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The karyotype of *Alouatta pigra* (Primates: Platyrrhini): mitotic and meiotic analyses

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Abstract. We describe for the first time the karyotype of the black howler monkey, *Alouatta pigra*. Conventional staining, G- and C-banding, and fluorescence in situ hybridization (FISH) with a peptide nucleic acid (PNA) pantelomeric probe were performed. Eight free ranging adult individuals, four males and four females, within the natural distribution of the species presented a diploid karyotype with 2n = 58. Mitotic analyses showed an autosomal complement composed of 6 submetacentric, 3 metacentric, and 19 acrocentric chromosome pairs for females, and 6 submetacentric, 3 metacentric, and 18 acrocentric pairs for males. Meiotic analyses in males revealed 27 autosomal bivalents and a quadrivalent composed of a submetacentric X_1 and acrocentric X_2, Y_1, and Y_2. The G-banded karyotype allowed us to identify pair #17 as the autosomal pair involved in the rearrangement and the morphology of the quadrivalent components. C-banding technique in metaphase I corroborated the structure of the quadrivalent showing four C+ centromeres. FISH analysis showed telomeric signals at the terminal regions of all chromosomes. No interstitial signals were detected. DNA sequence data were in accordance with those previously published for this species.

Howler monkeys, genus *Alouatta,* present the broadest geographic distribution among New World Primates reaching from northern Argentina to southern Mexico. They inhabit a wide variety of environments, from closed-canopy wet evergreen forests, including flooded swamp forests, to open, highly seasonal deciduous and semi-deciduous woodlands, and gallery forests (Crockett, 1998; Zunino et al., 2001). Traditionally, there have been six well recognized species: *A. palliata, A. seniculus, A. belzebul, A. guariba* (formerly *fusca*), *A. caraya* (Cabrera, 1957; Hill, 1962), and *A. pigra* (Smith, 1970). However, in the last ten years morphological and genetic data have provided evidence of a much more diverse genus (de Oliveira et al., 2002; Cortés-Ortiz et al., 2003; Gregorin, 2006). Two recent systematic revisions present differences in the number of recognized taxa: Ryland et al. (2000) accepted 21 taxa, with 9 species and 16 subspecies, and Groves (2001) distinguished 14 taxa, with 10 species and 7 subspecies.

Genetic studies have become an important and valuable tool to complement the traditional morphological and ecological analyses to delineate the taxonomy of primates.

The species of *Alouatta* have different diploid numbers (2n), ranging from 44 to 53 (e.g., Yunis et al., 1976 for *A. seniculus*; Armada et al., 1987 for *A. belzebul*; de Oliveira,

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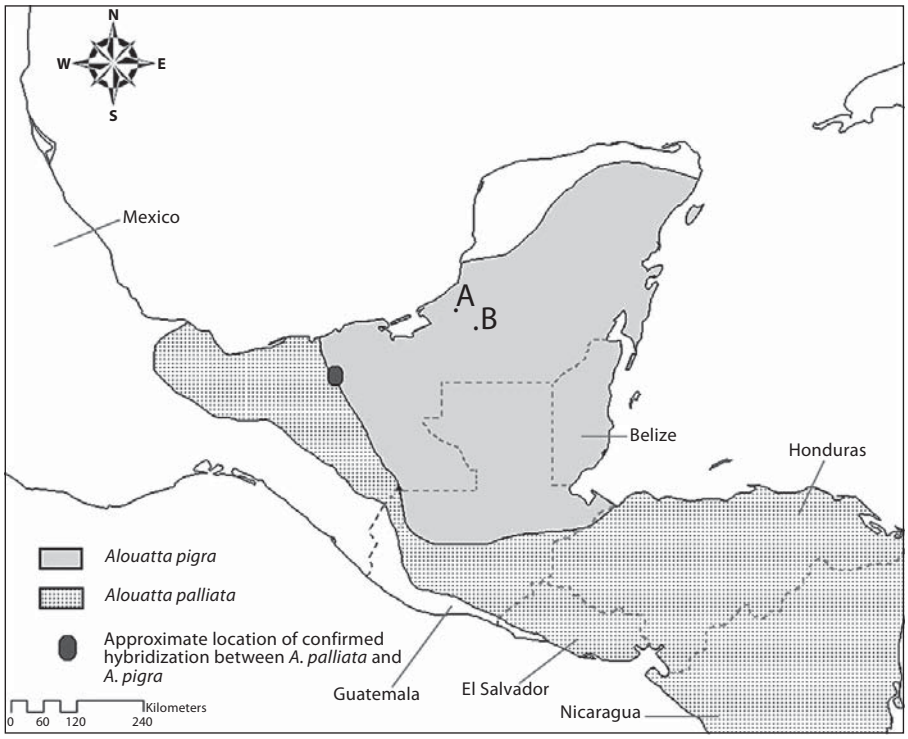


Fig. 1. Geographic distribution of *Alouatta pigra* and *A. palliata* and approximate location of collecting sites. Localities: A: 18°51′46.7″N, 90°46′08.6″W and B: 18°34′13.4″N, 90°26′18.0″W according to Global Positioning System (GPS) information. The dark shaded circle shows the location of the individuals that have been genetically characterized by Cortes-Ortiz et al. (2007) as hybrids/backcrosses *A. palliata*/*A. pigra*.

