Hystrix It. J. Mamm. (n.s.) 18 (1) (2007): 99-109

DISTRIBUTION AND GEOGRAPHIC LIMITS OF THE ALTERNATIVE CYTOTYPES OF TWO *MICROTUS* VOLES IN EUROPEAN RUSSIA

NINA BULATOVA[1], FEDOR GOLENISHCHEV[2], NATALIA BYSTRAKOVA[3], SVETLANA PAVLOVA[1], JULIA KOVAL'SKAYA[1], IGOR TIKHONOV[1], MARINA DIDORCHUK[4], KIRILL IVANOV[2]

[1]Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow 119071, Russia; e-mail: admin@sevin.ru
[2]Zoological Institute, Russian Academy of Sciences, St-Petersburg 199034, Russia
e-mail: microtus@zin.ru
[3]Penza State Pedagogical University, Penza 440602, Russia
e-mail: ermakov@penza.com.ru
[4]Oka State Biosphere Reserve, Lakash 391072, Ryazan region, Russia

Received 21 December 2006; accepted 24 May 2007

ABSTRACT - Chromosome data on the distributional limits of *Microtus* voles (*M. subterraneus*, *M. arvalis*) in the very east of Europe are presented. Wide border space reported formerly between the two *subterraneus* cytotypes (2n=54, 2n=52) as well as between the common vole cytotypes (*arvalis* and *obscurus,* both 2n=46) have been narrowed with the study progress, especially in the case of the common vole. For the first time, hybrid karyotypes were observed in a local sample, providing evidence of natural hybridization in the *arvalis – obscurus* contact zone.

Key words: karyotypes, contact zones, *Microtus arvalis*, *M. subterraneus*, European Russia

RIASSUNTO **– *Distribuzione e limiti geografici dei citotipi di due specie di* Microtus *nella Russia europea.*** I limiti dell'area di distribuzione dei due citotipi di *M. subterraneus* (2n=54, 2n=52) e di *M. arvalis* (*arvalis* e *obscurus,* entrambi con 2n=46) in Europa orientale sono stati aggiornati tramite analisi cromosomica, evidenziando, particolarmente per *M. arvalis*, una netta riduzione della distanza intercorrente tra i margini dei rispettivi areali. Per la prima volta, nella zona di contatto, è stata accertata l'ibridazione tra i citotipi *arvalis* e *obscurus*.

Parole chiave: cariotipi, zona di contatto, *Microtus arvalis*, *M. subterraneus*, Russia europea

INTRODUCTION

Since Meyer *et al*. (1972), the voles of the genus *Microtus* Schrank, 1798 have been a basic group for cytotaxonomic studies within the fauna of Russia and adjacent countries (Meyer *et al*., 1996). Recently, some vole species have become the object of extensive molecular studies with a particular emphasis on phylogenetic and phylogeographic aspects (Jaarola and Searle, 2002; Haynes *et al.*, 2003; Jaarola *et al.*, 2004). It is important, in this respect, to draw the distributional ranges of intraspecific forms, both molecular and cytogenetic ones, in order to obtain sound information on species recolonization histories.

In Eastern Europe, there are two species of voles - the pine vole *M. (Terricola) subterraneus* Pallas, 1773 and the common vole *Microtus (Microtus) arvalis* Pallas, 1778 -, which are of great interest for comparative cytogenetic and geographic studies due to their differentiation into two or more cytotypes each.

In European Russia, the common vole occurs in two main karyotypic forms differing in the centromere position of both six small autosome pairs and a larger autosome pair (No. 5), as well as in the size of the male Y-chromosome. The diploid number is the same (2n=46) in all populations, though the fundamental number (FN, or chromosome arms number) varies between 66 and 84 (Král *et al.*, 1980). After a series of more and more refined studies (Radjabli and Graphodatsky, 1977; Meyer *et al.*, 1985 and 1996; Mazurok *et al.*, 2001), this variation, which has been attributed to pericentric inversions, seems to be due to centric transposition and results in metacentric / acrocentric alterations in chromosome homologs. The two cytotypes, initially considered as “northern” and “southern” forms of a same species (Meyer *et al.,* 1972a), were assigned to as many subspecific forms, respectively “*arvalis*” and “*obscurus*” (Malygin, 1974), with the obvious tendency to debate two independent species. So far, however, there has not been any sort of consensus on whether these chromosomal forms are separate species (Mitchell-Jones *et al.,* 1999) or not (Meyer *et al.*, 1996).

