Biochemical Systematics and Ecology 50 (2013) 304–309

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Allozyme variation in bank vole, *Myodes glareolus* (Mammalia: Rodentia) in Northern Anatolia

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ARTICLE INFO

Article history:
Received 30 January 2013
Accepted 27 April 2013
Available online 5 June 2013

Keywords:
Bank vole
Biochemical markers
Genetic variation
Altitude
Turkey

ABSTRACT

A total of 94 specimens from 16 populations of *Myodes glareolus*, collected between 2004 and 2007, from different altitudinal distributions were analyzed, using 16 enzyme systems. We found that 10 out of 22 loci (*Idh-2*, *α-Gpdh*, *Me*, *Pgm*, *Pgd*, *Mdh-s*, *Ada*, *Est-1*, *Ldh-1*, and *Ldh-2*) were polymorphic. Group 1 included population from altitudes ranging from 27 to 605 m above sea level (ASL), and Group 2 were from altitudes ranging from 1003 to 1288 m ASL. The summaries of the genetic parameters also displayed differences between the 2 groups. The possible reasons of such fragmentation between *M. glareolus* populations were discussed.

1. Introduction

The bank vole, *Myodes glareolus*, is a rodent species widely distributed in the Palearctic region. Bank voles are distributed from the British Isles to Lake Baikal in Siberia (Raczynski, 1983). It lives discontinuously in Northern Turkey and has only 1 subspecies namely, *Clethrionomys glareolus ponticus* (Çolak and Kıvanç, 1991; Krystufek and Vohralik, 2005). This species mainly favors the deciduous and homogenous beech forests of the Black sea and Marmara regions of Turkey (Neuhauser, 1936; Osborn, 1962; Felten et al., 1971; Steiner, 1972; Çolak and Kıvanç, 1991; Çolak et al., 1997). The distance from the northwestern region (Bursa) to the northeastern region (Rize) of the distribution area is about 1500 km and it consists of very different habitats. *M. glareolus* occupies in an area extending from 27 m above sea level (ASL) to an altitude of about 1300 m ASL.

M. glareolus has specific habitat requirements and favors forests, woodlots, and hedgerows in Turkey. Natural (deep valleys, rivers, and high mountains) and manmade (dams, industrialization, roadways, highways, railways, and new settlements) barriers have resulted the in fragmentation of *M. glareolus* into small populations or the habitat loss of *M. glareolus* populations. The impact of such changes was studied in various *M. glareolus* populations. Aars et al. (1998) found that the gene flow was much more restricted in linear river bank habitats than in a 2-dimensional one, based on the analysis of DNA sequences for the mitochondrial D-loop region in southeastern Norway. Gerlach and Musolf (2000) studied the barrier effects of various roadways on the genetic subdivision of bank vole populations and reported an important effect of highways on the gene flow and the genetic substructuring of the populations, based on the polymorphism of 7 microsatellite markers. In

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0305-1978/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2013.04.013

Denmark, Redeker et al. (2006) examined the impact of a road as a barrier between 2 forests to analyze the genetic differentiation among bank voles, using microsatellite markers. They exhibited that the habitat fragmentation could be the reason for significant genetic differentiation among the 5 distinct localities. Some works have been focused on the phylogeography of *M. glareolus*, inferred from sequences of the cytochrome *b* gene on mtDNA. Deffontaine et al. (2005) suggested the existence of multiple continental refugees for *M. glareolus*, giving 5 lineages (Spanish, Italian, Balkan, Western European, and Eastern European). Kotlik et al. (2006) pointed out the Carpathian refuge of *M. glareolus*. Deffontaine et al. (2009) determined at least 3 distinct lineages in the Pyrenean region. Moreover, in those studies, the genetic structure and genetic variation of *M. glareolus* were studied by utilizing different molecular markers. Spitzenberger et al. (1999) found genetic differences between eastern and western populations of *M. glareolus* in Austria, based on allozyme and mtDNA analyses. High genetic diversity among 13 *M. glareolus* populations was determined by 51 allozyme loci in Eastern Austria (Leitner and Hartl, 1988). Similarly, based on an allozyme study, utilizing 21 loci, Gebczynski et al. (1993) documented the presence of high genetic variation between southern and eastern *M. glareolus* populations in Poland. Redeker et al. (2006) determined the loss of heterozygosity in 1 out of 5 *M. glareolus* populations, similar to the bottleneck effect, based on the analysis of 9 microsatellite loci in Denmark.

