Heterochromatin distribution and localization of NORs in the 2n = 48 cytotypes of Nannospalax xanthodon and N. ehrenbergi

Atilla ARSLAN1,*, Jan ZIMA2
1Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey
2Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Received: 22.02.2016 • Accepted/Published Online: 22.10.2016 • Final Version: 23.05.2017

Abstract: We compared the C-banding pattern and the NOR distribution in 3 populations of N. xanthodon and N. ehrenbergi with 48 chromosomes from Turkey, with the aim to reveal variations between and within species. We have found distinct variation in the C-banding and the NOR distribution pattern among the 3 populations compared; each of them possessed its own specific chromosomal features. However, it is not possible to distinguish between intra- and interspecific variation, and the observed patterns cannot be utilized for unequivocal species recognition.

Key words: Karyotype, chromosomal races, mole rats, Anatolia, Turkey

1. Introduction
The blind mole rats of the subfamily Spalacinae are remarkable because of extensive karyotypic variation, which produces many different chromosomal races or cytotypes. The core area of this variation within the subgenus Nannospalax (for current taxonomy, see Musser and Carleton, 2005; Kryštufek and Vohralík, 2009) is Asia Minor. In this area, 2 species are usually reported to occur, Nannospalax xanthodon and N. ehrenbergi, and 30 to 40 distinct cytotypes can be differentiated within them (Arslan and Zima, 2014; Arslan et al., 2016).

Both species have cytotypes possessing 48 chromosomes. The 48-chromosome populations of N. xanthodon were recorded in eastern Anatolia, in the provinces of Ağrı, Van, Muş, Iğdır, Erzurum, and Gümüşhane (Coşkun, 2003; Coşkun et al., 2009, 2012; Coşkun and Kaya, 2013; Sözen et al., 2006). This cytotype apparently occurs also in neighboring Armenia; it was recorded there in one of the earliest papers dealing with blind mole rat karyology (Matthey, 1959). Populations with the same diploid chromosome number were also found within the range of N. ehrenbergi in the southernmost parts of Asiatic Turkey (Hatay), between the Syrian border and the coast of the Mediterranean Sea (Coşkun, 2004).

In previous papers, we compared the C-banding pattern and the NOR distribution in allopatrically distributed cytotypes of blind mole rats sharing the same diploid number. We found that the karyotype structure revealed by banding can be differentiated between cytotypes belonging to different species (N. leucodon and N. xanthodon with 2n = 56, Arslan et al., 2014), but this interspecific variation was relatively small (N. xanthodon and N. ehrenbergi with 2n = 52, Arslan and Zima, 2015). Distinct differences were further demonstrated in comparisons of allopatric cytotypes with the same chromosome number within a single species (N. xanthodon with 2n = 58, Arslan and Zima, 2013; N. xanthodon with 2n = 50, Arslan and Zima, 2015).

In this study, we compare the C-banding pattern and the NOR distribution in 3 populations of N. xanthodon and N. ehrenbergi with 48 chromosomes from Turkey, with the aim to reveal variations between and within species.

2. Materials and methods
Cytogenetic analyses were performed in 6 specimens of N. xanthodon and N. ehrenbergi originating from Turkish populations collected in 3 localities. The specimens were caught with a metal pipe-type trap (Arslan, 2013), which enables obtaining live individuals without any injury. The number of specimens analyzed and the locations of the collection sites of the mole rats are shown in Figure 1 and the Table. Standard voucher specimens (skins and skulls) are deposited at Selçuk University, Department of Biology, Faculty of Science, Konya, Turkey.

Karyotype preparations were obtained in the field from bone marrow after colchicine treatment (Ford...
Air-dried preparations were stained conventionally using Giemsa stain. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by the techniques of C-banding (Sumner, 1972) and silver staining of nucleolar organizer regions (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 system of classification of chromosomes according to the centromere position was adopted after Hsu and Benirschke (1967–1977), and the biarmed (metacentric – M, submetacentric – SM, subtelocentric – ST) and uniarmed (acrocentric – A) chromosomes were distinguished. The fundamental number of autosomal arms (N Fa) and the number of all chromosomal arms in the female complement (NF) were calculated. We followed the arrangement of chromosomes in the karyotype applied in some previous papers (Ivanitskaya et al., 1997, 2008; Arslan et al., 2011).