1995; de Oliveira et al., 1998, 2002 for *A. guariba (= fusca)*; Mudry et al., 1998, 2001 for *A. caraya*; Ma et al., 1975 for *A. palliata*). An interesting feature in this genus is the presence of several sex chromosome systems among the recognized species. Mitotic studies have suggested the presence of trivalents and pentavalents in *A. guariba* (de Oliveira et al., 1998, 2002, respectively) and a quadrivalent in *A. sara* (Minezawa et al., 1985; Consigliere et al., 1996), while other studies suggest the presence of XY sex chromosome systems (*A. seniculus*: Yunis et al., 1976; *A. guariba*: de Oliveira, 1995; *A. palliata*: Torres and Ramírez, 2003). Multiple sex determination systems have only been confirmed through meiotic analysis for a small number of howler monkey species: trivalents $X_1X_2Y/X_1X_1X_2X_2$ for *A. belzebul* (Armada et al., 1987) and *A. palliata* (Solari and Rahn, 2005), and quadrivalents $X_1X_2Y_1Y_2/X_1X_1X_2X_2$ for *A. seniculus* (Lima and Seuánez, 1991) and *A. caraya* (Rahn et al., 1996; Mudry et al., 1998, 2001). Fluorescent in situ hybridization (FISH) analyses using human chromosome probes have revealed that the chromosomes involved in the sex chromosome systems hybridize with segments of human chromosomes #3 and #15 (Consigliere et al., 1996, 1998; Mudry et al., 2001; de Oliveira et al., 2002). Based on this finding de Oliveira et al. (2002) suggested that, in *Alouatta*, the diverse sex chromosome systems originated from the same autosomal pair and that this rearrangement may have occurred only once. To our knowledge, no cytogenetic analyses have been performed for *A. pigra*. In the present contribution we provide for the first time a cytogenetic characterization of this species using mitotic, meiotic, and FISH analyses.

Materials and methods

Sampled specimens

Four male and four female adult specimens of *A. pigra* showing the uniform black pelage coloration characteristic of the species were randomly collected in two localities of Campeche, Mexico (18°51′46.7″N, 90°46′08.6″W and 18°34′13.4″N, 90°26′18.0″W), within the natural distribution range of the species (Fig. 1). Weights and body measurements of individuals (not shown) fall within those reported for individuals of this species from different localities (Murie, 1935; Jungers, 1985; Reid, 1997; Cortés-Ortiz et al., unpublished data). In the field, animal immobilization was performed using Ketamine (7–10 mg/kg) with a Telinject (remote injection) system. Once the animals were captured, they were immediately transferred to the staging area where biological samples (see below) were taken. All the individuals were tattooed in the flanking abdominal area for identification purposes, as part of ongoing projects on population genetics for this species (Cortés-Ortiz, unpublished). After individuals were recovered from the anesthesia, they were released in the same capture location.

Mitotic studies

Peripheral blood samples were collected from all animals with disposable heparinized syringes. Lymphocyte cultures were performed for 72 h at 37°C following a modification of Buckton and Evans (1973). Metaphase spreads were treated by G-Wright banding (Seabright, 1971, modified as in Steinberg et al., 2007) and C-banding techniques (Sumner, 1972). At least 50 metaphases per animal were analyzed at 1000× to determine the diploid number (2n). Metaphases found among 1000 nuclei were used to determine the mitotic index (MI, %). At least 10 G- and C-banded metaphases were photographed. Photomicrographs were taken using a Leica DMLB microscope equipped with a photographic MPS 30 camera using an Ilforpan F 50 ASA black and white slide film.

Considering that telomeric sequences are frequently involved in chromosomal rearrangements, the analysis of their distribution among karyotypes is valuable for the study of chromosome evolution (Meyne et al., 1990; Slijepcevic, 1998; Bolzán and Bianchi, 2006; Mudry et al.,

2007). The distribution of the telomeric sequence $(TTAGGG)_n$ was analyzed on metaphase spreads by FISH using a Cy3-conjugated peptide nucleic acid (PNA) pantelomeric probe (DAKO Cytomation, Glostrup, Denmark). FISH was performed according to the instructions provided by the supplier. Signals were observed at 1000× using a Carl Zeiss (Germany) epifluorescence microscope equipped with an HBO 100 mercury lamp and filters for DAPI and Cy3 (Chroma Technology, USA). A Nikon DN100 digital camera was used for photography. Images were processed using the Adobe Photoshop CS® program.

Meiotic studies

Testicular biopsies were taken from the four males using Ketamine anesthesia. The cytogenetic protocol was performed following Evans et al. (1964) with modifications to maximize the performance of the meiotic analysis considering previous experiences in other Ceboidea species (Steinberg et al., 2007).

