

Banding Patterns of the Chromosomes of Two New Karyotypes of the Owl Monkey, *Aotus*, Captured in Panama

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Abstract. Two new chromosome complements of *Aotus trivirgatus griseimembra* are described making a total of five different karyotypes observed in this subspecies inhabiting Panama and the northwestern part of Colombia, South America. Detailed comparisons of the G-banded chromosomes of these five karyotypes suggest that the polymorphism of chromosome numbers 56 and 55 in Panamanian *Aotus* and 54, 53, and 52 in Colombian *Aotus* stems primarily from a Robertsonian translocation mechanism involving pairs B13 and B14 (or A1). A second Robertsonian translocation mechanism involving pairs B28 and B29 (or A2) constitutes the karyotypic differences between the two chromosomal races.

Introduction

In recent years, the South American owl monkey, *Aotus*, has become increasingly popular as a laboratory animal for various types of biomedical research. The demand for and paucity of this monkey prompted the establishment of breeding *Aotus* colonies in this country with proper genetic management. Although commonly regarded as a single species, this primate is actually comprised of a heterogeneous assemblage of karyotypically different animals with diploid numbers of chromosomes ranging from 46 to 54 [2-7, 9, 10, 13, 14, 20]. At the New England Regional Primate Center, we have studied the chromosome complements of *Aotus* by utilizing banding techniques. Seven different karyotypes have been identified from over 300 *Aotus*

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in our colony [14]. Based on variations of pelage coloration, karyotypes and geographic origin, we postulated that our samples of owl monkeys might represent at least four allopatric chromosome races. Examination of the chromosome complement of 500 additional *Aotus* substantiated our postulation and disclosed two additional karyotypes of *Aotus* from Panama, which possibly represent a fifth allopatric chromosome race. The present paper describes the chromosomal homology between these Panamanian *Aotus* and owl monkeys from the northwestern part of Colombia.

Materials and Methods

Twenty-one owl monkeys (nine males, 12 females) collected from Panama were among the 800 *Aotus* specimens which we analyzed cytogenetically at the New England Regional Primate Research Center. Ten *Aotus* (three males, seven females) were members of a colony of Panamanian owl monkeys captured east of the Panama Canal Zone. Seven *Aotus* (five males, two females) from the same colony were captured west of the Canal Zone. Four additional Panamanian *Aotus* specimens were housed at the Letterman Army Institute of Research, San Francisco. Their exact geographic origin is unknown.

From each monkey, 2 ml of peripheral blood were withdrawn from the femoral vein with a heparinized syringe and transferred immediately to a sterile vial containing an equal amount of F10 Nutrient Mixture. The specimen was packed inside an inner waterproof container within a sturdy mailing case and shipped promptly by priority air mail to our laboratory without refrigeration.

Standard chromosome preparations were then made from each specimen received following the leukocyte culture method [16]. Air-dried metaphase spreads were subjected to different staining treatments including conventional Wright and Giemsa's stains for routine chromosome counts, and the trypsin digestion technique [18] to obtain G-band patterns. Mitotic spreads were also treated to demonstrate C-band patterns [17]. Thirty-five differentially stained spreads from each monkey were photographed on high contrast film and printed. Chromosome counts were made directly from the prints, and well-spread metaphase chromosomes were karyotyped and analyzed. In accord with the system established in our laboratory, each *Aotus* karyotype was prepared by assigning autosomes to two morphological groups (A and B) and aligning the chromosomes in each group in order of decreasing size. Chromosomes included in group A had metacentric or submetacentric configurations, while those that had subtelocentric or acrocentric structures were placed in group B as described previously [14].

Results and Observations

Morphologically, all 21 Panamanian *Aotus* included in this report have the distinct pelage features characteristic of *Aotus trivirgatus griseimembra* Elliot, 1912, as described [12]. They are phenotypically similar to *Aotus* in-

habiting the northwestern part of Colombia; facial characteristics and intensity of the black head markings can usually be utilized for differentiation. *A. t. griseimembra* has light yellowish hair on its abdomen and chest. The underside of the neck and back are cinnamon in color with agouti hairs. Its face has a narrow white mantle around the periphery. The tail is brown at the base, shading to black at the tip.

