

## ABO BLOOD GROUPS IN TWO SPECIES OF PERUVIAN NIGHT MONKEYS (*Aotus nancymai* AND *Aotus vociferans*)

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### ABSTRACT

Blood and saliva samples were collected from 93 *Aotus nancymai* and 20 *A. vociferans* monkeys. Saliva samples were tested for human ABH antigens by a conventional hemagglutination inhibition test which permitted us to assign 21 *A. nancymai* and 10 *A. vociferans* specimens to the B group, and 72 *A. nancymai* and 10 *A. vociferans* specimens to the O group.

Serum samples were used to detect natural antibodies, and the results showed some discrepancies between sera and secretion phenotypes as well as suggested the existence of anti-A1 antibodies in these species.

### INTRODUCTION

Since Landsteiner and Miller (1925) first showed human ABO-like antigens in chimpanzees, several reports have been published on other non-human primates, especially the great apes and Old World monkeys. However, few New World monkey species have been studied, even though they are widely used in biomedical research. Among the 53 New World monkey species belonging to 16 genera, no more than 4 *Ateles*, 2 *Cebus* (including subspecies), 2 *Alouatta*, and 5 *Saguinus* have been studied, including some *Saimiri* specimens (Table I). Since some *Aotus* specimens have been used as biomedical models for the study of malaria, as well as in other areas of biomedical and behavior research, these animals have become extremely valuable.

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For this reason, it is most important to genetically characterize these species, especially with respect to those traits they share with man.

In this paper, we present the first report on the ABO blood groups in natural populations of *A. nancymai* and *A. vociferans*.

Table I - Distribution of ABO blood groups in New World Monkeys\*

Species	Blood Groups*				Number tested	Reference
	O	A	B	AB		
<b>Spider monkeys</b>						
<i>Ateles paniscus chamek</i>	0	6	0	0	6	Wiener <i>et al.</i> , 1942
<i>Ateles geoffroyi grisescens</i>	1	0	4	0	5	Wiener <i>et al.</i> , 1942
<i>Ateles marginatus</i>	0	3	0	0	3	Wiener <i>et al.</i> , 1966
<i>Ateles paniscus</i>	0	1	0	0	1	Wiener <i>et al.</i> , 1966
<i>Ateles fusciceps robustus</i>	0	0	1	0	1	Wiener <i>et al.</i> , 1966
<b>Squirrel monkeys</b>						
<i>Saimiri sp</i>	2	6	0	3	11	Socha and Ruffié, 1983
<b>Capuchins</b>						
<i>Cebus apella</i>	0	5	0	0	5	Socha and Ruffié, 1983
<i>Cebus apella apella</i>	3	9	5	2	19	Schneider <i>et al.</i> , 1985
<i>Cebus albifrons</i>	1	0	3	0	4	Socha and Ruffié, 1983
<i>Cebus apella paraguayanus</i>	0	55	0	0	55	Harada-Hamel <i>et al.</i> , 1988
<b>Howler monkeys</b>						
<i>Alouatta palliata</i>	0	0	52	0	52	Froehlich <i>et al.</i> , 1977
<i>Alouatta belzebul</i>	0	0	50	0	50	Corvelo <i>et al.</i> , 1985
<b>Tamarins</b>						
<i>Saguinus oedipus</i>	0	4	0	0	4	Wiener <i>et al.</i> , 1967
<i>Saguinus mistax</i>	0	3	0	0	3	Wiener <i>et al.</i> , 1967
<i>Saguinus nigricollis</i>	0	6	0	0	6	Wiener <i>et al.</i> , 1967
<i>Saguinus nigricollis</i>	0	8	0	0	8	Gengozian, 1964
<i>Saguinus fuscicollis</i>	0	18	0	0	18	Wiener <i>et al.</i> , 1967
<i>Saguinus midas niger</i>	0	11	45	25	81	Schneider <i>et al.</i> , 1987

\*ABO blood groups established by saliva inhibition and serum tests.

## MATERIAL AND METHODS

### *Geographic distribution of the specimens*

The genus *Aotus* shows a widespread geographic distribution from Panamá and the northern part of South America to South Paraguay, extending through the Amazonian tropical forest. In Peru, *A. nancymai* is found mostly South of the Amazonas-Solimões-Marañón river, while *A. vociferans* is encountered north of the same river system (Hershkovitz, 1983).

