

CHROMOSOME AND PROTEIN VARIATION IN RED HOWLER MONKEYS

Margarida M.C. Lima¹, Maria I.C. Sampaio¹, Maria P.C. Schneider¹,
Wolfgang Scheffrahn², Horacio Schneider¹ and Francisco M. Salzano³

ABSTRACT

Electrophoretic and karyotypic data were obtained for 16 specimens of *Alouatta seniculus* collected at the east margin of the Jari River, in Brazilian Amazonia. Diploid numbers of 47, 48 and 49 chromosomes were observed, this variation being due to the presence of 1 to 3 supernumerary microchromosomes. These and other karyotypic characteristics are at variance with results for this same species obtained in Colombia and Bolivia. Genetic variability in *A. seniculus* was estimated using 26 electrophoretically detectable protein loci. The average heterozygosity was calculated as 9.6%, similar to those observed among other Platyrrhini monkeys. Genetic distances between this species, *Alouatta belzebul* and *Cebus apella paraguayanus* yielded values compatible with those expected in intra and intergeneric primate comparisons.

INTRODUCTION

According to Neville *et al.* (1988) *Alouatta* is the most widely distributed genus of New World monkeys. There are six currently recognized species, two of them occurring in Amazonia: *Alouatta belzebul* and *Alouatta seniculus*.

¹ Departamento de Genética, Universidade Federal do Pará, 66059 Belém, Pará, Brasil.

² Anthropologisches Institut der Universität Zurich-Irchel, Winterthurer Str. 190, CH 8057 Zurich, Switzerland.

³ Departamento de Genética, Universidade Federal do Rio Grande do Sul, Caixa Postal 1953, 90001 Porto Alegre, RS, Brasil. Send correspondence to F.M.S.

Most authors recognize the existence of several subspecies for *A. seniculus*, but all agree that a taxonomic revision is needed. These monkeys live both north and south of the Amazonas river, in Brazil, the Guianas, Venezuela, Colombia, Peru and Bolivia (Hill, 1960; Wolfheim, 1983).

The karyotypes of *A. palliata*, *A. caraya*, *A. fusca*, *A. seniculus*, from Colombia and Bolivia, and *A. belzebul* have been previously described, and large differences among them have been reported. In *A. seniculus* from Colombia, Yunis et al. (1976) have found a complement of 43, 44 and 45 chromosomes, with 12 biarmed autosomes, 26 acrocentrics, and 3 to 5 metacentric microchromosomes with negative C bands; in addition, the X was an acrocentric chromosome, smaller than the X of most mammals, while the Y was represented by a small submetacentric. A heterozygous inversion was also found in pair 13, and the NOR band was in the short arm of pair 3, a large submetacentric. On the other hand, Minezawa et al. (1985) found in *A. seniculus* from Bolivia a diploid number ranging from $2n=48$ to 51, this variation being due to microchromosomes. The females presented 18 biarmed chromosomes (including the submetacentric X), while the males showed 14 biarmed and two unpaired biarmed chromosomes. The number of acrocentrics was 26 in females and 27 in males. A male specific acrocentric chromosome was found, probably derived from normal chromosome 6 by a centromeric fission and insertion of the Y-chromosome.

The karyotypic diversity found in *Alouatta*, where the diploid number ranges from 54 in *A. palliata* (Ma et al., 1975) to 43, 44 and 45 in *A. seniculus* (Yunis et al., 1976), suggests the occurrence of various rearrangements in the reduction of the diploid number during the evolution of the genus. De Boer (1974) considers the karyotype of *A. palliata* as ancestral in the genus. Similarly, Hershkovitz (1949), based on the development of the hooid apparatus, placed *A. palliata* and *A. caraya* at the beginning and *A. seniculus* at the end of a progressive specialization process. Minezawa et al. (1985) suggested that the Bolivian red howler could have split off from *A. seniculus* of Colombia as an independent species, because more than 10 chromosome rearrangements are necessary to reconstruct one karyotype from the other. High karyotypic variability has also been found among other cebids. In *Aotus* 12 karyotypes occur in 8 distinct species, probably derived by Robertsonian rearrangements (Ma et al., 1976; Pieczarka and Nagamachi, 1988a), while in *Callicebus* three species showed diploid numbers ranging from 20 to 50 (Pieczarka and Nagamachi, 1988b).

