# Allozyme Variations on Subspecies of *Meriones tristrami* (Rodentia: Gerbillinae) In Western Anatolia

## Batı Anadolu'da Yayılış Gösteren *Meriones tristrami* (Rodentia: Gerbillinae) Alttürlerinin Allozim Varyasyonları

Research Article / Araştırma Makalesi

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**ABSTRACT**

Allozyme variation was investigated by the electrophoretic analysis of 24 gene loci in three subspecies of *Meriones tristrami* from Western Anatolia. Twenty of the twenty-four loci were monomorphic among populations, whereas four loci were polymorphic. The mean value of the Wright's fixation index was $F_{ST}$ = 0.0671, suggesting the existence of a slightly distinct subspecies in the *Meriones tristrami* populations. Overall mean heterozygosity ($H_o$= direct count) for all populations was $H_o$= 0.017. Nei's measure of genetic distance was low and varied from D = 0.000 to 0.002 among populations. The number of migrants (Nm) equaled 3.48, which also suggests effective gene flow across populations..

**Key Words**
Allozyme, subspecies, *Meriones tristrami,* Turkey.

**ÖZET**

Allozim varyasyonları, Batı Anadolu'daki *Meriones tristrami*'nin üç alt popülasyonunda 24 gen lokusunun elektroforetik analiziyle incelendi. Yirmidört lokusun yirmisi monomorfiktir ve altpopülasyonlar arasında aynı allelde fikse olmuştur, dört lokus ise polimorfiktir. Wright'ın fiksasyon indeksinin ortalama değeri $F_{ST}$ = 0.0671, % 6.7'lik bir genetik varyasyon olduğunu göstermektedir ve bu da *Meriones tristrami* alttürlerinde biraz düşük bir farklılığın olduğuna işaret eder. Elde edilen $F_{ST}$ değeri *M. tristrami* altpopülasyonları için ise orta derecede bir genetik farklılığı göstermektedir. Tüm altpopülasyonlar için ortalama heterozigotluk (Ho= direk hesaplanan) $H_o$= 0.017'dir, 0.01 ve 0.029 arasında değişir. Nei'nin genetik mesafesi düşüktür ve altpopülasyonlar arasında D = 0.000 - 0.002 arasında değişiklik göstermiştir. Göç sayısı (Nm) 3.48 değerinde bulunmuştur ve bu değer altpopülasyonlar arasında etkin gen akışının olduğunu da göstermiştir.

**Anahtar Kelimeler**
Allozim, alttür, *Meriones tristrami*, Türkiye.

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## INTRODUCTION

*Meriones tristrami* Thomas, 1892, a polytypic species, is distributed in the Palearctic region. Of its subspecies, *Meriones blackleri blackleri* was first described from Izmir by Thomas (1903) [1], *Meriones blackleri lycaon* from Karadag (Karaman) by Thomas (1919) [2] and *Meriones blackleri intraponticus* from Tosya (Kastamonu) by Neuhäuser (1936) [3]. On the basis of the specimens in several museums, Neuhäuser (1936) [3] stated that *Meriones blackleri bogdanovi* lives in north-eastern Turkey. Later, *M. blackleri* was considered to be junior synonym of *M. tristrami* by Matthey (1957) [4], Baltazard et al. (1960) [5], Harrison (1972) [6] and Harrison & Bates (1991) [7]. Additionally, Yigit et al. (1998a) [8] gave the first record of *M.t. bodenheimeri*, and he also showed that the population from Kilis (Turkey) is a distinct taxon that described *M.t. kilisensis* [9]. Yigit et al. (1998a) [8] found different karyological data between *M.t. blackleri*, *M.t. intraponticus* and *M.t. lycaon*. The patterns of the blood serum proteins of Genus *Meriones* in Turkey were compared by SDS-PAGE [10], and did not show the diagnostic characters to distinguish the specimens of *Meriones*. Apart from this study [10], the others focused on karyology and morphology of *Meriones* in Turkey. Apart from these researches, the data obtained from allozyme electrophoresis have been used to separate species or to establish phylogenetic relationships of taxa. In this frame, some species of the genera *Microtus*, *Mesocricetus*, *Apodemus, Rattus, Spalax*, and *Dryomys* which are distributed in Turkey were investigated in respect to their allozyme variations [11-16]. In this connection we aimed to determine the level of genetic difference of three subspecies of *M. tristrami* in Western Anatolia: *lycaon, intraponticus* and *blackleri*.