Twenty specimens of *A. vociferans* were captured on the west bank of the Amazon river (Napo river) and 93 specimens of *A. nancymai* at several locations along the two banks of the Marañón-Amazon river (see Figure 1). Specimens were identified by the taxonomic criteria proposed by Hershkovitz (1983) to distinguish *Aotus* species (Aquino and Encarnacion, 1988). The animals were trapped by personnel of the Peruvian Primatological Project "Manuel Moro Sommo" and kept at the Centro de Reproducción y Conservación de Primates (CRCP) in Iquitos, Peru.

### *Sample collection*

All specimens were anesthetized with an intramuscular injection of ketamine hydrochloride (10 mg/kg of body weight). Salivation was induced by an intramuscular injection of 0.001 ml of pilocarpin chlorhydrate (Hertape). After collection, saliva was immediately heated to 100°C, on a water bath for 20 minutes to prevent glycoprotein hydrolysis. Five ml of blood were collected by puncture of the femoral vein using EDTA as an anticoagulant. Blood and saliva were centrifuged, and the supernatants stored on ice and shipped to the Belém laboratory.

### *Serological tests*

Saliva samples were tested for the presence of A, B and H substances by the hemagglutination inhibition test using human anti-A and anti-B antibodies from Ortho Corporation, both at 1:256 titers, and *Ulex europeus* lectin (anti-H) prepared in our laboratory at 1:64 titer and both read after incubation at room temperature (see Schneider *et al.*, 1987 for a detailed description of procedures). Anti-A and anti-B antibodies were diluted to about 8 agglutinating units for human A2 cells and B cells, while anti-H was adjusted to 4 units for O human cells. Cells from the same donor were used in all tests. Monkey sera were tested against human A1, A2, B and O cells by the hemagglutination test after absorption with human O red cells to remove non-specific antibodies.

