

## Intraspecific Chromosome Variability in a Lemur from the North of Madagascar: *Lepilemur septentrionalis*, species nova

YVES RUMPLER AND ROLLAND ALBIGNAC

Laboratoire d'Histologie, Embryologie-Cytogénétique, Faculté de Médecine,  
Université de Madagascar, B. P. 375, Tananarive, Malagasy Republic

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**ABSTRACT** A cytogenetical study of 25 *Lepilemur* from the north of Madagascar has given evidence for six karyotypes (with four diploid numbers). Four of the karyotypes with these diploid numbers —  $2N = 38$ ,  $2N = 36$ ,  $2N = 34$  — we believe evolved from one another by centric fusion; and two karyotypes resulted from the hybridization of two of these ( $2N = 37$  and  $2N = 35$ ). The different karyotypes distinguish themselves clearly from those of other species of *Lepilemur*. We have named a new species, *Lepilemur septentrionalis* (sp. nov.) with four subspecies: *L. s. andrafiamenensis* ( $2N = 38$ ), *L. s. ankaranensis* ( $2N = 36$ ), *L. s. sahafarensis* ( $2N = 36$ ), and *L. s. septentrionalis* ( $2N = 34$ ).

The genus *Lepilemur* or sportive lemurs are endemic Malagasy lemurs, common all over the island. The classification, specifically the nomenclature and taxonomy, of *Lepilemur* has been the subject of much controversy and discussion. We made a cytogenetical study of all lepilemurs to try to categorize each one clearly. In this paper, we present the karyotypes of different lepilemurs from the forested areas from the north of Madagascar in Ankarana, Andrafiarena, Montagne d'Ambre, and the forest of Sahafary.

### MATERIALS AND METHODS

We karyotyped 25 lepilemurs (10 males and 15 females) caught during six successive expeditions: three males in 1968, one on the first cliff on Ankarana, one south of Matsaborimanga, and one north of Andrafiabe; two females and one male on the Ankarana near Analamisondrota in 1969; two females and one male on Montagne d'Ambre in 1971; two females at the mouth of the Andrafiarena near Anjakely in 1972; and five males and nine females in the forest of Sahafary in 1972 and 1973.

Chromosome preparations were made, using the leucocyte cultivation method of Lejeune. For the karyotype analysis of

each animal we examined a minimum of ten mitotic metaphase plates in which the chromosomes were neither overlapping nor excessively contracted.

### RESULTS

The lepilemurs in this study were found to have six different karyotypes with five different diploid numbers:  $2N = 38$ ,  $2N = 37$ ,  $2N = 36$ ,  $2N = 35$  and  $2N = 34$  (table 1). A skin sample and a skull of each specimen have been left at the Museum d'Histoire Naturelle de Paris.<sup>1</sup>

The first karyotype,  $2N = 38$ , consists of one pair of medium sized submetacentric chromosomes, 17 pairs of acrocentric chromosomes, and one pair of sex chromosomes in which the X is a medium sized metacentric and the Y a very small acrocentric. The fourth pair of acrocentrics has a satellite on its distal extremities. The acrocentric chromosomes decrease in size regularly from the first to the seventh pair, from the eighth to the 13th, and from the 14th to the 17th (fig. 1).

<sup>1</sup> Numbers of the type specimens of *Lepilemur septentrionalis* in the "Catalogue Général, Magasin de Mammologie et d'Ornithologie" du Museum d'Histoire Naturelle de Paris.:

*L. s. ankaranensis*: 1974 — n° 77

*L. s. sahafarensis*: 1974 — n° 78

*L. s. andrafiamenensis*: 1974 — n° 79

*L. s. septentrionalis*: 1974 — n° 80

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Collection de Référence

n° M 7922 Zool.

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TABLE 1

Chromosome number and types in the *Lepilemur septentrionalis*

Subspecies	Number of specimens examined		Chromosomes				
	Male	Female	2N	S	A	X	Y
<i>L. s. andrafiamenensis</i>	2	2	38	2	34	M	A
<i>L. s. ankaranensis</i>	2	2	36	4	30	M	A
Hybrid <i>L. s. ankaranensis</i> × <i>L. s. andrafiamenensis</i>	1	2	37	3	32	M	A
<i>L. s. sahafarensis</i>	2	1	36	4	30	M	A
<i>L. s. septentrionalis</i>	1	3	34	6	26	M	A
Hybrid <i>L. s. sahafarensis</i> × <i>L. s. septentrionalis</i>	2	5	35	5	28	M	A

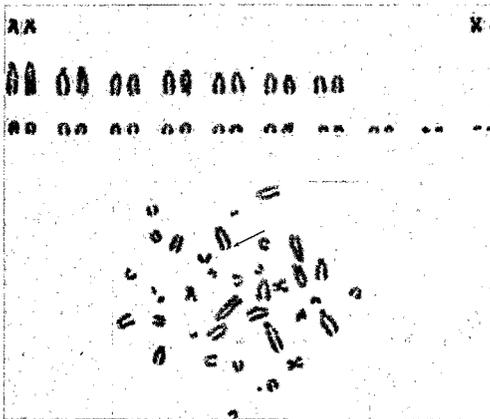


Fig. 1 Karyotype of *Lepilemur s. andrafiamenensis* ( $2N = 38$ ). Note the single pair of small, submetacentric chromosomes and the existence of satellites on the distal extremities of the fourth pair of acrocentric chromosomes (arrow).