In central European Russia, the two vicarious cytotypes get into contact. The overlap area is oriented longitudinally, extending for more than one thousand kilometers in length and hundreds of kilometers in width (Král and L’apunova, 1975; Král *et al.,* 1980; Bashenina and Sokolov, 1994). Special searches for the contact zone of the two forms were started by Meyer *et al.* (1997). Populations with mixed karyotypes were first discovered in 2000 (Golenischev *et al.,* 2001). In the pine vole the opposite karyological situation is observed. Two cytotypes differing in the diploid number, 2n=54 and 2n=52, reveal the same fundamental number (FN=60). A single Robertsonian alteration can explain this variation. So far, in European Russia the two cytotypes have been reported for only one site each (Baskevich *et al.*, 2000). The aim of this paper is to provide new data about the geographical distribution of the alternative cytotypes of both *Microtus* species in Eastern Europe.

MATERIALS AND METHODS

The area included between 50°46′ - 59°30′ N and 33°30′ - 48°56′ E was examined, considering voles from 16 different localities (Tab. 1). Chromosome preparations of pine voles were obtained from specimens occasionally captured in 2 sites (no. 2, and 4; Fig. 1) where the chromosome races of common shrew *Sorex araneus* have been studied in the last decade (Bulatova *et al.,* 2000; Bystrakova *et al.,* 2003) and, also, in an intermediate site (no. 3). Common voles from a total of 13 sites were analysed. Data from 8 localities already published in two preliminary reports in Russian (Bystrakova, 2003; Bystrakova *et al*., 2005) are included along with other 4 findings never reported before. Moreover, some data from previous studies on *M. subterraneus* and *M. arvalis* were also considered (Sablina *et al*., 1989; Baskevich, 1997; Baskevich *et al*., 2005). Standard techniques for chromosome analysis of small mammals, including routine and C-band staining (Graphodatsky and Radjabli ,1988) were applied.

RESULTS

The geographic limits of the alternative cytotypes of both pine voles and common voles are shown in Figure 1. At the north eastern and eastern limits of the species range, one specimen of *M. subterraneus* with 2n=54 and two specimens with 2n=52 were found. They all belong to the right bank of the Great

Table 1 - Current findings of alternative karyotypic forms of *Microtus* in European Russia. (*= approximate coordinates; no: see Fig. 1; N: number of speciements).

no	Geographic origin	Coordinates	Karyotype	N	References
		M. subterraneus: 2n=54*(I)*, 52*(II)*			
1	*St. Petersburg vic.	59°30’ N/33°50’ E	I	2	Sablina *et al*., 1989
2	Tver region, Lake Seliger	56°42′ N/33°30′ E	I	2	This paper
3	Ryazan region, Shatsk	53°50′ N/41°30′ E	II	1	This paper
4	Penza region, Zemetchino	53°20′ N/42°40′ E	II	1	This paper
5	Voronezh region, *Voronezh vic.	51°50′ N/39°20′ E	II	1	Baskevich, 1997
		M. arvalis: 2n=46			
6	Vladimir region, Kovrov vic.	56°20′ N/41°25′ E	NFa=80 (*arvalis*) NFa=74, 78 (*arvalis*× *obscurus)*	1 2	This paper This paper
7	Ryazan region, Spassk	54°39′ N/40°45′ E	NFa=80 (*arvalis)*	5	Bystrakova, 2003
8	Moscow region, Zaraisk	54°47′ N/38°42′ E	NFa=80 (*arvalis)*	2	This paper
9	Lipetsk region, Donskoye	52°37′ N/38°59′ E	NFa=80 (*arvalis)*	4	Bystrakova, 2003
10	Kirov region, Sovetsk	57°31′ N/48°56′ E	NFa=68 (*obscurus)*	3	Bystrakova, 2003
11	N. Novgorod region, Gorodets	56°33′ N/43°53′ E	NFa=68 (*obscurus)*	1	Bystrakova, 2003
4	Penza region, Zemetchino	53°20′ N/42°40′ E	NFa=68 (*obscurus)*	4	Bystrakova, 2003
12	Tambov region, Izberdei	52° 35′N/40°25′ E	NFa=68 (*obscurus)*	1	This paper
5	Voronezh region, *Voronezh vic.	51°50′ N/39°20′ E	NFa=68 (*obscurus)*	17	Baskevich *et al*., 2005
13	Voronezh region, Bobrov	51°14′ N/40°12′ E	NFa=68 (*obscurus)*	2	Bystrakova *et al*., 2005
14	Voronezh region, Liski	51°00′ N/39°18′ E	NFa=68 (*obscurus)*	2	Bystrakova *et al*., 2005
15	Voronezh region, Kamensk	50°46′ N/39°47′ E	NFa=68 (*obscurus)*	1	Bystrakova *et al*., 2005
16	Belgorod region, Novy Oskol	51°00′ N/38°00′ E	NFa=68 (*obscurus)*	1	This paper

