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Comparison of the chromosome banding patterns in *Dryomys laniger* and *D. nitedula* from Turkey

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Abstract: The karyotypes of *Dryomys laniger* and *D. nitedula* from Turkey were studied using C-banding and AgNOR staining. The standard karyotypes found in both species were fairly similar to previously published data (2n = 46, NF = 92 in *D. laniger*; 2n = 48, NF = 96 in *D. nitedula*). The C-banding pattern revealed a relatively small amount of heterochromatin in both karyotypes and C-heterochromatin was concentrated at centromeric areas of most autosomes and the X chromosome. Heterochromatin changes have apparently not been responsible for karyotypic divergences between the studied species. The AgNORs were recorded in the pericentromeric region of two autosome pairs in the complement of *D. laniger*, and at a single autosome pair of *D. nitedula*. The complement of *D. laniger* could be derived from that of *D. nitedula* after a tandem fusion of two autosomal pairs, and the assumed rearrangement also included the NOR region.

Key words: Dormice, karyotype, C-banding, AgNOR staining

1. Introduction

Dormice or glirids (Gliridae, Rodentia) represent one of the oldest rodent families with apparently ancient phylogenetic roots (Holden, 2005). Several species of glirids are currently living in Turkey, including newly discovered or recognized *Myomimus roachi*, *M. setzeri*, and *Dryomys laniger* (Kryštufek and Vohralík, 2005; Yiğit et al., 2006). *D. laniger* is one of the endemic species in Turkey. The distinct phylogenetic distances between the extant species of glirids are apparent also in differentiation of their chromosomal complements, which are usually specific and different between individual taxa.

The karyotypes of glirids from the Palearctic region are relatively well known and have been investigated in various regions (Zima and Král, 1984; Zima et al., 1995 for review). The glirid fauna of Turkey has also been karyologically studied rather intensively (Doğramacı and Kefelioğlu, 1990, 1992; Doğramacı and Tez, 1991; Civitelli et al., 1995; Kıvanç et al., 1997; Şekeroğlu and Şekeroğlu, 2011; Şekeroğlu et al., 2011; Arslan et al., 2013), and the only species in which the karyotype remains unknown is *Myomimus setzeri* (Arslan and Zima, 2014).

In this study, we report chromosome banding patterns in two species of the genus *Dryomys*, i.e. the forest dormouse *Dryomys nitedula* (Pallas, 1778) and the woolly dormouse *Dryomys laniger* Felten & Storch, 1968. The karyotype of *D. nitedula* was studied in various parts of its geographic range in Europe and Asia (see Zima et al., 1995 for review), and there are also cytogenetic records derived from Turkish populations (Doğramacı and Kefelioğlu, 1990; Civitelli et al., 1995; Şekeroğlu and Şekeroğlu, 2011). The karyotype of *D. laniger* was investigated in a single study (Kıvanç et al., 1997), and only the conventionally stained karyotype was described. We use C-banding and AgNOR staining to contribute to a better understanding of cytogenetic characteristics in both species. This detailed description of karyotypic features further enabled us to propose possible mechanisms responsible for the chromosomal divergence between the two species.

2. Materials and methods

Cytogenetic analyses were performed in six specimens of *D. laniger* and *D. nitedula* caught with live traps from three Turkish populations. The number of specimens analyzed

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and location of the collection sites are shown in Figure 1 and the Table. Standard voucher specimens (skins and skulls) are deposited in the Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey.

Karyotype preparations were obtained from bone marrow of animals treated with colchicine (Ford and Hamerton, 1956). After preparation of chromosome slides, conventional Giemsa staining was carried out. Constitutive heterochromatin and nucleolus organizer regions (NORs) were then detected in individual autosome and sex chromosome pairs via C-banding (Sumner, 1972) and AgNOR 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 system of classification of chromosomes according to the centromere position was adopted after Hsu and Benirschke (1967–1977). The fundamental number of autosomal arms (NFa) and the number of all chromosomal arms in the female complement (NF) were calculated.

3. Results

The chromosome pairs in both species were arranged into four groups. The first group includes metacentric or submetacentric autosomal pairs, the second group submetacentric or subtelocentric autosomal pairs, and the third group subtelocentric or acrocentric autosomal pairs. The fourth group is represented by the sex chromosomes.

