## Chromosomal and morphological diversity in 2 populations of Asian mountain vole, *Alticola lemminus* Miller (Rodentia, Cricetidae)

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Summary. Voles from Yakutia and Chukotka were found to differ in the morphology of 3 pairs of autosomes and sex chromosomes, as well as in  $M^3$ -structure and skull dimensions. This makes their conspecificity doubtful. A variation in short arm lengths of the X- and Y-chromosomes has been discovered.

The vole Alticola lemminus inhabits mountain tundra of North-Eastern Asia. Its taxonomic status is not yet clear. Some authors<sup>1,2</sup> consider A. lemminus to be a separate species, while others<sup>3,4</sup> regard it as a subspecies of the widespread A. macrotis. Up to now not much is known about the morphological variability of this vole and nothing about its chromosomes. The purpose of the present paper is to compare karyotypes and some morphological characters of A. lemminus of 2 isolated populations.

Materials and methods. The voles studied were captured in 2 remote localities, the Laptev Sea coast (Yakutia) and the Arctic coast of Chukotka (Chaun inlet). Teeth and skulls were examined in adult specimens only (23 from Yakutia, 17 from Chukotka). 3 animals from Yakutia (13, 299) and 8 from Chukotka (433, 499) were karyotyped. Chromosome preparations of the bone marrow and testes were made by conventional methods. G-bands were induced with the Seabright procedure<sup>5</sup>, for Chukotkan voles only. To diminish the possible effect of various chromosome contraction, the chromosomes to measure on the prints were chosen so that their average degree of contraction would be similar in the samples compared. For the same reason, in some cases relative chromosome sizes were estimated. G-banded chromosomes were measured if they could not be identified without banding.

*Terminology.* Metacentric and submetacentric chromosomes were identified according to Levan et al.<sup>6</sup>. Chromosomes possessing minute second arms were classified as subtelocentrics if the short arms were distinct, however high their degree of contraction. Chromosomes were considered as acrocentrics when their second arms were visible, only if the contraction was not too strong.

Results and discussion. Voles from the above localities exhibited different karyotypes, though the diploid number was the same in both forms (2n = 56). In Yakutian animals, autosomes consist of a pair of small metacentrics and 26 pairs of acrocentrics graded in size (figure 1). Meiosis study has shown that the X- and Y-chromosomes appear to be large acrocentrics (figure 3a). However, their exact identification is impossible without differential staining. No chromosome variability has been observed in Yakutian A. lemminus.

In Chukotkan voles the autosome set includes a pair of small metacentrics, a pair of medium-sized submetacentrics (No. 9), 2 pairs of large subtelocentrics (No. 1 and 5) and 23 pairs of acrocentrics (figure 2a). Their G-banding pat-

Relative size of arms of the  $Y_1$  and  $Y_2^*$ 

	Y <sub>1</sub>	Y <sub>2</sub>	р
Long arm Short arm n	$\begin{array}{c} 0.257 \pm 0.006 \\ 0.195 \pm 0.008 \\ 5 \end{array}$	$\begin{array}{c} 0.250 \pm 0.004 \\ 0.041 \pm 0.002 \\ 20 \end{array}$	> 0.95 < 0.001

\* To get relative sizes, arm lengths were normalized by the combined length of the X and 2 autosomes (pair 2) from the same cell.

terns are shown in figure 2b. The X-chromosome displays 2 morphological forms: a large submetacentric  $X_1$  with arm ratio of 1.6-2.2 and a smaller one  $X_2$  with arm ratio of 2.7-3.0 (figure 4a). One of the females was homozygous for the  $X_1$  and 3 were heterozygous. 3 males possessed the  $X_2$ -chromosome, and one male the  $X_1$ . To determine the chromosome mechanism responsible for this heteromor-



Fig. 1. Karyotype of a male from Yakutia.



b

Fig. 2. Male from Chukotka: karyotype (a) and G-banding (b).

phism, the size and G-bands of X-chromosomes were examined. The long arms of  $X_1$  and  $X_2$  proved identical both in G-banding and size (4.6±0.1 µm and 4.7±0.1 µm), while the lengths of their short arms were different (p < 0.001): 2.2±0.1 µm and 1.5±0.1 µm, respectively. After trypsin treatment, the short arms were faintly stained, with no bands being visible.

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The Y-chromosome of Chukotkan voles is seen to be also heteromorphic, occurring in 2 forms. One of the males had the metacentric  $Y_1$ , and the 3 others had the subtelocentric  $Y_2$  (figure 4b). G-banding patterns of the longer arms turned out to be the same for both  $Y_1$  and  $Y_2$ , their shorter arms being stained similarly to those of the X. The  $Y_1$ length is significantly higher than that of the  $Y_2$ : 7.1±0.4  $\mu$ m and 6.1±0.2  $\mu$ m, respectively (p < 0.05). As seen from the table, this diversity is exclusively due to the difference of short arm lengths.

Thus, similar polymorphism has been discovered for both sex chromosomes of Chukotkan *A. lemminus*. It is a new example of karyotypic orthoselection, one of the major principles of chromosomal evolution<sup>7</sup>. A length and Gbanding patterns constancy of longer arms of sex chromosomes and a variation in their shorter arms sizes are observed. This condition is supposed to be analogous to that seen in some species<sup>8-10</sup> where short heterochromatic arms varying in length have been found. To confirm this, however, C-banded karyotypes should be examined.





Fig. 3. Diakinesis in Yakutian male (a); Chukotkan male (b).

It is evident that there is considerable karyotype diversity between the Yakutian and Chukotkan A. lemminus. They proved to be different in the morphology of 3 pairs of autosomes (No. 1,5 and 9) and sex chromosomes. It seemed of interest to compare the degrees of chromosomal and morphological divergence of these forms. Skull dimensions and the mode of the 3rd upper molar variation were investigated.

Skulls of Yakutian voles are noticeably larger than those of Chukotkan ones. Meanwhile skull proportions were found to be identical in both forms. These data will be reported in detail elsewhere.

The populations compared turned out to differ in the M<sup>3</sup> crown variation; 11 patterns of the M<sup>3</sup> crown structure in Yakutian population and 8 in Chukotkan one were observed (figure 5a, b); not a single pattern is in common. The posterior loop of M<sup>3</sup> of Yakutian animals is elongated, and the 4th salient angles on both labial and lingual sides of the tooth are much smaller than those of Chukotkan voles. In the latter these angles are nearly as large as the first three. The number of dentinal areas on M<sup>3</sup> occlusal surface is one of the most important taxonomic characters in the Alticola group, and the forms studied proved to be quite different in regard to this trait. In Chukotkan animals, the M<sup>3</sup> re-entrant angles are far deeper and narrower than those of Yakutian form. In the former the M<sup>3</sup> occlusal surface is usually divided into 3-6 closed dentinal areas. while that of Yakutian voles displays only 1 or 2, rarely 3 dentinal areas.

Our findings show that *A. lemminus* from Yakutia and Chukotka are appreciably different in karyotypes and morphology. These populations have possibly reached the species level of differentiation. Further, the correlation between degrees of morphological diversity and karyotype divergence was observed. It is consistent with the evidence that in mammalian phylogenesis the rates of anatomical and chromosomal evolution are rather close<sup>11</sup>.



Fig. 4. Polymorphic X (a) and Y (b) chromosomes in voles from Chukotka, G-banding patterns (bottom line) and conventional stain.



Fig. 5.  $M^3$  crown variation patterns in Yakutian (a) and Chukotkan (b) voles.

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