
Sevilay Ustumbas, Reyhan Colak, Gul Olgun Karacan and Ercument Colak
Department of Biology, Faculty of Science, Ankara University, 06100 Ankara, Turkey

**Abstract:** Water vole, *Arvicola amphibius* is a rodent distributed widely in Palearctic region. Three subspecies of *A. amphibius* are distributed in Turkey. *A. amphibius* lives in Turkey located between Europe and Asia. In Turkey, there is no any study on this species at the level of genetic structure. The aim of the present study was to survey genetic structure based on DNA markers and to contribute to the taxonomic status, population genetics of *A. amphibius*, distributed in Thrace and Anatolia. A total of 38 specimens were collected from nine locations. In order to explore the extent of genetic variation in *A. amphibius* populations, a Randomly Amplified Polymorphic DNA (RAPD) marker system was used. The estimates of NEI’s standard genetic identity and standard genetic distance were calculated to show the genetic relationships between populations studied. UPGMA dendogram constructed with genetic distance data was clustered in 2 groups. The 1st group contains Thrace populations and the 2nd one including Anatolian populations was divided into 3 subgroups. Consequently, RAPD-PCR marker system confirmed the validity of *A.a. cerniavskii* and *A.a. persicus*.

**Key words:** Water vole, *Arvicola amphibius*, evolution, RAPD-PCR, populations, Turkey

**INTRODUCTION**

Water voles were once a familiar waterside animal often known locally as a water rat. Water voles are almost wholly vegetarian, feeding on a wide range of plants. They need luxurious bank side vegetation, particularly grasses and sedges to provide food and cover from predators. Although, water voles are widely distributed in palearctic region, they are one of the most rapid and serious declines of any mammal in recent years. This decline is attributed to habitat loss such as through river management and drainage. On this account beside morphological (Knatochvil, 1983; Nikolaeva, 1982; Krystufek and Tvrzovie, 1984; Ventura, 1991) and karyological studies (Raicu et al., 1971; Kulevi et al., 1978; Zima and Kral, 1984) there are several researches on metapopulation level (Stewart et al., 1999; Berthier et al., 2004, 2005, 2006; Aars et al., 2006).

Also mitochondrial genes were used for phylogeny researches (Martin et al., 2000; Pfunder et al., 2004; Piertney et al., 2005). Mursaloglu reported three subspecies of *Arvicola amphibius* in Turkey: *A. amphibius hintoni* from South Eastern Turkey, *A. amphibius persicus* from Anatolia and *A. amphibius cerniavskii* from Turkish Thrace. Morphological, karyological and biometric characters of *A. amphibius* were studied in Turkey (Ozkurt et al., 1999; Gozcelioglu et al., 2006). Although, blood proteins and allozyme profiles were investigated, these studies contained limited areas (Ilygın and Colak, 2004). In recent years to reveal intraspecific genetic differentiation and define the origin of the species, mtDNA and microsatellites have been usually used in Europe. But in Turkey, this species has not been studied on genetic level and this cause deficiency of data while considering *A. amphibius* population with other geographic forms in literatures. In this study, RAPD markers were elected for why this marker can provide an efficient assay for polymorphisms which should allow rapid identification and isolation of chromosome-specific DNA fragments (Williams et al., 1990).

**MATERIALS AND METHODS**

**Sampling localities:** We examined 38 individuals of *Arvicola amphibius* from nine localities of Turkey. As outgroups, we used Macedonian mouse *Mus macedonicus* from Kirklareli and Konya (N = 4), *Microtus levis* from Konya (N = 2) and *Microtus genntheri* from Kirklareli (N = 2). The sampling localities and sizes were as follows: Kirklareli (N = 6), Denizli (N = 4), Usak (N = 4), Afyon (Lake Eber) (N = 4), Konya (Lake Bayshehir) (N = 4), Eskisehir (N = 4), Bolu (N = 4), Ankara (N = 4), Kirsehir (N = 4) (Fig. 1).

**Corresponding Author:** Reyhan Colak, Department of Biology, Faculty of Science, Ankara University, 06100 Ankara, Turkey
Isolation procedure and amplification conditions: DNA was isolated from kidney tissue according to the CTAB method of Doyle and Doyle (1990). DNA was quantified using a spectrophotometer (Agilent, 2100 Bioanalyzer 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⁻¹); 2.5 μL of buffer (750 mM Tris-HCl pH 8.8, 200 mM (NH₄)₂SO₄; ferments); 0.3 μL of Taq DNA polymerase (100 unit ferments); 4 μL of deoxynucleotide triphosphate mix (200 μM each nucleotide); 1.5 μL of 2 mM MgCl₂; 1 μL of pmol primers (Thermo electron). The PCR steps were as follows: 95°C for 1 min, 45 cycles of 94°C for 1 min, 36°C for 2 min, 72°C for 2 and 15 min. Pre-screening of 23 random deamer primers revealed that 17 primers could be useful for further study and data collection (Table 1).

Agarose gel electrophoresis: The amplification products were separated on 1.7% agarose gels in 1X TAE (Tris, acetic acid, EDTA) buffer at 100 V 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, ferments).

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. We used POPGENE Version 1.31 (Yeh et al., 1997) software package to compute the intrapopulation and interpopulation variations. By this software, the percentage of polymorphic loci (P), observed number of alleles (N̄₀), effective number of alleles (Kimura and Crow 1964) (N̄ₑ) and Shannon's Information index (Lewontin, 1972) (I) were computed to display intrapopulation variations. The estimated parameters of interpopulation differentiation included total gene diversity (Hₚ), intrasample gene diversity (Hₛ), interpopulation gene diversity (Dₛ), coefficient of gene fixation (Gₛ), and coefficient of gene flow (the number of migrants per generation) Nm. Genetic distance matrix (Nei, 1972) was used to draw UPGMA tree by TFGPA software Version 1.3 (Miller, 1997) and MEGA software Version 4.0 (Tamura et al., 2007).

