American Journal of Primatology 54:65–78 (2001)

RESEARCH ARTICLES

Meiosis and Chromosome Painting of Sex Chromosome Systems in Ceboidea

M.D. MUDRY[1], I.M. RAHN[2], AND A.J. SOLARI[2]*
[1]*Grupo de Investigación en Biología Evolutiva (GIBE), Depto. de Biologçia, Facultad de Cs. Exactas y Naturales (FCEyN), Universidad de Buenos Aires (UBA), Ciudad Universitaria, Buenos Aires, Argentina*
[2]*Centro de Investigaciones de Reproducción (CIR), Facultad de Medicina, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina*

The identity of the chromosomes involved in the multiple sex system of *Alouatta caraya* (*Aca*) and the possible distribution of this system among other Ceboidea were investigated by chromosome painting of mitotic cells from five species and by analysis of meiosis at pachytene in two species. The identity of the autosome #7 (X_2) involved in the multiple system of *Aca* and its breakage points were demonstrated by both meiosis and chromosome painting. These features are identical to those described by Consigliere et al. [1996] in *Alouatta seniculus sara* (*Assa*) and *Alouatta seniculus arctoidea* (*Asar*). This multiple system was absent in the other four Ceboidea species studied here. However, data from the literature strongly suggest the presence of this multiple in other members of this genus. The presence of this multiple system among several species and subspecies that show high levels of chromosome rearrangements may suggest a special selective value of this multiple. The meiotic features of the sex systems of *Aca* and *Cebus apella paraguayanus* (*Cap*) are strikingly different at pachytene, as the latter system is similar to the sex pair of man and other primates. The relatively large genetic distances between species presently showing this multiple system suggest that its origin is not recent. Other members of the same genus should be investigated at meiosis and by chromosome painting in order to know the extent and distribution of this complex sex-chromosome system. Am. J. Primatol. 54:65–78, 2001.

Key words: multiple-sex-chromosome systems; meiosis; chromosome painting; chromosome conservation

INTRODUCTION

New World monkeys are much more karyologically variable than Old World monkeys and great apes [Wienberg & Stanyon, 1998]. Chromosome painting and

Contract grant sponsor: CONICET; Contract grant numbers: MDM-PIP 4431/97; AJS-PIP 4201; Contract grant sponsor: UBACYT; Contract grant numbers: MDM-EX 0022; AJS-01/TM54-M008.

*Correspondence to: Dr. Alberto J. Solari, CIR, Facultad de Medicina, Paraguay 2155 – p. 10, (1121) Buenos Aires, Argentina. E-mail: ajsolari@mail.retina.ar

Received 27 March 2000; revision accepted 22 February 2001

other cytogenetical data have added substantial support to this view. However, the number of species in some groups of New World monkeys, as well as their phylogenetic relationships, remain controversial.

The taxon Ceboidea harbors species with highly derived karyotypes, as exemplified by *Ateles geoffroyi* [Morescalchi et al., 1997] and several species of the genus *Alouatta* [Consigliere et al., 1996]. Despite the recognition of this wide spectra of chromosomal changes among Ceboidea, a striking multiple sex-chromosome system, of the $X_1X_2Y_1Y_2$ type, seems to be extended in at least several taxa of this family [Lima & Seuánez, 1991; Rahn et al., 1996; Consigliere et al., 1996; Mudry et al., 1998]. Meiotic studies have been a standard way to establish the identity and structure of multiple sex-chromosome systems in mammals [Fredga, 1970]. Such meiotic studies can be more informative when performed as synaptonemal complex analysis at pachytene in spreads studied with electron microscopy [Solari, 1998]. In these studies sex chromosomes are easily recognized and homology can be established by the chromosome-pairing patterns [Gillies, 1989]. However, fine structural, meiotic studies on New World monkeys are extremely scarce. The aim of the present work is to present both meiotic observations and chromosome paintings on two relatively distant species, *Cebus apella paraguayanus* (*Cap*) and *Alouatta caraya* (*Aca*), as well as provide some related data on three other species of Ceboidea. The identity of the sex-multiple $X_1X_2Y_1Y_2$ is confirmed, and its distribution on the genus *Alouatta* is discussed. On the other hand, the XY system found in *Cebus apella* has several features in common with the human XY pair and with other primates, and thus this XY system seems to be ancestral in Ceboidea. The conservation of the sex-multiple among Alouattinae is discussed, and the independent origin of other multiple sex-chromosome systems in Ceboidea is proposed.

METHODS

Animal Sampling

Adult specimens from five Ceboidea genera were used for cytogenetic studies, which included 10 blood samples. For Atelidae, we included three black howler monkeys (*Aca*) and one spider monkey (*Ateles paniscus* (*Apa*)). For Cebidae we included two owl monkeys (*Aotus azarae* (*Aza*)), two capuchins (*Cap*), and two squirrel monkeys (*Saimiri boliviensis* (*Sbo*)). Animals, from either the field or zoos, were anesthetized with Ketamine (10 mg/kg bw), and a maximum of 3 cc of blood was collected through femoral venipuncture with heparinized syringes (Heparin, Abbot, Buenos Aires, Argentina).

Chromosomal Studies

Mitotic analysis. Analysis was performed on lymphocytes following standard whole-blood culture techniques. The metaphase chromosomes were studied by modified G, C, and G/C sequential banding techniques [Sumner, 1972]. Fifteen G-banded karyotypes were prepared for each specimen. The banding identification of each chromosomal pair agreed with previously published reports [Mudry et al., 1998].

Chromosomal painting with human chromosome-specific DNA library probes (CAMBIO, Cambridge, UK) on chromosomes of five species (*Aca, Cap, Apa, Sbo*, and *Aza*) was performed as described in the manufacturer's instructions. Hybridizations were done using biotinylated painting kits for human chromosomes #3, #15, X, and Y (CAMBIO). After hybridization and washing of the slides, biotinylated DNA probes were detected with streptavidin coupled with fluores-

cein isothiocyanate (FITC) (CAMBIO). The counterstain was performed with propidium iodide. Photographs of hybridized metaphases were taken with an epifluorescence microscope (DM, Leika, using ProVia 400 ASA color slide film).

