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Cytogenetic characteristics of *Microtus dogramacii* (Mammalia: Rodentia) around Amasya, Turkey

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Abstract: The banding patterns of chromosomes of *Microtus dogramacii,* a recently described vole species endemic to Turkey, were studied. G-, C-, and Ag-NOR-banded patterns of this species are reported here for the first time. In this study, 2 karyotypical forms were determined. Each form had the same diploid chromosome numbers (2n = 48), but possessed different autosomal morphologies. For this reason, the samples collected from the research area were karyologically separated into 2 groups, cytotype-1 (NF = 50) and cytotype-2 (NF = 52). All chromosomes possessed centromeric/pericentromeric heterochromatin bands in both karyotypical forms. It was shown that the acrocentric chromosomes of pair 8 in cytotype-1 have been transformed into metacentric chromosomes in cytotype-2 through pericentric inversion. Variation in the number of active NORs was also observed, but the modal number of active NORs was 8. Due to the chromosomal variation found in *M. dogramacii*, the cytogenetic results presented in this study may represent a process of chromosomal speciation.

Key words: *Microtus dogramacii*, karyology, pericentric inversion, Turkey

Amasya (Türkiye) çevresindeki *Microtus dogramacii* (Mammalia: Rodentia)'nin sitogenetik özellikleri

Özet: Türkiye için endemik olan yeni tanımlanmış bir tarla faresi, *Microtus dogramacii*'nin kromozomlarının bantlı örnekleri çalışıldı. Bu türün G-, C- ve NOR-bantlı örnekleri ilk kez bu çalışmada rapor edildi. Kromozom sayısı aynı (2n = 48), fakat otozomların morfolojileri farklı olan iki karyotipik form belirlendi. Bu nedenle araştırma alanından toplanan örnekler karyolojik bakımdan sitotip-1 (NF = 50) ve sitotip-2 (NF = 52) olarak isimlendirilen iki gruba ayrıldı. Her iki karyotipik formdaki kromozomlar sentromerik/perisentromerik heterokromatin bantlara sahipti. Sitotip-1'e ait 8. akrosentrik kromozomların perisentrik inversiyona uğrayarak sitotip-2'deki metasentrik kromozomları oluşturduğu gözlendi. Nükleolar Organizatör Bölgelerin (NOR) sayısında varyasyon tespit edildi, fakat en sık görülen aktif NOR sayısı 8 olarak bulundu. Sitogenetik sonuçlarımız, kromozomal varyasyondan dolayı *M. dogramacii*'deki kromozomal bir türleşme sürecini gösteriyor olabilir.

Anahtar sözcükler: *Microtus dogramacii*, karyoloji, perisentrik inversiyon, Türkiye

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Introduction

The genus *Microtus* Schrank, 1798 represents one of the most speciose mammalian genera in the Holarctic. The genus represents an excellent case of rapid and extensive radiation in mammalian evolution and has produced about 65 extant species, which are distributed throughout the Palearctic and Nearctic regions (Musser and Carleton, 1993; Chaline et al., 1999; Nowak, 1999; Jaarola et al., 2004; Mitsainas et al., 2010). The rapid diversification of and variation in *Microtus* may be partially responsible for its complex taxonomic history (Anderson, 1985; Musser and Carleton, 1993; Conroy and Cook, 2000).

The genus *Microtus* is still in an ongoing speciation process and displays a number of features that make it ideal for evolutionary studies of speciation and phylogenetic research as a pattern taxon (Jaarola et al., 2004; Krystufek et al., 2009). However, the phylogenetic relationships among *Microtus* and its closest relatives are uncertain, and difficulties remain both in delimiting species and defining subgenera (Zagorodnyuk, 1990; Musser and Carleton, 1993; Jaarola et al., 2004). Recently, molecular approaches such as analyses of enzymes, alloenzymes, and certain mitochondrial DNA genes have greatly clarified the taxonomic status of the taxa.

