Revised karyotype of *Alouatta caraya* (Primates: Platyrrhini) based on synaptonemal complex and banding analyses

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Most primates studied have the usual XX/XY sex-chromosome system. However, exceptions to this rule among howler monkeys have been suggested by several authors. Recently a quadrivalent was discovered in male meiosis of *Alouatta caraya* and it was established that this species has an $X_1X_2Y_1Y_2$ sex chromosome system. On that basis, a cytogenetic analysis of 25 males of this species is described, showing the corrected karyotype of this species. Each chromosome involved in the particular sex-chromosome system of this species is identified on the basis of mitotic chromosome measurements, G and C-banding patterns as well as on the relative measurements of synaptonemal complexes. It is now established that *A. caraya* has a karyotype with 2n = 52 in both sexes, and that the male one shows a single autosome #7 (X_2) besides the X (X_1) and the two products of the reciprocal translocation between the second autosome #7 and the Y chromosome (Y_1 and Y_2), while females show a homomorphic pair #7 (X_2) and a pair of X_1. The evolutionary implications of the exceptional primate species having composite sex-chromosome systems are discussed.

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The usual sex-chromosome system among mammals is the XX/XY one (FREDGA 1970, 1988), and most primate species are no exceptions to this rule. However, it has been repeatedly reported that among New World monkeys there are species showing unusual features concerning sex chromosomes (MA 1981; PIECZARKA and NAGAMACHI 1988; VIEGAS-PEQUIGNOT et al. 1985) while in Old World monkeys only *Presbytis cristata*, 2n = 44, was also described with a Y autosome reciprocal translocation and an $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromsome system (BIGONI et al. 1997). Particularly among howler monkeys several reports had suggested the existence of exceptional sex chromosome systems (MA et al. 1975; ARMADA et al. 1987), and LIMA and SEUANEZ (1991) provided evidence that *Alouatta seniculus stramineus* has a multiple-sex chromosome system. Recently, firm evidence on the existence of a multiple sex chromosome system of the type $X_1X_2Y_1Y_2$ has been provided by two different approaches: by synaptonemal complex (SC)

analysis of pachytene spermatocytes RAHN et al. (1996) established that *Alouatta caraya* regularly forms a quadrivalent formed by the products of a Y-#7 reciprocal translocation, the X, and the usual #7; and on the other hand, using chromosome painting (CONSIGLIERE et al. 1996) it was showed that two supposed subspecies of *Alouatta seniculus*, *Alouatta seniculus sara* and *Alouatta seniculus arctoidea*, also share an $X_1X_2Y_1Y_2$ multiple sex-chromosome system, although they could not obtain signals with the human Y-chromosome painting probe.

On the basis of these observations, it is necessary to redefine the karyotype of *A. caraya*, which was originally assumed to have an XY sex chromosome pair and autosomal heteromorphisms (MUDRY et al. 1981, 1984, 1990, 1994). It is also necessary to survey specimens captured from different localities in order to assess the existence of chromosome polymorphisms in this species. Finally, comparative karyological data on the different species of this and other related genera should be analyzed in order to assess the extension of this very particular sex chromosome system and the phylogenetic relationships between these taxa.

