Turk J Biol
34 (2010) 247-252
© TÜBİTAK
doi:10.3906/biy-0903-20

Cytogenetic study on the European brown hare (*Lepus europaeus* Pallas, 1778) (Mammalia: Lagomorpha) in Turkey

Yasin DEMİRBAŞ, Nursel AŞAN, İrfan ALBAYRAK

University of Kırıkkale, Faculty of Science and Arts, Department of Biology, 71451 Yahşihan, Kırıkkale - TURKEY

Received: 30.03.2009

Abstract: Conventional and GTC, CTC, and $AgNO_3$ banded karyotypes of brown hares (*Lepus europaeus* Pallas, 1778) from Turkey are described for the first time. All specimens possessed a diploid number of 2n = 48 chromosomes and a fundamental autosomal number of NFa = 88. The chromosome set consisted of 8 metacentric and submetacentric pairs and 15 subtelocentric and acrocentric pairs decreasing in size from large to small. The X chromosome was a large submetacentric while the Y was a small acrocentric. In contrast with the other specimens, the Kilis specimen possessed a duplication in the long arm of one of the subtelocentric chromosomes. The G-banding pattern of the chromosomes was similar to that given for the genus. Variation was found in the C-banded karyotype and the distribution of NORs.

Key words: *Lepus europaeus*, G-banding, C-banding, NORs, Turkey

Türkiye'deki yabani tavşan (*Lepus europaeus* Pallas, 1778) üzerine sitogenetik çalışma (Mammalia: Lagomorpha)

Özet: Türkiye'deki yaban tavşanı (*Lepus europaeus* Pallas, 1778)'nın standart, GTC, CTG ve $AgNO_3$ bantlı karyotipleri ilk kez bu çalışma ile verilmiştir. Bütün örneklerde diploid kromozom sayısı 2n = 48 ve otozomal kromozomların kol sayısı ise NFa = 88'dir. Kromozom takımında sekiz metasentrik ve submetasentrik çift ile büyükten küçüğe doğru sıralanan 15 subtelosentrik ve akrosentrik çift bulunmaktadır. X kromozomu büyük submetasentrik iken, Y kromozomu küçük bir akrosentriktir. Diğer örneklerden ayrı olarak Kilis örneği, subtelosentrik kromozomunun uzun kolunda bir duplikasyona sahiptir. Kromozomların G-bantları cins için verilenlerle benzerdir. C-bantlı karyotiplerde ve NOR dağılımlarında varyasyon bulunmuştur.

Anahtar sözcükler: *Lepus europaeus*, G-bantlama, C-bantlama, NORs, Türkiye

Introduction

The order Lagomorpha is composed of 2 families: Ochotonidae (pikas) and Leporidae (hares, rabbits, and cottontails). Among mammals, *Lepus* Linnaeus, 1758 is one of the most widespread and diverse genera existing in the Palearctic, Nearctic, Ethiopian, and Oriental regions (1,2). *Lepus europaeus* Pallas, 1778 (European or brown hare) is distributed from western Europe (except large parts of the Iberian Peninsula) to the west Siberian lowlands, northern Israel, northern Syria, northern Iraq, and western Iran (3,4). According to Hoffmann and Smith (4) this species

was earlier placed in *Lepus capensis* Linnaeus, 1758 (Cape hare) distributed in Africa, the Middle East, and Central Asia. Angermann (5) considered the Anatolian hares as *L. europaeus.* To date, ecological characteristics of brown hares have been described by Oğurlu (6) and Yiğit et al. (7). Sert et al. (8,9) and Ben Slimen et al. (10,11) have examined the genetic diversity, allozyme, microsatellite, and mtDNA variation of the species in Anatolia. Despite distinct differentiation, a relatively close phylogenetic relationship between the brown hares from Europe and Anatolia was also detected, warranting their conspecificity.

The karyology of *Lepus europaeus* was studied by Schröder et al. (12), Zima and Kral (13), and Azzoroli Puccetti et al. (2) from Europe. The karyotype of the genus is characterized by a constant diploid number of 2n = 48 and a fundamental autosomal number of NFa = 88, as well as G- and C-banding patterns.