The *Microtus* karyotypes vary between 2n = 17 and 62. They exhibit one of the highest rates of karyotypic change in mammals (Maryama and Imai, 1981; Zima and Kral, 1984; Modi, 1987; Zagorodnyuk, 1990; Jaarola et al., 2004). Although some phylogenetic relationships can be deduced, especially from G-banded karyotypes, the overall picture is one of extensive karyotypic variation among closely related species with no apparent phylogenetic trends (Jaarola et al., 2004).

Microtus dogramacii from Amasya and Konya was described by Kefelioğlu and Krystufek (1999). The researchers determined 1 type of *M. dogramacii* karyotype in Konya and 3 types in Amasya. Jaarola et al. (2004) created a phylogenetic lineage tree, the result of analysis of the 1140 base pair in the mitochondrial cytochrome b gene from the *Microtus* species found in the Palearctic and North American regions. The analysis demonstrated a recent divergence of *M. dogramacii* from *Microtus guentheri*. Krystufek and Vohralik (2005) described the morphology of *M. dogramacii* in detail. Krystufek et al. (2009) established a cytochrome b phylogeny for 6 species of social voles, and *M. guentheri* and *M. dogramacii* lineages were strongly supported. Consequently, molecular analyses have shown that *M. dogramacii* is closely related to *M. guentheri*.

This study reports for the first time the C-, G-, and Ag-NOR-banded karyotypes of *M. dogramacii*. The aim of the present study is to describe the karyological characteristics of the new species using detailed cytogenetic techniques.

Materials and methods

Between 1999 and 2004, 74 specimens of *Microtus dogramacii* were collected from the type locality Boyalı village in Amasya Province, Turkey (41°40′N, 35°36′E) (Figure 1). Specimens were examined with respect to karyological characteristics. Chromosome preparations were obtained from the femur bone marrow cells of colchicine-treated animals (Ford and Hamerton, 1956). G-banding by trypsin treatment and Giemsa stain (GTG) was performed according to the methods of Seabright (1971). Constitutive heterochromatin and nucleolus organizer regions (NORs) were determined to identify pairs of each chromosome via C-banding (Sumner, 1972) and Ag-NOR staining (Howell and Black, 1980), respectively. From each specimen 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analyzed. The karyotype preparations were deposited at the Department of Biology, Faculty of Arts and Sciences, Ordu University, Turkey.

Results

The diploid number of chromosomes in all of the specimens was stable (2n = 48). However, the fundamental number of autosomes (NFa) and the number of fundamental arms (FN) varied. For this reason, it was established that 2 karyotypical forms are present in the area. The first karyotypical form, which we called cytotype-1, was 2n = 48, NFa = 46, and NF = 50. This karyotype was found in almost

Figure 1. Map of the study area indicating the locality *M. dogramacii* (▲).

70% of the samples. All autosomes were acrocentric in decreasing size. The X chromosome was medium-sized metacentric, while the Y chromosome was small-sized acrocentric. The other karyological form, which we called cytotype-2, was 2n = 48, NFa = 48, and NF = 52. The autosomal set consisted of 1 pair of metacentric and 22 pairs of acrocentric chromosomes. Morphologies of the sex chromosomes were of the same size and morphology as in cytotype-1 (Figure 2).

The localities of *M. dogramacii* species with different karyotypes overlapped, and we did not find any hybrid individual possessing the 2 karyotypical forms among the specimens collected from the research area. Moreover, the 2 karyotypical forms had similar morphological characters.

