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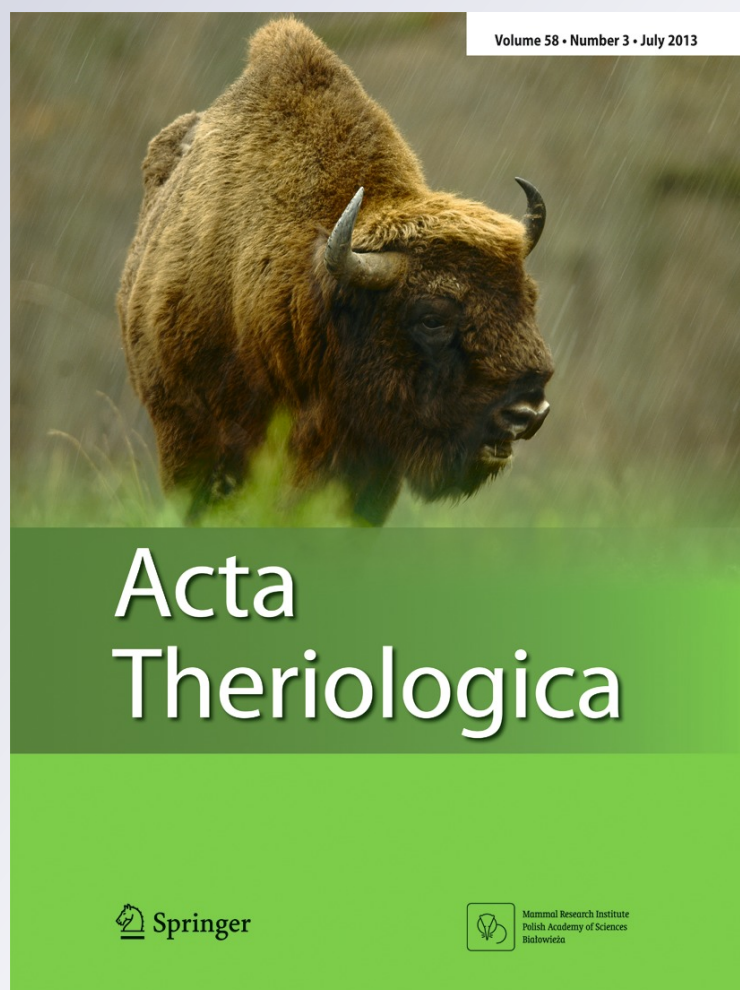
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Chromosomal variation in social voles: a Robertsonian fusion in Günther's vole

Jan Zima · Atilla Arslan · Petr Benda · Miloš Macholán · Boris Kryštufek

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Abstract The study reports on chromosomes in several populations of social voles from south-eastern Europe and the Middle East. The standard karyotypes of individuals of *Microtus hartingi* and *Microtus guentheri* originating from both south-eastern Europe and Asia Minor comprised 54 mostly acrocentric chromosomes. However, variation between populations was found in the amount and distribution of C-heterochromatin in certain autosomes and the sex chromosomes. Furthermore, a specific pattern of argyrophilic nucleolar organizer region distribution was recorded in different geographic populations. In a population from Asia Minor, a heterozygous centric fusion of two autosomes was found. The G-banded karyotypes of *M. guentheri* and

Microtus socialis were compared, and tandem fusions of autosomes were suggested as possible mechanism of the divergence. The karyotypes of the nine currently recognized species of social voles are reviewed, and implications of chromosomal data for systematics are evaluated.

Keywords Karyotypes · Systematics · *Microtus guentheri* · *M. hartingi* · *M. socialis* · C-banding · NOR distribution

Introduction

Social voles are small- to medium-sized voles that can be differentiated from related groups by five plantar pads, flat interorbital region and enlarged mastoid chamber (Kryštufek and Vohralík 2005). Social voles have been ranked as a subgenus *Sumeriomys* Argyropulo, 1933 particularly by Russian authors (e.g. Pavlinov and Rossolimo 1998; Golenishchev et al. 2002b); however, evidence from mitochondrial cytochrome *b* sequences leaves social voles within the subgenus *Microtus* (Jaarola et al. 2004). Ellerman and Morrison-Scott (1951) listed only three species, *Microtus guentheri* Danford et Alston, 1880; *Microtus socialis* Pallas, 1773; and *Microtus irani* Thomas, 1921 in their checklist, whereas Corbet (1978) lumped all these into *M. socialis* and recognized subspecific taxa only. In contrast, Musser and Carleton (2005) have currently identified as many as eight distinct species. Higher species richness has also been suggested by various morphological, chromosomal and molecular studies (Kryštufek and Kefelioğlu 2001b; Yiğit and Çolak 2002; Jaarola et al. 2004; Shehab et al. 2004; Kryštufek et al. 2009, 2012; Yiğit et al. 2012).