The species examined differ in the chromosome diploid number (2n = 46 in *D. laniger*, 2n = 48 in *D. nitedula*) as well as in the fundamental number of arms (NFa = 88, NF = 92 in *D. laniger*; NFa = 92, NF = 96 in *D. nitedula*). The autosomal pairs included in the first and the third groups are apparently similar in both species, whereas the composition of the second group of autosomes differs between them. The first group consists of two metacentric pair distinctly larger than the other pairs included in the group. There are further three medium-sized metacentric pairs and two small pairs with metacentric or

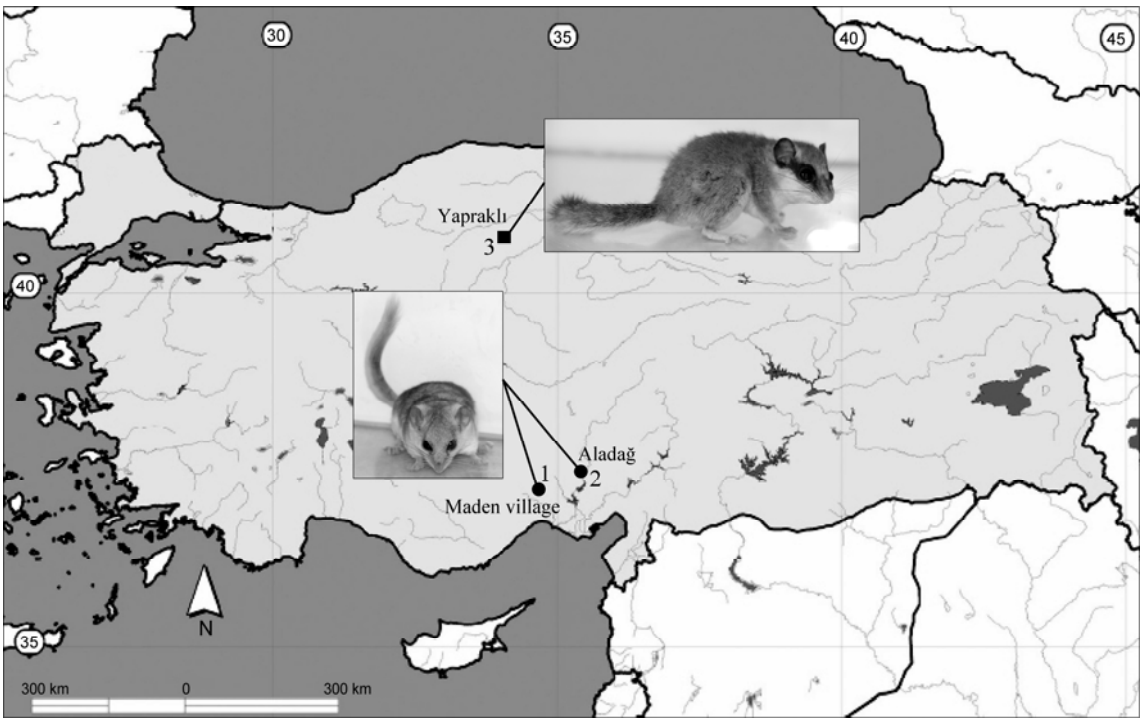

Figure 1. Collecting sites of *Dryomys laniger* (1, 2), and *Dryomys nitedula* (3) in Turkey. The numbering of sampling localities corresponds to data in the Table.

Table. Studied localities of two *Dryomys* species in Turkey. The numbering of the sampling sites corresponds to data in Figure 1.

No.	Species	No. of specimens		Locality/Province	Latitude, longitude	2n	NF	NFa	X	Y
		Male	Female							
1	*D. laniger*	2	-	Maden village / Niğde	37°27′N, 34°37′E	46	92	88	M	A
2	*D. laniger*	1	-	Aladağ / Adana	37°33′N, 35°23′E	46	92	88	M	A
3	*D. nitedula*	2	1	Yapraklı / Çankırı	40°45′N, 33°46′E	48	96	92	M	M

submetacentric position of the centromere. The number of autosomal pairs included in the second group differs between the species; 11 pairs are included in *D. laniger* and 12 pairs in *D. nitedula*. The largest pair of this group is submetacentric in *D. laniger* but metacentric, with a larger short arm, in *D. nitedula*. A small subtelocentric pair appears additionally in *D. nitedula*. The third group includes four medium-sized subtelocentric or acrocentric autosomal pairs (Figure 2). The short arms are always well visible in these pairs and they were included in calculation of the fundamental number. The chromosomes belonging to this group appear identical in the complements of both species. Secondary constrictions were observed in the short arm of autosomal pair no. 8 in the set of *D. laniger* and in the long arm of autosomal pair no. 18 in the set of *D. nitedula* (Figure 2).

