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An Electrophoretic Study on Esterase and Blood Serum Proteins of the Water Vole, *Arvicola terrestris* (L., 1758) (Mammalia: Rodentia), in Kırşehir Province*

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Abstract: Esterase enzymes from 30 specimens of *Arvicola terrestris* in 4 localities in Kırşehir province were examined using starch gel electrophoresis and blood serum proteins from 27 specimens using SDS-PAGE. It was determined that the esterase enzyme had 2 alleles (A and B), which were coded by 1 locus. A and B allele frequencies were 0.362 and 0.638, respectively. The heterozygosity level was 0.5. It was determined that there were variations in the globulin, prealbumin and postalbumin regions, while the albumin region was monomorphic. There were 6-13 bands in the globulin region, 1-2 bands in the postalbumin zone and 2-4 bands in the prealbumin zone.

Key Words: *Arvicola terrestris*, esterase, serum proteins, electrophoresis, Kırşehir

Kırşehir Yöresindeki Su Sıçanı *Arvicola terrestris* (L., 1758) (Mammalia: Rodentia)'in Esteraz ve Kan Serum Proteinleri Üzerine Elekroforetik Bir Çalışma

Özet: Kırşehir yöresinden dört lokaliteden *Arvicola terrestris*'e ait 30 örneğin esteraz enzimi nişasta jel elektroforez tekniği ve 27 örneğin kan serum proteinleri SDS-PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophoresis) tekniği ile incelendi. Esteraz enziminin bir lokus tarafından kodlanan iki alele (A ve B) sahip olduğu belirlendi. A alelinin frekansı 0.362, B alelinin frekansı ise 0.638 olarak belirlendi. Bu lokusta heterozigotluk oranı 0.5 olarak hesaplandı. Albumin bölgesi monomorfik iken globülin, postalbümin ve prealbumin bölgelerinde varyasyon belirlendi. Globülin bölgesinde 6-13 bant, postalbümin zonunda 1-2 bant ve prealbümin zonunda 2-4 bant belirlendi.

Anahtar Sözcükler: *Arvicola terrestris*, esteraz, serum proteinleri, elektroforez, Kırşehir

Introduction

The water vole, *Arvicola terrestris* (L., 1758), is distributed in Europe (1). According to Corbet (1), Ellermann (2), Ellerman and Morrison-Scott (3), Harrison and Bates (4), and Wilson and Reeder (5), *A. terrestris* lives in Europe, Russia, Turkey, Iran and Palestine. In Turkey, Steiner and Vauk (6) recorded *A. terrestris* from Lake Beyşehir, and Mursaloğlu (7) recorded *A. t. hintoni, A. t. persicus* and *A. t. cernjavskii*, and included specimens from Kırşehir in *A. t. persicus*. Özkurt et al. (8) gave karyotypic aspects of *A. terrestris* in Kırşehir. The studies above focused on the taxonomy and distribution of *A. terrestris*. However, there are no molecular studies on *A. terrestris* in Turkey. Recently studies on rodents have focused on isozyme and allozyme variations. On the basis of these studies, new taxa were described. In Turkey, allozyme variations of some rodent genera were determined by Filippucci et al. (9), and Macholan et al. (10). Blood serum proteins of the genera *Apodemus, Mesocricetus, Meriones, Rattus* and *Spermophilus* were recently investigated by Verimli et al. (11-13), Yiğit et al. (14), Çolak (15,16), Çolak et al. (17), and Çolak and Özkurt (18). Molecular studies contribute to the knowledge on the taxonomy, systematics and evolution of rodents as well as morphological ones.

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The aim of this study was to determine patterns of blood serum proteins and esterase of *A. terrestris* and to contribute to the knowledge of its population genetics.

Materials and Methods

Thirty specimens collected from 4 localities in Kırşehir province were used in the present study (Figure 1). Identification followed Mursaloğlu (7), and Harrison and Bates (4).

Muscle tissues kept at -70 °C were used in starch gel electrophoresis. After homogenization, homogenates were centrifuged at 12, 000 rpm for 3 min. Starch gel of 11% was prepared by boiling in gel buffer (19). Samples were loaded onto gel using a filter paper absorbed-homogenate of 0.3 x 0.4 cm (Whatmann No. 3). Gel was run, adjusting 8 V/cm (10 mA) for 5 h (19). Gels were incubated in 0.5 M boric acid for 15 min. After the boric acid was removed, gels were incubated for esterase in reaction solution (0.2 M Tris-HCl, pH: 7.0, 50 ml; substrate solution 3 ml; Fast Blue BB salt 0.05 g) at laboratory temperature until bands were visible (20). α and β-naphthyl acetate were used as substrate. Gel was fixed in solution [45 parts methanol, 55 parts acetic acid solution (1 acetic acid: 5 H_2O)].

