



Banded Karyotype Features of the Bitlis Chromosome Race ($2n=54$) of *Nannospalax xanthodon* (Nordmann, 1840) (Rodentia: Spalacidae) in Türkiye

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Abstract: *Nannospalax xanthodon* has five chromosome races of $2n=54$ in Türkiye. Until now, only C-banding and Ag-NOR staining analyses of Kırıkkale specimens of Yozgat chromosome race have been performed. In this study, we presented the karyotype characteristics of the Bitlis chromosome race with C-banding and Ag-NOR staining analyses. The numbers of bi-armed chromosomes in set of this race were found to be nine pairs and the numbers of acrocentric chromosomes were 16 pairs. This result is compatible with data on the previously studied Tatvan specimen. However, the Y chromosome of our specimens is different from that of the Tatvan specimen of this race. The Y chromosome is medium-sized subtelocentric (NFa=70). The dark centromeric C-bands were observed in all bi-armed and some acrocentric autosomes. The X chromosome had a centromeric band, while the Y chromosome was stained C-negative. The active NORs were determined in the telomeric regions of the short arms of two autosomes. The distribution pattern of the centromeric positive C-bands was generally similar between the Bitlis and Yozgat races. However, there were no C-heterochromatic short arms in the Bitlis race. The number and position of active NORs in both races were different.

Key words: mole-rat, Ag-NOR staining, C-band, Anatolia

Introduction

The species of the genus *Nannospalax* Palmer, 1903 are highly variable in relation to the karyotype structure and have 73 different chromosomal races. *Nannospalax xanthodon* (Nordmann, 1840) is distributed throughout most of Anatolia; according to ARSLAN et al. (2016), this species is represented by 28 chromosome races, with diploid numbers $2n=36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 60$. Additionally, with the

determination of a new $2n=58$ race (Uğurlu) from Karaman (AYBAKIR et al. 2021), the number of chromosome races in this taxon increased to 29. There are five chromosome races (Eflani, Yozgat, Tuncellicus, Bitlis and Adana) within the diploid chromosome number of $2n=54$ (ARSLAN et al. 2016). These chromosome races have been previously investigated by different researchers (SÖZEN 2004, SÖZEN et al. 2006, NEVO et al. 1995, COŞKUN 2004, COŞKUN et al. 2009, 2010, SÖZEN et al. 2015). The karyotypes

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of chromosome races with $2n=54$ vary in the proportion of bi-armed and acrocentric autosomes or in the morphology of marker chromosomes. While there are eight bi-armed chromosome pairs in the set of the Eflani race, there are nine bi-armed chromosome pairs in other races. Their NF value is 72–74. The complement of the Bitlis race includes a single large bi-armed marker autosome, whereas the Yozgat and Tuncelicus race has two distinctly large bi-armed pairs. The Adana race has no distinctively large bi-armed autosomes (COŞKUN 2004, SÖZEN et al. 2015, ARSLAN et al. 2016). Among the chromosome races, the C- and Ag-NOR banded chromosome features of the Yozgat chromosome race have been revealed with the studied Kırıkkale specimens (ARSLAN et al. 2011b). However, information about the different stained chromosomes and the detailed structure of the karyotype of the Bitlis race is still lacking.

The aim of this study is to provide a detailed description of the distribution pattern of Ag-NORs and C-heterochromatin regions in the karyotypes of mole-rats in Bitlis.

Materials and Methods

Cytogenetic analyses were performed in two specimens of mole-rats from Bitlis. The specimens were caught with Permission no. E-21264211-288.04-9978365 issued by the Ministry of Forest and Water Works of the Republic of Turkey. The specimens were caught using ARSLAN's (2013) metal tube type trap. Karyotype preparations were obtained in the field from the bone marrow after 0.025 % colchicine solution treatment (FORD & HAMERTON 1956). Air-dried slides were stained by Giemsa. Constitutive heterochromatin bands and nucleolus organizer regions (NORs) were detected according to SUMNER (1972) and HOWELL & BLACK (1980), respectively. A total of ten to 20 slides were prepared from each specimen, and at least 20 well-spread metaphase plates were analysed. The arrangement of chromosomes was made according the procedures described in previous articles (ARSLAN et al. 2011a, IVANITSKAYA et al. 1997, 2008). Slides are deposited at the Selçuk University, Biology Department, Faculty of Science, Konya, Türkiye.