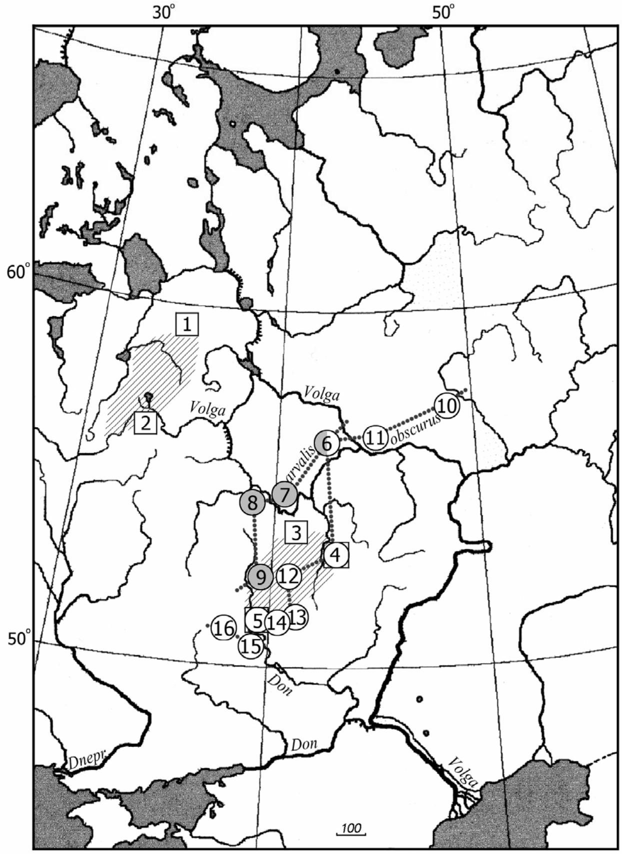

Figure 1 - Distribution limits of pine vole (2n=54, 2n=52) and common vole (*arvalis* and *obscurus*) cytotypes in European Russia. The numbers in squares (pine voles) and circles (common voles) correspond to site numbers in Table 1. Shades focus on the distribution limits of 54-chromosome (sites 1-2) and 52-chromosome (sites 3-5) pine voles. Current borders of each common vole cytotype are outlined by dotted lines connecting the corresponding finding sites (in grey for *arvalis* and in white for *obscurus*).

Russian River Volga, the first coming from a site of the Upper Volga (Tver region) and the other two from as many localities from the Middle Volga (Ryazan and Penza regions).

Considering former data, in European Russia overall 2 sites for the 54-chromosome karyotype and 3 sites for the 52-chromosome karyotype have been located (Fig. 1; Tab. 1).

