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Cytogenetic Characterization of *Alouatta belzebul* with Atypical Pelage Coloration

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Introduction

Pelage coloration is an important characteristic for the standard taxonomic identification of howler monkeys (Alouattinae). One of the two species that inhabits the Amazonian tropical forest, *Alouatta belzebul*, is currently characterized by its overall black pelage with rufuous extremities and tail, while the other, *Alouatta seniculus*, shows a characteristic brownish-copper tinge to a dark scarlet red coloration [1, 2]. Although the precise limits of their geographic distribution are unknown, there is general agreement that there is no overlap at the boundary between these two species. This is because *A. belzebul* is distributed to the south of the Amazonas-Madeira rivers, while the range of *A. seniculus* extends to the north of these rivers.

Previous karyotypic characterization of these species has shown that the chromosome diploid number in *A. belzebul* is 50 in the female and 49 in the male as a result of a Y-autosome translocation [3, 4], while the diploid number in *A. seniculus* is lower (2n = 43, 44 or 45), the different numbers resulting from a variable number of microchromosomes [5]. The presence of microchromosomes is, moreover, a unique characteristic of *A. seniculus* among the howler monkeys so far studied. Conversely, the Y-autosome translocation, present in *A. belzebul* and absent in *A. seniculus*, has also been reported in other howler monkey species [4, 6].

Previously published chromosome data for *A. belzebul* [3, 4] were based on the study of 11 wild-caught animals showing wholly or predominantly black coloration with rufuous extremities. Ten of these specimens, clearly identified as the species holotype, *Alouatta belzebul belzebul*, had been captured at the Tucurui dam reservoir, a region of the Amazonian tropical forest where this subspecies is normally distributed. Surprisingly, another specimen (a female; 2n = 50),

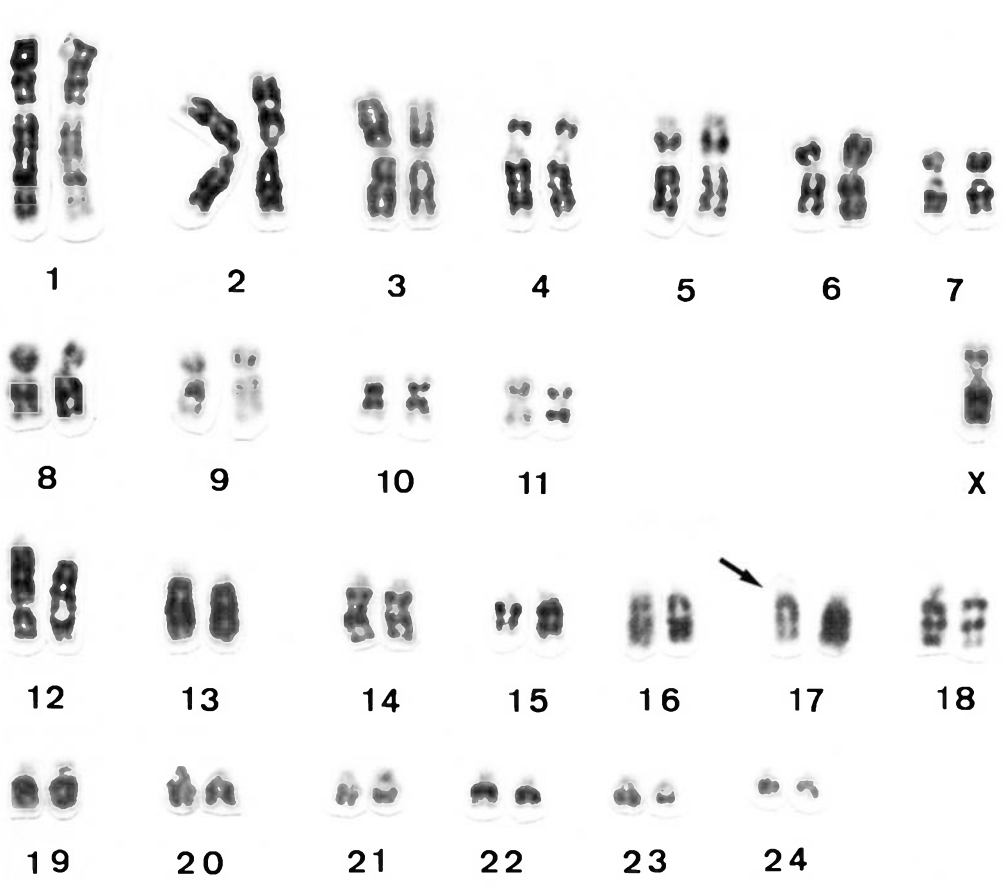

Fig. 1. G-banded karyotype of a male specimen (2n = 49). Arrow points to the translocated Y chromosome on chromosome 17.

tentatively identified as *A. belzebul nigerrima*, showed a chromosome complement different from that observed in the species holotype. This finding was illuminating in showing the existence of at least two karyotypically distinct types within the same species.