Results

The karyotype of two male mole-rats from Bitlis consisted of 54 chromosomes, including a distinctly large acrocentric (no. 1), a large submetacentric (no. 2), eight medium-sized bi-armed (nos. 3-10) and 16 acrocentric autosomal pairs of gradually diminishing

size (nos. 11-26) ($NFa = 70$). The large acrocentric autosomal pairs, which can be reliably recognised, were arranged as the first in the complement. The other bi-armed and acrocentric autosomes were arranged according to their size, respectively. The X chromosome was a large submetacentric and Y-chromosome medium-sized subtelocentric ($NF = 74$) (Fig. 1).

The dark centromeric C-bands were observed in nine bi-armed (nos. 2-10) and two acrocentric autosomes (nos. 11, 12). The X chromosome had a distinct centromeric band, and the Y chromosome was stained C-negatively (Fig. 2).

NORs were observed in the telomeric regions of the short arms of the autosomes 3 and 5. In one of the pairs (no. 5), the positive signal was detected in only one homologue (Fig. 3).

Discussion

N. xanthodon has five chromosome races in Türkiye with a diploid chromosome number of $2n=54$ (ARSLAN et al. 2016). The complements of these races, with the exception of the Eflani, contain nine pairs of bi-armed chromosomes. Eflani race contains eight pairs of bi-armed chromosomes. The morphologies of the bi-armed chromosomes are also different among these races. The bi-armed large marker chromosome pair is two in all, except for the Bitlis race. This marker chromosome was not found in the Adana race (ARSLAN et al. 2016). The Bitlis race was previously reported by COŞKUN et al. (2009) from Tatvan (Bitlis). Our specimens have one pair of large submetacentric chromosomes, nine pairs of bi-armed chromosomes and one large acrocentric chromosome, all features that are standard characteristics of the Tatvan specimens. However, the Y chromosome of our specimens is different from the small acrocentric Y chromosome of the Tatvan specimens. The Y chromosome is small subtelocentric or acrocentric in the Eflani, Yozgat and Tuncelicus races. In our specimens, the X chromosome is similar to those other races, where is medium-sized or large submetacentric. The Y chromosome of mole-rats in Türkiye is generally small acrocentric. Similarly to the present results, some chromosomal races have a medium-sized or Y chromosome as large as the X chromosome (ARSLAN et al. 2016). E.g., Y-chromosomes of the Turcicus race ($2n=56$) (*N. leucodon*), Yirce race ($2n=46$), Kula race ($2n=56$) and the Vasvari race ($2n=60$) are larger than those of other races (ARSLAN et al. 2011a, 2014 a, b). Variations among these geographically distant $2n=54$ chromosome races can be caused by a variety of reasons. The mechanisms of this variation are

