

Population Structure of Edible Dormouse, *Glis glis* (Linnaeus, 1766) in Turkey, Inferred from RAPD-PCR

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Abstract: The genetic structure of *Glis glis* (LINNAEUS, 1766) using RAPD-PCR technique was analysed. This study is conducted on 35 individuals collected from seven localities of Turkey. Twelve of the 20 RAPD markers tested yielded 74 polymorphic DNA bands. To show the genetic relation between the studied populations, genetic distance and similarity calculations of Nei were used. The total genetic diversity and genetic differentiation values were calculated as $H_T = 0.305$ and $G_{ST} = 0.864$, respectively. The dendrogram based on genetic distance data formed two clusters. The first one consisted of Kırklareli and Zonguldak populations. The second cluster was divided into two subgroups; the first one contained Ordu and Giresun populations, and the second subgroup was formed from Trabzon, Rize and Artvin populations. Populations from Thrace were not separated from those in Anatolia. All seven populations examined form North West Turkey and North East Turkey groups.

Key words: *Glis glis*, RAPD, Turkey, Taxonomy, Population genetics

Introduction

Specific genetic differentiations between populations or within populations may present a viewpoint to understand speciation and evolutionary changes (ALMEIDA *et al.* 2000). Many new species were described based on genetic researches (FILIPPUCCI *et al.* 1989, MEZHHERIN & ZAGORODNYUK 1989, VORONTSOV *et al.* 1992) and some subspecies were raised to species status (STORCH & LUTT 1989, MEZHHERIN 1991, VOGEL *et al.* 1991, VORONTSOV *et al.* 1992, ORLOV *et al.* 1996).

Edible dormouse, *Glis glis* (LINNAEUS, 1766) is an arboreal mammal living in deciduous and mixed forests in an area extending from Bulgarian border in the west to Georgian border in the east. It is distributed in Black sea, Marmara and Thrace regions of Turkey. In Turkey, *G. glis* has two subspecies; *G. g. orientalis* NEHRING (1903) in Anatolia and *G. g. pindicus* ONDRIAS (1966) in Thrace. WILSON & REEDER (2005) evaluated these subspecies as synonyms. Bosphorus and Dardanelles divide the distribution ar-

reas of species into two parts - Anatolia and Thrace. For all that, natural (deep valleys, rivers and high mountains) and manmade (dams, industrialization, roadways, highways, railways and new settlements) barriers fragment habitat of *G. glis*. These show that its Turkish populations are affected by anthropogenic activities and natural barriers such as Bosphorus and Dardanelles straits. Because of all these reasons it is important to determine genetic structure of *G. glis* in Turkey. This study can shed light on subsequent genetic studies on Turkish *G. glis*.

There are many comprehensive researches clarifying taxonomy, allozyme, morphometric and mitochondrial phylogeography. HURNER *et al.* (2010) reported two haplotypes in European sublineage of *G. glis* based on mtDNA (cytochrome b) and pointed out low genetic diversity of Turkish Thrace populations. In Turkey, Thrace and Anatolian populations of *G. glis* are distinguished using karyological, morphological and bacular features (DOGRAMACI & TEZ

1991). On the contrary, these populations would not be distinguished each other clearly based on allozymic loci (ÇOLAK *et al.* 2008).

Random amplified polymorphic DNA (RAPD) analyze is an informative method of population genetic analysis and they often show reasonably high levels of polymorphism that can be useful for inferring close genetic relationships (FREELAND 2005). RAPD markers were introduced as a method for genotyping individuals at multiple loci (WELSH & MCCLELLAND 1990, WILLIAMS *et al.* 1990). Also, this technique was used as a useful tool to determine genetic differentiation between subspecies and geographic populations of some rodents; *A. agrarius* (ATOPKIN *et al.* 2007, DOKUCHAEV *et al.* 2008), *Mus musculus* (SPIRIDONOVA *et al.* 2008) and *A. mystacinus* (OLGUN *et al.* 2009).

The aims of this study are: 1) to reveal genetic differentiations between two subspecies of *G. glis*; 2) to discuss impact of the straits on gen flow between Turkish Thrace and Anatolian populations; 3) to asses distribution pattern of populations in Anatolia; 4) define population specific RAPD patterns; 5) to determine phylogenetic relationships between populations.