Molecular studies

With the aim of comparing the DNA sequence identity of the sampled individuals with previously published sequences for this species, 1 ml of blood was collected from each individual and stored in Lysis buffer (100 mM Tris/HCl pH 8, 100 mM EDTA pH 8, 10 mM NaCl, 0.5% SDS) (Seutin et al., 1991; Frantzen et al., 1998) at a ratio of 1:5. Genomic DNA was extracted from blood samples using the DNeasy Tissue kit (Qiagen, Valencia, CA) and primers CB1-5′ and CB2-3′ (Palumbi, 1996) were used to amplify and sequence a region of the mitochondrial cytochrome *b (cytb)* gene, under the conditions described in Cortés-Ortiz et al. (2003).

Results

Molecular analysis

Sequences of a 307-bp region of the mitochondrial *cytb* gene were obtained from each individual. All individuals presented identical mitochondrial sequences, which have been observed in other individuals from different geographic locations within the distribution range of *A. pigra* in Mexico (see GenBank accession number DQ875696).

Mitotic studies

All peripheral blood samples showed good in vitro proliferation allowing us to determine a modal number. The mitotic index ranged from 1.39 to 5.3% with a medium of 3.51 ± 1.62%. The diploid number for all specimens was 2n = 58, observed in 68.8 ± 7.07% of the analyzed metaphases. The fundamental number (NF) was 78 for females and 77 for males due to the presence of a multiple sex determination system in males (see below).

The female autosomal complement was composed of 6 submetacentric, 3 metacentric and 19 acrocentric pairs. In males the autosomal complement was composed of 6 submetacentric, 3 metacentric and 18 acrocentric pairs. The G-banding pattern obtained for *A. pigra* (Fig. 2) showed the presence of an XX sex determination system in females and suggested a multiple sex determination system $X_1X_2Y_1Y_2$ in males, which could have been produced by the translocation between the Y chromosome and chromosome pair #17. This multiple sex determination system seems to be composed of a submetacentric X_1 and acrocentric X_2, Y_1, and Y_2 chromosomes. In the C-banded metaphases (Fig. 3), centromeric heterochromatin was detected in all chromosome pairs.

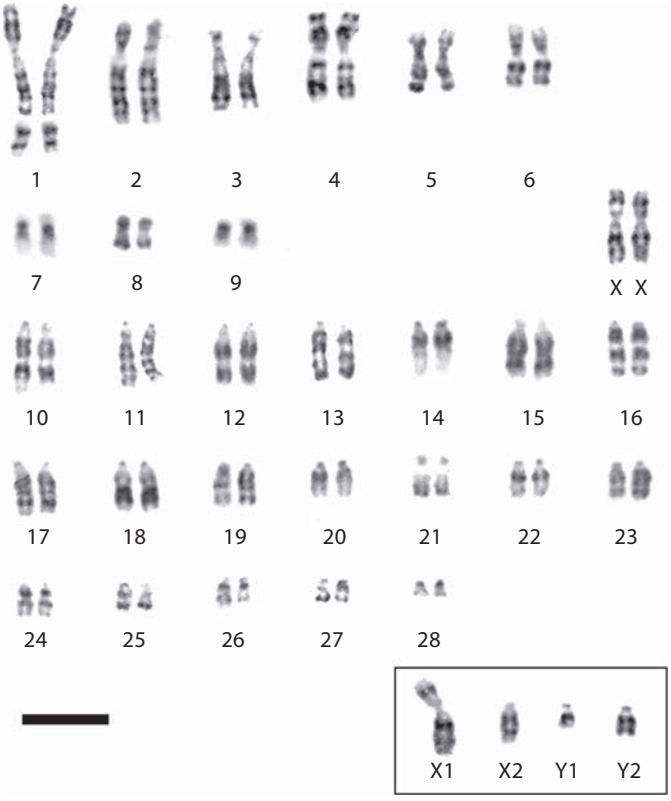


Fig. 2. G-banded karyotype of a female *Alouatta pigra* (bar = 10 μm). Inset: sex chromosomes from a male *A. pigra*.

