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Cytogenetic recognition of the common wood mouse, *Sylvaemus sylvaticus* s. l. (Mammalia: Rodentia: Muridae), in European Russia

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Abstract. A male mouse singled out from the sample of 10 wood mice trapped in Kursk Province (Central European Russia) has been studied by means of karyological analysis. This mouse was allotted by a presence of distinguished chest spot of outstretched shape. There was another one specimen with the distinct chest spot of the same shape, while all the rest born very small and hardly discerned chest spot or didn't bear it at all. The diploid number and general chromosomes morphology of this individual (2n=48, all are acrocentrics) are common to all representatives of the genus *Sylvaemus* Ognev, 1924. Prominent block of constitutive heterochromatin is concentrated mainly in telomeric regions of several small and medium-sized autosomes. The centromeric heterochromatin is observed only in the largest autosome No.1 and X-chromosome. Y-chromosome is entirely heterochromatic small acrocentric. The nucleolar organizers (AgNOR-staining) are distributed in centromeric or telomeric regions of several autosomes. The wood mice with similar karyotype peculiarities were already reported from the spacious European territory between Austria in the west, Bulgaria in the south-east and Ukraine and the neighboring Russian region (Bryansk Prov.) in the east. New data proved that Kursk Prov. is the second of known regions where the supposed superspecies *Sylvaemus sylvaticus* (Linnaeus, 1758) (Orlov et al., 1996) occurs in European Russia. At the moment it is the easternmost point of the *S. sylvaticus* s. l. distributional range.

Key words: wood mouse, *Sylvaemus sylvaticus*, cytotaxonomy, C-banding, Europe, Russia.

Introduction

Since the last decade of XX century genus *Sylvaemus* Ognev, 1924 (considered initially as a subgenus of the genus *Apodemus* Kaup, 1829) from Eastern Europe and Caucasus has been substantially revised after carrying out allozyme analysis (Vorontsov et al., 1992) along with the new karyological studies (Kozlovsky et al., 1990). It caused not only the increasing of a number of described taxa within this subgenus, but also affected the interpretation of the species borders and relationships. Taking into account all available genetical, karyological as well as molecular data, each of these newly established taxa can be considered as an independent species or "semispecies" in frameworks of the superspecies concept. As it was assumed by Orlov et al. (1996a, b), the total number of such "taxa" is at least 8 and may be assigned to four different superspecies – *S. flavicollis* (Melchior, 1834) s.l., *S. sylvaticus* (Linnaeus, 1758) s. l., *S. fulvipectus* (Ognev,

1924) and *S. uralensis* (Pallas, 1811) s. l. In these publications the name "vohlynensis" has been applied to a local group of populations within the superspecies *S. sylvaticus*. It was initially used as a name for classical morphological subspecies *Sylvaemus sylvaticus vohlynensis* Migulin, 1938 from western Ukraine. Afterwards, it was erected to the species rank (Orlov et al., 1996) due to heterochromatin peculiarities which distinguish it from the other species of the same superspecies, *S. sylvaticus* s. str. Mice with similar heterochromatin pattern were described from some regions of Central and South Europe – Austria, Czech Republic, Bulgaria, Ukraine and neighboring Bryansk Prov. of Russia (Gamperl et al., 1982; Nadjafova et al., 1993; Orlov et al., 1996a, b; Nová et al., 2002). The data presented in this paper allow us to expand the geographical range of *S. vohlynensis* from Dnepr basin by western boundary farther to the center of Russia.

Material and methods

For chromosome analysis, one male of the wood mouse was caught in Kurchatov Distr. (Kursk Prov., 51°39'N/35°36'E) in the summer of 1996. Chromosome preparations were made in accordance with standard procedure developed purposely for cytogenetical studies of wood mice in the laboratory of Microevolution of mammals, A.N. Severtsov Institute of the Russian Academy of Sciences (Kozlovsky et al., 1990; Nadjafova et al., 1993). Unfortunately, we failed to obtain satisfactory G-banding necessary for chromosomes identification, but it was possible to define sex chromosomes and locate the heterochromatin and NOR positions which proved to be very important markers in cytotaxonomy of European wood mice.

Results

Karyotype of the studied male demonstrated the characteristics usual for all species of the genus *Sylvaemus*: 2n=48, all chromosomes are acrocentrics gradually descending in size. No chromosome specific markers were revealed before applying the differential staining. C-banding showed certain differences in the constitutive heterochromatin distribution over chromosomes.

As it can be seen on Fig. 1, centromeric heterochromatin is absent in almost all acrocentrics with only a few exceptions. A small C-block is located in pericentromeric area of the largest autosome pair. More distinguishable centomeric heterochromatin block is present in the X-chromosome. At the same time, there are prominent heterochromatic blocks located in the distal regions of at least 6 pairs of medium-sized and small autosomes. Also there is one entirely heterochromatic chromosome which might be identified as Y-chromosome.

NORs are located in centromeric or telomeric areas of several autosomes which can not be identified without G-banding.

