

KARYOLOGICAL COMPARISONS OF THE EUROPEAN HARE (*LEPUS EUROPAEUS* PALLAS, 1778) FROM THE ASIAN PART OF TURKEY, WITH MORPHOLOGICAL CONTRIBUTIONS

C. TEZ^{1*}, Y. ÖZKUL² and O. İBİŞ³

¹Department of Biology, Faculty of Sciences, Erciyes University, 38039 Kayseri, Turkey

²Department of Medical Genetics, Faculty of Medicine, Erciyes University, 38039 Kayseri, Turkey

³Graduate School of Natural and Applied Sciences, Erciyes University, 38039 Kayseri, Turkey

Abstract - We focused on a comparison of karyological data for the European brown hare *Lepus europaeus*, from the Asian part of Turkey. In Turkish *L. europaeus*, the diploid number (2n), the fundamental number of chromosomal arms (FN) and the number of autosomal arms (FNa) were determined to be 2n=48, 84 and 80, respectively. The autosomes are composed of three pairs of metacentric chromosomes, four pairs of submetacentric chromosomes, ten pairs of subtelocentric chromosomes and six pairs of acrocentric chromosomes. The X chromosome was a medium-large submetacentric and the Y chromosome was a very small acrocentric. This is the third report for *L. europaeus* from Turkey and confirmed the previous results with regard to 2n. However, when comparing our findings with those of other authors, there were karyotypic differences among the chromosomes. These differences were related to the number of chromosome arms. Additionally, we present the skull measurements of seven samples for the Turkish brown hare collected from three localities, and these measurements conformed to those of previous studies.

Key words: *Lepus europaeus*, karyotype, skull measurement, Turkey

INTRODUCTION

The family Leporidae includes more than 47 extant rabbit, jackrabbit and hare species. Members of this family occur on all continents, except for Antarctica. The Leporidae is subdivided into 11 genera: jackrabbits and hares, *Lepus*; and rabbits, *Pentalagus*, *Pronolagus*, *Romerolagus*, *Caprolagus*, *Oryctolagus*, *Sylvilagus*, *Brachylagus*, *Bunolagus*, *Poelagus* and *Nesolagus*. Of these genera, the genus *Lepus* L., 1758 is represented by 23-30 species and widely distributed in the world (Chapman and Flux, 1990; Corbet and Hill, 1991; Burkitt, 1995; Wilson and Reeder, 1993, 2005). Currently, there is a good amount of studies about these species (Harrison, 1972; Halanych et al., 1999; Pierpaoli et al., 1999; Mamuris et al., 2001; Alves et

al., 2000, 2003; Wu et al., 2005; Fredsted et al., 2006; Djan et al., 2006; Slimen et al., 2006, 2008; Stamatis et al., 2009; Koh and Jang, 2010).

Lepus europaeus Pallas 1778 was firstly defined as a subspecies of *L. capensis* L. 1758 and both taxa are currently accepted as two different species (Flux and Angermann, 1990; Wilson and Reeder, 1993, 2005). The European hare, *L. europaeus*, is widely distributed throughout Palaearctic and other regions, except for Antarctica (Chapman and Flux, 1990; Burkitt, 1995; Wilson and Reeder, 1993, 2005). Although there are 30 known subspecies belonging to this species today, the status of these subspecies is considered to be very variable (Flux and Angermann, 1990; Wilson and Reeder, 1993, 2005).

The Turkish *Lepus* populations have been reported as *L. capensis* by some authors (Harrison, 1972; Dođramacı, 1989; Çanakçiođlu and Mol, 1996; Kurtunur et al., 1996), but in recent years, some other authors have defined it as *L. europaeus* and this species is a common and widespread leporid in Turkey (Chapman and Flux, 1990; Corbet and Hill, 1991; Ođurlu, 1997; Demirsoy, 1996; Krystufek and Vohralik, 2001, 2009; Yiđit et al., 2001, 2002; Wilson and Reeder, 1993, 2005).

In the last decade, the European brown hare (*L. europaeus*) from different regions has been investigated genetically and biochemically. Numerous genetic polymorphisms have been described by various authors (Halanych et al., 1999; Pierpaoli et al., 1999; Mamuris et al., 2001; Alves et al., 2000, 2003; Sert et al., 2005, 2008; Slimen et al., 2006; Djan et al., 2006; Fredsted et al., 2006; Vapa et al., 2007; Stamatis et al., 2009).