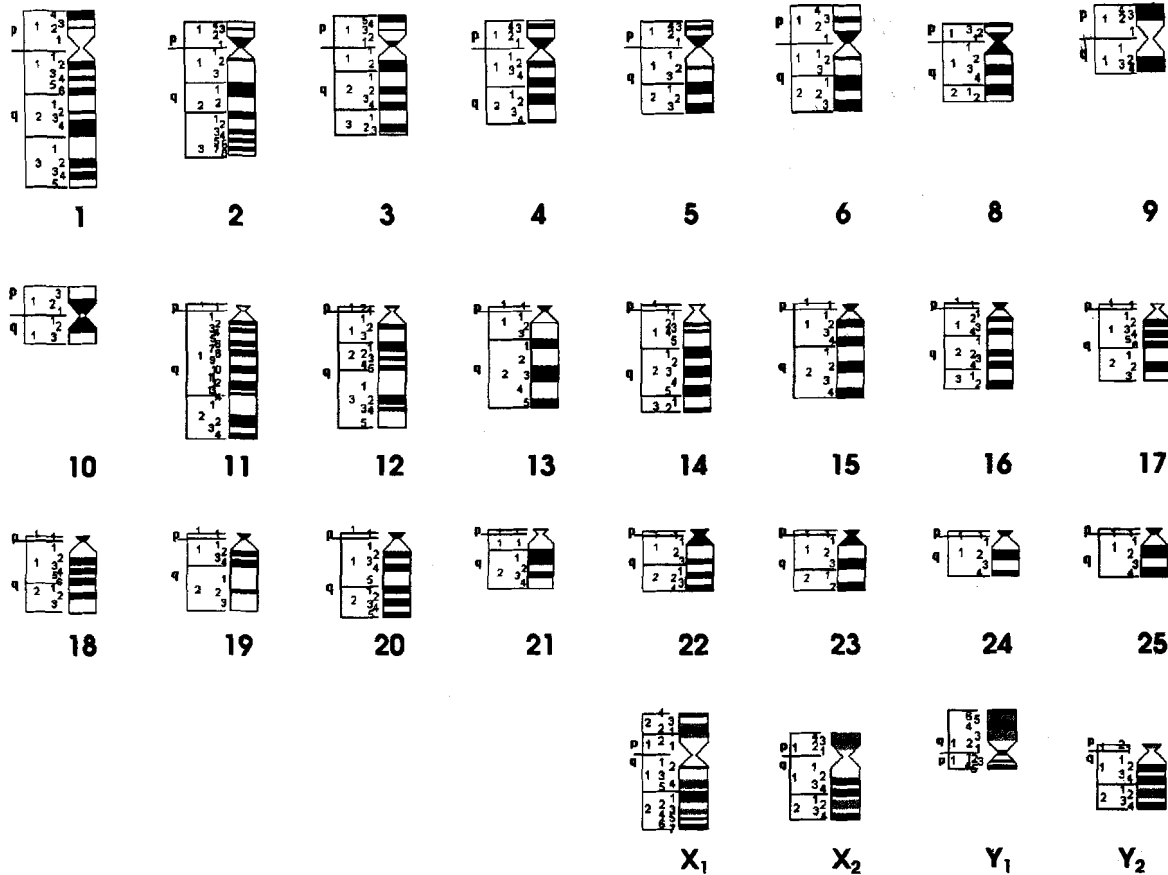This paper presents our results and discussion on these subjects.

Fig. 1. Idiogram of G-banded chromosomes of *Alouatta caraya*. A multiple sex-chromosome system is given below showing each element involved in the characteristic $X_1X_2Y_1Y_2$ translocation.

Fig. 2. Idiogram illustrating the G-banding pattern of *Alouatta caraya* multiple sex-chromsome system arranged according to the proposed $X_1X_2Y_1Y_2$ where X_1 = original X chromosome; X_2 = autosome #7; $Y_1 = Y_7$ as a translocation product; and $Y_2 = 7_Y$ as the other translocation product.

MATERIAL AND METHODS

Mitotic analyses were conducted on lymphocytes from peripheral blood samples following conventional culture methods (96 h in culture, 30 min in colcemid, modified from BUCKTON and EVANS 1973). Samples were obtained from 25 male and 22 female specimens coming from different localities in the geographical distribution of this species (Chaco and Corrientes, Argentina).

Slides were subjected to routine G-banding (WANG and FEDOROFF 1972) and C-banding (SUMNER 1972). At least 25 metaphases were analyzed to determine the modal chromosome number (2n). Mitotic mea-

