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Cytogenetic Characteristics of *Microtus guentheri*, *Microtus arvalis* sensu lato and *Microtus majori* (Mammalia: Rodentia) From Turkey: Constitutive Heterochromatin Distribution

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ABSTRACT

Conventionally stained and C- banded karyotypes of Guenther's vole (*Microtus guentheri*), Major's pine vole (*Microtus majori*) and Common vole (*Microtus arvalis*) were studied from Turkey. Diploid chromosome numbers of *M. guentheri, M. arvalis* and *M. majori* were found as 2n=54 and NFa=52, 2n=46 and NFa=68 and 2n=54 and NFa=56, respectively. All chromosomes of *M. guentheri* were pericentromeric C- band. In *Microtus arvalis* (*obscurus* cytotype) and *Microtus majori* karyotypes, autosomal chromosomes were heterochromatin C band positive and negative band. In *M. arvalis* (*obscurus* cytotype), sex chromosome was C band negative. In this study, heterozygote chromosome was not found in the obtained autosomal chromosome set of *M. arvalis*. *M. majori* has enlarged heterochromatin block from centromere to telomere on the long arm of X chromosome. Y chromosome was completely heterochromatin.

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Microtus guentheri, *Microtus arvalis* ve *Microtus majori* (Mammalia: Rodentia) Türlerinin Sitogenetik Özellikleri: Konstitütif Heterokromatin Dağılımı

ÖZET

Bu çalışmada, Güentheri tarla faresi (*Microtus guentheri*), Kısa kulaklı kır faresi (*Microtus majori*) ve Yaygın tarla faresi (*Microtus arvalis*) türlerinin standart karyotipleri ve kromozomların C-bant özellikleri belirlendi. *M. guentheri* türünün diploid kromozom sayısı (2n) = 54 ve otozomal kromozomların kol sayısı (NFa) = 52, *M. arvalis* (*obscurus* sitotip) türünün 2n = 46 ve NFa = 68, *M.majori* türünün 2n = 54 ve NFa = 56 şeklindedir. *M. guentheri* otozomal ve eşey kromozomlarda pericentromerik C-bant olduğu belirlendi. *Microtus arvalis* (*obscurus* sitotip) ve *Microtus majori* karyotiplerinde otozomal kromozomlar C bant pozitif ve negatif şeklindedir. *M. arvalis* türünde X ve Y kromozomu C bant negatif özelliktedir. *M. majori* karyotipinde X kromozomunun uzun kolunda sentromerden telomere doğru genişlemiş heterokromatin blok bulunmaktadır. Y kromozomu ise tamamen heterokromatindir.

Araştırma Makalesi

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Anahtar Kelimeler
C-bantlama
Guenther tarla faresi
Kısa kulaklı kır faresi
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INTRODUCTION

Microtus is the most branched species genus among the rodents distributed in the Palearctic (Shenbrot and Krasnov, 2005). It is very difficult to morphologically distinguish the *Microtus*, which is represented by 65 species, from each other (Musser and Carleton, 1993; Jaarola et al., 2004). However, they are frequently used in comparative cytotaxonomy studies since they differ from each other in terms of diploid chromosome numbers (2n=17-62) despite their morphological similarities (Zima and Král, 1984; Modi, 1987; Zagorodnyuk, 1990; Lemskaya et al., 2010). Twelve of the vole species, *M. dogramacii*, *M. anatolicus*, *M. hartingi*, *M. levis*, *M. arvalis*, *M. subterraneus*, *M. daghestanicus*, *M. irani*, *M. schidlovskii*, *M. majori*, *M. guentheri* and *M. socialis* have been reported from Turkey (Jaarola et al., 2004; Kryštufek and Vohralik, 2005; Kryštufek et al., 2012; Arslan et al., 2016; Selçuk and Kefelioğlu, 2018; Demirtaş and Gürler, 2019). These species distributed in Turkey are classified in three main groups as 'pine voles', 'social voles' and 'arvalis group' (Kryštufek and Vohralik, 2005).