As in all *Lepus*, the karyotype of *L. europaeus* has 48 chromosomes over this taxon's range (Flux and Angermann, 1990) and was described by Gustavsson (1971) from Sweden, Schröder et al. (1978a,b) from Finland, and different researchers (see Zima and Kral, 1984) from Germany, (former) Yugoslavia, Spain and (former) Czechoslovakia. Recently, data on the karyology of the European hare was reported by Arslan (2010) and Demirbař et al. (2010) from Turkey,

In the present study, we aimed to report on the cranial measurements and to confirm the karyotype of *L. europaeus* from the Asian part of Turkey.

MATERIALS AND METHODS

In this study, we examined seven samples of *L. europaeus* from three different localities (Kayseri, 3; Çankırı–Ilgaz, 3; Nevşehir–Gülşehir, 1) in the Asian part of Turkey (Fig. 1). All samples were obtained from hunters during legal hunting seasons in 1998–1999. Of these samples, blood samples of two individuals were collected from Kayseri and Nevşehir and used to prepare karyotypes. The

blood was taken from the marginal ear vein and by cardiac puncture, and stored in heparinized tubes at +4°C until treatment. Chromosome analysis was carried out in accordance with the method of Rooney and Czepulkowski (1987). Nomenclature for the centromeric position on chromosomes follows Levan et al. (1964): metacentric (m); submetacentric (sm); subtelocentric (st); and acrocentric (a).

Twenty-one measurements (mm) of the skull were obtained for each animal, following the method of Riga et al. (2001), with digital calipers to the nearest 0.01 mm: ANW – anterior nasal width; ENL – external nasal length; FIL – foramen incisivum; FIW – foramen incisiva width; FTL – facial tubercle length; HPMM – height of processus muscularis mandibulae; INL – internal nasal length; LCTRL – lower cheek-tooth row length; UCTRL – upper cheek-tooth row length; MH – mandible height; ML – mandible length; PL – palatal length; PNW – posterior nasal width; PPW – post palatal width; PZW – posterior zygomatic width; RW – rostral width; SFW – smallest frontal width; TBL – tympanic bulla length; TBW – tympanic bulla width; TL – total length; WFT – width between facial tubercles. The skulls and karyotype slides are deposited at the Department of Biology, Faculty of Sciences, Erciyes University in Kayseri.

RESULTS

Habitat: European brown hares are found around cultivated lands (Kayseri, Nevşehir–Gülşehir) and tend to live in shrubberies, conifers and broad-leaved forests (Serçeler Plateau, Ilgaz–Çankırı), and steppes and bushes (Kayseri).

Skull characteristics: The skull measurements of Turkish brown hares are given in Table 1. The skull of Turkish brown hare is shown in Fig. 2.

Status: In “IUCN Red List of Threatened Species”, *L. europaeus* was evaluated to be of “Least Concern (LC)” by Smith and Johnston (2008). The population density of this species has gradually been decreasing

Table 1. The skull measurements (mm) of the Turkish *Lepus europaeus* (N - number of specimens; SD - standard deviation; SE - standard error).

Measurements	N	Min-Max	Mean	St. D.	SE Mean
ANW	7	12.39 – 15.49	13.781	1.123	0.378
ENL	7	32.69 – 39.90	36.499	2.547	0.378
FIL	7	21.41 – 26.37	23.744	1.796	0.378
FIW	7	9.11 – 11.08	10.316	0.765	0.378
FTL	7	5.83 – 7.90	6.939	0.798	0.378
HPMM	7	3.64 – 4.84	4.183	0.471	0.378
INL	7	29.20 – 35.45	33.084	1.985	0.378
LCTRL	7	15.26 – 17.71	16.606	0.872	0.378
UCTRL	7	14.29 – 17.13	15.753	1.096	0.378
MH	7	34.82 – 43.17	40.474	2.760	0.378
ML	7	58.85 – 71.05	67.114	4.432	0.378
PL	7	32.05 – 38.86	35.716	2.431	0.378
PNW	7	18.75 – 21.79	20.351	1.168	0.378
PPW	7	9.00 – 11.71	10.030	0.959	0.378
PZW	7	40.61 – 45.88	43.780	1.724	0.378
RW	7	24.02 – 26.95	25.340	1.114	0.378
SFW	7	12.95 – 14.70	13.854	0.745	0.378
TBL	6	12.40 – 15.38	11.826	5.300	0.378
TBW	6	7.61 – 8.46	6.940	3.071	0.378
TL	6	88.52 – 97.22	78.763	34.891	0.378
WFT	7	37.85 – 41.95	39.806	1.651	0.378

Table 2. Karyotypic records of *Lepus europaeus* from Turkey (m – metacentric; sm –submetacentric; st – subtelocentric; a – acrocentric).