Chromosome analysis of these Panamanian *Aotus* revealed two diploid numbers. Nine *Aotus*, four males and five females (three captured east of the Canal Zone, three captured west of the Canal Zone and three at the Letterman Army Institute) had a diploid count of 56, while the remaining 12 animals, four males, eight females (seven from east of the Canal Zone, four from west of the Canal Zone and one at the Letterman Army Institute) had a chromosome complement of $2n = 55$. The karyotype with 56 chromosomes (designated as K-IX, $2n = 56$) consists of four pairs of meta- and submetacentrics (group A, 3-6) and 23 pairs of subtelo- and acrocentric autosomes (group B, 7-29). The X chromosome is medium sized and submetacentric, and the Y is a small metacentric, the smallest of the chromosome complement. Homologous chromosomes can be identified by their distinctive G-band patterns and paired readily as shown in figure 1a. To compare the degree of chromosomal homology between karyotypes of the Panamanian *Aotus* and the Colombian *Aotus*, each G-band chromosome pair of the Panamanian complement was assigned a number and placed in accord with the chromosomal arrangement established for the Colombian complements (K-II, $2n = 54$; K-III, $2n = 53$, and K-IV, $2n = 52$) previously described [14]. A composite karyotype of K-II, $2n = 54$; K-III, $2n = 53$, and K-IV, $2n = 52$ is shown in figure 2 [14].

On the karyogram of Panamanian *Aotus* with 56 chromosomes (K-IX, $2n = 56$), the positions assigned for A1 and A2 pairs are left vacant (fig. 1a). Twenty-six chromosome pairs (A 3-6, B 7-27), including the sex chromosomes, have identical matching elements with those of K-II, $2n = 54$. Autosomal pairs B28 and B29 are characteristic only of the karyotypes of Panamanian monkeys.

After the C-band treatment, pericentromeric heterochromatin bands of various sizes are present on all chromosomes of these animals as illustrated in figure 1b. Six pairs of autosomes: A3, B7, B8, B9, B11 and B12 have an additional heterochromatin band on the telomeric end of their short arm while pairs A5 and A6 have a telomeric C-band on their long arm. The short arm of the remaining subtelo- and acrocentric pairs appears to be completely heterochromatic.