Protein polymorphism studies in natural populations of primates have been widely performed in Old world monkeys, where the average heterozygosity ranges from 0 to 10%, the highest values being found in *Macaca* (Kawamoto et al., 1982). In the New World monkeys heterozygosity levels range from 1 to 8%, being highest in *Alouatta* (Malmgren, 1979; Forman et al., 1986; Schneider, 1988).

In this paper we present the karyotypic description and an evaluation of the genetic variability in a sample of 16 animals derived from a natural population of *Alouatta seniculus* captured at the margins of the Jari river, in eastern Amazonia, Pará State, Brazil.

MATERIAL AND METHODS

The sample studied was composed of three males and 13 females of *Alouatta seniculus* (Hill, 1960) from the eastern bank of the Jari river, a northern tributary of the Amazonas (Figure 1). This sample was collected during an expedition performed in 1986, with the purpose of screening the primate fauna around the Jari river, where a hydroelectric dam exists. All the sacrificed animals were preserved taxidermically, and the skulls and skins included in the mammals collection of the Museu Paraense Emilio Goeldi.

Two blood samples were collected from each animal, one with EDTA, for electrophoretic studies, and the other with heparin, for cytogenetic analysis. The blood for electrophoresis was centrifuged at 3,000 rpm for 10 minutes and the plasmas separated. The red blood cells were glycerolized and then stored together with the plasma at -20°C . Kidney fragments were collected for enzyme studies.

For chromosome studies lymphocytes were cultivated for 72 hours in TC 199 medium, enriched with fetal calf serum at 20% and Phytohemagglutinin at 2%. The chromosome analyses were performed by G-C-NOR banding procedures (Seabright, 1971; Sumner, 1972; Howel and Black, 1980).

For the study of protein variation the horizontal electrophoretic method was used, and 20 proteins coded by 26 genetic loci were screened (Table I).

The average heterozygosity and genetic distances were calculated according to Nei (1978). All gene frequencies and differences between the observed and expected distributions were estimated using the maximum likelihood method with the MAXLIK program developed by Reed and Schull (1968). Genetic relationships were evaluated considering each population as an operational unit (OTU), and the similarity coefficient of Nei (1978) was estimated for all paired comparisons of OTUS using the BIOSYS program of Swofford and Selander (1981).

RESULTS AND DISCUSSION

The cytogenetic analysis of two males and nine females showed a diploid number of 47, 48 and 49 chromosomes, this variation being due to the presence of

