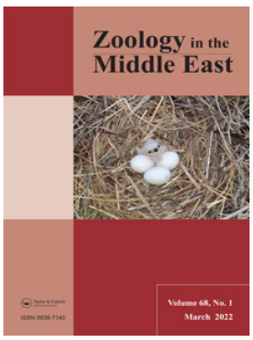

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Allozyme variation in *Rattus rattus* (Rodentia: Muridae) in Turkey, with particular emphasis on the taxonomy

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Allozyme variation in *Rattus rattus* (Rodentia: Muridae) in Turkey, with particular emphasis on the taxonomy

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Abstract. The Turkish black rat "*Rattus rattus*" shows variation in coat colour corresponding to the occurrence of three subspecies with intermediate colour stages: *Rattus rattus rattus, Rattus r. alexandrinus* and *Rattus r. frugivorus*. Turkish black rat populations were divided geographically into six sub-populations: Rr1= Northwest Anatolia, Rr2= Central Anatolia, Rr3= Eastern Mediterranean, Rr4= Western Mediterranean, Rr5= Turkish Thrace, and Rr6= Black Sea region. Genetic variation was assessed using twenty two isoenzyme systems. Seven of twenty-two loci (*Pgm-1, Hk, Me-M, G3pdh*, *Gpdh-1, Gpi, Fum-1*) were found to be polymorphic. The mean value of F_{ST} is found to be 0.073, indicating 7.3 % genetic variation among groups and suggesting the existence of a moderate differentiation between sub-populations of the Turkish black rat. Overall mean heterozygosity (*Ho*= direct count) for sub-populations was *Ho*= 0.020, ranging from 0.008 to 0.031. Nei's measure of genetic distance showed that Rr2 and Rr6 were the most identical and sub-populations Rr1 and Rr5 had diverged the most.

Key words. Allozyme, morphology, *Rattus rattus,* Turkey, Middle East.

Introduction

The genus *Rattus* is known to be a widely distributed and taxonomically mixed group that includes many species and subspecies throughout the world (WILSON & REEDER 1993). There are only a few distribution records of *R. rattus* Linnaeus, 1758 and *R. norvegicus* Berkenhout, 1769 in Turkey (AHARONI 1932, NEUHAUSER 1936, LEHMANN 1969, YIĞIT et al. 1998). Taxonomic studies performed on this genus in eastern and south-eastern Asia and Europe have mainly focused on karyology.

The black rat has been the subject of numerous cytogenetic studies (CAPANNA et al. 1970, CAPANNA & CIVITELLI 1971, YOSIDA 1973, 1980, YOSIDA et al. 1971, YIĞIT et al. 1998, KANKILIÇ et al. 2006). These studies have revealed a great wealth of chromosomal diversity within the species, including pericentric inversions, centric fusions, centric fissions, C-band polymorphisms and supernumerary chromosomes. YIĞIT et al. (1998) described the karyotypes of the Turkish populations of *R. rattus* and *R. norvegicus* along with colour variations. According to YIĞIT et al. (1998) there are four common colour morphs in the Turkish black rat, with intermediate colour stages.

The patterns of the blood serum proteins of the genus *Rattus* in Turkey were compared with SDS-PAGE (YIĞIT et al. 2001), but did not show the diagnostic characteristics related to *R. rattus*. Although extensively studied from a karyological point of view, few estimates of genetic variability in black rats are available. Overall levels of intrapopulation allozyme polymorphism are relatively low, ranging from 0.0 % to 10.2 % in mainland populations (BAVERSTOCK et al. 1983, GEMMEKE & NIETHAMMER 1984, PATTON et al. 1975), but values

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reach up to 16% in island populations which have been more thoroughly investigated (CHEYLAN et al. 1998, GEMMEKE & NIETHAMMER 1984, PASTEUR et al. 1982, PATTON et al. 1975). In the present study, we examined allozymic variation and genetic differentiation in *R. rattus* throughout Turkey in order to compare geographic populations.

