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Chromosome polymorphism in Bulgarian populations of the striped field mouse (*Apodemus agrarius* Pallas 1771)

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Abstract. Chromosome polymorphism in Bulgarian populations of the striped field mouse (*Apodemus agrarius* Pallas, 1771) has been described. The diploid chromosome number is 2n=48 (NFa = 54). In the karyotype of 3 specimens from the Iskar region, the presence of an additional B chromosome has been established for the first time. The autosomes are 19 acrocentric pairs, continuously decreasing in size, and 4 pairs of be-armed chromosomes, barely distinguishable by size and location of the centromere. Specimens with 3 pairs of metacentric chromosomes were firstly described in Bulgaria for the regions of Iskar and Omurtag. The localization of heterochromatin in the centromeric regions of the chromosomes, blocks of heterochromatin of different sizes, as well as intercalated bands, distinguishable in weakly spiraled chromosomes are found. Telomeric heterochromatin is present in the largest autosomal pair and in two of the middle–sized autosomal pairs. The largest and smallest pairs of be-armed chromosomes do not have centromeric heterochromatin, whereas all the other autosomal pairs do. The presence of a NOR in 6 chromosomal pairs is established. Two of the pairs exhibited pericentromeric NORs, whereas the other 4 displayed telomeric NORs. The karyotype analysis illustrates the chromosome and genome polymorphism of *A. agrarius* in Bulgarian populations.

Key words: *Apodemus agrarius*, karyotype, chromosomal polymorphism, B chromosomes.

Introduction

The field mouse *Apodemus agrarius* (Pallas, 1771) is widely spread in the temperate zone of Eurasia (Corbet, 1978). The Palearctic range of *A. agrarius* consists of two isolated tracts of land: European–Siberian and Far Eastern-Chinese (Karaseva et al., 1992; Panteleyev, 1998; Gliwicz, Kryštufek, 1999). Wide steppe areas separate these two regions. The species is not found in Western Europe and has an unstable and dynamic western border of its European distribution (Kratochvil, 1976), reaching to the northern part of the Balaton lake and Eastern Austria, according to the latest studies (Spitzenberger, 1997).

The southern border of the European area of the field mouse passes through Bulgaria. It is a relatively rare species in Bulgaria, with sparse populations that have a mosaic spread.

The karyotype of *A. agrarius* in Eastern Europe was firstly described by Matthey (1936) – 2n=48, NF=56, and was later characterized for populations in the former Republic of Yugoslavia (2n=48), with a description of the morphology of the chromosomes as follows: 40 acrocentric, of gradually decreasing

size, and 8 small to averagely - sized metacentric (Kral, 1970, 1972; Soldatović et al., 1971, 1975; Gamperl et al., 1982). A chromosomal form with 6 metacentric autosomes (NF=54) was firstly described for populations in Bosnia (Soldatović et al., 1971), Moldova, Romania (Raicu et al., 1972), and Hungary (Zima, Kral, 1984). In populations found in the European parts of Turkey (Yigit et al., 2000), Azerbaijan (Kuliev, Nadjafova, 1986), and China (Tsuchiya, 1979), a chromosomal form with 10 metacentric chromosomes (NF=58) has been established. According to Kartavtseva (2002), the decrease in number of the small metacentric chromosomes (from 8 to 6) is linked to a pericentric inversion, whereas the rise to 10 metracentrics is probably due to a duplication of the centromeric chromatin of the acrocentric chromosomes, as a result of which additional arms are formed. The literature on the geographic distribution of the 3 established chromosomal forms (cytotypes) shows that the form with 4 pairs of metacentric autosomes is more widely spread in Europe, whereas the one with 3 pairs of metacentric autosomes is found in Hungary and Southeastern Europe.

For the European populations of the field mouse, variability in the localization and amount of C-bands has been established. The constitutive heterochromatin is mainly located in the centromeric regions. Interstitial C-blocks in some autosomal pairs also are described. The Y chromosome is made up entirely of heterochromatin (Gamperl et al., 1982; Vujošević et al., 1984; Lungeanu et al., 1986), whereas the heterochromatin in the X chromosome is pericentromeric. For populations of the field mouse in Estonia, Russia, and the Ukraine (Boeskorov et al., 1995), the localization of the nuclear organizer is in 8 chromosomes: telomeric in 4 large acrocentric autosomes and pericentromeric in 4 small ones.

