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Karyotypic relationships of the Tatra vole (*Microtus tatricus*)

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A b s t r a c t . This study reports for the first time the banding pattern on chromosomes of the Tatra vole, *Microtus tatricus*, as revealed by G-, C-, and Ag-NOR staining procedures. The banded karyotype of *M. tatricus* was compared with *Microtus (Terricola) subterraneus, M. (Stenocranius) gregalis,* and *M. (Blanfordimys) aghanus*. The karyotype of *M. tatricus* possesses highly derived features, e.g., the low diploid number of chromosomes or unique combinations of arms in the biarmed autosomes. It is almost impossible to find clear relationships of *M. tatricus* with other extant vole species from the point of view of comparative karyology. The karyotypic changes in voles are apparently not accompanied by adequate divergence in morphological and genetic traits.

Key words: chromosomes, banding pattern, phylogeny, *M. subterraneus, M. gregalis, M. afghanus*

Introduction

The Tatra vole, *Microtus tatricus* (Kratochvíl, 1952) is the only mammalian species endemic to the Carpathian Mountains. The small distribution range of the species extends over Carpathian mountain ranges in Slovakia, Poland, Ukraine, and Romania (M i t c h e l l - J o n e s et al. 1999, M a r t í n k o v á & D u d i c h 2003). The populations of the Tatra vole are distributed in a patchy pattern in often isolated mountain ranges, and their densities in specific habitats are usually very low (M a r t í n k o v á & D u d i c h 2003). The phylogenetic relationships of this species are rather poorly known. K r a t o c h v í l (1970) provided a re-description and systematic revision of the Tatra vole, and he concluded that this is an endemic Carpathian species with possible relationships to certain species of the subgenus *Terricola* inhabiting the Alps, particularly to *M. multiplex* and *M. bavaricus*. This suggestion found a confirmation in phylogenies derived from mitochondrial genes sequences (H a r i n g et al. 2001, J a a r o l a et al. 2004). However, the molecular data also showed that the species' relationships in the western Palaearctic subgenus *Terricola* are poorly resolved, and rapid radiation involving nearly simultaneous diversification of many lineages was indicated in this group (J a a r o l a et al. 2004).

The first report on the karyotype of the Tatra vole (M a t t h e y 1964, K r á l 1972) showed that the diploid number of chromosomes and the basic karyotype characteristics (2N=32, NF=46) are unique among the European pine voles of the subgenus *Terricola*, as well as among the other voles of the genus *Microtus*. The same karyotype was recorded in

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various geographic populations of the Tatra vole, and no distinct chromosomal variation within the species was revealed (Zagorodnyuk & Zima 1992).

This study reports for the first time the banding pattern on chromosomes of the Tatra vole. Our aim was to use the results of the detailed cytogenetic analysis to look for similarities with other species that could indicate possible phylogenetic relationships.

Material and Methods

The specimens of the Tatra vole were collected in the High Tatra Mts in Slovakia in June and September 2000. Two females originated from the Veľká Studená Valley and a single male from Lake Tretie Roháčské pleso. The capture of these animals was allowed by the permission of the Ministry of Environment of the Slovak Republic no. 3287/1137/1999 issued on January 27, 2000. For comparative purposes, we used previously published data on banded chromosomes of *Microtus (Terricola) subterraneus* from Slovakia (Zima 1984), and new data derived from original chromosomal preparations of *M. (Stenocranius) gregalis* from Siberia, and *M. (Blanfordimys) afghanus* from the Kopetdag Mts in Turkmenistan.

The direct treatment of bone marrow cells was used for karyotype analysis, followed by flame-drying of slides. The slides were conventionally stained by Giemsa or G- and C-banded following the procedures by Seabright (1971) and Sumner (1972). The Ag-NOR staining was performed according to the modified method of Howell & Black (1980). The specimens examined and the microscopic slides analyzed are deposited in the collections of the Institute of Vertebrate Biology AS CR (Brno).

Results and Discussion

The karyotype of the Tatra voles examined was the same (2N=32, NF=46) as reported for this species previously (Fig. 1). C-banding revealed rather low content of C-heterochromatin. The C-positively stained segments were observed in centromeric regions of several autosomes and the X chromosome. The Y chromosome also stained positively but its dark staining was not as intense as in the centromeric regions (Fig. 2). The small amount of C-heterochromatin in *M. tatricus* corresponds with the similar state reported in *M. subterraneus* (Gamperl et al. 1982), *M. duodecimcostatus* (Burgos et al. 1991), *M. majori* (Macholán et al. 2001) or *M. sikkimensis* (Mekada et al. 2002). On the other hand, distinctly higher amount of C-heterochromatin was described in the centromeric regions of chromosomes of *M. savii* (Galleni et al. 1992) and *M. multiplex* (Brunet-Lecomte & Volobouev 1994), a species that is grouped with the Tatra vole by molecular analysis (Haring et al. 2001, Jaarola et al. 2004).