## MATERIALS AND METHODS

Thirty-five *Meriones tristrami* samples were collected from three type localities in Western Anatolia (Figure 1). Ten of those are *M.t. lycaon* from Karadag (Karaman), thirteen are *M.t. intraponticus* from Tosya (Kastamonu), and twelve are *M.t. blackleri* from Turgutlu (Manisa). Specimens were caught with Sherman live traps and transferred to the laboratory alive. Specimens were sacrificed in the laboratory, and liver, heart, kidney, muscle were removed to the deep-freezer at -70°C until homogenized. According to our modified method, the samples were homogenized in approximately 450 µl of distilled water with a glass homogenizer. The electrophoretic procedures were carried out as described by Shaw & Prasad (1970) [17] and Harris & Hopkinson (1976) [18]; the gel percentage was 10 %, and the running of samples was performed during 3-5 hours with 120 V. The different buffers for gel, running and dying were used in accordance with enzyme systems described by Shaw & Prasad (1970) [17] and Harris & Hopkinson (1976) [18].

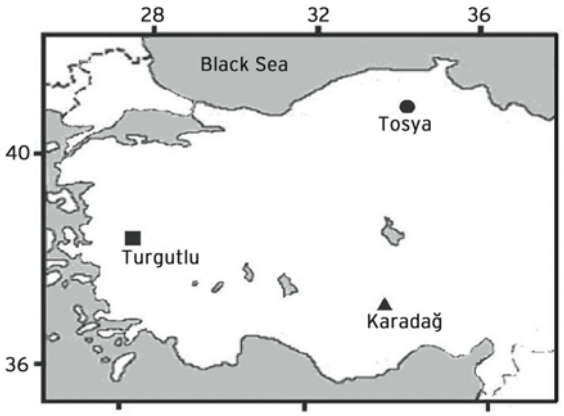


**Figure 1.** The sampling locations of the subspecies of *M. tristrami* in western Anatolia (▲ *M.t. lycaon*; ● *M.t. intraponticus*; ■ *M.t. blackleri*).

Genetic variation was assessed using standard horizontal gel electrophoresis and 15 enzymes coding for 24 loci were analysed. Homogenates obtained from muscle were processed for the following enzymatic proteins: Glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12; $G_3$pdh), α-Glycerophosphate dehydrogenase (E.C. 1.1.1.8; α-Gpdh-1 and α-Gpdh-2), Hexokinase (E.C. 2.7.1.1; Hk), Aconidase (E.C. 4.2.1.3; Acon-1 and Acon-2), Superoxide dismutase (E.C. 1.15.1.1; Sod-1), Phosphogluconate Dehydrogenase (E.C. 1.1.1.44, Pgd), Phosphoglucomutase (E.C. 2.5.7.1; Pgm-1), Mannose phosphate isomerase (E.C. 5.3.1.8; Mpi), Aldolase (E.C. 4.1.2.13; Aldo), Malic enzyme (E.C. 1.1.1.40; Me-1), Lactate dehydrogenase (E.C. 1.1.1.37; Ldh-1, Ldh-2, Ldh-3, Ldh-4 and Ldh-5), Isocitrate dehydrogenase (E.C. 1.1.1.42; Idh-1 and Idh-2),

Glucose phosphate isomerase (E.C. 5.3.1.9; Gpi-1 and Gpi-2), Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; $G_6$pdh-1 and $G_6$pdh-2), Fumarase (E.C. 4.2.1.2, Fum).

The obtained electrophoretic band patterns were analyzed following the method by Harris & Hopkinson (1976) [18]. Presumptive alleles were designated alphabetically by their relative mobility, with the allele variant migrating farthest towards the anode denoted as A.

Allozymic data were analyzed as allele frequencies with BIOSYS-2 (Black 1997 [19]; original version BIOSYS-1 Release 1.7 program and modifications to HDYWBG and FSTAT by William C. Black IV). Intrapopulational genetic variation was estimated by the mean heterozygosity per locus (expected: He, and observed: Ho), the proportion of polymorphic loci in the population (a locus is considered polymorphic if the frequency of the common allele is not greater than 0.95), and the mean number of alleles per locus. The departure from Hardy–Weinberg equilibrium was tested by three methods (the chi-square for goodness of fit and an exact probability test [20]; because our samples were sometimes small, we also used a chi-square test with Levene (1949) [21] correction for small sample sizes). The program FSTAT was used to calculate overall and population-specific Wright's F-statistics estimators of $F_{ST}$ value. Fixation index (F-statistics; [22,23]) was used to summarize the distribution of genetic variation within and between populations. According to the Nei & Chesser (1983) [24] correction, negative values were considered as 0. Estimates of overall gene flow between populations (Nm) were derived from the approximation $F_{ST} = 1 / (1 + 4Nm)$ as recommended by Slatkin & Barton (1989) [25]. The amount of genetic divergence between species was estimated with the indices of standard genetic identity (I) and distance (D, Nei unbiased distance) proposed by Nei (1978) [26]. A dendrogram of the genetic relationships among the populations was obtained using the Unweighted Pair Group Method with Arithmetic Mean UPGMA [27,28]