Recently, Ledevin et al. (2010), using an outline analysis of occlusal surfaces of the 1st and 3rd upper molars, and the 1st lower molar, determined a decreasing trend in the size of 3 teeth towards high latitudes. According to Ledevin et al. (2010), this decreasing in size is interpreted as the result of a balance between metabolic efficiency and food availability, favoring a small body size in cold regions.

Although *M. glareolus* has been widely studied in different countries in Europe, there is no information on the genetic structure of *M. glareolus* populations in Turkey. The aim of this study was to assess the extent of the genetic variations in *M. glareolus* populations, based on a biochemical marker system.

2. Materials and methods

A total of 94 *Myodes glareolus* samples were collected from 16 localities having different altitudes, ranging from 27 to 1288 m ASL, between 2004 and 2007 (Fig. 1 and Table 1).

All of the animals were collected in the frame of a project supported by BAPRO (20030705077). After the animals were killed by an overdose of ether, their tissues were removed. *M. glareolus* is considered as a species of least concern by the IUCN Red List (2009).

Starch gel electrophoresis was conducted and 16 enzyme systems were screened using muscle extracts (stored at −86 °C until use), according to the method of Harris and Hopkinson (1976). The names of the enzyme systems were (the abbreviation and EC numbers are provided within parenthesis) esterase (Est, E.C. 3.1.1.1), aconitase (Aco, E.C. 4.2.1.3), glucose-6-phosphate dehydrogenase (G6pdh, E.C. 1.1.1.49), glucose-6-phosphate isomerase (Gpi, E.C. 5.3.1.9), α-glycerophophate dehydrogenase (α-Gpdh, E.C. 1.1.1.8), isocitrate dehydrogenase (Idh, E.C. 1.1.1.42), malate dehydrogenase (Mdh, E.C. 1.1.1.37), malic enzyme (Me, E.C. 1.1.1.40), phosphoglucomutase (Pgm, E.C. 5.4.2.2), superoxide dismutase (Sod, E.C. 1.15.1.1), fumarase (Fum, E.C. 4.2.1.2), phosphogluconate dehydrogenase (Pgd, E.C. 1.1.1.44), adenosine deaminase (ADA, E.C. 3.5.4.4), nucleoside phosphorylase (Np, E.C. hexokinase 2.4.2.1), (Hk, E.C. 2.7.1.1), and lactate dehydrogenase (Ldh, E.C. 1.1.1.27).

Band profiles were considered based on their flow speeds on the gel as A, B, and C, after staining. Population genetic parameters and genetic differentiation among the populations (F*st*) were estimated using ARLEQUIN (Excoffier et al., 2005). The UPGMA dendrogram was constructed using POPGENE (Yeh et al., 1999), based on Nei's (1972) genetic distance. In

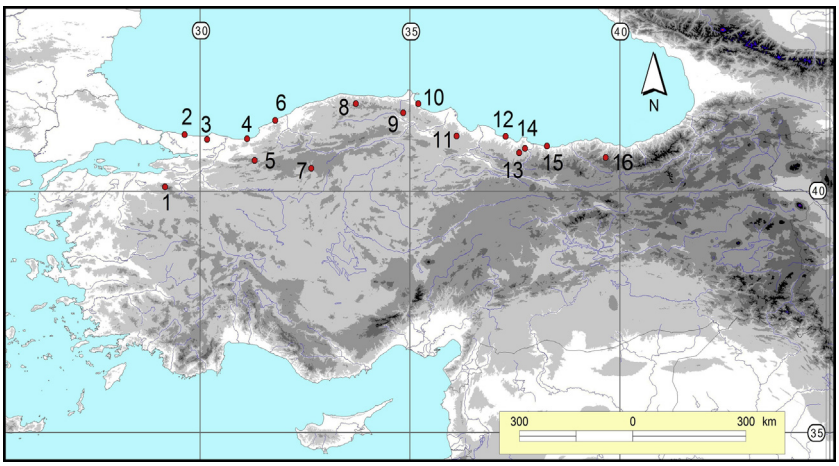

Fig. 1. *Myodes glareolus* sampling localities: 1, Uludağ-Bursa; 2, Sile-İstanbul; 3, Kandıra-İzmit; 4, Akçakoca-Düzce; 5, Abant-Bolu; 6, Zonguldak; 7, Kızılcahamam-Ankara; 8, Küre- Kastamonu; 9, Bürnük-Sinop; 10, Göktepe-Sinop; 11, Çakallı-Samsun; 12, Ünye-Ordu; 13, Gürgentepe-Ordu; 14, Ulubey-Ordu; 15, Giresun; and 16, Sümela-Trabzon.

Table 1
Mean values of the genetic variability of 22 loci in 16 populations of *Myodes glareolus*.