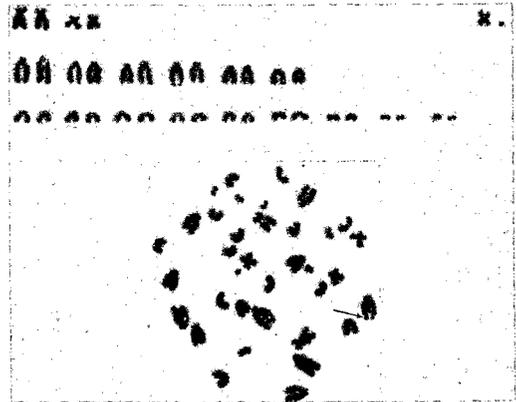


Fig. 2 Karyotype of *Lepilemur s. ankaranensis* ( $2N = 36$ ). Note one pair of supplementary large submetacentric chromosomes and the lack of a pair of large and a pair of small acrocentric chromosomes. The fourth pair of acrocentric chromosomes has satellites on its distal extremities (arrow).

The second karyotype,  $2N = 36$ , consists of two pairs of submetacentric chromosomes, one of which is similar to the aforementioned pair in the first karyotype and the other is larger, 15 pairs of acrocentric chromosomes, and one pair of sex chromosomes in which the X is also a metacentric of medium size. The fourth pair of acrocentrics has a satellite on the distal extremities. The acrocentric chromosomes regularly decrease in size from the first to the sixth pair, from the seventh to the 12th pair, and from the 13th to the 15th pair (fig. 2).

The third karyotype,  $2N = 37$ , consists of three submetacentric chromosomes, 15 pairs of acrocentric chromosomes, two unpaired acrocentric chromosomes, and one pair of sex chromosomes, X and Y, similar

to those existing in the karyotype  $2N = 36$ . Among the submetacentric chromosomes is one pair of chromosomes similar to the only pair which exists in the first karyotype ( $2N = 38$ ) and one isolated chromosome similar to the chromosomes found in the second karyotype ( $2N = 36$ ) (fig. 3). Among the two unpaired acrocentric chromosomes one is a large chromosome similar to the chromosomes in the seventh pair and the other is a small chromosome similar to the chromosomes in the 15th pair that are found in the karyotype  $2N = 38$ .

The fourth karyotype,  $2N = 36$ , contains two pairs of submetacentric chromosomes of medium size: one is similar to the smallest submetacentric pair existing

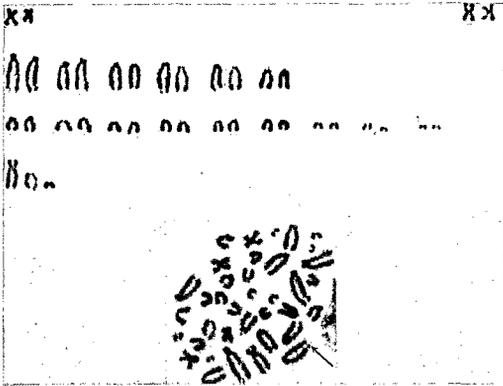


Fig. 3 Karyotype of a natural hybrid,  $2N = 37$ . The satellites are clearly visible on the long arms of the fourth pair of acrocentric chromosomes (arrow). Note one submetacentric and two acrocentric chromosomes each of which is unpaired.

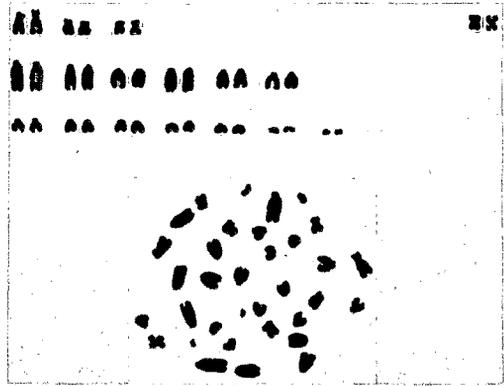


Fig. 5 Karyotype of *L. s. septentrionalis* ( $2N = 34$ ). Note the presence of the pairs of submetacentric chromosomes. Two pairs that are found also in the *L. s. sahafarensis* and one pair of large supplementary submetacentric chromosomes that are found also in the *L. s. ankaranensis*.