Social voles inhabit dry steppes and semi-deserts of Eastern Europe, the Balkans, Cyrenaica in Libya and the vast area from the Middle East to Central Asia (Kryštufek and Vohralík 2005; Musser and Carleton 2005). Despite this wide range, the majority of species occurs in Anatolia, the

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Table 1 A synopsis of known karyotypes of social voles. Chromosomes are characterized as acrocentric (A), subtelocentric (ST), submetacentric (SM) and metacentric (M) according to their centromere position

Species	2n	NFa	X	Reference	Origin
<i>M. hartingi</i>	54	52	A	Živković and Petrov (1975)	FYR Macedonia
	54	52	A/SM	Belcheva et al. (1980), Chassovnikarova et al. (2008)	Strandja Mts., Bulgaria
	54	52	A/M	Kefelioğlu (1995)	Turkish Thrace
	54	52	SM	Golenishchev et al. (2002b)	Sozopol, Bulgaria
	54	52	A	Mitsainas et al. (2010)	Greece
	54	52	A/ST	This paper	Macedonia, Bulgaria
<i>M. guentheri</i>	54	52–54	M	Matthey (1952)	Not known
	54	52	A	Kefelioğlu (1995)	Type locality, Maraş, Turkey
	54	52–54	A/M	Çolak et al. (1997)	SE Anatolia, Turkey
	54	52	A	Çolak et al. (1998)	Central Anatolia, Turkey
	54	–	–	Modi (1993)	Not known
	54	52	A/M	Kefelioğlu and Kryštufek (1999)	Anatolia, Turkey
	54	52	A	Golenishchev et al. (2002b)	Israel
	54	52	A/M	Yiğit and Çolak (2002)	Anatolia, Turkey
	54	52	A	Yiğit and Çolak (2002)	Ankara, Turkey
	54	52	A	O'Brien et al. (2006)	Not specified
	60	58	A	O'Brien et al. (2006)	Not specified
	54	52	A	Gözütök and Albayrak (2009)	Kırıkkale, Anatolia, Turkey
	54	52	A/SM	Aşan Baydemir et al. (2011)	Kırıkkale, Nevşehir, Gaziantep, Kahramanmaraş; Turkey
	53–54	52	A/ST	This paper	Harput, Anatolia, Turkey
	54	52	A/ST	This paper	Syria
<i>M. dogramacii</i>	48	46–50		Kefelioğlu and Kryštufek (1999)	Amasya, Konya; Turkey
	48	46, 48		Şekeroğlu et al. (2011)	Amasya, Turkey
<i>M. socialis</i>	62	60	A	Matthey (1952)	Not known
	62	60	A	Orlov (1970)	Armenia
	62	60	A	Gaichenko (1973)	Armenia
	62	60	A	Kuliev (1979)	Azerbaijan
	62	60	A	Ayrumyan et al. (1986)	Armenia
	62	60	A	Zykov and Zagorodnyuk (1988)	Ukraine, S Russia
	62	60	A	Kefelioğlu (1995)	Iran, Azerbaijan
	62	60	A	Golenishchev et al. (1999)	Iran
	62	60	A	Kefelioğlu and Kryštufek (1999)	E Anatolia, Turkey
	62	60	A	Golenishchev et al. (2002b)	Ukraine, S Russia, Daghestan, Georgia
	62	60	A	O'Brien et al. (2006)	Not specified
	62	60	A	Yiğit et al. (2006)	Zanjan, Iran
	62	60	A	This paper	Ukraine, Armenia
<i>M. anatolicus</i>	60	60	A	Kefelioğlu and Kryštufek (1999), Kryštufek and Kefelioğlu (2001a)	Konya, Anatolia, Turkey
	60	58	A	Yavuz et al. (2009)	Antalya, Anatolia, Turkey
<i>M. irani</i>	54			Matthey (1954)	Iran
	60–64			Matthey (1954)	Iran
	46	46	M	Çolak et al. (1997)	Kilis, SE Anatolia, Turkey
	62	60	A	Golenishchev et al. (1999)	Type locality, Shiraz, Fars; Iran
	60	58	A	Kryštufek et al. (2010)	Balkusan, Turkey
<i>M. schidlovskii</i>	62	60	A	Ayrumyan et al. (1986)	W Armenia
	60	58	A	Akhverdyan et al. (1990)	Armenia
	60	58	A	Akhverdyan and Lyapunova (1990)	Armenia
	60	58	A	Golenishchev et al. (2002b)	Armenia

Table 1 (continued)

Species	2n	NFa	X	Reference	Origin
	60	58	A	O'Brien et al. (2006)	Not specified
	60	58	A	Yiğit et al. (2006)	Van, Hakkari; Turkey
<i>M. paradoxus</i>	62	60	A	Zykov and Zagorodnyuk (1988)	Kopetdag Mts., Turkmenistan
<i>M. qazvinensis</i>	54	52	ST	Golenishchev et al. (1999), 2002a	Qazvin, Iran

Caucasus and Iran (Shenbrot and Krasnov 2005; Aulagnier et al. 2009). Thus, the south-western Asia is probably a centre of speciation and diversification of this group.

