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# Population Genetic Variability of *Myodes glareolus* (Schreber, 1780) (Mammalia: Rodentia) Distributed in Northern Anatolia as Revealed by RAPD – PCR Analysis

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**Abstract**: The bank vole, *Myodes glareolus*, lives in deciduous forests in Northern Anatolia. Eighty-four individuals collected from 17 localities in Turkey were studied to reveal the differentiation of *M. glareolus* by RAPD markers. Fifteen RAPD markers were tested and they yielded 142 DNA bands. Nei's (1978) genetic distance and similarity calculations were used in order to show the genetic relationships between the populations studied. The total genetic diversity and genetic differentiation values were calculated as H=0.1571 and $G_{ST}$=0.4170, respectively. Nei's genetic distance (D) ranged from 0.026 to 0.124. Thus the most genetically distant populations were Kandıra and Sümela with D=0.124, while the closest ones were Şile and Kandıra with D=0.026. The dendrogram based on the genetic distance data showed two main groups. The first group, including western populations, was divided into two subgroups; the first subgroup contained the Uludağ population and the second subgroup the remaining western populations. The second group, including eastern populations, was also separated into two subgroups. The first subgroup consisted of the Sümela population while the other eastern populations formed the second subgroup.

**Key words:** *Myodes glareolus,* genetic differentiation, RAPD-PCR, Turkey.

## Introduction

The bank vole, *Myodes glareolus*, is a rodent species widespread in the Palearctic region. Bank voles are distributed from the British Isles to Lake Baikal in Siberia (Raczynski 1983). There are 18 subspecies (Shenbrot, Krasnov 2005) but only one of them, *M. glareolus ponticus*, occurs in Turkey (Ellerman, Morrison-Scott 1951, Corbet 1978, Çolak, Kivanç 1991). Mixed and deciduous forests and bush woods are the preferred habitat of bank voles. Accordingly, habitat fragmentation caused by habitat barriers (human-induced or natural) – such as motorways and rivers – of the bank vole populations may cause a sub-structuring of the populations (Redeker *et al.* 2005).

The highways and dams in northern Anatolia divide up the habitat of *M. glareolus* and lead to the intermittent spread of populations. Accordingly, habitat fragmentation, over time, as in many other species (*Apodemus argenteus, Microtus arvalis*), may lead to changes in the genetic structure of the bank vole populations. Habitat barriers divide populations into smaller parts and lead to local reductions in genetic diversity or genetic bottlenecks. Thus, the isolated populations might differentiate genetically by inbreeding (Aars *et al.* 1998, Gerlach, Musolf 2000, Redeker *et al*. 2005, Kozakiewicz *et al*. 2009).

Morphological (Neuhauser 1936, Osborn 1962, Felten *et al.* 1971, Çolak, Kivanç 1991, Demirsoy *et*

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*al*. 2006), karyological (Çolak *et al.* 1997), and allozyme characters (Çolak *et al.* 2013) of *M. glareolus* in Turkey were studied. According to Osborn (1962) and Çolak, Kivanç (1991), *M. glareolus* in Turkey has a discontinuous distribution. This causes an interruption of the gene flow between different Turkish populations of *M. glareolus*. In addition, Çolak, Kivanç (1991) revealed some morphological differences between the western and eastern populations of northern Anatolia. Furthermore, the genetic differentiations in bank voles were studied in Europe (Tegelström, Jaarola 1998, Matson, Baker 2001, Redeker *et al.* 2005, Potapov *et al*. 2007, Kozakiewicz *et al*. 2009).

Redeker *et al.* (2005) analyzed 9 microsatellite loci in the Danish bank voles and observed significant genetic differentiation among the five distinct localities studied. According to the mentioned study, the habitat fragmentation or different tree composition of the forests could be the causes of differentiation. Kozakiewicz *et al*. (2009) studied the effect of the distance and habitat barriers on the spatial genetic structure of *M. glareolus* and *Apodemus flavicollis* populations. They used microsatellite markers and studied two island populations and two mainland populations living on opposite lakeshores. They found that the differences among mainland populations are small and the island populations are genetically more distinct. The genetic diversity of mainland populations is higher than that of the island populations in the two species separated by the water barrier (Kozakiewicz *et al*. 2009).

