

COMPARISON OF ABO BLOOD GROUPS FROM TWO SPECIES OF PERUVIAN SQUIRREL MONKEYS (*Saimiri sciureus macrodon*, *Saimiri boliviensis peruviansis*) WITH A NATURAL POPULATION OF *S. b. peruviansis*-*S. s. macrodon* HYBRIDS

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ABSTRACT

Blood and saliva samples were collected from 44 *Saimiri boliviensis peruviansis*, eight *S. sciureus macrodon* and 41 *S. b. peruviansis* - *S. s. macrodon* hybrids. Saliva samples were tested for human ABH antigens by a conventional hemagglutination inhibition test. The results were the following for *S. b. peruviansis* and *S. s. macrodon*, respectively: A - 32, 2; B - 1.0; AB - 11, 6. Sera samples were used for detecting natural antibodies. A high frequency of discrepancies was found among the two species in relation to serum and saliva phenotypes.

INTRODUCTION

Since Landsteiner and Miller (1925) showed human ABO-like antigens in chimpanzees, several reports have been published for other non-human primates. Great apes (chimpanzee, gorilla, orangutan) present ABH antigens in red blood cells and fluids and antibodies in sera exactly as in humans. However in Old and New World monkeys ABH antigens occur only in body fluids, being absent in red blood cells.

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In New World monkeys the ABO system has been studied in several species: *Alouatta palliata* (Froehlich *et al.*, 1977); *Saguinus nigricollis* (Gengozian, 1964) and *S. fuscicollis* (Wiener *et al.*, 1967); *Saimiri boliviensis* (Terao *et al.*, 1988); *Cebus apella* (Schneider *et al.*, 1985; Hamel *et al.*, 1988); *Saguinus midas niger* (Schneider *et al.*, 1987); *Alouatta belzebul* (Corvelo *et al.*, 1985); *Aotus nancymai* and *Aotus vociferans* (Harada-Hamel *et al.*, 1990b).

In this paper we describe and compare ABH saliva phenotypes of two natural populations of Peruvian Amazonian *Saimiri sciureus macrodon*, *Saimiri boliviensis peruviansis* with a population considered as a hybrid between *S. s. macrodon* and *S. b. peruviansis*.

MATERIAL AND METHODS

Forty four *S. boliviensis peruviansis*, eight *S. sciureus macrodon* and 41 natural hybrids between *S. b. peruviansis* and *S. s. macrodon* were captured in the Peruvian Amazon (see Figure 1) and maintained at the "Centro de Reproduccion y Conservacion de Primates" - CRCP (Iquitos, Peru). According the taxonomic criteria proposed by Hershkovits (1984), *S. b. peruviansis* belongs to the roman group, the principal characteristics of which are the roman supraciliar arch and a hair pencil thin tail. On the other hand, *S. s. macrodon* belongs to the gothic form, the main features of which are the presence of a gothic supraciliar arch and a comparatively bushy tail. The hybrids captured in a sympatry zone between *S. sciureus macrodon* and *S. boliviensis peruviansis* showed the supraciliar arch and tail patterns intermediate between gothic and roman groups. Furthermore the hybrid status was confirmed by karyotypic and biochemical data (See Silva *et al.*, in press, for details).

ABH substances were detected in the saliva by the hemagglutination inhibition test, using commercial anti-A and anti-B reagents (Ortho Corporation), both at 1:256 titer, and *Ulex europaeus* lectin (anti-H) prepared in our laboratory at a 1:32 titer. Anti-A and anti-B reagents used in these tests were diluted to eight agglutinating units for human A2 and B cells, while the titer of anti-H lectin was adjusted to four units for human O cells. ABO agglutinins in all sera were investigated by hemagglutination tests after previous absorption with human O cells. All tests were done with blood from the same donors and on the same day to reduce the influence of environmental factors. Details about collection of blood and saliva are reported elsewhere (Schneider *et al.*, 1987; Hamel *et al.*, 1988). The gene frequencies were estimated by the maximum likelihood method, using the MAXLIK program developed by Reed and Schull (1968). Unbiased single loci heterozygosities ($h_{s1} = (2n/2n-1) (1 - \sum x_i^2)$) and standard deviations (square root of single locus variance: $V_{s1} = 2[\sum x_i^3 - (\sum x_i^2)^2/n]$) were estimated as suggested by Nei and Roychoudhury (1974).