Chromosomes of social voles have been extensively studied (see Zima and Král (1984) for a review). The karyotype of *M. guentheri* was first described by Matthey as early as 1952. Later, Živković and Petrov (1975) examined chromosomes of this species from Macedonia and found 54 telo- and acrocentric chromosomes in the diploid complement. Heteromorphism of the centromeric position on the X chromosome was reported by Belcheva et al. (1980) and Chassovnikarova et al. (2008) who studied C- and G-banded chromosomes in a Bulgarian population. C-heterochromatin distribution was also investigated in the karyotype of Greek populations by Mitsainas et al. (2010). In Asia Minor, chromosomes of this species were studied by Kefelioğlu (1995) who investigated also topotypes from Maraş (Kahramanmaraş). Further investigations were performed by Çolak et al. (1997, 1998), Yiğit and Çolak (2002), Gözütok and Albayrak (2009) and Aşan Baydemir et al. (2011). The karyotype of *M. guentheri* (including *Microtus hartingi*, recently distinguished as a species distinct from *M.*

guentheri by Kryštufek et al. 2012), is rather conservative, with the only exception of the subspecies *M. guentheri arm* in which O'Brien et al. (2006) found 60 chromosomes (Table 1).

The karyotype of *M. socialis* was first described by Matthey (1952, 1954) from Iran, who treated the examined specimens as *M. socialis irani* or *M. irani*. In these pioneer papers, the diploid number was not assessed uniformly, with $2n=62$, 54 or 60–64 being reported in the individual specimens studied. This contributed to confusion on the taxonomic status of *M. irani*. Subsequent studies carried out in Russia showed that the diploid number of 62 chromosomes is standard in various geographical populations of *M. socialis* (Orlov 1970; Gaichenko 1973; Kuliev 1979; Ayrumyan et al. 1986; Golenishchev et al. 2002b), and the same karyotype was recorded also in a sibling species, *Microtus paradoxus*, from the Kopetdag Mts. in Turkmenistan (Zykov and Zagorodnyuk 1988). Akhverdyan et al. (1990) and Akhverdyan and Lyapunova (1990) found $2n=60$ in populations of *M. socialis schidlovskii* (now considered a distinct species *Microtus schidlovskii*) from Armenia. The lower diploid number in *M. schidlovskii* was explained by a centromeric–telomeric



Fig. 1 Geographic location of the sites studied. *M. hartingi* (triangles), *M. guentheri* (squares), *M. socialis* (circle)

tandem fusion of two autosomal pairs (Golenishchev et al. 2002b). Golenishchev et al. (2002b) provided an analysis of the G-banding pattern in *M. socialis*, *M. schidlovskii* and *M. hartingi* and proposed possible mechanisms of karyotypic divergence between these species. Other cytogenetic studies in social voles were aimed at the pattern of X–Y chromosomes pairing (Borodin et al. 1995) and the occurrence of repeated DNA sequences in the Y chromosome (Marchall et al. 2004). Karyological studies were performed also in other populations that have been subsequently recognized as separate species (Kefelioğlu and Kryštufek 1999; Kryštufek and Kefelioğlu 2001a; Golenishchev et al. 2002a; Table 1).

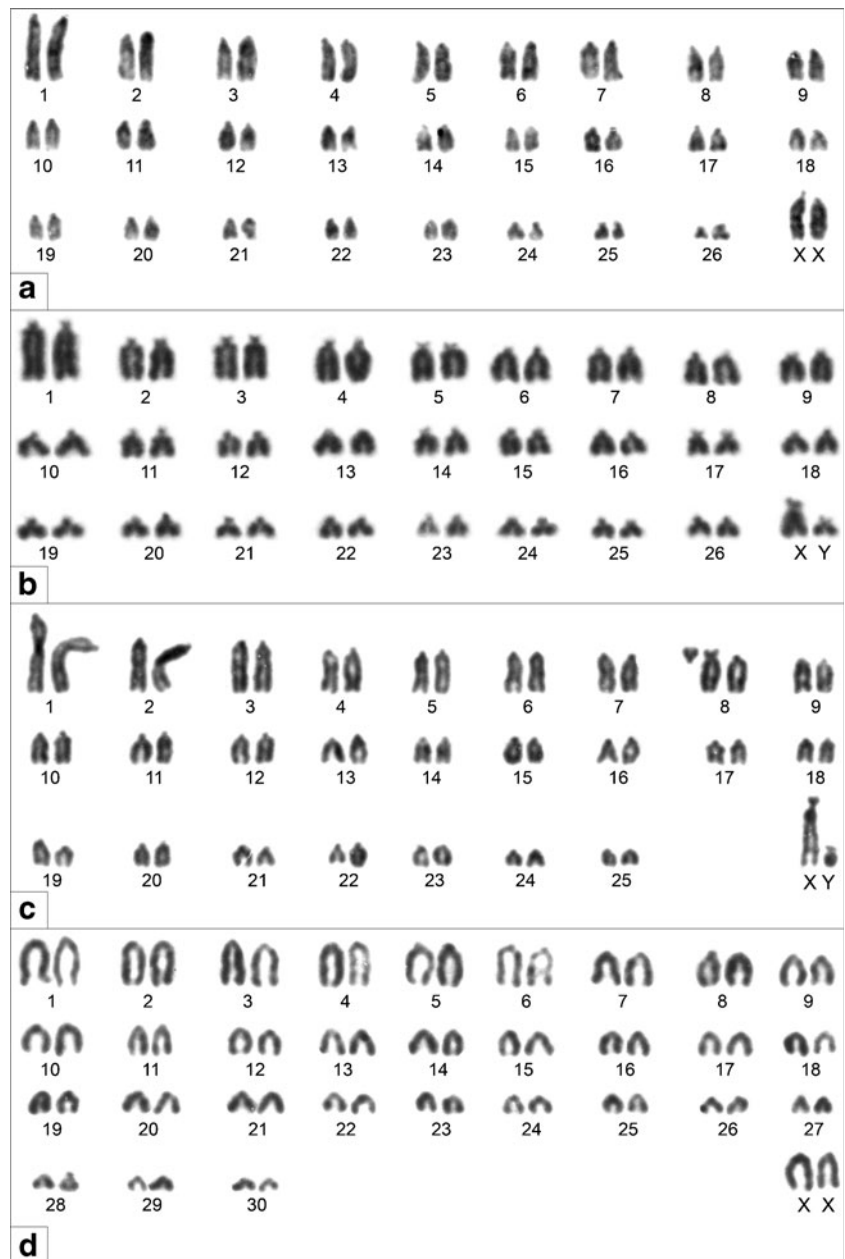
In this paper, we examined the karyotype of diverse populations of social voles from a vast area spanning from