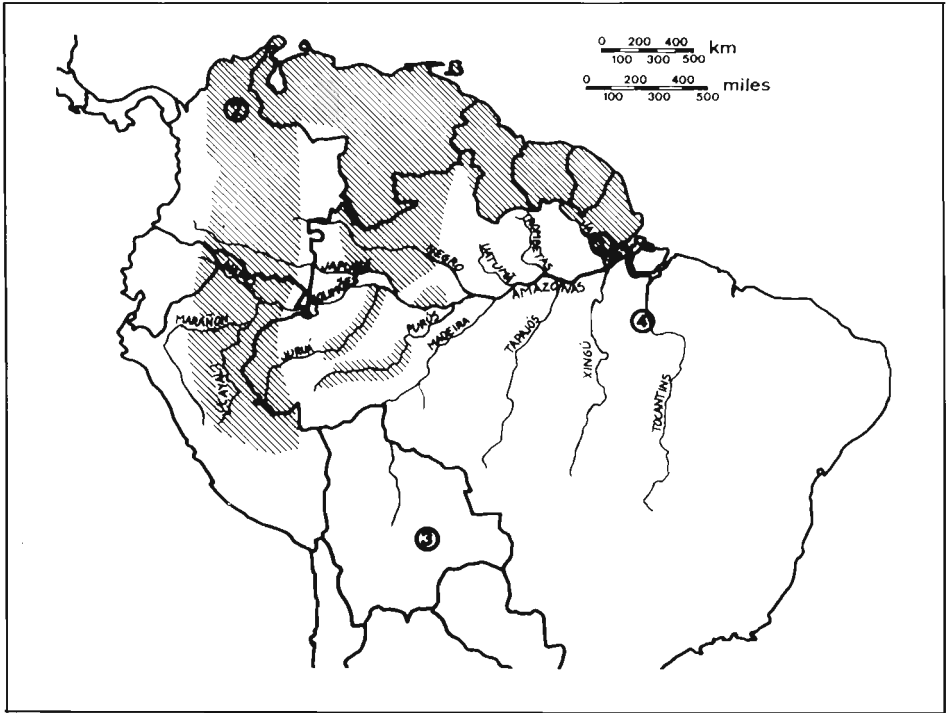


Figure 1 - Map showing the geographical distribution of *A. seniculus*, according to Hill (1960). Number (1) shows the place where the sample studied here (with $2n = 48$ to 51) was collected; (2) indicates the origin of the sample of *A. seniculus* studied by Yunis et al. (1976) ($2n = 43$ to 45); (3) indicates the collection sites of *A. seniculus* investigated by Minezawa et al. (1985) ($2n = 48$ to 51); and (4) the place where the *A. belzebul* samples studied by Armada et al. (1987) ($2n = 49$ and 50) and Schneider (1988) were collected.

one, two or three supernumerary B microchromosomes. Twenty-two biarmed chromosomes and 24 acrocentrics were found in males, contrasting with the findings of 24 biarmed chromosomes and 22 acrocentrics in females. B microchromosomes were found in both sexes (Figure 2).

As can be seen in the square inset of Figure 2, G-banding patterns suggest that, in males, one of the homologues of autosome pair number 7 has lost a short arm segment which is probably translocated to the long arm of the small Y-chromosome. In consequence, one biarmed chromosome (pair number 7) was converted into an acrocentric and the Y remained acrocentric, but larger. To confirm these findings other studies, especially of meiosis, which were not possible in this work, will be necessary.

Table I - Electrophoretic procedures used in this investigation.

Systems (loci)	Tissue ¹	Buffer ²	Support	Staining mixture (ref ³)
Acid phosphatase (ACP1)	RBC	PCE	Agarose 1%	1
Adenosine deaminase (ADA)	RBC	TEB1	Agarose 1%	2
Albumin (ALB)	PL	TEB2	Starch 11%	3
Carbonic anhydrase 2 (CA2)	RBC	TEMM	Agarose 1%	4
Cell esterases (ES1, ES2, ES3)	RBC	PCE	Starch 11%	2
Esterase D	RBC	PCE	Agarose 1%	1
Glyoxalase (GLO)	RBC	PCE	Starch 11%	2
Glucose phosphate isomerase (GPI)	RBC	TEB1	Agarose 1%	2
Glutamic-oxalacetate transaminase (GOT1)	RBC, K	TEMM	Starch 11%	2
Glutamic-pyruvate transaminase (GPT)	RBC	TEMM	Starch 11%	2
Haptoglobin (HP)	PL	Borate	Starch 11%	5
Hemoglobin (HBA, HBB)	RBC	TEB3	Acetate	6
Isocitrate dehydrogenase (IDH1, IDH2)	RBC, K	PC	Starch 11%	2
Lactate dehydrogenase (LDHA, LDHB)	RBC	PC	Starch 11%	2
Malate dehydrogenase (MDH1, MDH2)	RBC, K	PC	Starch 11%	2
Nucleoside phosphorylase (NP)	RBC	TEMM	Agarose 1%	2
Phosphoglucomutase (PGM1)	RBC	TEMM	Agarose 1%	2
Phosphogluconate dehydrogenase (PGD)	RBC	PCE	Starch 11%	2
Properdin factor B (BF)	PL	Barbital	Agarose 1%	7
Superoxide dismutase (SOD1)	RBC	PCE	Starch 11%	2

¹ *Tissues*: RBC = red blood cells; PL = plasma; K = kidney.