We analyzed the bank voles by using RAPD markers. The intrapopulation genetic polymorphism, genetic differentiation and phylogenetic relationships between populations can be detected by the RAPD technique (Spiridonova *et al*. 2008). In this context, the aim of the present study was to ascertain the genetic differentiation among Turkish *M. glareolus* populations by RAPD-PCR method.

# Materials and Methods

Eighty-four specimens from 17 localities in Turkey were analyzed to explore the extent of genetic variation in *M. glareolus* (*n*=84). Specimens of *Arvicola amphibius* (*n*=2) and *Glis glis* (*n*=2) were used as an outgroup (Fig. 1).

Total DNA was extracted from kidney tissues according to the CTAB DNA isolation method described by Doyle & Doyle (1991). The kidney tissues were kept at -80°C until the isolation of total

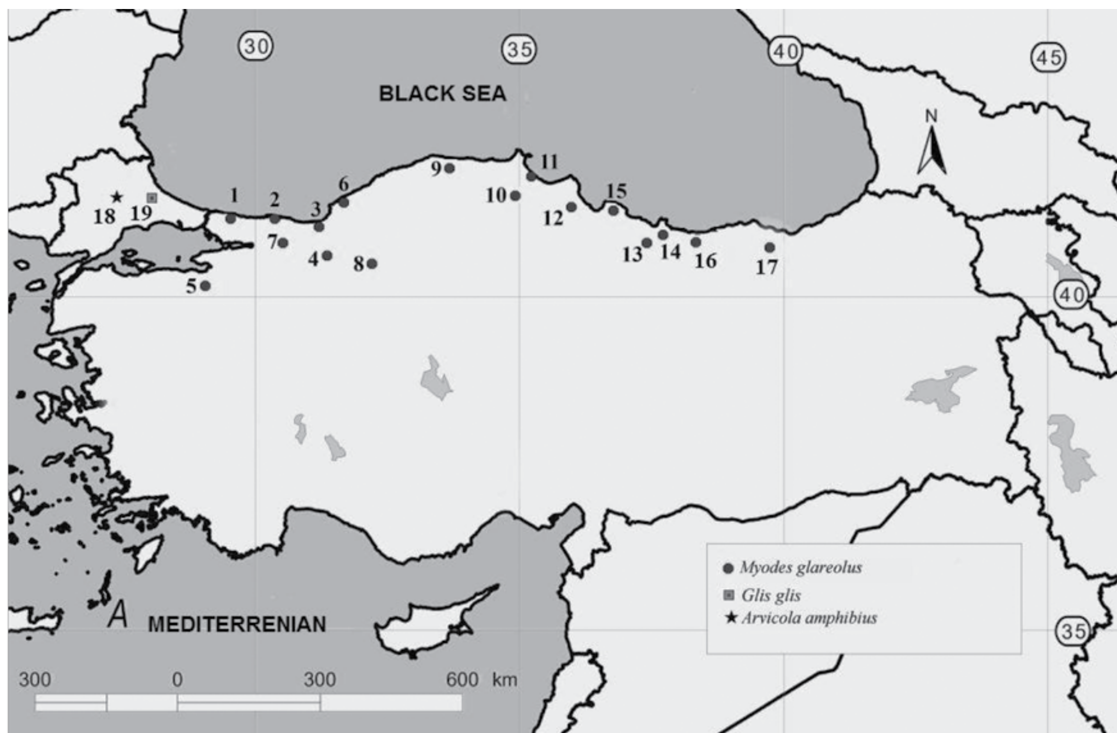


**Fig. 1**. Map showing the collection localities of *M. glareolus, A. amphibibius* and *G. glis* populations. 1-19: *M. glareolus*; 1. Şile (7), 2. Kandıra (6), 3. Akçakoca (6), 4. Abant (7), 5. Uludağ (3), 6. Zonguldak (7), 7. Kartepe (5), 8. Kızılcahamam (2), 9. Küre (6), 10. Bürnük (5), 11. Göktepe (6), 12. Çakallı (2), 13. Gürgentepe (3), 14. Ulubey (6), 15. Ünye (4), 16. Ordu (5), 17. Sümela (4), 18: *A. amphibius*; İnece (2). 19: *G. glis*; Demirköy (2)