The Ag-NOR positive staining was observed in the telomeric regions of two large biarmed autosomal pairs of *M. tatricus*. This number of NOR sites found is lower than reported in most other vole species. Zagorodnyuk (1992) reviewed available data on the distribution of NORs in the karyotypes of voles, and concluded that the NOR sites occurred in 5–10 chromosomal pairs in 11 species examined. The NOR sites in two or three autosomal pairs were only observed in *M. oeconomus*, *M. montebelli*, and *M. montanus*.

G-banding revealed the distinct pattern in individual chromosomal pairs (Fig. 3). The comparison of banded chromosomes between *M. tatricus* and *M. subterraneus* (2N=52) showed an extensive homology between large segments represented by individual chromosomal arms. Most of the chromosomal arms from the karyotype of *M. subterraneus*

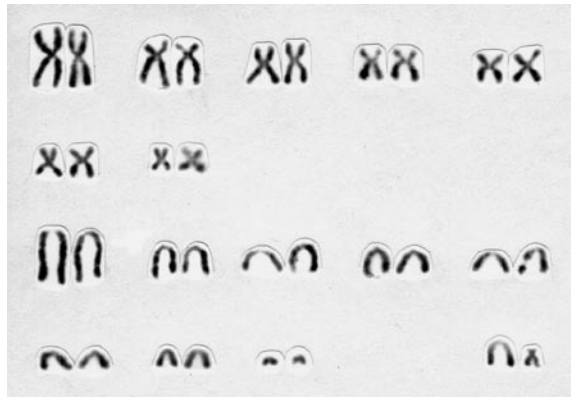

Fig. 1. Conventionally stained karyotype of *Microtus tatricus*.

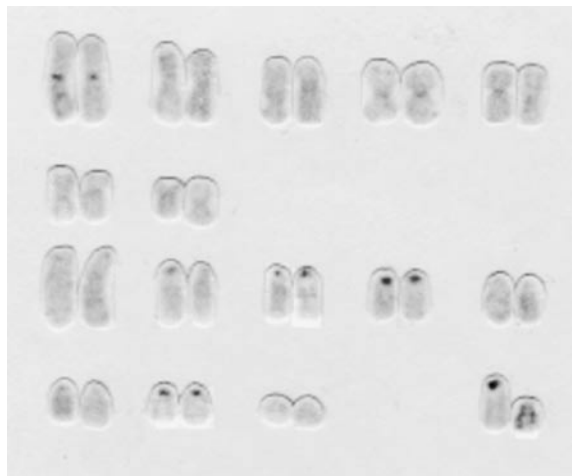

Fig. 2. C-banded karyotype of *M. tatricus*.

have their homologues in the complement of *M. tatricus*. The karyotypes of both the species can be derived from each other through a series of autosomal centric and tandem fusions and, possibly, centromeric shifts. The shorter arm of the large submetacentric autosome of *M. subterraneus* is apparently involved in a different autosomal fusion in *M. tatricus*, whereas the longer arm of this autosome of *M. subterraneus* appears as a free acrocentric autosome in *M. tatricus*. The largest metacentric autosome of *M. tatricus* could be derived after two successive fusions of acrocentric autosomes of *M. subterraneus*. A pericentric inversion could explain the difference in the centromeric position between the largest acrocentric autosome of *M. tatricus* and the largest subtelocentric autosome of *M. subterraneus*. No homology was identified in the *M. tatricus* karyotype for the small metacentric autosome of *M. subterraneus* (Fig. 4).

The karyotype of *M. gregalis* with 36 chromosomes resembles that of *M. tatricus* in the low diploid number and in the similar ratio between the uniarmed and the biarmed autosomes. However, the G-banding pattern showed that the five large biarmed autosomes from the karyotype of *M. gregalis* have a different combination of presumably fused arms compared to the five large biarmed autosomes of *M. tatricus* (Fig. 5). The largest

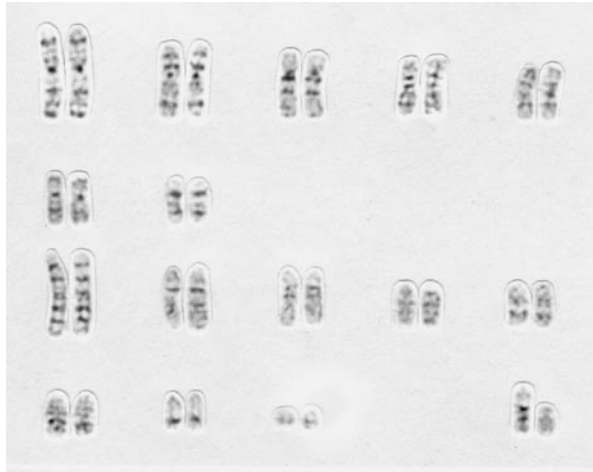

Fig. 3. G-banded karyotype of *M. tatricus*.

