

Electrophoretic Aspects of Blood-Serum Proteins of *Apodemus mystacinus* and *Apodemus agrarius* (Mammalia: Rodentia) in Turkey

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Abstract: In this study, globulin and albumin proteins of *Apodemus mystacinus* and *Apodemus agrarius* were examined using the SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) technique. In *A. mystacinus*, there were 7-8 bands in the globulin region and 2-3 bands in the prealbumin zone. It was determined that there were variations in both the globulin region and prealbumin zone in populations of *A. mystacinus*. In specimens of *A. agrarius*, nine bands appeared in the globulin region, and two bands were observed in the prealbumin zone. These findings showed that *A. mystacinus* differs from *A. agrarius* in respect to electrophoretic aspects of albumin and globulin proteins.

Key Words: *Apodemus mystacinus*, *Apodemus agrarius*, albumin, globulin, SDS-PAGE.

Türkiye'deki *Apodemus mystacinus* ve *Apodemus agrarius* (Mammalia: Rodentia)'un Kan-Serum Proteinlerinin Elektroforetik Özellikleri

Özet: Bu çalışmada, *Apodemus mystacinus* ve *Apodemus agrarius*'un albumin ve globulin proteinleri SDS-PAGE (sodyum dodesil sülfat-poliakrilamid jel elektroforez) tekniği ile incelendi. *A. mystacinus*'da globulin bölgesinde 7-8 bant ve prealbumin zonunda ise 2-3 bant tespit edildi. *A. mystacinus* populasyonlarında hem globulin bölgesinde hem de prealbumin zonunda varyasyonlar görüldü. *A. agrarius* örneklerinde globulin bölgesinde dokuz bant prealbumin zonunda ise iki bant tespit edildi. Bu bulgular albumin ve globulin proteinlerinin elektroforetik özelliklerine göre *A. mystacinus*'un *A. agrarius*'tan ayrıldığını gösterdi.

Anahtar Sözcükler: *Apodemus mystacinus*, *Apodemus agrarius*, albumin, globulin, SDS-PAGE.

Introduction

Serum proteins as well as their clinical importance have been studied intensively in both man and in mice (*Mus musculus*) (1, 2). Electrophoretic techniques have permitted the separation of major groups such as albumin and globulin in serum (2).

The genus *Apodemus* occurs throughout the Palaearctic region. Four species of the genus *Apodemus*; *Apodemus mystacinus*, *Apodemus sylvaticus*, *Apodemus flavicollis*, and *Apodemus agrarius*, are distributed in Turkey (3, 4). On the basis of morphological, biometric and allozyme aspects, *Apodemus hermonensis* and *Apodemus uralensis* were recently recorded by Filippucci *et al.* (5) from western Anatolia. Fragedakis-Tsolis *et al.* (6), and Fragedakis-Tsolis and Chondropoulos (7) examined electrophoretic aspects of the blood serum proteins of *A. sylvaticus* in Greece and noted that this species is different from *Mus musculus* and *Pitymys taticus* on the basis of different electrophoretic bands.

Electrophoresis has permitted the verification of the validity of morphological characters to be diagnostic (8). Electrophoretic analysis has also allowed the detection of sibling species and the clarification of the taxonomic position of several *Apodemus* taxa.

This paper is a preliminary report of work on the blood serum proteins of Turkish rodents, and presents electrophoretic aspects of the blood serum proteins of *A. mystacinus* and *A. agrarius*.

Material and Methods

Electrophoretic analysis was performed on 35 live specimens collected from eight localities in the distribution areas of *Apodemus mystacinus* and *Apodemus agrarius* in Turkey (Fig. 1).

Blood was taken by cardiac puncture from the animals, which had been anaesthetised with ether. After blood clotting, the separated sera were centrifuged at

12000 rpm for 3 min. The sera were mixed with a sample buffer containing 0.0625 M Tris Cl, pH 6.8, 2 % SDS, 10 % Glycerol, 5 % 2-Mercaptoethanol and 0.01 % bromphenol blue (9), and the final concentration of the sera was adjusted to 5 %. The samples were boiled for 3 min and stored at -70 °C until electrophoresis. The amount of protein loaded onto the gel was qualitatively determined according to the method of Esen (10). Samples of 10 to 15 µl were applied to gels in different experiments. Electrophoresis was carried out using Consort E 863 model vertical slab gel electrophoresis apparatus. SDS-polyacrylamide denaturing gels, separating gels (7.5 %) and stacking gels (4 %) were prepared as described by Sambrook *et al.* (11). The electrode buffer solution used contained 0.025 M Tris, 0.192 M Glycine, 0.1 % SDS at pH 8.3 (9). The Molecular Weight Marker (Sigma MW-SDS-200) consisted 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 (8 V/cm) was applied to the stacking gel. After the separating gel was obtained through tracing the dye, the voltage was adjusted to 15 V/cm. After electrophoresis, the gels were stained with 0.25 % Coomassie Brilliant Blue R250 in 90 ml of methanol: water (1: 1 v/v) and 10 ml glacial acetic acid, and were destained in methanol: water: acetic acid (45: 45: 10) (11).