References	2n	NF	NFa	m/sm	m	sm	st/a	st	a	X	Y
Arslan (2010)	48	90	86	-	6	10	-	24	6	sm	a
Demirbaş et al. (2010)	48	92	88	16	-	-	30			sm	a
This study	48	84	80	-	6	8	-	20	12	sm	a

because of extreme hunting and habitat spoiling in Turkey. Therefore, in the near future, we predict that it will be listed as “Vulnerable” for Turkey.

Karyology: Our results confirm previous findings in regards to diploid number, and the karyotype consisted of 48 chromosomes ($2n = 48$, $FN = 84$,

$FNa=80$). The autosomal set can be divided into four groups: three pairs of metacentric (1-3); four pairs of submetacentric (4-7); ten pairs of subtelocentric (8-17) and six pairs of acrocentric (18-23). The X chromosome was a medium-large submetacentric, and the Y chromosome was a very small acrocentric (Fig. 3; Table 2).



Fig. 1. Map showing the locations of *Lepus europaeus* from central Turkey (A – Kayseri; B – Nevşehir-Gülşehir; C – Çankırı-Ilgaz).

DISCUSSION

Lagomorph mammals known as “hares, rabbits and pikas” arose in the Paleocene (Harrison, 1972; Burkitt, 1995). The genus *Lepus* is represented by 11 to 15 species in Eurasia (Flux and Angermann, 1990; Wilson and Reeder, 1993, 2005). The taxonomic status of these species has been discussed in detail by various authors (Riga et al., 2001; Pierpaoli et al., 1999; Suchentrunk et al., 2000). *L. europaeus* was previously evaluated to be a subspecies of *L. capensis*, a cline in morphological features over most of its range (Wilson and Reeder, 1993, 2005). A cline in morphological characteristics may reflect genetic differences originating from the divergence time between two species, or adaptation to different environments (see Riga et al., 2001). According to Wilson and Reeder (1993, 2005), *L. europaeus* is larger in terms of its size than *L. capensis*. This size difference was also noted by Gramov and Erbajeva (1995) who mapped the distribution of the genus *Lepus* in Russia. According to the distributional map of Gramov

and Erbajeva (1995), *L. europaeus* was also distributed in Asia Minor. In this study, the skull characteristics of the Turkish samples were similar to those of the Russian *L. europaeus* reported by Gramov and Erbajeva (1995).

Recent molecular genetic studies, including the mtDNA control region and cytochrome *b*, have also maintained that *L. europaeus* and *L. capensis* are two separate species (Halanych et al., 1999; Pierpaoli et al., 1999; Slimen et al., 2006, 2008). Even though all species of the genus *Lepus* have the same $2n=48$, the fundamental number of chromosomal arms (FN) or number of autosomal arms (FN_a) and G banded patterns, are very similar. This may imply that karyotypic conservatism in the genus *Lepus* has occurred in the course of its chromosomal evolution (see Robinson 1981 in Lorenzo et al., 2003).

Karyotypes of animals that are known as chromosome complements exhibit a great diversity in their number and morphology (Ferguson-Smith and Tri-