DNA. A spectrophotometric method (Agilent 2100 BioanalyserNanoDropND-1000Spectrophotometer) and agarose gel electrophoresis were used to determine DNA quantity and quality, respectively.

The RAPD protocol of Kaya, Neale (1995) was modified as follows: 1 µl of the DNA samples (200 ng/µl), 2.5 µl of buffer (750 mM Tris-HCl pH: 8.8, 200 mM $(NH_4)_2 SO_4$; Fermentas), 0.3 µl (100 unit) of *Taq* DNA Polymerase (Fermentas), 4 µl of deoxynucleotide triphospate mix (200 µM of each nucleotide), 2 µl of 2 mM $MgCl_2$, and 1 µl of 1 pmol primers (Thermo Electron). In order to detect any DNA contamination, negative control reactions without genomic DNA were used.

The amplifications were performed using a Thermo Electron Px2 Thermal Cycler that was programmed for 95°C for 1 min, 45 cycles of 94°C for 1 min, 36°C for 2 min, 72°C for 2 min, and 72°C for 15 min. Sixty decamer primers were prescreened (Thermo Electron) and 15 of them were chosen with regard to band pattern quality and reproducibility for further analysis (Table 1). The amplification products were separated by 1.5% agarose gels in 1X TAE (Tris, acetic acid, EDTA) buffer at 70 V over 4 h. A 100 base pair ladder was used as a size standard marker (Generuler™ 100 base pair DNA Ladder).

The primers of OPA, OPC, and OPD series (Thermo Electron), including decamers, were used. The amplification products were evaluated visually. For each of them the haploid bands were scored as present ‘1’ or absent ‘0’. The UPGMA dendrogram was constructed by POPGENE Microsoft Window-based Freeware for Population Genetics Analysis, Version 1.31 (YEH *et al*. 1999) and the bootstrap values were calculated by TFPGA Version 1.3 (Miller 1997) for this dendrogram. NTSYS-pc v. 2.2 (Rohlf 2000) was used to evaluate the eigenvalues obtained from the RAPD bands and these values were used to generate PCA by SPSS 13 (NORUSIS 1994). The observed number of alleles ($N_a$), effective number of alleles ($N_e$), Nei’s genetic diversity (1987) (H), and Shannon information index (I) were calculated for each specimen. In addition, the expected heterozygosity of an individual in a particular population ($H_S$) and the expected heterozygosity of an individual in all populations ($H_T$) were estimated for conformity to with the Hardy-Weinberg expectations. The relative magnitude of genetic differentiation among the subpopulations ($G_{ST}$) was obtained according to NEI (1987) and the gene flows ($N_m$) were derived from the $G_{ST}$ value.