## RESULTS AND DISCUSSION

### Allele frequency and genetic variation

Twenty of the 24 loci analyzed were monomorphic and fixed for the same allele in all the populations of *M. tristrami*, and four loci ($G_3$pdh, α-Gpdh-1, α-Gpdh-2 and Sod) were found to be polymorphic. Of these loci, $G_3$pdh, α-Gpdh-1 and α-Gpdh-2 are polymorphic in The Karadag and Tosya populations. The population of *M. tristrami blackleri* from Turgutlu was polymorphic at only Sod locus. The allelic frequencies at the polymorphic loci are given in Table 1.

**Table 1.** The allelic frequencies at the polymorphic loci of the populations of *M. tristrami* (N= number of specimens).

| Locus and alleles | 1. Tosya (*M. t. intraponticus*) N: 13 | 2. Karadag (*M. t. lycaon*) N: 10 | 3. Turgutlu (*M. t. blackleri*) N: 12 |
|---|---|---|---|
| $G_3$pdh | | | |
| A | 0.923 | 0.850 | 1.000 |
| B | 0.077 | 0.150 | - |
| α-Gpdh-1 | | | |
| A | 0.923 | 0.950 | 1.000 |
| B | 0.077 | 0.050 | - |
| α-Gpdh-2 | | | |
| A | 1.000 | 0.850 | 1.000 |
| B | - | - | - |
| C | - | 0.150 | - |
| Sod | | | |
| A | 1.000 | 1.000 | 0.875 |
| B | - | - | 0.125 |

**Table 2.** Levels of genetic variation based on 20 loci in all populations (1. *M. t. intraponticus*, 2. *M. t. lycaon*, 3. *M. t. blackleri*) (standard errors in parentheses).

| Population | Mean sample size per locus | Mean number of alleles per locus | Percentage of polymorphic loci* | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | Direct count (Ho) (He) | HydWbg Expected |
| 1. Tosya | 13 | 1.1 | 8.3 | 0.013 | 0.012 |
| | | (0.1) | | (0.009) | (0.009) |
| 2. Karadag | 10 | 1.1 | 12.3 | 0.029 | 0.027 |
| | | (0.1) | | (0.018) | (0.016) |
| 3. Turgutlu | 12 | 1.1 | 4.2 | 0.010 | 0.010 |
| | | (0.0) | | (0.010) | (0.010) |

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

Levels of genetic variation within each population are shown in Table 2. The expected frequency of heterozygotes (He) under Hardy-Weinberg equilibrium for all populations of the three subspecies was 0.016, and ranged from 0.01 (Turgutlu) to 0.027 (Karadag). However, direct counts of frequency of heterozygotes (Ho) were found to be varied from 0.01 to 0.029. The mean percentage of polymorphic loci was 8.23, which is quite low. The Karadag population had the highest polymorphism with 12.5 percent but the lowest one was Turgutlu with 4.2 percent. We expected the observed polymorphism values due to habitat differences subspecies of *M. tristrami*.

The habitat of *blackleri* has a warmer climate and lower altitude from the other habitats, thus we thought this caused the neutral population for *blackleri*. In a review on the genetic variation in natural populations, Nevo (1978) [29] estimated Ho value for 44 small rodents to be 0.038, with values ranging from 0 to 0.106. Our findings are half of that value because the present study was performed within one population of *M. tristrami*. Mean number of alleles per locus was 1.1 and the same for all populations. Estimates of *F*-statistics for four leading loci were calculated with BIOSYS-2 (Table 3). Negative values were observed both for mean $F_{IS}$ =-0.1320 and for mean $F_{IT}$ = -0.0560 indicating the deficiency of heterozygotes both within the population and within the species. The mean value of the fixation index was $F_{ST}$ =0.0671, indicating that 6.7 % of the genetic variation in *M. tristrami* is due to differentiation existing among populations. The loci $G_3$pdh, α-Gpdh-1, α-Gpdh-2, and Sod are the ones which significantly contribute to the differentiation between populations observed (Table 3). Wright (1978) [30] used the following groupings for the evaluation of $F_{ST}$ values: the range 0 to 0.05 is considered to reflect little genetic differentiation, 0.05 to 0.15 is indicative of moderate differentiation, 0.15 to 0.25 indicates great genetic differentiation, and values greater than 0.25 reflect very great genetic differentiation. In the present study, the mean fixation index was $F_{ST}$ =0.0671, indicating moderate differentiation. Yigit et al. (2007) [16] found that the mean $F_{ST}$ value was 0.0748 among Turkish populations of *Mesocricetus brandti*. It can be proposed that $F_{ST}$ values between *M. brandti* and *M. tristrami* were so close due to habitat similarity - both share the steppe habitats. An estimation of gene flow, Nm, between the populations studied was done by using the formula $F_{ST}$ = 1/ (4Nm + 1) . The mean gene flow value (Nm) was 3.48 among