south-eastern Europe to Syria to provide a reliable cytogenetic comparative standards of *M. guentheri*, *M. hartingi* and *M. socialis*, using a combination of three differential staining techniques, G-banding, C-banding and argyrophilic nucleolar organizer region (AgNOR) staining. Since the systematics of social voles has become a hot topic, we review karyological data on social voles and evaluate their significance for current taxonomy.

Material and methods

In the species nomenclature, we follow here the system proposed by Kryštufek et al. (2009, 2012). Since the eastern

Fig. 2 Conventionally stained karyotypes. **a** *M. hartingi*, Macedonia. **b** *M. guentheri*, Konya, Turkey. **c** *M. guentheri*, Harput, Turkey. **d** *M. socialis*, Armenia



border of *M. hartingi* in Turkey remains unresolved (Kryštufek and Vohralík 2009), we provisionally treat all individuals originating from the Asiatic part of Turkey as *M. guentheri sensu lato*. In total, we examined karyotypes of 21 specimens of *M. hartingi*, *M. guentheri* and *M. socialis* collected from natural populations at six localities and two breeding colonies of known origin (Fig. 1):

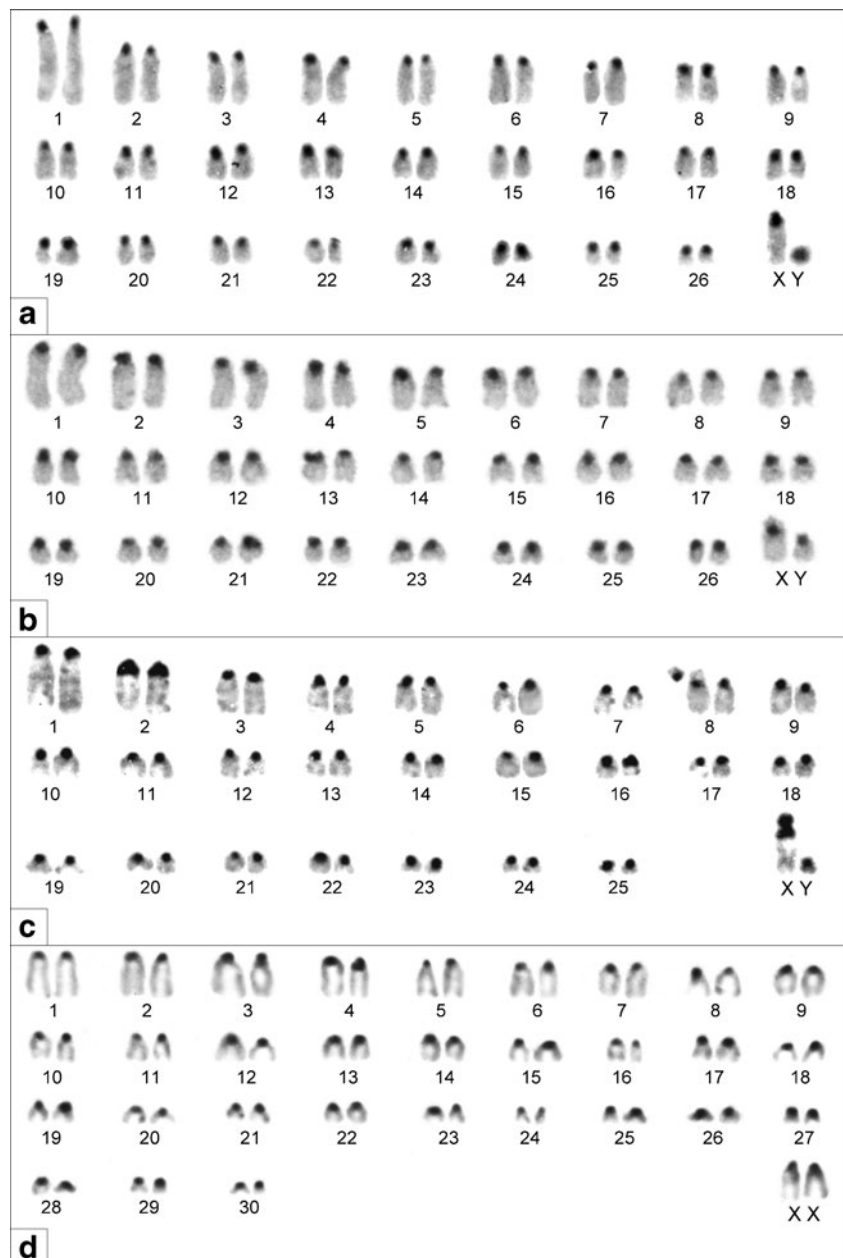
- *M. hartingi*: two F, three M, east of Veles, Macedonia (41°45' N, 21°50' E); two F, Bulgaria—a breeding colony kept at the Biological Faculty of University of South Bohemia in České Budějovice, Czech Republic
- *M. guentheri*: two F, three M, Beyşehir, Konya Province, central Anatolia, Turkey (37°40' N, 31°45'