sures were performed in G-banded metaphases using a Sigma-Scan program (Jandel Sci, San Rafael, C.A.) with a Genitizer GT-1212B digitizer. Three adult males, one of them kept near CAPRIM in Apipé Island and two remaining at the ECAS (Estación de Criz de Animales Salvajes; their geographic origin is unknown), were employed to perform meiotic studies. Testicular biopsy was performed under anesthesia. The biopsies (3 mm^3) were divided in three pieces, one of them was fixed in 2.5% glutartaldehyde in 0.1M phosphate buffer (pH 7) for thin serial sections for electron microscopy (EM) and the other two pieces were used for synaptonemal complex (SC) prepara-
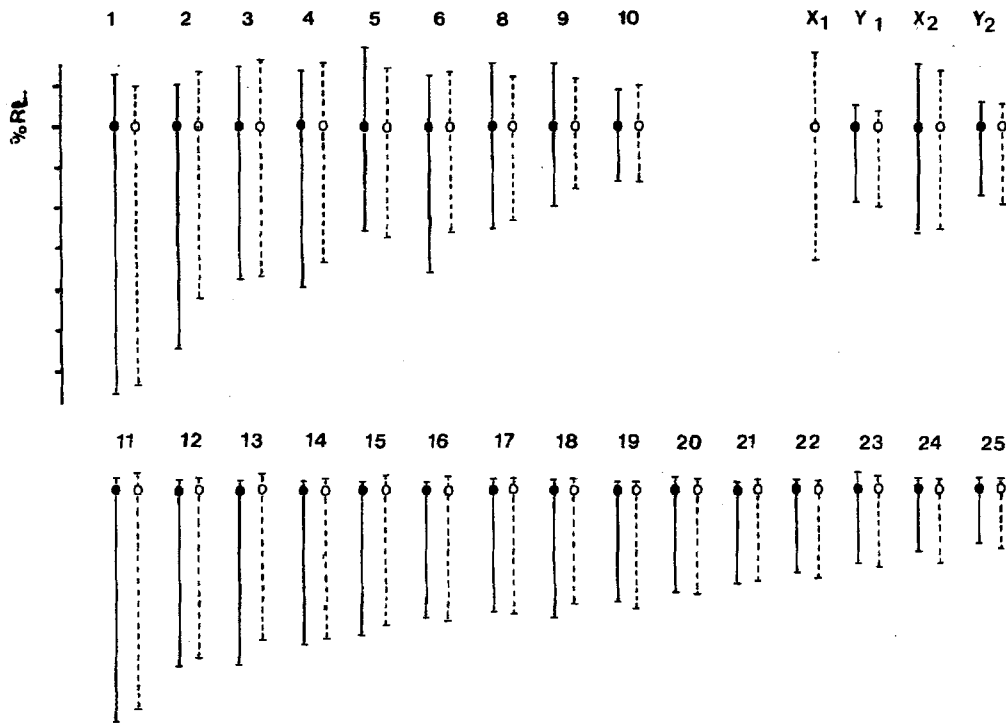
Fig. 3. *Alouatta caraya* SC and mitotic idiogram based on the mean values of 16 pachytene spermatocytes (–) and 20 G-banded metaphases (---).
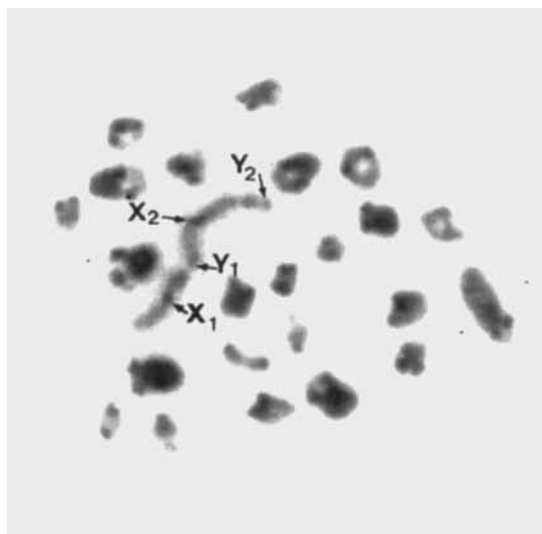

Fig. 4. *Alouatta caraya* C-banded M-I cells. In the quadrivalent, the arrows show four centromeric blocks corresponding to alternate elements: X_1-Y_1-X_2-Y_2.

tions and for meiotic analyses for light microscopy. For SC preparations the pieces were kept in Hank's solution and used for SC preparations immediately after testicular biopsy. Meiotic studies were carried out with the microspreading technique (SOLARI 1980). The SCs were stained with the method of HOWELL and BLACK (1980) or with phosphotungstic

acid for the kinetochore location.

