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Distribution of constitutive heterochromatin and nucleolar organizer regions (NORs) in *Mustela nivalis* Linnaeus, 1766 (Carnivora, Mustelidae) in Turkey

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C- and Ag-NOR banding of the least weasel, *Mustela nivalis*, from the central and Mediterranean regions of Turkey was examined for the first time in this study. The chromosome set was composed of 18 biarmed and three acrocentric autosome pairs. The X chromosome was a medium-sized submetacentric, while the Y was a small metacentric. A distinct secondary constriction was observed on the juxta centromeric region of the long arm of an autosome that was negatively C-banded. Large heterochromatic C-blocks, stained densely, were detected in six biarmed pairs, while ones stained faintly were recorded in four biarmed autosome pairs. Active NORs were located in the secondary constriction of the acrocentric autosome. The numbers of C-banded autosomes of Turkish specimens were similar to those reported previously from the Palearctic region.

Keywords: C-banding; Ag-NOR banding; heterochromatin; *Mustela nivalis*; Turkey

Introduction

Mustela is a speciose genus of the family Mustelidae and is represented by 17 species (Abramov 2000; Wozencraft 2005). Of these, the least weasel, *Mustela nivalis* (Linnaeus, 1776), is widely distributed in the Holoarctic region including most of Europe, North Africa, North America, and Asia as well as on many islands. The detailed taxonomic status of the species is discussed by Abramov and Baryshnikov (2000). Mandahl and Fredga (1980) examined the chromosomes of two subspecies, *M. n. vulgaris* (weasel) from southern Sweden and *M. n. nivalis* (pygmy weasel) from northern Sweden. However, recently Wozencraft (2005) recorded the subspecies *vulgaris* as a synonym of *M. nivalis*. An increase in the length of the body from north to south is reported for the least weasel (Abramov and Baryshnikov 2000; Broekhuizen et al. 2007).

Many studies regarding the morphological, cranial, bacular, taxonomic, cytogenetic, and biochemical characteristics of the genus *Mustela* in the Palearctic region have been performed by various authors as summarized in Abramov (2000). Individual, seasonal, and geographic variations were detected in *M. nivalis* (Zima and Cenevová 2002). The winter and summer coat coloration of the least weasel was reviewed and examined by Abramov (2000) and Zima and Cenevová (2002), respectively. Summer pelage of the species was divided into *nivalis* and *vulgaris* type by the authors. According to Zima and Cenevová (2002), only *vulgaris* type summer coat coloration is observed in specimens in the Czech Republic.

The diploid chromosome number in the genus *Mustela* ranges between $2n = 38$ and $2n = 44$ (Mandahl and Fredga 1980). The karyotype of *Mustela nivalis* in the Palearctic region was examined and reviewed by Fredga and Mandahl (1973), Graphodatsky et al. (1977), Mandahl and Fredga (1980), Jarrel (1983), Zima and Král (1984), Zima and Graphodatsky (1985), Peshev et al. (1985), Obara (1991), Baker et al. (1996), Zima and Cenevová (2002), and Lin et al. (2010). According to Mandahl and Fredga (1980), Zima and Cenevová (2002), and Franco-de-Sa et al. (2007), the karyotype of the mustelid species was polytypic with regard to the diploid number of autosomes.

The first record of the species in Turkey was given by Danford and Alston (1877) from the Taurus mountains. According to Çolak et al. (1999), *Mustela nivalis* occurs in many parts of Turkey. In addition, the first cytogenetic study based on a conventionally stained karyotype was carried out by these authors. The diploid number, fundamental number, and number of autosomal arms of the karyotype were found to be $2n = 42$, NF = 76, and NFa = 72, respectively.

The aims of the present study were to examine the C- and Ag-NOR banding of *Mustela nivalis* from the central and Mediterranean regions of Turkey and to determine the distribution of the constitutive heterochromatin and active Ag-NORs in the karyotype of Turkish specimens.

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Material and methods

Three male least weasel specimens from the central and Mediterranean regions of Turkey were karyologically studied. A male specimen examined from Isparta province (district Aksu, 37°47′56.74″ N, 31°04′22.02″ E) and two male specimens from Kırıkkale province (district Yahşihan, 39°51′01.00″ N, 33°27′13.00″ E) were examined. After peripheral blood was obtained, the specimens were released. Mitotic chromosomes were prepared from lymphocyte cultures according to Hillis et al. (1996). Constitutive heterochromatin and nucleolar organizer regions (NORs) were detected as described by Sumner (1972) and Howell and Black (1980), respectively. For each individual, 15 slides were prepared and at least 10 well-stained and C- and Ag-NOR banded metaphases were examined and photographed. The morphology of the chromosomes was arranged as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) in order of decreasing size. Centromeric index was calculated according to Levan et al. (1964). The slides are deposited at the Department of Biology, Faculty of Science, University of Kırıkkale.