In the 54-chromosome variant, the largest subtelocentric pair and a very small metacentric pair represent a group of bi-armed autosomes. The X-chromosome is a medium metacentric, whilst the other members of the chromosome complement are all acrocentric. In the 52-chromosome karyotype, an extra metacentric autosome of medium size occurs. Its presence, together with the reduced 2n value, is considered as the result of a single rearrangement - namely, a centric fusion of two acrocentrics of the original 54-chromosome. By means of C-banding, the Y-chromosome appears as a fully heterochromatic element among numerous acrocentrics with poor centromeric heterochromatin and shows a similar size (exceeding the length of any arm of the metacentric X-chromosome) in males of both cytotypes (Fig. 2).

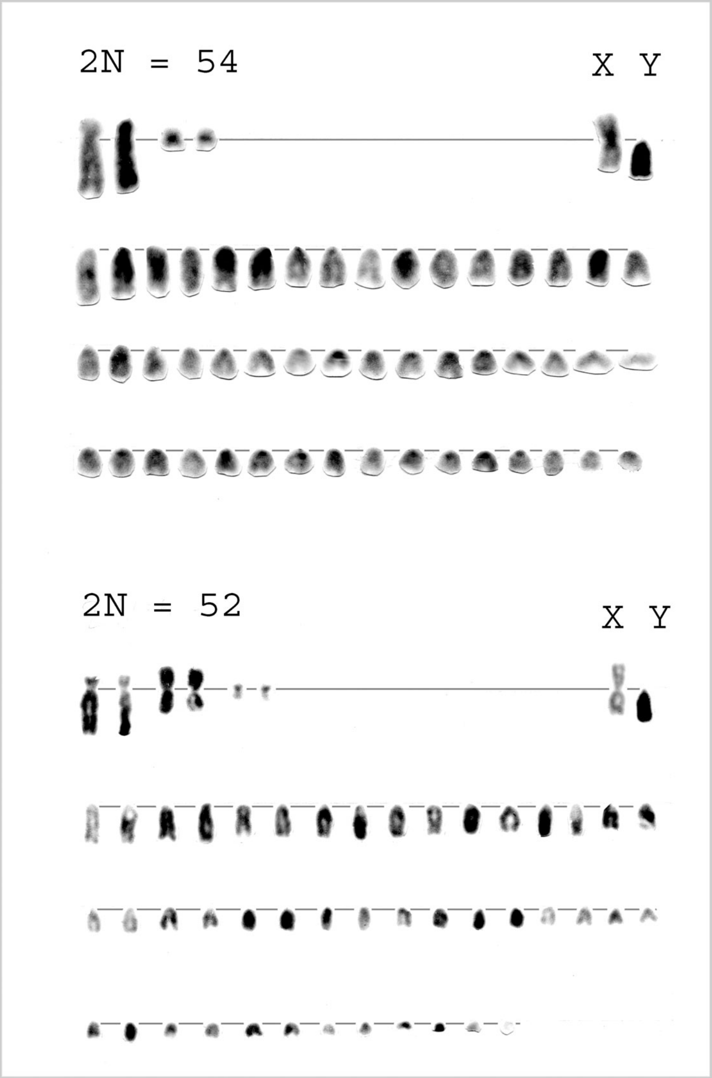

Figure 2 - C-banded karyograms of pine voles. Top: Cytotype 2n=54 (male, Tver region, site 2). Bottom: Cytotype 2n=52 (male, Penza region, site 4).

The cytotype *arvalis* was defined in 12 common voles from 4 new sites, which move eastwards the formerly known limit in this part of the country (Fig. 1). The chromosome complements never contain more than 4 pairs of small acrocentrics, whilst the Y chromosome is very small and C-neutral following heterochromatin staining (Fig. 3-A).