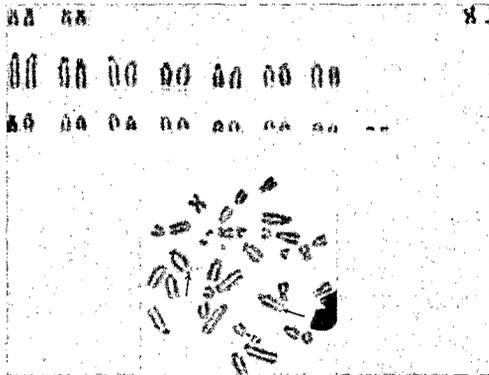


Fig. 4 Karyotype of *Lepilemur s. sahafarensis* ( $2N = 36$ ). Note two pairs of submetacentric chromosomes, the smallest similar to those of *L. s. andraflamenensis* and *L. s. ankaranensis*. The satellites are particularly visible on the long arms of the fourth pair of acrocentric chromosomes (arrow).

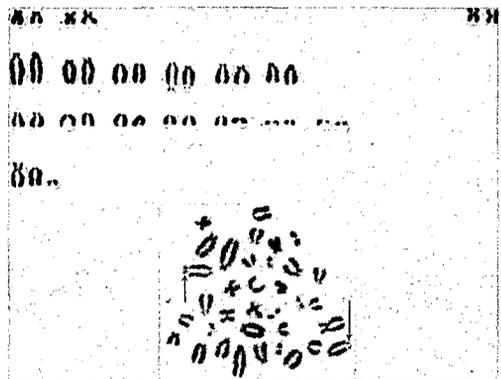


Fig. 6 Karyotype of natural hybrid,  $2N = 35$ . Note the submetacentric and two acrocentric chromosomes, each of which is unpaired. The arrows indicate as previously the satellites of the fourth pair of acrocentric chromosomes.

in the two karyotypes,  $2N = 38$  and  $2N = 36$ , previously described, 15 pairs of acrocentric chromosomes, and one pair of sex chromosomes, X and Y, similar to those described previously. The fourth pair of acrocentrics has a satellite on its distal extremities. The acrocentric chromosomes regularly decrease in size from the first to the seventh pair, from the eighth to the 12th pair, and from the 13th to the 15th pair (fig. 4).

The fifth karyotype,  $2N = 34$ , contains three pairs of submetacentric chromosomes, 13 pairs of acrocentric chromosomes, and

one pair of sex chromosomes, X and Y, similar to those described previously. Among the submetacentric chromosomes are two pairs of medium size similar to those existing in the fourth karyotype,  $2N = 36$ , and a large pair similar to the largest pair of the second karyotype,  $2N = 36$ . The fourth pair of acrocentrics has a satellite on the distal extremities. The acrocentric chromosomes regularly decrease in size from the first to the sixth pair, and from the seventh to the 11th pair, where the 13th pair is more reduced in size and the 14th pair is punctiform (fig. 5).

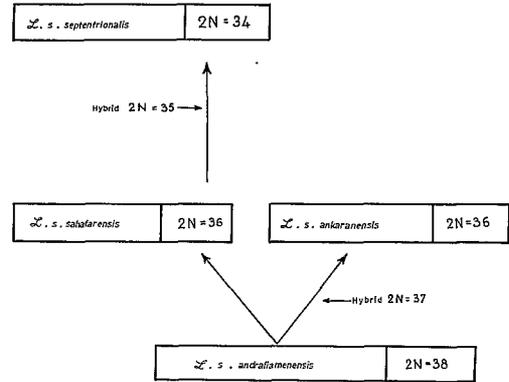
The sixth karyotype,  $2N = 35$ , con-

tains two pairs of submetacentric chromosomes of medium size, one unpaired submetacentric chromosome, 13 pairs of acrocentric chromosomes, two unpaired acrocentric chromosomes, and two sex chromosomes, X and Y, similar to those described previously. Among the unpaired chromosomes, the submetacentric of large size is similar to those of the largest pair of the fifth karyotype. Among the two unpaired acrocentric chromosomes the largest is similar to those of the seventh pair where the smallest is similar to those of the 14th pair of the fifth karyotype (fig. 6)

#### DISCUSSION

The results lead us to consider the systematics of these animals and their evolution.