Results

The least weasel showed a diploid number of $2n = 42$, including four metacentric, 14 submetacentric and subtelocentric, and three acrocentric autosomal pairs, as well as a large submetacentric X chromosome and a small metacentric Y chromosome. The Y chromosome was the smallest one of the set. The fundamental number (NF) was 78 and the number of autosomal arms (NFa) was 74. One of the acrocentric chromosomes (no. 17) possessed a distinct heteromorphic secondary constriction in all examined metaphase plates (Figure 1).

The quantity of C-bands in the chromosomes was variable. Large constitutive heterochromatic short arms were detected in six biarmed pairs (nos. 1, 2, 4, 6, 8, and 11). In addition, faint telomeric C- positive bands were also observed in the short arms of four submetacentric and subtelocentric pairs (Nos. 5, 7, 12, and 13). Faint pericentromeric C-positive bands were detected in the X chromosome, while the Y chromosome was entirely heterochromatic but C-band staining was less dense than it was in autosome pairs. However, the secondary constriction was C-banded negative (Figure 2).

Active Ag-NORs were located in the secondary constriction of the acrocentric autosome (Figure 2).

Discussion

Peshev et al. (1985) and Baker et al. (1996) determined the karyotypes of Bulgarian and Belarusian specimens, and the 2*n*, NF and NFa of the karyotypes were found to be 42, 84, and 80, respectively. No acrocentric chromosomes were reported in the chromosome sets. Abramov and Baryshnikov (2000) recorded that the higher NF and NFa of the karyotypes were probably due to the additional heterochromatic arms. In addition, Zima and Cenevová (2002) stated that karyotypic divergence between weasel populations revealed a polytypic heterochromatin variation. According to Graphodatsky et al. (1977), Zima and Graphodatsky (1985), and Obara (1985), changes in constitutive heterochromatin amount, Robertsonian fusions, and tandem translocations played a major role in the karyotypic evolution of the family Mustelidae.

The first karyological study of *Mustela nivalis* distributed in Turkey was conducted by Çolak et al. (1999). The chromosome set was composed of 16 pairs of

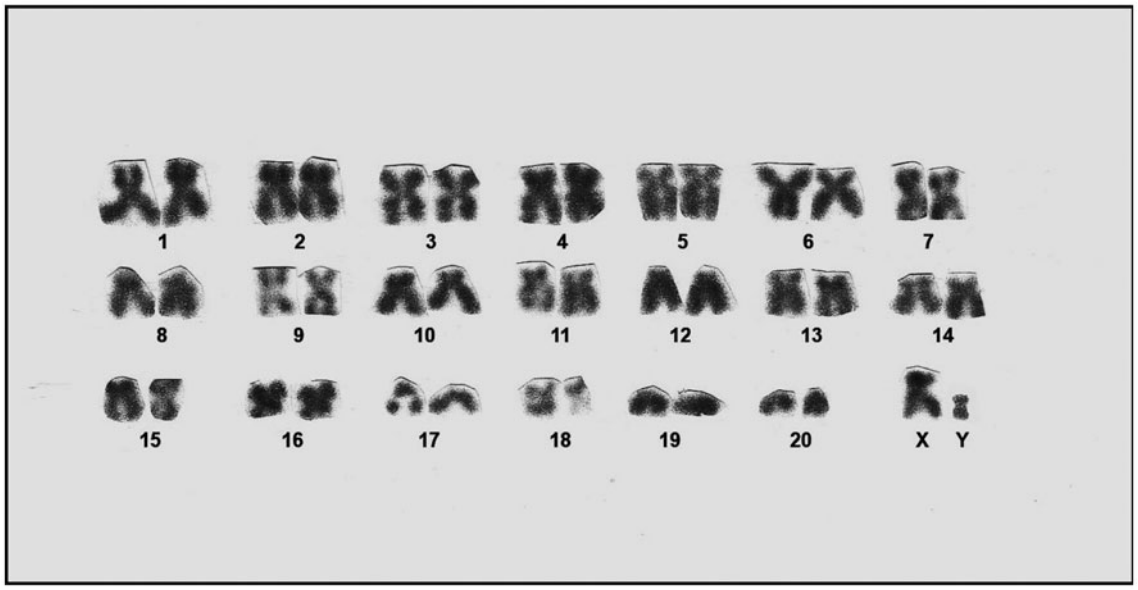

Figure 1. Conventionally Giemsa stained karyotype of a male *Mustela nivalis* from Central Anatolia.