Blood samples were obtained from cardiac punctures of animals anaesthetized by ether. Sera were centrifuged at 12,000 rpm for 3 min. Sample and electrode buffers were prepared in accordance with Laemmli (21). Final concentrations of the sera were 5%. Samples were denatured by boiling for 3 min. In electrophoresis, a Consort E 863 model vertical slab gel

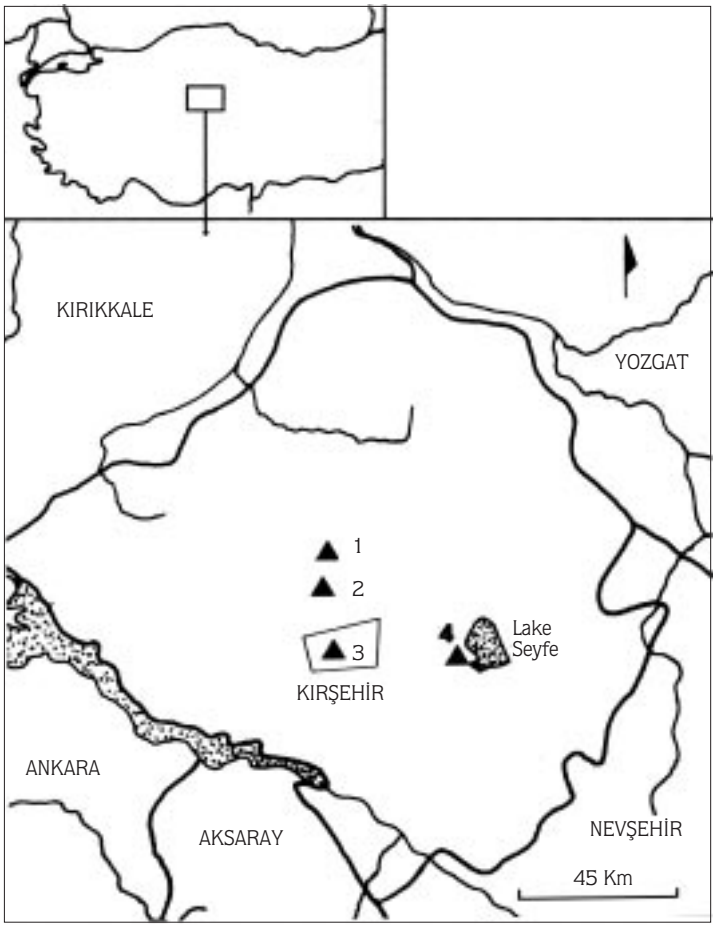

Figure 1. Kırşehir map showing localities of *Arvicola terrestris*. 1. 12 km along Kırşehir-Ankara road (n = 3), 2. 10 km along Kırşehir-Ankara road (n = 3), 3. Kırşehir center (n = 23), 4. Lake Seyfe (n = 1).

electrophoresis device was used. SDS-polyacrylamide denatured-gels were prepared based on Sambrook et al. (22). The amount of protein loaded onto the gel was semiquantitatively determined (23). The marker (Sigma MW-SDS-200) used in the present study was composed of carbonic anhydrase (29,000), egg albumin (45,000), bovine albumin (66,000), phosphorylase B (97,400), β-galactosidase (116,000), and myosin (205,000). A constant voltage of 80 V was applied to the stacking gel. When the tracing stain reached the resolving gel, the voltage was increased to 150 V. After electrophoresis, gels were fixed in a solution of methanol:water:acetic acid (45:45:10) along with 0.25% Coomassie Brilliant Blue R 250 overnight. Stained gels were destained with methanol:water:acetic acid (45:45:10). The globulin and albumin regions were evaluated.

Both esterase and SDS-PAGE gels were photographed, and each gel was drawn.

Results

Esterase Isozyme

α and β-naphthyl acetate were used as substrate in electrophoretic analysis. β- naphthyl acetate did not give a successful result. When α-naphthyl acetate was applied, a single esterase locus migrating to the anode was observed (Figure 2). This locus had 2 alleles: a fast (A) and slow one (B). One specimen had no band, and it was designated as 0. The frequency of A and B alleles was 0.36 and 0.64, respectively (Table 1).

Blood Serum Proteins

There was no difference between females and males with respect to the blood serum protein profiles of *A. terrestris* in Kırşehir (Figures 3 and 4). Albumin regions were divided into 3 sub-zones: postalbumin, albumin and prealbumin. The globulin region has 6-13 bands. The postalbumin and prealbumin zones are polymorphic, the postalbumin zone has 1-2 bands, and the prealbumin zone has 2-4 bands (Table 2).