The X chromosome is a large or medium-sized submetacentric or metacentric in both species. The Y chromosome is the smallest element of the complement in both species, and its centromeric position appears acrocentric in *D. laniger* and metacentric in *D. nitedula* (Figure 2).

The C-banding pattern reveals a relatively small amount of heterochromatin in both karyotypes. C-heterochromatin is concentrated at centromeric areas of most autosomes and the X chromosome. C-negative staining was detected in about seven autosomal pairs of *D. laniger* and in ten pairs of *D. nitedula*. The largest autosome (no. 8) of the second group in *D. laniger* reveals a distinct dark centromeric C-band but the first autosome of the same group in *D. nitedula* is stained negatively. The Y chromosome of *D. laniger* has a C-positively stained centromeric region, whereas the Y chromosome of *D. nitedula* is completely C-negative (Figure 3).

The AgNORs were recorded in the pericentromeric region of two autosome pairs (8, 19) in the complement

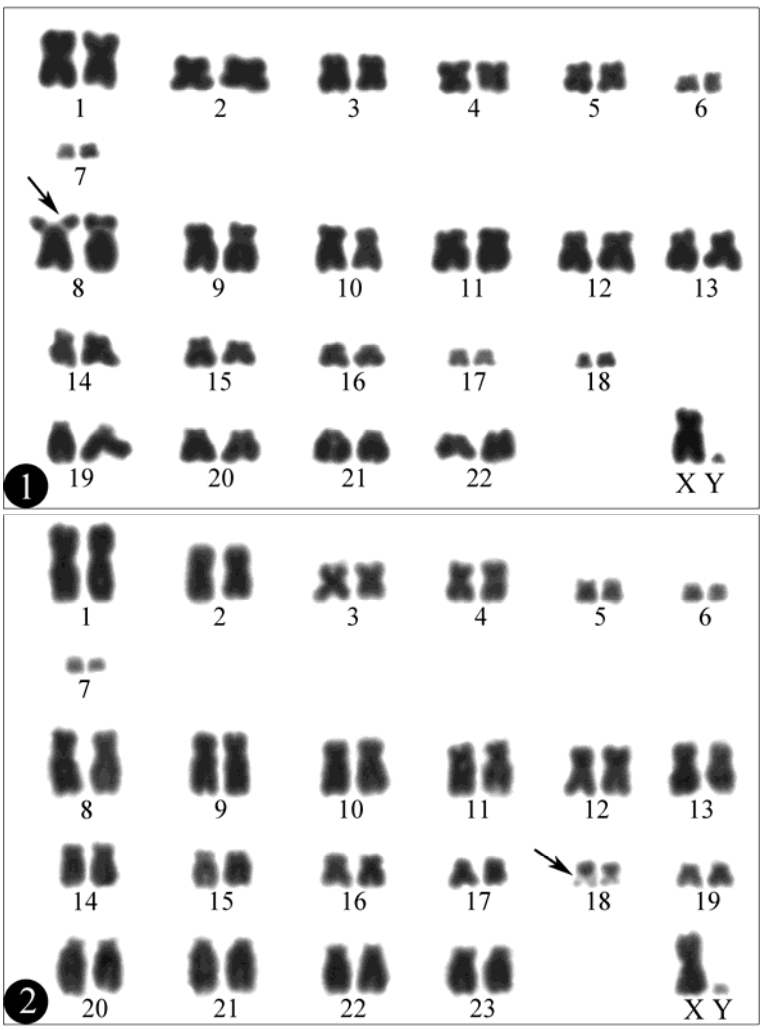

Figure 2. Standard karyotypes of *Dryomys laniger* (1) and *Dryomys nitedula* (2). Arrows indicate the position of secondary constrictions.