Material and methods

159 specimens of *Rattus rattus* were collected from 29 localities in Turkey (Fig. 1 and Table 1). The sub-populations were mainly assigned according to geographic proximity and also the colour patterns of the dorsal fur. Specimens were caught with Sherman live traps, transferred to the laboratory and then killed. The liver, heart, kidney and muscles were removed and immediately stored frozen at -70°C until homogenized. All specimens were skinned in the standard museum manner, the colours of animals were carefully noted, and the skulls were examined in order to identify species. The identification was also performed in accordance with YIĞIT et al. (1998).

The tissue 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) and HARRIS & HOPKINSON (1976); the gel percentage was 12 % and samples were run at 120 V for a period of 4–6 hours. Different buffer systems were used in accordance with enzyme systems in different parts of the procedure (gel preparation, sample running and staining). Genetic variation was assessed using standard horizontal gel electrophoresis and 16 enzymes coded for 22 presumptive loci were analysed. Presumptive alleles were designated alphabetically by their relative mobility, with the allele variant migrating furthest anodically denoted as A.

Homogenates obtained from muscle were processed for the following enzymatic proteins: *a-Glycerophosphate dehydrogenase* (E.C. 1.1.1.8; *a-Gpdh-1* and *a-Gpdh-2*), *Sorbitol dehydrogenase* (E.C.1.1.1.14; *Sdh*), *Lactate dehydrogenase* (E.C. 1.1.1.37; *Ldh*), *Malate dehydrogenase* (E.C. 1.1.1.37; *Mdh-1* and *Mdh-2*), *Malic enzyme* (E.C. 1.1.1.40; *Me-1* and *Me-2*), *Isocitrate dehydrogenase* (E.C. 1.1.1.42; *Idh-1* and *Idh-2*), *Glucose-6-phosphate dehydrogenase* (E.C. 1.1.1.49; *G6pdh*), *Glyceraldehyde-3-phosphate dehydrogenase* (E.C. 1.2.1.12; *G3pdh*), *Superoxide dismutase* (E.C. 1.15.1.1; *Sod-1* and *Sod-2*), *Hexokinase* (E.C. 2.7.1.1; *Hk*), *Adenylate kinase* (E.C. 2.7.4.3; *Adk-1* and *Adk-2*), *Phosphoglucomutase* (E.C. 2.5.7.1; *Pgm-1*), *Aldolase* (E.C. 4.1.2.13; *Aldo*), *Fumarase* (E.C. 4.2.1.2, *Fum*), *Mannose phosphate isomerase* (E.C. 5.3.1.8; *Mpi*), and *Glucose phosphate isomerase* (E.C. 5.3.1.9; *Gpi*).

Allozymic data were analysed as allele frequencies with BIOSYS-2 (BLACK 1997; original version BIOSYS-1 Release 1.7 program of SWOFFORD & SELANDER 1981, and modifications to HDYWBG and FSTAT by WILLIAM C. BLACK IV). The genetic variation between intra-specific sub-populations was estimated as the mean heterozygosity per locus "*Ho*= observed (direct count) and *He*= expected and frequencies of heterozygotes" under Hardy-Weinberg equilibrium (NEI 1978), 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 program FSTAT was used to calculate overall and population-specific Wright's F-statistics estimators of F_{ST}, F_{IT} and F_{IS}. Fixation indices (F-statistics; WRIGHT, 1951, 1965) were used to summarize the distribution of genetic variation within and between populations. According to the NEI & CHESSER (1983) correction, negative values were considered as 0. Estimates of overall gene flow between populations (Nm) were derived from the approximation $F_{ST} = 1 / (1 + 4\,Nm)$ as recommended by SLATKIN & BARTON (1989). The amount of genetic divergence between sub-populations was estimated with the indices of standard genetic identity (I) and distance (D, Nei unbiased distance) proposed by NEI (1978). A dendrogram of the genetic relationships among the sub-populations was constructed using the Unweighted Pair Group Method with Arithmetic Mean UPGMA (SOKAL & SNEATH 1963, ROHLF 2000).