The aim of the present study is to characterize and evaluate the chromosome polymorphism in populations of the striped field mouse in Bulgaria.

Material and methods

67 specimens (35 male and 32 female) of the Bulgarian field mouse were studied in 9 different locations (Fig. 1). Preparations of chromosomes from bone marrow cells were made using a conventional technique (Rothfels, Siminovitch, 1958), and differential G-, C-, and NOR-staining was performed via standard methods (Sumner, 1972; Pellicciari et al., 1990; Bulatova et al., 1991; Seabright, 1971).

Results

The established diploid chromosome number of *A. agrarius* in Bulgaria is 2n=48 (NFa = 54). The autosomes are 19 acrocentric pairs, continuously decreasing in size and 4 pairs of two-armed chromosomes, barely distinguishable by size and location of the centromere (Fig. 2). In Bulgaria, the karyotype of the field mouse with 3 pairs of metacentric chromosomes was first described for the regions of Iskar (8 specimens) and Omurtag (3 specimens). For 3 specimens from the Iskar region, the presence of an additional B chromosome was established for the first time (Fig. 3).

For specimens from the regions of Rupite (7 specimens), Plovdiv (8 specimens), Gramatikovo (6 specimens), and Simeonovgrad (5 specimens), the first autosomal pair is heteromorphic: one of the homologues has clearly distinguishable short arms and may be classified as subtelocentric (ST), whereas the other homologue is acrocentric. This is clearly distinguishable with differential G-staining (Fig. 4). The first autosomal pair is homologous in field mice from the regions of Botevgrad (4 specimens) and Ivanovo (7 specimens) – both homologues are subtelocentric, whereas, for

Fig. 1. Localities of the cytogenetic studied populations of *Apodemus agrarius* in Bulgaria. (Black square - individuals with 4 metacentrics; asterisk - individuals with 3 metacentrics).

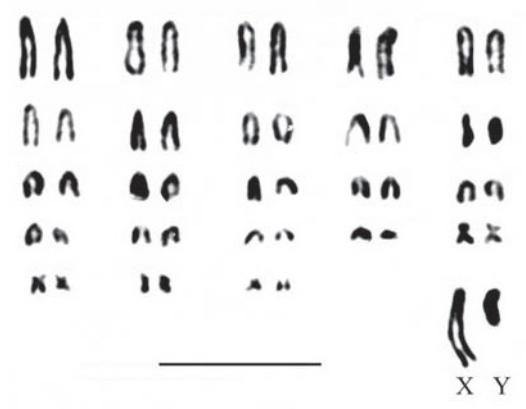

Fig. 2. Male karyotype of the striped field mouse (*A. agrarius*) from Plovdiv region. Bar = 10µm.

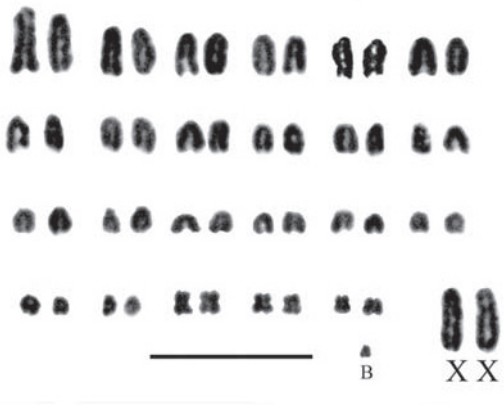

Fig. 3. Female karyotype of the striped field mouse (*A. agrarius*) from Iskar region (Pleven district). Bar =10µm.

specimens from Sinemorets (Strandzha) (19 specimens), the two homologues are areocentric (7 specimens). Therefore, the field mouse from Bulgaria can be characterized by variability in the morphology of the chromosomes in the first autosomal pair, the heteromorphic chromosomal pair, made up of a subtelocentric and an acrocentric, being predominant.