Meiotic analysis. Testicular biopsies were performed under anesthesia in two males of *Cap* from the Centro de Estudios Médicos e Investigaciones Científicas (CEMIC), in one male of *Apa* from the Estación de Cría de Animales Silvestres (ECAS), and in three males of *Aca* from the Centro Argentino de Primates (CAPRIM) and ECAS. Testicular biopsies (2 × 2 mm), were kept in modified Eagle's medium (MEM) and divided into two pieces: one was used for synaptonemal complex (SC) preparations and the other for light microscopy. Pieces of unfixed tissue kept in MEM solution were used for SC preparations immediately after the testicular biopsy. Preparations were performed by the microspreading technique [Solari, 1980] and stained with silver nitrate [Howell & Black, 1980] or with ethanolic phosphotungstic acid (PTA). For the study of metaphases I and II the testicular tissue was fixed in Carnoy (3:1) after hypotonic treatment with sodium citrate [Evans et al., 1964]. C-banding was performed as previously described [Sumner, 1972].

RESULTS

Synaptonemal Complex Analysis in *Aca*

The pachytene nuclei of spermatocytes from *Aca* show an outstanding quadrivalent that involves the sex chromosomes. The structure of the quadrivalent has been previously described by us [Rahn et al., 1996; Mudry et al., 1998], and it is represented in Fig. 1. The sequential changes of this quadrivalent during pachytene will be presented here. The nomenclature for pachytene substages is that of the human spermatocytes [Solari, 1980]. In the first stage (Fig. 2a) the quadrivalent is already visible. The original X axis does not show differentiations except for a large and dense nodule located near the pairing end of the X axis. There is not yet a definite SC in this pairing region, as synapsis between Y_1 and X_1—involving the original pseudoautosomal region—is delayed. On the other hand, there are SCs between both Y_2 and #7 (X_2) and the other end of #7 and Y_1.

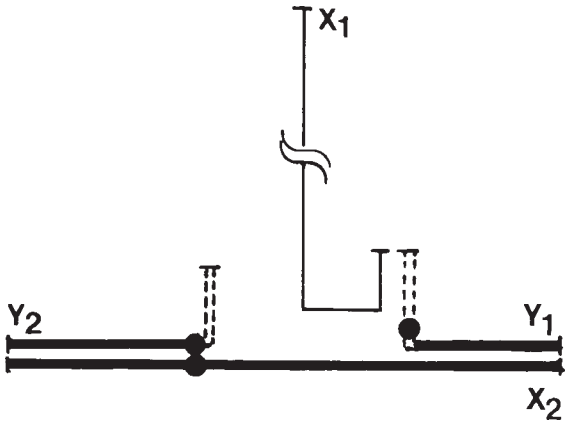

Fig. 1. Structure of the quadrivalent of *Aca*. The original X chromosome is labeled X_1 and is paired with a small region (segmented line) of a compound chromosome, Y_1. The autosomal part of Y_1 is black-filled and the segmented line corresponds to the original Y. The nondisrupted autosome (#7) is labeled X_2. Y_2 is the other translocation product that bears the autosomal centromere.