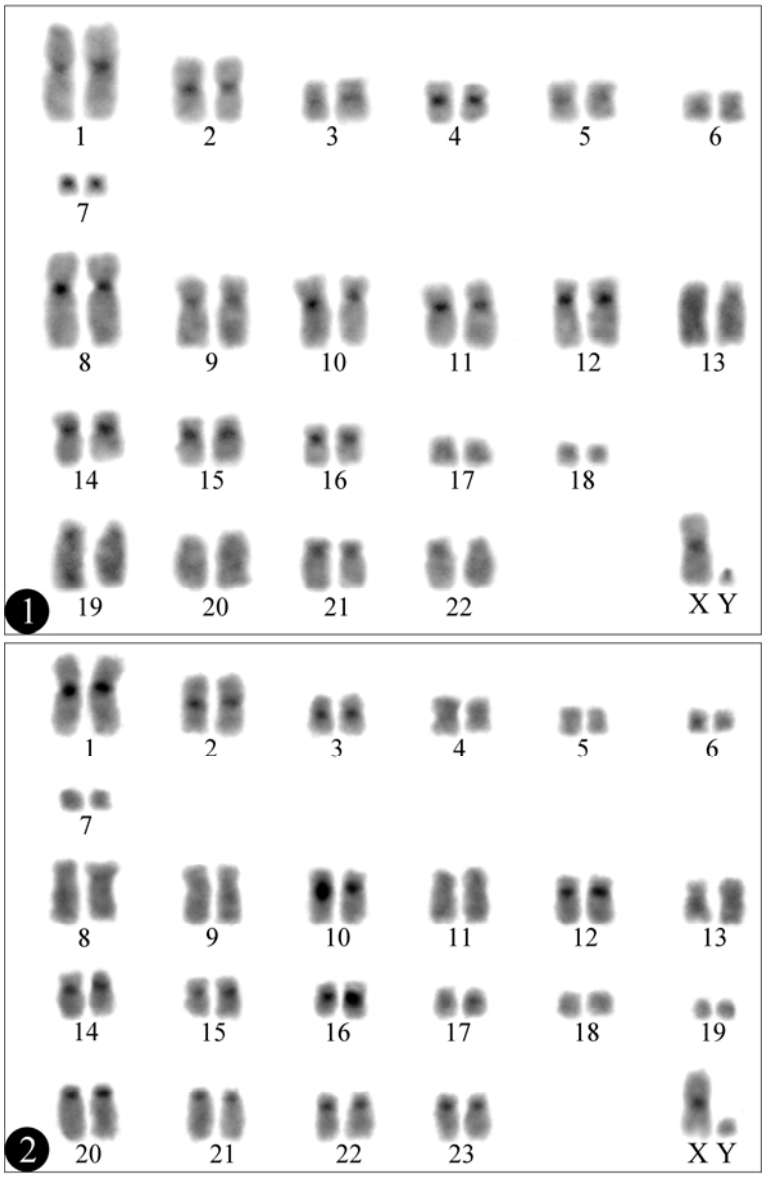

Figure 3. C-banded karyotypes of *Dryomys laniger* (1) and *Dryomys nitedula* (2).

of *D. laniger*. In some cells, only one homologue of pair 19 bore the positive silver signal. In *D. nitedula*, the AgNORs were localized interstitially in a single autosome pair no. 18 (Figure 4).

4. Discussion

The standard karyotypes recorded in the specimens examined are fairly similar to those described in previous papers studying Turkish or other geographic populations (Doğramacı and Kefelioğlu, 1990; Civitelli et al., 1995; Zima et al., 1995 for review; Şekeroğlu and Şekeroğlu, 2011). There is little intraspecific chromosomal variation reported in *D. nitedula*. Zima et al. (1995) found a pericentric inversion in an individual from the Lesser Caucasus and somatic cells mosaic 2n = 46/48 in an individual from Tajikistan.

In the C-banded karyotype of *D. nitedula*, we have not observed any interstitial or distal blocks of C-heterochromatin and/or C-heterochromatic short arms as recorded by Mitsainas et al. (2008) and Şekeroğlu and Şekeroğlu (2011). Our results are fairly similar to the C-banding pattern described by Filippucci et al. (1985) in specimens from Massif du Pollino in Italy. However, the karyotypes of specimens examined by Filippucci et al. (1985) had slightly larger centromeric dark blocks of C-heterochromatin compared to the results of this study,