Table. Localities studied in Turkey. The numbering of the sampling sites corresponds to Figure 1. See Section 2 for abbreviations.

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Locality/province</th>
<th>Latitude, longitude</th>
<th>No. of specimens</th>
<th>2n</th>
<th>NF</th>
<th>N Fa</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>N. xanthodon</em></td>
<td>Şamanlı/Gümüşhane</td>
<td>40°36′N, 39°28′E</td>
<td>1</td>
<td>1</td>
<td>48</td>
<td>70</td>
<td>66</td>
<td>SM</td>
</tr>
<tr>
<td>2</td>
<td><em>N. xanthodon</em></td>
<td>Malazgirt/Muş</td>
<td>39°08′N, 42°26′E</td>
<td>2</td>
<td>-</td>
<td>48</td>
<td>72</td>
<td>68</td>
<td>SM</td>
</tr>
<tr>
<td>3</td>
<td><em>N. ehrenbergi</em></td>
<td>Yayladağı/Hatay</td>
<td>35°53′N, 36°05′E</td>
<td>2</td>
<td>-</td>
<td>48</td>
<td>73</td>
<td>69</td>
<td>M</td>
</tr>
</tbody>
</table>
3. Results

3.1. Şamanlı population, Gümüşhane Province

The karyotype of 1 male and 1 female of *N. xanthodon* consisted of 48 chromosomes, including a distinctly large acrocentric (no. 1), a large subtelocentric (2), a large submetacentric (3), 8 medium-sized biarmed (4–11), and 12 acrocentric autosomal pairs of gradually diminishing size (12–23) (NFa = 66). The X chromosome was large submetacentric (NF = 70); the Y small acrocentric (Figure 2, inset 1). Dark centromeric C-bands were observed in 10 biarmed (2 to 11) and some acrocentric autosomes (1 and 17). In the metacentric autosomal pair 6, dark C-staining included a large proximal part of the long arm. C-heterochromatic short arms were not found in any autosomal pair. The X chromosome possessed a centromeric C-positive band, and the Y chromosome was entirely C-positive (Figure 2, inset 2). NORs were observed in the telomeric regions of the short arms of autosomes 1, 2, 5, and 9 (Figure 2, inset 3).

3.2. Malazgirt population, Muş Province

The karyotype of the 2 males of *N. xanthodon* consisted of 48 chromosomes, including a distinctly large acrocentric (no. 1), a large subtelocentric (2), a large submetacentric (3), 9 medium-sized biarmed (4–12), and 11 acrocentric autosomal pairs of gradually diminishing size (13–23) (NFa = 68). The X chromosome was large submetacentric (NF = 72); the Y small acrocentric (Figure 3, inset 1). Distinct dark C-bands were observed in the centromeric or pericentromeric areas of all of the biarmed autosomes. C-heterochromatic short arms were found in 2 autosomal pairs (2, 9). In the metacentric autosomal pair 6, a large dark C-band appeared in the proximal part of the long arm. An interstitial dark C-band was apparent also on the long arm of the acrocentric autosomal pair 17. Tiny centromeric bands were observed in some acrocentric pairs, but no positive C-staining could be found in pairs 20, 22, and 23. The X chromosome had a centromeric C-positive area; the Y chromosome was entirely C-positive (Figure 3, inset 2). NORs were observed in the telomeric regions of the short heterochromatic arms of autosomes 2 and 9 (Figure 3, inset 3).