There was a band between marker proteins of 205,000 and 116,000 kDa in the globulin region thicker than the other bands.

The slow band in the postalbumin zone was the strongest in all specimens, while the fast one was weak. The slow band in postalbumin was 77,500 kDa (Rf = 0.45), and the fast one was 71.000 kDa (Rf = 0.5). The albumin region contained a thick monomorphic band, having a molecular weight of 60,000 kDa (Rf = 0.6). In the prealbumin zone, in 2 banded phenotypes, the fast band was stronger in some specimens, and the slow one

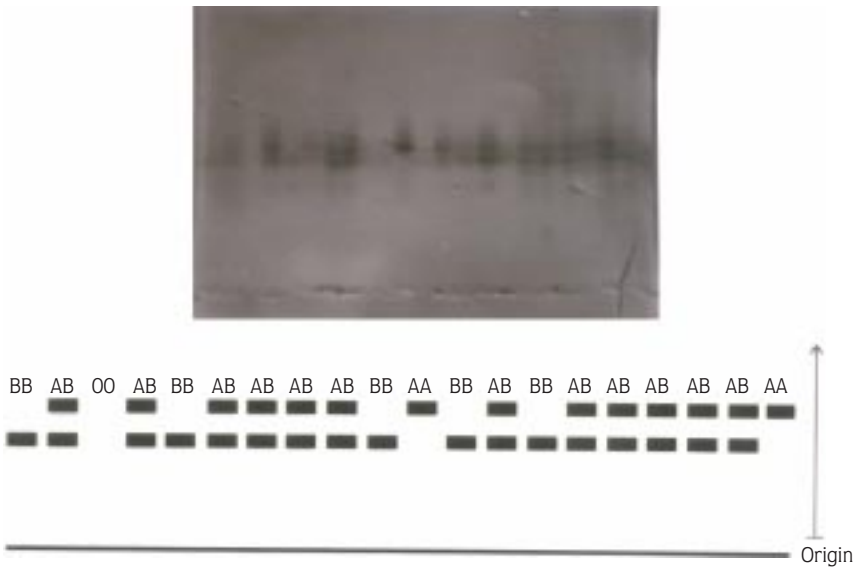

Figure 2. Patterns of esterase locus of *Arvicola terrestris* (a) Zymogram (b). +: anode, AA, BB, AB, 00: genotypes.

Table 1. Allele and genotype frequencies of esterase in *Arvicola terrestris* in Kırşehir province. Locality 1: 12. km along Kırşehir-Ankara road, Locality 2: 10. km along Kırşehir-Ankara road, Locality 3: Kırşehir center, Locality 4: Lake Seyfe. n = number of specimens.

		Genotype frequency				Allele frequency	
Locality No	n	AA	AB	BB	OO	A	B
1	3	0.00 (n=0)	0.67 (n=2)	0.33 (n=1)	0.00 (n=0)	0.33	0.67
2	3	0.33 (n=1)	0.00 (n=0)	0.67 (n=2)	0.00 (n=0)	0.33	0.67
3	23	0.09 (n=2)	0.57 (n=13)	0.30 (n=7)	0.04 (n=1)	0.39	0.61
4	1	0.00 (n=0)	0.00 (n=0)	1.00 (n=1)	0.00 (n=0)	0.00	1.00
Total	30	0.10 (n=3)	0.50 (n=15)	0.37 (n=11)	0.03 (n=1)	0.36	0.64

Table 2. The band numbers of the 4 main blood serum proteins of *Arvicola terrestris* along with a comparison of the other species in Turkey (G = Globulin, PsA = Postalbumin, A = Albumin, PA = Prealbumin).