Results

Blood serum proteins from 31 specimens of *Apodemus mystacinus* collected from eight localities were examined by SDS-PAGE. In the specimens from Akseki, the globulin region consisted of seven bands, of which the first and the second were larger than the others, and the

prealbumin zone had three bands in two specimens and four bands in one specimen (Fig. 2). However, with ten specimens of *A. mystacinus* from Sebil (Tarsus, Mersin), eight bands appeared in the globulin region, while the prealbumin zone contained three bands. In the specimens from Beyşehir (Konya), Buharkent (Aydın), Burdur, and Kemalpaşa (İzmir), seven bands were determined, of which the second was larger in the globulin region, and two bands appeared in the prealbumin zone (Fig. 3). In the specimens from Ardanuç, there were seven bands in the globulin region, and the third and the fourth bands were larger than the others. There were four bands in the prealbumin zone in the specimens from Ardanuç (Fig. 4).

The blood serum proteins of four specimens of *Apodemus agrarius* collected from Iğneada (Kırklareli) were examined. Nine electrophoretic bands were detected in the globulin region; all the bands except the fourth one were quite weak. The albumin region was composed of postalbumin, albumin, and prealbumin zones. The prealbumin zone had two bands; the first band was weak and slow, and the second band was prominent (Fig. 5).

Discussion

Franguedakis-Tsolis *et al.* (6) stated that there is one band in the prealbumin zone and two bands in the albumin zone in *A. flavicollis*, one prealbumin band and one albumin band in *A. sylvaticus*, and a fast prealbumin and two albumin bands in *A. mystacinus*. These results show that the electrophoretic features of the prealbumin and albumin proteins of three *Apodemus* species in Greece are different from those of *A. agrarius* and *A. mystacinus* in Turkey.

According to Franguedakis-Tsolis and Chondropoulos (7), two prealbumin zones were found in *Mus musculus*. Although one prealbumin zone was constantly present in *A. flavicollis*, no prealbumin was observed in *Ptymys*

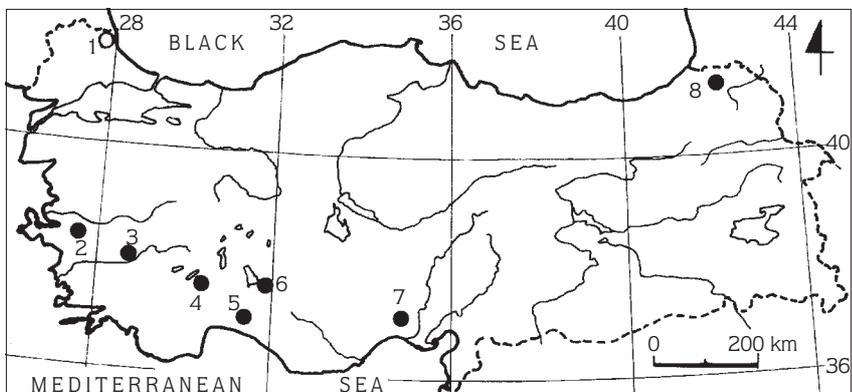


Figure 1. Map showing the localities of the specimens examined. *A. mystacinus* (●) *A. agrarius* (○) 1. Iğneada, 2. Kemalpaşa, 3. Buharkent, 4. Burdur, 5. Akseki, 6. Beyşehir, 7. Sebil, 8. Ardanuç.

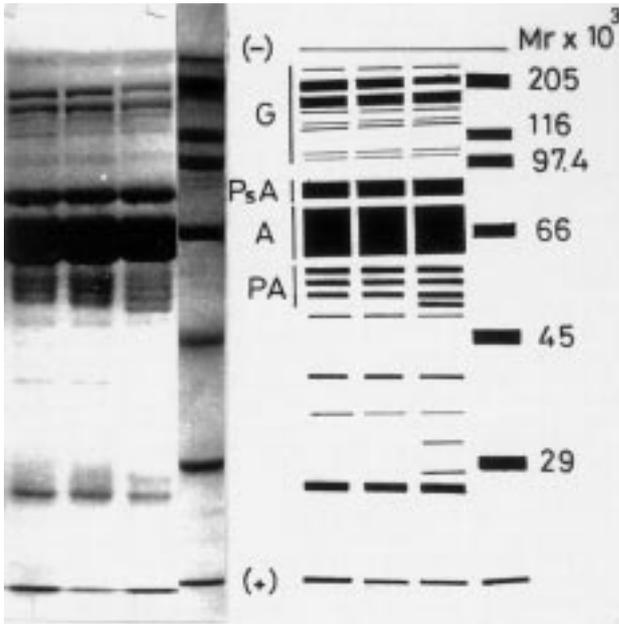


Figure 2. SDS-PAGE patterns of serum proteins of *A. mystacinus* from Akseki. G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker.

