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The karyotype of *Alouatta fusca clamitans* from Rio de Janeiro, Brazil: Evidence for a y-autosome translocation

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ABSTRACT

The chromosome complements of four males of *Alouatta fusca clamitans*, caught in Rio de Janeiro State, Brazil, were analyzed by G-, C-, and NOR-banding techniques. The diploid number found was 49 in all the specimens. The presence of a heteromorphic pair of submetacentric chromosomes in the analyzed specimens, not present in males and females with 2n = 50 previously reported, and its G-banding pattern, led us to assume that this pair is involved in a Y-autosome translocation. Thus, the sex determination system appears modified to $X_1X_1X_2X_2$ /X_1X_2Y. Heterochromatic segments were found in the pericentromeric region of all the chromosomes, in the telomeric region of the short arm in pair 2, in the complete length of the short arm of pairs 5 and 6 and in the intercalary region of the long arm in pair 17. The nucleolar organizer regions were situated in the intercalary region of the long arm in two small acrocentric pairs.

INTRODUCTION

The six species of the genus *Alouatta* (Primates, Atelidae) currently recognized by most authorities have a widespread geographic distribution in the New World, from southern Mexico to northern Argentina (Wolfheim, 1983; Crockett, 1987).

Although previous karyological studies of this genus are scarce, many interesting rearrangements have been reported, with inter and intra-specific chromosomal variations detected. Five species have been analyzed karyologically, and different Y-autosome translocations have been reported in four of them: *A. palliata* (Ma *et al.*, 1975), *A. belzebul* (Armada *et al.*, 1987; Lima and Seuanez, 1989), *A. seniculus* (Yunis *et al.*, 1976; Minezawa *et al.*, 1985; Lima *et al.*, 1990; Lima and Seuanez, 1991) and *A. fusca* (Koiffmann, 1977).

Brown howlers (*Alouatta fusca*) are endemic to the coastal Atlantic forest of eastern and southeastern Brazil and northeastern Argentina, with two subspecies: *A. f. fusca* and *A. f. clamitans*, with the latter having a distribution from Rio de Janeiro and Minas Gerais to Argentina (Wolfheim, 1983). This subspecies has shown a diploid number ranging from 48 to 50 in specimens from São Paulo. The only specimen that showed 2n = 48 was a male caught in Registro city (southern São Paulo), being heterozygotic for two Robertsonian autosomic rearrangements. In addition, two males were found with 2n = 49, differing from 2n = 50 due to a Y-autosome translocation (Koiffmann, 1977).

MATERIAL AND METHODS

Blood samples were collected from four males of *Alouatta fusca clamitans*, caught in Rio de Janeiro State (Teresópolis, Nova Friburgo and Cachoeira do Macacu, 22° to 23° S and 43° to 42° W) and kept at Centro de Primatologia do Rio de Janeiro and Fundação Rio Zoo. The samples were collected in disposable plastic syringes, previously heparinized (Liquemin, Roche). Whole blood cultures were made in RPMI medium, with 20% fetal calf serum, 2% PHA (Cultilab) for 72 h at 37°C. Colcemid (0.2 mg/ml) was added during the last 60 min of incubation before harvesting (Moorhead *et al*., 1960). Well-spread preparations showing elongated chromosomes were stained with G-, C- and NOR-banding techniques, performed according to standard procedures (Seabright, 1971; Sumner, 1972; Howell and Black, 1980). Sequential G- C-band was performed to identify clearly the position of the heterochromatic segments. Chromosome spreads were photographed for karyotyping, and the autosomes were divided into two groups on the basis of their morphological attributes (biarmed or acrocentric) and homologue pairs were arranged within each group in decreasing size.

The present study describes the karyotypes of four specimens of *Alouatta fusca clamitans* from Rio de Janeiro, using G-, C- and NOR-banding staining for the first time. We also demonstrate a Y-autosome translocation in all the analyzed animals, by comparing the G-banding pattern to a male and a female with 2n = 50 (Koiffmann, 1977).

RESULTS

The four specimens analyzed in this study had a diploid number of 49 chromosomes, consisting of 17 biarmed and 32 acrocentric chromosomes. Of the sex chromosomes, the X was submetacentric and the Y apparently absent (Figure 1). G-banding patterns allowed us to identify clearly one heteromorphic pair of submetacentric chromosomes, and the X chromosome showed a pattern similar to that of most Plathyrrhini (Figure 2). Constitutive heterochromatin, identified by C-banding, was present in the pericentromeric region of all the chromosomes, in the telomeric region of the short arm in chromosome 2, in the short arm of chromosomes 5 and 6 and in the intercalary region of the long arm in chromosome 17 (Figure 3). NOR staining showed four small chromosomes marked in the proximal region of the long arm in 87% of the analyzed cells (Figure 4); in the rest, the number of marked chromosomes was two or three.