E); one F, three M, Harput, Elazığ Province, eastern Anatolia, Turkey (38°40' N, 39°15' E); one M, Aqrabat, Idlib Province, Syria (36°16' N, 36°43' E); one M, Qattinah, Homs Province, Syria (34°40' N, 36°37' E)

- *M. socialis*: one F, one M, Askania-Nova, Ukraine (46°27' N, 33°52' E); one F, Armenia—a breeding colony kept at the Institute of Cytology and Genetics, Russian Academy of Sciences in Novosibirsk

The specimens examined are deposited as skulls and skins at collections of the Institute of Vertebrate Biology AS CR in Brno, National Museum (Natural History) in

Fig. 3 C-banded karyotypes. **a** *M. hartingi*, Bulgaria. **b** *M. guentheri*, Konya, Turkey. **c** *M. guentheri*, Harput, Turkey. **d** *M. socialis*, Armenia



Prague, Czech Republic; Slovenian Museum of Natural History in Ljubljana, Slovenia; and Selçuk University in Konya, Turkey. Chromosome preparations were obtained using a standard technique of direct colchicine/hypotonic treatment of bone marrow. G-banding followed the procedure of Seabright (1971); C-banding, that of Sumner (1972); and AgNOR staining, that of Howell and Black (1980).

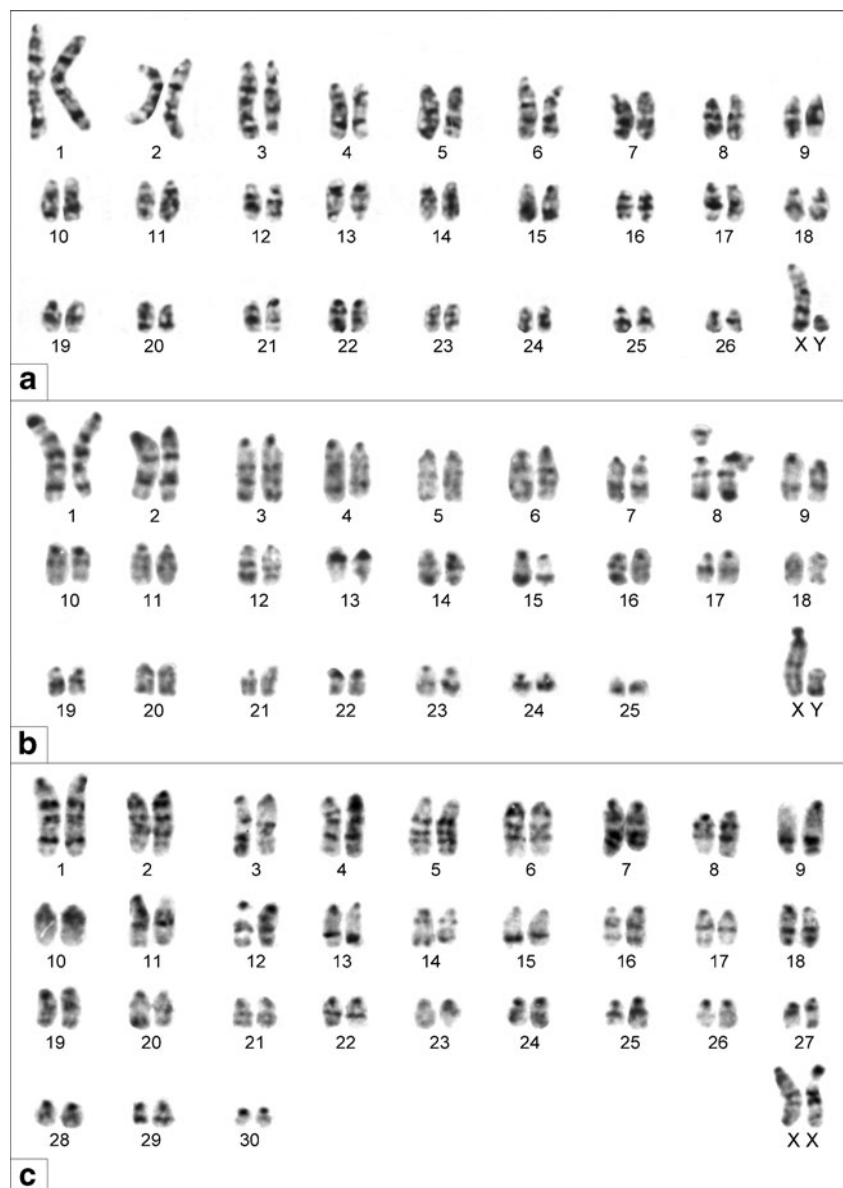
Results

In most of the *M. hartingi* and *M. guentheri* individuals examined, 54 chromosomes were found except for two males from Harput (Turkey) with 53 chromosomes in the karyotype. In conventionally stained preparations, most of the chromosomes appeared acrocentric, with short arms prominent to

various degrees. The two specimens with 53 chromosomes were heterozygous for a centric fusion between two nonhomologous autosomes of different sizes (Fig. 2a–c). All the studied specimens of *M. socialis* had 62 acrocentric chromosomes in the diploid complement (Fig. 2d).