The aims of this study were to determine the conventional karyotype, G- and C-banding patterns, and NORs localization of *Lepus europaeus* by evaluating specimens having different pelage colorations from Turkey for the first time and to make a contribution to the knowledge of karyological evolution of the brown hares in the Palearctic region.

Materials and methods

Specimens examined (27 ♂♂, 4 ♀♀): Edirne (41°33′N 26°49′E) (2 ♂♂), Tekirdağ (41°01′N 27°22′E) (2 ♂♂), Kastamonu (41°01′N 34°02′E) (2 ♂♂, 1 ♀), Kırıkkale (39°50′N 33°27′E) (6 ♂♂, 2 ♀♀), Çorum (40°09′N 34°22′E) (2 ♂♂), Kırşehir (39°03′N 34°22′E) (1 ♂, 1 ♀), Kayseri (38°43′N 35°29′E) (2 ♂♂), Niğde (37°49′N, 34°59′E) (1 ♂), Iğdır (39°52′N, 44°31′E) (2 ♂♂), Ağrı (39°43′N, 43°03′E) (2 ♂♂), and Kilis (36°40′N, 37°27′E) (5 ♂♂) provinces (Figure 1).

Live animals were transported to the laboratory in order to prepare conventional karyotypes from bone marrow according to Ford and Hamerton (14). Lymphocyte cultures were achieved according to Hillis et al. (15). G-banding was performed by digestion with 0.25% trypsin according to Seabright (16). Nucleolar organizer regions (NORs) and C-banded patterns were detected with the methods of Howell and Black (17) and Sumner (18), respectively. For each individual 20 slides were prepared and at least 25 well stained and G-, C-, and Ag-NOR banded metaphases were examined. Centromeric indexes were calculated for establishing the chromosome morphologies including X and Y.

Permission was obtained for hunting brown hares from the Turkish National Parks, Hunting, and Wildlife Directorate (Turkish Forestry Ministry).

Voucher specimens, skulls, and karyotype slides are deposited in the mammal collection at the Department of Biology, Kırıkkale University.

Results and discussion

All examined specimens of *Lepus europaeus* had a diploid number of 2n = 48 and fundamental autosomal number of NFa = 88 as characterized for the genus. The chromosome set consisted of 8 metacentric and submetacentric pairs (nos. 1-8) and 15 subtelocentric and acrocentric pairs (nos. 9-23) decreasing in size from large to small. The X chromosome was a large submetacentric and the Y was a small acrocentric (Figure 2).

Specimens from Edirne, Tekirdağ, Kastamonu, Kırıkkale, Çorum, Kayseri, Kırşehir, Niğde, Iğdır, and Ağrı had karyotypes similar to that stated for the genus. However, in Kilis we determined 2 populations having different pelage colors from each other. In all 3 male specimens with a yellowish pelage color, one of the subtelocentric chromosomes possessed a duplication on the long arm. Two specimens from the other population possessed a karyotype similar to those from other localities (Figure 3).

The karyotype of the brown hares examined in this study conforms to the conservative karyotype of the genus except for the Kilis specimens.

The distribution of the constitutive heterochromatin was pericentromeric in 14 pairs as well as in the X chromosome. In addition, one of the metacentric pairs possessed telomeric C-bands. The Y chromosome was largely heterochromatic and stained dark. Eight pairs of autosomes were devoid of any C-bands. The C-banded karyotype of the specimens from Kilis is given Figure 4, which shows that the duplication on chromosome 14 stained C-positive.