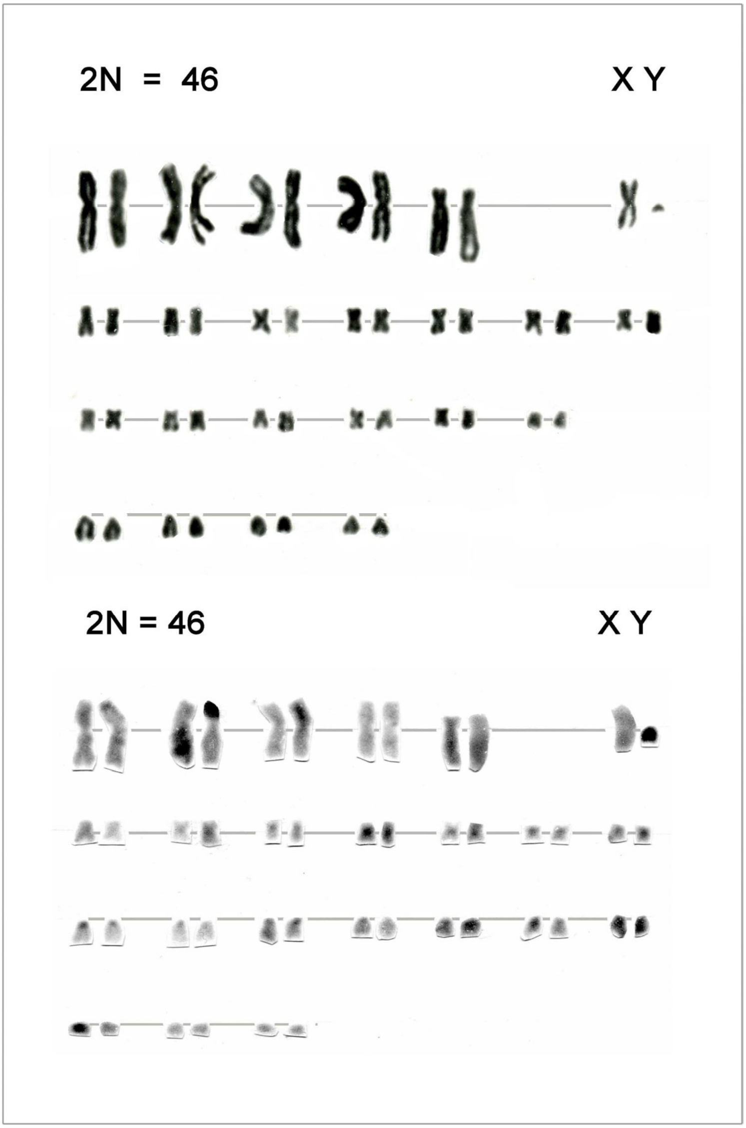

Figure 3 - Typical karyograms of common voles from the Eastern European boundary of their range. Top: *arvalis* (male, Lipetsk region, site 9, routine staining). Bottom: *obscurus* (male, Belgorod region, site 16, C-banding).

The boundary of the cytotype *obscurus* ran from north-east (57°31′ N / 48°56′ E) to south-west (50°46′ N / 39°47′ E). According to former data (Meyer *et al.,* 1997 and 1999; Golenishchev *et al.,* 2001), the area lying near Kovrov, in Vladimir region (56°20′ N / 41°25′ E), should be included into the range of both *arvalis* and *obscurus* (Fig. 1). In the karyotypes of common voles from 10 different localities, small acrocentrics predominate over metacentrics (10 vs. 7). The Y chromosome is bigger than that of *arvalis* and C-positive after staining (Fig. 3 *B*).

Specimens captured in the same field in the vicinity of Kovrov were detected by karyotypic analysis as pure *arvalis* (1 female), F1 hybrid *arvalis* × *obscurus* (1 female), and backcross (1 male) (Tab. 1). The karyotype constitution of the F1 hybrid female - 20 metacentrics and 14 acrocentrics (Fig. 4, top) – clearly derives from the haploid numbers typical for pure cytotypes – respectively, 13/4 in *arvalis* and 7/10 in *obscurus*. The backcross male vole shows a big Y-chromosome (*obscurus* type) and, contemporarily, a proportion of small elements (24/10) roughly reflecting those of cytotype *arvalis* (26/8) or F1 (20/14) rather than that of *obscurus* (14/20) (Fig. 4, bottom).