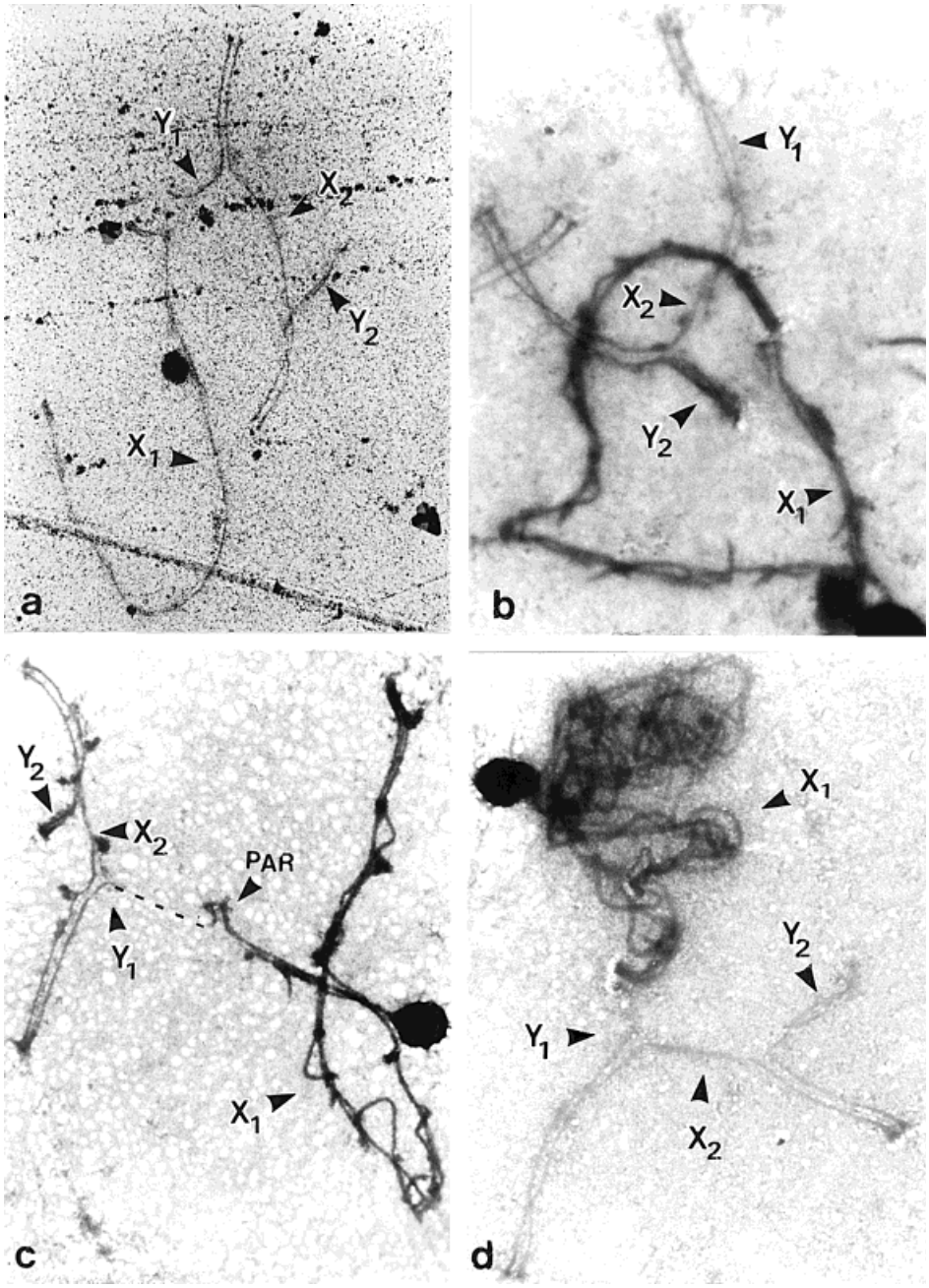

Fig. 2. Electron micrographs of the sequential substages of the sex quadrivalent of *Aca* during pachytene. **a:** At very early pachytene the axes are thin and lack differentiations. **b:** At the second substage the differential region of X_1 and the Y- stemming part of Y_2 are thickened. **c:** The X axis is split at several places but remains thin at the short PAR. **d:** The X axis is forming a net-like arrangement. **e:** At late pachytene the autosomal regions remain paired but shortened. **f:** The beginning of diplotene is shown by the desynapsing autosomes (upper part). The net-like arrangement of the quadrivalent remains visible (lower part).

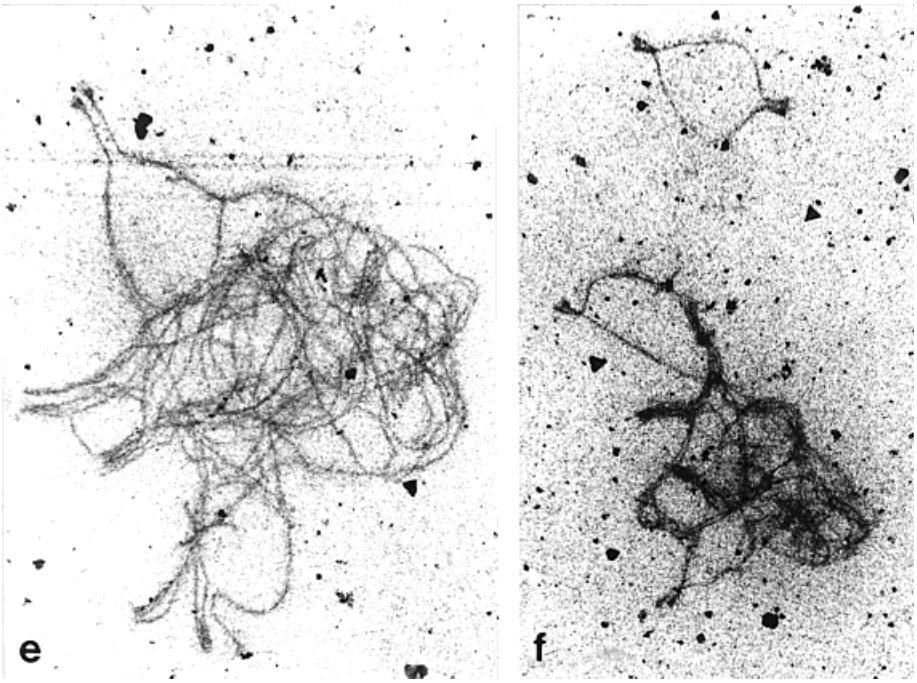

Figure 2 (Continued).

In a second stage (Fig. 2b) the segments coming from the original sex chromosomes are clearly differentiated from those of autosomal origin. While the latter remain thin and form long stretches of SCs, the Y-stemming region of Y_2 is thickened and unpaired. Most of the X axis is thickened and partially split into two filaments, which may emerge like short branches from the main axis. The pseudoautosomal region forms a short SC. In a third stage (Fig. 2c) the split regions along the X axis become widened. The SC at the pseudoautosomal region (PAR) is slightly shortened in relation to stage 2. In the fourth stage the X axis forms a net-like arrangement through the fusion of its branchings. The three SCs remain visible, although that formed at the PAR is very short (Fig. 2d). The dense nodule of the X axis remains at the same location throughout these stages and does not change significantly in shape or size.

At stage 5 the net-like arrangement is extended to part of the autosomal segments, and thus the SCs are shortened (Fig. 2e). The nodule on the X axis disappears. The beginning of diplotene is marked by the partial separation of the autosomal SCs (Fig. 2f). The quadrivalent with the net-like array is easily recognized, although the SCs have become reduced to terminal segments. Thus, the sequential order of the changes is validated by the appearance of the quadrivalent at early diplotene.

Synaptonemal Complex Analysis in *Cap*

The pachytene nuclei of *Cap* show a typical XY body. The structural changes of this XY pair are presented in Fig. 3a–d. The earlier substage shows the X and Y axes unpaired, without significant differentiations but thicker than the auto-