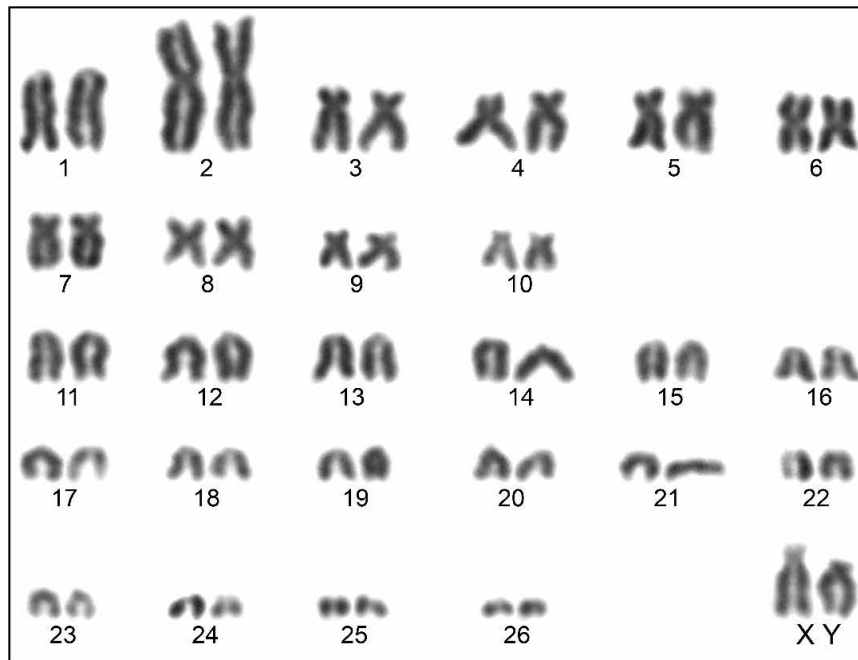


Fig. 1. Standard karyotype of Bitlis chromosome race.

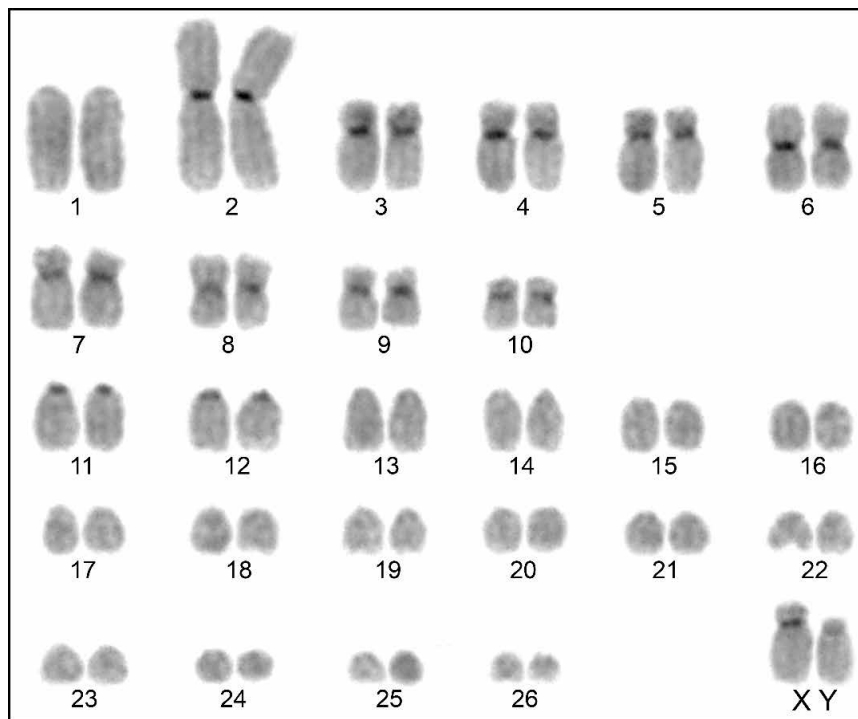


Fig. 2. C-banded karyotype of Bitlis chromosome race.

not always clear and various types of chromosomal rearrangements may be involved. Moreover, differences in reported values of NFA may also be due to subjective assessment of chromosome morphology by individual authors.

Banded karyotype characteristics of chromosomal races of mole-rats have been studied by differ-

ent researchers (for review, see ARSLAN et al. 2016). Among these, the C-banded and Ag-NOR staining characteristics of the Yozgat race from Kırıkkale only were determined (ARSLAN et al. 2011b). There are some differences between the standard karyotypes of these two races. The largest chromosome in the Bitlis race is submetacentric (no. 2), while it is sub-

