

Karyotypic Study of Titi Monkeys, *Callicebus moloch brunneus*

MITSURU MINEZAWA,
Kyoto University
OTTO CARLOS JORDAN C.,
Zoo Municipal Santa Cruz
and C. JAIME VALDIVIA B.
Instituto Bioclinico Central

ABSTRACT. A karyotypic study on a subspecies of the dusky titi, *Callicebus moloch brunneus*, was carried out and a third karyotype of *C. moloch* was discovered. The chromosome number of this subspecies is 48. The autosomes consist of 5 subtelocentric, 5 submetacentric or metacentric, and 13 acrocentric chromosome pairs. The X chromosome and the Y chromosome are submetacentric and metacentric, respectively.

A comparative study with other subspecies of the *C. moloch* group (i.e., *C. m. cupreus* and *C. m. ornatus* with $2n = 46$ and *C. m. donacophilus* with $2n = 50$) suggests that the karyotype of *brunneus* occupies a position intermediate between the two other karyotypes of *C. moloch*, but nearer to that of $2n = 50$. The presumed total differences between *brunneus* and *cupreus* comprise one Robertsonian rearrangement, one centromeric transposition and four pericentric inversions, and those between *brunneus* and *donacophilus* involve one translocation or breakage (possibly corresponding to two events, that is, one Robertsonian rearrangement and one centromeric transposition).

Key Words: *Callicebus moloch brunneus*; New World monkey; Karyotype; Comparative cytogenetics; Differentiation.

INTRODUCTION

Based on morphological characters, HERSHKOVITZ (1963) distinguished seven subspecies of the dusky titi, *Callicebus moloch*. Five of these are distributed from Columbia to Paraguay along the base of the Andes (*C. m. ornatus*, *C. m. discolor*, *C. m. cupreus*, *C. m. brunneus*, and *C. m. donacophilus*) and two extend downstream to the middle courses along the tributaries of the Amazonas (*C. m. hoffmannsi* and *C. m. moloch*).

Two karyotypes of *C. moloch* have previously been reported: two subspecies, *C. m. cupreus* and *C. m. ornatus*, had 46 chromosomes (EGOZCUE, 1969; BENIRSCHKE & BOGART, 1976), while *C. m. donacophilus* had 50 chromosomes (DE BOER, 1974; MINEZAWA & VALDIVIA, 1984). A comparison using G- and C-band techniques revealed that the two karyotypes differed from each other by two Robertsonian rearrangements and four pericentric inversions (MINEZAWA & VALDIVIA, 1984). These two karyotypes are distributed geographically in the northern (*C. m. ornatus*) and southern (*C. m. donacophilus*) extremes of *Callicebus*'s habitat, and the differences between them are rather large in terms of karyotypic differences between ordinary conspecific subspecies. Therefore, in the mid-area between the habitats of the two *Callicebus* karyotypic races ($2n = 46$ and $2n = 50$), we could expect to discover a new karyotype intermediate between the two.

This paper give details of the G- and C-bands of a third karyotype of *Callicebus moloch*

collected at Montecarlo, Pando, Bolivia, where *brunneus* titi is expected to occur according to the descriptions of HERSHKOVITZ (1963).

MATERIALS AND METHODS

Peripheral blood samples were taken from two female and two male dusky titi monkeys, *Callicebus moloch*, captured on the south bank of Rio Manuripi, within a radius of 4 km from Montecarlo, Pando, Bolivia (Fig. 1). HERSHKOVITZ (1963) reported that *C. moloch brunneus* was distributed within the area between the two rivers, Rio Madre de Dios and Rio Manuripi, and the titi monkeys captured there by the present authors showed a coat color similar to the descriptions of the *C. m. brunneus* subspecies given by HERSHKOVITZ (1963).

Whole blood samples were conserved with ice for three to five days before culture for 72 hr in RPMI-1640 (containing PHA-M, FCS, and antibiotics). Cells were fixed by standard methods at the Instituto Bioclinico Central, Santa Cruz, Bolivia, and stored at -20°C in Carnoy's acetic acid-alcohol fluid. The fixed samples were processed at the Primate Research Institute, Kyoto University, Japan. The metaphase chromosome was sequentially stained by the standard Giemsa, G- and C-bands with ASG (SUMNER et al., 1971) and BSG (SUMNER, 1972) methods.