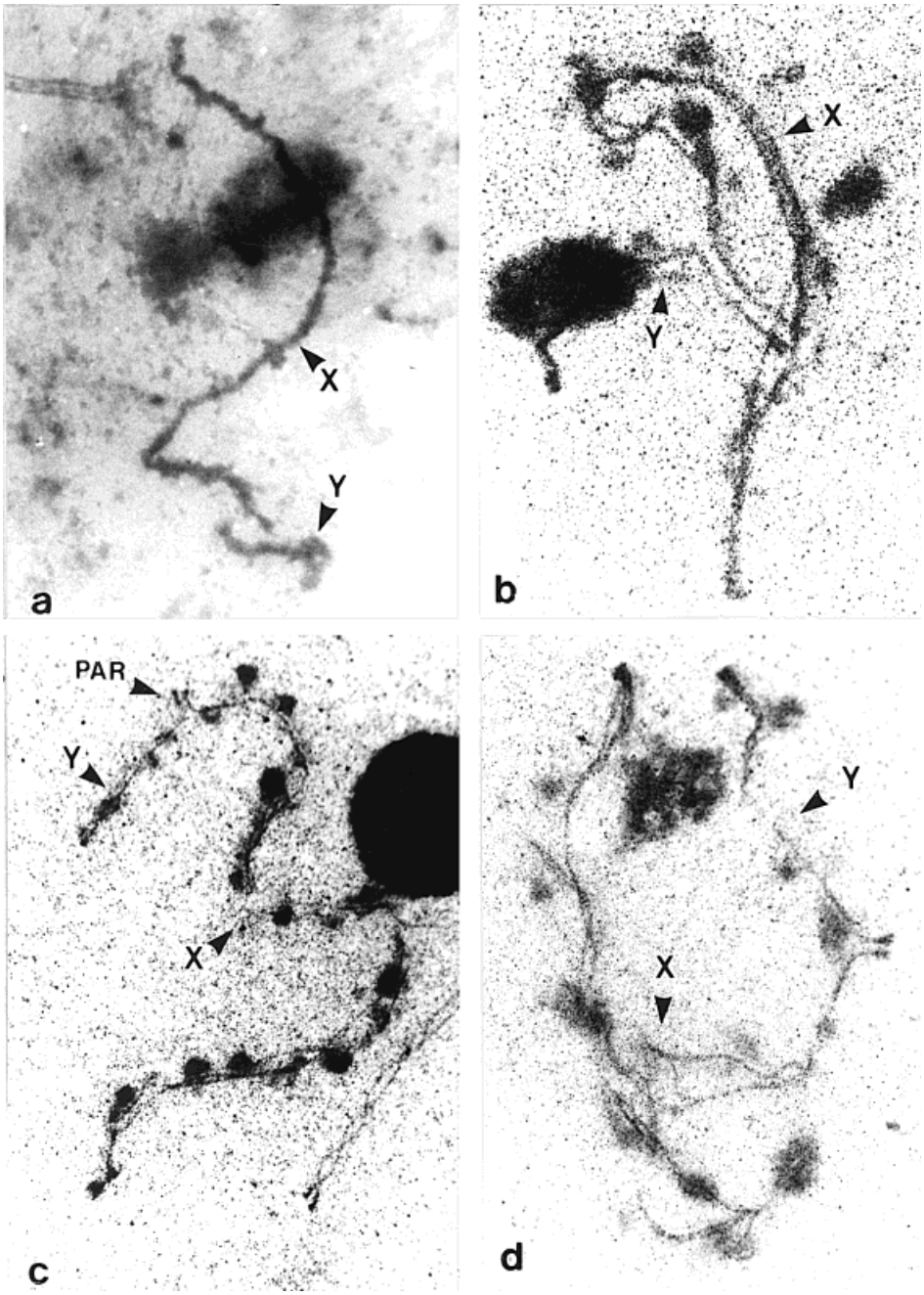

Fig. 3. Sequential substages of the XY pair of *Cap.* **a:** At early pachytene the X and Y axes are already thickened. **b:** The pairing region between the X and Y axes attains the maximum length. **c:** Typical "excrescences" are developed along the X and Y axes. **d:** The pairing region is minimal and there is some axial splitting.

somal axes (Fig. 3a). In the second substage (Fig. 3b) a clear SC is already formed at the PAR and its length is maximal. This SC is significantly longer than that of the PAR in *Aca*. A few large nodules are formed along the X and Y axes. Near the nonsynaptic end of the Y a typical, large nodule is located. In the third substage the SC is shortened and a number of "excrescences" develop along the sex axes, in a way similar to that of the human XY axis. Some splitting also occurs along the differential regions. In the fourth substage (Fig. 3c) the number of excrescences is increased, the SC becomes minimal, and there is no development of a filamentous net. In the final stages (Fig. 3d) the shortened SC remains visible. The excrescences become loosened from the axes and diminish in size while some branchings develop at the sites of the excrescences. Thus the developmental changes in the XY pair of *Cap* are generally similar to those of the human XY pair and strikingly different from those of *Aca.*

Chromosome Painting in *Aca*

In metaphases from *Aca*, which has an $X_1X_2Y_1Y_2$ multiple sex system, the human chromosome X-paint hybridizes on one submetacentric chromosome (Fig. 4a), and human Y-paint does not give any signal. Human chromosome paint #3 labels two of the components of the multiple sex-chromosome system: the autosome #7, which becomes a "secondary sex chromosome" ("X_2"), and the translocation product between the original Y and the disrupted autosome (Fig. 4b). As this latter product holds the autosomal centromere it is called "Y_2." Besides these two components of the sex system, the human paint #3 also labels a pair of medium-sized acrocentric chromosomes (putative chromosome pair #15) which are not involved in the organization of the sex system. The labeled segments in the sex system are: the distal 2/3 of the X_2 element (84% of X_2q) and practically all the long arm of the acrocentric Y_2 (73% of this chromosome). Human chromosome paint #15 labels the short arm of the "secondary sex chromosome" X_2 (the nondisrupted autosome #7) and approximately the distal half (58%) of the other translocation product, which bears the original Y centromere and thus is called Y_1 (Fig. 4c). Besides these two elements of the sex system, the human paint #15 labels segments of two pairs of autosomes that are not involved in the sex system: a tiny acrocentric (the label is in the distal half of putative chromosome #22) and a medium-sized acrocentric pair (the label is in an interstitial, proximal segment of the putative #18) (Fig. 5).