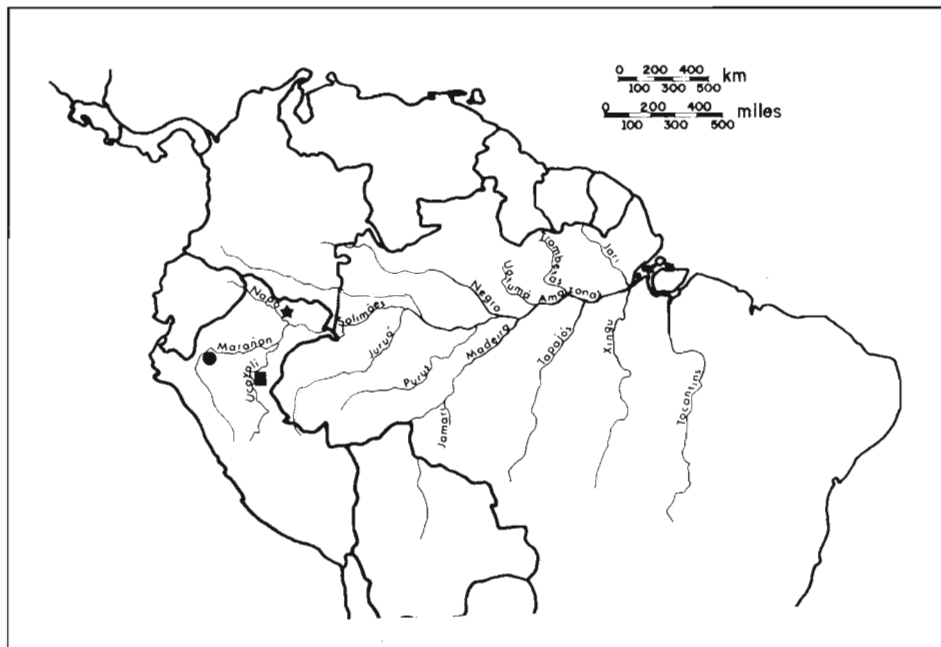


Figure 1 - The small circles, large circles and squares indicate collection areas of *Saimiri sciureus macrodon*, *Saimiri boliviensis peruviansis* and *S. b. peruviansis* - *S. s. macrodon* hybrids, respectively.

RESULTS AND DISCUSSION

As shown in Table I, three phenotypes were detected by saliva tests in *S. boliviensis peruviansis* suggesting the occurrence of two alleles: I^A and I^B . The observed distribution fits well the Hardy-Weinberg equilibrium ($P > 0.50$). Our results are somewhat similar to those reported by Terao *et al.* (1988) who also found in *S. boliviensis* from Bolivia, two alleles (I^A and I^B) in one sample and three alleles in two others. In all cases Terao *et al.* (1988) found that the ABO trait was transmitted in a Mendelian fashion. Furthermore, Schneider and Harada (1991) also found only two phenotypes (A and AB) and suggested the hypothesis of two alleles in *S. boliviensis boliviensis* from Santa Cruz de La Sierra (Bolivia), with very similar frequencies (See Table II).

In *S. sciureus macrodon* only two phenotypes were observed (A = 2); AB = 6), suggesting the presence of two alleles I^A and I^B . In spite of the small sample size, the phenotype distribution fits the expected one, assuming three phenotypes and a two allele model in Hardy Weinberg equilibrium. There is no data available for comparison with other subspecies.

Table I - Observed and expected phenotypic distributions assuming a two allele model.

Class	Observed	Expected	χ^2	Allele	Freq. \pm Sd
<i>S. boliviensis peruvienis</i>					
A	32	31.96	0.000	I ^A	0.852 \pm 0.037
AB	11	11.08	0.000	I ^B	0.148 \pm 0.037
B	1	0.96	0.002		
Total	44	44.00	0.002		1.00
<i>S. sciureus macrodon</i>					
A	2	3.12	0.404	I ^A	0.625 \pm 0.121
AB	6	3.75	1.349	I ^B	0.375 \pm 0.121
B	0	1.13	1.126		
Total	8	8.00	2.879		1.00
<i>S. b. peruvienis</i> - <i>S. s. macrodon</i> hybrids					
A	26	24.20	0.133	I ^A	0.768 \pm 0.046
AB	11	14.60	0.887	I ^B	0.232 \pm 0.046
B	4	2.20	1.470		
Total	41	41.00	2.490		1.00

Sd - standard deviation.