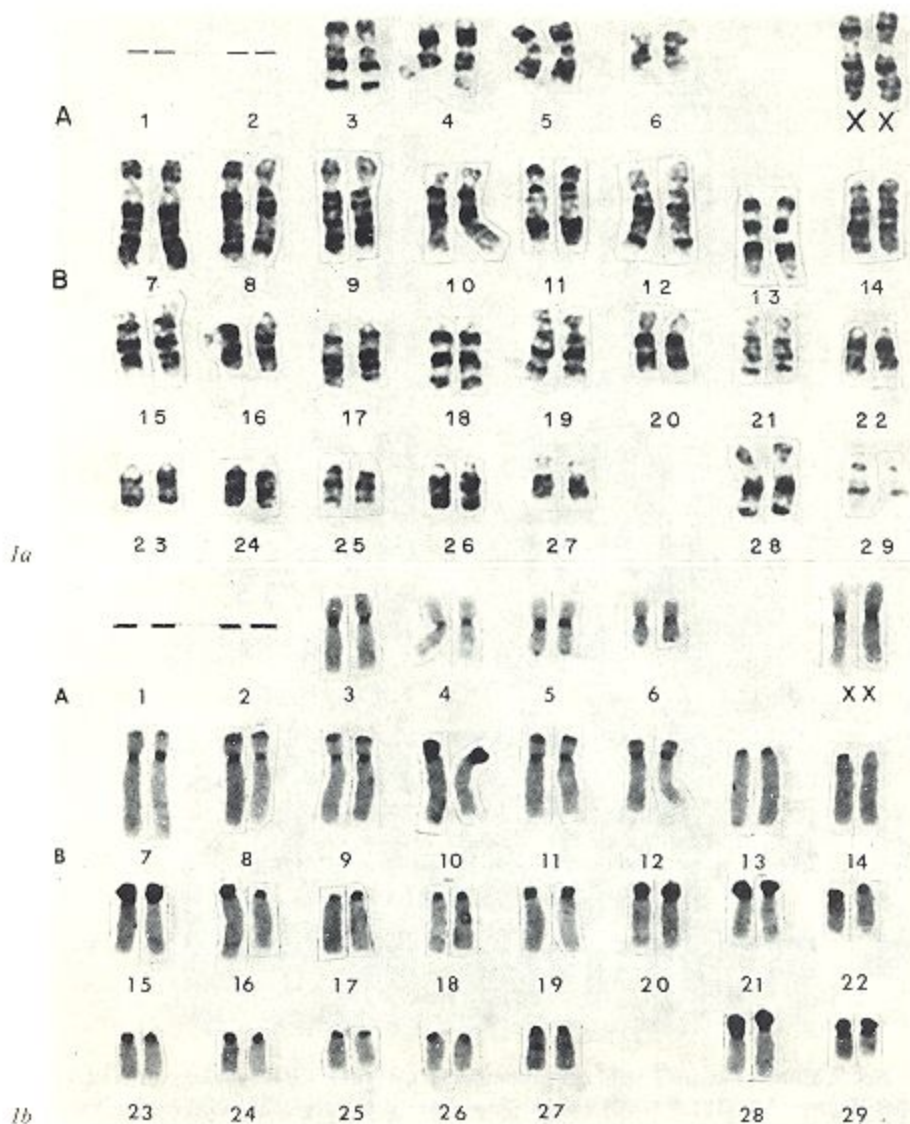


Fig. 1. Karyotypes from G-band (a) and C-band (b) preparations of a Panamanian *Aotus* with a diploid number of 56 (K-IX, $2n = 56$). The chromosomes are arranged following the system established for the Colombian complement (K-II, $2n = 54$; K-III, $2n = 53$; and K-IV, $2n = 52$). Group A includes all metacentric and submetacentric autosomes. Group B includes all subtelo-centric and acrocentric autosomes. In this karyotype, the positions assigned for A1 and A2 pairs are left vacant.



Fig. 2. Composite of G-band chromosomes of Colombian owl monkeys with karyotypes II ($2n = 54$), III ($2n = 53$) and IV ($2n = 52$). Note three unmatched chromosomes in K-III ($2n = 53$). Long and short arms, respectively, of A1 are identical to B13 and B14 [14].

Eleven of the 12 animals with a chromosome complement of $2n = 55$ share a common karyotype (designated as K-VIII, $2n = 55$) consisting of four-and-one-half pairs of meta- and submetacentrics (A1, 3-6), 21 pairs of subtelo- and acrocentrics (B 7-12, 15-29) and two unpaired acrocentric