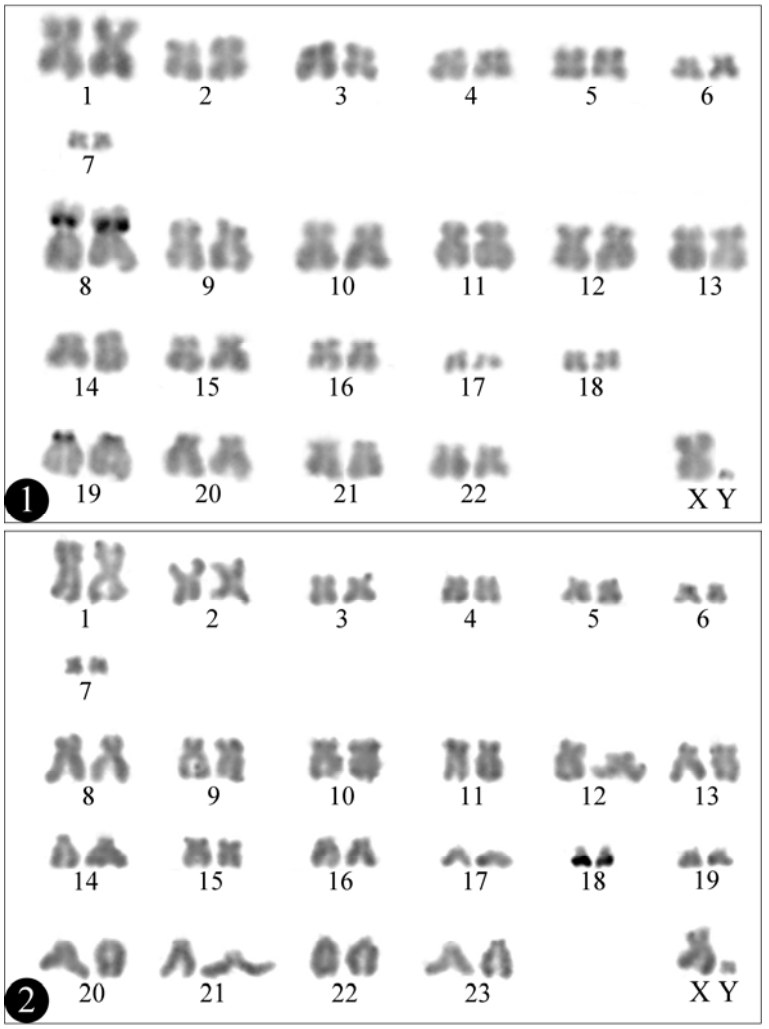

Figure 4. Silver stained karyotypes of *Dryomys laniger* (1) and *Dryomys nitedula* (2).

and we have not observed double dark C-bands in the X chromosome and the largest autosome.

The karyotype of both species studied differ primarily in their chromosome diploid number (2n = 46 and 2n = 48, respectively). This difference results from the absence of one pair of biarmed autosomes in the complement of *D. laniger* compared to that of *D. nitedula*. The C-banding pattern is rather similar in both species, showing relatively low amounts of C-heterochromatin. Therefore, heterochromatin changes have apparently not been responsible for karyotypic divergences between the species. The AgNOR distribution pattern is distinctly different between the two species, with two NORs found in *D. laniger* and a single site in *D. nitedula*. The location of the NORs is specific in each species. This difference may indicate a possible mechanism of karyotypic divergence between the species. In *D. laniger*, the second group of autosomes includes 11 pairs compared to 12 pairs in *D. nitedula*. The complement of *D. laniger* could thus be derived from that of *D. nitedula* after a tandem fusion of two autosomes (pairs no. 8 and 18 in the karyotype of *D. nitedula*), and subsequent silencing of one centromere. It is remarkable that the assumed rearrangement also includes the NOR, and a C-positive centromeric band appeared after the fusion in the complement of *D. laniger*.

The NOR sites have been found in the karyotype of glirids at one, two, or exceptionally three autosomal pairs that usually carry a prominent secondary constriction (Zima et al., 1995 for review). The NOR-bearing autosomes in various glirid species have a differing G-banding pattern, and Graphodatsky and Fokin (1993) assumed that they might not be homologous. Filippucci et al. (1983) and Mitsainas et al. (2008) detected only a single NOR site in specimens of *D. nitedula* examined in Italy and Greece,

and the NOR-bearing pair was quite similar to the small biarmed pair carrying a secondary constriction with NOR described in this study. Graphodatsky and Fokin (1993) found this NOR-bearing pair in specimens collected in Transcaucasia, but it was absent in specimens from Central Asia. These authors observed additional NOR sites in telomeric areas of the short arms of two autosomal pairs. One of these NOR sites is located in an autosome appearing quite similar to pair no. 19 of *D. laniger*.

The direction of chromosomal divergence that evolved between the two *Dryomys* species cannot be decided unequivocally. The lowering of the chromosome number in *D. laniger* during karyotypic divergence from ancestral *D. nitedula* seems to be the more feasible scenario. This view can be supported by generally more frequent appearance of chromosome fusions compared to chromosome dissociations in karyotype evolution (e.g., Zima, 2000) as well as by geographic comparison of the ranges of both species. *D. nitedula* possesses a relatively large range spreading from central Europe to central Asia, whereas the range of *D. laniger* is restricted to a small area in Turkey (Holden, 2005). This indicates a position of *D. nitedula* as an ancestral and that of *D. laniger* as a derived lineage.

The proposed hypothesis of the mechanism of karyotypic divergence between *D. laniger* and *D. nitedula* should further be tested with the use of sequential chromosome banding or FISH analyses, which are currently not available.

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