At least ten metaphase karyoplasts from each specimen were observed under a microscope in order to count the chromosome number and record their gross morphology. More than two G- and C-banded karyoplasts of three individuals (two males and one female) were photographed, and they were compared with each other and with the two other karyotypes reported previously (BENIRSCHKE & BOGART, 1976; MINEZAWA & VALDIVIA, 1984).

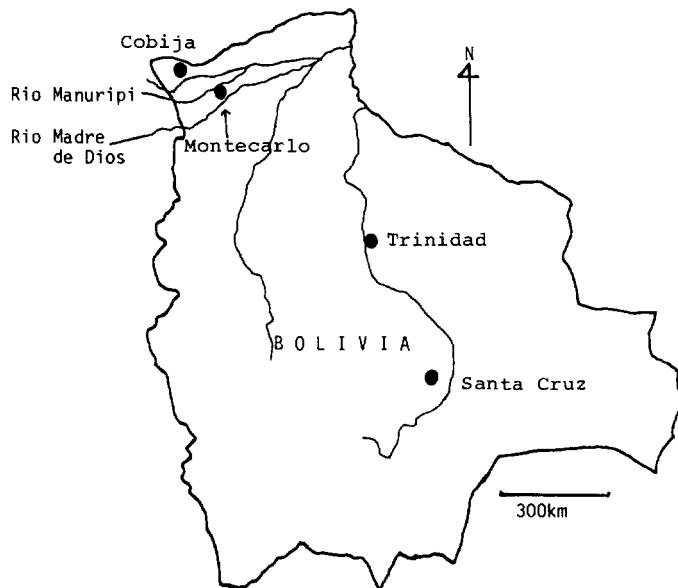


Fig. 1. Map of Bolivia showing the location of Montecarlo (indicated by the arrow).

RESULTS

The chromosome number of all the specimens studied was 48. Their autosomes consisted of 5 pairs of subtelocentric, 5 pairs of submetacentric or metacentric, and 13 pairs of acrocentric chromosomes. The X-chromosome and the Y-chromosome of this karyotype were submetacentric and metacentric, respectively.

G- and C-band karyotypes are illustrated in Figure 2. Centromeric C-bands are observed on all chromosomes. Telomeric C-bands are also noted on the short arms of chromosomes Nos. 4, 8, and 9 and no interstitial C-band is observed. The Y-chromosome stains entirely dark. Using the G-band technique, we could distinguish all the chromosomes. By comparing the G-banded karyotype of *brunneus* with the two other types of the *Callicebus moloch*