**Table 3.** F-statistics of variable loci in the populations of *M. tristrami* calculated using the method of Wright (1978) [30].

| Locus | $F_{IS}$ | $F_{ST}$ |
|---|---|---|
| $G_3$pdh | -0.1374 | 0.0531 |
| α-Gpdh-1 | -0.0729 | 0.0262 |
| α-Gpdh-2 | -0.1765 | 0.1119 |
| Sod | -0.1429 | 0.0858 |
| Mean | -0.1320 | 0.0671 |

the populations. This value suggests that gene flow is high among the populations of *M. tristrami* studied. That means there are no geographic barriers between the subspecies of *M. tristrami*.

**Genetic distance and evolutionary remarks**

In order to observe the genetic relationships among the populations studied, Nei's (1978) [26] values of genetic identity (*I*) and distance (*D*) were calculated on the basis of 24 loci (Table 4). These values between populations of *M. tristrami* varied from D= 0.000 to D= 0.002. The highest value of genetic distance was found between Karadag and Tosya (D = 0.002). The lowest value of genetic distance was between the populations from Tosya and Karaman (*D*=0.000). Kankılıc (2005) [15] determined the genetic distance between populations of *Rattus sp.* in Turkish Trace and reported that Nei's genetic distances were low among *R. rattus* populations (D= 0.0001 to 0.011). It was reported to vary from D= 0.006 to D= 0.026 in *M. brandti* [16]. Comparing to *R. rattus* and *M. brandti*, the genetic distance between populations of *M. tristrami* was strictly lower than in these taxa. An UPGMA dendrogram summarizing the genetic relationships found among the populations of *M. tristrami* is given in Fig. 2. The UPGMA dendrogram shows that the Turgutlu population is separate from the other populations. The Tosya and Karadag populations are close populations, which is an expected result because the populations of *intraponticus* and *lycaon* are connected.

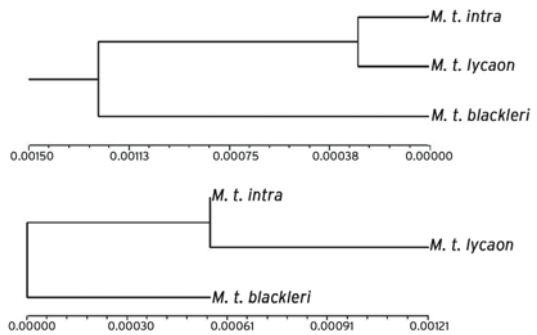


**Figure 2.** UPGMA (above) and neighbour joining (below) dendrogrammes summarizing the genetic relationships among the *M. tristrami* populations studied (D= Nei's (1978) unbiased genetic distance, based on 24 enzyme loci). The coefficient of cophenetic correlation is 1.00.

**Table 4.** Values of NEI's (1978) unbiased genetic identity (I; below the diagonal) and distance (D; above the diagonal) among the *M. tristrami* populations.

| Populations | 1 | 2 | 3 |
|---|---|---|---|
| 1. Tosya | ***** | 0.000 | 0.001 |
| 2. Karadag | 1.000 | ***** | 0.002 |
| 3. Turgutlu | 0.999 | 0.998 | ***** |

Western Anatolia is considered as a scene of evolutionary theater by many authors [11,31,32]. The karyological differences among rodent species might be related to ecological factors, and a low diploid number of chromosomes among species is usually indicative of an ancestral population [11,31]. Nevo (1994) [31] also suggested that speciation and adaptation of Turkish *Spalax* is positively correlated with stress and climatic unpredictability, and that 2n values *Spalax leucodon* increase toward the ecologically arid, climatically unpredictable, and geologically young central Anatolian Plateau from all directions. Yigit et al. (2005) [32] similarly noted that 2n values of Turkish ground squirrel increased toward central Anatolia from the Southwest Mountains, and *Spermoplilus* spp. colonized central Anatolia during the Pleistocene after the retreat of the extensive inner sea system. Yigit et al. (1998*a*) [8] found that the fundamental number of chromosomes (FN) of *M. tristrami* increased from the western coast (*M.t. blackleri*, FN=78.) to central Anatolia (*M.t. lycaon*, FN= 82). The heterozygosity of allozymes among *M. tristrami* populations also showed an increase towards central Anatolia. Therefore ancestor of *M.t. lycaon* might have originated from the peripheral populations, and these subspecies are assumed to be an early stage of speciation. Support for this scenario requires further researches covering a wider range of *M. tristrami* populations.

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