Table 1. Sampling sites, number of specimens (N) and abbreviations for geographic localities (groups) of *Rattus rattus* studied. See Fig. 1 for the distribution of localities; Rr: *Rattus rattus*.

Group	N	Locations
Rr1	23	Beykoz (Istanbul), Gönen (Balıkesir) (Northwest Anatolia)
Rr2	13	Central Anatolia; Ankara, Düzce, Gerede
Rr3	32	Mediterrenean 1; Mersin, Nizip, Pozantı, Adana, Nusaybin, Birecik, Ceylanpınar
Rr4	13	Mediterrenean 2; Kemalpaşa, Bayındır, Akseki, Alanya, Belek, Dalaman
Rr5	59	Turkish Tharce; Demirköy, Pınarhisar, Saray, İpsala, Eceabat
Rr6	19	The Black Sea region; Bartın, Zonguldak, Fatsa, Ordu, Trabzon, Artvin

Results

Morphological peculiarities and allozymic patterns: Turkish black rats showed marked colour variation in both dorsal and ventral fur. In this connection, the Rr1 and Rr5 sub-populations have dark brown dorsal fur with an off-white / yellow belly. The Rr2 sub-population has both black and brown specimens with grey ventral fur. The Rr3 sub-population covers the specimens from the southeastern Mediterranean and has dark brown dorsal fur with white or off-white ventral fur. The specimens captured from the Rr3 sub-population prefer to live in the ground under thistle bushes. The Rr4 sub-population from the southwest Mediterranean and Aegean coasts has black and jet black dorsal fur with a white belly and is usually captured from attics in buildings. The Rr6 sub-population also has black and jet black dorsal fur with grey or pale grey ventral fur. Thus the samples within Rr1, Rr3, Rr4, Rr5 and Rr6 are highly homogenous in respect to the colours on the dorsal and ventral of the body. The specimens of Rr2 were a more heterogeneous group in their dorsal colours than were the other sub-populations described above.

In electrophoresis, 22 loci were analysed in the six sub-populations of *R. rattus*. Seven of 22 loci were polymorphic among the sub-populations. Other loci were found to be monomorphic and fixed for the same allele. The frequencies of polymorphic loci (*Pgm-1, Hk, Me-M, G3pdh, Gpdh-1, Gpi, Fum-1*) are summarised in Table 2. *Pgm-1* and *Fum-1* were variable in all sub-populations, *Hk* in Rr2, Rr3 and Rr4, *Me-M* in Rr1, Rr4, Rr5 and Rr6, *Gpdh-1* in Rr1, Rr2, Rr3, Rr4 and Rr6, *Gpi* in Rr3, Rr5, Rr6. Allozymic patterns do not appear to be location-specific or to relate to the geographic proximity of groups. The polymorphic loci were found to be spread through all sub-populations.

Genetic variation: The mean average of genetic variation (*He*) in the sub-populations studied was found to be 0.044, ranging from 0.022 to 0.059, whilst the mean value of *Ho* was 0.020. The values of *Ho* and *He* in all sub-populations studied showed differences (Table 3). The overall mean percentage of polymorphic loci for all sub-populations was 15.1 and ranged from 4.5 to 27.3. The highest value was found in the Rr6 (the Black Sea) and the lowest in the Rr1 sub-population. The percentages of polymorphic loci showed correspondence with the geographic proximity of the sub-populations studied. This percentage was found to be closest in sub-populations Rr1 (4.5) and Rr5 (9.1), followed by Rr3–Rr4 (13.6), then by Rr2 (22.7) and Rr6 (27.3).