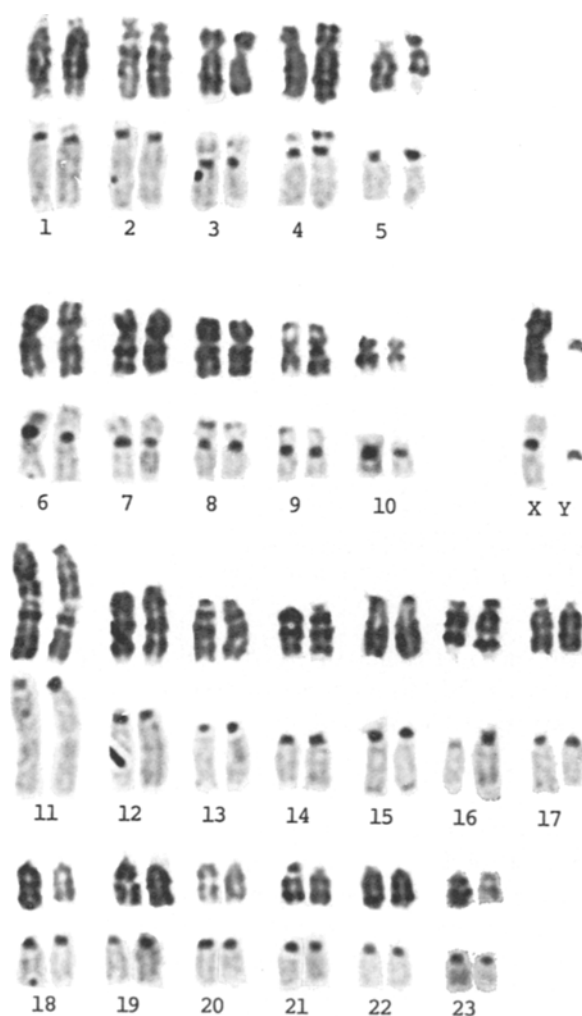


Fig. 2. G- (above) and C-band (below) karyotypes of male *Callicebus moloch brunneus* analyzed in the present study.

Table 1. Chromosome studies on *Callicebus moloch*.

Species	2n	Chromosomes			
		Non-acrocentric	Acrocentric	X	Y
<i>C. m. brunneus</i>	48	20	26	SM	M
<i>C. m. donacophilus</i>	50	22	26	SM	M
<i>C. m. ornatus</i> and <i>cupreus</i>	46	20	24	SM	M

Table 2. Comparison among the three karyotypes of *Callicebus moloch* based on G-band karyotypes.

<i>Callicebus moloch donacophilus</i> (2n = 50)	<i>Callicebus moloch brunneus</i> (2n = 48)	<i>Callicebus moloch cupreus</i> (2n = 46)
1	1	3
2	2	4
3	12 ¹⁾	11 ¹⁾
4	3	12 ²⁾
5	4	14 ²⁾
6	5	16 ²⁾
7	6	5
8	7	8
9	8	7
10	9	9
11	10	10
12	15	2q
13	11-prox	1q
14	13	13
15	14	15
16	17	22
17	16	6 ²⁾
18	18	17
19	11-dist	1p
20	19	18
21	20	2p
22	21	19
23	22	20
24	23	21
X	X	X
Y	Y	Y

1) Small pericentric inversion or growth of a C-negative element; 2) pericentric inversion. p: Short arm; q: long arm; dist: distal part of arm; prox: proximal part of arm.

group, i.e., 2n = 46 (BENIRSCHKE & BOGART, 1976) and 2n = 50 (MINEZAWA & VALDIVIA, 1984), it can be seen that all the chromosomes of *brunneus* possess homologues in the two previously reported karyotypes (Tables 1 & 2).

brunneus vs. *cupreus*

The longest metacentric chromosome pair of the 2n = 46 karyotype corresponds to the longest acrocentric pair of the 2n = 48 karyotype, and the difference between these two chromosomes is only a transposition of the centromere or centromere-shift without inversion. The second largest bi-arm chromosome pair (No. 2) of the 2n = 46 corresponds to two acrocentric chromosomes (Nos. 15 and 20) of the 2n = 48. Three acrocentric chromosomes (Nos. 12, 14, and 16) of the 2n = 46 karyotype are changed into three bi-arm chromosomes in the 2n = 48 karyotype (Nos. 3, 4, and 5, respectively). On the other hand, one bi-arm chromosome (No. 6) of *cupreus* forms the homologue of an acrocentric chromosome (No. 16) of *brunneus*. The total karyotypic differences between the two karyotypes comprise one Robertsonian rearrangement, one centromeric transposition and four pericentric inversions.

brunneus vs. *donacophilus*

The longest acrocentric chromosome (No. 11) of the $2n = 48$ corresponds to two acrocentric chromosomes (Nos. 13 and 19) of the $2n = 50$ karyotype. One acrocentric chromosome (No. 12) of *brunneus* is changed into a submetacentric chromosome (No. 3) in *donacophilus* by a small pericentric inversion or growth of a C-negative element. The total differences between *brunneus* and *donacophilus* consist of one translocation or breakage (possibly corresponding to two events; that is, one Robertsonian rearrangement and one centromeric transposition), and the existence of a C-negative small short arm.

DISCUSSION

Among the karyotypes of *Callicebus*, that of *torquatus* is special: it has the smallest chromosome number ($2n = 20$) in primates (ARDITO, 1979). Morphologically, *C. torquatus* also has distinct characteristics within the *Callicebus* group (HERSHKOVITZ, 1963). The three karyotypes of *C. moloch* are similar to each other, when compared with that of *C. torquatus*. The karyotypic differences among the *Callicebus moloch* subspecies are, however, much larger than those among conspecific subspecies of Old World monkeys.