² *Buffers*: (1) PCE: Tank: monobasic sodium phosphate 0.11 M - trisodium citrate 0.075 M - EDTA 0.0025 M, pH 6.9, adjusted with NaOH 10 N; Gel: dilution 1:20. (2) PC: Tank: monobasic sodium phosphate 0.245 M - citric acid 0.15 M, pH 5.9; Gel: dilution 1:40. (3) TEB1: Tank: tris 0.18 M - boric acid 0.1 M - EDTA 0.004 M, pH 8.6; Gel: dilution 1:10. (4) TEB2: Tank: tris 0.03 M - boric acid 0.3 M - EDTA 0.0043 M, pH 6.9; Gel: dilution 1:6.2. (5) TEB3: Tank: tris 0.1 M - boric acid 0.05 M - EDTA 0.0016 M - urea 6 M, pH 8.6. (6) Borate: Tank: boric acid 0.3 M, adjusted to pH 8.6 with NaOH 10 N; Gel: tris 0.076 M - citric acid 0.005 M, pH 8.6. (7) TEMM: Tank: tris 0.1 M - maleic anhydride 0.1 M - EDTA 0.01 M - magnesium chloride 0.01 M, pH 7.4, adjusted with NaOH 10 N; Gel: dilution 1:15. (8) Barbital: Tank: sodium barbital 0.0425 M - barbital 0.0075 M - calcium lactate 0.0018 M, pH 8.6; Gel: dilution 1:2.

³ *References*: (1) Sampaio and Schneider (1986); (2) Harris and Hopkinson (1976); (3) Franco and Salzano (1985); (4) Sampaio *et al.* (1986); (5) Franco *et al.* (1981); (6) P.C. Naoum (personal communication); (7) W. Scheffrahn (personal communication).

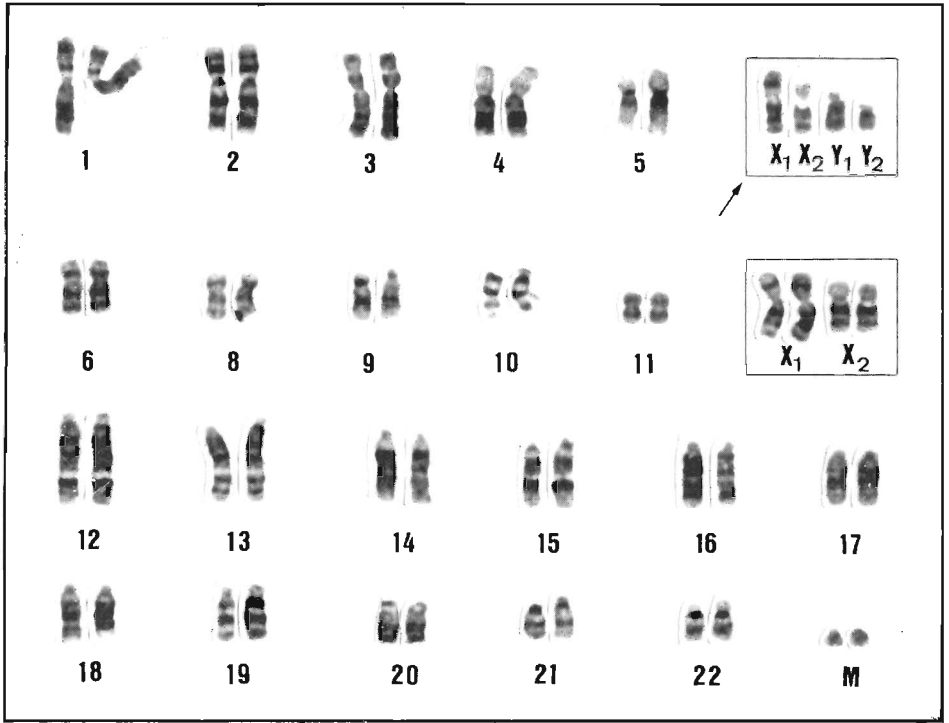


Figure 2 - G-banding patterns of *A. seniculus*. The X and Y appear in the square inset. The arrow shows the male complement.

In *Alouatta* chromosome rearrangements involving the Y-chromosome are common. One which occurred in *A. seniculus* from Bolivia has already been mentioned. In addition, Armada *et al.* (1987) and Lima and Seuanetz (1989) found in *Alouatta belzebul* a chromosome complement of 49 in males and 50 in females, this difference being due to a Y-autosome translocation. Similarly, Ma *et al.* (1975) found a Y-chromosome translocated to an autosome in *A. palliata* from Panama. This type of change has been also observed in other New World monkeys, such as *Callimico goeldi* (De Boer, 1974), *Cacajao calvus rubicundus* (Koiffmann and Saldanha, 1981), *Aotus sp.* (Ma *et al.*, 1976), and *Alouatta sp.* (Ma *et al.*, 1975).