**Table 1.** List of the best yielding primers and corresponding base sequences in *M. glareolus* specimens

| Primers | Sequences | Number of Polymorphic Bands |
|---|---|---|
| OPA -06 | 5’-GTT CCC TGAC-3’ | 10 |
| OPA-08 | 5’-GTG ACG TAGG-3’ | 14 |
| OPA-09 | 5’-GGG TAA CGCC-3’ | 8 |
| OPA-10 | 5’-GTG ATC GCAG-3’ | 9 |
| OPA-16 | 5’-AGC CAG CGAA-3’ | 8 |
| OPA-20 | 5’-GTT GCG ATCC-3’ | 9 |
| OPB-01 | 5’-GTT TCG CTCC-3’ | 16 |
| OPB-02 | 5’-TGA TCC CTGG-3’ | 9 |
| OPB-04 | 5’-GGA CTG GAGT-3’ | 4 |
| OPB-06 | 5’-TGC TCT GCCC-3’ | 4 |
| OPB-17 | 5’-AGG GAA CGAG-3’ | 13 |
| OPD-01 | 5’-ACC GCG AAGG-3’ | 9 |
| OPD-07 | 5’-TTG GCA CGGG-3’ | 3 |
| OPD-12 | 5’-CAC CGT ATCC-3’ | 15 |
| OPD-15 | 5’-CAT CCG TGCT-3’ | 11 |

# Results

Fifteen RAPD primers revealed a total of 339 bands; with 142 out of the 339 bands belonging to specimens of *Myodes glareolus*. For all the specimens the lowest band number was obtained from OPB-6 primer (15), while the highest band number was obtained from OPD-12 primer (30) (Fig. 2). If only *M. glareolus* specimens were considered, the lowest band number was obtained from OPD-7 (3) and the highest from OPB-01 (16) (Table 1).

For all specimens, Nei’s genetic diversity or heterozygosity was H=0.1571 (P% =66.37). When only the populations of *M. glareolus* were considered the $G_{ST}$ value was 0.4170 and $N_m$ was 0.6990. Nei’s H and P% values ranged from 0.0525 to 0.1246 and from 12.68 to 35.10, respectively. According to these values, the highest polymorphism was detected in the Zonguldak population, while the lowest one in the Çakallı population. Nei’s genetic distance (D) ranged from 0.026 to 0.124. Thus the most genetically distant populations were Kandıra and Sümela with D=0.124 (distance 1027 km), while the closest ones were Şile and Kandıra with D=0.026 (distance 67 km).

The constructed dendrogram based on genetic distance data showed two clusters. The first cluster that included western populations was divided into two subgroups: the first subgroup contained the Uludağ population, while the second subgroup the other western populations. The second cluster that included eastern populations was also separated into two subgroups. The first subgroup consisted of the