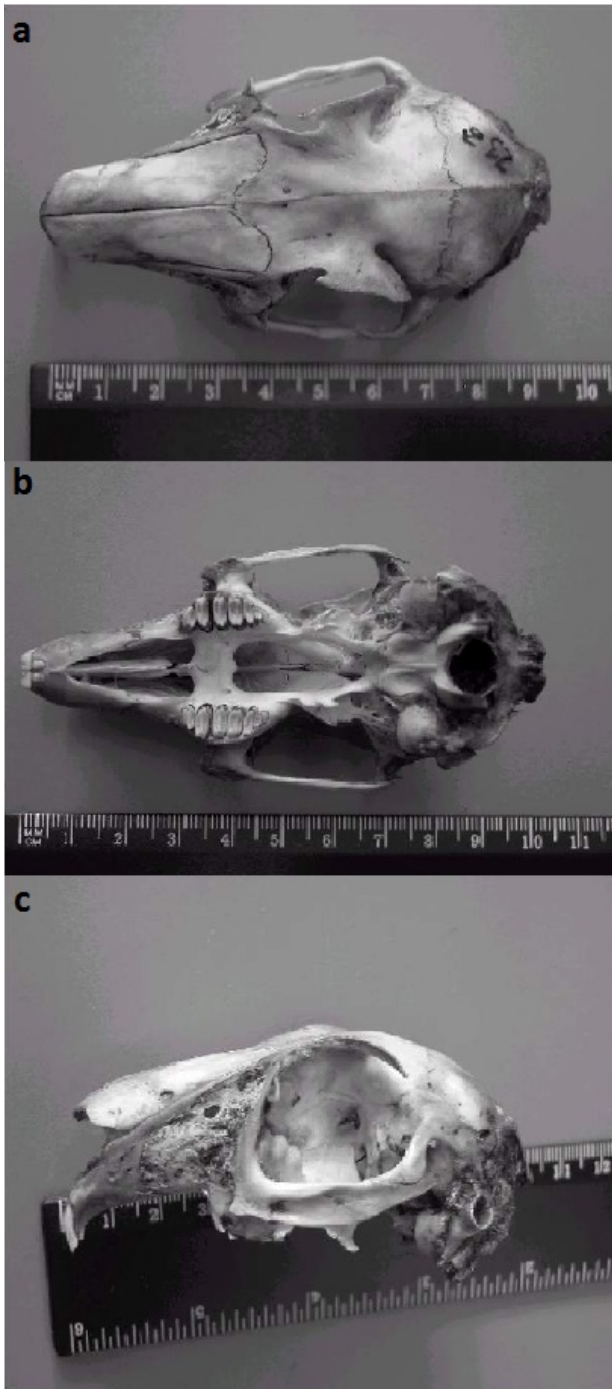


Fig. 2. Skull of *Lepus europaeus* from central Turkey: a – dorsal; b – ventral; c – lateral.

fonov, 2007; O'Brien et al., 2006). In all karyotyped *Lepus* species, they have closely similar or identical karyotypes, showing $2n = 48$ (Flux and Angermann, 1990). This appears to be conserved within the genus *Lepus*. It is indicated by some authors that the lack of chromosomal diversity within the genus *Lepus* points to the mechanisms of speciation that is not valid in the chromosomal models proposed for other mammals. These researchers have stated that isolation mechanisms may be the reason for speciation within the genus *Lepus* (see Halanych et al., 1999) because all species of the genus *Lepus* have very similar karyotypes with the same diploid number of chromosomes ($2n=48$) (Gustavsson, 1971; see Schröder et al., 1978b; see Zima and Kral, 1984; Flux and Angermann, 1990), although there are minor differences resulting from the amount of C-heterochromatin among the karyotypes of species belonging to the genus *Lepus*. Centric fusion and heterochromatin addition are regarded as the causes of these differences (see Zima and Kral, 1984).

The first record on the karyotypes of European brown hare was presented by Gustavsson (1971) from Sweden. In the Swedish *L. europaeus*, Gustavsson (1971) described five submetacentric pairs, three metacentric pairs, nine subtelo-centric pairs and six telocentric pairs of chromosomes, as well as identical results between hybrids of *L. europaeus* and *L. timidus*.

Schröder et al. (1978a) compared the karyotype of European brown hare (*L. europaeus*) with that of the jackrabbit (*L. californicus deserticola*) and observed that both taxa have identical karyotypes. However, these authors stated that there are differences in terms of heterochromatin staining on two chromosomes due to paracentric inversions. Furthermore, Schröder et al., (1978b) compared karyotypes obtained from the lymphocytes of rabbit (*Oryctolagus cuniculus*) and two hare species (*L. europaeus* and *L. timidus*), and reported that the karyotypes of both hare species were the same, ($2n=48$) (except for chromosomal rearrangements; especially deletions and duplications), but differed in the rabbit ($2n=44$). The lower $2n$ in the rabbit (*O. cuniculus*)