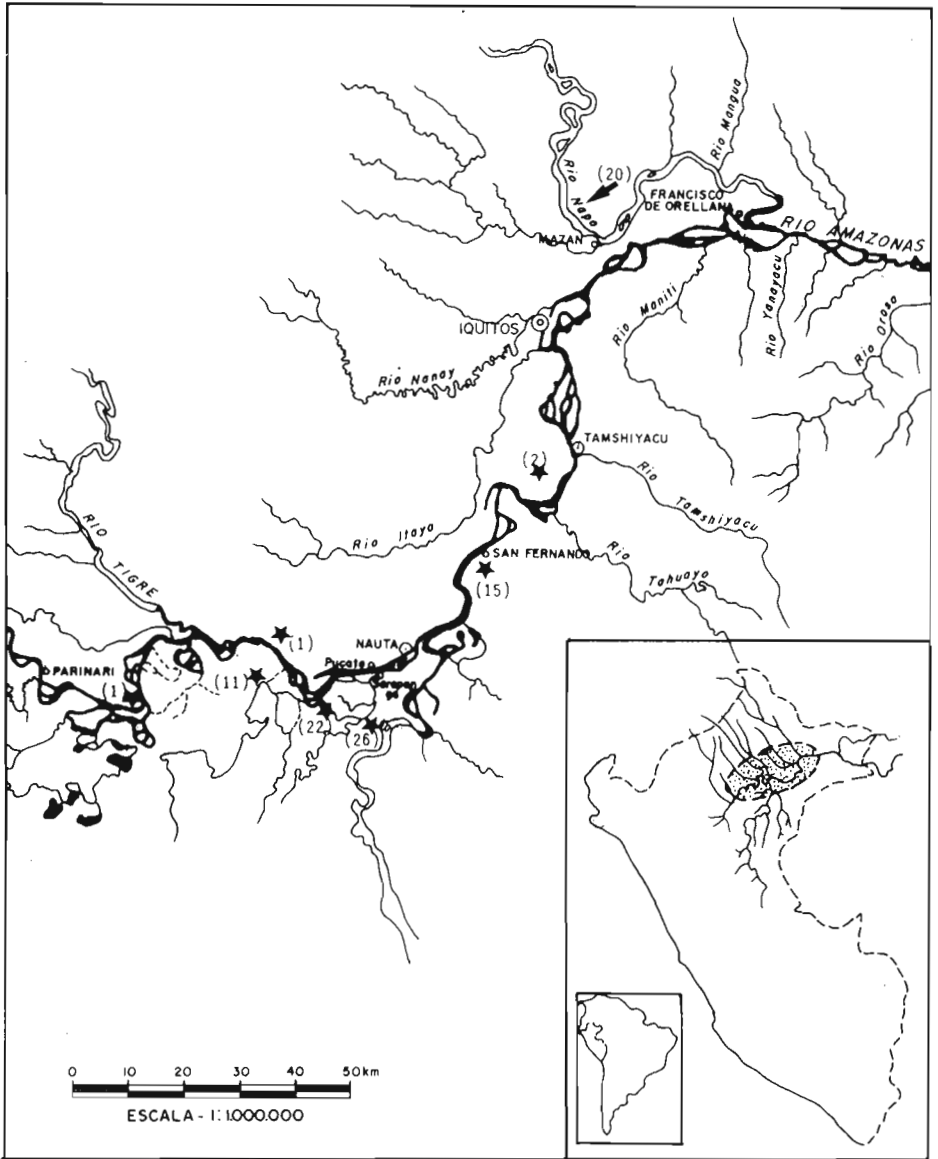


Figure 1 - Map showing the collecting sites of the specimens investigated, black stars corresponding to those where *Aotus nancymai* and arrow to that where *Aotus vociferans* were captured. Numbers in parenthesis indicate sample sizes. No information about the collecting site of 15 *A. nancymai* specimens from the east bank is available.

### Statistical analysis

All gene frequencies were estimated by the maximum likelihood method using the MAXLIK program prepared by Reed and Schull (1968). Fisher's test or the G test were used to verify differences of phenotypic frequencies between the two species. Differences between means were examined by the Student t-test using the SPSS program (Statistical Package for Social Sciences).

## RESULTS AND DISCUSSION

Figure 1 shows the four collecting sites of *A. nancymai* on the east bank of the Marañón river, where the O and B phenotype numbers were: (11) = 9 and 2; (22) = 18 and 4; (26) = 21 and 5; (15) = 13 and 2, respectively. No statistical differences were observed in the distribution of B and O phenotypes among these sites ( $\chi^2 = 3.948$ ;  $df = 4$ ;  $P > 0.05$ ) or even when the farthest site (15) was compared with the others ( $\chi^2 = 3.082$ ;  $df = 1$ ;  $P > 0.05$ ).

As is indicated in Table II, only two phenotypes (B and O) were observed in the two species by saliva tests. In *A. nancymai*, 72 specimens were classified as O, and 21 as B. In *A. vociferans*, 10 belonged to the O group, and 10 to the B group. These results suggest that only two alleles ( $I^O$  and  $I^B$ ) were present in these populations, producing two different phenotypes whose frequencies were significantly different

Table II - ABO phenotypes, antigen titers and gene frequencies in night monkeys.

Species	Salivary		N	Antigen titers*		Gene frequencies and standard deviation
	Phenotype	Antigen		X	SD	
<i>Aotus nancymai</i>	O	none	21			
	O	H	51	3.4 ± 1.6		$I^O = 0.88$ ; $sd = 0.025$
	B (without H)	B	11	2.5 ± 1.0		$I^B = 0.12$ ; $sd = 0.025$
	B (with H)	B	10	3.1 ± 1.6		
		H	10	4.4 ± 1.6		
<i>Aotus vociferans</i>	O	H	10	1.7 ± 1.7		$I^O = 0.71$ ; $sd = 0.079$
	B <sup>1</sup>	B	10	2.2 ± 1.2		$I^B = 0.29$ ; $sd = 0.079$
		H	8	3.2 ± 1.6		

\*The inhibition titers were converted to logarithms, base two.

<sup>1</sup>Two samples without H substance

(Fisher's test;  $P = 0.012$ ). The allele frequencies observed were:  $I^O = 0.88$  and  $0.71$ ;  $I^B = 0.12$  and  $0.29$  in *A. nancymai* and in *A. vociferans*, respectively. Hardy-Weinberg equilibrium could not be tested due to the presence of only two phenotypes and two alleles, which results in a number of degrees of freedom equal to zero. Moreover, we could not extend our comparisons because no previous reports on *Aotus* are available. A review of the literature, however, indicates that the family Cebidae is very heterogeneous, with species showing one, two or even three alleles, though several of these reports are based on very few animals. It is therefore necessary to extend these studies and to include animals from natural populations to learn more precisely how these genes are distributed.

The H substance was not detected in all saliva samples. Twenty-one samples of *A. nancymai* did not present ABH substances and 11 presented only B substance. In *A. vociferans* 2 samples presented only B substance. So, the criteria used to classify the O phenotype was the absence of A and B substances in saliva. Somewhat similar results have been reported for other New World primates (Gengozian, 1964; Wiener *et al.*, 1964). Wiener *et al.* (1967), when testing specimens of the genus *Saguinus* showed that saliva samples inhibited the antibody only when the anti-H reagent was highly diluted, suggesting that the H substance was qualitatively or quantitatively different in these species in comparison to man.

Comparison between the substances titers in distinct phenotypes did not show statistically significant differences.