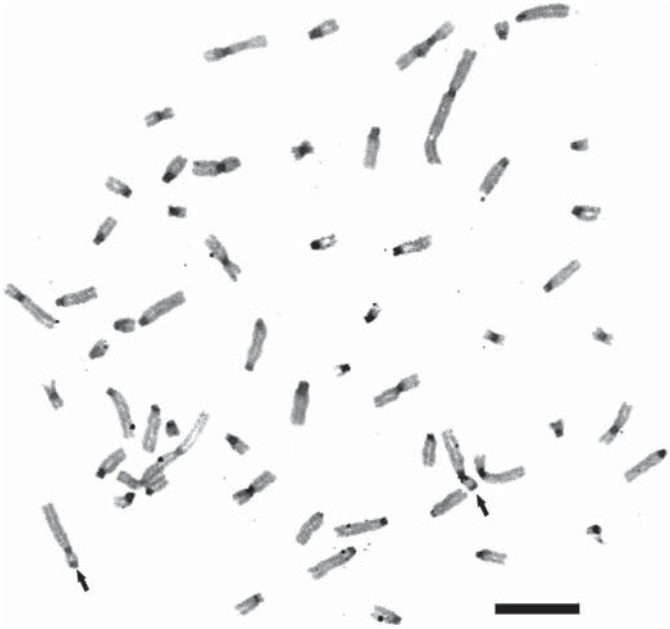
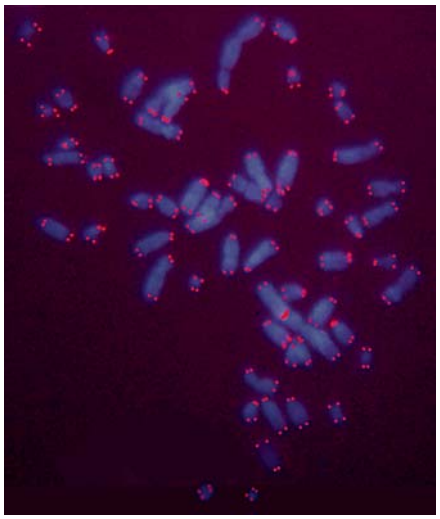


Fig. 3. C-banded metaphase of a male *Alouatta pigra* showing centromeric C+ heterochromatin bands in all chromosome pairs. The arrows indicate the telomeric heterochromatic C+ bands only detected in pair #2 (bar = 10 μm).



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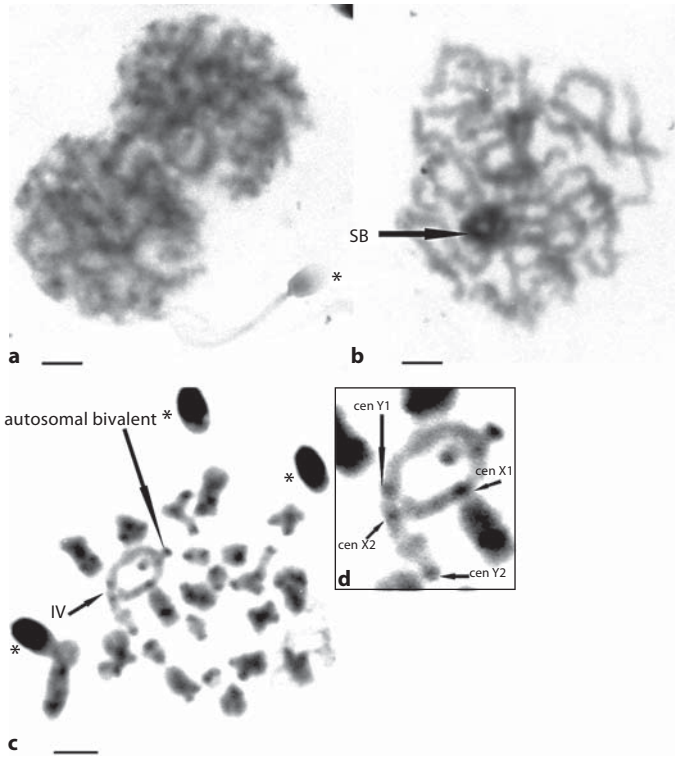


Fig. 4. Location of the telomeric sequence $(TTAGGG)_n$ after hybridization with a Cy3-labeled PNA telomeric probe. The chromosomes were counterstained with DAPI.

Fig. 5. Spermatocytes of *Alouatta pigra* at different stages of the meiotic cycle (bars = 5 μm). (**a**) Leptotene showing the chromatin masses formed by the autosomes and gonosomes, indistinguishable from each other. The asterisk indicates the presence of a sperm cell in the spread of the biopsy material. (**b**) Late zygotene showing the bivalents with nearly complete synapsis. The arrow shows the sex body (SB) as a heteropycnotic bulk. (**c**) C-banded spermatocyte of *A. pigra* at metaphase I showing the location of the heterochromatic regions (C+). The arrows indicate the quadrivalent $X_1X_2Y_1Y_2$ and an autosomal bivalent located under the quadrivalent. The asterisks indicate the presence of spermatozoa in the testicular material. (**d**) Close-up of the quadrivalent. The centromeres of the four components of the quadrivalent are indicated with arrows.

Telomeric heterochromatic bands were observed in one chromosome pair (#2). No interstitial bands were detected.

To determine if the rearranged regions involved in the multiple sex chromosome system have conserved interstitial telomeric repeat sequences, and to characterize the chromosomal distribution of the telomeric sequence $(TTAGGG)_n$ in this species, FISH with a PNA telomeric probe was performed. Male and female metaphases of *A. pigra* showed telomeric signals only at the terminal regions of all chromosomes (Fig. 4). A total of 116 signals were observed in both males and females confirming that all chromosomes of the complement are biarmed. No interstitial signals were detected. A more intense hybridization signal was observed in the telomeres of the short arms of ten chromosome pairs (data not shown).