atticus. There were two albumin zones in *A. flavicollis*, and one albumin zone in *M. musculus* and *P. atticus*. These results show that the electrophoretic aspects of the albumin and prealbumin proteins are different in the

above species. A comparison of the albumin and prealbumin proteins of *A. mystacinus* and *A. agrarius* in Turkey, and those of *A. flavicollis* in Greece showed that *A. mystacinus* and *A. agrarius* differ from *A. flavicollis* in terms of both prealbumin and albumin proteins.

According to Gemmeke *et al.*, (12), there are 1 to 5 electrophoretic bands in the postalbumin zone of 21 populations of European *A. sylvaticus*, whereas there is one electrophoretic band in the postalbumin zone in both *A. mystacinus* and *A. agrarius*.

In this study, it was determined that there was considerable variation in the prealbumin zone of *A. mystacinus*; that is, there were two bands in the specimens from western Anatolia, three bands in those from Sebil and Akseki, and four bands in the Ardanuç specimens. Furthermore, eight globulin bands were found in the specimens of *A. mystacinus* from Sebil, whereas seven bands were determined for the other localities. The reason for this difference in the prealbumin zone of *A. mystacinus* may lie in the isolation of three localities.

According to Reuter and Kennes (13), who used the horizontal starch gel electrophoresis technique, the electrophoretic pattern of female mice is significantly different from that of the males with respect to the prealbumin zone, due to the presence of three bands in the prealbumin zone. In contrast, we used the SDS-PAGE technique and did not observe any difference between

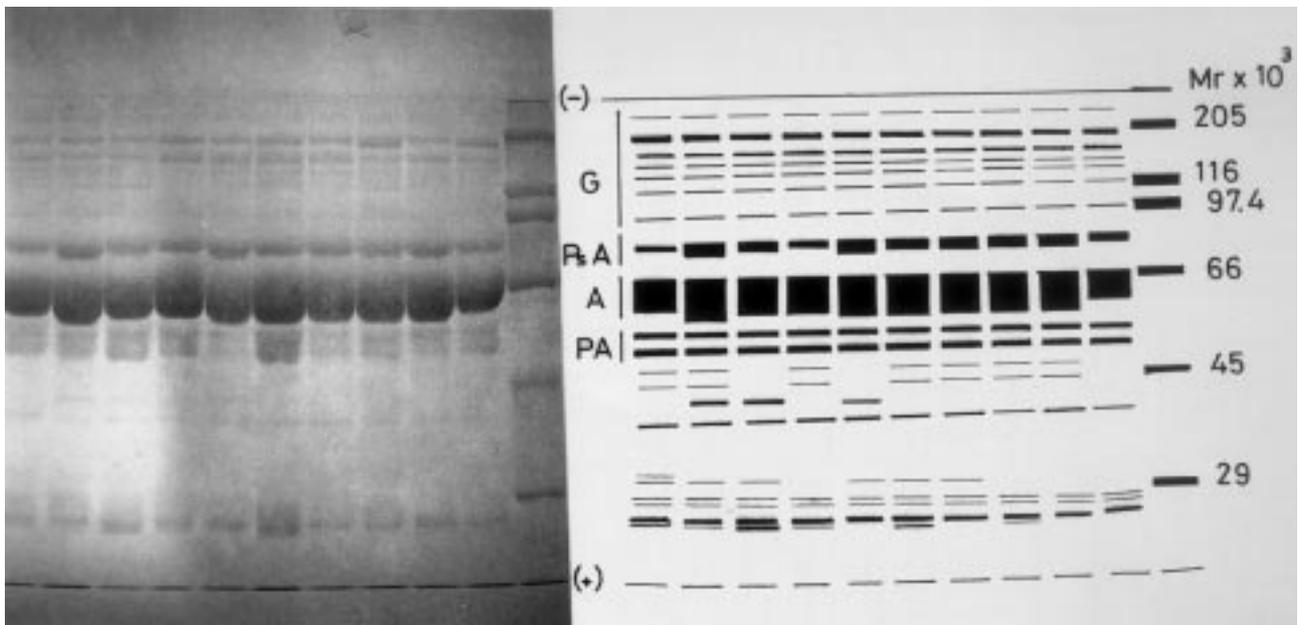


Figure 3. SDS-PAGE patterns of serum proteins of *A. mystacinus* from western Anatolia. G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker.