Populations	Sample size	Altitude	*A*	*P*	*H*	
					H_e	H_o
1. Şile	11	27	1.18	18.18	0.0480	0.0413
2. Kandıra	6	67	1.18	18.18	0.0675	0.0455
3. Akçakoca	7	40	1.27	27.27	0.0884	0.0584
4. Uludağ	2	1127	1.09	9.09	0.0530	0.0682
5. Zonguldak	7	300	1.14	13.64	0.0330	0.0130
6. Abant	10	1020	1.36	31.82	0.1060	0.0864
7.Kızılcahamam	2	1200	1.09	9.09	0.0530	0.0682
8. Küre	7	1022	1.14	13.64	0.0544	0.0065
9. Bürnük	6	1288	1.27	27.27	0.0813	0.0455
10. Göktepe	11	1003	1.27	22.73	0.0775	0.0496
11. Çakallı	2	485	1.14	13.64	0.0833	0.0682
12. Ünye	4	73	1.14	13.64	0.0828	0.0795
13. Gürgentepe	3	605	1.23	18.18	0.0636	0.0455
14. Ulubey	6	500	1.23	22.73	0.0773	0.0576
15. Giresun	6	518	1.36	31.82	0.1067	0.0682
16. Sümela	4	1130	1.18	18.18	0.0763	0.0568

addition, allele frequencies were calculated using POPGENE (Yeh et al., 1999). Factorial correspondence analysis (FCA) was performed using GENETIX (Belkhir et al., 2004).

2.1. Geographic and floral features of the localities

The altitude of the localities, ranging from 27 to 605 m ASL, formed Group 1 (Şile, Kandıra, Akçakoca, Gürgentepe, Çakallı, Ulubey, Ünye, and Giresun) (Fig. 1). The localities in this group have a milder climate and were covered with young homogeneous beech forests, closer to Black Sea coast. The beech forests are in the form of patches surrounded by pine forests and the hazelnut plantations due to anthropogenic activities. Group 2 comprised altitude localities ranging from 1003 to 1288 m ASL (Uludağ, Abant, Sümela, Bürnük, Göktepe, Küre, and Kızılcahamam). However, Zonguldak specimens collected from 300 m ASL were classified with Group 2 (Fig. 1). The localities in this group have a harsh climate and were mostly covered with snow for 5–6 months. These areas are covered with mixed forests, including mainly pine and old beech trees, and are heavily influenced by human activities.

3. Results

The genetic variation present in the *Myodes glareolus* populations in Turkey was assessed for the first time using biochemical markers. Of the 22 loci examined, 10 were polymorphic: *Idh-2*, *α-Gpdh*, *Me*, *Pgm*, *Pgd*, *Mdh-s*, *Ada*, *Est-1*, *Ldh-1*, and *Ldh-2*. The allele frequencies of the polymorphic enzyme loci for the 2 main groups were given in Table 2. The remaining 12 loci (*Aco-1*, *Aco-2*, *G6pdh*, *Gpi*, *Sod*, *Fum*, *Np*, *Mdh-m*, *Idh-1*, *Est-2*, *Est-3*, and *Hk*) were monomorphic, and were fixed for the same alleles in the 16 populations of *M. glareolus* in Turkey.

The statistical estimates of the population genetic parameters, sample size and altitude of the localities, average number of alleles per locus (*A*), proportion of polymorphic loci (*P*), mean heterozygosity (*H*) represented as the observed heterozygosity (H_o), and expected heterozygosity (H_e) are visualized in Table 1. The lowest numbers of the alleles were documented in Kızılcahamam and Uludağ (1.09) and the highest were in Abant and Giresun (1.36), respectively. The proportion of polymorphic loci (*P*) ranged between 9.09% (in Uludağ and Kızılcahamam) and 31.82% (in Giresun and Abant). The lowest H_o was found in Küre (0.0065) and the highest H_o was estimated in Abant (0.0864) (Table 1).

The *M. glareolus* populations were divided into 2 main groups (Group 1 and Group 2) in the UPGMA dendrogram constructed using the data obtained from the enzyme allele frequencies (Fig. 2). Group 1 (Şile, Kandıra, Akçakoca, Çakallı, Gürgentepe, Ulubey, Ünye, and Giresun) consisted of populations coming from low elevation localities (altitudes were between 27 and 605 m ASL) in the Black Sea and Marmara regions. On the other hand, Group 2 included populations from higher elevations (altitudes were over 1000 m ASL). Substructuring was observed within each of the 2 main groups. The 1st group had 3 subgroups: Şile-Kandıra, Gürgentepe-Ulubey-Ünye-Giresun-Akçakoca, and Çakallı; and the 2nd group contained populations from Uludağ, Abant, Sümela, Bürnük, Göktepe, Küre, Kızılcahamam, and Zonguldak. These localities were also clustered into 3 subgroups as follows: Uludağ-Abant-Sümela-Bürnük-Göktepe, Küre-Kızılcahamam, and Zonguldak.