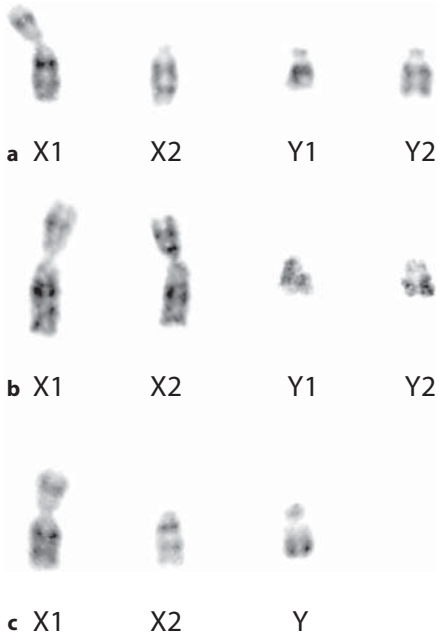
Meiotic studies

The analysis of spermatocytes in different stages of meiosis was used to evaluate the behavior of the male sex chromosomes and to corroborate the presence of a multiple sex chromosome system detected in the mitotic analyses. The observation of cells at early prophase I showed that the sex body is undistinguishable from the autosomes during leptotene (Fig. 5a). By the onset of zygotene and pachytene (Fig. 5b) the sex body is observed as a heteropycnotic bulk in the periphery of the nucleus. The 27 autosomal bivalents and the quadrivalent observed in at least ten diakinesis/metaphase I per individual corresponded to $2n = 58,X_1X_1X_2X_2/X_1X_2Y_1Y_2$. When the C-banding technique was applied in metaphase I from meiotic cells, the location of the C+ heterochromatic regions evidenced the presence of four centromeres, thus confirming the proposed structure of a quadrivalent (Fig. 5c, d).

Discussion

Taxonomic implications

In a revision of the Mesoamerican howler monkeys, Lawrence (1933) considered *A. pigra* a subspecies of *A. palliata*, highlighting the 'definitive' differences between *A. p. pigra* and the rest of the *A. palliata* subspecies. Smith (1970), analyzing a larger number of specimens from different localities, also found remarkable morphological differences between *A. p. pigra* and the rest of the *A. palliata* subspecies. This and the presence of a sympatric area between *A. palliata mexicana* and *A. pigra* in Mexico made Smith recommend that *A. pigra* should be considered a species distinct from *A. palliata* (Smith, 1970; Wolfheim, 1983). Molecular genetic data presented by Cortés-Ortiz et al. (2003) also offered evidence that *A. pigra* is a distinct species. The present study offers new supporting evidence of the taxonomic distinction between *A. pigra* (API) and *A. palliata* (APA). Whereas *A. palliata* has 2n = 53 with an X_1X_2Y trivalent in males (Ma et al., 1975; Solari and Rahn, 2005), *A. pigra* has 2n = 58 and an $X_1X_2Y_1Y_2$ quadrivalent in males, *A. pigra*'s modal number being the largest one described so far for the genus (see introduction). Differences in the chromosome



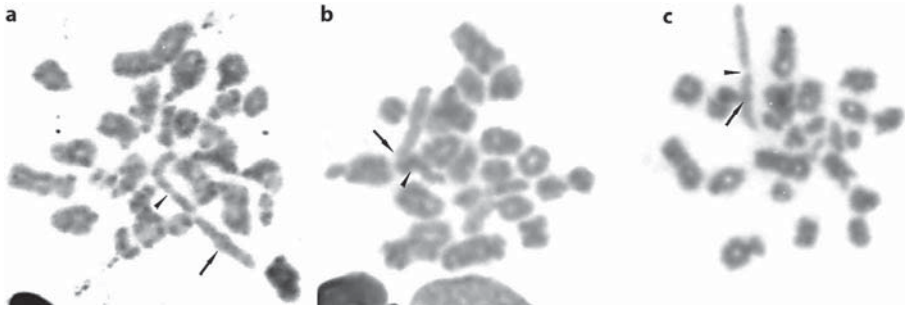


Fig. 6. Comparison of the sex chromosomes from mitotic metaphases of three *Alouatta* species. (**a**) *A. pigra*, (**b**) *A. caraya*, (**c**) *A. palliata*.

Fig. 7. Comparison of the sex chromosomes in metaphase I of three *Alouatta* species. The arrows indicate interstitial chiasmata while the arrowheads indicate end-to-end joining. (**a**) *A. pigra,* (**b**) *A. caraya*, (**c**) *A. palliata*.