DISCUSSION

New chromosome data add substantial information on the Eastern European limits of the karyotypic forms of both vole species, showing that no gap could exist between the distribution areas of common vole cytotypes, as already suggested by the early findings of Meyer *et al.* (1997). The monitoring of this contact zone during the last decade has revealed its vulnerability and dependence on human activities. The decline of agriculture in the region could make fields overgrow, becoming more suitable for root voles rather than for common voles (Golenishchev, unpublished data).

The occurrence of the cytotype *arvalis* on the left bank of the Upper Don (site 9), together with the presence of the cytotype *obscurus* far westwards from the right bank of the Middle Don (site 16), let us infer that main rivers do not act as geographic barriers for the two alternative forms of common voles.

Apart from the two variants from Kovrov which were interpreted as hybrids, the karyotypes of *arvalis* and *obscurus* looked invariant, even though a certain degree of variability has been reported for both cytotypes (Král *et al.*, 1980; Bashenina and Sokolov, 1994; Meyer *et al.*, 1996). The inversion in the pair No. 5 of *obscurus* (subtelocentric vs. acrocentric), which is widespread from Transcaucasia to West Siberia and Middle Volga (Radjabli and Graphodatsky, 1977; Akhverdian *et al.,* 1999; Bystrakova, 2003), remarkably was never observed in our samples. It must be noticed that the chromosome markers of the cytotype *arvalis* are distributed only in the eastern part of the European range of *M. arvalis*. As Meyer *et al.* (1996) summarized, the populations of European Russia and those from the eastern part of Germany show the same chromosome features, whereas, further westwards, the karyotypes under the name *arvalis* may differ in some autosome features and in the morphology of the Y sex-chromosome. Particularly, the Y-chromosome of *M. a. arvalis* (Austria) and *M. a. asturianus* (Spain) is about as large as that of the easterly *obscurus* cytotype (Gamperl, 1982; Gamperl *et al.*, 1982). Curiously, the proportion of the diagnostic metacentrics/acrocentrics ratio in *M. a. asturianus* (Gamperl, 1982) is very similar to that of our F1 hybrid, though only numerically, before G-bands identification. These observations suggest that the sound karyotype identification of common

Figure 4 - Karyotypes from the *arvalis*/*obscurus* contact zone (Kovrov, site 6). Top: F1 hybrid (female). Bottom: interpretation of F1 × *arvalis* backcross (male).

voles from the opposite extremes of the species ranges needs the support of comparative molecular research to confirm it.

Available information about pine voles karyotypes remains rather scanty, requiring further studies. The range of the so-called “northern” karyotypic form of *M. subterraneus* (2n=54) coincides with the area where disputable subspecies were repeatedly described, particularly *M. (T.) s. transvolgensis* Schaposchnicov et Schanev, 1958 (Gromov and Erbaeva, 1995). It is worthy of note that no chromosomal variation has been referred to this karyotype of the pine vole (for details see Sablina *et al*., 1989).

The findings of the alternative cytotype (2n=52) refer to the very eastern extreme of the species range in the Middle Volga. On the whole, our findings of both cytotypes reduce the distance between their distribution areas from about 1000 km (sites 1, 5) to approximately 600 km (sites 2-4). The right bank of the Upper Volga (about 55ºN) could be proposed as the boundary between the two Eastern European chromosome forms of *M. subterraneus*.

In contrast to the conservative cytotype 2n=54, at least two modes of variation exist in the 52-chromosome variant, concerning the size of the heterochromatic Y-chromosome and the uni-armed / bi-armed condition of the largest autosome (Sablina *et al.,* 1989). In our material, this polymorphic pair looks uniformly subtelocentric. The large Y-chromosome from Ryazan region (site 3) is comparable with that described for the Austrian Alps (Gamperl *et al*., 1982). In contrast, voles with smaller Y-chromosome occur in the middle part of the European range, including Czech Tatras, Ukrainian Carpathians in the west and Voronezh region (Russia, Upper Don) in the east (Baskevich *et al*., 2000).

Based on molecular data, *M. arvalis* is splitting into clear mt-DNA lineages, which occur in western, central and eastern Europe, correspondingly (Haynes *et al*., 2003). Similarly, the geographical partition of *M. subterraneus* cytotypes should reflect diverged mt-DNA lineages. For both species comparative studies on the opposite edges of their European range are of primary interest.