More recently, six wild-living adult specimens with brown-copper pelage were caught at the Tucurui dam reservoir and later transferred to the National Primate Centre (Belem, Para). These specimens were phenotypically similar to *A. seniculus*, but they had been captured in a region where *A. belzebul* is normally distributed, far to the south of the range limit of the former. A karyotypic analysis of these specimens seemed appropriate in view of (1) the apparent contradiction between phenotypic characteristics and geographic origin and (2) the already known karyotypic variability between *A. belzebul* subspecies [4].

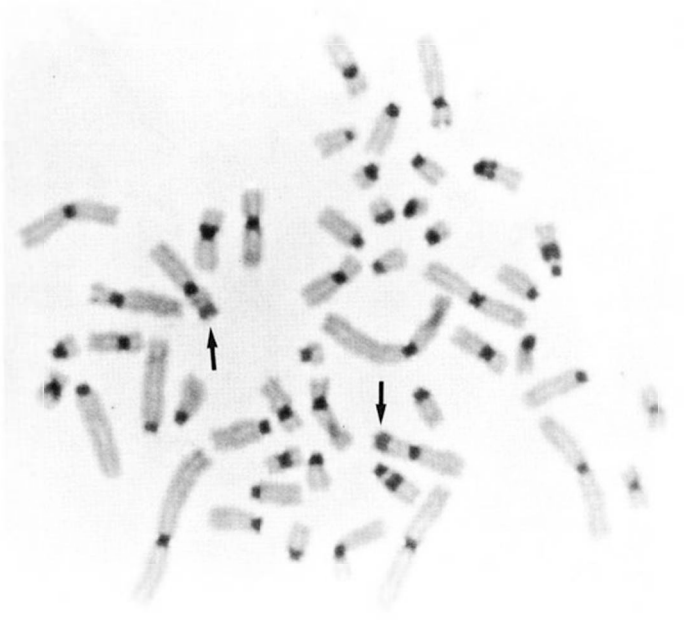

Fig. 2. C-banded metaphase showing constitutive heterochromatic regions. Note variable regions in the short arm of chromosome 5 (arrowed).

Material and Methods

Animals and Region of Capture

Six adult howler monkeys of red copper pelage (3 males and 3 females), phenotypically similar to *A. seniculus*, were wild-caught in the Tucurui dam reservoir (latitude 4° S; longitude 49° W, State of Para, Brazil). Two of these specimens were caught on the east bank of the Tocantins river. The remaining four were caught either on Tocantins island or on the east bank of this river.

Cytogenetic Analysis

Chromosome preparations were obtained with whole blood cultures in TC 199 supplemented with 20% fetal calf serum and 5% PHA. Colchicine was added after 92 h of incubation at 37 °C, to a final concentration of 0.5×10^{-5} *M*, for a further period of 4 h. Mitotic metaphase spreads were obtained with a hypotonic solution (KCl 0.075 *M*) followed by three fixations in methanol:acetic acid (3:1). Chromosome preparations were G-, C- and NOR-banded following the standard procedures described previously [7–9].

Results and Discussion

The diploid chromosome number in these specimens was 50 in females and 49 in males. In the male, one acrocentric pair (n = 17) was heteromorphic, showing a proximal G-positive region in one chromosome that was absent in its counterpart (fig. 1). In the female, this pair was homomorphic, with both homologues lacking the proximal G-positive region. The numbers of biarmed and acrocentric chromosomes, as well as the G- and C-banding patterns (fig. 1, 2), were identical with those of *A. belzebul belzebul* [4]. Although a meiotic analysis of the male has not been carried out, we propose that the difference in diploid number between the sexes is due to a Y-autosome translocation, as previously described. The distribution of constitutive heterochromatin was mainly centromeric, with intercalar blocks in two