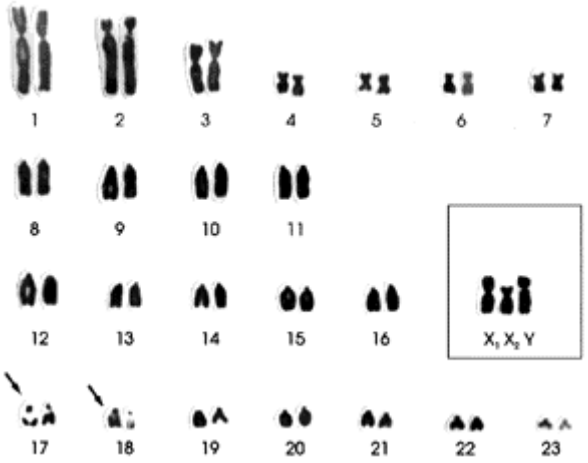

Figure 1 - The karyotype of *Alouatta fusca clamitans*, male, 2n = 49. The arrows show the secondary constrictions.

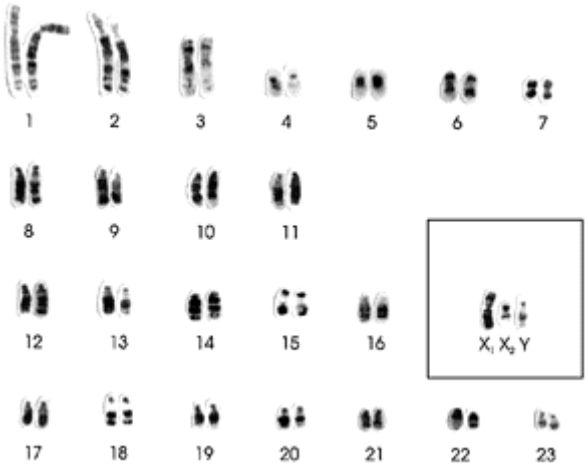

Figure 2 - G-banded karyotype of *Alouatta fusca clamitans*, male.

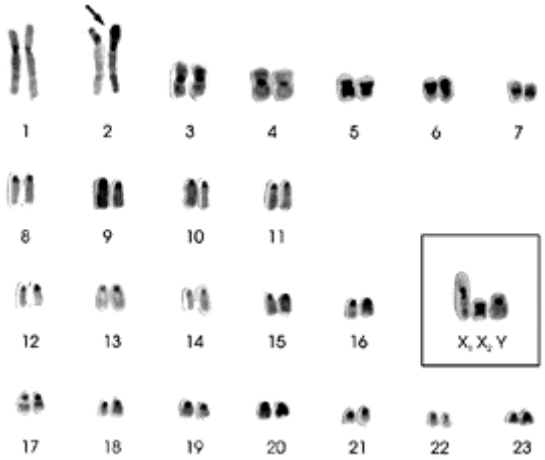

Figure 3 - C-banded karyotype of *Alouatta fusca clamitans*, male. The arrow shows a heteromorphism found in one of the specimens.

Figure 4 - Nucleolar organizer regions (arrows) found in the karyotype of *Alouatta fusca clamitans*.

DISCUSSION

Chromosome studies in *Alouatta fusca clamitans* from Rio de Janeiro have shown that all the analyzed males had 2n = 49 due to a Y-autosome translocation. This fact has been also observed in males caught in São Paulo (Koiffmann, 1977). However, the existence of a Y-autosome translocation in this subspecies does not seem to be constant, with two reported males with 2n = 50 (Koiffmann and Saldanha, 1974). The existence of a heteromorphic submetacentric pair in a male with 2n = 49 from São Paulo led Koiffmann (1977) to propose a translocation. Although meiotic studies were not possible to have been done in this study (the authors were not allowed to collect the necessary material for it), the presence of a heteromorphic submetacentric pair in all the analyzed males, and not observed in males and females with 2n = 50, with the G-banding pattern obtained, shows unequivocally that this pair is involved in the rearrangement with the Y chromosome. According to the G-banding pattern, and comparing it with the acrocentric Y chromosome reported for this species (Koiffmann, 1977), we agree with the proposed rearrangement, and assume the occurrence of two breaks in the process: one in the long arm of the submetacentric chromosome at the junction of the positive band, and one in the short arm of the Y chromosome, near the centromere. Subsequently, the submetacentric chromosome fused with the Y chromosome (Figure 5).