RESULTS

In this study, 17 of 23 RAPD primers were choice to analyze Arvicola specimens. While these 17 RAPD primers constituted 147 bands for all individuals, only 95 bands were observed in Arvicola specimens. Four primers (OPA-2, OPB-6, OPB-19, and OPD-10) were diagnostic between Anatolian and Thrace populations (Fig. 2).

Inference of genetic variation and differentiation analysis: Genetic distance matrix that was computed according to Nei (1972) showed that while the closest populations were Eskisehir and Bolu (D = 0.057), the most distant populations were Kırklareli ve Bolu (D = 0.187) (Table 2).

The mean observed Number of alleles (Na) was 1.561. When all populations were considered, the mean Ne value was 1.266. Nei’s genetic diversity or Heterozygosity (H) was the lowest in Denizli (0.051) and the highest in Afyon (0.094). For all populations, the genetic diversity was calculated as 0.160.

The high Gₛ value of 0.496 indicated that genetic differentiation among the studied populations was substantial. The total gene diversity (Hₚ) was 0.146 in Arvicola amphibius populations but 50.3% of this was within population variation (Hₛ = 0.0736). UPGMA tree was constructed using TFGPA and MEGA software.

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Table 1: Sequences and polymorphism percentage of all primers

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
<th>P (%)</th>
</tr>
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<tbody>
<tr>
<td>OPA-02</td>
<td>5'-TGC CGA GCT G-3'</td>
<td>4.1</td>
</tr>
<tr>
<td>OPA-03</td>
<td>5'-AGT CAG CCA C-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>OPA-04</td>
<td>5'-AAT CGG GCT G-3'</td>
<td>9.5</td>
</tr>
<tr>
<td>OPA-07</td>
<td>5'-GAA ACG GGT G-3'</td>
<td>8.2</td>
</tr>
<tr>
<td>OPA-08</td>
<td>5'-GTG ACG TAG G-3'</td>
<td>7.5</td>
</tr>
<tr>
<td>OPA-10</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>OPA-16</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>OPA-18</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>OPA-19</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>Opb-20</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
<tr>
<td>Opb-08</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
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<td>Opb-09</td>
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<td>Opb-10</td>
<td>5'-GGA GGG CTG T-3'</td>
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<td>Opb-11</td>
<td>5'-GGA GGG CTG T-3'</td>
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<td>Opb-12</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
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<tr>
<td>Opb-14</td>
<td>5'-GGA GGG CTG T-3'</td>
<td>5.4</td>
</tr>
</tbody>
</table>
populations called Thrace, the 2nd group contained three sub-groups as Kirsehir-Usak-Ankara-Eskisehir-Bolu, Konya-Afyon and Denizli called Anatolian group (Fig. 3).

**DISCUSSION**

Geographical variations in subspecific level of *A. amphibius* were investigated in Turkey. Ozkurt et al. (1999) and Gozoeligolu et al. (2006) separated *A.a. cernjavskii* in Thrace from *A.a. persicus* in Anatolia based on karyotype analysis. In this study, RAPD analyses of Anatolian and Thrace populations supported the existence of these two subspecies. In addition, soyun and Colak (2004) proved high genetic diversity in Kirsehir populations of Turkey due to absence of the bottleneck based on their esterase and SDS-PAGE studies. Contrarily, RAPD loci did not show obvious disparity in heterozygosity of Kirsehir populations, possibly owing to the difference of the markers between two studies.

Afyon population and Konya population were formed from Lake Eber and Lake Beysehir, respectively. These two populations clustered together in UPGMA dendrogram in consequence of the lakes have very similar water vole habitats. Similarly, populations from rivers and brooks were clustered in same group (Kirsehir, Usak, Ankara, Eskisehir, Bolu). This habitat similarity reduces the differentiation between populations. Although, Denizli specimens were collected from riverside too, genetic drift and fluctuation in population density may cause evolution of the populations differently therefore, Denizli population might differ from Anatolian group in this way. According to the RAPD data while there is gene flow between *A. amphibius* populations in Anatolia, presence of subpopulations might be depended on river and lake habitats. In additional, Bosphorus and Marmara sea seem to interrupt gene flow between Thrace and Anatolia populations. This barrier effect may cause differentiation of *A.a. cernjavskii* and *A.a. persicus*.

RAPD-PCR is widely used in rodents (Atopkin et al., 2007; Spirdonova et al., 2008; Dokuchaev et al., 2008; Olgun et al., 2009). RAPD assay may in some instances detect single base changes in genomic DNA. Most single nucleotide changes in a primer sequence caused a complete change in the pattern of amplified DNA segments (Williams et al., 1990).

**CONCLUSION**

This study was the first molecular study of Arvicola in Turkey on DNA level. As a result, there is a significant genetic differentiation between Thrace and Anatolian
populations. In order to discover evolution and population dynamics of *A. amphibius* much more molecular technique should be used to reach definitive conclusion in DNA level.

**ACKNOWLEDGEMENT**

Specimens used in this study were collected between 2008-2010 funded by the Scientific Research Office of Ankara University (BAPRO No: 2008-07-05-003).

**REFERENCES**