Material and Methods

Localities of samples

35 individuals of *Glis glis* from 7 localities of Turkey were examined. The samples from the regions situated to the west (Thrace) and the east (Anatolia) of Istanbul Bosphorus received as the disjunction zone. As outgroups, we used forest dormouse *Dryomys nitedula* from Çamlıhemsin-Rize (N = 2), woolly dormouse *Dryomys laniger* from Elmalı-Antalya (one individual) and Macedonian mouse *Mus macedonicus* from Edirne (N = 3).

The sampling localities and sizes were as follows: 1) Dereköy-Kırklareli (N = 7); 2) Demirköy-Kırklareli (N = 10); 3) Zonguldak (N = 3); 4) Ordu (N = 3); 5) Giresun (N = 1); 6) Trabzon (N = 4); 7) Çayeli-Rize (N = 3); 8) Borçka-Artvin (N = 2); 9) Şavşat-Artvin (N = 2) (Fig. 1).

Isolation procedure and amplification conditions

These samplings were demoted to 7 populations as Demirköy with Dereköy (Kırklareli) and Borçka with Şavşat (Artvin) grouped together.

DNA was isolated from kidney tissue according to the CTAB method of DOYLE & DOYLE (1991).

DNA was quantified using a spectrophotometer (Agilent 2100 Bioanalyser NanoDrop ND-1000 Spectrophotometer). The PCR was run in 25 µL of a reaction mixture containing 1 µL of the DNA samples (200 ng/µL); 2.5 µL of buffer (750 mM Tris-HCl pH: 8.8, 200 mM (NH₄)₂ SO₄; Fermentas); 0.3 µL of *Taq* DNA Polymerase (100 unit Fermentas); 4 µL of deoxynucleoside triphosphate mix (200 µM of each nucleotide); 1.5 µL of 2 mM MgCl₂; 1 µL of 1 pmol primers (Thermo Electron).

The PCR steps were as follows; 95 °C for 1 min, 45 cycle of '94 °C for 1 min, 36 °C for 2 min, 72 °C for 2 min' and 72 °C for 15 min. Prescreening of 20 random decamer primers revealed that 12 primers could be useful for further study and data collection (Table 1).

Table 1. Polymorphism percentage of all primers.

Primer	Sequence 5'→3'	P %
OPA-01	CAGGCCCTTC	36.3
OPA-02	TGCCGAGCTG	28.5
OPA-03	AGTCAGCCAC	62.5
OPA-04	AATCGGGCTG	66.6
OPA-08	GTGACGTAGG	60
OPB-01	GTTTCGCTCC	66.6
OPB-02	TGATCCCTGG	11.1
OPB-03	CATCCCCCTG	12.5
OPB-04	GGACTGGAGT	66.6
OPB-06	TGCTCTGCCC	10
OPD-02	GGACCCAACC	27.2
OPD-04	TCTGGTGAGG	100

Agarose gel electrophoresis

The amplification products were separated on 1.7% agarose gels in 1X TAE (Tris, acetic acid, EDTA) buffer at 100 volts for 4 h and visualized by staining with ethidium bromide. A 100 base pair ladder was used as a size standard marker (DNA Ladder Plus, Fermentas).

Analyzing of amplified products

All visible bands on gels were considered as RAPD loci and all loci were scored as presence (1) and absence (0) of the bands. RAPD patterns that could not be seen on agarose gels were out of evaluation so they were not amplified (Fig. 2). POPGENE Version

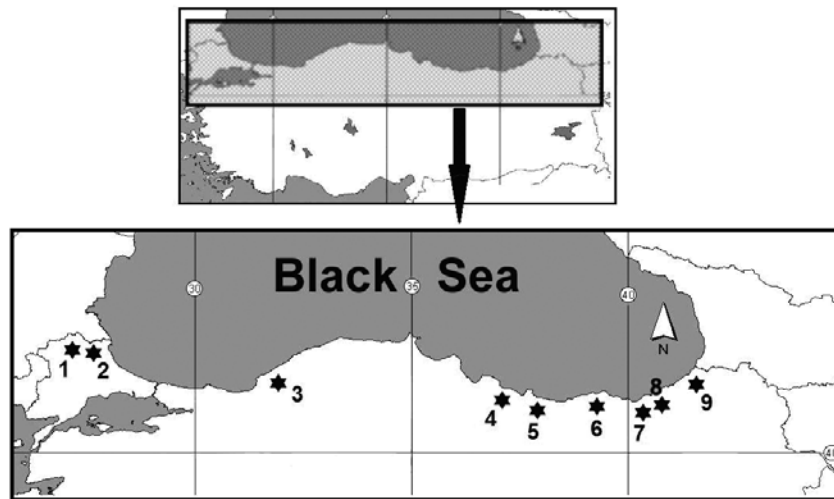


Fig. 1. Collecting localities of specimens (Numbers referring to localities are presented in Materials and Methods).