ACKNOWLEDGEMENTS

The final portion of the study was supported by the Russian Fund of Basic Research (RFBR grants 04-04-48412, 07-04-00102) and the program Biodiversity of the Russian Academy of Sciences. This paper is dedicated to the memory of Marina N. Meyer and Sevil I. Radjabli.

REFERENCES

Bashenina N.N. and Sokolov V.E. 1994. The Common Vole. Nauka Publ. House, Moscow, 432 pp. [In Russian].

Baskevich M. 1997. A comparative analysis of structural features of spermatozoa and karyotypes in three species of shrub voles: *Terricola majori, T. daghestanicus and T. subterraneus* (Rodentia, Cricetidae) from the former USSR. *Zool. Zhurnal*, 76: 597-607. [In Russian, with English summary].

Baskevich M.I., Kozlovskii A.I. and Mitev D.B. 2000. New data on chromosome variability in the pine vole *Terricola subterraneus* (Rodentia, Cricetidae). *Zool. Zhurnal,* 79: 1355-1360. [In Russian, with English summary].

Baskevich M., Okulova N., Sapel'nikov S., Balakirev A., Ryabina S. and Malygin V. 2005. Cytogenetic and electrophoretic differentiation of the common vole, *Microtus arvalis* sensu lato, at the Voronezhskii State Reserve and adjacent territories of Central Chernozem region. *Zool. Zhurnal*, 84(10): 1298-1309. [In Russian, with English summary].

Bulatova N., Searle J.B., Bystrakova N., Nadjafova R., Shchipanov N. and Orlov V. 2000. The diversity of chromosome races in *Sorex araneus* from European Russia. *Acta Theriol.*, 45 (Suppl. 1): 33-46.

Bystrakova N., Bulatova N., Koval'skaya Y., Shchipanov N., Kalinin A., Nadjafova R. and Searle J.B. 2003. Geographical limits of chromosome races of common shrew *Sorex araneus* L. in the Middle Volga (East European Russia). *Mammalia*, 67(2): 187-191.

Bystrakova N.V. 2003. The geographical ranges of chromosomal sibling species of common voles (Rodentia, Cricetidae, *Microtus*) in Middle Volga Region. *Theriological Investigations*. Issue III. Zoological Institute RAS, St. Petersburg: 94-104. [In Russian, with English summary].

Bystrakova N., Ermakov O. and Titov S. 2005. Chromosome routes by Middle Don. *Vestnik VOGIS (Novosibirsk),* 9 (1): 67-69. [In Russian].

Gamperl R. 1982. Die Chromosomen von *Microtus arvalis* (Rodentia, Microtinae). *Z. Säugetierkunde,* 47: 356-363.

Gamperl R., Ehmann Ch. and Backmann K. 1982. Genome size and heterochromatin variation in rodents. *Genetica*, 58: 199-212.

Golenischev F.N., Meyer M.N. and Bulatova N.Sh. 2001. The hybrid zone between two karyomorphs of *Microtus arvalis* (Rodentia, Arvicolidae). *Proc. of the Zoological Institute RAS*, 289: 89-94.

Graphodatsky A.S., Radjabli S.I. 1988. Chromosomes of farming and laboratory mammals. Nauka Publ. House Siberian Branch, Novosibirsk: 14-16.

Gromov I.M. and Erbaeva M.A. 1995. The Mammals of Russia and adjacent territories. Lagomorphs and Rodents. Rus. Acad. Sci., Zool. Inst., St.-Petersburg: 472-473.

Haynes S., Jaarola M. and Searle J.B. 2003. Phylogeography of the common vole (*Microtus arvalis*) with particular emphasis on the colonization of the Orkney archipelago. *Molecular Ecology*, 12: 951 – 956.

Jaarola M., Martínková N., Gündüz İ., Brunhoff C., Zima J., Nadachowski A., Amori G., Bulatova N., Chondropoulos B., Fraguedakis-Tsolis S., González-Esteban J., Lopez-Fuster M. J., Kandaurov A., Kefelioğlu H., da Luz Mathias M., Villate I. and Searle J. B. 2004. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution (MPEV)*, 33: 647-663.