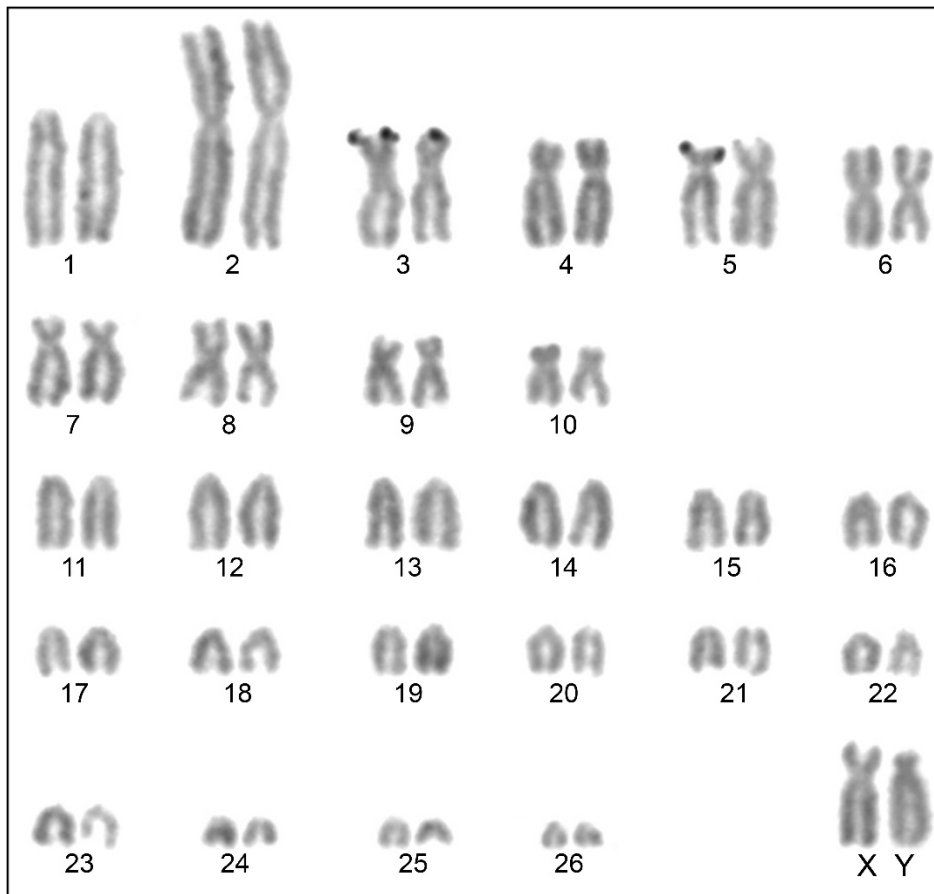


Fig. 3. Silver-stained karyotype of Bitlis chromosome race.

telocentric in the Yozgat race. In addition, the largest acrocentric chromosome (no. 1) in the chromosome set of the Bitlis race is not found in the Yozgat race. The heterochromatin distribution in both autosomes and sex chromosomes of the Bitlis chromosome race is similar to the characteristics of the Yozgat race. However, there are no C-heterochromatic short arms in the Bitlis race. The number of active NORs in the Bitlis race is lower than that in the Yozgat race. Moreover, the active NORs in the Bitlis race are not associated with heterochromatin regions as it is in the Yozgat race. The number of active NORs is highly variable within mole-rats. The published reports show that Ag-NOR regions were observed in the telomeric areas of the short arms of two, three, four or five pairs of chromosomes in complements found in the chromosome races of various *N. xanthodon* populations in Türkiye (ARSLAN & ZIMA 2013, 2015, 2017, ARSLAN et al. 2014a, b, 2016).

The diversity of the karyotypic variations among species and genera of blind mole-rats is still remarkable. Some differences between races belonging to the same diploid chromosome forms, as in this study, have recently been detected in the banded kar-

yotype studies. In comprehensive studies conducted in Türkiye, it has been assumed that one of the main regions of these chromosome differences is Anatolia (ARSLAN et al. 2016). SAVIĆ & SOLDATOVIĆ (1979) argued that karyotype changes in Balkan species occur due to Robertsonian fusion; as a result, these rearrangements are reflected as a decrease in the number of acrocentric autosomes and diploid values. IVANITSKAYA et al. (2005) also argued that chromosomal fusion is responsible for karyotype change in mole-rats. Contrary to these statements, NEVO et al. (1994, 2000) suggested that mole-rats in Israel and Türkiye have increased diploid chromosomes due to chromosomal rearrangements.

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