The overall mean number of alleles per locus was 1.25, ranging from 1.2 to 1.3 among the sub-populations (Table 3). The mean heterozygosity of the polymorphic loci in *R. rattus*

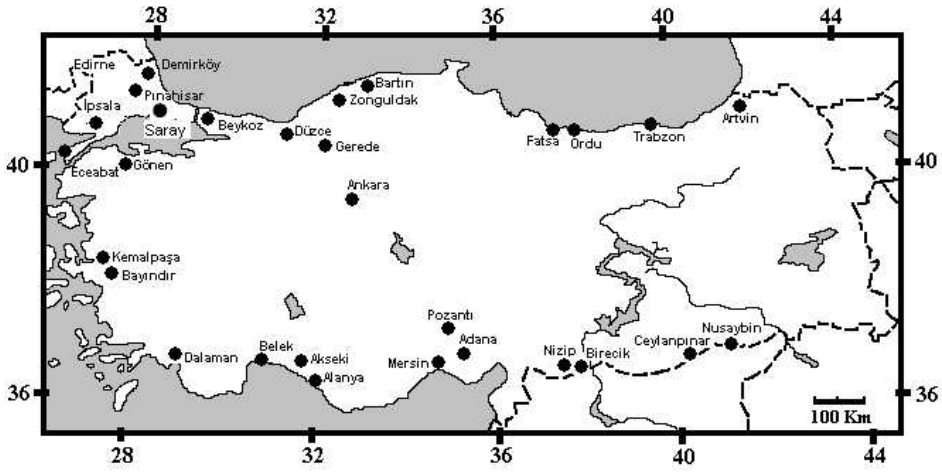

Fig. 1. Map of the study area, indicating the collecting localities for *Rattus rattus* in Turkey

sub-populations shows significant deviation from Hardy-Weinberg equilibrium. Apart from this, significant deviations from H-W expectation were observed in all of the sub-populations. *Fum-1* deviated significantly in all of the sub-populations except for Rr4. *Hk* deviated from H-W equilibrium in Rr2, Rr3 and Rr4; *Gpi* in Rr3, Rr5 and Rr6; *G3pdh* in Rr2 and Rr6; *Me-M* in Rr6. Contingency Chi-square analysis at all polymorphic loci indicated that there is substantial differentiation among sub-populations. All polymorphic loci except *Gpi* were found to have significant differences between *R. rattus* sub-populations.

Estimates of *F*-statistics for the sub-populations are summarised in Table 4. The mean value for the individual inbreeding coefficient was F_{IS} = 0.56 which reflects the moderate level of the deviation of the total-population from the genotype frequency via non-random breeding between sub-populations of *R. rattus*. The mean value of the overall individual coefficient was F_{IT} = 0.595, and this value corresponds to moderate levels of gene flow between sub-populations. The mean value of the fixation index was found to be F_{ST} = 0.073, indicating that 7.3 % of the genetic variation was due to the differentiation among the sub-populations. This value reflects moderate genetic differentiation in *R. rattus* sub-populations. Apart from F_{ST}, WRIGHT (1943) produced a formula (F_{ST} = 1/ (4Nm + 1)) used to estimate gene flow between the sub-populations (where Nm= number of migrants). According to this formula, the gene flow was determined to range from 2.4 (*Hk*) to 9.4 (*Gpi*) with a mean of 3.2 among the sub-populations. This value suggests that gene flow is very high between sub-populations and also indicates that there are no geographic barriers preventing the flow of genes among the sub-populations of *R. rattus* studied.

Genetic distance: The values of NEI's (1978) unbiased genetic identity (I) and distance (D) were calculated among the sub-populations of the black rat for all pair-wise comparisons from the allele frequencies at the 22 loci (see Fig. 2). The genetic distance was found to be low among sub-populations of the Turkish black rat. This result supports reliable evidence for gene flow between the Turkish black rat sub-populations and is also consistent with the F_{ST} value.

Table 2. Allele frequencies of seven polymorphic loci analyzed in *Rattus rattus* populations in Turkey.