Jaarola M. and Searle J.B. 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. *Molecular Ecology*, 11: 2613 – 2621.

Král B., Bel'anin A., Zima J., Malygin V., Gajcenko V. and Orlov V. 1980. Distribution of *Microtus arvalis* and *M. epiroticus*. *Acta Sc. Nat. Brno*, 14 (9): 1-31.

Král B. and L'apunova E. 1975. Karyotypes of 46-chromosome *Microtus arvalis* (Microtidae, Rodentia). *Zool. Listy*, 24: 1-11.

Malygin V.M. 1974. A comparative morphometric analysis of karyotipes in two geographical forms of 46-chromosome common vole, *Microtus arvalis*. *Zool. Zhurnal,* 53: 769-778. [In Russian, with English summary].

Mazurok N., Rubtsova N., Isaenko A., Pavlova M., Slobodyanyuk S., Nesterova T. and Zakian S. 2001. Comparative chromosome and mitochondrial DNA analyses and phylogenetic relationships within common voles (*Microtus*, Arvicolidae). *Chromosome Research,* 9: 107 – 120.

Meyer M.N., Orlov V.N. and Scholl E.D. 1972. Sibling species in the group *Microtus arvalis* (Rodentia, Cricetidae). *Zool. Zhurnal,* 51: 724-737. [In Russian, with English summary].

Meyer M.N., Golenishchev F.N. and Bulatova N.Sh. 1999. Peculiarities of geographic distribution of two karyomorphs of *Microtus arvalis* Pallas, 1779 (Rodentia, Arvicolinae) in European Russia. *Proc. Zool. Inst. RAS,* St. Petersburg, 281: 33-38.

Meyer M.N., Golenishchev F.N., Bulatova N.Sh. and Artobolevsky G.V. 1997. On distribution of two *Microtus arvalis* chromosomal forms in European Russia. *Zool. Zhurnal,* 76 (4): 487-493. [In Russian, with English summary].

Meyer M.N., Golenishchev F.N., Radjabli S.I. and Sablina O.V. 1996. Voles (subgenus *Microtus* Schrank) of Russia and adjacent territories. *Proc. Zool. Institute,* St. Petersburg, 232: 319. [In Russian].

Meyer M.N., Radjabli S.I., Bulatova N.Sh. and Golenishchev F.N. 1985. Karyological peculiarities and probable relations of common voles of the group "arvalis" (Rodentia, Cricetidae, *Microtus*). *Zool. Zhurnal*, 64(3): 417-428. [In Russian, with English summary].

Mitchell-Jones A.J., Amori G., Bogdanowicz W., Krystufek B., Reijnders P.J.H., Spitzenberger F., Stubbe M., Thissen J.B.M., Vohralik V. and Zima J. 1999. The Atlas of European Mammals. Academic Press, T. & A.D.Poyser Ltd., London, 484 pp.

Pavlova S., Bystrakova N., Kowalskaya Y., Golenishchev F. and Bulatova N. 2006. The boundaries of some chromosome forms of small mammals in European Russia. In: Prigioni C, Nieder L. and Colli L. (eds), Proc. 10th Rodents & Spatium. The International conference on rodent biology. *Hystrix It. J. Mamm.* (n.s.) supp. (2006): 102.

Radjabli S.I. and Graphodatsky A.S. 1977. Karyotype evolution in mammals (chromosome rearrangements and heterochromatin). In: Khvostova V.V. (ed.) The cytogenetics of hybrids, mutations and karyotype evolution. Chapter 13. Nauka Publ. House Siberian Branch, Novosibirsk: 231-248. [In Russian].

Sablina O.V., Zima J., Radjabli S.I., Kryštufek B. and Goleniščev F.N. 1989. New data on karyotype variation in the pine vole, *Pitymys subterraneus* (Rodentia, Arvicolidae). *Věst. čs. Společ. zool.*, 53: 295-299.