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RAPD-PCR Analysis of Water Vole, *Arvicola amphibius* (Linnaeus, 1758) (Mammalia: Rodentia) Distributed in Turkey

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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 (RAPD) DNA 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. cernjavskii* 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 (Kratochvil, 1983; Nikolaeva, 1982; Krystufek and Tvrtkovic, 1984; Ventura, 1991) and karyological studies (Raicu *et al*., 1971; Kuliev *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 cernjavskii* 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 (Iyigun 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 forwhy 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 guentheri* 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 Beysehir) (N = 4), Eskisehir (N = 4), Bolu (N = 4), Ankara (N = 4), Kirsehir (N = 4) (Fig. 1).

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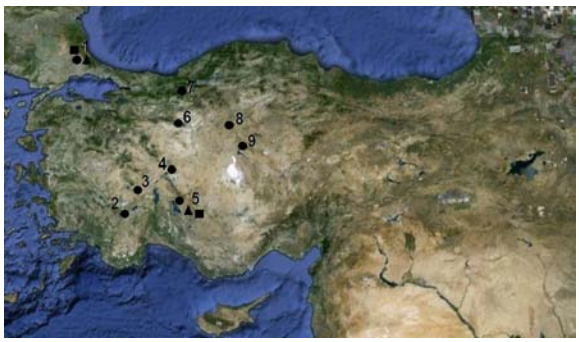

Fig. 1: Collecting localities of specimens (Numbers refer to localities were presented in materials and methods;●: *Arvicola*, ■: *Microtus*, ▲: *Mus*)

Table 1: Sequences and polymorphism percentage of all primers

Primers	Sequences	P (%)
OPA-02	5'-TGC CGA GCT G-3'	4.1
OPA-03	5'-AGT CAG CCA C-3'	5.4
OPA-04	5'-AAT CGG GCT G-3'	9.5
OPA-07	5'-GAA ACG GGT G-3'	8.2
OPA-08	5'-GTG ACG TAG G-3'	7.5
OPA-16	5'-AGC CAG CGA A-3'	7.5
OPB-15	5'-GGA GGG TGT T-3'	5.4
OPB-16	5'-TTT GCC CGG A-3'	5.4
OPB-18	5'-CCA CAG CAG T-3'	4.1
OPB-19	5'-ACC CCC GAA G-3'	5.4
OPB-20	5'-GGA CCC TTA C-3'	4.1
OPD-08	5'-GTG TGC CCC A-3'	4.1
OPD-09	5'-CTC TGG AGA C-3'	5.4
OPD-10	5'-GGT CTA CAC C-3'	6.1
OPD-11	5'-AGC GCC ATT G-3'	5.4
OPD-12	5'-CAC CGT ATC C-3'	6.1
OPD-14	5'-CTT CCC CAA G-3'	5.4

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 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^{-1}); 2.5 μL of buffer (750 mM Tris-HCl ph: 8.8, 200 mM $(NH_4)_2 SO_4$; fermentas); 0.3 μL of Taq DNA polymerase (100 unit fermentas); 4 μL of deoxynucleotide triphosphate mix (200 μM of each nucleotide); 1.5 μL of 2 mM $MgCl_2$; 1 μL of 1 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 decamer 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, 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. 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 (N_a), effective number of alleles (Kimura and Crow 1964) (N_e) and Nei (1972)'s 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 TFPGA 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 ($OPA\text{-}2_{650}$, $OPB\text{-}16_{700}$, $OPB\text{-}19_{2000}$ and $OPD\text{-}10_{550}$) 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 Kirklareli 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 G_{ST} value of 0.496 indicated that genetic differentiation among the studied populations was substantial. The total gene diversity (H_T) was 0.146 in *Arvicola amphibius* populations but 50.3% of this was within population variation (H_S = 0.0736). UPGMA tree was constructed using TFPGA and MEGA software

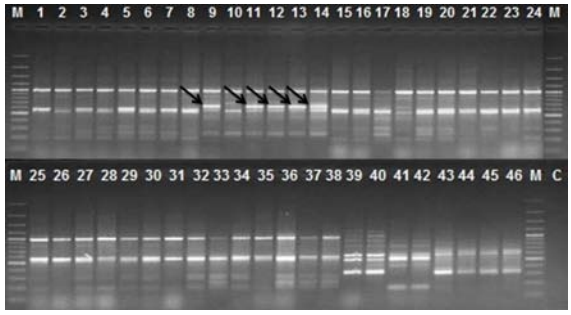

Fig. 2: Amplification products of Primer OPA-02. The arrows show diagnostic RAPD loci in Kirklareli specimens. M: marker (100 bp DNA ladder, fermentas), C: negative control. 1-4: Kirsehir, 5-8: Ankara, 9-14: Kirklareli, 15-18: Konya, 19-22: Afyon, 23-26: Denizli, 27-30: Usak, 31-34: Eskisehir, 35-38: Bolu (1-38: *A. amphibius*), 39-40: Kirklareli *(Microtus guentheri)*, 41-42: Konya *(Microtus levis)*, 43-44: Konya *(Mus macedonicus)*, 45-46: Kirklareli *(Mus macedonicus)*

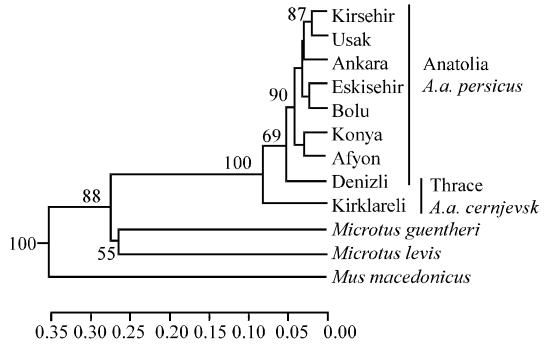

Fig. 3: Genetic similarity of *A. amphibius* from different localities based on RAPD data and generated using the UPGMA method, respectively within the MEGA software program. At the branching nodes, the BP values exceeding (50% are presented)

Table 2: Pairwise dissimilarity matrix based on Nei (1972) between *Arvicola amphibius* populations

	1	2	3	4	5	6	7	8	9
Kirsehir	-								
Ankara	0.0681	-							
Kirklareli	0.1338	0.1683	-						
Konya	0.1070	0.1084	0.1488	-					
Afyon	0.0847	0.1067	0.1389	0.0885	-				
Denizli	0.1122	0.1295	0.1626	0.0906	0.1042	-			
Usak	0.0623	0.0785	0.1670	0.0812	0.0969	0.1033	-		
Eskisehir	0.0776	0.0794	0.1516	0.0799	0.0773	0.0992	0.0665	-	
Bolu	0.0957	0.0744	0.1879	0.1301	0.1126	0.1141	0.0959	0.0571	-

packages. All specimens analyzed were divided into two major groups while the 1st group contained only Kirklareli 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 Gozcelioglu *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 Iyigun 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 heterozigosity 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 dendogram 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, Bosporus 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; Spiridonova *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.

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