According to Tougard et al., (2013) in the *Microtus arvalis* sensu lato, cytogenetic studies revealed that there were two distinct cytotypes in the *Microtus arvalis* sensu lato; the common (*arvalis* cytotype) and Altai (*obscurus* cytotype) voles. These cytotypes were attributed to the common (arvalis) and Altai (obscurus) voles having, respectively, fundamental number of autosomes (NFa)=80 in Caucasian populations; NFa=68–70 (Meyer et al., 1996). Within the distribution areas of populations of *M. majori*, *M. guentheri* and *obscurus* cytotype, various researchers have conducted karyological studies in the form of conventional chromosome staining (Kefelioğlu, 1995; Çolak et al., 1997a, b; Çolak et al., 1998; Kefelioğlu and Kryštufek, 1999; Yiğit and Çolak, 2002; Arslan and Zima, 2014), G- banding (Macholan et al., 2001; Zima et al., 2013), C and Ag-NOR banding (Yiğit and Çolak, 2002; Baydemir et al., 2011; Tougard et al.,2013; Yorulmaz et al., 2013; Zima et al., 2013). According to these studies, variations was found in the autosomal and sex chromosome morphologies of *M. guentheri*, *M. arvalis* (*obscurus* cytotype) and *M. majori* species.

The objective of this study was to compare the conventional karyotypes of *M. majori*, *M. guentheri* and *M. arvalis* (*obscurus* cytotype) species and their constitutive heterochromatin regions, which is a karyotypic characteristic, with previously conducted studies and thus contribute to future karyological studies.

MATERIAL and METHOD

Chromosome preparations were obtained from the femoral bone marrow cells of colchicine treated animals (Ford and Hamerton, 1956). Two samples (two females) of *M. guentheri* species were obtained from Tokat province (N40°21'-E36°37', Central Anatolia), two samples (two males) of *M. majori* species were obtained from Artvin province (N41°13'-E41°59', Northeast Anatolia, Caucasia region) and one sample of *M. arvalis* (*obscurus* cytotype) was obtained from Ardahan-Kars border province (N40°48'-E42°52', Northeast Anatolia) by using live animal traps. Diploid chromosome number (2n) and fundamental number of autosomal arms (NFa) and sex chromosomes of small mammals used in the study were defined as metacentric, acrocentric, submetacentric and subtelocentric. The constitutive heterochromatin distribution was determined by using techniques from Summer (1972). From each specimen, 10 to 20 slides were prepared and at minimum of 10 well-spread metaphase plates were analysed. Karyotype slides and chromosome-fixative solution, which did not undergo diffusion procedure, are being kept at Ondokuz Mayıs University Cytogenetic Laboratory under -20 degrees for future studies.

RESULT

Karyotype is 2n=54 and NFa=52 in Tokat samples of *M. guentheri*. The karyotype has 26 pairs of acrocentric autosomal chromosome in different sizes (chromosomes no: 1-26). In sex chromosomes, X chromosome is large subtelocentric. In the C-banding pattern of *M. guentheri*, positive constitutive heterochromatins are in centromere region. The X chromosome is positive C-band (Figure 1).