The C-band technique to identify heterochromatic distribution was also performed in meiotic chromosomes prepared with the standard technique (EVANS et al. 1964).

RESULTS

Chromosome counts conducted in 906 cells from males and 1136 from females confirmed that the *Alouatta caraya* diploid number is 2n = 52. Among the 25 males investigated, 13 were selected for identification of the translocation products involving one autosome and the sex chromosomes, and in all of these specimens the 4 involved chromosomes ($\#7$, $\#7_Y$, $\#Y_7$ and X) were identified in banded metaphases. Taking into account the G-banding pattern obtained from 5 metaphases from 4 different male individuals, a standard G-idiogram was constructed (Fig. 1). Chromosome $\#7$ in this idiogram is the same as the pair $\#7$ previously described in females. In all the male specimens analyzed, the second $\#7$ is replaced by the 7 – Y translocation products, which show partial banding homologies with 7q and 7p (Fig. 2).

These observations were confirmed by measurements made in metaphases from three males coming from different geographic origins. The mitotic rela-
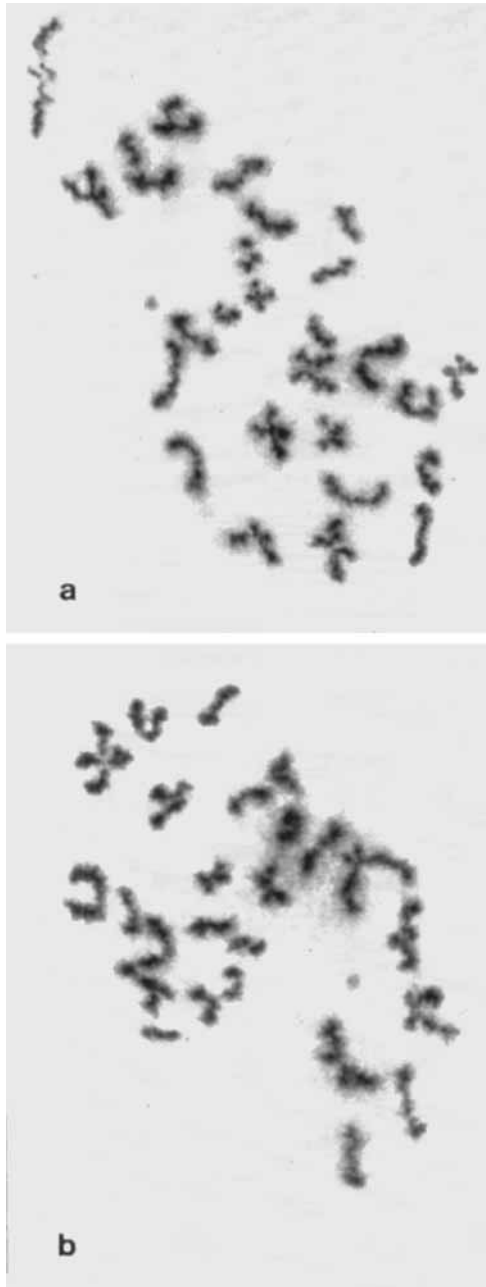
Fig. 5a and b. *Alouatta caraya* M-II cells showing 26 chromosomes with 11 biarmed and 15 acrocentric chromosomes (a) and 9 biarmed and 17 acrocentric chromosomes (b).

tive lengths of these elements (expressed in % of the total length of the chromosome complement) are: chromosome $7 = 3.98\%$; $7_Y = 2.56\%$; $Y_7 = 2.31\%$; $X = 4.36\%$. These relative measurements agree with those obtained from the measurements of meiotic axes in electron micrographs of spread spermatocytes. Thus, a comparative idiogram of SCs and mitotic chromosomes (Fig. 3) shows that each mitotic chromosome can be identified (by its arm ratio and relative length), with the corresponding SC.

C-banding was used in meiotic cells at metaphase I to identify the elements involved in their rearrangement with the sex chromosomes. A chain multivalent consisting of four elements was always present in C-banded M-I cells (Fig. 4). Four centromeric blocks were clearly identified in all M-I cells. The quadrivalent is formed by two longer submetacentric elements and two shorter, acrocentric ones, which alternate with each other in this order: X_1-Y_1-X_2-Y_2 (Fig. 4).