1.31 (YEH *et al.* 1997) software package was used to compute the intrapopulation and interpopulation variations. By this software the percentage of polymorphic loci (P), observed number of alleles (N_a), effective number of alleles (KIMURA & CROW 1964) (N_e), NEI's (1972) gene diversity (H) and Shannon's Information index (LEWONTIN 1972) (I) were computed to display intrapopulation variations. The estimated parameters of interpopulation differentiation included total gene diversity (H_T), intrasample gene diversity (H_S), interpopulation gene diversity (DST), coefficient of gene fixation (G_{ST}) and coefficient of gene flow (the number of migrants per generation) Nm. Genetic distance matrix (NEI 1972) was used to draw UPGMA tree by MEGA software version 4.0 (TAMURA *et al.* 2007).

To further examine the patterns of variation among individual samples, a principal coordinated analysis (PCoA) was performed with NTSYS-pc v. 2.2 (ROHLF 2000). Pairwise genetic distance were calculated using Jaccard's coefficient (JACCARD, 1908) for binary data. EIGEN values was used to compute principal coordinates along all axes. The resulting first three axes were latter used to 3D-Plot with PAST v. 1.99 (HAMMER *et al.* 2001).

Results

Variation of RAPD patterns

In this study 12 of 20 RAPD primers were chosen to analyze *Glis* specimens. While these 12 RAPD primers constituted 169 bands for all individuals,

only 74 bands were observed in *Glis* specimens. The most polymorphic primer was OPD-04, which was diagnostic between Black Sea and Thrace populations. Three primers (OPA-04, OPB-01 and OPB-04) shared the polymorphism of 66.6%. Also the primer OPB-02 has the least polymorphism as 11.1% (Table 1). RAPD band profiles could seem most regular in OPD-02 (Fig. 2).

Inference of genetic variation and differentiation analysis

Genetic distance matrix that was computed belong to NEI (1972) showed that while the closest population were Kırklareli and Zonguldak ($D = 0.0553$), the most distant (furthest) populations were Zonguldak and Rize ($D = 0.2713$) (Table 2).

Glis glis was subsequently analyzed with a principal coordinated analysis (PCoA) on 169 RAPD loci. The three axes obtained in the PCoA explained 45.6% of the total variation. The proportions of variation explained by the first, the second and the third axis were 23.9, 36.4, and 45.6%, respectively. Kırklareli and Zonguldak specimens formed a group distant from the other localities (Fig. 3).

The mean observed number of alleles (N_a) was 2.00. When all populations were considered, the mean N_e value was 1.412. NEI's genetic diversity or heterozygosity (H) was the lowest in Zonguldak and Giresun (0.000) and the highest in Trabzon (0.104). For all populations, the genetic diversity was calculated as 0.2577. The high G_{ST} value of 0.864 indicated that genetic differentiation among the studied populations was substantial.

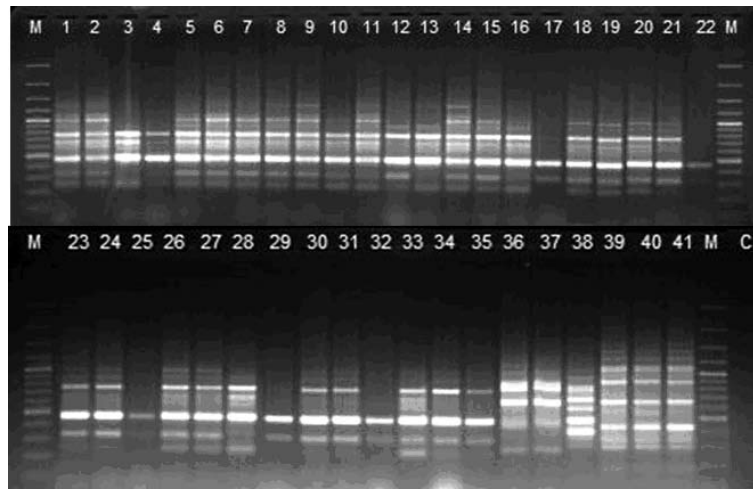


Fig. 2. Amplification products of Primer OPD-02. M: marker (100 bp DNA Ladder, Fermentas), 1-35: *Glis glis* (1-17: Kırklareli, 18-20: Zonguldak, 21-23: Ordu 24: Giresun, 25-28: Trabzon, 29-31: Rize, 32-35: Artvin), 36-37: *Dryomys nitedula*, 38: *Dryomys laniger*, 39-41: *Mus macedonicus*, C: Negative control.