The karyotype of *brunneus* occupies a position intermediate between those of *donacophilus* and *cupreus*, but nearer to that of *donacophilus* than that of *cupreus* (Fig. 3). From the morphological viewpoint, several taxonomists have discussed the relationships among these three kinds of *Callicebus moloch* titi (Table 3). CABRERA (1958) assigned *C. cupreus toppini* to the titi monkey of the area presently studied. HILL (1960) also placed this animal in *C. cupreus* as *C. c. brunneus*. HERSHKOVITZ (1963) regarded *brunneus* as one of the subspecies of *C. moloch* with the other two subspecies currently under discussion, but the key characters, which distinguish the subspecies of *C. moloch*, indicate a relatively closer relationship of *C. m. brunneus* to *C. m. cupreus* than to *C. m. donacophilus*. Regardless of whether the differences between *donacophilus* and the other two dusky titi monkeys were at the species level or subspecies level, all three taxonomists have placed *donacophilus* in the most remote

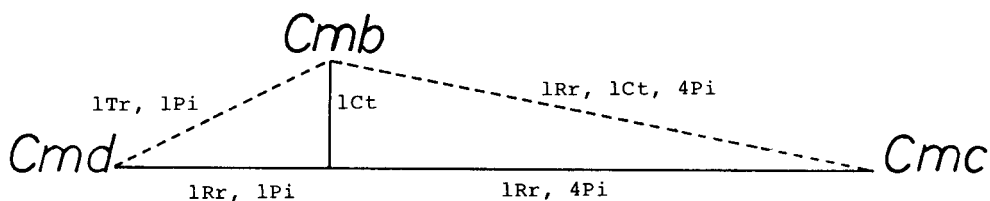


Fig. 3. Relationships among the three karyotypes of *Callicebus moloch*. Rearrangement (including chromosomes No. 11 of *brunneus*, No. 1 of *cupreus* and Nos. 13 and 19 of *donacophilus*) could have occurred independently (dashed line) or sequentially (solid line). *Cmb*: *C. m. brunneus*; *Cmc*: *C. m. cupreus*; *Cmd*: *C. m. donacophilus*; *Rr*: Robertsonian rearrangement; *Tr*: translocation; *Pi*: pericentric inversion; *Ct*: centromere transposition.

Table 3. Comparison of earlier classifications of *Callicebus moloch*.

CABRERA (1958)	HILL (1960)	HERSHKOVITZ (1963)
<i>C. ornatus</i>	<i>C. cupreus ornatus</i>	<i>C. m. ornatus</i>
<i>C. cupreus</i>	<i>C. cupreus cupreus</i>	<i>C. m. cupreus</i>
<i>C. cupreus toppini</i>	<i>C. cupreus brunneus</i>	<i>C. m. brunneus</i>
<i>C. moloch donacophilus</i>	<i>C. gigot donacophilus</i>	<i>C. m. donacophilus</i>

position among these three *C. moloch* subspecies. As mentioned above, the karyotypic results are contradictory to the morphological observations. Similar contradictions between classification and karyotype are frequently observed among primates. For example, many congeneric species of *Saguinus* share almost the same karyotypes as $2n = 46$ (HERSHKOVITZ, 1977). Although previously all owl monkeys were classified as one species, *Aotus trivirgatus*, many widely differentiated karyotypes were discovered in succession (BRUMBACK, 1974, 1975; REUMER & DE BOER, 1980; MA, 1981). As a result of these karyotypic findings, HERSHKOVITZ (1983) split the genus *Aotus* from one species to nine species. Similarly, the genus *Saimiri* was divided from one or two species into four species (HERSHKOVITZ, 1984). Although, all species of the genus *Saimiri* show the same chromosome number $2n = 44$, the regional populations differ from each other by two pericentric inversions (JONES & MA, 1975). When considered against the examples described above, the cytogenetic differences among the three subspecies of *C. moloch* are clearly large enough to split them into, at least, three species. Moreover, the morphological key characters for distinguishing the conspecific subspecies of *Callicebus moloch* show almost the same degree of morphological difference to those of congeneric species of *Saimiri* and *Aotus* (HERSHKOVITZ, 1963, 1983, 1984). It is necessary therefore to reconstruct the interrelationships among the subspecies of *C. moloch* by cytogenetic methods.