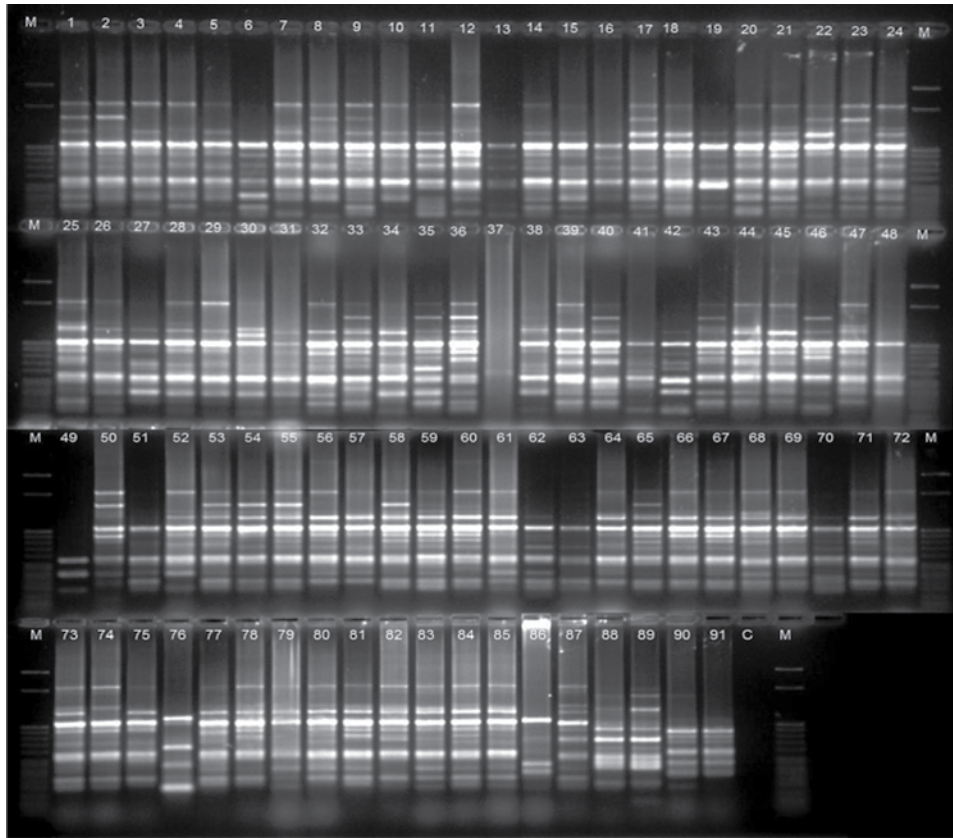


**Fig. 2.** Amplification products of primer OPD-12. C: negative control M: marker (100 bp DNA Ladder, Generuler™) 1-87: *M. glareolus* (1-7: Şile, 8-13: Kandıra, 14-19: Akçakoca, 20-26: Abant, 27-29: Uludağ, 30-36: Zonguldak, 37-41: Kartepe, 42-43: Kızılcahamam, 44-49: Küre, 50-54: Bürnük, 55-60: Göktepe, 61-62: Çakallı, 63-65: Gürgentepe, 66-71: Ulubey, 72-75: Ünye, 77-81: Ordu, 82-85: Sümela) 88-89: *A. amphibius* - İnece, 90-91: *G. glis* – Demirköy. Samples numbered 76, 86, and 87 belonged to other species and were excluded from the study

Sümela population, while the other eastern populations formed the second subgroup (Fig. 3).

*M. glareolus* was subsequently analyzed using principal component analysis (PCA) on 339 RAPD loci (Fig. 4). The PCA corresponded to the UPGMA dendrogram as the eastern and western Black Sea populations were separated from each other.

## Discussion

Some rodent studies have shown that the number of genetically distinct groups can vary over geographic distances (Patton, Smith 1990). The species that occupy broad areas show higher genetic variation than those that occupy narrow areas (Nevo 1978, 1983). It is assumed that the gene flow is efficient in homogenizing populations only over short distances. It is possible that the ability of the species to migrate and inhabit different territories may affect the rate of gene flow (Gebczynski *et al*. 1993). In a Polish forest population P%=30 was determined (Fedyk, Gebczynski 1980). P%=12 was reported in a Danish population (Gebczynski *et al*. 1986) and P%=8-22 in an Austrian population (Leitner, Hartl 1988). Genetic variation was described in certain Swedish populations, and the P% and H values were found to be different between geographically close populations, as well as between distant populations (Graf, Scholl 1975, Graf 1982). In the present study, by using the RAPD technique P% was determined to be 66.37. An association between the geographical distance and genetic variation was found. According to the dendrogram and values of the geographic distance, the genetic distance increases with the increase in the geographic distance among populations. A comparison between the European and Turkish populations showed that the range of polymorphism within the Turkish bank vole populations (66.37%) is greater than that of the European bank vole populations. This difference may be ascribed to the use of different techniques or to the wide dispersion of Turkish populations.

According to Çolak *et al*. (2013), the fur of the Şile population is different from that of the others