Among mammals, the diploid numbers range from 6 to 102 (see Ferguson-Smith and Tri-

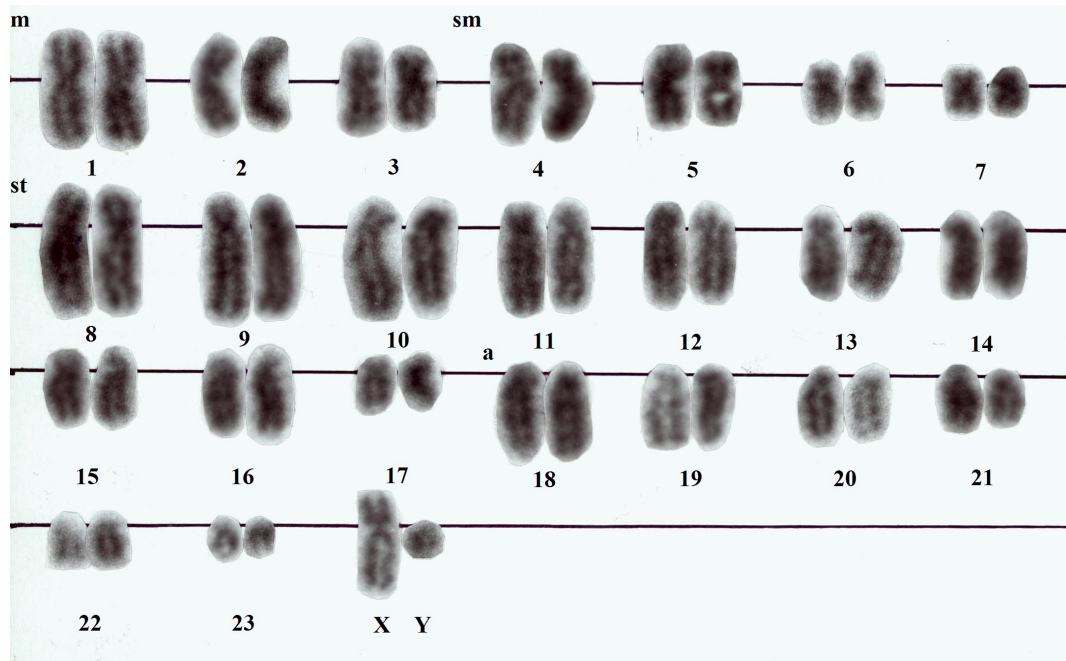


Fig. 3. The karyotype of *Lepus europaeus* ($2n=48$) from central Turkey.

had resulted from two centric fusions (see Schröder et al., 1978b). In spite of great differences regarding $2n$ between rabbit and hares species, Schröder et al. (1978b) stated that great similarities were found in the G-banding of the chromosomes of *L. europaeus*, *L. timidus* and *O. cuniculus*.

It was also reported by Zima and Kral (1984) that the karyotype of *L. europaeus* consists of 48 chromosomes, and 3 metacentric, 5 submetacentric, 9 subtelocentric and six acrocentric pairs. According to Zima and Kral (1984), the X chromosome is submetacentric or metacentric, but the Y is a very small acrocentric chromosome. In addition, the same karyotype was described differently from country to country in Europe (Germany, Spain, (the former) Yugoslavia and (the former) Czechoslovakia) (see Zima and Kral, 1984).

In this study, the conventional karyotype of *L. europaeus* is presented for the third time from Turkey. In two studies by Arslan (2010 and Demirbaş et al. (2010), as expected, the $2n$ of brown hares occurring

in Turkey was the same. However, comparing the values of the FN and FNa for Turkish brown hares, the present study had a lower number of chromosomal arms and was therefore different from previous studies (Arslan, 2010; Demirbaş et al., 2010) (Table 2). Furthermore, Demirbaş et al. (2010) reported that the karyotype of the Kilis sample had a duplication in one of the long arms in a pair of 14.

In conclusion, the karyotypes reported here and by Arslan (2010) and Demirbaş et al. (2010) may be representatives of the karyological forms of *L. europaeus* in Turkey (Table 2); however, the differences between karyotypes of Turkish brown hare could have resulted from the classification system used by each author; they could also be a reflection of inter-individual variations in the procedure used for chromosomal measurements.

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