All autosomes and both sex chromosomes were identified in the G-banding patterns. When the G-bands were analyzed (Figure 3), it was shown

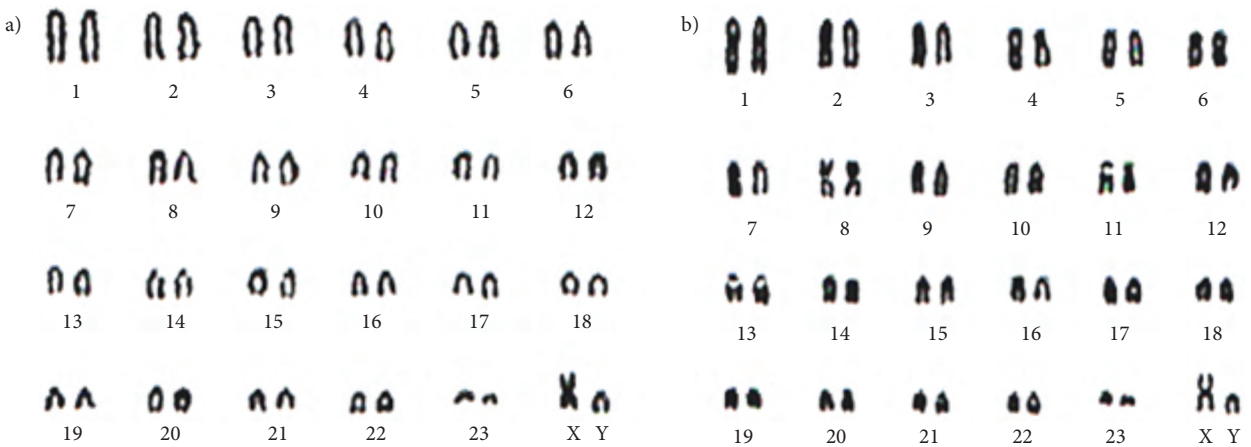

Figure 2. Karyotypes of cytotype-1 (a) and cytotype-2 (b) of *M. dogramacii*.

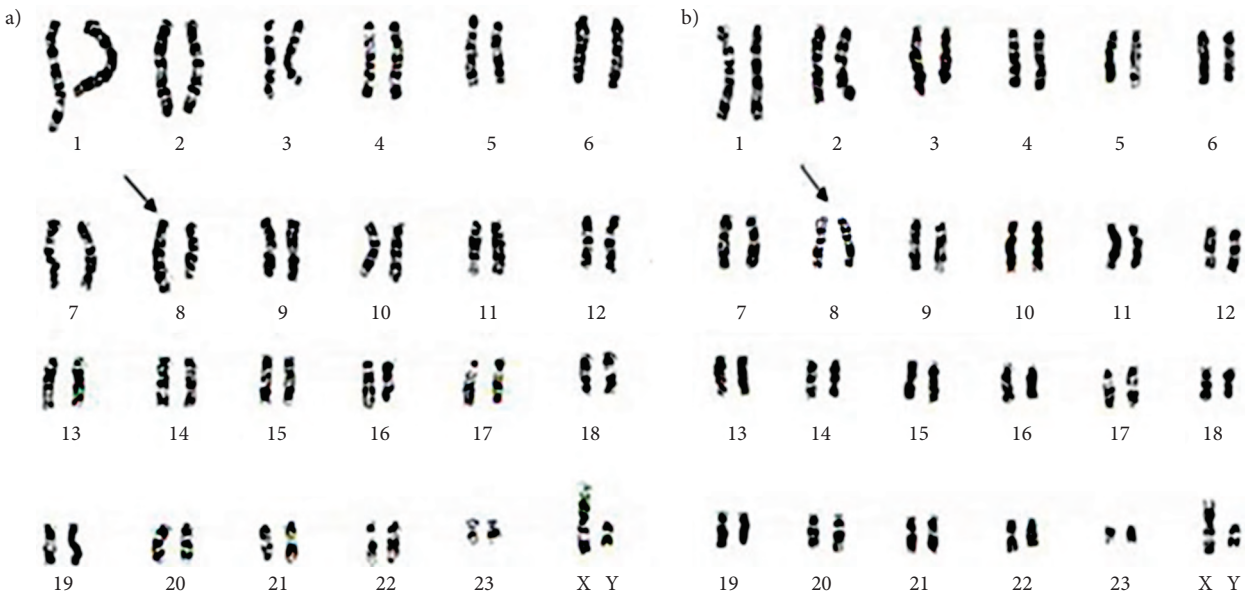

Figure 3. G-banded karyotypes of cytotype-1 (a) and cytotype-2 (b) of *M. dogramacii*.

that the acrocentric chromosomes of pair 8 in cytotype-1 had been transformed into metacentric chromosomes in cytotype-2. Although the diploid chromosome number remained unchanged, autosome morphologies changed and NF number increased through pericentric inversion.