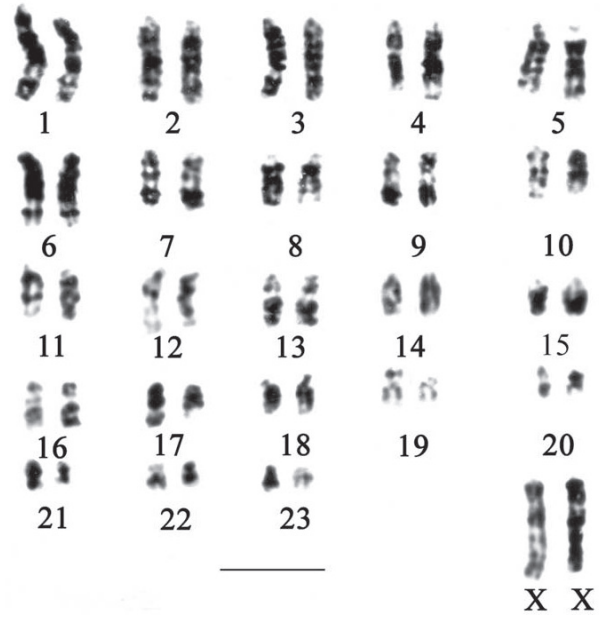

Fig. 4. Female G-banded karyotype of the striped field mouse (*A. agrarius*) from Plovdiv region. Bar =10μm.

Fig. 5. Male C-banded karyotype of the striped field mouse (*A. agrarius*) from Plovdiv region. Bar =10μm.

In a study of the localization of heterochromatin (C-banding) in the centromeric regions of the chromosomes, blocks of heterochromatin of different sizes, as well as intercalated bands, distinguishable in weakly spiraled chromosomes, are found (Fig. 5). The short arms of the subtelocentric chromosomes always have clear blocks of heterochromatin. The largest blocks of heterochromain and distinct intercalated bands are found in the first autosomal pair. The block of heterochromatin of the subtelocentric homologue in the heteromorphic pair is twice as big as that of the acrocentric one (Fig. 5). For specimens from Sinemorets (7 specimens), the largest autosomal pair is homologous and is made up of two acrocentrics, whose pericentromeric blocks of heterochromatin do not differ in size (Fig. 6). Intercalated bands of heterochromain are easily distinguished in chromosomes of the first and third autosomal pairs. Telomeric heterochromatin is present in the largest autosomal pair and in two of the middle-sized autosomal pairs. The largest and smallest pairs of biarmed chromosomes do not have centromeric heterochromatin, whereas all the other autosomal pairs do.

Analysis of the distribution of heterochromatin blocks in the metacentric chromosomes showed that the largest pair of metacentrics has a weak diffused staining along the entire length of the chromosomes and a barely visible C-staining in the pericentromeric region. The second largest metacentric pair has a clear C-block in the centromeric region. In the third metacentric pair, pericentromeric C-blocks are strongly expressed. The smallest pair of metacentric chromosomes is variable in the spread of heterochromatin. In the groups from Sinemorets, Gramatikovo, and Rupite, it is C-negative – there is no localization of heterochromatin, whereas in specimens from Plovdiv, Botevgrad, and Ivanovo, it is C-positive. For specimens from Simeonovgrad, the smallest pair of metacentrics is heteromorphic, regarding the distribution of heterochromatin (+/-) – one chromosome has a pericentromeric block of heterochromatin, whereas the other one does not. The additional B chromosome

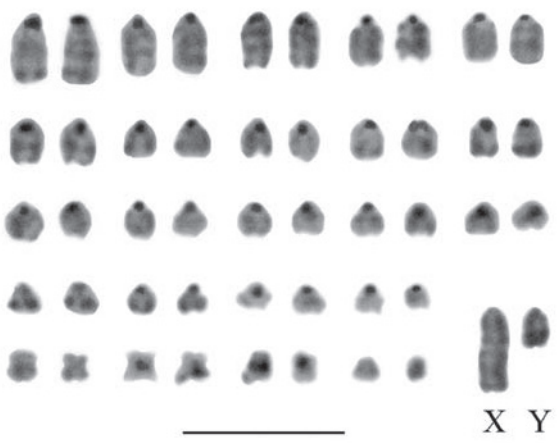

Fig. 6. Male C-banded karyotype of the striped field mouse (*A. agrarius*) from Sinemorets region. Bar =10μm.

