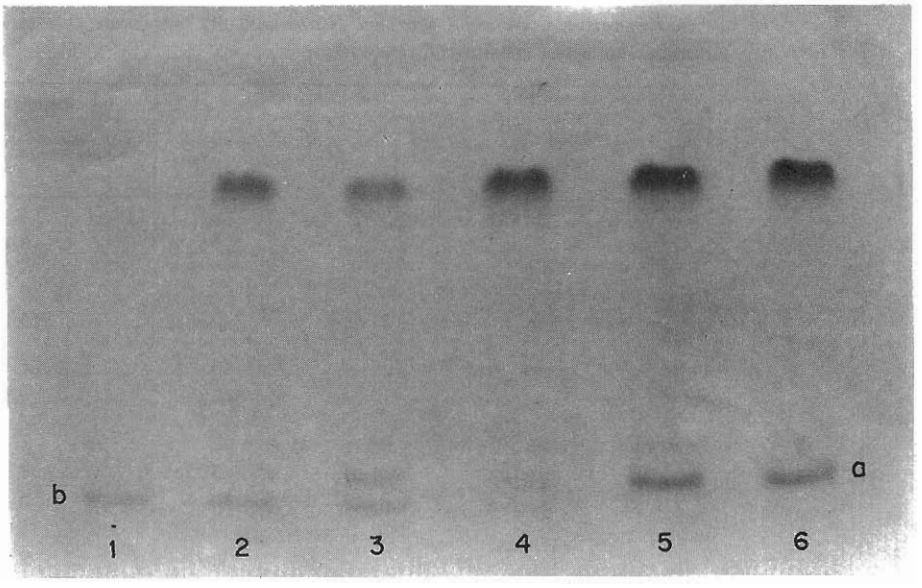

Figure 2 - Transferrin genotypes of Amazon turtle (*Podocnemis expansa*) separated by starch gel electrophoresis. The genotypes are: samples 1, 2 (Tf^bTf^b), samples 3, 4 (Tf^aTf^b) and samples 5, 6 (Tf^aTf^a).

Tf allele distribution in development stages

Contingency tests applied to the allele frequencies observed in different stages of development

Table I - The genotypes and transferrin allele frequencies distribution in seven population samples of Amazon turtle (*Podocnemis expansa*). Expectations of genotype frequencies assuming Hardy-Weinberg equilibrium are presented in parentheses.

Locality	Stage of development	Date collected	No. of specimens tested	Tf genotypes			Allele frequency		d.f.	χ^2	Probability
				Tf^aTf^a	Tf^aTf^b	Tf^bTf^b	Tf^a	Tf^b			
1. Costa Marques, Rio Guaporé, RO	Hatchling	/1989	28	22 (22.33)	6 (5.35)	0 (0.32)	0.893	0.107	1	0.404	0.70-0.50
2. Abufari, Rio Purus, AM	Adult	04-05/09/1990	36	31 (30.27)	4 (5.48)	1 (0.25)	0.917	0.083	1	2.668	0.20-0.10
3. Balbina, Rio Uatumã, AM	Adult	13/04-22/05/1988	30	16 (17.65)	14 (10.72)	0 (1.63)	0.767	0.233	1	2.788	0.10-0.05
4. Reserva Biológica do Rio Trombetas, PA	(a) Juvenile	/1984	26	19 (19.45)	7 (6.07)	0 (0.47)	0.865	0.135	1	0.622	0.50-0.30
	(b) Adult	24/11/1988	6	4 (4.16)	2 (1.67)	0 (0.17)	0.833	0.167	1	0.241	0.70-0.50
	(c) Hatchling	10/01/1990	19	11 (11.83)	8 (6.33)	0 (0.85)	0.789	0.211	1	1.349	0.30-0.20
5. Monte Cristo, Rio Tapajós, PA	Hatchling	04/01/1992	60	41 (40.05)	16 (17.94)	3 (2.01)	0.817	0.183	1	0.722	0.50-0.30
Total			205	144 (144.99)	57 (54.82)	4 (5.18)	0.841	0.159	1	0.363	0.70-0.50
Sum of χ^2									7	8.794	0.30-0.20
Heterogeneity χ^2 (by difference)									6	8.431	0.30-0.20

Table II - Contingency tests used to examine the distribution of transferrin allele frequencies in the Amazon turthe population samples. No statistically significant difference was found. Expected numbers of alleles are shown in parentheses.

Population samples		Transferrin alleles	
		Tf^a	Tf^b
Costa Marques, Rio Guaporé, RO		50	6
		(47.122)	(8.878)
Abufari, Rio Purus, AM		66	6
		(60.586)	(11.415)
Balbina, Rio Uatumã, AM		46	14
		(50.488)	(9.512)
Reserva Biológica do Trombetas, PA	(a)	45	7
		(43.756)	(8.244)
	(b)	10	2
		(10.098)	(1.902)
	(c)	30	8
		(31.976)	(6.024)
Monte Cristo, Rio Tapajós, PA		98	22
		(100.980)	(19.024)
Total		345	65

	d.f.	Value	Probability
Chi-square (χ^2)	6	8.229	0.222
Likelihood ratio (G^2)	6	8.547	0.201

Table III - Forty-two contingency tests were applied to verify the transferrin allele frequencies among the seven population samples of Amazon turtle (*Podocnemis expansa*). Chi-square (χ^2) and log-likelihood ratio (G^2) statistic values are shown for each pair-wise comparison.