As can be seen in Figure 3, the distribution of the constitutive heterochromatin is centromeric, except for pairs four and eight, where no heterochromatin was detected by the CGB method. Furthermore, no interstitial or telomeric C-bands were found in any chromosome, as has been reported for other species of *Alouatta*.

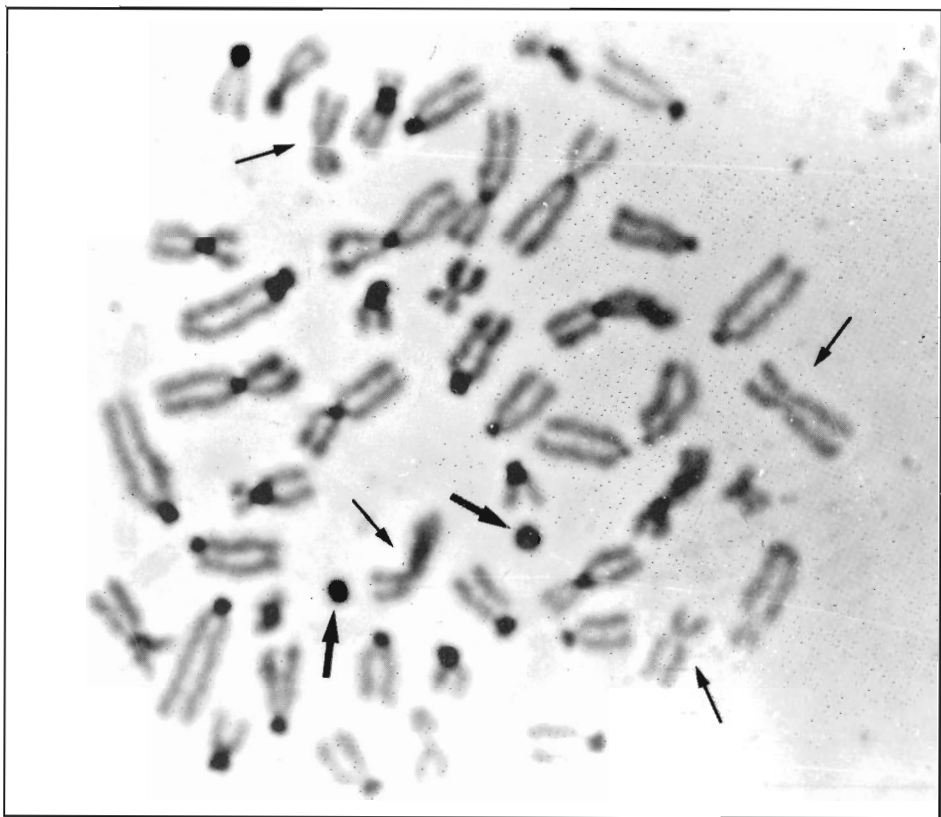


Figure 3 - C-banding patterns of *A. seniculus*. The large arrows show the C-positive microchromosomes, while the narrow arrows indicate pairs 4 and 8, with negative C-banding patterns.

Interestingly, the microchromosomes we found in *Alouatta seniculus* appear to be clearly C-positive, very small acrocentrics (Figure 3; large arrows). This contrasts with the findings of Yunis *et al.* (1976) and Minezawa *et al.* (1985), who observed in this same species C-band negative microchromosomes. *A. seniculus* is the only New World primate which presents these supernumerary chromosomes, and this could be a consequence of the great karyotypic modification which has occurred in this group.

NOR bands were encountered in the long arm of autosome pair number 10, being found in duplicate in some animals (Figure 4A and B). In addition, Figure 4C shows an association between two NOR regions. These results differ from those of Yunis *et al.* (1976) for *A. seniculus* from Colombia, in which the NOR bands possibly were in the biarmed autosome pair number 3.