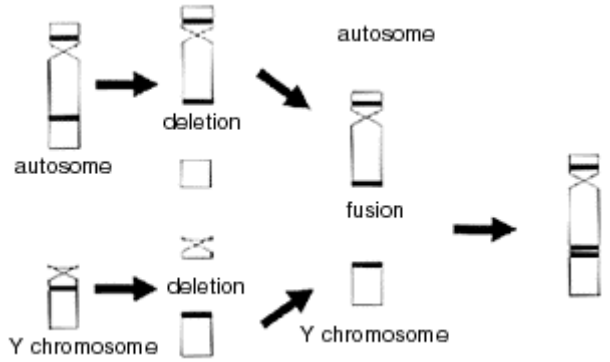

Figure 5 - Scheme of the Y-autosome translocation found in *Alouatta fusca clamitans*.

Although the sex determination system among mammals follows almost invariably the XX/XY system, it appears modified in the case of a Y-autosome translocation to $X_1X_1X_2X_2$ /X_1X_2Y. In this system, the true X chromosome which was not involved in the translocation is designated as the X_1. The Y-carrying autosome is called Y while its

non-carrying homologue, which occurs unpaired in the male and as a pair in the female, is called X_2. Y-autosome translocations have been reported in other platyrrhini primates: *Callimico* (Hsu and Hampton, 1970), *Aotus* (Ma *et al.*, 1976) and *Cacajao* (Koiffmann and Saldanha, 1981). However, in *Alouatta* this rearrangement seems to be quite common. Of the five species analyzed so far, only *Alouatta caraya* showed a constant XX/XY chromosomal sex-determining system. Armada *et al.* (1987) showed in males of *Alouatta belzebul belzebul* with 2n = 49 that the meiotic cells were comprised of a trivalent formed by the X chromosome, the Y-carrying autosome and the original pair of this autosome. We assume that this is probably what happens in the meiotic cells of *Alouatta fusca clamitans*, with a Y-autosome translocation similar to the one reported in *Alouatta belzebul belzebul*. However, although in *Alouatta belzebul belzebul* it seems constant, in *Alouatta fusca clamitans* Koiffmann (1977) and Koifmann and Saldanha (1974) have reported males with 2n = 50.

Pair 2 was also heteromorphic in one of the specimens studied in this report. C-banding demonstrated that this heteromorphism was due to a difference in the length of the heterochromatic segment (Figure 3). This technique could not help in the identification of the Y-autosome translocation, since studies in other species of *Alouatta* have demonstrated that the Y chromosome has only a centromeric segment of heterochromatin (Lima and Seuanez, 1991; Mudry *et al.*, 1994). The distribution of the heterochromatic segments in *A. fusca clamitans* resembles the one observed in *A. belzebul belzebul*, with the latter having positive C-bands in the centromeric region of all the chromosomes, in the telomeric region of a pair of submetacentric and in the intercalary region of two pairs of acrocentric chromosomes. However, pair 6 of *A. fusca clamitans* showed the complete short arm with constitutive heterochromatin. This fact is not observed in other species of this genus.

The nuclear organizer regions were situated in the long arm of two pairs of acrocentric chromosomes, probably corresponding to pairs 21 and 22, which possessed secondary constrictions when analyzed by conventional staining technique. The position of NORs was similar to the one described in *Alouatta caraya*, although this species showed six small acrocentric chromosomes possessing nucleolar organizer regions (Mudry *et al.*, 1994).

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RESUMO

Os cariótipos referentes a quatro machos de *Alouatta fusca clamitans* oriundos do Rio de Janeiro foram analisados através de técnicas de bandamento G, C e NOR. O número diplóide em todos os espécimes foi igual a 49, com a presença de três cromossomos não pareados. A comparação dos padrões de bandamento G com espécimes previamente

descritos com 2n = 50 revelou a ocorrência de uma translocação do tipo Y-autossomo, modificando o sistema cromossômico de determinação sexual para o tipo múltiplo, $X_1X_2Y/X_1X_1\ X_2X_2$. Os blocos de heterocromatina constitutiva se distribuíram na região pericentromérica de todos os cromossomos; segmentos intercalares e teloméricos foram visualizados em um par acrocêntrico e em outro submetacêntrico, respectivamente. As regiões organizadoras de nucléolo se localizaram no braço longo de dois pares de pequenos acrocêntricos.

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