Chromosome Painting in Other Ceboidea Species

In order to characterize sex chromosome systems we compared them in Atelines (two species, *Aca* and *Apa*) and Cebines (three species, *Cap, Sbo*, and *Aza*). Painting probes for human autosomes #3 and #15 and from chromosomes X and Y, which are involved in the sex chromosome system previously found in *Aca,* were used in all these specimens. In all of them the human chromosome X-paint labels the same chromosome (the "original" X) and the Y-paint fails to give any signal (Fig. 4a).

In *Cap* cells, human chromosome paint #15 labels segments of two autosomal pairs which are morphologically different from the ones involved in the multiple sex system of *Aca* (Fig. 4d). This label is located in the distal half of a tiny acrocentric (putative chromosome pair #26) and in a medium-size submetacentric (putative chromosome pair #6) in which the whole of the short arm appeared labeled as well as the proximal region of the long arm (Fig. 4d).

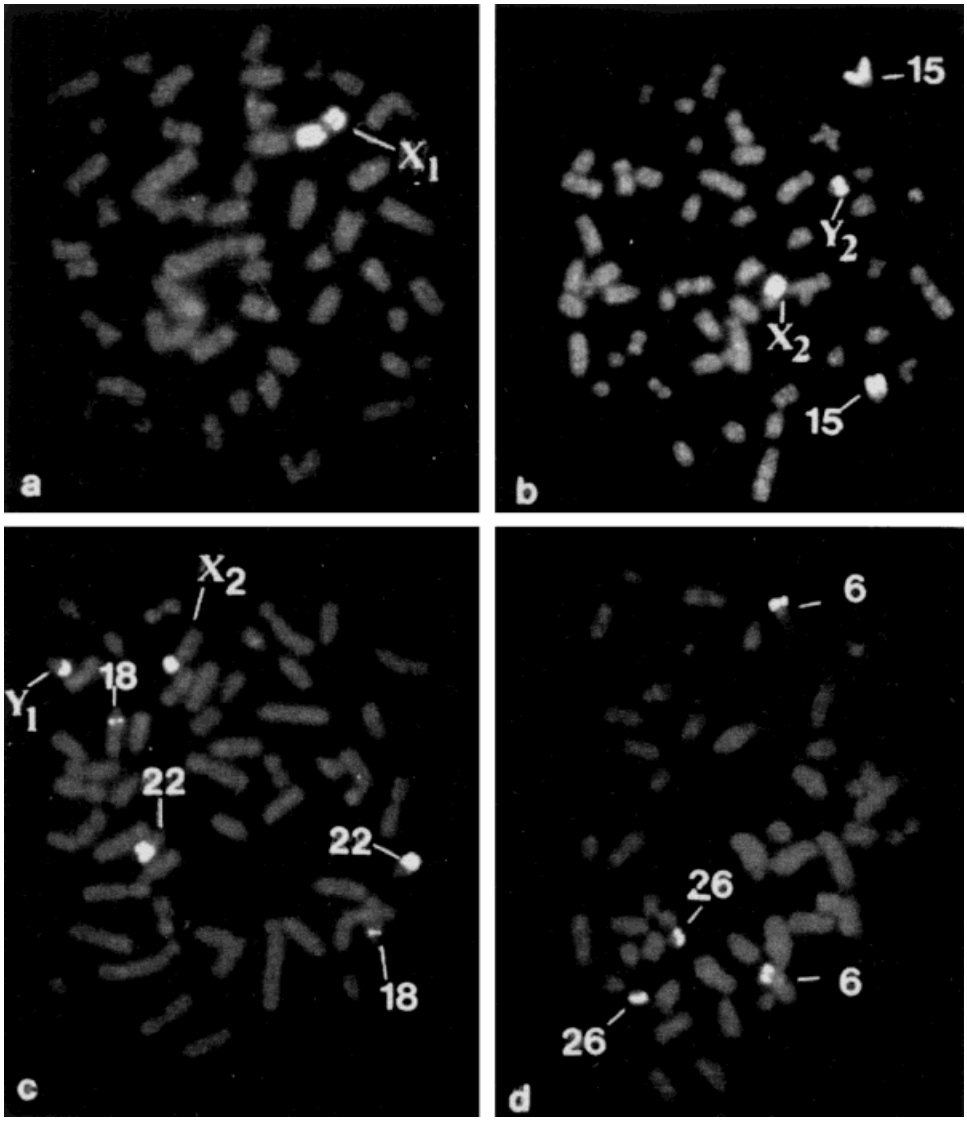

Fig. 4. Chromosome painting with human chromosome probes. **a:** The human X probe paints the original X of *Aca* (X_1). **b:** The human probe #3 paints two components of the multiple sex-chromosome system of *Aca:* the X_2q and almost all the Y_2q. The arrows also show the label on an *Aca* autosomic pair (putative #15). **c:** The human probe #15 labels two components of the multiple sex chromosome of *Aca:* the X_2p and the distal half of Y1. The arrows also show the labels on two autosomic pairs (putative #18 and #22). **d:** The human probe #15 paints two autosomal pairs in *Cap* (putative chromosomes #6 and #26).

In *Apa* metaphases, human chromosome #15 provides fragmented signals on two submetacentric chromosome pairs. There are two signals on #2q: one of them is paracentromeric and the other telomeric. The other signal is located on a large metacentric chromosome, which originates from a 4/12 translocation (Fig. 5).