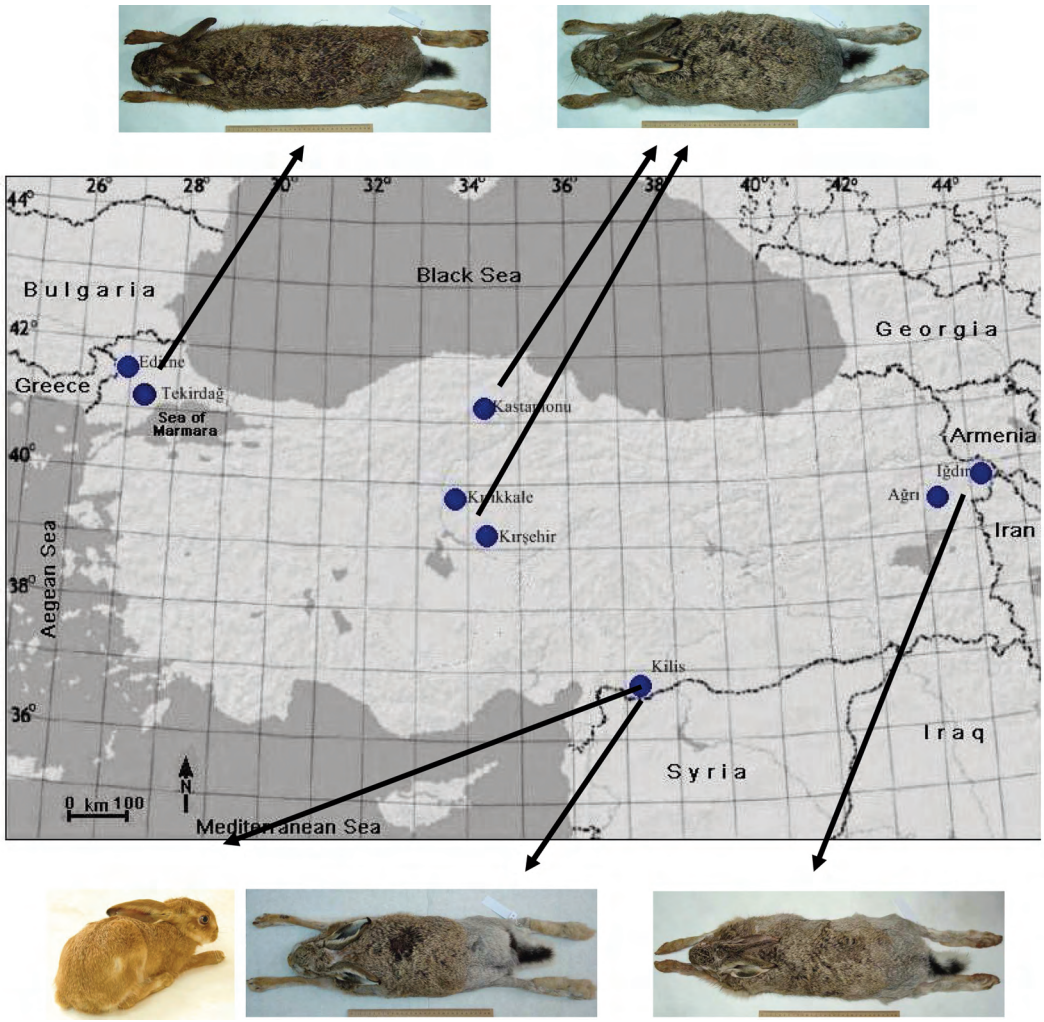

Figure 1. Localities and pelage color of *Lepus europaeus* specimens collected from Turkey.

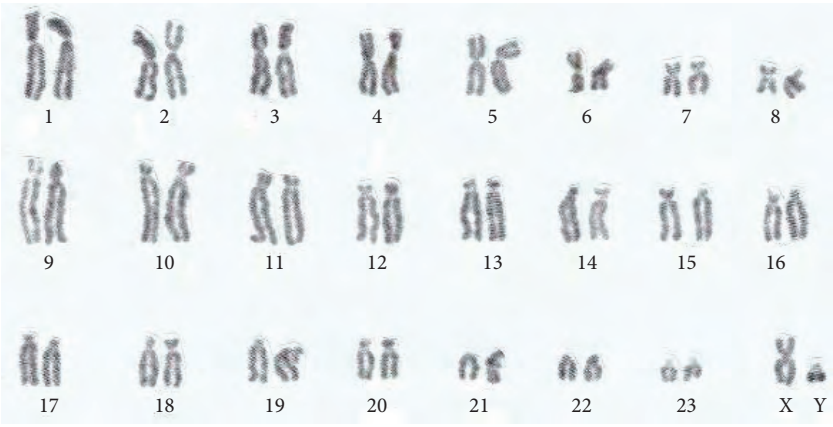

Figure 2. Conventionally stained male karyotype of *Lepus europaeus* (2n = 48, NFa = 88).