The studied male bore chest spot of outstretched and slightly curved shape. The body length was 91.5 mm, tail length – 91.0 mm, foot length – 20.2 mm, ear length – 16.1 mm. All other specimens captured at the same location were mostly juvenile and demonstrated very close morphometric parameters. 8 mice (5 males and 3 females) had very small and hardly discerned chest spot or did not bear it at all.

Preliminary information considering this finding was published in the abstracts of the All-Russian Theriological Conference (Nadjafova, Bulatova, 2000), but without the publication of a karyogram and some other details.

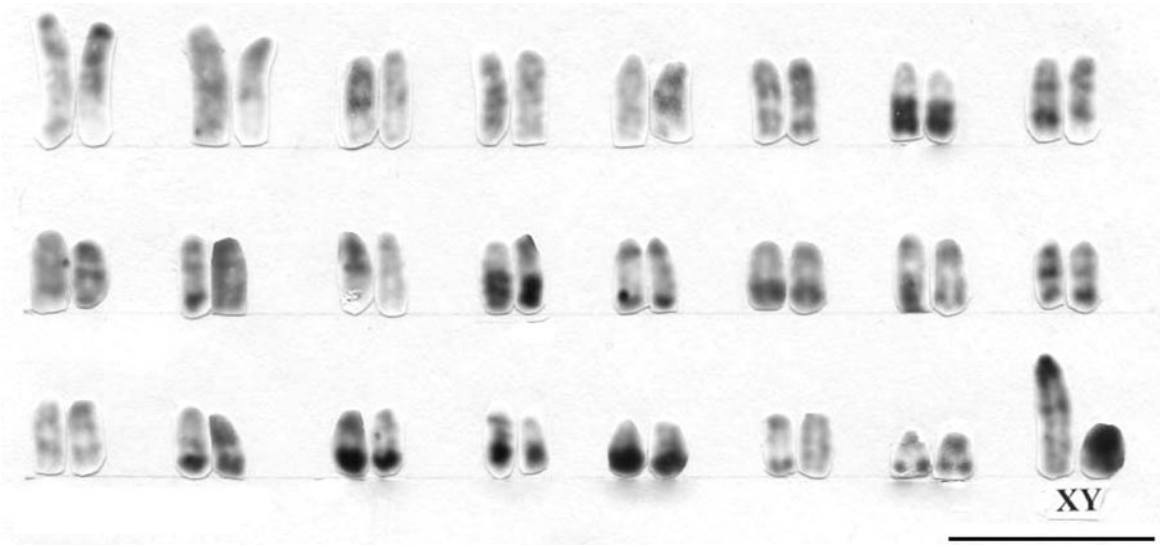

Fig. 1. C-banded karyogram of the male wood mouse from Kurchatov Distr., Kursk Prov. (Central European Russia). XY – sex chromosomes. Bar = 10 μm.

Discussion

Karyotype. Contrasted heterochromatin localization has been repeatedly reported for two most common and sympatric European wood mice – *S. sylvaticus* and *S. flavicollis*. Exclusively C-positive heterochromatic centromeric blocks mark the wholly acrocentric karyotype of the large yellow-necked wood mouse (S. flavicollis), while smaller and morphologically distinct species *S. sylvaticus* bears telomeric heterochromatin in the karyotype as well. Such striking differences in heterochromatin localization have been initially registered in *S. sylvaticus* / *S. flavicollis* comparative studies from western parts of their widely overlapping ranges, namely, from Freiburg, Germany (Engel et al., 1973) and Graz, Austria (Gamperl et al., 1982). Similar peculiarities have been registered in wood mice from Bulgaria, which is in concordance with the species diagnosis based on the presence (*S. sylvaticus*) or absence (*S. flavicollis*) of prominent telomeric C-blocks in chromosomes (Nadjafova et al., 1993).

Molecular study (filter and *in situ* hybridization of DNA) conducted on the chromosomes of these two species demonstrated similar genome features. It was concluded that telomeric heterochromatin of *S. sylvaticus* most likely evolved by transportation and amplification of centromeric satellite DNA elements after these species were separated (Hirning et al., 1989). The synopsis of all available data has led us to the preliminary conclusion about the polytypicism within *S. sylvaticus*: specimens from one site (Freiburg) born centromeric and telomeric C-bands and were attributed to the cytotype "sylvaticus-E1", while the karyologocally studied mice from another localities revealed no considerable centromeric C-blocks (cytotype "sylvaticus-E2") (Nadjafova et al., 1993).

Cytogenetical data have shown rather continuous distribution of wood mice with both cytotypes E1 and E2 marked by the presence of prominent telomeric blocks on territories of several countries from Germany and Austria in the west through Bulgaria in the south-east

Table 1. The geographical list of *Sylvaemus sylvaticus* s. l. karyological examination (* provisional geographical coordinates are map defined; ** the semispecies names are cited accordingly to Orlov et al., 1996a, b).