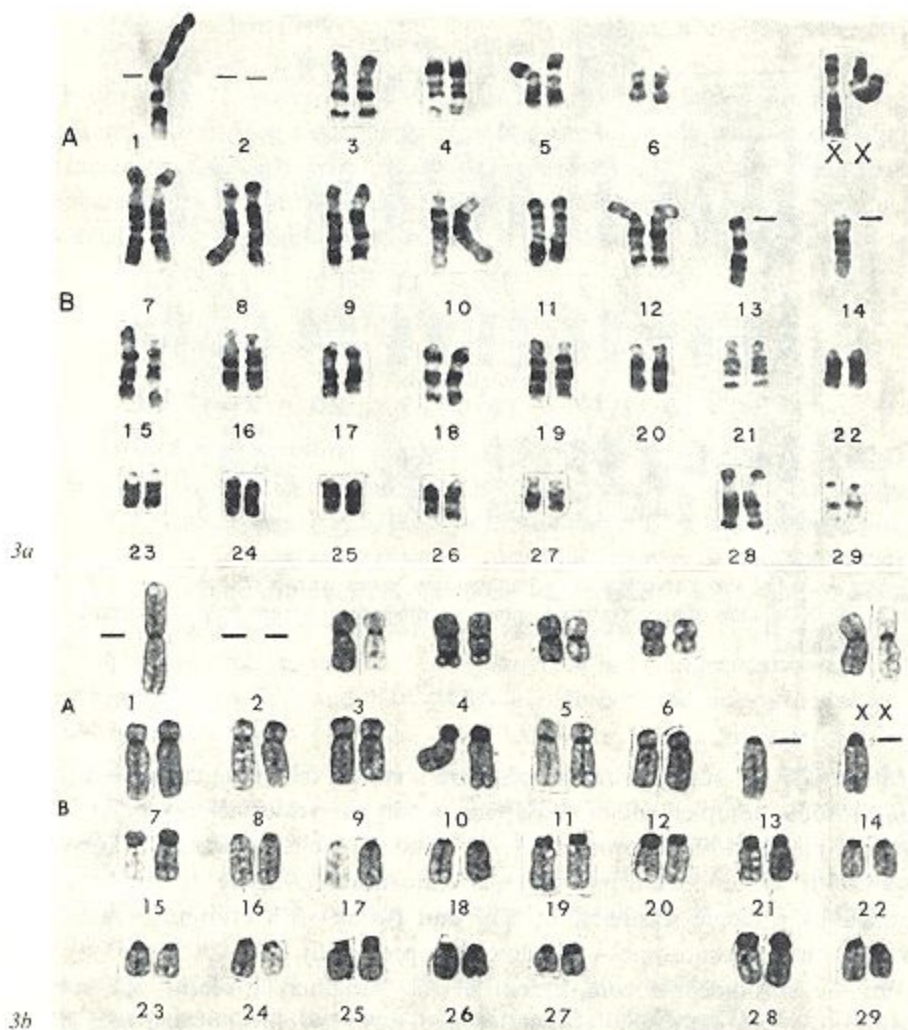


Fig. 3. *a* A G-band karyotype and *b* a C-band karyotype of a Panamanian *Aotus* with 55 chromosome count (K-VIII, $2n = 55$). The arrangement of chromosomes is the same as described in figure 1. Notice that this chromosome complement of $2n = 55$ has identical matching elements with the complement of K-IX ($2n = 56$) except for the unpaired A1, B13 and B14 chromosomes.

autosomes (B13, B14). The X and Y chromosomes are identical to sex chromosomes of owl monkeys with 56 chromosomes. Examples of G-band and C-band karyotypes from an *Aotus* with a chromosome complement of $2n = 55$ are shown in figures 3a and 3b, respectively. Except for pairs

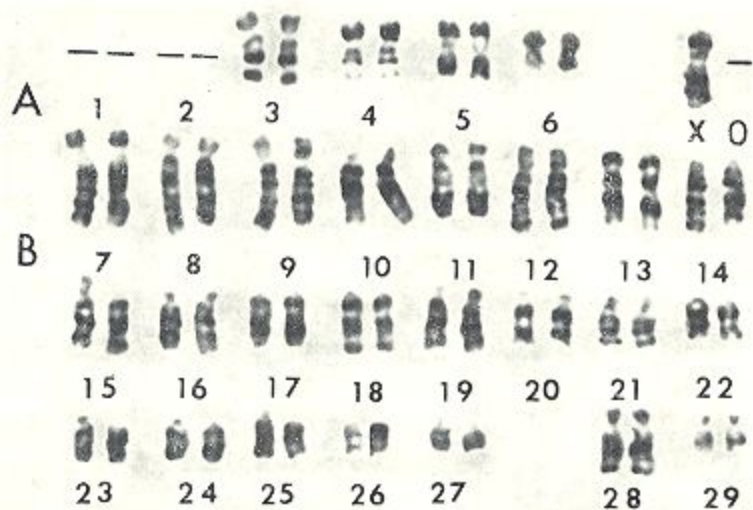


Fig. 4. A G-band karyotype of a Panamanian *Aotus* with a complement of K-IX ($2n = 55$; XO). One of the X chromosomes is consistently absent from all metaphase spreads studied.

B28 and B29, all other chromosomes have identical matching elements with chromosome complement of K-III, $2n = 53$. The relationship among the three unmatched chromosomes A1, B13 and B14, characteristic of karyotypes with 55 or 53 chromosomes, as illustrated in figure 2, can be explained by a single inversion of B14 and fusion with chromosome B13 to constitute chromosome A1 as described previously [14].