Table 1. Karyotype comparison between *Alouatta pigra*, *A. palliata* and *A. caraya*

	A. pigra (API)	*A. palliata* (APA)	*A. caraya* (ACA)
Modal number	♀: 2n = 58; ♂: 2n = 58	♀: 2n = 54; ♂: 2n = 53	♀: 2n = 52; ♂: 2n = 52
Fundamental number (FN)	♀: FN = 78; ♂: FN = 77	♀: FN = 78; ♂: FN = 76	♀: FN = 74; ♂: FN = 72
Chromosome formulae for males[a]	12 SM + 6 M + 36 A + X_1 SM + X_2 A + Y_1 A + Y_2 A	16 SM + 6 M + 28 A + X_1 SM + X_2 A + Y A	14 SM + 4 M + 30 A + X_1 SM + X_2 SM + Y_1 A + Y_2 A
Sex determination system	$X_1X_1X_2X_2/X_1X_2Y_1Y_2$	$X_1X_1X_2X_2/X_1X_2Y$	$X_1X_1X_2X_2/X_1X_2Y_1Y_2$

[a] Chromosome morphology: SM = submetacentric, M = metacentric, A = acrocentric (see text for details).

formulae were also found between these two species (Table 1): *A. pigra* possesses two pairs of submetacentric chromosomes less and four pairs of acrocentric chromosomes more than *A. palliata* (Ma et al., 1975; Solari and Rahn, 2005).

Comparing the proposed karyotype of *A. pigra* with the one previously described for *A. caraya* (ACA) (Rahn et al., 1996; Mudry et al., 1998, 2001) several differences also appear (Table 1). *A. caraya* possesses one pair of submetacentric chromosomes more, one pair of metacentrics less and three pairs of acrocentric chromosomes less than *A. pigra*. Comparative G- and C-banding studies and FISH studies with human probes for characterization of the chromosomes involved in these rearrangements are ongoing projects in our research group.

The application of G-banding allowed us to identify the acrocentric pair #17 as the putative autosomal pair involved in the formation of the quadrivalent in *A. pigra* (Fig. 6). In *A. caraya* the pair involved in its sex chromosome system (ACA7) has submetacentric morphology. These two chromosome pairs, ACA7 and API17 have different size, centromeric index, %RLC and G-banding pattern, as can be observed in Fig. 6, suggesting a different origin for these two multivalents.

Sex chromosome systems in Alouatta

A comparison of the structure of the three different multivalents observed by meiotic studies in the genus showed some marked differences among them. *A. pigra* exhibits one interstitial chiasma formed between Y_2 and X_2 (Fig. 7a), while in *A. caraya* the interstitial chiasma is formed between Y_1 and X_2 (Fig. 7b). Moreover, in *A. pigra* a terminal chiasma is formed between Y_1 and X_2, with the characteristic end-to-end joining between Y_1 and X_1 following. This end-to-end joining is similar in *A. pigra* and *A. caraya* quadrivalents. In the trivalent of *A. palliata* (Fig. 7c) the interstitial chiasma occurs between Y_1 and X_2 with the end-to-end joining also similar to the distribution detected for *A. pigra* sex chromosome structure.

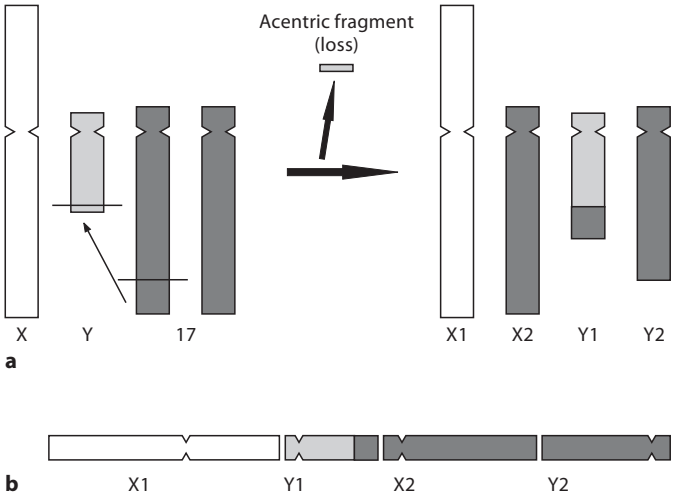


Fig. 8. Hypothesis on the origin of the quadrivalent. (**a**) One of the homologs of the autosomal pair 17 breaks in the distal region of the long arm whereas the original small Y breaks in its terminal region. 17qter becomes Y_1qter while the unprotected ends of API17q develop functional telomeres and this chromosome becomes Y_2. (**b**) Structure of the quadrivalent at metaphase I. The longest element (X_1) is located at one end, with Y_2 at the other end. Y_1 and X_2 are located interstitially.

Telomeric $(TTAGGG)_n$ sequences in Alouatta pigra

In this study telomeric signals were found only at the terminal regions of chromosomes. This only-telomeres distribution pattern of the $(TTAGGG)_n$ sequence was previously also observed in *A. caraya*, *A. palliata* and *A. guariba clamitans* as well as in other neotropical primates (Mudry et al., 2007). This finding strongly suggests that interstitial telomeric repeat sequences are not involved in the chromosomal rearrangements present in the *A. pigra* karyotype.