Table II - ABO gene frequencies, standard deviations and single locus heterozygosities in New world monkeys (%).

Species	N	I ^A	I ^B	I ^O	H _{s1}	Ref.
<i>Cebus</i>						
<i>C. apella</i>	19	36 \pm 9	21 \pm 7	43 \pm 9	68 \pm 3	1
<i>C. a. apella</i>	95	50 \pm 4	22 \pm 3	28 \pm 4	63 \pm 2	2
<i>C. a. paraguayanus</i>	55	100				3
<i>Alouatta</i>						
<i>A. palliata</i>	52		100			4
<i>A. belzebul</i>	50		100			5

Continued

Table II - Continued.

Species	N	I ^A	I ^B	I ^O	H _{s1}	Ref.
<i>Saguinus</i>						
<i>S. midas niger</i>	81	26 ± 3	66 ± 4	8 ± 4	49 ± 3	6
<i>S. fuscicollis</i>	18	100				7
<i>S. nigricollis</i>	8	100				8
<i>S. f. weddelli</i>	88	100				9
<i>Callithrix</i>						
<i>C. emiliae</i>	47	100				9
<i>C. jacchus jacchus</i>	35	100				9
<i>Chiropotes</i>						
<i>C. satanas</i>	72	100				10
<i>Aotus</i>						
<i>A. nancymai</i>	93		12 ± 3	88 ± 3	21 ± 4	11
<i>A. vociferans</i>	20		29 ± 8	71 ± 8	42 ± 6	11
<i>Saimiri</i>						
<i>S. boliviensis*</i>	17	46 ± 10	28 ± 8	26 ± 10	66 ± 3	12
<i>S. boliviensis*</i>	18	52 ± 10	28 ± 8	20 ± 9	63 ± 4	12
<i>S. boliviensis*</i>	22	86 ± 5	14 ± 5		25 ± 7	12
<i>S. b. boliviensis</i>	76	89 ± 3	11 ± 3		20 ± 4	13
<i>S. s. macrodon</i>	8	62 ± 12	38 ± 12		50 ± 6	a
<i>S. b. peruviansis</i>	44	85 ± 4	15 ± 4		25 ± 5	a
Sbp-SSm hybrids	41	77 ± 4	23 ± 4		36 ± 5	a

*The species *S. sciureus* first named by Terao *et al.* (1988) was renamed by the authors as *S. boliviensis*, based on Hershkovitz (1984). Standard deviations and gene frequencies were re-estimated through maximum likelihood method from the phenotypic frequencies published for this species.

H_{s1}: unbiased single locus heterozygosity.

References: (1) Schneider *et al.* (1985); (2) Corvelo *et al.* (1987); (3) Hamel *et al.* (1988); (4) Froelich *et al.* (1977); (5) Corvelo *et al.* (1985); (6) Schneider *et al.* (1987); (7) Wiener *et al.* (1967); (8) Gengozian (1964); (9) Rocha *et al.* (1990); (10) Harada-Hamel *et al.* (1990a); (11) Harada-Hamel *et al.* (1990b); (12) Terao *et al.* (1988); (13) Schneider and Harada (1991); (a) Present work.

In the hybrid sample three phenotypes were observed, implying the occurrence of two alleles I^A and I^B . In spite of the small differences between allele frequencies, the hybrids showed intermediate frequencies between those estimated for *S. s. macrodon* and *S. b. peruviansis*. The sample is in Hardy-Weinberg equilibrium in spite of a slight, but not significant, homozygous excess as expected in the case of recent hybridization. Our results suggest that the taxonomy of *Saimiri* genus needs a revision, as proposed by Silva *et al.* (in press).

