

Cytogenetic Studies on European Brown Hare, *Lepus europaeus* Pallas, 1778 (Lagomorpha: Leporidae) in Turkey

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Summary

In this study, the karyotype, C-heterochromatin blocks, and nucleolar organizer regions (NORs) of four specimens of *Lepus europaeus* from Turkey were examined. The diploid number of chromosomes (2n), the fundamental number of chromosome arms (FN), and the number of autosomal arms (FNa) were determined as 48, 90, and 86, respectively. All of the chromosomes have been determined to be possessed of centromeric constitutive heterochromatin, except of the pair no. 10. The X chromosome had centromeric heterochromatin and Y chromosome had pericentromeric heterochromatin. There were no distal C-bands in any chromosome of samples. The NORs have been determined to be located in the terminal regions of the long arms of three subtelocentric chromosomes (nos. 15, 16, and 18). Any relation was not determined between the localization of the NORs and C-positive regions of autosomes.

Keywords: Brown hare, *Lepus europaeus*, C-banding, NOR localization, Turkey

Türkiye'deki Avrupa Kahverengi Tavşanı, *Lepus europaeus* Pallas, 1778 (Lagomorpha: Leporidae) Üzerine Sitogenetik Çalışmalar

Özet

Bu çalışmada, Türkiye'deki *Lepus europaeus*'a ait dört örneğin karyotipi, C-heterokromatin blokları ve nükleolar organizatör bölgeleri çalışıldı. Diploid kromozom sayısı (2n), temel kromozom kol sayısı (FN) ve otozomal kromozom kol sayısı (FNa) sırasıyla 48, 90 ve 86 olduğu belirlendi. On noluk kromozom çifti hariç, kromozomların tamamı sentromerik konstitütif heterokromatine sahip olduğu belirlendi. X kromozom sentromerik ve Y kromozom perisentromerik heterokromatine sahipti. Örneklerin hiçbir kromozomunda distal C-bantlar yoktu. NOR'lar üç çift subtelosentrik kromozomun uzun kolun terminalinde lokalize olduğu belirlendi (no. 15, 16, ve 18). NOR'ların lokalizasyonu ve otozomların C-pozitif bölgeleri arasında herhangi bir ilişki belirlenmedi.


Anahtar sözcükler: Kahverengi tavşan, *Lepus europaeus*, C-band, NOR lokalizasyonu, Türkiye


INTRODUCTION

The genus *Lepus* L., 1758 was represented about by 30 species in the world is distributed in vast areas. One of these species is *Lepus europaeus* Pallas, 1778 classified as a subspecies of *Lepus capensis* L., 1758, in the past. It is now accepted that *L. capensis* is nonconspesific with *L. europaeus*. They are regarded as different species today ^{1,2}. The European or Brown hare, *L. europaeus*, is the most widespread (Palearctic and Nearctic regions) and best known hare species in the world ³.

C- and Ag-NOR banding, used to establish heterochromatin regions on the chromosomes is frequently used in animals specially in mammals and fish and thus is useful for examining intra and interspecific chromosomal differences between closely related species ^{4,5}. The karyotype of *L. europaeus* was described by Gustavsson ⁶ from Sweden, by Höhn and Herzog ⁷ from Germany, by Schröder et al. ⁸, by Palacios ⁹ from Spain and Richard et al. ¹⁰ from France. Sert et al. ¹¹ studied on genetic diversity of

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Anatolian and European populations of *L. europaeus* by using electrophoresis, biochemical staining of proteins and genotyping methods. Unfortunately, information on the conventional, C- and Ag-NOR-banded karyotypes of the European hare were unreported from the European and Anatolia up to now. Therefore, in this study, we presented conventional, C- and Ag-NOR banded karyotypical data on *L. europaeus* from Turkey.

MATERIAL and METHODS

The animals studied (two female and two males) were collected in their habitat from İçel and Konya Provinces. Karyotype preparations were obtained from the bone marrow of the colchicined animal¹². After these preparations, conventional Giemsa-staining was carried out. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected in individual autosomal and sex chromosome pairs via C-banding¹³ and Ag-NOR staining¹⁴. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analysed. Chromosome morphologies were determined after calculating centromeric indexes. Standard voucher specimens (skins and skulls) are deposited in the Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey.