These results were supported by FCA, which is a multivariate method of analysis of allele frequency data (Fig. 3).

Estimates of Wright's F-statistics are given in Table 3. Heterozygosity was low within Group 1 ($F_{IS} = 0.1406$) due to inbreeding, whereas virtually no inbreeding was apparent in Group 2 ($F_{IS} = 0.2287$). The value of the fixation index was nearly the same in Groups 1 and 2 ($F_{ST} = 0.2432$ and $F_{ST} = 0.2575$, respectively). These values indicate that about 24%–25% of the genetic variation is due to differentiation among the populations in the groups.

Table 2
Allelic frequencies of the 2 main groups of *Myodes glareolus* in Turkey.

Loci	Alleles					
	A		B		C	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Idh-1	1.000	1.000	–	–	–	–
Idh-2	–	0.0222	0.9778	1.000	–	–
α-Gpdh	0.0444	0.0204	0.9444	0.9796	0.0111	
Me	–	0.0204	1.000	0.9796	–	–
Pgm	0.0222	0.0204	0.9556	0.9592	0.0222	0.0204
SOD-1	1.000	–	1.000	–	1.000	–
SOD-2	1.000	–	1.000	–	1.000	–
Aco-1	1.000	–	1.000	–	1.000	–
Aco-2	1.000	–	1.000	–	1.000	–
Pgd	0.0111	0.1122	0.9889	0.8878	–	–
NP	1.000	1.000	–	–	–	–
Fum	1.000	1.000	–	–	–	–
Mdh-S	0.0222	0.0306	0.9778	0.9592	–	0.0102
Mdh-M	1.000	1.000	–	–	–	–
ADA	0.4444	0.4898	0.5556	0.5102	–	–
G6pdh	1.000	1.000	–	–	–	–
Hk	1.000	1.000	–	–	–	–
Gpi	1.000	1.000	–	–	–	–
Est-1	0.5778	0.7041	0.4000	0.2755	0.0222	0.0204
Est-3	1.000	1.000	–	–	–	–
Ldh-1	0.6477	0.8878	0.3523	0.1122	–	–
Ldh-2	0.0909	0.8367	0.9091	0.1633	–	–

4. Discussion

In *M. glareolus*, the main source of population genetic variation is related to environmental patchiness, habitat loss, and its disjunctive distribution (Gebczynski et al., 1986; Leither and Hartl, 1988; Gebczynski et al., 1993; Aars et al., 1998; Gerlach and Musolf, 2000; Redeker et al., 2006). Gebczynski et al. (1993) indicated that allele distribution patterns in polymorphic loci varied among 5 geographically distinct Polish populations of *M. glareolus* and found that *Ldh-1*, *Mdh-1*, and *Sod* were polymorphic in all of the populations. We demonstrated that the Turkish *M. glareolus* populations were divided into 2 main groups on the basis of altitudinal distribution based on biochemical co-dominant markers. We determined genetic variation among *M. glareolus* populations related to its altitudinal distribution and found that the *Idh-2*, *α-Gpdh*, *Me*, and *Mdh-s* loci were effective for separating Groups 1 and 2. Both Group 1 and Group 2 have different climate aspects. The genetic differentiation between the groups based on allozyme variation may have resulted from an adaptation to cold conditions (Ledevin et al., 2010). In this study, we found that the lowest *D* value was 0.0027, between Şile and Kandıra, and the highest *D* value was 0.1551, between Kızılcahamam and Çakallı. The *D* value between Şile in the most western part of the distributional area and