3.3. Yayladağı population, Hatay Province

The karyotype of the 2 males of *N. ehrenbergi* consisted of 48 chromosomes, including a distinctly large acrocentric (no. 1), a large subtelocentric (2), a large submetacentric (3), 9 medium-sized submetacentric or subtelocentric (4–12), and 11 acrocentric autosomal pairs of gradually diminishing size (13–23). The X chromosome was large submetacentric; the Y was small meta- or submetacentric. The autosomal pair 15 was heteromorphic in both studied specimens, consisting of a subtelocentric and an acrocentric chromosome (NFa = 69, NF = 73) (Figure 4, inset 1). The dark C-bands were observed in the centromeric or pericentromeric areas of 5 biarmed (3–6 and 8) and all acrocentric autosomes. The subtelocentric pair 2 had only a telomeric C-band on the short arm, and the submetacentric pair 6 had an interstitial C-band on the long arm. The subtelocentric element from the heteromorphic autosomal pair 15 was almost entirely C-positive, except for a narrow distal region on the long arm; only a centromeric dark C-band was observed in its homologue. The X chromosome revealed no positive band, and the Y chromosome had a dark centromeric C-band (Figure 4, inset 2). NORs were observed in the telomeric regions of the C-heterochromatic short arms of autosomes 2, 9 and 15. The acrocentric homologue of pair 15 showed no AgNOR-positive staining. In pair 9, the positive signal was observed in only 1 homologue of the pair in some cells (Figure 4, inset 3).

4. Discussion

The numbers of chromosomal arms differed among populations despite the identical 2n. We found that variation between the conventionally stained karyotypes was manifested mainly in the different proportion of biarmed and acrocentric autosomes. Variations in the fundamental number of autosomal arms ascertained in the present paper fall within the extent reported in both *N. xanthodon* and *N. ehrenbergi*. Previous studies of Turkish blind mole rat populations with 48 chromosomes revealed the presence of 9, 10, or 11 pairs of biarmed autosomes in populations of *N. xanthodon*, which resulted in variation in NFa from 64 to 68 (Coşkun, 2003; Sözen et al., 2006; Coşkun et al., 2009, 2012). In a population of *N. ehrenbergi* from Yayladağı, 12 pairs of biarmed autosomes were distinguished with resulting NFa = 70 (Coşkun, 2004). The presence of a heteromorphic pair in the studied specimens of *N. ehrenbergi* suggests heterochromatin changes as possible mechanisms that may produce this kind of variation.

Among the samples studied, distinct variation was observed in the number of C-positive areas and their positions. The C-banding pattern revealed in the studied samples is generally similar to other published data on C-banded karyotypes in Turkish blind mole rats (Ivanitskaya et al., 1997, 2008; Arslan et al., 2014; Arslan and Zima, 2015a, 2015b). C-heterochromatin mostly occurs in the centromeric chromosome areas, whereas the occurrence of whole-heterochromatic short arms or interstitial bands is rare. An unusual case of variation was revealed in the compared samples in the C-banding of autosome 6. In both samples of *N. xanthodon*, a relatively large block of C-heterochromatin occurred in the proximal part and the centromeric area of the long arm. In the sample of *N. ehrenbergi*, this conspicuous block was
Figure 2. Standard karyotype (1), C-banded karyotype (2), and silver-stained karyotype (3) of Nannospalax xanthodon from Şamanlı.
Figure 3. Standard karyotype (1), C-banded karyotype (2), and silver-stained karyotype (3) of Nannospalax xanthodon from Malazgirt.
Figure 4. Standard karyotype (1), C-banded karyotype (2), and silver-stained karyotype (3) of *Nannospalax ehrenbergi* from Yayladağı. The heteromorphic chromosome pair is within the frame.
divided into distinct centromeric and intercalary dark bands.

Two, 3, or 4 chromosomes bearing the positive NOR signal were found in karyotypes of the 3 populations studied. Two NOR-bearing autosomes (pairs 2 and 9) were probably identical in all specimens and populations; the other NORs produced the difference between samples. The observed pattern of variation is consistent with previous data on the NOR distribution in blind mole rat populations from Anatolia (Ivanitskaya et al., 1997, 2008; Arslan and Zima, 2015a, 2015b). The number of NOR found per individual usually varied from 2 to 5, and their terminal position on the short arm was quite common.

The recorded karyotypes of 48 chromosome populations of N. xanthodon and N. ehrenbergi were remarkably similar, in spite of the very large geographic distance that separates them. The overall similarity of basic karyotype characteristics contrasted with the distinct variation that we found in the C-banding and the NOR distribution patterns among the 3 populations compared. Each of these populations possessed its own specific chromosomal features. However, it was not possible to distinguish between intra- and interspecific variation, and the observed patterns could hardly be utilized for unequivocal species recognition.

Acknowledgment
This study was supported by grants from the Coordination Committee of Scientific Research Projects (BAP No: 16401010) of Selçuk University.

References