Species	G	PsA	A	PA	References
Rattus rattus	8-12	1-2	1	1-4	Yiğit et al. 2001
Rattus norvegicus	8-11	1-2	1	3-4	Yiğit et al. 2001
Apodemus mystacinus	7-8	1	1	2-4	Verimli et al. 2000b
Apodemus flavicollis	6-9	1	1	2	Verimli et al. 2001
Apodemus hermonensis	7-9	1	1	2	Verimli et al. 2001
Apodemus uralensis	7-9	1	1	1-2	Çolak 2002
Apodemus agrarius	9	1	1	2	Verimli et al. 2000b
Mesocricetus auratus	7	1	1	2	Verimli et al. 2000a
Mesocricetus brandti	7	1	1	2	Verimli et al. 2000a
Meriones meridianus	9-10	1	1	1-2	Çolak et al. 2002
Meriones crassus	9	1	1	2	Çolak et al. 2002
Meriones persicus	7-8	1	1	2	Çolak et al. 2002
Meriones tristrami	7	1	1	2	Çolak et al. 2002
Spermohilus citellus	8	1	1	1	Çolak and Özkurt, 2002
Spermohilus xanthoprymnus	9-10	1-4	1	1	Çolak and Özkurt, 2002
Arvicola terrestris					This study
Kırşehir-Ankara 12 km (n = 3)	11-13	1-2	1	3	
Kırşehir-Ankara 10 km (n = 3)	10-11	1-2	1	3	
Kırşehir center (n = 20)	6-10	1-2	1	2-4	
Lake Seyfe (n = 1)	13	1	1	3	

was stronger in other specimens (Figure 3). In phenotypes with 3 bands, the second band was stronger than the others. In phenotypes with 4 bands, all bands were similar (Figure 4).

Discussion

According to Semeonoff (24), in *Microtus ochrogaster* Es-1 has 3 different phenotypes: F (a single band), D (pair band) and O (no band). In this study, there was no band in the esterase locus in one specimen. Semeonoff (24) revealed that Es-1 was dimeric in *M. ochrogaster*. In contrast, the locus of esterase studied in this work gave heterozygosity with 2 bands. This showed that this enzyme was monomeric. Gebczyñski (25) recorded 2 alleles (A and B) in the Es-1 locus of *Clethrionomys glareolus* in Poland. Gebczyñski (25) stated that some

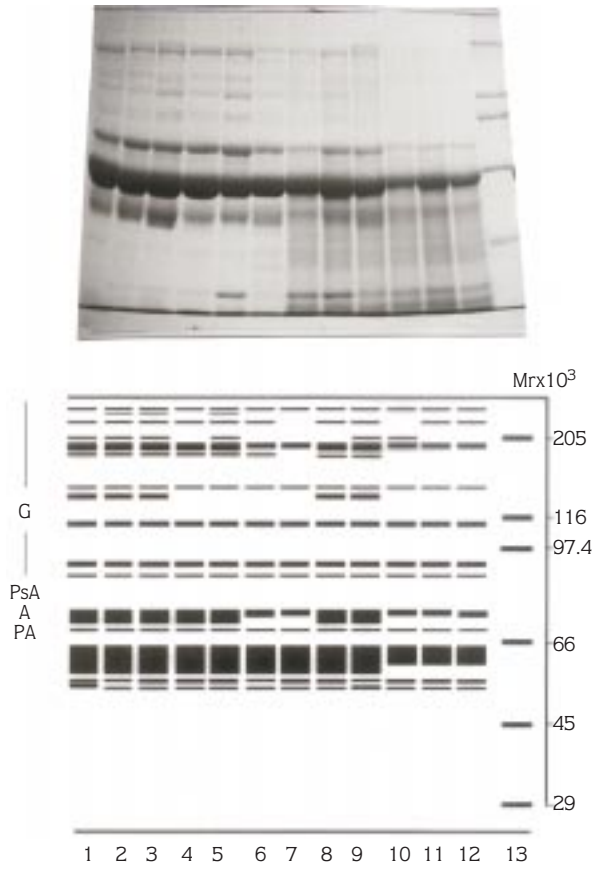

Figure 3. SDS-PAGE patterns of blood serum proteins of *Arvicola terrestris* with 2 banded PA phenotype. (1-12), Mr: Marker (13). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin.