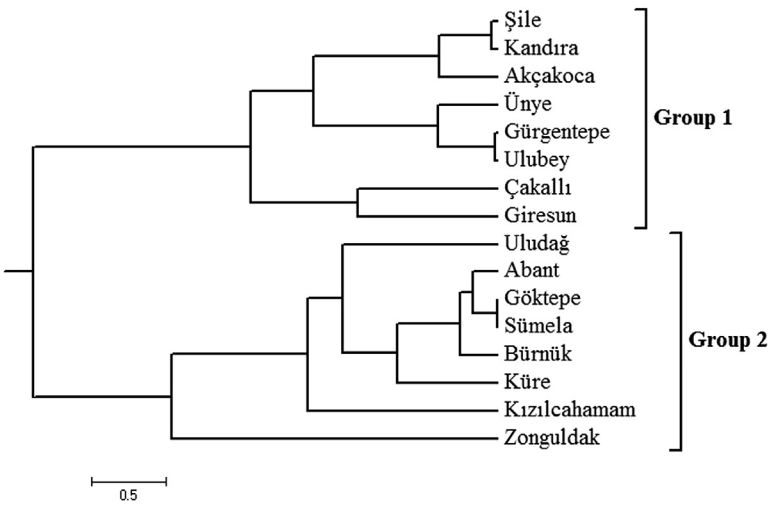

Fig. 2. The UPGMA dendrogram constructed based on Nei 1972, among 16 populations of *M. glareolus* (Group 1: low altitude, Group 2: high altitude).

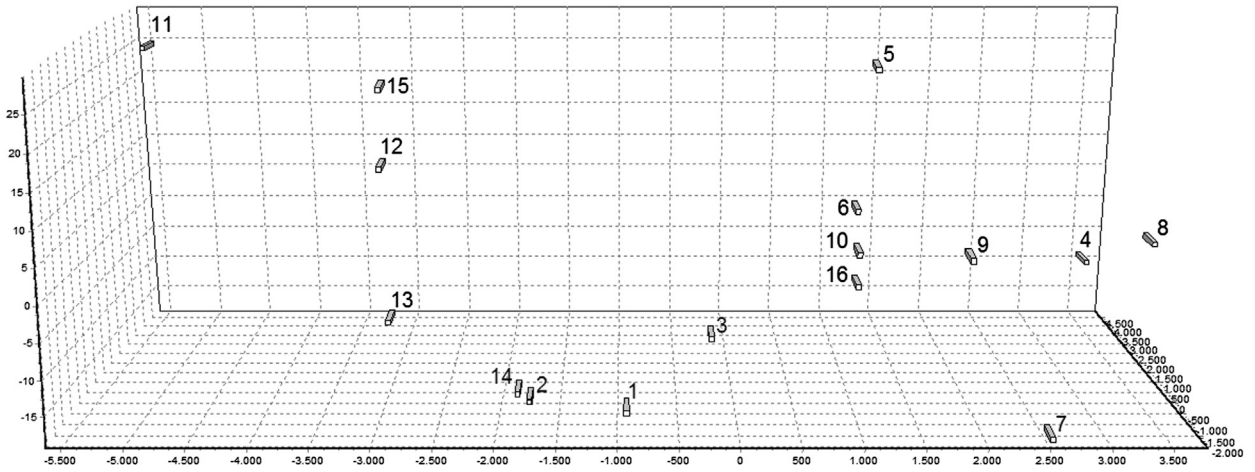

Fig. 3. FCA of *M. glareolus* populations (Group 1: 1, Sile; 2, Kandıra; 3, Akçakoca; 11, Çakallı; 12, Ünye; 13, Gürgentepe; 14, Ulubey; and 15, Giresun. Group 2: 4, Uludağ; 5, Zonguldak; 6, Abant; 7, Kızılcahamam; 8, Küre; 9, Bürnük; 10, Göktepe; and 16, Sümela).

Table 3
Mean values of coefficients of Wright's F statistics for Group 1 and 2.

	F(IS)	*F*(IT)	*F*(ST)	*Nm*
Group 1	0.1406	0.3496	0.2432	0.7779
Group 2	0.2287	0.4273	0.2575	0.7208

Sümela, in the most eastern part, was 0.0305. However, the *D* value was 0.0783 between Şile and Zonguldak, which is very close to that of the 2 localities in this study. Thus, this shows that there is no relation between the genetic distance values and the geographic distance among populations, as previously reported by Gebczynski et al. (1993).

During field collections, we observed that anthropogenic impacts on the natural habitat of *M. glareolus* such as roads, highways, and forest damage (especially beech trees) lead to habitat fragmentation. Moreover, aside from newly formed hurdles; there were many natural barriers, such as rivers and mountain chains that can interfere with the gene flow among *M. glareolus* populations. In many areas on the Ankara-İstanbul highway, which was built about 20 years ago, the distribution of *M. glareolus* is fragmentized. We also determined great geographical gaps between *M. glareolus* populations during the sampling period of 3 years. Habitat fragmentations and habitat losses, as well as natural barriers, affect the genetic structure of *M. glareolus* populations in Turkey. However, further studies are necessary in order to investigate and document the effect of habitat fragmentation and habitat loss on *M. glareolus* populations using other genetic markers.

Acknowledgments

This study was sponsored by BAPRO (NO: 2003-07-05-077) of Ankara University.

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