All cells at metaphase II showed 26 chromosomes, but there are two distinct classes of cells: (a) cells with 11 biarmed elements and 15 acrocentric chromosomes (which contain the intact 7 and the X chromosome); and (b) cells with 9 biarmed chromosomes and 17 acrocentric chromosomes (these cells include the two rearranged products 7_Y and Y_7) (Fig. 5a and b).

SC preparations at pachytene show an outstanding quadrivalent (Fig. 6). The four axes involved in this quadrivalent are those formed by the X chromosome, the intact autosome #7, and the translocation products, 7_Y and Y_7. The relative lengths and the kinetochore positions in these meiotic axes forming the quadrivalent agree with the relative lengths and arm ratios of the corresponding mitotic chromosomes.

No microchromosomes have been found in any specimen and no other polymorphism has been noticed in the specimens studied.

DISCUSSION

Karyological variability among New World monkeys

The order Primates shows species having variable chromosomal numbers, between $2n = 20$ and $2n = 72$ (EGOZCUE 1975). In the older literature many reports have suggested the presence of wide variations in chromosome morphology and in diploid numbers among related species (BENDER and CHU 1963; EGOZCUE and EGOZCUE 1966; EGOZCUE et al. 1968; MA et al. 1975, 1985; YUNIS et al. 1976; KOIFFMANN 1982; DUTRILLAUX et al. 1986; ARMADA et al. 1987; LIMA and SEUANEZ 1989). Some of this karyological variation is associated with the geographical origin of individuals, for instance in the genus *Saimiri* (MOORE et al. 1990; GARCIA et al. 1995) or in the species *Alouatta fusca* (OLIVEIRA et al. 1995). The sources of this karyological variability have been attributed to a high frequency of chromosomal rearrangements, involving Robertsonian rearrangements (fusion/fission), reciprocal translocations, inversions, and complex rearrangements (DE BOER and BRUIJN 1990; OLIVEIRA et al. 1995). However, the scarcity of systematic banding analyses and the virtual lack of meiotic studies in both sexes of each species have hindered a phylogenetic interpretation of this kary-
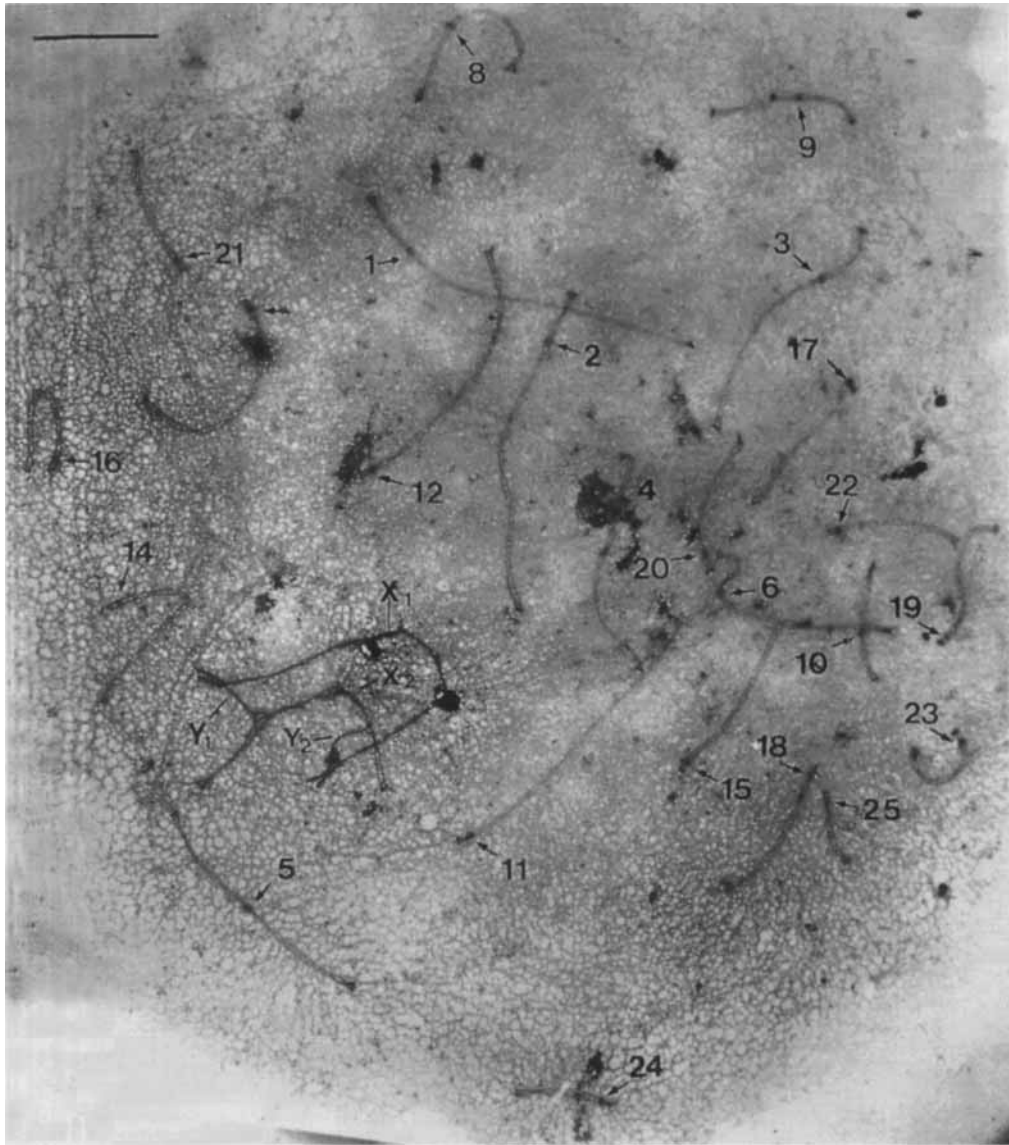
Fig. 6. *Alouatta caraya* pachytene spermatocyte nucleus. The arrows show the kinetochores of the bivalents (identified with a number) Magnification × 3.500. Bar = 5 μm.