In *Sbo* metaphases, human chromosome paint #3 hybridizes on two mid-sized metacentric pairs (putative #A_3 and #A_4), and in both cases the label is

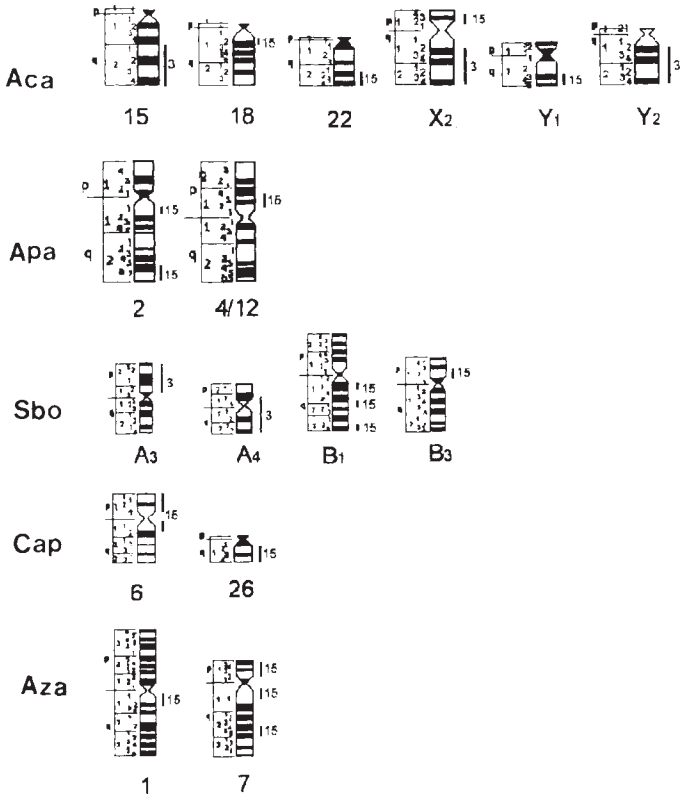

Fig. 5. Scheme showing a summary of the in situ hybridization results with human chromosome paints #3 and #15 on G-banded idiograms of five Ceboidea species: *Aca*, *Apa*, *Sbo*, *Cap*, and *Aza*. The Ceboidea chromosomes are numbered below, and the regions of homology with human chromosome segments are on the right.

located on one entire arm. Chromosome #15 labels three regions of the same arm of the metacentric chromosome #B_1 and one region on the submetacentric chromosome #B_3p. On this pair the signal is distal in one homologue and telomeric in the other, showing that in the studied individual this pair is heteromorphic (Fig. 5). This heteromorphism was confirmed by G-banding.

In *Aza* metaphases, the human chromosome paint #15 gives one paracentromeric signal on one arm of the metacentric chromosome #1, and three signals on an acrocentric, medium-sized pair. In this acrocentric pair one of the signals is on a short arm and the other two are on the long arm (Fig. 5).

DISCUSSION

Identity of the $X_1X_2Y_1Y_2$ Multiple Sex-Chromosome System in *Aca*

While the existence of a sex-multiple in the genus *Alouatta* was previously proposed [Lima & Seuánez, 1991] on the basis of the observation of a quadrivalent at metaphase I from *A. seniculus stramineus* (*Asst*), the identification of the elements involved in the quadrivalent, as well as its possible presence in other species of the same genus, remained unknown until the identity of the autosome involved in the sex-multiple in *Aca* was demonstrated [Rahn et al., 1996] and a report [Consigliere et al., 1996] showed by chromosome painting in mitotic chro-

mosomes of *A. seniculus sara* (*Assa*) and *A. seniculus arctoidea* (*Asar*) that human paint #3 labeled homologous regions in the putative X_2q and Y_2q, while X_2p and Y_1q were labeled by human paint #15.

The present work shows that the same pattern of chromosome painting is present in the distant species *Aca*, and that the chromosome fracture points deduced from the meiotic measurements [Rahn et al., 1996] agree with the discontinuities of painting in the mitotic chromosomes. Thus, the identity of the four elements involved in this multiple is now demonstrated, at least for this species (*Aca*).

Possible Distribution of the Sex-Multiple Among the Species of the Genus *Alouatta*

A very striking similarity is found when comparing the four elements described as the putative sex-multiple in *Assa* and *Asar* [Consigliere et al., 1996] and the meiotic quadrivalent described in *Aca* [Rahn et al., 1996]. The present observations show that in the three taxa the involved chromosomes are in fact the same ones. A previous report on *Asst* [Lima & Seuánez, 1991] described a multivalent at metaphase I in which the elements were located in the order $X_1Y_1X_2Y_2$, which is the same order in which the elements were identified by the meiotic and fine structural studies of *Aca* [Rahn et al., 1996]. When the multiple depicted at metaphase I in *Asst* is compared with the three precedent cases, the similarities are also striking. Thus, it can be suggested that at least in the four mentioned taxa (in three putative subspecies of *A. seniculus* and in *Aca*) the sex multiple is the same. There are other reports on the possible presence of sex multiples in other species of the genus *Alouatta,* but they remain poorly supported by the lack of meiotic data and molecular observations. Thus, the possible existence of a sex multiple in *A. palliata* has been reported [Ma et al., 1975], and at least one similar report has been presented on *A. fusca* [de Oliveira et al., 1995]. Thus, at the present time the distribution of the presently identified sex-multiple is demonstrated among several taxa of the genus *Alouatta,* but other sex-chromosome systems may be present in this genus.

It seems remarkable that the demonstrated presence of the sex-multiple occurs between two widely different species of the genus *Alouatta*: *Aca* and *A. seniculus*. Molecular data [Meireles et al., 1999] show that *Aca* diverges substantially from *Assa* and from *Asar*, three taxa that undoubtedly share this sex multiple (see Results). Thus, the sequences of the gamma-1-globin pseudogene suggest a divergence time of distinct *Aca* and *A. seniculus* groups at 2.4×10^6 years [Meireles et al., 1999]. Other members of the genus Alouatta are even more genetically distant from *Aca*. Genetic distances between *A. seniculus* and *A. palliata* are reported to range between 7.94% and 8.38% [Figueiredo et al., 1998]. Morphological studies [Hill, 1962] suggest that *Aca* is closer to *A. seniculus* when compared to *A. palliata*. However, meiotic and molecular evidences on the sex system of *A. palliata* are lacking.