Table II shows the allele frequencies estimated in the present study and those obtained from previous surveys in natural populations of New World monkeys. Four cebid genera (*Cebus*, *Alouatta*, *Saimiri*, *Aotus*, *Chiropotes*) and two Callitrichid genera (*Saguinus* and *Callithrix*) were studied. In the genus *Saimiri*, all species studied until now were found to be polymorphic. In *Saguinus* only *S. m. niger* was polymorphic, other species and the genus *Callithrix* being monomorphic. Both species from the genus *Alouatta* were monomorphic.

The I^A allele has not yet been found in *Aotus* and *Alouatta*. Among the Callitrichidae only three species of *Saguinus* and two of *Callithrix* were investigated, all of them being monomorphic for the I^A allele, except for *S. midas niger* which was polymorphic. These differences in *Saguinus* are probably due to geographical diversity, since Rocha *et al.* (1990) investigated a large sample of *S. fuscicollis weddelli* and confirmed the results previously described for this species (Wiener *et al.*, 1967). In addition, differences in gene frequencies among some species are very large. The I^B allele was the most frequent in *Alouatta* and *Saguinus midas niger*; conversely, I^O was the most frequent in *Aotus*, while I^A was the commonest in *Cebus apella paraguayanus* (and in all species of *Saimiri* tested).

The results of sera are presented in Table III. *S. b. peruviansis* and *S. s. macrodon* presented anomalous agglutinins. The incidence of anomalies detected here is clearly different from that found by Dracopoli and Jolly (1983) in baboons. They detected 0.03% anti-A anomalous positive and 6.9% anti-B anomalous negative.

Landsteiner's rule generally applies to all New and Old World monkeys, but exceptions to this rule have been reported for humans, Old World monkeys and New World monkeys (see Hamel *et al.*, 1988 for details).

Moor-Jankowski *et al.* (1964) and Wiener *et al.* (1974) suggested that anomalies in baboons are due to the existence of two antigens with differential cell specificity. The authors postulated that a specific Ac (or Bc) antigen occurs exclusively in red cells while another, As (or Bs) antigen must be common to both red cells and saliva. Conversely, Dracopoli and Jolly (1983) in order to explain the anomalies found in *Cercopithecus aethiops*, considered two kinds of serum-saliva discrepancies: one comprising positive alterations (i.e., saliva with A and serum with anti-A) and another encompassing negative alterations (i.e., saliva with A and serum without anti-B). These authors proposed a model

Table III - Reactions of squirrel monkey sera against human erythrocytes.

Salivar phenotype	Number	Serum reactions				Classification ^a
		O	A1	A2	B	
<i>S. b. peruviansis</i> (N=44)						
A	21	-	-	-	+	Normal
A	1	-	-	-	-	Negative anomaly
A	9	-	+	+	+	Positive anomaly
A	1	-	+	+	-	Double anomaly
B	1	-	+	+	+	Positive anomaly
AB	6	-	-	-	-	Normal
AB	1	-	-	-	+	Positive anomaly
AB	2	-	+	+	+	Double anomaly
AB	2	-	+	+	-	Positive anomaly
<i>S. s. macrodon</i> (n=8)						
A	1	-	-	-	+	Normal
A	1	-	+	+	+	Positive anomaly
AB	2	-	-	-	-	Normal
AB	1	-	-	-	+	Positive anomaly
AB	3	-	+	+	+	Double anomaly

^aAccording to Dracopoli and Jolly (1983).

based on six alleles, each having a specific function: Am and Ao (or Bm and Bo), inhibiting the production of anti-A (or anti-B) and As (or Bs), stimulating the production of anti-A (or anti-B). But, the high frequency of anomalous antibodies in New World monkeys cannot be explained by any of the above proposals and therefore remains as an open question.

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RESUMO

Foram coletadas amostras de sangue e saliva de 44 *Saimiri boliviensis peruvienis*, 8 *S. sciureus macrodon* e 41 híbridos de *S. b. peruvienis* - *S. s. macrodon*. Amostras de saliva foram investigadas para antígenos ABH humanos pela técnica convencional de inibição da hemaglutinação. Os resultados foram os seguintes para *S. s. peruvienis* e *S. s. macrodon*, respectivamente: A - 32, 2; B - 1, 0; AB - 11, 6. Amostras de soro foram utilizadas para detectar aglutininas naturais. As duas espécies apresentaram uma alta frequência de discrepâncias entre os fenótipos sérico e salivar.

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