Landsteiner's rule states that if a given antigen is present in one individual, its corresponding agglutinin should be absent. This rule is commonly used when testing serum agglutinins to confirm detection in secretions and/or red cells. When testing the available sera from 89 *A. nancymai* and 13 *A. vociferans*, we found that all samples presented anti-A, agglutinating both A1 and A2 red cells, while some showed very weak anti-B reactions. These results are presented in Table III, showing a high proportion of cases where Landsteiner's rule is not valid: 72% in *A. nancymai* and 54% in *A. vociferans*.

Several discrepancies between sera and secretions phenotypes had been described in previous reports testing non-human primates (Wiener *et al.*, 1942; Gengozian, 1964; Moor-Jankowski *et al.*, 1964; Wiener *et al.*, 1966; Wiener *et al.*, 1967; Nakajima *et al.*, 1970; Socha *et al.*, 1972; Downing *et al.*, 1973; Wiener *et al.*, 1974; Froehlich *et al.*, 1977; Socha *et al.*, 1977, 1981; Terao *et al.*, 1981; Dracopoli and Jolly, 1983; Schneider *et al.*, 1985; Harada-Hamel *et al.*, 1988).

Some authors have proposed alternative models to explain these differences. However, an investigation dealing with this subject is necessary involving a more detailed analysis of the nature and stability of the antibodies after collection as well as its monitoring through the time.

To test whether these specimens had an anti-A fraction capable of reacting specifically with A1 red cells, we selected 5 serum samples from *A. nancymai* and 3

Table III - Distribution of serum agglutinins and salivary phenotypes in night monkeys.

Salivary phenotypes	Serum agglutinins		Total
	An ti-A only	Anti-A and Anti-B	
<i>Aotus nancymai</i>			
O	60 (58.9)	9 (10.1)	69
B	16 (17.1)	4 (2.9)	20
Total	76	13	89
<i>Aotus vociferans</i>			
O	5 (4.6)	1 (1.4)	6
B	5 (5.4)	2 (1.6)	7
Total	10	3	13

$G = 0.167$ ;  $df = 1$ ;  $P > 0.05$  (*A. nancymai*)

$G = 0.023$ ;  $df = 1$ ;  $P > 0.05$  (*A. vociferans*)

The expected number of random antigen and agglutinin association are in parentheses. If results perfectly fitted Landsteiner's rule, all individuals would lie on the diagonal B-anti A/O-anti A anti B. Those in O-anti A were classified as negative anomalous ( $N = 60$  and  $5$ ), and those in B-anti A anti B ( $N = 4$  and  $2$ ) as positive anomalous.

from *A. vociferans* which were strongly reactive with human A red cells. After extensive absorption with A2 erythrocytes, these sera were retested with A1 red cells and three samples of *A. nancymai* produced a positive reaction, suggesting that anti-A1 should exist in this species. A similar result was obtained by Schneider *et al.*, (1985) in *Cebus apella*, leading us to propose that anti-A1 must be present in other species of New World monkeys. Though *A. vociferans* sera did not produce a positive reaction, the existence of anti-A1 antibody in this species can't be excluded because our results are based on a small number of specimens.

### ACKNOWLEDGMENTS

The authors thank Mr. Lenor Mandu for technical assistance and Dr. Hector Seuánez for revising the manuscript.

This work was supported by the following Brazilian Institutions: UFPA (Universidade Federal do Pará), FINEP (Financiadora de Estudos e Projetos), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPESP (Fundação de Amparo e Desenvolvimento da Pesquisa); as well as by the Peruvian Proyecto Peruano de Primatología "Manuel Moro Sommo", which is developed by the Dirección General Forestal y de Fauna of the Ministry of Agriculture in collaboration with the Instituto Veterinario de Investigaciones Tropicales y de Altura of the Universidad Nacional Mayor de San Marcos, and the Instituto Nacional de Salud of the Ministry of Health. Additional support came

from the Pan American Health Organization/World Health Organization, and the National Institutes of Health (USA).

## RESUMO

Foram coletadas amostras de sangue e saliva de 93 animais da espécie *Aotus nancymai* e 20 de *A. vociferans*. As amostras de saliva foram testadas para a presença de antígenos ABH humanos, através do teste convencional de inibição da hemaglutinação, o qual nos permitiu classificar 21 *A. nancymai* e 10 *A. vociferans* como sendo do grupo B e 72 *A. nancymai* e 10 *A. vociferans* como pertencentes ao grupo O.

Amostras de soro foram utilizadas para detectar aglutininas naturais do sistema ABO. Os resultados mostraram algumas discrepâncias entre os fenótipos séricos e salivares, como também sugeriram a existência de anticorpos anti-A1 nessas espécies.

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(Received June 21, 1988)