In the chromosome complement of the Panamanian *Aotus*, the substitution of two autosomal pairs, B28 and B29, for chromosome pair A2 is the chief difference from the chromosome complement of the Colombian *Aotus*.

The existence of the polymorphism and the origin of the A2 chromosome pair in the Colombian *Aotus* as well as the B28 and B29 pairs in Panamanian *Aotus* can be explained by the occurrence of a fusion or fission in the subspecies. A postulated telomeric-centromeric fusion of chromosome B29 to chromosome B28 would form the A2 chromosome pair found in K-II, K-III and K-IV. Such an assumption is supported by C-band preparations in which the A2 chromosome is characterized by a C-band on the short arm of the A2 chromosome, probably representing the inactivated

pericentromeric heterochromatin band of chromosome B29 (K-VIII, $2n = 55$ and K-IX, $2n = 56$).

From these 21 specimens of Panamanian *Aotus*, we have identified one apparently normal female having a diploid count of 55 but with a karyotype characteristic for K-IX, $2n = 56$. In 100 spreads, a medium submetacentric chromosome consistent with the second X sex chromosome was absent. An example of the karyotype of this individual with the X-O anomaly is shown in figure 4.

Discussion

Owl monkeys inhabiting Central America and the northwestern part of South America are commonly combined under the subspecies *Aotus trivirgatus griseimembra* [11, 12]. Previous studies on the chromosome complement of *A. t. griseimembra* from the northwestern part of South America demonstrated three different chromosome numbers of 54, 53, and 52 [14]. These karyotypes were designated by us as K-II, $2n = 54$; K-III, $2n = 53$; and K-IV, $2n = 52$, respectively. K-II, $2n = 54$ is distinguished by having homologous pairs B13 and B14 which are absent and substituted by a homologous A1 pair in K-IV, $2n = 52$. K-III, $2n = 53$ is characterized by having a haploid set from both K-II, $2n = 54$ and K-IV, $2n = 52$ complements. Chromosomal analysis of a random sample of 363 Colombian *Aotus* showed 148 animals with the K-II, $2n = 54$ complement, 168 had the K-III, $2n = 53$ complement and 47 of them had the K-IV, $2n = 52$ complement. The frequencies of these three types are consistent with the Hardy-Weinberg equilibrium.

Since chromosome complements with 55 and 56 diploid counts resulted from rearrangement of the same two pairs of chromosomes (B13 and B14), these two additional karyotypes representing *Aotus*, captured in Panama, disclose the existence of another chromosomal race in the subspecies *A. t. griseimembra*. Although we have not yet observed the karyotype with a 54 diploid count and characterized by having a homologous A1 pair, we anticipate its existence. Further samples of *Aotus* from Panama may establish this point.

Our finding of true polymorphism of chromosome number in two separate chromosomal races of *A. t. griseimembra* substantiates the characterization and stabilization of such a chromosome rearrangement (involving B13 and B14 pairs) in the subspecies. The rearrangement involving chromo-

some pairs B28 and B29 probably is of a more recent origin, and combined with geographic isolation, constituted two separate chromosomal races within this subspecies.

In man, the X-O chromosome anomaly occurs once in 2,500 female births [15] and is usually associated with a clinical entity known as Turner's syndrome. In mammals, this sex chromosome aberration has also been reported in the mouse, horse [1] and rhesus monkey [19]. Except for the mouse, an X-O chromosomal condition usually confers infertility upon the bearer. In our samples of 800 *Aotus*, two were found to have such a chromosomal anomaly. One is a Colombian *Aotus* with a complement of K-11, $2n = 53$; XO previously reported [14], while the second, which we report here, is a Panamanian *Aotus* with a K-IX, $2n = 55$; XO complement. Phenotypically, both of these 'X-O' animals appeared normal in size and body weight, and with no obvious distinguishing features. Further studies on the development and fertility of these animals are in progress.

The high frequency of X-O carrier in our samples may be coincidental. It is also possible that in this lower form of primate, an X-O condition is less deleterious for the survival of the carrier than in man, where 5% of the common chromosomal aberrations encountered in aborted fetuses had the X-O complement of sex chromosomes [8, 15].