Possible origin of the quadrivalent

Taking into consideration the G-banding patterns and the chromosomal morphology, the quadrivalent observed at metaphase I of *A. pigra* could have originated from an ancestral XY system by a reciprocal translocation between autosome 17 (API17) and the ancestral Y (Fig. 8). Simultaneous breaks in the distal region of the long arm of one homolog of API17 and in the terminal part of the ancestral Y would give rise to the chromosomes Y_1 and Y_2. Under this hypothesis, API17qter would have joined the remainder of the ancestral Y chromosome to form Y_1. The now unprotected ends of API17q would have developed functional telomeres and this chromosome would have become Y_2. This development of functional telomeres could have arisen from three different pathways (Meyne et al., 1990; Bouffler et al., 1996; Azzalin et al., 2001; Nergadze et al., 2004; Bolzán and Bianchi, 2006; Lin and Yan, 2008): de novo formation of functional telomeres; amplification of pre-existing low copy telomeric sequences at the site of breakage and posterior acquisition of full telomeric functionality; or pre-existence of interstitial telomeric repeats ('latent telomeres') that acquire telomeric functionality after the breakage. Considering the evidence observed in our results, the second pathway seems to be the most feasible to explain the configuration observed at metaphase I and the sex chromosomes G-banding pattern.

Future directions

The study of a larger sample of *A. pigra* from different locations would allow us to explore possible chromosomal variation within the species, as has been noted for other *Alouatta* species (Ma et al., 1975; Yunis et al., 1976; Minezawa et al., 1985; Armada et al., 1987; de Oliveira, 1995; Stanyon et al., 1995; Consigliere et al., 1996, 1998; de Oliveira et al., 1998, 2002; Torres and Ramirez, 2003). It is worth noting that although three different haplotypes (with one or two nucleotide differences among them) have been observed in *A. pigra* for the *cytb* mitochondrial fragment used in this study (Cortés-Ortiz et al., 2003), only one of them was observed in the individuals analyzed here. Furthermore, mitotic, meiotic, and FISH analyses of other members of the genus *Alouatta* will allow us to reconstruct the evolutionary pathways that led to the diversity within this genus.

This study also establishes the basis for future comparative cytogenetic studies of purebred and hybrid *A. palliata*/*A. pigra* individuals, such as those previously reported by Cortés-Ortiz et al. (2007). Those studies would allow us to understand the genetic interaction of the parental species at chromosomal level, as well as to explore possible reproductive isolating mechanisms that have been suggested by means of molecular data.

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References

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

Azzalin CM, Nergadze SG, Giulotto E: Human intrachromosomal telomeric-like repeats: sequence organization and mechanisms of origin. Chromosoma 110:75–82 (2001).

Baumgarten A, Williamson GB: The distributions of howling monkeys *(Alouatta pigra* and *A. palliata)* in southeastern Mexico and Central America. Primates 48:310–315 (2007).

Bolzán AD, Bianchi MS: Telomeres, interstitial telomeric repeat sequences and chromosomal aberrations. Mutat Res 612:189–214 (2006).

Bouffler SD, Morgan WF, Pandita TK, Slijepcevic P: The involvement of telomeric sequences in chromosomal aberrations. Mutat Res 366:129–135 (1996).

Buckton K, Evans H: Method for the Analysis of Human Chromosome Aberrations (WHO Publications, Ginebra 1973).

Cabrera A: Catálogo de los mamíferos Sudamericanos. Rev Mus Arg Cien Nat 'Bernardino Rivadavia', Zoología IV:1–732 (1957).

Consigliere S, Stanyon R, Koehler U, Agoramoorthy G, Wienberg J: Chromosome painting defines genome rearrangements between red howler monkeys subspecies. Chromosome Res 4:264–270 (1996).

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

Cortés-Ortiz L, Bermingham E, Rico C, Rodríguez-Luna E, Sampaio I, Ruiz-García M: Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Mol Phylogenet Evol 26:64–81 (2003).

Cortés-Ortiz L, Duda Jr TF, Canales-Espinosa D, García-Orduña F, Rodríguez-Luna E, Bermingham E: Hybridization in large-bodied New World primates. Genetics 176:2421–2425 (2007).

Crockett CM: Conservation biology of the genus *Alouatta*. Int J Primatol 19:549–578 (1998).

de Oliveira EHC: Chromosomal variation in *Alouatta fusca*. Neotropical Primates 3:181–183 (1995).

de Oliveira EHC, Lima MMC, Sbalqueiro IJ, Pissinati A: The karyotype of *Alouatta fusca clamitans* from Rio de Janeiro, Brazil: Evidence for a Y-autosome translocation. Gen Mol Biol 21 [online, http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47571998000300012&lng=en&nrm=iso] (1998).

de Oliveira EHC, Neusser M, Figueiredo WB, Nagamach C, Pieczarka JC, et al: The phylogeny of howler monkeys (*Alouatta*, Platyrrhini): reconstruction by multicolor or cross-species chromosome painting. Chromosome Res 10:669–683 (2002).