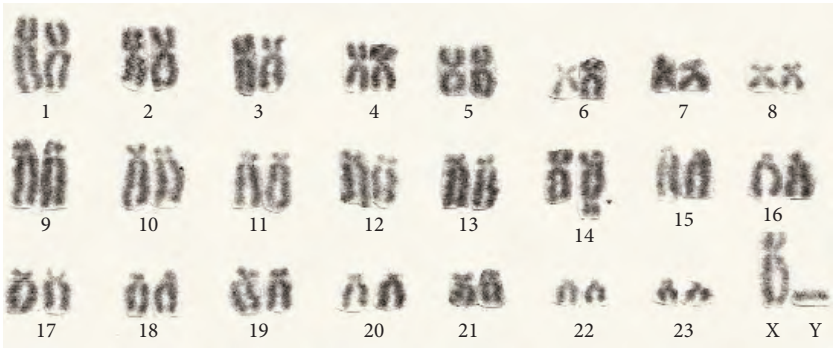

Figure 3. Conventionally stained karyotype of male *Lepus europaeus* with a duplication on the long arm of chromosome 14.

Schröder et al. (12) stated that both chromosomes 7 and 8 had distinct darkly stained heterochromatin at the centromere. In addition, Zima and Kral (13) determined C-positive areas around the centromeres of some chromosomes. Lorenzo et al. (19) examined C-banded chromosomes of the genus *Lepus* and determined small amounts of pericentromeric heterochromatin in the chromosomes. No telomeric C-bands were detected by the authors in the specimens examined. In *Lepus californicus*, heterochromatin in the pericentric area was present on chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 11, 15, 16, 18, and 19. Telomeric constitutive heterochromatin was observed in chromosome pairs 3, 4, 10, 11, 18, and 19 (20). Turkish specimens also possessed small amounts of pericentromeric heterochromatin. Nevertheless, our results are in accordance with those reported by Cervantes et al. (20) with respect to pericentric constitutive heterochromatin amounts and telomeric heterochromatin blocks.

Stock (21) reported that centric fusions and the addition of heterochromatin were the most important features of karyotype change in the lagomorphs. Schröder et al. (12) reported duplication in *Lepus europaeus* and stated that this rearrangement was a result of lymphocyte culture. However, in the Kilis specimens an additional heterochromatin region was also detected in bone marrow preparations. Therefore, we accepted that the kind of preparation did not affect the rearrangements such as duplication.

The G-band pattern of the chromosomes in *Lepus europaeus* examined in this study was similar to that given for the genus (Figure 5).

NORs are located in the telomeric regions of the short and long arms of metacentric and subtelocentric

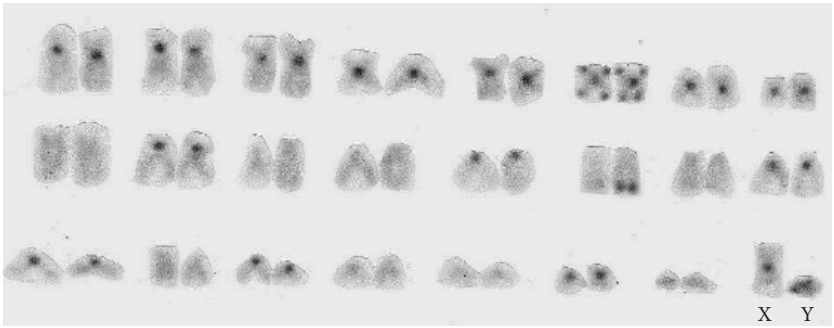

Figure 4. C-banding karyotype of male *Lepus europaeus* from Kilis province.

Figure 5. G-banded karyotype of male *Lepus europaeus* from Kilis province.

pairs as well as in the paracentric region of one of the subtelocentric pairs (Figure 6).