RESULTS

The karyotype of *L. europaeus* contains 48 chromosomes. The number of autosomal arms (FN_a) is 86 and the number

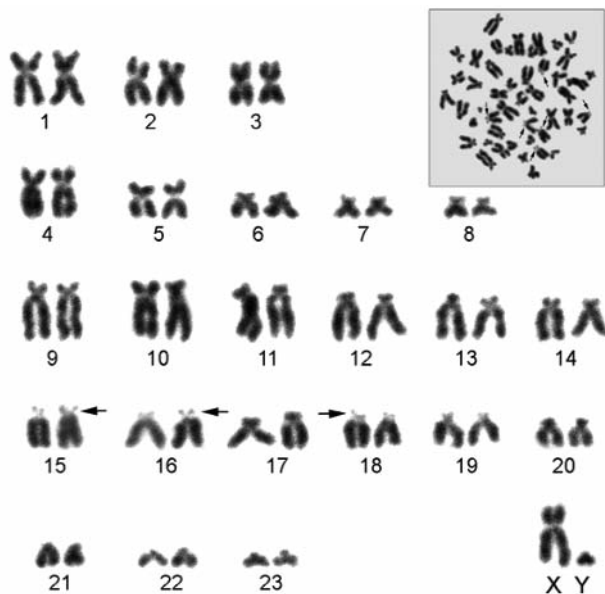


Fig 1. Metaphase spread and karyotype of *Lepus europaeus* from İçel. Arrows indicate the NORs

Şekil 1. İçel'deki *Lepus europaeus*'un karyotipi ve metafaz plağı. Oklar NOR'ları göstermektedir

of fundamental arms (FN) is 90. Three autosomal pairs are large metacentric (nos. 1-3), five pairs are submetacentric (nos. 4-8) and chromosome no. 4 is larger than others. Also six pairs are large and five pairs are medium-sized. Besides one pair is small subtelocentric (nos. 9-20), and three pairs are small acrocentric (nos. 21-23). The X chromosome is the large submetacentric and the Y chromosome is acrocentric and the smallest in the set (Fig. 1).

The C-banded karyotype is shown in Fig. 2. All autosomes of *L. europaeus* contained centromeric and pericentromeric constitutive heterochromatin, except for chromosome pair no. 10. In addition, the whole of short arm of pairs nos. 14 and 17 are heterochromatic. All autosomes containing heterochromatin are stained strongly, except for pairs nos. 4, 11, 15, 16, and 18. The X chromosome has centromeric heterochromatin and Y chromosome has pericentromeric heterochromatin.

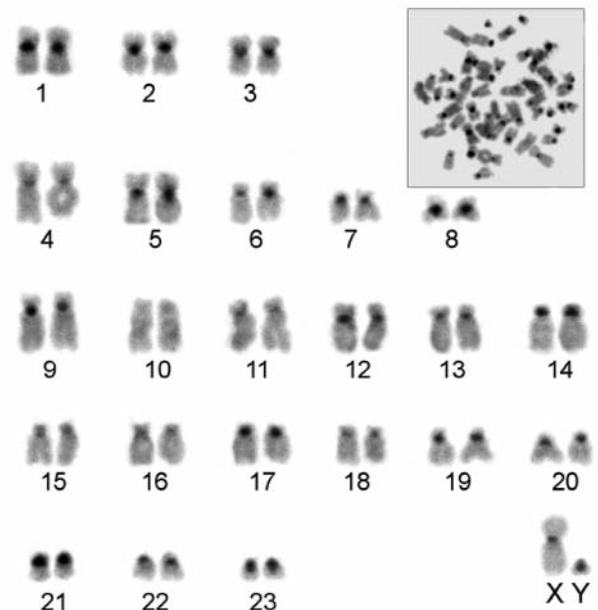


Fig 2. Metaphase spread and C-banded karyotype of *Lepus europaeus* from İçel

Şekil 2. İçel'deki *Lepus europaeus*'un C-bantlı karyotipi ve metafaz plağı

By using silver-nitrate staining, we determined that nucleolar organizer regions (NORs) which had localised at satellite sites of three pairs subtelocentric chromosomes (nos. 15, 16, and 18). All signals of active NORs are homomorphic. Two (nos. 16 and 18) of them are smaller and the other (no. 15) is larger size in Ag-stained metaphase (Fig. 3). All active NORs were detected by the conventional karyotype (Fig. 1). The number of active NORs ranged from 4 to 6 per cell (with an average of 5.2) in the metaphase.