The C-banded karyotypes of *M. dogramacii* are illustrated in Figure 4. All autosomes possess heterochromatin bands in the centromeric/pericentromeric regions. Some autosomal pairs have slightly stained heterochromatin bands (numbers 2 and 23); the others have strongly stained heterochromatin bands. One pair (number 2) is small and faintly stained and displays an additional interstitial heterochromatin band. The X chromosome has centromeric heterochromatin, and the Y chromosome has a large centromeric/pericentromeric heterochromatin block (Figure 4).

With silver nitrate staining, the number of active NORs varied from 6 to 10 per cell in the metaphase of *M. dogramacii*. Nevertheless, the modal number of active NORs was 8, because 8 active NORs were found

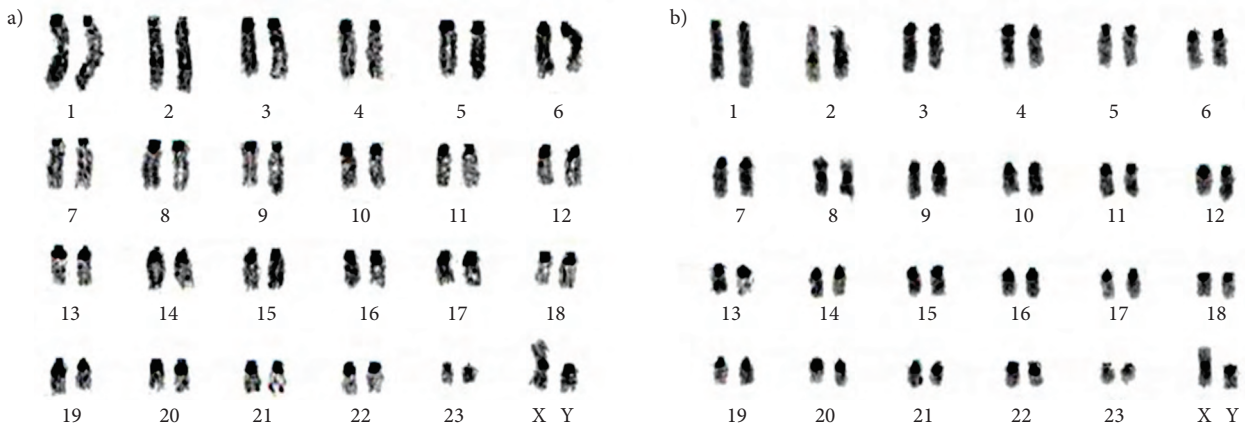

Figure 4. C-banded karyotypes of cytotype-1 (a) and cytotype-2 (b) of *M. dogramacii*.

in most metaphases in both cytotypes. In cytotype-1 and cytotype-2, the active NORs were localized in the centromeric regions of 4 acrocentric pairs (numbers 12, 18, 19, and 22). The NORs are homomorphic and medium-sized in both karyotypes (Figure 5).

Discussion

Kefelioğlu and Krystufek (1999) carried out conventional Giemsa-stain karyotypes of *M. dogramacii* and determined 1 type of karyotype from Konya and 3 types of karyotypes from Amasya (Table). Cytotype-1 (2n = 48, NFa = 46, NF = 50) showed the same chromosomal characters, both in number and morphology, as 1 of the 3 karyotypes from Amasya. In cytotype-1, on the other hand, only the morphology of the Y chromosome was different from the Konya karyotype. For cytotype-2 (2n = 48, NFa = 48, NF = 52), only the morphology of the Y chromosome differed from 1 of the 3 karyotypes obtained from Amasya. The third karyotype described by these researchers, which had 4 metacentric autosomes, is not found in this study.