Ag-NORs occurred at the telomeres of the short arms of 3 pairs of medium and small subtelocentric chromosomes (nos. 13, 16, and 20) in *Lepus starckii* examined from Ethiopia (2). NOR distribution in our specimens differed from that reported by Azzoroli Puccetti et al. (2). Miller et al. (22) and Croce et al. (23) demonstrated that only functional NORs on chromosomes that were active in the preceding interphase could be detected by silver staining. Therefore, the NOR-carrying chromosomes of the species examined from Turkey were not consistent with the published data.

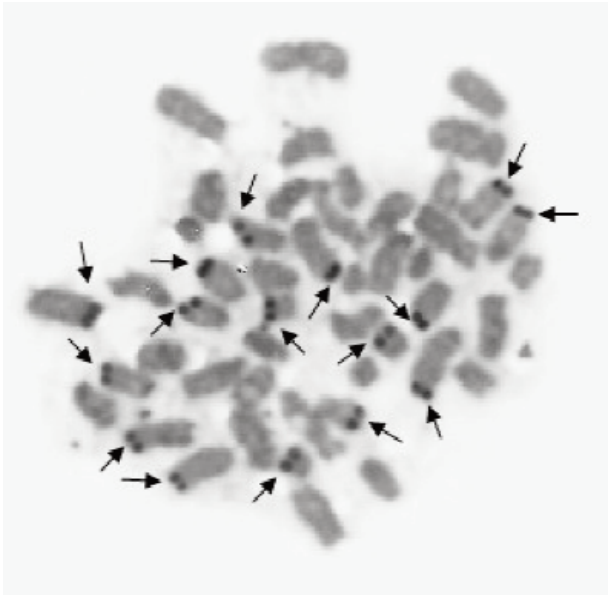

Figure 6. $AgNO_3$ stained metaphase plate of *Lepus europaeus*. Arrows indicate NOR-bearing chromosomes.

Mitchell-Jones et al. (3) stated that several subspecies of *Lepus europaeus* are recognized in Europe with different color patterns. Suchentrunk et al. (24) argued that the pronounced regional variation in the external appearance such as pelage color, body size, and ear length of Israeli hares is caused by ecogenetic factors. More recently, Sert et al. (8) examined the genetic diversity of *L. europaeus* from Turkey and determined that hares inhabiting Asia Minor might have received substantial gene flow from the Caucasus region, Iran, Syria, Iraq, Lebanon, and Israel, where *L. capensis* occurred. Apart from the hares collected from Gaziantep and Şanlıurfa, close to the Syrian border, all hares are recorded as having the typical pelage color of brown hares in Europe. In addition, no pronounced genetic divergence was detected between the specimens examined. Gaziantep neighbors Kilis and we collected the specimens with yellowish pelage from Kilis province in this study.

Chromosomes are used as a taxonomic feature in many animal groups (25). The conventional and banded karyotypes of the European brown hare have not been reported from Turkey before the conventional and banded karyotypes of the European brown hare have not been reported from Turkey

before. Our results support the conservative nature of the karyotype of the genus *Lepus* except for the differences due to differential amounts of heterochromatin. We accepted the Kilis population, with yellowish pelage color, as *L. europaeus,* as did Sert et al. (8,9), although it had a different pelage color and a karyotype with a duplication on a subtelocentric pair.

Corresponding author:
Nursel AŞAN
University of Kırıkkale,
Faculty of Science and Arts,
Department of Biology,
71451 Yahşihan, Kırıkkale - TURKEY
E-mail: nurselasan@yahoo.com