Table 2. Pairwise dissimilarity matrix based on NEI (1972) between *Glis glis* populations.

	1	2	3	4	5	6	7
1. Kırklareli	-						
2. Zonguldak	0,0553	-					
3. Ordu	0,2111	0,2255	-				
4. Giresun	0,1506	0,1538	0,1036	-			
5. Trabzon	0,0861	0,1057	0,1996	0,142	-		
6. Rize	0,2387	0,2713	0,1603	0,1792	0,1355	-	
7. Artvin	0,1716	0,2003	0,171	0,1548	0,0905	0,1244	-

The total gene diversity (H_T) was 0.305 in *Glis glis* populations, but 13.5 % of this was within population variation ($H_S = 0.0414$).

Phylogenetic tree constructed based on NEI's genetic distance matrix clustered seven populations of *G. glis* into two main groups; North Western Turkey and North Eastern Turkey. The first group includes Kırklareli and Zonguldak populations, which are the closest populations each other. The second one was split to two subgroups as Ordu-Giresun and Rize-Trabzon-Artvin (Fig. 4).

The genetic differentiation computed using coefficient of gene fixations and gene flow values was meaningful. Gene flow value was low ($Nm = 0.3244$) among *G. glis* populations.

When Thrace and Anatolian populations of *G. glis* were compared, even if Zonguldak is grouped with Kırklareli, gene flow between North Eastern and North Western Turkey populations was very low ($Nm = 0.0815$).

Discussion

Geographical variations on subspecific level of *G. glis* were investigated in its distribution area in Turkey. DOGRAMACI & TEZ (1991) and ÇOLAK *et al.* (2008) separated morphologically and morphometrically *G. g. pindicus* in Thrace from *G. g. orientalis* in Anatolia. In contrast, analysis of 28 allozyme loci did not reveal any differentiation between two subspecies (ÇOLAK *et al.* 2008). On the basis of RAPD-PCR analysis in this study, *G. g. pindicus* was not clearly separated from *G. g. orientalis*. It seems to be a contradiction between genetic and morphological data in distinguishing of subspecies of *G. glis* in Turkey. According to FILIPPUCI & KOTSAKIS (1995), the reason of the contradiction between genetic and morphological data among Glirid species is the ancient origin and separation of its genera. In addition to FILIPPUCI & KOTSAKIS (1995), a comparison of this study to morphological study (DOGRAMACI & TEZ

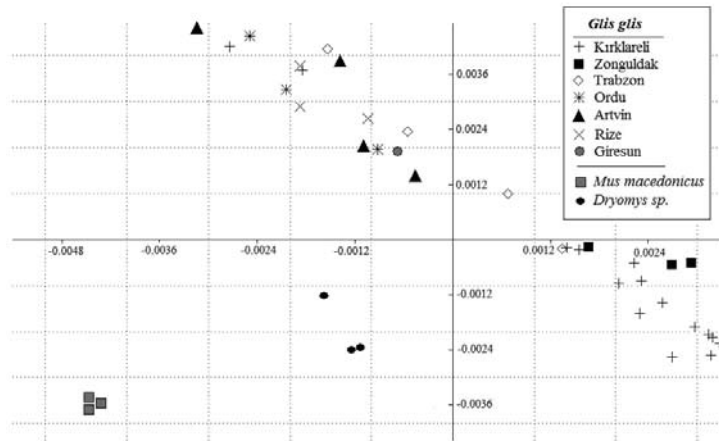


Fig. 3. Principal coordinated analysis (PCoA) of *Glis glis*.

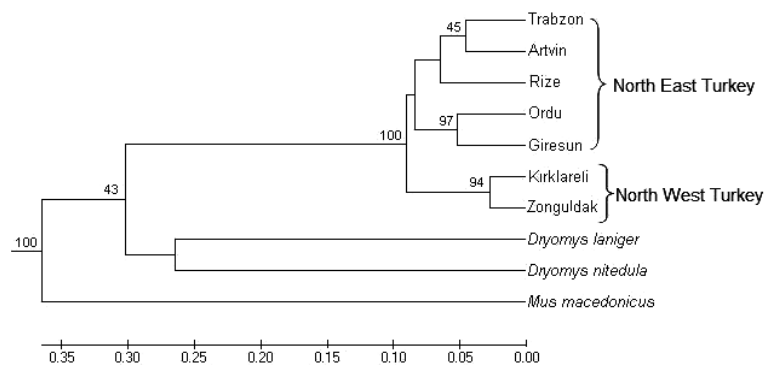


Fig. 4. UPGMA tree of seven populations of *Glis glis* depend on Nei (1972).