Locus		Groups					
		Rr1	**Rr2**	**Rr3**	**Rr4**	**Rr5**	**Rr6**
Pgm-1	A:	0.957	0.731	0.078	0.962	0.983	0.868
	B:	0.043	0.269	0.922	0.038	0.017	0.132
Hk	A:	-	-	-	0.077	-	-
	B:	1.000	0.923	0.844	0.769	1.000	1.000
	C:	-	0.077	0.156	0.154	-	-
Me-M	A:	0.022	-	-	0.038	0.076	-
	B:	0.978	1.000	1.000	0.962	0.924	0.947
	C:	-	-	-	-	-	0.053
G3pdh	A:	-	0.077	-	-	-	0.053
	B:	1.000	0.923	1.000	1.000	1.000	0.947
Gpdh1	A:	0.022	0.077	0.047	0.077	-	0.132
	B:	0.978	0.923	0.953	0.923	1.000	0.868
Gpi	A:	-	-	0.031	-	-	0.053
	B:	1.000	1.000	0.969	1.000	0.983	0.947
	C:	-	-	-	-	0.017	-
Fum-1	A:	0.696	0.731	0.578	0.423	0.831	0.763
	B:	-	0.077	-	-	-	-
	C:	0.304	0.192	0.422	0.577	0.169	0.237

Table 3. Genetic variability at 22 loci in analysed geographic samplings of *Rattus rattus* in Turkey. Mean sample size per locus, mean number of alleles, locus, percentage of polymorphic loci, and observed (*Ho*) and expected (*He*) heterozygosities based on NEI (1978). Standard errors in parentheses. *A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95; **Unbiased estimate (NEI 1978).

	Sample size per locus	**Mean number of alleles per locus**	**Percentage of polymorphic loci***	**Mean Heterozygosity**	
				Ho	**He****
Rr1	23	1.2 (0.1)	4.5	0.016 (0.009)	0.027 (0.020)
Rr2	13	1.3 (0.1)	22.7	0.021 (0.013)	0.059 (0.028)
Rr3	32	1.2 (0.1)	13.6	0.016 (0.009)	0.048 (0.025)
Rr4	13	1.3 (0.1)	13.6	0.031 (0.019)	0.055 (0.029)
Rr5	59	1.2 (0.1)	9.1	0.008 (0.007)	0.022 (0.014)
Rr6	19	1.3 (0.1)	27.3	0.026 (0.017)	0.052 (0.022)

The genetic distances were found to range from <0.00001 to 0.0004 among the sub-populations of *R. rattus* (Rr1–Rr6). The highest value of genetic distance among *R. rattus* sub-populations was found to be D= 0.0233 between Rr4 and Rr5 which are geographically far from each other. A UPGMA dendrogram summarises the genetic relationship found between the sub-populations (Fig. 2). The sub-populations of *R. rattus* in Turkish Thrace and Beykoz, Gönen (Asiatic Turkey) established the first sub-cluster and were close to each