Area		Costa Marques	Abufari	Balbina	Trombetas (a)	Trombetas (b)	Trombetas (c)
Abufari	$\chi^2_{(1)}$ / p	0.210/0.647					
	$G^2_{(1)}$ / p	0.209/0.648					
Balbina	$\chi^2_{(1)}$ / p	3.233/0.072	5.728/0.017				
	$G^2_{(1)}$ / p	3.320/0.068	5.790/0.016				
Trombetas (a)	$\chi^2_{(1)}$ / p	0.192/0.661	0.846/0.358	1.782/0.182			
	$G^2_{(1)}$ / p	0.192/0.661	0.834/0.361	1.818/0.178			
Trombetas (b)	$\chi^2_{(1)}$ / p	0.337/0.561	0.829/0.363	0.257/0.612	0.083/0.773		
	$G^2_{(1)}$ / p	0.331/0.577	0.717/0.397	0.271/0.602	0.080/0.777		
Trombetas (c)	$\chi^2_{(1)}$ / p	1.909/0.167	3.623/0.057	0.070/0.792	0.911/0.340	0.110/0.741	
	$G^2_{(1)}$ / p	1.872/0.171	3.439/0.064	0.070/0.791	0.900/0.343	0.113/0.737	
Monte Cristo	$\chi^2_{(1)}$ / p	1.657/0.198	3.613/0.057	0.625/0.429	0.614/0.433	0.020/0.887	0.139/0.710
	$G^2_{(1)}$ / p	1.758/0.185	3.876/0.049	0.614/0.433	0.636/0.425	0.021/0.885	0.136/0.712

Table IV - Contingency tests applied to investigate the distribution of Amazon turtle transferrin allele frequencies, in relation to the stage of development of the specimens examined. Expected numbers of alleles are shown in parentheses.

Stage of development	Transferrin alleles	
	Tf^a	Tf^b
Adult	122 (121.170)	22 (22.829)
Juvenile	45 (43.756)	7 (8.244)
Hatchling	178 (180.070)	36 (33.927)
Total	345	65

	d.f.	Value	Probability
Chi-square (χ^2)	2	0.409	0.815
Likelihood ratio (G^2)	2	0.417	0.812

(adult, juvenile and hatchling) of the Amazon turtle specimens examined revealed no significant difference (Table IV). Similar results were obtained when the three possible pair-wise comparisons were performed.

DISCUSSION

The main spawning areas of the Amazon turtle (*P. expansa*) have been recognized in almost 200 tabuleiros[1] found along the main rivers of the hydrographic Amazon basin. However, the stock structure of this species is not defined as yet.

Although the Amazon turtle population samples were taken hundreds of kilometers apart ($\overline{X}$ = 860 km), they appear to be genetically very similar, this being the main finding of the present study. A good genetic balance according to Hardy-Weinberg expectations was shown by all population samples, and supports the hypothesis of a single stock for this species in the geographical area examined. Additional protein and DNA variants should be used as genetic markers to test this hypothesis in the near future. If this is confirmed, IBAMA could implement a conservation and management policy for this species, by transporting the animals to different locations in the Amazon region without much concern for mixing of genetic stocks.

[1] The most elevated points at the sand beaches where the Amazon turtle females lay eggs during the nesting seasons.

The non-association between the Tf allele distributions and developmental stages of the Amazon turtle validates the use of the Tf gene locus as a genetic marker for determining the population structure of this species.

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RESUMO

O locus gênico (Tf) controlador das moléculas de transferrinas foi examinado em sete amostras populacionais da tartaruga da Amazônia (*Podocnemis expansa*) coletadas de cinco áreas geográficas na região amazônica. Esse locus mostrou-se polimórfico, com três genótipos (Tf^aTf^a, Tf^aTf^b and Tf^bTf^b), provavelmente codificados por dois alelos co-dominantes (Tf^a and Tf^b). Todas as populações mostraram um bom balanço genético de acordo com a lei de Hardy-Weinberg, sustentando portanto a hipótese de um único estoque da espécie na área estudada. Testes do χ^2 e G^2 foram aplicados para se investigar a distribuição total dos alelos de transferrinas. Dos 21 pares de combinações teoricamente possíveis nas sete amostras populacionais investigadas, detectou-se uma única diferença estatística significante entre as amostras do Abufari e Balbina, que pode ser interpretada como um artefato estatístico. Os dados apresentados sustentam a hipótese de fluxo livre de genes entre as amostras populacionais investigadas.

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