On the basis of observations using human chromosomes paints on cultured fibroblasts from *A. belzebul* (*Abe*), it has been suggested that *Abe* has a more conserved karyotype compared to *Assa* and *Asar* [Consigliere et al., 1998]. Regarding the sex system, these authors consider that *Abe* has a Y-autosome insertion, most probably in chromosome #17, although Y labeling could not be demonstrated. The same authors consider that both *Abe* and the *A. seniculus* subspecies are all characterized by Y-autosome translocations, and that of *Abe* is the ancestral one [Consigliere et al., 1998]. The meiotic analysis of one specimen of *Alouatta belzebul belzebul* at metaphases I and II has been reported as show-

ing a sex trivalent formed by the X chromosome, a Y-carrying chromosome #17, and the normal #17, that is: X_1-Y-X_2 [Armada et al., 1987]. However, that report did not describe the pairing pattern at pachytene and thus the actual homologous structures of this multivalent remain unknown. It may be concluded that additional meiotic studies are needed to settle the status of the sex-multiple in the genus *Alouatta*.

Ancestral XY Type of *Cebus apella*

On the other hand, the sex-chromosome system of *Cap* is completely different from that of the mentioned howler monkeys. The XY system of *Cap* is not only a classical sex pair, but it is also similar in many respects to that of the human species. Thus, the XY of *Cap* and that of the human have a short PAR which attains a maximum SC length at the second pachytene substage. In both species there is a similar development of excrescences, and in the human there is the development of a net-like structure, with disappearance of large nodules. The human chromosome paints show that the autosomes involved in the sex multiple in *Aca* are not involved in rearrangements with sex chromosomes in *Cap*, and do not show any similarity in pattern in the other species mentioned here. In fact, *Cap* seems to have a much less rearranged karyotype, as regards human paints. It has been suggested that *Cebus* is more proximal to the ancestral stem of platyrrhines compared to Alouattinae and other taxa.

Mechanisms of Conservation of the Sex-Multiple, and the Possible Evolutionary Pressures for the Development of Multiple-Sex-Systems

Given that the sex-multiple presented here has been definitely identified in several members of the genus *Alouatta,* which is also rich in chromosomal rearrangements even between related species, it is mandatory to think about the mechanisms that allowed the persistence and extent of this sex-multiple. It is suggested that in a taxon with high chromosomal plasticity, such as *Alouatta*, there must be a selective advantage in the presence of this multiple. The present results suggest one of the possible advantages, as it has been shown that the pairing region of the primitive sex pair in *Alouatta* is extremely short, and also the primitive Y is very short. Thus, the acquisition of a new chromatin mass through a chromosomal rearrangement of the primitive Y with autosome #7 may have added some dynamic stability throughout meiosis to the ancestral tiny Y chromosome. It is known that tiny chromosomes are more prone to segregation errors through division. The avoidance of these errors leading to sterility or inviability may have assisted the fixation of this striking sex-multiple among different species of *Alouatta*. Other functions of the autosomal chromatin added to the original Y may be related to male fertility, as the harboring of male-related features may form a selectively beneficial linkage-block. The conservation of this sex-multiple is also related to the regular presence of alternate segregation in this multivalent at the first meiotic division [Rahn et al., 1996], which is the only segregation type in translocation carriers that warrants fertile gamete production. It remains for additional studies to search for the original source of this multiple and its approximate time in the evolution of platyrrhines.

Presence of Sex Multiples Among Other Primates

There are a number of studies reporting the existence of multiple-sex-chromosome systems among primates [reviewed in Rahn et al., 1996; Consigliere et

al., 1996]. However, there is a striking difference between platyrrhines and catarrhines in that respect: among all catarrhines, there is a single species in which a sex-multiple has been demonstrated: *Presbytis cristata* [Bigoni et al., 1997]. This system is totally different from the one present in the genus *Alouatta,* as shown by the involvement of the homologue of human chromosome #5 instead of human chromosomes #3 and #15. Furthermore, among great apes there are no instances of sex-multiples.

On the other hand, several reports have assumed the presence of sex-multiples among platyrrhines [Benirschke et al., 1976; Koiffmann & Saldanha, 1981; Dutrillaux et al., 1981; Armada et al., 1987; Pieczarka & Nagamachi, 1988; de Oliveira et al., 1995; Vassart et al., 1996]. These reports are mainly based on the analysis of mitotic karyotypes after solid staining and sometimes C- and G-banding, without the analysis of pachytene spermatocytes or chromosome painting. Despite these limitations, it is obvious from most of these reports that they do not show quadrivalents similar to those of *Aca*, and that some of them may be other types of sex multiples, XY_1Y_2 types, which are well known in chiroptera [reviewed in Solari, 1993]. It is known that the transition between a typical XY system and a multiple-sex-chromosome system can be attained through a simple reciprocal translocation between an autosome and one of the sex chromosomes [Fredga, 1970; Solari, 1993]. Despite the strength with which the synteny in the X chromosome is protected, a large number of instances of X-autosome translocations have been reported among some mammalian taxa, such as chiroptera and insectivora [Fredga, 1970; Solari, 1993]. Furthermore, when the Y chromosome is the one involved in an exchange with an autosome, an $X_1X_2Y_1Y_2$ system of the *Alouatta* type can be originated. Thus, sex multiples might not be exceptional in a karyologically variable taxon such as platyrrhines. However, when the presence of the same multiple is identified among different species, it probably stems from a common ancestor, and it may become a useful chromosomal marker.

CONCLUSIONS

The identity of the chromosomes involved in the $X_1X_2Y_1Y_2$ sex system of *Aca* has been confirmed by chromosome painting. The involved autosome and the fracture points are the same as those described by chromosome painting in *Assa* and *Asar* by other authors. The pattern of meiotic changes in the sex multivalent of *Aca* at pachytene is widely different from that of the XY pair of *Cap,* and the latter is more similar to that of the XY pair in humans. The possible presence of the same sex-multiple in other species of the genus *Alouatta* is raised, and the conservation of the same sex-multiple among at least four taxa of the genus *Alouatta*, which show many different chromosomal rearrangements, suggests a high selective value of this sex-multiple. From the presently known data no other instance of the same sex system as *Alouatta* is present in other platyrrhine genera. However, as stated above, more chromosome studies—especially meiotic ones—are needed among these primates in order to establish the origin of this remarkably stable sex system.