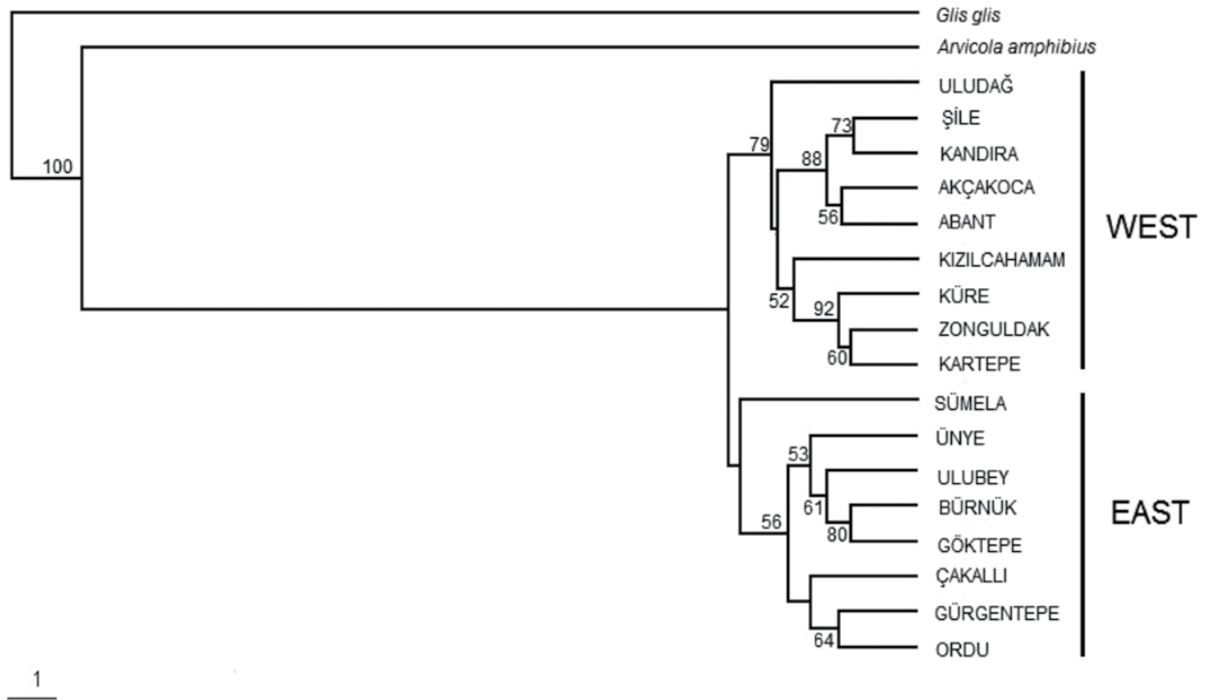


**Fig. 3.** Generated dendrogram based on the genetic distance of *M. glareolus* from different localities using the UPGMA method. At the branching nodes the BP (bootstrap) values exceeding 50% are presented