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

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH: Empirical evaluation of preservation methods for faecal DNA. Mol Ecol 7:1423–1428 (1998).

Gregorin R: Taxonomia e variação geográfica das espécies do gênero *Alouatta* Lacépedè (Primates, Atelidae) no Brasil. Rev Bras Zool 23: 64–144 (2006).

Groves CP: Primate Taxonomy (Smithsonian Institution Press, Washington and London 2001).

Hill WCO: Primates: Comparative Anatomy and Taxonomy. Vol 5, Cebidae, Part B (Edinburgh University Press, Edinburgh 1962).

Jungers WL: Body size and scaling of limb proportions in primates, in Jungers WL (ed): Size and Scaling in Primate Biology, pp 345–381 (Plenum Press, New York 1985).

Lawrence B: Howler monkeys of the *palliata* group. Bull Mus Comp Zool 75:314–354 (1933).

Lima MMC, Seuánez HN: Chromosome studies in the red howler monkey, *Alouatta seniculus stramineus* (Platyrrhini, 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 (1991).

Lin KW, Yan J: Endings in the middle: Current knowledge of interstitial telomeric sequences. Mutat Res 658:95–110 (2008).

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

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, et al: Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99:3–10 (1990).

Minezawa M, Harada M, Jordan Oc, Borda CJV: Cytogenetics of Bolivian endemic red howler monkeys *(Alouatta seniculus sara)*: accessory chromosomes and Y-autosome translocation-related numerical variations. Kyoto Univ Overseas Res Rep New World Monkeys 5:7–16 (1985).

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

Mudry M, Rahn IM, Solari AJ: Meiosis and chromosome painting of sex chromosome systems in Ceboidea. Am J Primatol 54:65–78 (2001).

Mudry MD, Nieves M, Bolzán AD: Chromosomal localization of the telomeric $(TTAGGG)_n$ sequence in eight species of New World primates (Neotropical Primates, Platyrrhini). Cytogenet Genome Res 119:221–224 (2007).

Murie A: Mammals from Guatemala and British Honduras. Univ Mich Mus Zool Misc Publ 26: 5–33 (1935).

Nergadze SG, Rocchi M, Azzalin CM, Mondello C, Giulotto E: Insertion of telomeric repeats at intrachromosomal break sites during primate evolution. Genome Res 14:1704–1710 (2004).

Palumbi SR: Nucleic acids II: The polymerase chain reaction, in Hillis DM, Moritz C, Mable BK (eds): Molecular Systematics, pp 205–247 (Sinauer, Sunderland 1996).

Rahn MI, Mudry MD, Merani MS, Solari AJ: Meiotic behavior of the $X_1X_2Y_1Y_2$ quadrivalent of the primate *Alouatta caraya*. Chromosome Res 4:350–356 (1996).

Reid FA: A Field Guide to the Mammals of Central America and Southeast Mexico (Oxford University Press, New York 1997).

Ryland AB, Schneider H, Langguth A, Mittermeier RA, Groves CP, Rodríguez-Luna E: An assessment of the diversity of New World Primates. Neotropical Primates 8:61–93 (2000).

Seabright M: A rapid banding technique for human chromosomes. Lancet 2:971–972 (1971).

Seutin G, White BN, Boag PT: Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82–90 (1991).

Slijepcevic P: Telomeres and mechanisms of Robertsonian fusions. Chromosoma 107:136–140 (1998).

Smith JD: The systematic status of the black howler monkey, *Alouatta pigra* Lawrence. J Mammal 51:358–369 (1970).

Solari AJ, Rahn MI: Fine structure and meiotic behavior of the male multiple sex chromosomes in the genus *Alouatta*. Cytogenet Genome Res 108:262–267 (2005).

Stanyon R, Tofanelli S, Morescalchi MA, Agoramoorthy G, Ryder OA, Wienberg J: Cytogenetic analysis shows extensive genomic rearrangements between red howler (*Alouatta seniculus*, Linnaeus) subspecies. Am J Primatol 35:171–183 (1995).

Steinberg ER, Nieves M, Mudry MD: Meiotic characterization and sex determination system of Neotropical primates: Bolivians squirrel monkey *Saimiri boliviensis* (Primates: Cebidae). Am J Primatol 69:1–6 (2007).

Summer AT: A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75: 304–305 (1972).

Torres OM, Ramírez C: Estudio citogenético de *Alouatta palliata* (Cebidae). Caldasia 25:193–198 (2003).

Wolfheim JH: Primates of the World (University of Washington Press, Seattle 1983).

Yunis EJ, Torres de Caballero OM, Ramirez C, Ramirez ZE: Chromosomal variation in the primate *Alouatta seniculus seniculus*. Folia Primatol 25:215–224 (1976).

Zunino GE, González V, Kowaleski M, Bravo S: *Alouatta caraya*. Relationships among habitat, density and social organization. Primate Report 61:37–46 (2001).