C-banding showed distinct centromeric dark bands in all chromosomes in the karyotype of the studied individuals of *M. hartingi* and *M. guentheri*. The extent of the C-positively stained centromeric regions was in general larger in the individuals from Harput than in the specimens from the Balkans and central Anatolia (Fig. 3a–c). The difference was particularly prominent in autosomal pair no. 2 and the X chromosome. The distinct short arm as well as the pericentromeric region of the long arm of the X chromosome in the specimens from Harput was completely heterochromatic. In the European specimens, the whole short arm of the X

Fig. 4 G-banded karyotypes. **a** *M. hartingi*, Bulgaria. **b** *M. guentheri*, Harput, Turkey. **c** *M. socialis*, Armenia



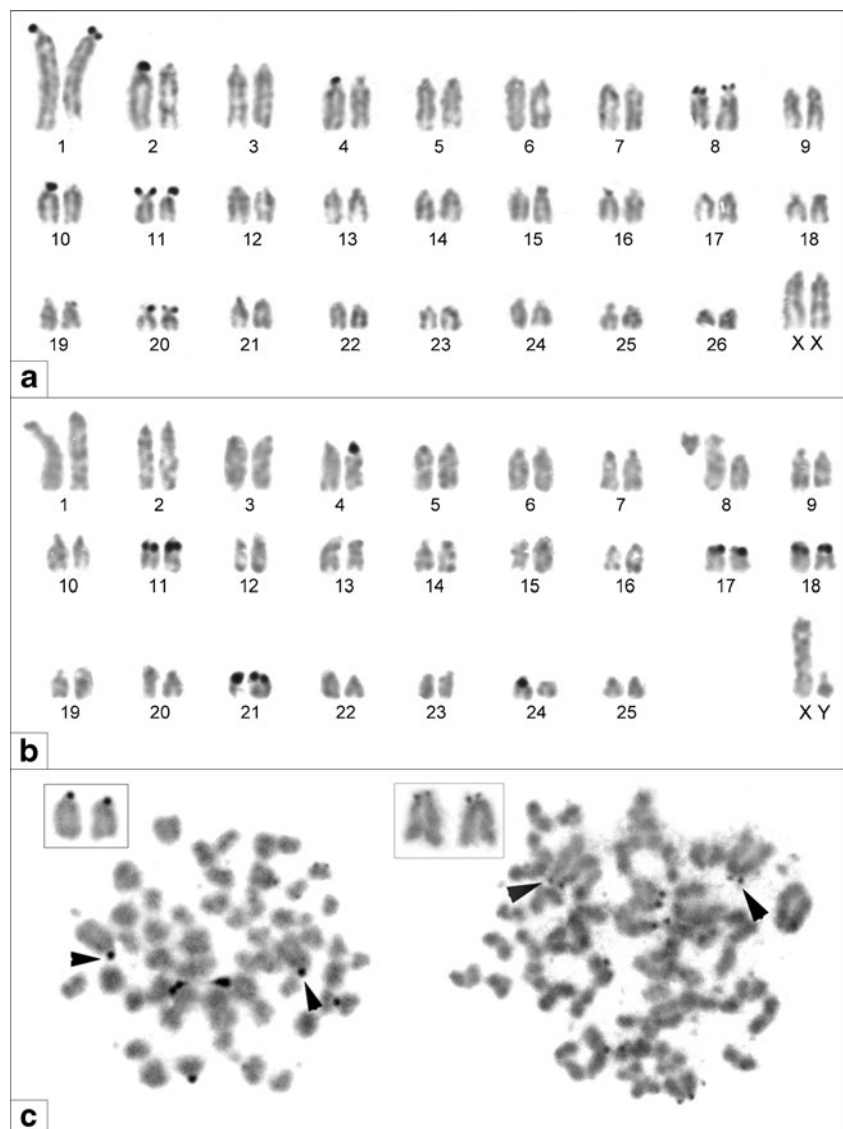
chromosome was also positively stained, but it was rather tiny. Finally, the X chromosome in the Konya specimens possessed only small pericentromeric C-block with the rest of the short arm unstained. The size of the Y chromosome was slightly larger in the Anatolian males than in the European ones. However, whereas the Y chromosome in males from Harput was completely heterochromatic, that in males from Konya had only a tiny pericentromeric C-band. In Syrian voles, centromeric C-bands were rather faint and their size was similar to that of the karyotypes from Macedonia. In the four largest autosomal pairs and in the X chromosome, an interstitial dark C-band was observed near the centromere which was not present in individuals from other localities studied. Pericentromeric dark C-bands were observed in all the autosomes in the karyotype of *M. socialis*; however, they were not distinctly apparent in the X chromosome (Fig. 3d).