References

1. Robinson TJ, Elder FFB, Chapman JA. Karyotypic conservatism in the genus *Lepus* (Order Lagomorpha). Can J Genet Cytol 25: 540-544, 1983.
2. Azzoroli Puccetti ML, Corti M, Scanzani A. et al. Karyotypes of two endemic species of hare from Ethiopia *Lepus habessinicus* and *L. starckii* (Lagomorpha, Leporidae). A comparison with *L. europaeus*. Mammalia 60: 223-230, 1996.
3. Mitchell-Jones AJ, Amori G, Bogdanowicz W et al. The Atlas of European Mammals. Academic Press, California; 1999.
4. Hoffmann RS, Smith AT. Order Lagomorpha. In: Mammal Species of the World. A Taxonomic and Geographic Reference, Volume 1. The Johns Hopkins University Press, Baltimore; 2005: pp. 185-211.
5. Angermann R. The taxonomy of old world *Lepus*. Acta Zool. Fennica 174: 17-21, 1983.
6. Oğurlu I. Habitat use and food habits of Brown hare (*Lepus europaeus* Pallas) in a woodland. Turk J Zool 21: 381-398, 1997.
7. Yiğit N, Demirsoy A, Karataş A et al. Notes on the mammals found in Kazdağı National Park and its environs. Turk J Zool 30: 73-82, 2006.
8. Sert H, Suchentrunk F, Erdoğan A. Genetic diversity within Anatolian Brown hares (*Lepus europaeus* Pallas, 1778) and differentiation among Anatolian and European populations. Mamm Biol 70: 171-186, 2005.
9. Sert H, Ben Slimen H, Erdoğan A et al. Mitochondrial HVI sequence variation in Anatolian Hares (*Lepus europaeus* Pallas, 1778). Mamm Biol 74: 286-297, 2009.
10. Ben Slimen H, Suchentrunk F, Memmi A et al. Evolutionary relationships among hares from North Africa *(Lepus* sp. or *Lepus* spp.) cape hares *(L. capensis*) from South Africa, and Brown hares *(L. europaeus*) as inferred from mtDNA PCR-RFLP and allozyme data. J Zool Syst Evol Res 44: 88-99, 2006.
11. Ben Slimen H, Suchentrunk F, Stamatis C et al. Population genetics of cape and brown hares (*Lepus capensis* and *L. europaeus*): A test of Petter's hypothesis of conspecificity. Biochem Syst Ecol 36: 22-39, 2008.
12. Schröder J, Suomalainen van der WL, Schröder E. Karyotypes in lymphocytes of two strains of rabbit, and two species of hare. Hereditas 83: 183-188, 1978.
13. Zima J, Kral B. Karyotypes of European mammals. Acta Sci Nat Acad Scien 7: 1-53, 1984.
14. Ford CE, Hamerton JL. A colchicines, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technology 31: 247-251, 1956.
15. Hillis DM, Moritz C, Mable BK. Molecular Systematics. Sinauer Associates, Massachusetts, 655 pp; 1996.
16. Seabright M. A rapid banding technique for human chromosomes. Lancet 2: 971-972, 1971.
17. Howell WM, Black DA. Controlled silver staining for nucleolus organizer regions with a protective colloidal developer: A 1-step method. Experientia 36: 1014-1015, 1980.
18. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304-306, 1972.
19. Lorenzo C, Cervantes FA, Francisco Y et al. Bandas chromosomicas c de Los generos *Romerolagus*, *Sylvilagus* y *Lepus* (Mammalia: Lagomorpha) de Mexico. Revista Mexicana de Mastozoologica 4: 84-94, 2000.
20. Cervantes FA, Rojas-Vilaria A, Lorenzo C et al. Chromosomal differentiation between the jackrabbits *Lepus insularis* and *Lepus californicus* from Baja California Sur, Mexico. Revista Mexicana de Mastozoologica 4: 40-52, 2000.
21. Stock AD. Chromosome banding pattern relationships of hares, rabbits and pikas (order Lagomorpha). Cytogenet Cell Genet 17: 78-88, 1976.
22. Miller OJ, Miller DA, Dev VG et al. Expression of human and suppression of Mouse nucleolar organizer activity in Mouse-human somatic cell hybrids. P Natl Acad Sci USA 73: 4532-4535, 1976.
23. Croce CM, Talavera A, Basilico C et al. Suppression of production of mouse 28 S ribosomal RNA in Mouse-human hybrids segregating mouse chromosomes. P Natl Acad Sci USA 74: 694-697, 1977.
24. Suchentrunk F, Alkon PU, Willing R et al. Epigenetic dental variability of Israeli hares (*Lepus* sp.) ecogenetic or phylogenetic causation. J of Zool Lond 252: 503-515, 2000.
25. Yüksel E, Gülkaç MD. The Cytogenetical Comparisons of *Spalax* (Rodenta: Spalacidae) populations from Middle Kızılırmak Basin, Turkey. Turk J Biol 25: 17-24, 2001.