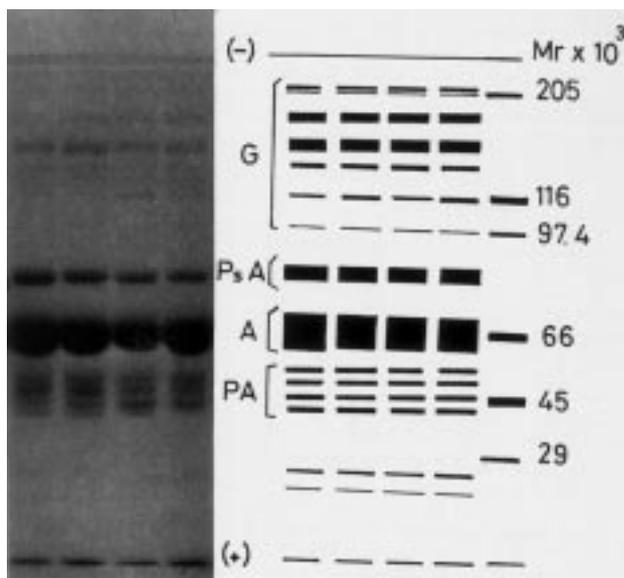


Figure 4. SDS-PAGE patterns of serum proteins of *A. mystacinus* from Ardanuç. G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker.

males and females. Pantelorius and Hale (14) stated that plasma from adult female mice at various stages of pregnancy produced 21 bands on starch gel after electrophoresis. However, we detected 17-20 bands in the serum of *A. mystacinus* and *A. agrarius*, with considerable variation. This difference may result from the differences between the techniques used.

In *A. agrarius*, there were two bands in the prealbumin zone; one is strong and the other is slight. There were eight bands in the globulin region, whereas 3-4 bands were detected in the prealbumin zone, and 7-8 bands were observed in the globulin region of *A. mystacinus*. In *A. mystacinus*, in the globulin region, two large bands were observed, while in *A. agrarius*, one large band was observed. *A. mystacinus* and *A. agrarius*, which are two easily distinguished species of the genus *Apodemus*, showed different electrophoretic patterns for albumin and globulin proteins.

Acknowledgments

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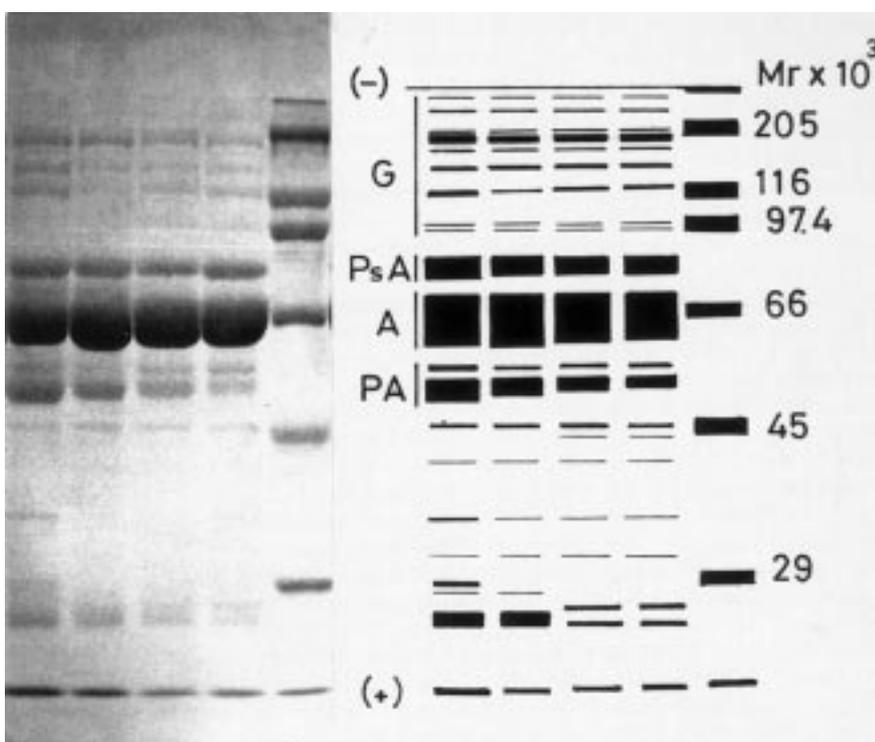


Figure 5. SDS-PAGE patterns of serum proteins of *A. mystacinus* from Iğneada. G: Globulin, PsA: Postalbumin, A: Albumin, PA: Prealbumin, Mr: Marker.

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