1991) reveals a contradiction with respect to separating of *G. glis* subspecies.

Seven populations examined were clustered into two main groups (Fig. 4) - the first is North Western Anatolia and the second is North Eastern Anatolia with high bootstrap value. This grouping may be resulted from climate conditions changing from the West to the East of Black Sea coast, Eurosiberian climate gradually increases in seasonality (AKMAN 1995, 2011).

According to KRYŠTUFEK & VOHRALĀK (2005), Turkish range of *G. glis* has two parts, European and Asiatic. But, in present study, population from Zonguldak (N = 3) are genetically closer to those in Thrace. This shows that, although Bosphorus and Dardanelle straits seem to be a geographic barrier, as formed about 10 000 years ago (HOSEY 1982), gene flow continues between populations in Thrace and those in Anatolia. Also, present results confirm those given by MICHAUX *et al.* (2003, 2004) who suggest the population of wood mice (*Apodemus*

sylvaticus) in western Turkey and yellow-necked field mice (*Apodemus flavicollis*) in Turkey are very similar to that from the Balkans. The reason of this gene flow may be introduction of *G. glis* by lorries carrying wood and logs of oak from Turkish Thrace (Istranca forests) to several cities in Anatolia. HURNER *et al.* (2010) reported a possible introduction of *G. glis* from mainland Italy to Sicily by human traffic, based on mtDNA haplotype. During the Neolithic, the transportation of chert and obsidian across the Tyrrhenian Sea might be given dormice the opportunity to spread into the islands (Italy) (CARPANETO & CRISTALDI 1995). In Çakallı (Samsun), a dormouse was captured among logs in a lorry coming from Ordu (Çolak, E. pers. comm.). These show that *G. glis* can be easily transported to one place to another one.

Genetic differentiation was low among Ordu, Giresun, Trabzon, Rize and Artvin populations ($H_s = 0.0414$). This indicates a relationship between genetic similarity and geographic proximity.

Number of polymorphic loci was low in the Turkish Thrace population (FILIPPUCCI & KOTSAKIS 1995, ÇOLAK *et al.* 2008) and Anatolia populations (ÇOLAK *et al.* 2008), as in this study. This low polymorphism shows the low variability in Turkish lineage of *G. glis*, and genetic relationship between Turkish and European lineages. The possibly reason of the low diversity has been recently explained by HURNER *et al.* (2010). According to HURNER *et al.* (2010), in European lineage of *G. glis*, the reason of the low diversity is probably the result of a recent expansion (dated around 2000 years ago) from a single refugee in where *G. glis* may be reinforced into genetic bottlenecks by some factors.

With respect to the reason of low genetic differentiation between *G. glis* populations, it may be due to discontinues distribution of this species. During this study, we observed that *G. glis* lives in patches (hazel and beech trees) in tea gardens and ruin houses away from pure beech and mixed forests, which are favourable habitats of *G. glis*. Also, ÇOLAK *et al.* (1994) found a breeding colony including 8 females and their pups of *G. glis* in a honey hive at a linden tree within tea garden. This confirms that there is

a continuously gene flow between *G. glis* populations in Anatolia and habitat fragmentation (due to tea plantation) does not stop gen flow between local populations of *G. glis*.

In conclusion, this species needs specific habitats such as mixed and old beech forests. Habitat fragmentation and habitat loss by anthropogenic activities impact population structure of *G. glis*, but not gene flow. Also, there is a habitat shift from favourable habitat to urban areas. Besides, RAPD markers revealed low polymorphism in *G. glis* populations. We analyzed three specimens from Zonguldak. This sample size may be less for RAPD analysis. It is important to determine if Anatolia is a refugium for *G. glis* or not. It needs more specimens, especially from Marmara region and various molecular markers, such as microsatellites, to examine the effect of Istanbul Bosphorus and Dardanelles on genetic differentiation between Thrace and Anatolian populations, and to contribute to conservation biology of *G. glis*.

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