According to HERSHKOVITZ (1963), *C. moloch* may have originated in the highlands of Southern Brazil, whence it spread to the present habitat, and *C. torquatus* evolved from *C. moloch* in the area between the upper Rios Napo and Guaviare. *C. personatus* also evolved from *C. moloch* in the course of dispersal to the coastal forests of Southeastern Brazil. HERSHKOVITZ also suggested on the basis of his "centripetal dispersal" concept that the major Amazonian tributaries acted as barriers between populations spreading downstream along the gallery forests, and racial divergence increased with downstream spread. However, KINZEY (1982) found that the distribution pattern of *Callicebus* was largely the product of Pleistocene climatic fluctuations and repeated disruption of the forest, and it fitted in with HAFFER's model for the neotropical forest biota (1982). The discussions of the speciation process of *Callicebus* given by HERSHKOVITZ (1963) and KINZEY (1982) were made without detailed cytogenetic information. If data on the karyotypes of other subspecies were available, the discussion of the process of differentiation of *Callicebus* should become more meaningful and clear-cut.

HERSHKOVITZ (1963) noted that *Callicebus olallae* LÖNNBERG, 1939 had all the important diagnostic characters of *Callicebus moloch brunneus* and others which suggested intergradation with *C. m. donacophilus*. He also indicated that *Callicebus modestus* LÖNNBERG, 1939 had intermediate characters between these two subspecies, being more similar to *brunneus* than to *donacophilus*, and that the existence of this morphologically intermediate type was a result of intergradation between the two subspecies. The possibility must be considered, however, that these individuals could really show endemic characters of regional populations, inhabiting the region between those of *donacophilus* and *brunneus*. By using the discrete karyotypic differences which exist between *donacophilus* and *brunneus*, we should be able to clarify whether LÖNNBERG's two *Callicebus* species form part of a stable endemic population or represent only an unstable product of intergradation.

The discovery of a third karyotype of *Callicebus moloch* in the present study strongly

suggests the need for a detailed re-examination of this species from the morphological as well as genetic standpoint.

Acknowledgements. We are especially grateful to Drs. Y. NOGAMI, T. SETOGUCHI, K. NOZAWA, and T. SHOTAKE, Primate Research Institute, Kyoto University, and CNL. R. MENDEZ JORDAN, Cobija, Pando, Bolivia, for their encouragement and advice during this study. We express our sincere thanks to the late Prof. N. KEMPF MERCADO for his kind advice and also our deep sorrow at his death. We are much obliged to Dr. D. PESSOA REA and other members of the Instituto Bioclinico Central, Santa Cruz, Bolivia, for their kind collaboration. We are greatly indebted to Mr. A. MIASHIRO B., Montecarlo, Pando, Bolivia, for his helpful support and cooperation in collecting samples. This work was supported by the Overseas Scientific Research Funds of the Ministry of Education, Science, and Culture, Japan (No. 6141045).