We think that cytotype-1, in which all autosomes are acrocentric, is the ancestral karyotype and that it is more adapted to the habitat than cytotype-2. On the other hand, reproductive isolation between these karyological forms can be assumed, because no hybrids were found.

Rearrangement of chromosomes played a role in mammalian chromosomal evolution and is important as an initial event in phyletic divergence (King, 1993; Qumsiyeh, 1994). Changes in chromosome morphology may occur due to pericentric inversions, translocations, and deletion/amplification events (Qumsiyeh, 1994; Zima, 2000). The fundamental number of chromosomes may change through pericentric inversions without changing the chromosome number. In the present study, evidence of pericentric inversion was shown clearly with

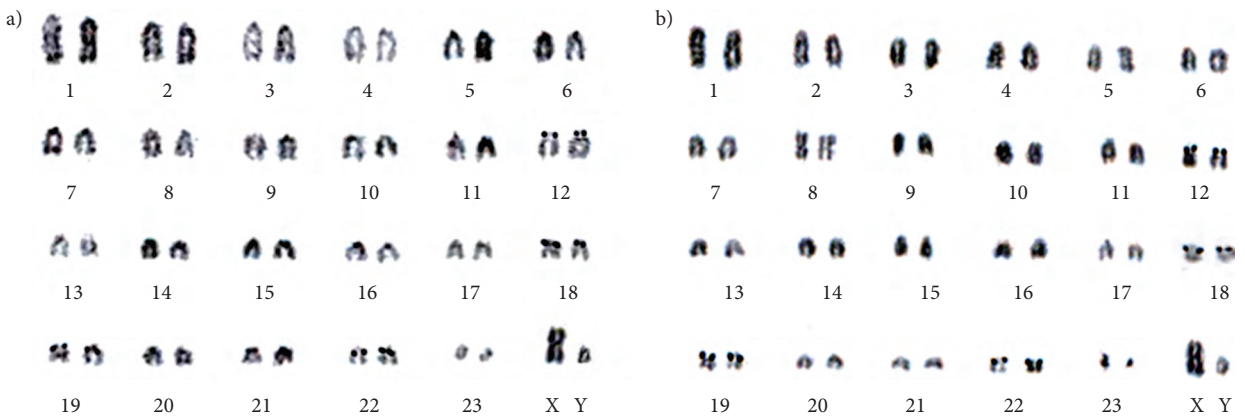

Figure 5. Silver-stained karyotypes of cytotype-1 (a) and cytotype-2 (b) of *M. dogramacii*.

Table. Sampling localities and karyotypic data of *M. dogramacii*.

2n	NFa	X	Y	Localities	References
48	46	m	sm	Cihanbeyli, Konya	Kefelioğlu and Krystufek (1999)
48	46	m	a	Boyalı, Amasya	Kefelioğlu and Krystufek (1999); in this paper
48	48	m	sm	Boyalı, Amasya	Kefelioğlu and Krystufek (1999)
48	50	m	sm	Boyalı, Amasya	Kefelioğlu and Krystufek (1999)
48	48	m	a	Boyalı, Amasya	In this paper

G-banding (Figure 3). Additionally, variation in the number of active NORs was observed even though the model number of NORs in *M. dogramacii* was found to be 8. The polymorphism of the number of NORs is observed in many other species (Yonenaga-Yassuda et al., 1987; Sanchez et al., 1989; Sanchez et al., 1995; Arslan et al., 2010)

Our cytogenetic findings clearly indicate intraspecific karyotype variation in *M. dogramacii*. In light of these results, *M. dogramacii* may be undergoing a chromosomal speciation process due to the karyotypic rearrangement resulting from pericentric inversion.

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