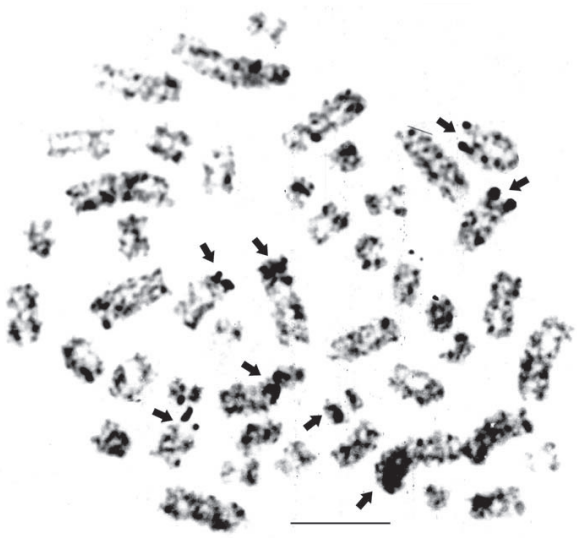

Fig. 7. Male Ag-banded karyotype of the striped field mouse (*A. agrarius*). Bar =10μm.

found in three specimens from the Iskar region stains C-positive.

In the current study, the presence of a NOR in 6 chromosomal pairs is established. Two of the pairs exhibited pericentromeric NORs, whereas the other 4 displayed telomeric NORs (Fig. 7). The localization of NORs on the short arms of the subtelocentric autosome from the first pair is established, whereas the acrocentric homologue does not possess such a structure. The NOR activation of the subtelocentric autosome from the first pair is linked to the duplication of the section of the short arm. In three acrocentric pairs NORs are situated in the telomeric regions, whereas, in the other two acrocentric autosomal pairs they are situated in the pericentromeric regions. NORs are also established in the telomeric regions of one of the biarmed autosomal pairs. This localization of NORs differs from the one previously described in karyological studies of *A. agrarius*. The presence of NORs on the subtelocentric homologue of the first autosomal pair and on one of the metacentric autosomes is established, for the first time, for specimens from Bulgarian populations.

The sex chromosomes are acrocentric: the X chromosome is slightly larger than the largest autosomal pair; the Y chromosome is middle-sized acrocentric and is not easily distinguishable with routine staining. This calls for the use of additional identification techniques, such as G- and C-banding (Fig. 4, 5, 6). The Y chromosome in all studied male specimens of the field mouse is a middle-sized acrocentric chromosome, with heterochromatin along its entire length and clear blocks in the pericentromeric and intercalated regions.

The X chromosome is larger, and its size can be compared to that of the largest autosomal pair. It has a very large pericentromeric block of heterochromatin, encompassing ¼ of its length, a clear intercalated band near the telomeric region, and fainter bands along the length of the chromosome in-between the centromeric blocks and the intensively colored bands near the telomeres (Fig. 4). The C-banding reveals two blocks of heterochromatin: a large, pericentromeric block, occupying nearly ¼ of the length of the chromosome, and clear

intercalated bands, situated between the pericentromeric heterochromatin and the clearly distinguishable telomeric C-block. Pericentromeric heterochromatin is described for the X chromosomes of all studied female specimens of the field mouse, whereas the telomeric C-block is described for the first time. This characteristic C-banding of the X chromosome can be used as an X chromosome marker.