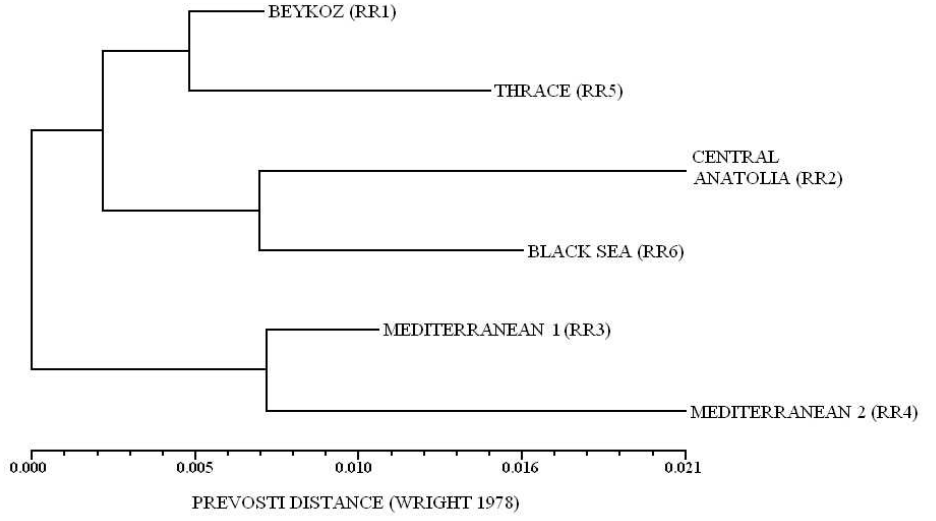

Fig. 2. Neighbour Joining dendrogram summarising the genetic relationships (Prevosti Distance) based on 22 enzyme loci among the *Rattus rattus* populations studied.

other (D= 0.0006). Central Anatolia and the Black Sea sub-populations formed the second sub-cluster and were connected to the first cluster with a genetic distance of D= 0.0016. The Mediterranean sub-populations were gradually connected with previous clusters. The sub-population of Mediterranean 1 (Rr4) appeared to be the most diverged sub-population of the Turkish black rat.

Taxonomic remarks: According to 159 specimens of the Turkish black rat examined, there is no colour uniformity in the geographically separated sub-populations. As in the conventional taxonomy, the specimens which have jet black or black dorsal fur and grey ventral fur are referred to as *Rattus rattus rattus*. Some of the specimens from Central Anatolia (Rr2), Western Mediterranean (Rr4) and the Black Sea region (Rr6) showed this morphotype. The specimens which have dark brown dorsal fur with yellow / white ventral fur are known as *Rattus rattus alexandrinus* E. Geoffroy, 1803. Rr1 and Rr5 sub-populations have these colour characteristics. The specimens with dark brown dorsal fur and pure white ventral fur are referred to as *Rattus rattus frugivorus* Rafinesque, 1814 and are known in the Mediterranean region. However, the specimens with jet black dorsal fur and yellow or off-white ventral fur were also captured from the Mediterranean region. Hence, when considering only colouration it is difficult to assign all the sub-populations (Rr1–Rr6) of the Turkish black rat to any subspecies except for Rr1 and Rr5 which show similarity to the colouration of *R. r. alexandrinus* and Rr3 which has the colour characteristics of *R. r. frugivorus*. The genetic distance calculated from allozymic data does not support a differentiation at the subspecific level between the sub-populations studied. The colour-based relationship of sub-populations in the Turkish black rat requires further research focused on the genetic markers of the species in order to resolve these issues.

Table 4. Summary of F-statistics at all variable loci in the geographic samplings of *Rattus rattus.*

Locus	F_{IS}	F_{ST}	F_{IT}	Nm
Pgm-1	0.0480	0.0767	0.1210	2.7
Hk	1.0000	0.1098	1.0000	2.4
Me-M	0.0945	0.0311	0.1226	7.7
G3pdh	1.0000	0.0529	1.0000	5.1
Gpdh1	-0.0950	0.0478	-0.0426	7.4
Gpi	1.0000	0.0192	1.0000	9.4
Fum-1	0.7693	0.0800	0.7877	2.6
Mean	**0.5637**	**0.0725**	**0.5953**	**3.2**