We have not found any apparent differences between G-banded karyotypes of individuals of *M. hartingi* and *M.*

guentheri. The males from Harput with $2n=53$ appeared to be heterozygous for a Robertsonian fusion of autosome nos. 8 and 25 (Fig. 4a, b). Furthermore, the G-banding pattern suggested that tandem fusions are apparently responsible for the difference in the diploid number between *M. guentheri* and *M. socialis* (Fig. 4c).

Silver-stained NORs were distributed in the telomeric areas of the short arms in certain autosomes and, exceptionally, also in pericentromeric regions (Fig. 5). In the karyotype of the *M. hartingi* individuals, active NORs were located in telomeres of the short arms of the two largest autosomes and five autosomes of medium or small size. A similar AgNOR pattern was also revealed in individuals of *M. guentheri* from Konya resembling thus the pattern found in the European samples. There were apparently other NORs localized in smaller autosomes of the Konya individuals, but their exact number could not be reliably estimated because of the deficient quality of preparations. In contrast

Fig. 5 AgNOR-stained karyotypes. **a** *M. hartingi*, Macedonia. **b** *M. guentheri*, Harput, Turkey. **c** Inset of the two largest autosomal pairs of *M. guentheri* from Konya, Turkey. Arrowheads indicate the AgNORs located on the largest autosomal pair



to voles from Europe and central Anatolia, no active NORs were observed in the two largest autosomal pairs of individuals from Harput. Instead, NORs were identified in six autosomal pairs, most of them of small or medium size. A medium-sized pair (no. 11) carried a NOR site on the long arm near the centromere contrary to the same pair in the European individuals with the telomeric NOR position. The centromeric rather than telomeric position is apparent also in other smaller NOR-bearing autosomes in the complement of specimens from Harput. In *M. socialis*, NOR-possessing chromosomes were more numerous than in *M. guentheri* and *M. hartingi*. In the individuals examined, up to 16 NOR sites were observed in the pericentromeric region of the long autosomal arms.

Discussion

The heterozygous centric fusion found in two males from Harput is the first Robertsonian translocation hitherto reported in any social vole species. This type of chromosomal rearrangement has only occasionally been recorded in some populations of voles of the *Microtus (Terricola) daghestanicus-nasarovi* group from the Caucasus (Akhverdyan et al. 1992) and *Microtus agrestis* from Slovakia (Zima et al. 1990). Other cases of centric fusions in the genus *Microtus* were reported by Macholán et al. (2001) and Rovatsos et al. (2011). Such a type of polymorphism is apparently rare in the genus. Golenishchev et al. (2002b) recognized no centric fusions between species of social voles but proposed tandem fusions as the major chromosomal rearrangement in karyotypic evolution in the group.

The comparison of banding patterns indicates that variation exists even among morphologically similar standard karyotypes, particularly so among the C-banded and AgNOR-stained karyotypes of *M. hartingi* and *M. guentheri*. This variation includes the amount and distribution of C-heterochromatin in autosomes and the sex chromosomes, and the number and distribution of NORs in autosomes. The population of *M. guentheri* from eastern Anatolia (Harput) differs from populations of *M. hartingi* in Europe and *M. guentheri* in central Anatolia (Konya) by the presence of a distinct heterochromatic block on the second largest autosome and the C-banding pattern of the X chromosome. Variation in the X chromosome was reported from individuals of *M. hartingi* studied in Bulgaria (Belcheva et al. 1980; Chassovnikarova et al. 2008), and random heterochromatin amplification was suggested as the responsible mechanism. Similar variation in the X chromosome was recorded also in *M. guentheri*, and it is probably associated with the differently reported centromeric position (see Table 1). Aşan Baydemir et al. (2011) studied specimens from central Anatolia (Kırıkkale and Nevşehir provinces) and found a submetacentric X

chromosome and a centromeric C-block on the second largest autosome. The same authors recorded in samples from south-eastern Anatolia (Kahramanmaraş and Gaziantep provinces) the acrocentric X chromosome with only moderate amount of centromeric C-heterochromatin. In our sample from the same area, the amount of C-heterochromatin on the X chromosome was distinctly higher. The amount and distribution of C-heterochromatin were different between individuals from Syria and those from the other populations studied. The interstitial dark C-bands recorded in our sample from Syria were previously found also in specimens studied by Modi (1993), Chassovnikarova et al. (2008) and Aşan Baydemir et al. (2011).