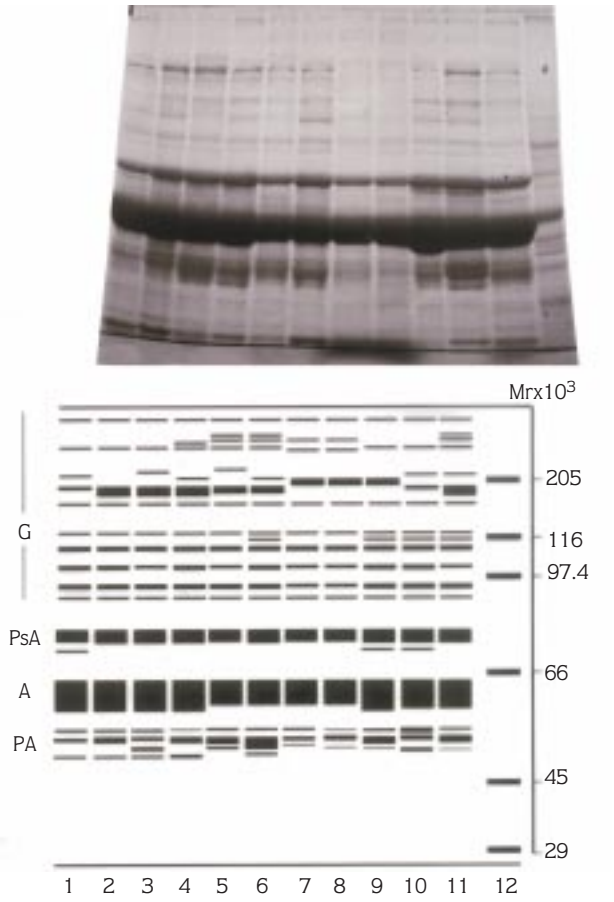

Figure 4. SDS-PAGE patterns of blood serum proteins of *Arvicola terrestris* with 3 and 4 banded PA phenotype. (1-11), Mr: Marker (12). G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin.

populations in southern Poland were fixed to the A allele. In this study, esterase enzyme was polymorphic in 3 localities, and a specimen examined in one locality had a B allele. In America, Klaus et al. (26) revealed that Es-1 in *Microtus richardsoni* was polymorphic, similar to specimens of *A. terrestris* from Kırşehir. In Asia, Europe and Africa, Bonhomme et al. (27) determined that Es-3 in the genus *Mus* had considerable alleles. There was no allelic confusion in the esterase enzyme of *A. terrestris* in Kırşehir.

According to Yamada et al. (28), in the wild *R. rattus*, based on polyacrylamide gel electrophoresis, Es-3 was monomorphic, Es-1 and Es-2 contain 2 alleles, and Es-4 consists of 3 alleles. By using starch gel electrophoresis, we found a single locus in esterase enzyme in *A. terrestris*. This difference may have resulted from the

methods and tissues used. Verimli et al. (29) determined Es-4 and Es-10 in muscle, heart, kidney and liver tissues; Es-3 and Es-17 in the kidney and liver; Es-18 in the liver; Es-15 in the kidney and liver; Es-2 in the muscle and heart; and Es-16 in the heart in *R. norvegicus*, depending on horizontal starch gel electrophoresis with α-naphthyl acetate substrate. We determined a single locus of esterase in the muscle of *A. terrestris* using α-naphthyl acetate substrate. According to Hartl et al. (30), esterase enzymes are considerably polymorphic, and this locus is impossible to evaluate; therefore this locus is not suitable for separating taxa. In our study, esterase enzyme in *A. terrestris* had 2 alleles, and therefore it is possible to evaluate this locus.

In Poland, Dobrowolska (31) examined the transferrin zone of *Microtus arvalis* by starch gel electrophoresis, and determined 2 alleles. Dobrowolska (31) stated that *M. arvalis* was considerably heterozygotic with respect to the transferrin zone. According to Dobrowolska (31), variations in the gamma-globulin level resulted from individual diversity and inner physiological conditions. In our study, SDS-PAGE gave 6-13 bands in the globulin region. The individual variation observed in this study is similar to that of *M. arvalis*.

In Greece, Freguedakis-Tsolis and Chondropoulos (32) stated that *Pitymys atticus* has no band in the prealbumin and one band in albumin region, depending on polyacrylamide gel electrophoresis. *P. atticus* shows similarity to *A. terrestris* with respect to the albumin region, and is different from *A. terrestris* with respect to the prealbumin zone.

According to Yiğit et al. (14), in the prealbumin zone, there are 1-4 bands in *Rattus rattus*, and 3-4 bands in *R. norvegicus* based on SDS-PAGE. The prealbumin values of these species are consistent with those of *A. terrestris* (Table 2).

Çolak (15) determined a slow and fast band in the postalbumin zone in *Apodemus* in the Black Sea region; their electrophoretic mobilities were Rf = 0.38 and 0.35, respectively. These values are different from Rf = 0.45-0.6 in *A. terrestris*.

According to Çolak et al. (17), in the genus *Meriones*, the globulin region has 7-10 bands, the prealbumin has 1-2 bands, and the postalbumin and albumin zones have 1 band (Table 2). In our study, the prealbumin and postalbumin values of *A. terrestris* are different from those of the genus *Meriones*.

The findings of this study showed that there was variation in blood serum proteins and esterase enzyme in *A. terrestris*, the level of heterozygosity was high in the esterase, and the prealbumin and postalbumin zones and the globulin region were polymorphic. This also indicated the presence of variation in Kırşehir populations, and there is no genetic bottleneck in the *A. terrestris* population in Kırşehir with respect to esterase locus or blood serum proteins.

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