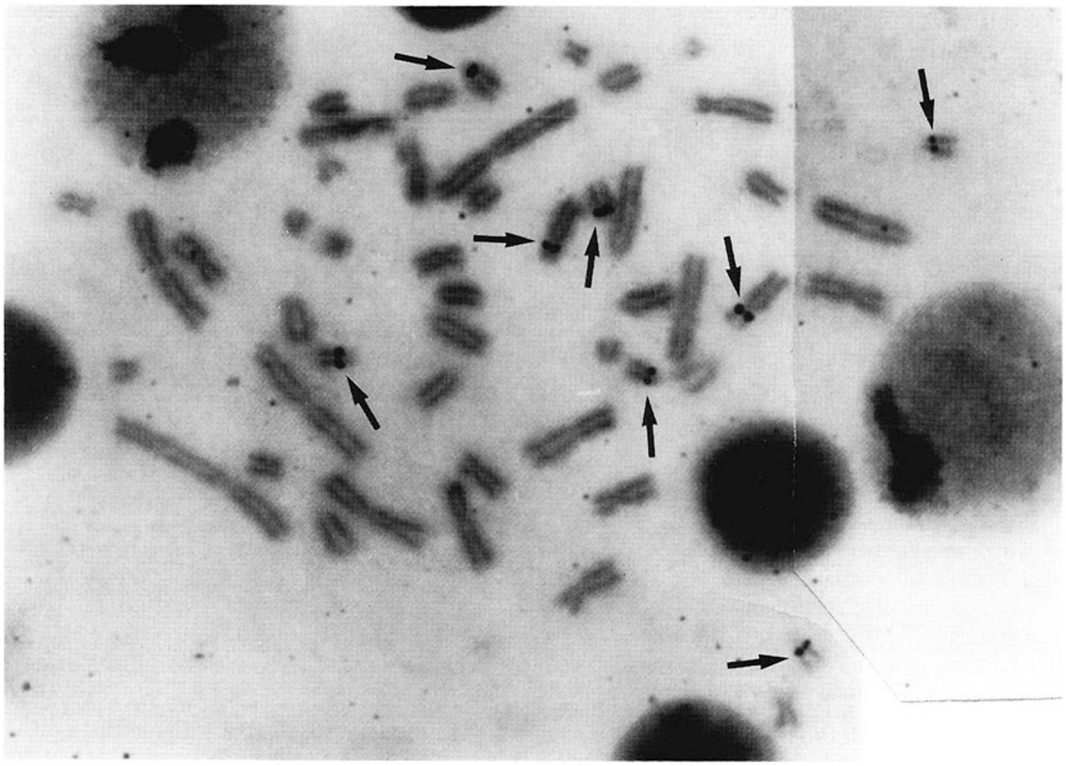

Fig. 3. Silver-stained nucleolus organizer regions (arrowed) in four chromosome pairs.

acrocentric pairs and terminal blocks in the short-arm region of two biarmed chromosomes. These regions were occasionally absent in one member of the pair or were variable in size, especially in the short-arm region of chromosome 5 (fig. 2), as a result of polymorphic variation. Silver staining showed eight nucleolus organizer regions (NORs), in chromosomes 6, 18, 19, and in a small acrocentric chromosome pair (fig. 3).

These data show that these new specimens were chromosomally identical with *A. belzebul belzebul*, except for an extra number of NORs in the brown-copper specimens. Diploid chromosome number, presence of Y-autosome translocation in the male, chromosome morphology, and G- and C-banding patterns allowed us to distinguish these specimens from *A. seniculus* and from all other species of the Alouattinae so far reported [4]. The new specimens are, though phenotypically different, practically identical with the species holotype at the chromosome level. They probably represent a small number of variant specimens contained in a large sample of some 1,000 animals captured at the Tucurui dam reservoir, where some degree of gradual chromatic variation had been observed between animals on the west bank of the Tocantins, on Tocantins island, and on the east bank of this river (H. Schneider, unpubl. data). As pelage may be genetically

determined by a few loci in the genome, such an observation would not be surprising when analysing large population samples. However, a difference of this kind may be misleading for taxonomists who commonly rely on gross external morphological traits as distinctive attributes for species (or subspecies) identification, especially when no reliable data are available on the geographic origin of the specimens under study. This is likely to be the case in zoos, research laboratories or animal colonies where data on captive specimens might be incomplete or fragmentary. Moreover, as pelage coloration might be an unreliable trait for taxonomic identification, field observations must also take this into consideration when delimiting the geographic range of natural populations. If pelage coloration were used as a criterion for the identification of species or subspecies in the wild, serious misinterpretations could result in the estimation of population numbers and geographic distribution. Characterization by karyotypic analysis might therefore be useful for a more reliable assessment of primate populations and for establishing new taxonomic schemes unbiased by phenotypic variability.

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