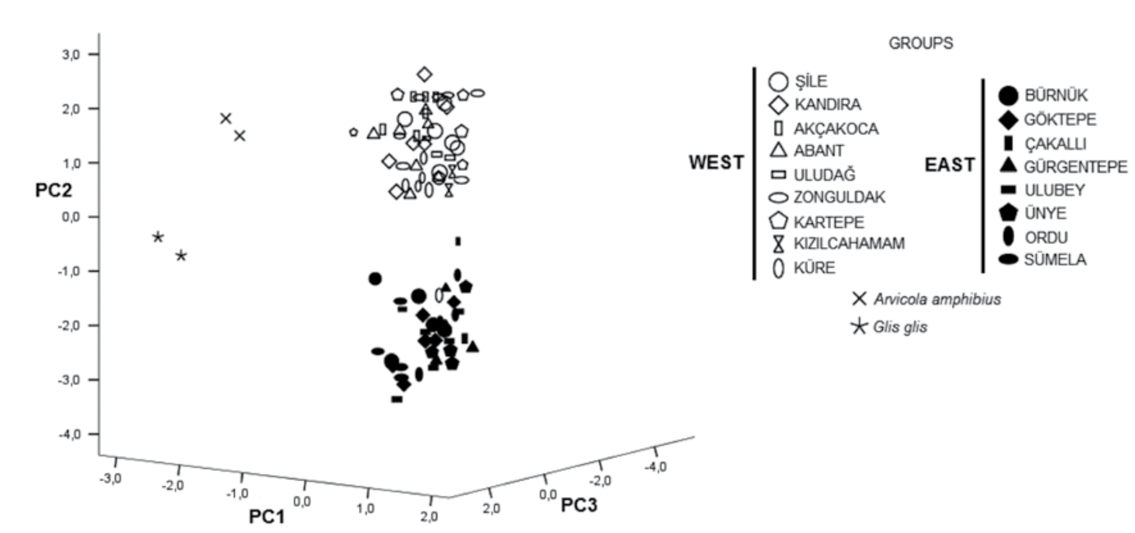


**Fig. 4.** Principal component analysis of *Myodes glareolus*

and the dorsal parts of the furs are darkish. In an allozyme study (Çolak *et al.* 2013), 24 loci belonging to 16 enzyme system were analyzed. Twelve out of the 24 loci were polymorphic, revealing P=50%, $N_m$=0.7213. NEI (1972)'s D values ranged from 0.0022 to 0.1465. According to the genetic distance values and the dendrogram based on the Cavalli–Sforza coefficient (NJ: Neighbor-joining tree), 8 populations of *M. glareolus* constituted 3 groups. Dispersion to clusters of populations differed in the two studies. In the present study, the dendrogram showed two main clusters; the first cluster included western populations and the second included eastern populations. The western and the eastern sampling areas have different geographical and vegetation characteristics. The localities in the western sampling areas (Şile, Kandıra, Akçakoca, Kızılcahamam, Abant, Uludağ, Küre, Zonguldak, Kartepe) have a harsh climate. These areas are covered with mixed forests including pine and old beech trees. The localities in the eastern sampling areas (Sümela, Ünye, Ulubey, Bürnük, Göktepe, Çakallı, Gürgentepe, Ordu) have a

milder climate and are closer to the Black Sea coast, being covered with young beech forests. The genetic differentiation among the eastern and western areas may be on the account of the adaptation of bank voles to different environmental conditions.

The main reasons for genetic differentiation are habitat patchiness and separate distribution of the species and these factors may help maintain genetic variation in species (Lande 1988). In the study of Kozakiewicz *et al*. (2009), the spatial genetic structures of *M. glareolus* and *Apodemus flavicollis* populations were detected. The authors found that the island populations were more divergent than the other populations. The effect of geographical isolation was greater in the island populations of *M. glareolus* in comparison to the populations of *A. flavicollis* because of the lower mobility in the bank voles and differences in behavior.

In the present study the Uludağ population was found to be more genetically distinct among the other western Anatolian populations, because there is a difference in altitude between the Uludağ and the other western populations' habitats. As the locality of Uludağ is higher than the other western localities, the Uludağ population appeared to have different adaptation value which may cause the genetic separation from the others. The separation of the Uludağ population is an indication of adaptation to the ecological conditions of high elevation.

Redeker *et al*. (2005) studied the effect of habitat fragmentation on bank vole populations. They reported that forest damage (natural or human-induced) caused sub-structuring of bank vole populations and limitation of gene flow between populations. Therefore, the intrapopulation genetic diversity would decrease and genetic bottlenecks would be seen in populations. Redeker *et al*. (2005) detected a genetic bottleneck in one out of the 5 populations studied. We did not observe a genetic bottleneck effect in our study. The gene flow was high enough to show that it was still continuing ($N_m$=0.6990).

In conclusion, the genetic differentiation detected among the populations of *M. glareolus* distributed in northern Anatolia and the constructed dendrogram based on genetic distance data showed two clusters. The first cluster included western populations and the second one represented eastern populations. The genetic differentiations of populations are consistent with geographic distances. The gene flow was found to be high. According to the RAPD data, it appears that the gene flow between bank vole populations is continuing. Bank vole populations can evolve in different ways depending on the genetic diversity and genetic drift. Even though *M. glareolus* in Northern Anatolia is divided into eastern and western groups, the consensus in the literature regarding the dispersion of a single subspecies (*M. glareolus ponticus*) in Turkey appears to be supported on the grounds of the high level of gene flow between them. In the future, increasing the number of specimens and examining more polymorphic loci may be useful in gaining insights into the taxonomy of bank voles and the genetics of populations.

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