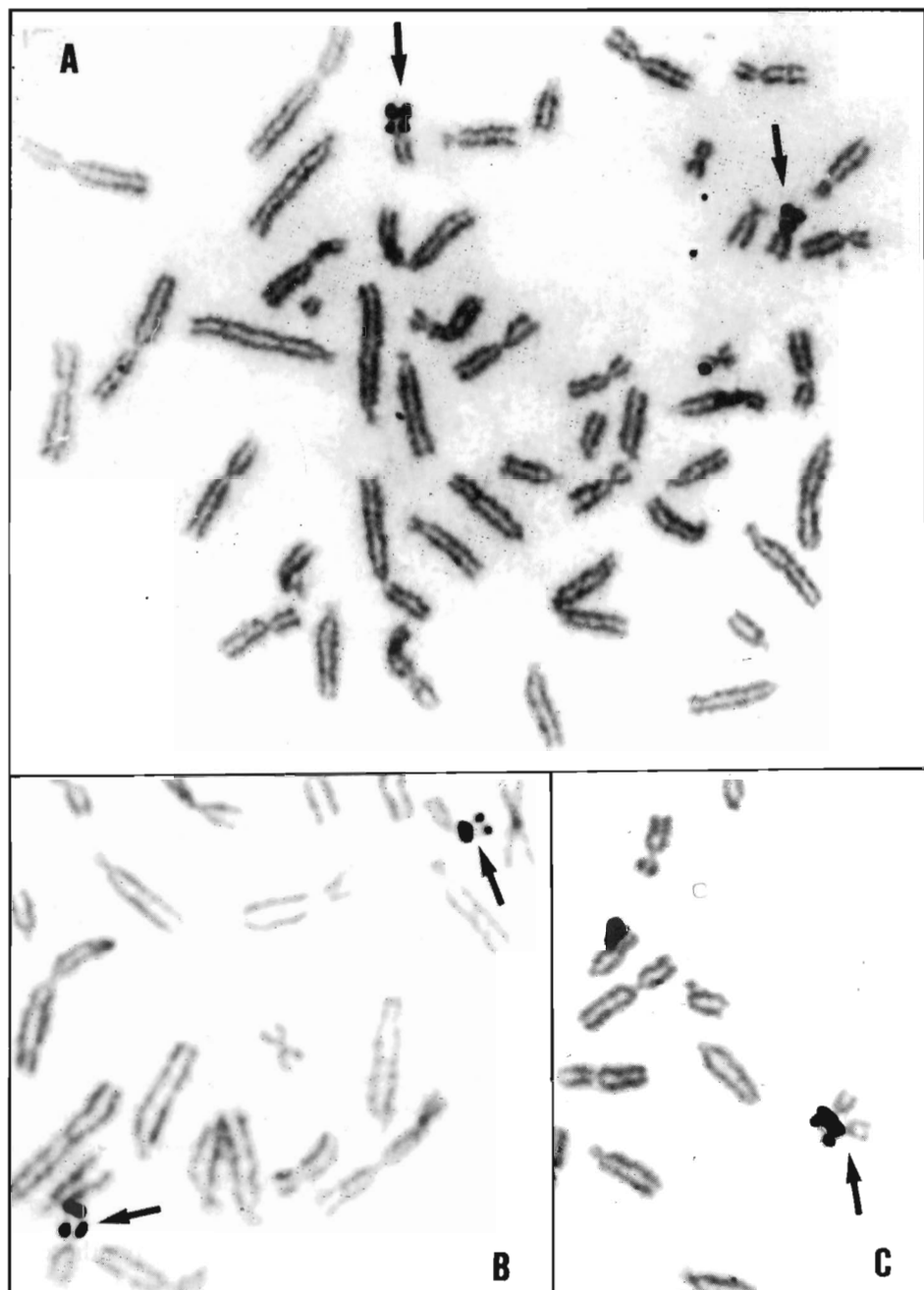


Figure 4 - NOR-banding patterns in *A. seniculus*. A and B show duplicated nor-banding patterns and C shows an association between two NOR regions.

In general, our data differ from those of *A. seniculus* from Colombia (Yunis *et al.*, 1976) and from Bolivia (Minezawa *et al.*, 1985) in relation to diploid number, chromosome morphology, banding pattern (C, G and NOR), sexual chromosome patterns and microchromosome heterochromatin, raising the question as to whether we are really dealing with the same taxonomic entity.