References

- 1 BENIRSCHKE, K.: Cytogenetic abnormalities in reproduction; in Progress in Infertility (Little, Brown, Boston 1975).
- 2 BOER, L.E.M. DE: A case of abnormal chromosome number in a female *Aotus trivirgatus* (Humboldt, 1811 (Cebidae; primates). Genen Phaenen 14: 51-55 (1971).
- 3 BOER, L.E.M. DE: Chromosome studies in primates from zoological gardens in The Netherlands. Genen Phaenen 15: 41-64 (1972).
- 4 BOER, L.E.M. DE: Cytotaxonomy of the Platyrrhini (Primates). Genen Phaenen 17: 1-115 (1974).
- 5 BRUMBACK, R.A.: Two distinctive types of owl monkeys (*Aotus*). J. med. Primatol. 2: 284-289 (1973).
- 6 BRUMBACK, R.A.: A third species of the owl monkey (*Aotus*). J. Hered. 65: 321-323 (1974).
- 7 BRUMBACK, R.A.; STATON, R.D.; BENJAMIN, S.A., and LANG, C.M.: The chromosomes of *Aotus trivirgatus* Humboldt 1812. Folia primatol. 15: 264-273 (1971).
- 8 CARR, D.H.: Chromosome anomalies as a cause of spontaneous abortion. Am. J. Obstet. Gynec. 97: 283 (1967).
- 9 CHIARELLI, B. and BARBERIS, L.: Some data on the chromosomes of Prosimiae and of New World monkeys. Mamm. Chrom. Newsl. 22: 216 (1966).

- 10 EGOZCUE, J.; PERKINS, E.M., and HAGEMENAS, F.: The chromosomes of *Saguinus fuscicollis illigrei* (Pucheran, 1845) and *Aotus trivirgatus* (Humboldt, 1811). *Folia primatol.* 10: 154-159 (1969).
- 11 HERSKOVITZ, P.: Mammals of northern Colombia. Preliminary report No. 4. Monkeys (primates) with taxonomic revisions of some forms. *Proc. US natn. Mus.* 98: 323-427 (1949).
- 12 HILL, W.C.O.: Primates: comparative anatomy and taxonomy. 4. *Cebidae*; part A (University Press, Edinburgh 1960).
- 13 KOIFFMANN, C.P. and SALDANHA, P.H.: Cytogenetics of Brazilian monkeys. *J. hum. Evol.* 3: 275-282 (1974).
- 14 MA, N.S.F.; JONES, T.C.; MILLER, A.C.; MORGAN, L.M., and ADAMS, E.A.: Chromosome polymorphism and banding patterns in the owl monkey (*Aotus*). *Lab. Anim. Sci.* 26: 1022-1036 (1976).
- 15 MACLEAN, N.; HARNDEN, D.G.; COURT BROWN, W.M.; BOND, J., and MANTLE, D.J.: Sex-chromosome abnormalities in newborn babies. *Lancet* i: 286-290 (1964).
- 16 MOORHEAD, P.S.; NOWELL, P.C.; MELLMAN, W.J.; BATTIPS, D.M., and HUNGERFORD, D.A.: Chromosome preparations of leukocytes cultured from human peripheral blood. *Expl Cell Res.* 20: 613-616 (1960).
- 17 STEFOS, K. and ARRIGHI, F.E.: Heterochromatic nature of W chromosome in birds. *Expl Cell Res.* 68: 228-231 (1971).
- 18 WANG, H.C. and FEDEROFF, S.: Banding in human chromosomes treated with trypsin. *Nature, Lond.* 235: 52-53 (1972).
- 19 WEISS, G.; WEICK, R.F.; KNOBIL, E.; WOLMAN, S.R., and GORSTEIN, F.: An X-O anomaly and ovarian dysgenesis in a rhesus monkey. *Folia primatol.* 19: 24-27 (1973).
- 20 YUNIS, E.; CABALLERO, O.M.T. DE, and RAMIREZ, C.: Genus *Aotus* Q- and G-band karyotypes and natural hybrids. *Folia primatol.* 27: 165-177 (1977).