ACKNOWLEDGMENTS

We thank the Buenos Aires Zoo; La Plata Zoo, Buenos Aires; CEM, Roque Saenz Peña, Chaco; ECAS, La Plata, Buenos Aires; CAPRIM; Dr. C. Nagle, who provided the *Cebus* samples from CEMIC, Buenos Aires; Instituto de Investiga-

ciones Científicas y de la Salud (IICS), Asunción, Paraguay; Dr. G. Zunino from Museo Argentino. de Cs. Nat.; Bernardino Rivadavia, who provided the blood samples from the field; and V. Szapkievich, M. Gorostiaga, I. Marchesini, and M. Nieves for their technical assistance.

REFERENCES

Armada JLA, Barroso CML, Lima MMC, Muniz JAP, Seuánez HN. 1987. Chromosome studies in *Alouatta belzebul*. Am J Primatol 13:283–296.

Benirschke K, de Boer LEM, Bogart M. 1976. The karyotype of two uakari species *Cacajao calvus* and *C. rubicundus* (Primates, Platyrrhini). Genen Phaenen 19:1–6.

Bigoni F, Koehler U, Stanyon R, Ishida T, Wienberg J. 1997. Fluorescence *in situ* hybridization establishes homology between human and silvered leaf monkey chromosomes, reveals reciprocal translocations between chromosomes homologous to human Y/5, 1/19 and 6/16, and delineates an $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system. Am J Phys Anthropol 98:315–328.

Consigliere S, Stanyon R, Koelher U, Agoramoorthy G, Weinberg J. 1996. Chromosome painting defines genome rearrangement between red howler monkey subspecies. Chrom Res 4:264–270.

Consigliere S, Stanyon R, Koehler U, Arnold N, Wienberg J. 1998. In situ hybridization (FISH) maps chromosomal homologies between *Alouatta belzebul* (Platyrrhini, Cebidae) and other primates and reveals extensive interchromosomal rearrangements between howler monkey genomes. Am J Primatol 46:119–133.

de Oliveira OH, de Lima MM, Sbalqueiro IJ. 1995. Chromosomal variation in *Alouatta fusca*. Neotrop Prim 3:181–183.

Dutrillaux B, Descailleaux J, Viégas-Péquignot E, Couturier J. 1981. Y-autosome translocation in *Cacajao calvus rubicundus* (Platyrrhini). Ann Genet 24:197–201.

Evans EP, Breckon G, Ford CE. 1964. An air-drying method for meiotic preparations from mammalian testes. Cytogenetics 3:289–294.

Figueiredo WB, Carvalho-Filho N, Schneider H, Sampaio I. 1998. Mitochondrial DNA sequences and the taxonomic status of *Alouatta seniculus* populations in Northeastern Amazonia. Neotrop Prim 6:73–77.

Fredga K. 1970. Unusual sex chromosome inheritance in mammals. Philos Trans R Soc B, Lond 322:83–95.

Gillies CB. 1989. Fertility and chromosome pairing: recent studies in plants and animals. Boca Raton: CRC Press. 182 p.

Hill WCO. 1962. Primates: comparative anatomy and taxonomy. 4. Cebidae, Part A. Edinburgh: Edinburgh University Press.

Howell WM, Black DA. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1 step method. Experientia 31:260–262.

Koiffmann CP, Saldanha PH. 1981. The karyotype of *Cacajao melanocephalus* (Platyrrhini, Primates). Folia Primatol 36:150–155.

Lima MMC, Seuánez HN. 1991. Chromosome studies in the red howler monkey, *Alouatta seniculus stramineus* (Platyrrhyni, Primates): description of an $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ sex-chromosome system and karyological comparisons with other subspecies. Cytogenet Cell Genet 57:151–156.

Ma NSF, Jones TC, Thorington RW, Miller A, Morgan L. 1975. Y-autosome translocation in the howler monkey, *Alouatta palliata*. J Med Primatol 4:299–307.

Meireles CM, Czelusniak J, Ferrari SF, Schneider MPC, Goodman M. 1999. Phylogenetic relationships among Brazilian howler monkeys, genus *Alouatta* (Platyrrhini, Atelidae), based on γ^1-globin pseudogene sequences. Genet Mol Biol 22:373–344.

Morescalchi MA, Schempp W, Consigliere S, Bignoni F, Wienberg J, Stanyon R. 1997. Mapping chromosomal homology between humans and black-handed spider monkey by fluorescence in situ hybridization. Chrom Res 5:527–536.

Mudry MD, Rahn IM, Gorostiaga M, Hick A, Merani MS, Solari AJ. 1998. Revised karyotype of *Alouatta caraya* (Primates: Platyrrhini) based on synaptonemal complex and banding analyses. Hereditas 128:9–16.

Pieczarka JC, Nagamachi CY. 1988. Cytogenetic studies of *Aotus* from eastern Amazonia: Y-autosome rearrangement. Am J Primatol 14:255–263.

Rahn M, Mudry M, Merani SM, Solari AJ. 1996. Meiotic behavior of the $X_1X_2Y_1Y_2$ quadrivalent of the primate *Alouatta caraya.* Chrom Res 4:350–356.

Solari AJ. 1980. Synaptonemal complexes and associated structures in microspread human spermatocytes. Chromosoma 81:315–337.

Solari AJ. 1993. Sex chromosomes and sex determination in vertebrates. Boca Raton: CRC Press. 308 p.

Solari AJ. 1998. Structural analysis of meiotic chromosomes and synaptonemal complexes in higher vertebrates. In: Berrios M, editor. Nuclear structure and function. San Diego: Academic Press. p 235–256.

Sumner AT. 1972. A simple technique for

demonstrating centromeric heterochromatin. Exp Cell Res 75:304–306.
Vassart M, Guédant A, Vié JC, Kéravec J, Séguéla A, Volobouev VT. 1996. Chromosomes of *Alouatta seniculus* (Platyrrhini, Primates) from French Guiana. J Hered 87:331–334.
Wienberg J, Stanyon R. 1998. Comparative chromosome painting of primate genomes. ILAR J 39:77–91.