Discussion

According to MILLER (1912), there are two main colour types in *R. rattus*; the first has moderately jet black dorsal fur (*R. r. rattus*) whilst the second has brown dorsal fur (*R. r. alexandrinus*). The ventral fur of *R. r. alexandrinus* has been reported as grey or light grey (MILLER 1912, ONDRIAS 1966). *R. r. alexandrinus* with off-white or yellow ventral fur has also been reported from Arabia (HARRISON & BATES 1991). These findings showed that specimens with two different ventral colours are assigned to the same subspecies: *R. r. alexandrinus*. HARRISON & BATES (1991) have suggested rejecting the subspecies categorisation, proposing instead that all these forms are colour phases. ONDRIAS (1966) stated that there are two subspecies in Greece, *R. r. alexandrinus* and *R. r. frugivorus*. Specimens of Rr3 have brown dorsal and pure white ventral colours. This colour morph was first considered as a separate species "*Rattus frugivorus*" from the same region by NEUHAUSER (1936). This taxon was then assigned as a subspecies of *R. rattus* in earlier reviews (ONDRIAS 1966, CORBET 1978, WILSON & REEDER 1993). Specimens of Rr3 may be assigned to *R. r. frugivorus* which is distributed in the coastal part of the Mediterranean region in Turkey. According to our observations specimens of *R. r. frugivorus* in coastal areas at low elevations were caught at ground level, suggesting a potential preference for this kind of habitat. However we also captured specimens with jet black dorsal fur and pure white ventral fur which occupy the attics of houses and whose distribution tends towards the Mediterranean Mountains. The specimens of Rr4 mostly have jet black dorsal fur. Even though these two colour morphs seem to coexist in the same region, their niches are highly different as indicated above. Hence, we conclude that three subspecies (*R. r. rattus, R. r. alexandrinus*, *R. r. frugivorus*) with intermediate colour stages are distributed across Turkey.

Apart from the morphological evaluation, allozyme variations were also considered as a molecular marker to elucidate geographic and colour-based variations. Our results show that there is a considerable amount of genetic variability (7.3%) in the Turkish black rat. This result is consistent with those of CHEYLAN (1986), CHEYLAN et al. (1998), GEMMEKE & NIETHAMMER (1984), and PATTON et al. (1975). However, higher genetic variability was also reported for some island populations by GEMMEKE & NIETHAMMER (1984) and CHEYLAN et al. (1998). The overall mean heterozygosity (*He*: 0.044) for Turkish black rat subpopulations was found to be relatively higher than those reported for different populations: western Mediterranean populations (*He*: 0.025, CHEYLAN et al. 1998), the Galapagos Archipelago (*He*: 0.03, PATTON et al. 1975), and the Oceanian form with 2n=38 (*He*: 0.01 BAVER-

STOCK et al. 1983, 1986). The mean value of polymorphic loci in the black rat populations (p = 15.1) is less than those measured in *Mus musculus domesticus*, p= 25 (BRITTON-DAVIDIAN 1990) and *Mesocricetus brandti*, p= 29.4 (YIĞIT et al. 2007), but higher than in the genus *Sylvaemus* in western Anatolia (FILIPUCCI et al. 1996) and in *Mesocricetus auratus* (YIĞIT et al. 2007). NEVO (1978) estimated the mean heterozygosity value for 44 small rodents to be 0.038, with values ranging from 0 to 0.106. In this study, the heterozygosity values (*He*) for *R. rattus* were found to be in the range of small rodents, varying from 0.022 to 0.059.

Estimates of *F*-statistics for the sub-populations indicate the deviation of genotype frequency via non-random breeding between sub-populations (F_{IS}), the level of non-random mating within the sub-populations (F_{IT}) and genetic differentiation via genetic drift among the sub-populations (F_{ST}). WRIGHT (1978) 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. According to these criteria, our F_{ST} value (0.073) between the sub-populations of the Turkish black rat is indicative of moderate differentiation. F_{ST} and Nm values also support the effective gene flow between the sub-populations.

The genetic distances (NEI 1978) between the sub-populations of the Turkish black rat are also very low. The genetic distance is more marked in respect to geographic proximity than to colour morphs. As a result, allozyme differences in the six sub-populations of the Turkish black rat do not provide a certain taxonomic criterion for distinguishing populations at the subspecies level. In this connection, confirming the validity of the subspecies and determining the level of intra-specific variation requires further research focusing on the use of genetic markers such as nuclear or mitochondrial DNA, RFLP's, DNA sequencing or microsatellites.

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