As for protein variation, 26 loci were investigated, and electrophoretic variation was detected in ADA, BF, CA2, ESD, ES3, GPI, IDH1, PGD and SOD1. The patterns found and their interpretation are presented in Figure 5 and Table II. The following additional information is also in order: (a) *Adenosine deaminase*: The ADA*2 and ADA*4 alleles are the same as those previously reported for *Alouatta belzebul* by Barroso *et al.* (1988). The ADA*5 allele occurs in *Alouatta seniculus* only. All the patterns reported in this genus differ from the usual human ADA 1, which is more cathodal; (b) *Carbonic anhydrase 2*: CA2 occurs also in *A. belzebul* (Schneider, 1988). The four alleles detected in *Alouatta* are different from those reported in *Cebus apella* (Sampaio *et al.*, 1986) and *Chiropotes satanas* (Sampaio and Schneider, 1986), being also distinct from the common human CA2 pattern; (c) *Esterase D*: ESD 1 occurs also in *A. belzebul* (Schneider, 1988); (d) *Esterase 3*: Besides esterase D three other zones of esterase activity were investigated (using β -naphthyl acetate as a substrate). The less anodic zone, ES3 showed polymorphism, presenting three phenotypes, ES3 1, ES3 1-2 e ES3 2, probably coded by two alleles, ES3*1 and ES3*2;

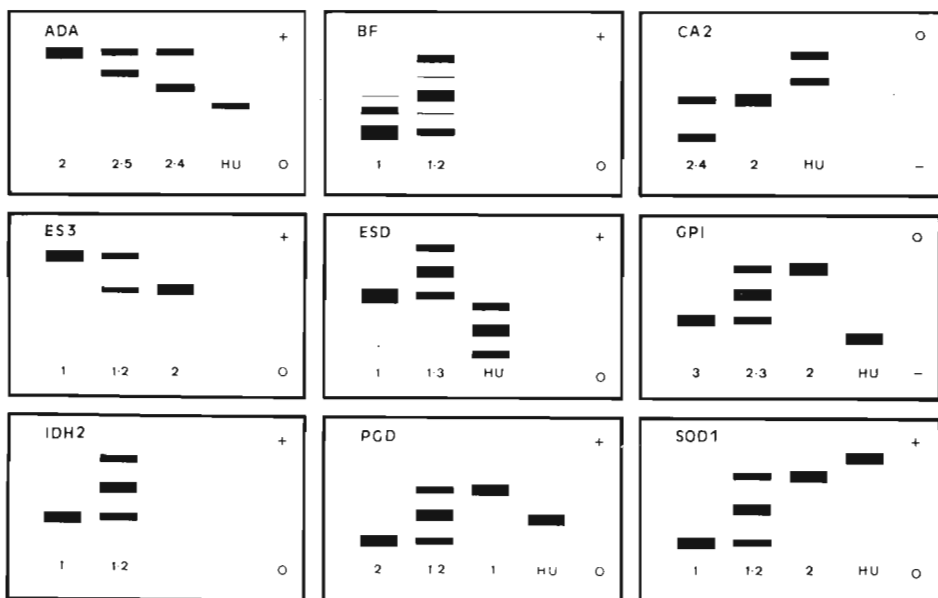


Figure 5 - Diagrams showing the electrophoretic patterns observed for the nine polymorphic systems detected in *A. seniculus*. HU = human control; o = origin.

(e) *Glucose phosphate isomerase*: GPI 2-3 presents the same mobility as the pattern with the same notation observed in *A. belzebul* (Schneider, 1988); (f) *Phosphogluconate dehydrogenase*: The alleles responsible for the observed phenotypes are probably the same as those reported with the same notation for *A. belzebul* (Schneider, 1988); (g) *Superoxide dismutase 1*: All bands were less anodic than those of the usual human SOD 1 pattern.

Table II - Phenotype distribution and allele frequencies of the nine polymorphic loci in *Alouatta seniculus*.

Loci	Phenotype	Number observed	Number expected	Allele frequencies and chi-squares	DF	P (%)
ADA	2	13	13.1	$ADA^*2 = 0.91$	3	99
	2-4	1	0.9	$ADA^*4 = 0.03$		
	2-5	2	1.9	$ADA^*5 = 0.06$		
	4	0	0.0	$\chi^2 = 0.11$		
	4-5	0	0.1			
	5	0	0.0			
BF*	1	13	13.0	$BF^*1 = 0.96$	1	100
	1-2	1	1.0	$BF^*2 = 0.04$		
	2	0	0.0	$\chi^2 = 0.00$		
CA2	2	14	14.0	$CA2^*2 = 0.94$	1	85
	2-4	2	1.9	$Ca2^*4 = 0.06$		
	4	0	0.1	$\chi^2 = 0.03$		
ESD	1	12	12.2	$ESD^*1 = 0.88$	1	63
	1-3	4	3.6	$ESD^*3 = 0.12$		
	3	0	0.2	$\chi^2 = 0.24$		
ES3**	1	9	8.0	$ES3^*1 = 0.73$	1	25
	1-2	4	5.9	$ES3^*2 = 0.27$		
	2	2	1.1	$\chi^2 = 1.47$		
GPI	2	2	3.4	$GPI^*2 = 0.47$	1	16
	2-3	11	8.2	$GPI^*3 = 0.53$		
	3	3	4.4	$\chi^2 = 1.94$		

Continued

Table II - Continued.