Variation was observed also in the AgNOR distribution pattern which differentiated specimens of *M. hartingi* from south-eastern Europe and putative *M. guentheri* from central Anatolia compared with *M. guentheri* specimens from eastern Anatolia (Harput). These data show that specimens from south-eastern Europe and from central Anatolia (Konya) may be cytogenetically closer to each other than to specimens from eastern Anatolia (Harput) and Syria. The splitting of European and Anatolian populations of social voles into two separate species, *M. hartingi* and *M. guentheri* (cf. Kryštufek et al. 2012) seems plausible in this respect, whereas the taxonomic separation of populations from western Anatolia from those in Europe (Yiğit et al. 2012) is apparently not strongly supported by cytogenetic data. We should note that the detection of the NORs has often random

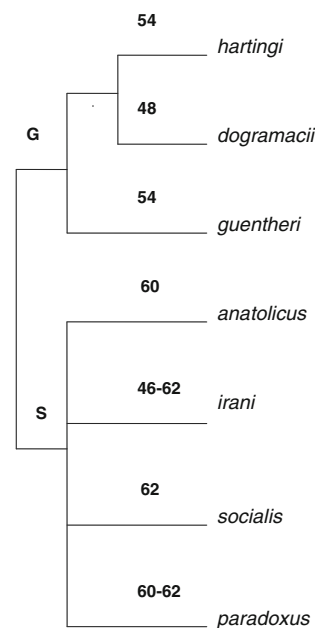


Fig. 6 A schematic view of the Bayesian inference tree reconstructed from cytochrome *b* sequences of social voles. The known diploid chromosome numbers are shown on individual branches (see Table 1 for details). *M. guentheri* lineage (G); *M. socialis* lineage (S). (Kryštufek et al. 2012)

topology among individuals and populations (Burgos et al. 1990; Sánchez et al. 1990) and the NORs distribution may not be an entirely reliable marker of phylogenetic relationships.

The social voles appear to be a rather exceptional group within the genus *Microtus* because of low karyological variability that could only exceptionally contribute to the individual species recognition and taxonomy. There is clear distinction between the karyotype with 54 chromosomes, typical for *M. guentheri*, and the karyotype with 60–62 chromosomes, typical for *M. socialis* and related forms (Fig. 6). The single report of $2n=60$ in *M. guentheri* by O'Brien et al. (2006) may be associated with misidentification of the material studied.

The karyological status of *M. irani* and *Microtus dogramacii* remains uncertain. The diploid numbers of 46, 54, 60 and 62 were reported for *M. irani* (not considering the older work by Matthey in 1952 and 1954 reporting even 64 chromosomes in the diploid complement of this species). This variation appears rather enigmatic and could hardly be explained as a result of rapid chromosomal evolution. We can rather assume that a possible reason for these differences between published results may be an incorrect determination of the diploid number in some older papers and/or the uncertain taxonomic classification of the specimens examined. The record of $2n=46$ in a population ascribed to *M. irani* (Çolak et al. 1997) is probably related to social voles of unresolved taxonomic affiliation (Kryštufek et al. 2010). Golenishchev et al. (1999) studied animals from the type locality of *M. irani* and found the diploid number of 62 chromosomes. Kryštufek et al. (2010) examined specimens from the type series of a newly described subspecies *M. irani karamani* from eastern Anatolia and found the karyotype with 60 chromosomes.

M. dogramacii is the only species of social voles with the chromosome number ($2n=48$) distinctly deviating from those commonly observed in other species ($2n=54$ or 60–62). From the point of view of comparative karyology, this species represents a separate evolutionary lineage within social voles. However, Jaarola et al. (2004) and Kryštufek et al. (2009, 2012) included this species within the *M. guentheri* clade.

We conclude that comparative cytogenetics using classical staining methods is obviously of limited value for resolving the taxonomic questions within the group of social voles. The evolutionary processes at phenotypic, chromosomal and molecular levels seem to be independent, particularly in the initial stages of the process. The results of the studies attempting to correlate the processes at various levels are often not unambiguous (e.g. Wójcik et al. 2000; Macholán et al. 2001; Polly 2007; Horn et al. 2012). The possible reason for this may be in varying rate and mode of evolution at various levels. It is important that all

karyologically studied specimens should be reliably taxonomically identified and subjected to parallel molecular studies. The future solution of the systematic questions related to the social voles will obviously be based on the use of various research approaches.

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