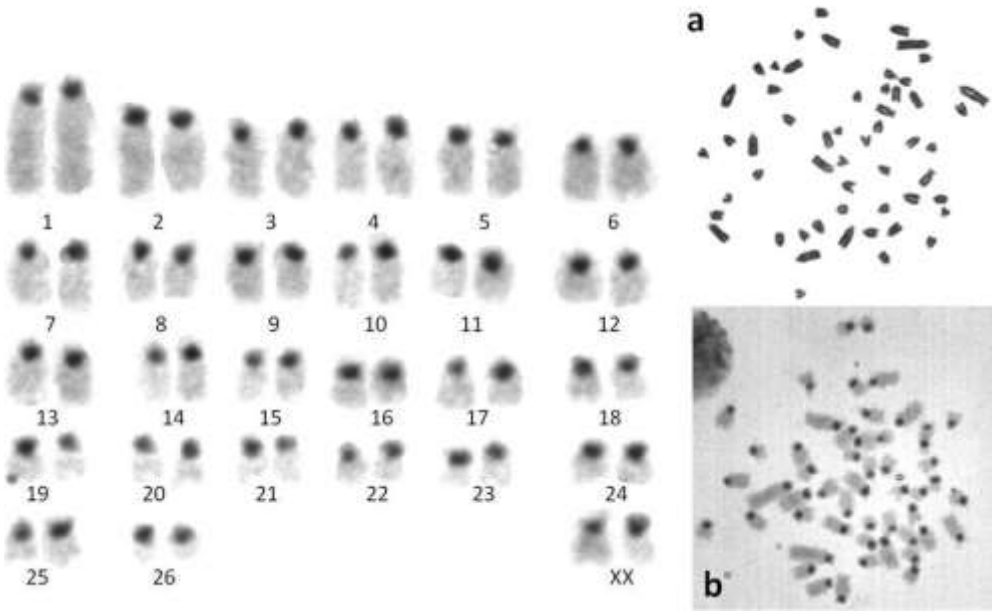

Figure 1. Metephase plate (a) and C-banded karyotype (b) of *Microtus guentheri* (female) from Tokat (Central Anatolia)

Karyotype is 2n=46 and NFa=68 in Kars sample of *M. arvalis* (*obscurus* cytotype). The karyotype has four pairs of different sizes of metacentric and submetacentric (chromosomes no: 1-4), one pair of subtelocentric (chromosome no: 5), seven pairs of small metacentric and submetacentric (chromosomes no: 6-12) and 10 pairs of different sizes of acrocentric (chromosomes no: 13-22) autosomal chromosome. The X chromosome is large metacentric and the Y chromosome is small metacentric (Figure 2).

The karyotype has positive and negative constitutive heterochromatin. While small two-armed chromosomes (chromosomes no: 6-11) have pericentromeric C- positive banding pattern, only chromosomes: 13-15-16-19-22 have pericentromeric C-positive banding pattern in acrocentric chromosomes. Sex chromosomes (X and Y) are C- band negative (Figure 3).

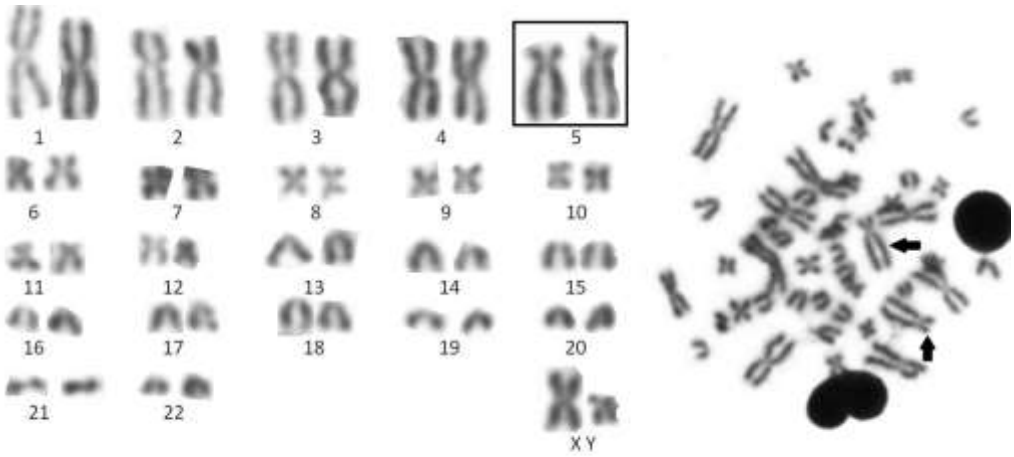

Figure 2. Conventional karyotype and metaphase plate of *M. arvalis* (*obscurus* cytotype) (male) from Kars. In box: Homomorphic subtelocentric autosomal chromosome, arrows: homomorphic subtelocentric chromosome