Discussion

The established karyotype of the field mouse in Bulgaria (2n=48+1B) does not differ from the one described for the Palearctic area of the species in Yugoslavia (Kral, 1970, 1972; Soldatović et al., 1971, 1975; Gamperl et al., 1982), Greece (Brittan-Davidian et al., 1991; Giagia et al., 1985), Poland, Czechoslovakia, (Soldatović et al., 1975), Hungary (Zima, Kral, 1984), Estonia (Boeskorov et al., 1995), Caucasus (Nadjafova, 1989; Bulatova et al., 1991), East Siberia (Kral, 1971), the Far East, the Primorski region (Kral, 1971; Bekasova et al., 1980), China (Wang et al., 1993), and Korea (Makino, 1951; Kang et al., 1974; Koh, 1982, 1987a, b). Additional chromosomes in the field mouse have only been established in the Far East and Russian populations so far (Kartavtseva, 1994; Kartavtseva, Pavlenko, 2000). For the first time in the current study, the presence of an additional B chromosome in the *A. agrarius* species in Europe is described.

The variability in the morphology of the large autosomes from the first and second pairs (variant ST/A) has been described for species in Korea (Kang, Koh, 1976; Koh, 1982), Azerbaijan (Bulatova et al., 1991), and the Far East (Kartavtseva, 2002). The appearance of short arms in the last study can be described with an increase in the amount of heterochromatin in the centromeric region of the largest acrocentric chromosome. The Bulgarian field mouse resembles those in Azerbaijan in that the short arms of the subtelocentric chromosomes are composed of brightly colored heterochromatin. An increase in the amount of centromeric heterochromatin can be due to a local amplification, leading to differences in the amount of centromeric heterochromatin. The variability in the amount and distribution of heterochromatin in recent studies has been used in intra- and interspecies differentiation of the species in *Sylvaemus subgenera* (Orlov et al., 1996). According to several authors, such variability in the short arms of the chromosomes in *A. agrarius* might be linked to the different functional state of the organism. (Prokof'eva-Belgovskaya, 1977, 1979, 1986; Kartavtseva et al., 1998).

For 11 species of the genus *Apodemus*, the number and localization of NORs differ (Boeskorov et al., 1995). In the striped field mice from Estonia, Russia, the Ukraine, Dagestan, North Ossetia (Boeskorov et al., 1995), and Azerbaijan (Bulatova et al., 1991), the NORs are localized in 8 chromosomes (4 pairs): in the 4 large acrocentric autosomes the localization is telomeric, and in the 4 small ones, it is pericentromeric. Only the species on the Estonian island Saaremaa have two pericentromeric NORs less, which, according to authors (Boeskorov et al., 1995), is, possibly, evidence for their earlier geographic isolation from the continental populations. In the field mice from the Primorski region of Russia, the NORs are localized in the telomeric area of two large acrocentric pairs of autosomes and a small, possibly subtelocentric, autosomal pair in the pericentromeric region of the short arm (Kartavtseva, 2002).

For specimens from the region of Iskarski prolom (Belcheva et al., 1987), the NOR is localized in the telomeric region of one of the large acrocentric autosomal pairs and in the short arms of one of the metacentric pairs of chromosomes. In the current study, the pres-

ence of NOR in 6 chromosomal pairs is established. In two of the pairs, the localization is pericentromeric, and in the other 4 it is telomeric. This localization of NOR differs from the one described for the representatives of *A. agrarius*. The presence of NOR in the subtelocentric homologue of the first autosomal pair and in one of the metacentric autosomes is established for the first time for specimens from the studied Bulgarian populations.

The chromosome form with 4 pairs of metacentric chromosomes can be described as prevailed for the Bulgarian populations and the one with 3 pairs of metacentric chromosomes as a rarer variant. It is determined in only two of nine cytogenetically studied locations of the field mouse in Bulgaria.

The review of geographic distribution of the chromosome forms of *A. agrarius* in its European area shows that the chromosome form with 3 metacentric chromosomes has only been described in Moldova, Romania, Southeast Hungary, and North Bulgaria so far. A spread area that borders on the Carpathian Mountains to the north is formed. Proceeding from the orthogenetic concept, regarding evolution of the karyotype (Imai, 1976), this means that populations of the field mouse that are more evolutionarily ancient are preserved here, and probably the more recently evolved chromosomal form with 4 metacentrics has originated from them. According to Griffiths et al. (2004), recent chromosome diversity models in the Balkan small mammalian fauna confirm the presence of endemic populations with ancestral characters, which differ from the populations distributed northern in their species area.

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