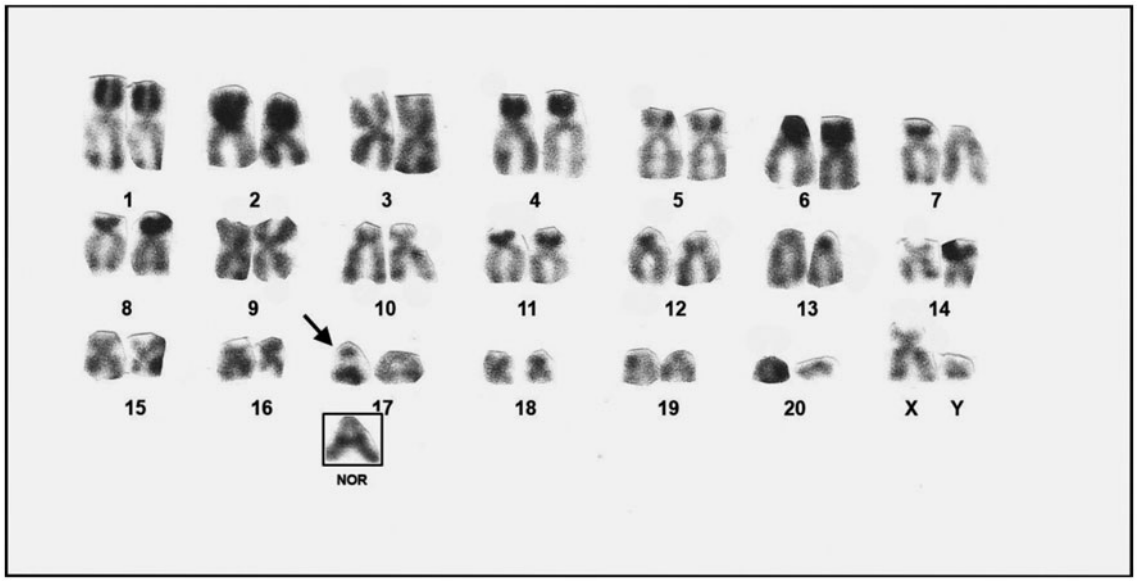

Figure 2. C-banded karyotype of a male *Mustela nivalis* from Mediterranean region of Turkey. The arrow indicates the secondary constriction in one autosome no 17. The frame indicates the NOR bearing chromosome with secondary constriction.

biarmed and four pairs of acrocentric chromosomes. The X chromosome was a submetacentric, while the Y was a small metacentric. However, the karyotype of the species was given in the form of a hand-drawn illustration; therefore, the secondary constriction was not shown in the ideogram. In addition, the dissimilarity in the number of acrocentric autosome pairs between the authors' study and this study was probably due to the different methods used for classification of the chromosomes.

According to Zima and Cenevová (2002), 5–7 autosomal pairs possessing large heterochromatic arms were recorded from Sweden, Siberia, Japan, Alaska, and Slovakia. Dense and distinct C-positive bands were determined in six biarmed chromosome pairs from the Czech Republic and Turkish Thrace. In addition, faint telomeric C-bands were also detected in the short arm of two subtelocentric autosomes. An intercalar C-positive band was also recorded in the short arm of one metacentric pair by the authors. The karyotypes reported from Central Europe and Istranca Mountains are rather similar to the complement described in the present study with respect to basic karyotypic characteristics (2*n*, the shape of X and Y chromosomes, the number of whole heterochromatic arms). However, in contrast to Zima and Cenevová (2002), dark telomeric C-bands in some autosomes (nos. 2 and 4) were recorded in this study and we also did not detect an intercalar C-positive band in the set.

Homomorphic or heteromorphic secondary constrictions were recorded for various species of the genus *Mustela* (Fredga and Mandahl 1973; Graphodatsky et al. 1976, 1977; Graphodatsky and Ternovskaya 1977; Graphodatsky and Radjabli 1980; Obara 1985; Kurose et al. 2000; Zima and Cenevová 2002; Lin et al. 2010). The secondary constrictions of *Mustela nivalis* determined by Mandahl and Fredga (1980), Zima and Cenevová (2002) and Lin et al. (2010) were homomorphic and located on the acrocentric chromosome no. 14 or 18. In contrast, the secondary constrictions of Turkish specimens were heteromorphic and located on acrocentric chromosome no. 17.

Furthermore, in agreement with Mandahl and Fredga (1980), we determined active NORs located in the secondary constriction in this study. Although Ag-NORs can be variable, even in specimens within a population, and Ag-staining does not always reveal active NORs as stated by Mandal and Fredga (1980) and Dobigny et al. (2002), the number of NOR-bearing chromosomes is still frequently used in the cytotaxonomy of mammalian species.

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