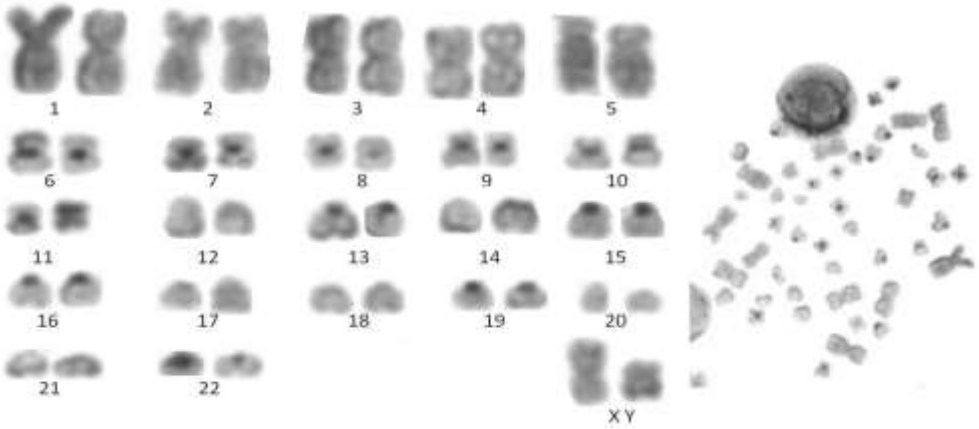

Figure 3. C-banded karyotype and metaphase plate of *M. arvalis* (*obscurus* cytotype)

In *Microtus majori* Artvin samples, the karyotype is 2n=54, NFa=56. While X chromosome is large submetacentric, Y chromosome is acrocentric (Figure 4). The karyotype has two pairs of submetacentric (chromosomes no: 1-2) and 24 pairs of acrocentric autosomal chromosome (chromosomes no: 3-26). The karyotype has positive (chromosomes no: 2, 3, 4, 8, 20, 22, 25) and negative C- banding pattern (Figure 4). The long arm of the X chromosome has heterochromatin block enlarged from centromere to telomere. The Y chromosome is completely heterochromatin (Figure 4).

DISCUSSION

Microtus guentheri karyotype (2n=54) was found to be compatible in similar with the studies conducted in the Anatolia and neighbouring regions (Kefelioğlu, 1995; Baydemir et al., 2011; Zima et al., 2013). *M. guentheri* shows variations in terms of sex chromosome morphology (X=acrocentric, metacentric, submetacentric, subtelocentric). X chromosome is metacentric in studies conducted in South Anatolia (Çolak et al., 1997a) and in various regions of Anatolia

(Kefelioğlu, 1995; Yiğit and Çolak, 2002); acrocentric in studies conducted in Central and Southern Anatolia (Çolak et al., 1998; Baydemir et al., 2011); submetacentric in studies conducted in Central Anatolia (Baydemir et al., 2011); and subtelocentric in Southern Anatolia and Syria population (Zima et al., 2013) and the present study (central Anatolia). Constitutive heterochromatin distribution is homomorphic and pericentromeric in autosomal chromosomes (O'Brien, 2006; Baydemir et al., 2011; Zima et al., 2013; in this study). In Harput (Southern Anatolia) samples, while the short arm of the X chromosome consists of completely heterochromatin block (Zima et al., 2013), constitutive heterochromatin is in the pericentromeric region of subtelocentric or submetacentric X chromosome in Central Anatolia sample (Nevşehir samples, Baydemir et al., 2011; Konya samples, Zima et al., 2013; Tokat samples in the present study).