ological variability. In a recent study of two subspecies of *Alouatta seniculus* performed with banding analysis, the different diploid numbers in females (44) and males (45) of *A. seniculus arctoidea* were attributed to differences in the numbers of microchromosomes (STANYON et al. 1995). On the other hand, differences in the diploid numbers of other species have been attributed to the presence of multiple sex-chromosome systems (MA 1981). However, in the lack of meiotic studies, the latter interpretation is hypothetical.

Recently, the use of chromosome painting in mitotic chromosomes has allowed a detailed description of chromosomal rearrangements in some species of

Howler monkeys. Thus, CONSIGLIERE et al. (1996) showed that the two subspecies *Alouatta seniculus sara* and *Alouatta seniculus arctoidea* differ from each other in at least 16 chromosomal rearrangements, and microchromosomes were reported to vary between 1 and 5 in number and having no hybridization with any human chromosome paint, suggesting that they are composed of highly repetitive DNA. It has been suggested that these microchromosomes could be transient products of Robertsonian translocations, thus suggesting their recent appearance in these subspecies (CONSIGLIERE et al. 1996).

In the face of these wide chromosomal variations occurring among subspecies in the genus *Alouatta*

(which contains only 6 species, EMMONS 1990) the present results showing a highly constant karyotype in the species *Alouatta caraya* are remarkable, and confirm the previous reports on the stable karyotype of this species (MUDRY et al. 1994; TORRES et al. personal commun.). More interestingly, the present observations — gathering data from 25 males and 22 females from different geographical origins — show the same standard karyotype. Thus, while chromosomal speciation mechanisms may be acting in *Alouatta seniculus*, there is no evidence that these mechanisms are present in *Alouatta caraya*.

