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[1,2] consider *A. lemminus* to be a separate species, while others[3,4] 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 (1♂, 2♀♀) and 8 from Chukotka (4♂♂, 4♀♀) were karyotyped. Chromosome preparations of the bone marrow and testes were made by conventional methods. G-bands were induced with the Seabright procedure[5], 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.[6]. 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 patterns 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-

Relative size of arms of the Y_1 and Y_2*

	Y_1	Y_2	p
Long arm	0.257±0.006	0.250±0.004	>0.95
Short arm	0.195±0.008	0.041±0.002	<0.001
n	5	20	

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

Fig. 1. Karyotype of a male from Yakutia.

a

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.

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 µm and 6.1±0.2 µm, 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[7]. A length and G-banding 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[8-10] where short heterochromatic arms varying in length have been found. To confirm this, however, C-banded karyotypes should be examined.

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^3 crown variation; 11 patterns of the M^3 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^3 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^3 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^3 re-entrant angles are far deeper and narrower than those of Yakutian form. In the former the M^3 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[11].

a

b

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

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

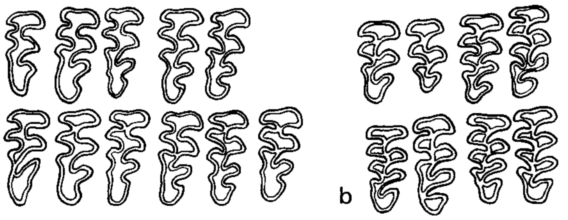

Fig. 5. M^3 crown variation patterns in Yakutian (a) and Chukotkan (b) voles.

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'Boost' in gibberellin response by water-stress in seedling growth[1]

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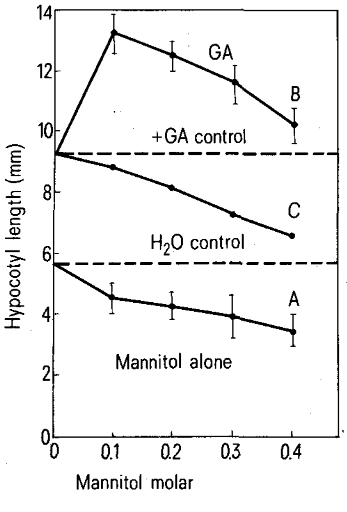
Summary. The seedling growth of *Brassica campestris* var. *varuna,* has been studied, as affected by water-stress and gibberellin treatments. A 'boost' in the net GA response due to water-stress, has been observed. Thus presence of GA can overcome the water-stress effects.

Longitudinal growth caused either by auxin or gibberellin is always associated with increase in water uptake[2]; the same is true for leaf expansion caused by Kinetin[3]. Role of this water uptake has been examined in some detail in auxin-induced growth, by use of respiratory inhibitors[4], and also by providing osmoticum by mannitol, and studying the permeability of the membrane to water[5], and other solutes[6]. The present experiment was designed to study the interaction of osmoticum provided by mannitol and gibberellic acid (GA)-induced growth in *Brassica campestris* seedlings. It has been noticed that, in presence of mannitol and GA together, both the hormone-induced growth and also the stress-induced inhibition are only partially expressed. So to elaborate their role in such an interaction, the following experiment was performed. Basic methods have been described earlier[7].

Germinated seeds of *B. campestris* were given 24 h of the stress treatment using the different concentrations of mannitol (0.1-0.4 M) and from each treatment half the seedlings were transferred to water and the other half transferred to GA (10 mg/l) solution, to study the recovery of the stress effects in water or in GA. Growth measurements were taken 48 h after transfer from mannitol solution, and hypocotyl length is the mean of 20 seedlings. A separate control was run for comparison, which comprised of the first 24 h in water and then half the seedlings to water (water control) and the other half to GA (GA control). Respective mannitol control refers to half the seedlings given stress (0.1-0.4 M) and then transferred to water, as compared to other half transferred to GA.

Hypocotyl length measured after 48 h of recovery is plotted in the figure, where curve A represents the hypocotyl length of seedlings transferred from different mannitol concentrations to water against the water control, thus showing the inhibition caused by the pre-stress treatment. The curve B shows the hypocotyl length of seedlings transferred from different mannitol concentrations to GA, showing a net promotion in GA response, and against GA control, whereas the curve C is plotted as growth of the seedlings transferred from different mannitol concentrations to GA solutions, minus the mannitol alone seedlings, showing that the pre-stress still interfers in GA alone response.

It is clear from the figure that the stress-treatment of the seedlings is reflected in an inhibition even at later periods of the growth (curve A), and also that a stress pre-treatment interferes in the net GA response of a normal seedling (curve C). However, when we plot the growth of the stress-pretreated seedling in GA, a very significant picture arises, since all these points keep values higher than the GA control itself. This shows that the pre-treatment with stress in some way makes the seedlings more responsive to GA, thus a sort of 'boost' is observed in GA response. This boost may also reflect that GA in some way helps in the recovery of the injury caused by mannitol stress. A similar pattern has also been observed in *B. nigra* seedlings.



Showing the hypocotyl growth in length of seedlings of *B. campestris* pretreated with different concentrations of mannitol for 24 h and then transferred to water or GA solution. For explanation of curves see text. Vertical bars show SD. The calculated and table values of significance at 5% levels are 397.589 and 2.10 for treatments, and 1.751 and 2.10 for replications.

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