The existence of these particular karyotypes, different from that of the other lepilemurs (Rumpler et al., '72; Buettner-Janusch et al., '73; Rumpler, in press) led us to put this group of *Lepilemur* into a new species: *Lepilemur septentrionalis*. The latest classification did not mention a new and particular type in this area of Madagascar (Petter and Petter-Rousseaux, '60). This proposition, based purely on the cytogenetical characteristics, was later justified by examination of the skull of these animals. In fact, although *L. septentrionalis* resembles *L. dorsalis* in size, the color of its pelage, and the length of its tail to a great extent, it is clearly distinctive in the shape of its skull which is smaller. We await proof of the fertility or sterility of the hybrids,  $2N = 37$ , but the hybrids  $2N = 35$  are fertile; in our last expedition we caught nine pregnant females of which three had a karyotype  $2N = 35$ . In the absence of noticeable morphological differences between *L. septentrionalis* which possess different karyotypes, it seems reasonable to consider them as subspecies: *L. s. andrafiamenensis* ( $2N = 38$ ), *L. s. ankaranaensis* ( $2N = 36$ ), *L. s. sahafarensis* ( $2N = 36$ ), and *L. s. septentrionalis* ( $2N = 34$ ). This position appears to us justified since *Lemur fulvus collaris* ( $2N = 48$ ) and *Lemur fulvus rufus* ( $2N = 60$ ), in spite of their very dissimilar karyotypes, have given birth to fertile hybrids showing that they at least belong to the same species (Rumpler, in press).



Chromosomal evolution of the *Lepilemur septentrionalis*

Fig. 7 Design of hypothetical course of chromosomal evolution of *Lepilemur septentrionalis*.

The chromosomal evolution of numerous lemurs has been by centric fusion, especially in the Lemurinae. The comparison of the karyotypes with  $2N = 38$ ,  $2N = 36$ , and  $2N = 34$ , where the fundamental number is the same, allows us to envisage the same phenomenon in *Lepilemur*. At the origin of this evolutionary process is the karyotype of *L. s. andrafiamenensis* ( $2N = 38$ ) which evolved in two different ways to give the karyotype of *L. s. ankaranaensis* ( $2N = 36$ ) and *L. s. sahafarensis* ( $2N = 36$ ) (fig. 7). The transformation of the karyotype  $2N = 38$  to the karyotype of *L. s. ankaranaensis* ( $2N = 36$ ) requires the centric fusion of two pairs of acrocentric chromosomes, the seventh and the 15th pairs. This hypothesis is supported by the existence of animals with  $2N = 37$ , natural hybrids of the two former ones. Evolution from the karyotype  $2N = 38$  to the karyotype  $2N = 36$  of *L. s. sahafarensis* requires the centric fusion of two other pairs of acrocentric chromosomes, probably the ninth or tenth pair and the 16th pair (fig. 7).

From the karyotype  $2N = 36$  of *L. s. sahafarensis* karyotype  $2N = 34$  of *L. s. septentrionalis* arose by centric fusion of the same pair of acrocentric chromosomes as that which had arisen between the karyotypes of *L. s. andrafiamenensis* ( $2N = 38$ ) and *L. s. ankaranaensis* ( $2N = 36$ ). The existence of natural hybrids at  $2N = 35$  supports this hypothesis.

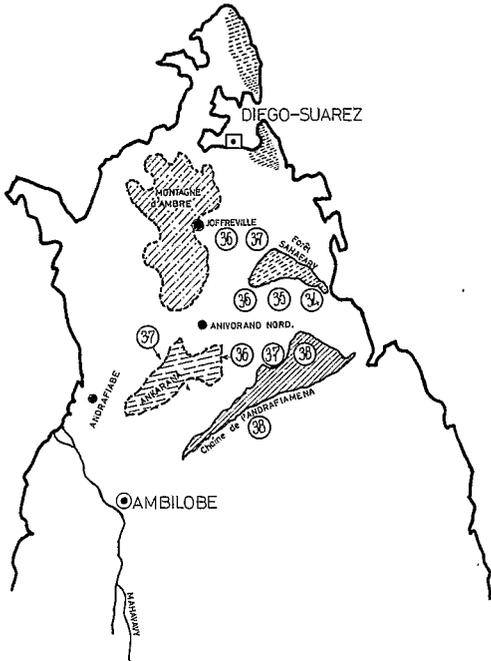


Fig. 8 Map of distribution in the north of Madagascar of the different subspecies of *Lepilemur septentrionalis*. The circled numbers indicate the diploid number of the animals and the place of capture. Blacked areas mean areas with forest.

The absence of notable differences between the four subspecies of *Lepilemur*, as in the African mouse *Leggada* (Mathey, '64), shows that chromosomal transformations can precede morphological changes. The chromosomal polymorphism observed in *L. septentrionalis* and the great number of natural hybrids suggest that this group is still evolving.

The distribution of these animals is shown on the map of the northern part of Madagascar (fig. 8).

#### CONCLUSION

The discovery of a particular karyotype in *lepilemurs* from the north of Madagascar has led us to propose a new species: *L. septentrionalis*. The existence of an important chromosomal polymorphism in *L. septentrionalis* and of numerous natural hybrids between the different populations in the absence of coexisting morphological differences indicate a still evolving group.

#### ACKNOWLEDGMENT

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