The extent of multiple sex-chromosome systems among New World monkeys

Multiple sex-chromosome systems have been assumed to be present in some species of New World monkeys (DE BOER 1974; MINEZAWA et al. 1985). These claims were generally based on the study of mitotic chromosomes, with or without banding procedures, and on the finding of different diploid numbers in both sexes. However, the possible presence of microchromosomes, the lack of analysis of meiotic cells and the absence of data on synaptonemal complex behavior during meiosis in these species make these claims to remain largely hypothetical. The first convincing evidence on the existence of sex multiples was the reported presence of a quadrivalent at metaphase-I in the subspecies *Alouatta seniculus stramineus* ($2n = 46 + 1 - 3$ microchromosomes) (LIMA and SEUANEZ 1991). According to these authors, the metaphase-I quadrivalent showed the presence of an $X_1X_2Y_1Y_2$ multiple. However, LIMA and SEUANEZ (1991) did not study the pachytene stage and the synaptonemal complex complement, and thus, their interpretation of the quadrivalent at metaphase-I rested solely on morphological grounds.

Furthermore, the presence of variable numbers of microchromosomes in this subspecies complicates the interpretation presented by LIMA and SEUANEZ (1991).

Our previous paper (RAHN et al. 1996) was the first analysis of synaptonemal complexes in a member of the genus *Alouatta*: in 3 males of *A. caraya* the quadrivalent at pachytene was analyzed and measured, and each of its components was identified: chromosomes 7 and X, and the two translocation products 7_Y and Y_7 (RAHN et al. 1996).

The present paper shows that this $X_1X_2Y_1Y_2$ multiple sex-chromosome system (X_1 = original X chromosome; X_2 = chromosome #7; $Y_1 = Y_7$ product bearing the Y centromere; $Y_2 = 7_Y$ product bearing an autosomal centromere) is a general and constant feature of all the 25 males studied of *Alouatta caraya*, discarding any association with specific geographical origins of the individuals studied.

The present observations also allow the presentation of a corrected, standard male mitotic karyotype in this species (Fig. 1), in which the older assignment of a heteromorphism in autosome 7 is replaced by the single intact chromosome X_2 and the rearranged elements Y_1 and Y_2, which may be useful for the study of karyological relationships with the other species and subspecies of the genus *Alouatta*.

In that respect it is interesting that the subspecies *Alouatta seniculus sara* ($2n = 50$) and *Alouatta seniculus arctoidea* ($2n = 45$) analyzed with chromosome painting (CONSIGLIERE et al. 1996) also share an $X_1X_2Y_1Y_2$ multiple sex chromosome system, which is virtually the same in both subspecies, despite their difference in at least 16 autosomal rearrangements (CONSIGLIERE et al. 1996). Human chromosome paint #3 hybridizes with X_2q, and human chromosome paint #15 hybridizes with X_2p, while human chromosome paint #3 hybridizes most of Y_2q and human chromosome paint #15 hybridizes most of Y_1; however, no signal from the human Y was consistently detected in both *A. seniculus* subspecies (CONSIGLIERE et al. 1996).

From the published reports it is very probable that the same $X_1X_2Y_1Y_2$ multiple is present in *Alouatta caraya* (RAHN et al. 1996; and present report), in *Alouatta seniculus stramineus* (LIMA and SEUANEZ 1991) and in *Alouatta seniculus sara* and *Alouatta seniculus arctoidea* (CONSIGLIERE et al. 1996). Although they lack the strength of the above cited cases, previous reports on *Alouatta palliata* (MA et al. 1975) and on *Alouatta fusca clamitans* (LIMA and SEUANEZ 1991) suggest that in these two species also a sex chromosome multiple is present. Thus, at the present time, this particular $X_1X_2Y_1Y_2$ sex chromosome system may be shared by most of the species in the genus *Alouatta*, despite the large degree of chromosomal variation present in this genus. On a larger taxonomical scale, sex chromosome multiples are not a shared feature in New World monkeys; for instance, among the Ceboidea, the species *Cebus apella* has a "usual" XY/XX sex chromosome system, as convincingly shown by both high resolution mitotic G-banding (MATAYOSHI et al. 1986; PONSA et al. 1995) and meiotic and synaptonemal complex analysis (SOLARI, unpublished observations). Thus, the phylogenetic origin and spreading of the $X_1X_2Y_1Y_2$ system in the genus *Alouatta* remains to be resolved.

ACKNOWLEDGEMENTS

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