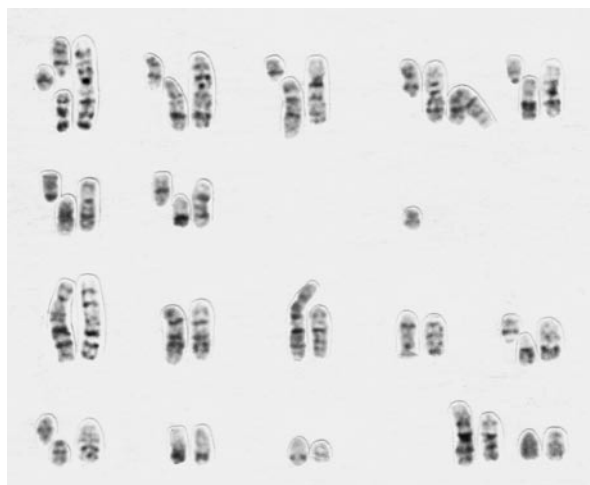

Fig. 4. Comparison of G-banded karyotypes of *Microtus subterraneus* (left) and *M. tatricus* (right)

subtelocentric autosome of *M. gregalis* is partly identical with the largest acrocentric autosome of *M. tatricus*. The difference between these autosomes of both species can be explained by a pericentric inversion and addition of a new segment to the autosome of *M. gregalis*. Other autosomal arms also revealed an extensive homology between the karyotypes of *M. gregalis* and *M. tatricus*. However, the observed structural differences in the arm composition of the metacentrics in both the species seem to exclude close karyotypic relationships between them.

An extensive homology between chromosomal segments of various vole species was apparent also in the comparison with *M. afghanus*. *M. afghanus* belongs to *Allophaiomys*-like species group which members are considered old Pleistocene relicts (N a d a c h o w s k i & Z a g o r o d n y u k 1996). The somatic cells of this species have 54 chromosomes and this diploid number can be considered a primitive feature within the genus *Microtus*, because it is occurring in various distantly related species, and even in certain other genera of arvicolid rodents (Z i m a & K r á l 1984, for review). The G-banded chromosomes of *M. afghanus*

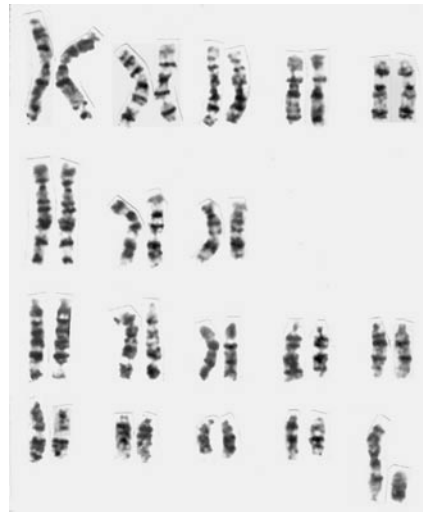

Fig. 5. G-banded karyotype of *Microtus gregalis*.

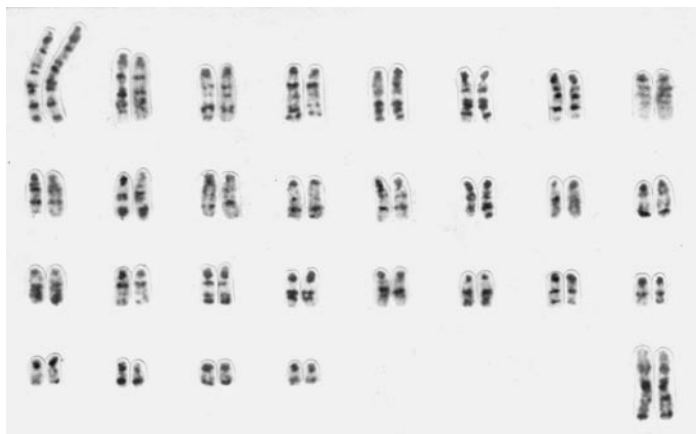

Fig. 6. G-banded karyotype of *Microtus afghanus*.

(Fig. 6) are often quite similar to individual chromosomal arms from the karyotypes of other species examined in this study, even though a detailed assessment of homology seems speculative in respect of the resolution level of banding achieved.

Our results show that chromosomal homology can be extensive even between the vole species belonging to distant phylogenetic lineages. On the other hand, homology between chromosomes and large segments can sometimes be followed only with difficulties even between related species, and the attempts to find such homologies completely failed in some cases (e.g. *M. maximowiczi*, M e y e r et al. 1996). This indicates that chromosomal evolution in microtines may be characteristic by highly variable rates of karyotypic change in individual phylogenetic lineages. Furthermore, it is obvious that karyotypic changes were not usually accompanied by adequate and comparable divergence in morphological and genetic traits. The Tatra vole represents a derived lineage within evolution of the karyotype of the microtine voles, and it is difficult to find its clear relationships with other extant species.

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