Loci	Phenotype	Number observed	Number expected	Allele frequencies and chi-squares	DF	P (%)
IDH2*	1	12	12.1	$IDH2*1 = 0.93$	1	84
	1-2	2	1.9	$IDH2*2 = 0.07$		
	2	0	0.0	$\chi^2 = 0.04$		
PGD	1	7	6.1	$PGD*1 = 0.63$	1	35
	1-2	6	7.7	$PGD*2 = 0.37$		
	2	3	2.2	$\chi^2 = 0.87$		
SOD1	1	2	1.5	$SOD1*1 = 0.31$	1	52
	1-2	6	7.1	$SOD1*2 = 0.69$		
	2	8	7.4	$\chi^2 = 0.41$		

In some systems only 14 (*) or 15 (**) animals were examined.

Average heterozygosity calculated for 26 loci of *A. seniculus* was 9.6%, with a standard error of 3.4%. if we consider only those systems previously studied in New and Old World primates as well as here (13 and 14 loci, respectively), no significant differences in heterozygosity levels are observed. Comparison with *A. palliata* was not possible, because of the 20 loci studied by Malmgrem (1979) only 5 are common to the systems investigated here.

A. seniculus from Jari has a karyotype more similar to *A. belzebul* from Pará, Brazil than to *A. seniculus* from Colombia or Bolivia. In spite of this karyotypic resemblance, the genetic distances between *A. seniculus* and *A. belzebul* (Table III) varied from 0.128 to 0.136, these values being similar to the variation reported for other interspecific, intragenetic distances. The distance between *C. apella paraguayanus* and *A. seniculus* (0.565) is also of the same magnitude as those reported for other intergeneric comparisons (such as between man and chimpanzees - 0.60; King and Wilson, 1975).

Table III - Matrix of Nei's unbiased genetic identity (I) above the diagonal and Nei's unbiased genetic distance (D) below the diagonal.

Population	WB	TI	EB	CAP	AS
<i>Alouatta belzebul</i> (WB)	****	0.999	0.999	0.529	0.880
<i>Alouatta belzebul</i> (TI)	0.001	****	0.998	0.523	0.873
<i>Alouatta belzebul</i> (EB)	0.001	0.002	****	0.530	0.879
<i>Cebus apella paraguayanus</i>	0.637	0.647	0.635	****	0.568
<i>Alouatta seniculus</i>	0.128	0.136	0.129	0.565	****

WB = West bank; TI = Tocantins island; EB = East bank; CAP = *Cebus apella paraguayanus*; AS = *Alouatta seniculus*.

Loci considered: ALB, HP, GPI, PGD, PGM1, ADA, ACP1, MDH1, LDHA, LDHB, IDH1, ESD and CA2.

Source of data for *A. belzebul*: Schneider (1988); for *C. apella paraguayanus*: Schneider et al. (1988).

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RESUMO

Dados eletroforéticos e citogenéticos foram obtidos de 16 animais de *Alouatta seniculus* coletados na margem oriental do rio Jari, na Amazônia brasileira. Foram observados números diplóides de 47, 48 e 49, sendo esta variação devida à presença de 1 a 3 microcromossomos supernumerários. Essas e outras características cariotípicas apresentaram-se diferentes de resultados alcançados com esta mesma espécie na Colômbia e Bolívia. A variabilidade genética de *A. seniculus* foi estimada usando-se 26 locos protéicos detectáveis eletroforeticamente. A heterozigose média foi calculada em 9,6%, similar às observadas em outros Platyrríneos. Distâncias genéticas entre esta espécie, *A. belzebul* e *Cebus apella paraguayanus* forneceram valores compatíveis com aqueles esperados em comparações intra e intergenéticas em primatas.

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