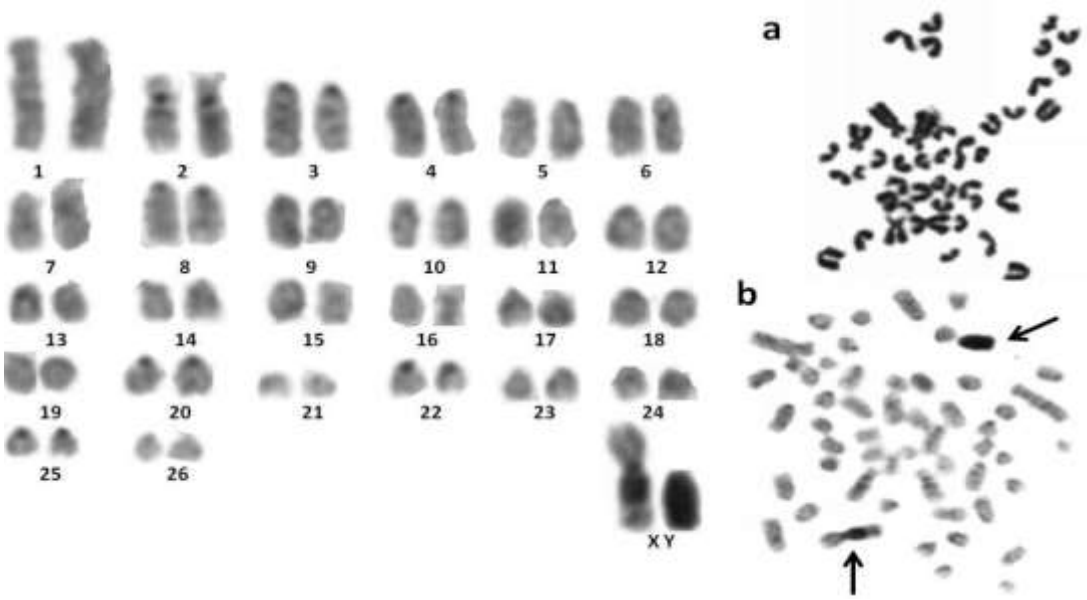

Figure 4. Metephase plate (a) and C-banded karyotype (b) of *Microtus majori* (male) from Artvin (Northeast Anatolia, Caucasia region). Arrows: X and Y chromosome

In terms of *M. arvalis* (*obscurus* cytotype) diploid chromosome number, this study was similar to previously conducted (Zima and Král, 1984; Kefelioğlu, 1995; Yorulmaz et al., 2013) studies. However, due to pericentric inversion, NFa number can differ (Gileva and Rakitin, 2006; Baskevich et al., 2016). Due to pericentric inversion, there may be one pair of the heteromorphic chromosome (subtelocentric and acrocentric) in autosomal chromosome set of *obscurus* cytotype (Gileva and Rakitin, 2006; Yorulmaz et al., 2013; Baskevich et al., 2016). In this study, (chromosome no: 5) and in Kefelioğlu (1995)'s study conducted in Turkish populations, heterozygote chromosome was not found in the obtained autosomal chromosome set. However, Heteromorphic chromosome was found in the Artvin (Turkey) sample (Yorulmaz et al., 2013).While X chromosome is metacentric in *obscurus* cytotype (Zima and Král, 1984; Kozlovskii et al., 1988; Kefelioğlu 1995; Tougard et al., 2013; Yorulmaz et al., 2013; Baskevich et al., 2016), variations can be seen in the centromere position of Y chromosome (Baskevich, 1996). While Y chromosome was found to be acrocentric in a study conducted in Turkey by Kefelioğlu (1995) and in Chinese by Tougard et al., (2013), it is metacentric in the present study. Constitutive heterochromatin distribution generally shows negative C- banding pattern in autosomal chromosomes and this is similar to a study conducted by Yorulmaz et al., (2013) from Turkey. In addition, X chromosome has negative C-banding pattern in our study.

Microtus majori diploid chromosome number and sex chromosome morphology was similar in the studies conducted previously in Turkey and Caucasia (Zima and Král, 1984; Macholan et al., 2001; O'Brien, 2006; Kuliev and Bickham, 2010; Arslan and Zima, 2014; Baskevich et al., 2015). As an exception to this result, in studies conducted by Çolak et al., (1997b) on samples of *M. majori* species, X chromosome was reported to be subtelocentric. While constitutive heterochromatin distribution is obscurely C- positive banded in autosomal chromosomes, autosomal chromosomes are generally C- negative banded. A similar result was found in a study conducted by Kuliev and Bickham (2010) on the Greater Caucasus Mountains and Lesser Caucasia Mountains. Autosomal chromosome set generally shows C- band negative characteristic (Kuliev and Bickham, 2010; in this study). A wide heterochromatin block extending down the centromere from the long arm of the X

chromosome is similar to samples obtained from Greater Caucasus Mountains by Kuliev and Bickham (2010) and samples obtained by Macholan et al., (2001) from Turkey. However, in samples obtained from Lesser Caucasia Mountains, there was not a wide heterochromatin block in the long arm of the X chromosome (Kuliev and Bickham, 2010). The Y chromosome consists of completely heterochromatin block in both Caucasian and Turkish population (Macholan et al., 2001; Kuliev and Bickham, 2010; in this study).

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