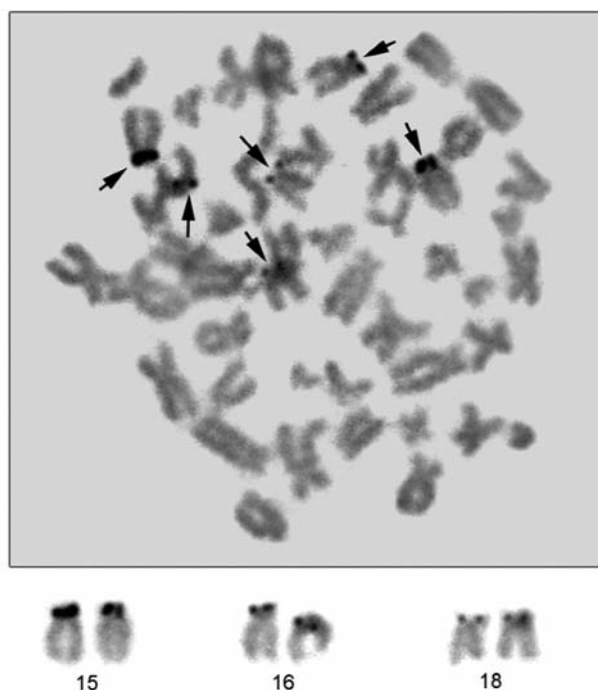


Fig 3. Silver-stained metaphase spread and positive chromosomes of *Lepus europaeus* from İçel. Arrows indicate the Ag-NOR

Şekil 3. İçel'deki *Lepus europaeus*'un Gümüş boyalı metafaz plağı ve pozitif kromozomlar. Oklar Ag-NOR'ları göstermektedir

DISCUSSION

The first study on the European hare karyotypes was done by Gustavsson 6 from Sweden. In Swedish *L. europaeus*, the researcher has described five pairs submetacentrics, three pairs metacentrics, nine pairs subtelocentrics and six pairs telocentrics. Besides he showed identical results between hybrid forms of Lepus. The diploid number of chromosomes of the European example of this species as well as in our examples is 48. Also, it is known that all the species belonging to this genus have 48 chromosomes¹⁵. However, the number of the autosomal arms changes both among the different populations of this species and different species of genus. For example, the number of the chromosome types that Robinson¹⁶ defined in *Lepus capensis* and Chen et al.¹⁷ in *Lepus comus* are in different from our samples. While the FN value of *L. europaeus* (88) and that of *L. californicus*, *L. alleni* and *L. flavigularis*¹⁸ were similar, it is different from that of *L. callotis* (FN = 90)¹⁹. While the sex chromosomes of our samples and the number of metacentric and submetacentric chromosome numbers in autosomes were similar to the samples of *L. europaeus* in Europe studied by Gustavsson⁶, Höhn and Herzog⁷, Palacios⁹ and Schröder et al.²⁰, the numbers of subtelocentric and acrocentric chromosomes were different from theirs. Probably, these differences in

autosomes of specimens might either result from methodical inconsistencies in karyotype preparation and the arrangement of the pairs in the diploid complement or indicate possible geographic variation in the karyotype of *L. europaeus*.

A comparison of the karyotypes in the jack rabbit (*Lepus californicus deserticola*) with the European hare (*L. europaeus*) was done by Schroder et al.⁸. They have found that the karyotypes of both species are almost identical. Nevertheless, in respect of staining heterochromatin on the long arm at the centromere, chromosomes 7 and 8 of *L. europaeus* are different than those of *L. californicus deserticola*. On the contrary, chromosomes 7 and 8 of *L. californicus deserticola* have a dark band distally on the long arms. Those researchers have been stating that these differences in chromosomes banding mentioned in both species could be a result of paracentric inversions. According to Robinson et al.²¹, *L. capensis* appears to possess similar C-positive material to other *Lepus* species analyzed, with most chromosomes showing small amounts of pericentromeric heterochromatin. The C-banding patterns of the X chromosome of this species are similar to those of the submetacentric autosomes of comparable size, while the Y chromosome appears to be largely heterochromatic in most preparations studied, although it is not intensely staining as the pericentromeric autosomal heterochromatin. Chen et al.¹⁷ determined that Yunnan hare, *L. comus* had centromeric heterochromatin in all chromosomes from China, except for four pairs chromosomes. Besides, this researcher recorded that as in the some chromosome pairs (nos. 4, 11, 15 and 18) of the examples of *L. europaeus* in Turkey, centromeric bands of the three pairs of chromosomes of Yunnan Hare were very slightly stained. However, different from Yunnan hare, only one pair chromosome of our samples does not have heterochromatin.

Banding patterns of rabbit, *Oryctolagus cuniculus* (2n = 44) were described by Switonski et al.²². According to these researchers, the amount of heterochromatin in the set of *O. cuniculus* is small. Only in a few pairs are there clear juxtacentromeric dark C-bands, most chromosomes have a small amount of centromeric heterochromatin, and the short arm of two pairs of autosomes have dark telomeric bands. Included in our examples, the other species belong to *Lepus* which does not have telomeric C-bands.

Consequently, the conventional karyotype of hares and rabbits are usually very similar, with only minor differences in the amount of heterochromatin, and the main mechanism of chromosomal evolution of leporids is centric fusion and heterochromatin addition. Moreover,

in this study, any active NOR of the long arms of chromosomes of *L. europaeus* was not established as in *O. cuniculus*²³.

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