No.	Locality	Geographical Coordinates*	Cytotype	Taxon**	Data source
1	Germany, Freiburg	47°00'N/7°30'E	sylvaticus-E1	*S. sylvaticus*	Engel et al., 1973
2	Czech Republic, Libéchov	50°25'N/14°26'E	sylvaticus-E1	*S. sylvaticus*	Nová et al., 2002
3	Austria, Graz	47°00'N/15°30'E	sylvaticus-E2	*S. vohlynensis*	Gamperl et al., 1982
4	Bulgaria, Thrace lowland	42°20'N/24°30'E	sylvaticus-E2	*S. vohlynensis*	Nadjafova et al., 1993
5	Bulgaria, Stara Planina	42°32'N/23°00'E	sylvaticus-E2	*S. vohlynensis*	Nadjafova et al., 1993
6	Ukraine, Kiev vicinity	51°00'N/30°40'E	sylvaticus-E2	*S. vohlynensis*	Orlov et al., 1996 a, b
7	Russia, Bryansk Prov.	52°30'N/32°00'E	sylvaticus-E2	*S. vohlynensis*	Orlov et al., 1996 a, b
8	Russia, Kursk Prov.	51°39'N/35°36'E	sylvaticus-E2	*S. vohlynensis*	Present paper

and up to Ukraine and neighboring Russia in the east (Table 1). Mice with centromeric/telomeric heterochromatin distribution and X-chromosome with characteristic large proximal heterochromatin block were reported recently from South Bohemia, Czech Republic (Nová et al., 2002). Basing on the data of the present study, we may move the outmost eastern border of this taxonomical complex farther into Russia till the point in Kursk region with the geographical coordinates specified as 51º39´N/35º36´E. Thus, chromosome analysis proves the presence of Linnean wood mouse (*S. sylvaticus* s.l.) in Russia in spite of certain systematic confusion taken place after the taxonomic revision of East-European wood mice based on allozyme analysis (Mezhzherin, Zykov, 1991; Mezzherin, Lashkova, 1992). As a consequence, the separation of *S. uralensis* had virtually broke the old system and led almost to the full exclusion of *S. sylvaticus* from national systematic lists (Pavlinov, Rossolimo, 1998). For wood mice with cytotype "sylvaticus-E2", Orlov et al. (1996) proposed the species rank with the only valid name *S. vohlynensis*. Data obtained from Kursk Prov. showed that the outmost eastern points for both species and superspecies coincide. This finding well corresponds to the view developed on the base of complex allozyme and morphometry data (Mezhzherin, Lashkova, 1992) and means that the probable distributional range of the common wood mouse should be extended farther on from Ukraine to Bryansk and certainly to Kursk region of Russia. Predictions made by the above cited authors regarding neighboring regions in the south (Belgorod) and east (Lipetsk) are to be examined.

Separation of two cytotypes within the former unified Linnean wood mouse may serve as an evidence of major phylogeographic subdivision probably connected to the different postglacial history of populations occupied southern and northern Europe. To be certain of this supposition, adequate molecular (mtDNA and nuclear DNA) studies are required. Like all others well investigated zoological objects such as grasshoppers, hedgehogs or bears (Hewitt, 2000), wood mice with cytotype "sylvaticus-E2" could definitely contribute to one of the South European refugial areas. Molecular study of the phylogeographic structure in two European sympatric species indicates Iberian Peninsula as the most probable start of recolonization routes for *S. sylvaticus* and the Italo-Balkan area for *S. flavicollis* (Michaux et al., 2005). If the presence of mice with another cytotype, "sylvaticus-E1" is proved for northern Europe, then the paradigm of common postglacial colonization from southern Europe might be broken. The idea that these two cytotypes had survived separately outside (E2) and within the glaciations zone (E1) seems to be more correct. A possibility of survival in some ice free isolates within the borders of the last ice sheet has been recently supposed for chromosome races of another large mammalian species, the common shrew (*Sorex araneus* Linnaeus, 1758), in northern Russia (Orlov et al., 2007).

Morphology. The wood and yellow-necked mice look very similar, but the last ones are bigger and in about one and a half times heavily. As a rule, they have a bright yellow chest spot either in a collar or round shape. Wood mice also reported to have a chest spot, but in smaller size and never collar-shaped, more often it has outstretched shape. Distinct karyotype characteristics might serve a guide in taxonomical recognition if measurements and other morphological data are scored for the specimens and samples involved in karyological study. Unfortunately, in the case of wood mice there are poor sources of such combined data. Morphological measurements of karyologically studied specimens of *S. flavicollis* from Bulgaria showed the length for head-to-body, foot and ear within limits 91.0-115.0 mm, 22.0-24.5 mm and 15.4-19.0 mm, respectively. Tails were damaged too often to be taken into account (Nadjafova et al., 1993). The majority of wood mice with cytotype "sylvaticus-E2" from Bulgaria (Nadjafova et al., 1993), Bryansk Prov. (Orlov et al., 1996a, b) and Kurchatov (present study) had outstretched chest spot (27 specimens out of total 29). The body length does not exceed 113 mm, the tail is shorter than body, the foot length is within 19-24.3 mm, the ear length varies between 13.5 and 18.0 mm. The specimen described in this paper can be placed in the middle of the range.

Unfortunately, there are no data on morphology of wood mice with "sylvaticus-E1" cytotype in the corresponding publications cited above.

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