REFERENCES

- ARDITO, G., 1979. Primate chromosome atlas. In: *Comparative Karyology of Primates*, B. CHIARELLI, A. L. KOEN, & G. ARDITO (eds.), Mouton Publisher, The Hague, Paris & New York, pp. 213–281.
- BENIRSCHKE, K. & M. H. BOGART, 1976. Chromosomes of the tan-handed titi (*Callicebus torquatus*, HOFFMANNSEGG, 1807). *Folia Primatol.*, 25: 25–34.
- BRUMBACK, R. A., 1974. A third species of the owl monkey (*Aotus*). *J. Hered.*, 65: 321–323.
- , 1975. Giemsa banding pattern of the karyotype of *Aotus griseimembra* ELLIOT 1912: A preliminary study. *J. Human Evol.*, 4: 385–386.
- CABRERA, A., 1958. Catalogo de los Mamiferos de America del Sur. *Rev. Mus. Argentino Cienc. Nat. "Bernardino Rivadavia,"* 4: 1–307.
- DE BOER, L. E. M., 1974. Cytotaxonomy of the Platyrrhini (Primates) *Genen Phaenen*, 17: 1–115. Cited in G. ARDITO, 1979. Primate chromosome atlas. In: *Comparative Karyology of Primates*, B. CHIARELLI, A. L. KOEN, & G. ARDITO (eds.), Mouton Publisher, The Hague, Paris & New York, pp. 213–281.
- EGOZCUE, J., 1969. Primates. In: *Comparative Mammalian Cytogenetics*, K. BENIRSCHKE (ed.), Springer, New York, pp. 357–389.
- HAFFER, J., 1982. General aspects of the refuge theory. In: *Biological Diversification in the Tropics*, G. T. PRANCE (ed.), Columbia Univ. Press, New York, pp. 6–22.
- HERSHKOVITZ, P., 1963. A systematic and zoogeographic account of the monkeys of the genus *Callicebus* (Cebidae) of the Amazonas and Orinoco River Basins. *Mammalia*, 27: 1–80.
- , 1977. *Living New World Monkeys (Platyrrhini)*. Vol. 1. Univ. of Chicago Press, Chicago.
- , 1983. Two new species of night monkeys, genus *Aotus* (Cebidae, Platyrrhini): A preliminary report on *Aotus* taxonomy. *Amer. J. Primatol.*, 4: 209–243.
- , 1984. Taxonomy of squirrel monkeys, genus *Saimiri* (Cebidae, Platyrrhini): A preliminary report with description of a hitherto unnamed form. *Amer. J. Primatol.*, 7: 155–210.
- HILL, W. C. O., 1960. *Primates, Comparative Anatomy and Taxonomy. IV. Cebidae Part A*. Wiley Interscience, New York.
- JONES, T. C. & N. S. F. MA, 1975. Cytogenetics of the squirrel monkey (*Saimiri sciureus*). *Fed. Proc.*, 34: 1646–1650.
- KINZEY, W. G., 1982. Distribution of primates and forest refuges. In: *Biological Diversification in the Tropics*, G. T. PRANCE (ed.), Columbia Univ. Press, New York, pp. 455–482.
- LÖNNBERG, E., 1939. Notes on some members of the genus *Callicebus*. *Ark. Zool.*, (Stockholm), 31A: 1–18, Cited by P. HERSHKOVITZ, 1963. A systematic and zoogeographic account of the monkeys of the genus *Callicebus* (Cebidae) of the Amazonas and Orinoco River Basins. *Mammalia*, 27: 1–80.
- MA, N. S. F., 1981. Chromosomal evolution in the owl monkey, *Aotus*. *Amer. J. Phys. Anthropol.*, 54: 293–303.
- MINEZAWA, M. & C. J. VALDIVIA, 1984. Cytogenetic study of the Bolivian titi and revision of its

- cytotaxonomic state. *Kyoto Univ. Overseas Research Report of New World Monkeys*, 4: 39–45.
- REUMER, J. W. F. & L. E. M. DE BOER, 1980. Standardization of *Aotus* chromosome nomenclature, with description of $2n = 49-50$ karyotype and that of a new hybrid. *J. Human Evol.*, 9: 461–482.
- SUMNER, A. T., 1972. A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.*, 75: 304–306.
- , H. J. EVANS, & R. A. BACKLAND, 1971. A new technique for distinguishing between human chromosomes. *Nature New Biol.*, 232: 31–32.

—Received February 22, 1988; Accepted June 13, 1988

Authors' Names and Present Addresses: MITSURU MINEZAWA, *Laboratory of Animal Germplasma Research and Evaluation, Department of Genetic Resources I, National Institute of Agrobiological Resources, Kannondai, Tsukuba-shi, Ibaraki, 305 Japan*; OTTO CARLOS JORDAN C., *Zoo Municipal Santa Cruz, Fauna Sudamerica, Santa Cruz, Bolivia*; C. JAIME VALDIVIA B., *Instituto Bioclinico Central, Santa Cruz, Bolivia*.