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

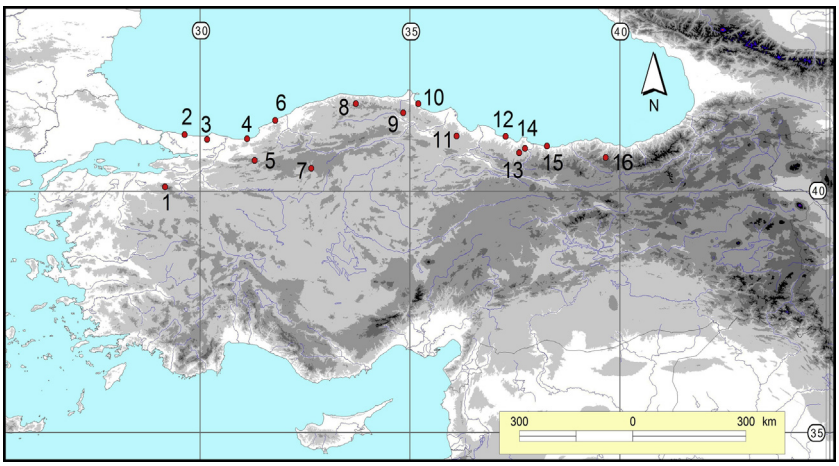

Fig. 1. *Myodes glareolus* sampling localities: 1, Uludağ-Bursa; 2, Sile-İstanbul; 3, Kandıra-İzmit; 4, Akçakoca-Düzce; 5, Abant-Bolu; 6, Zonguldak; 7, Kızılcahamam-Ankara; 8, Küre- Kastamonu; 9, Bürnük-Sinop; 10, Göktepe-Sinop; 11, Çakallı-Samsun; 12, Ünye-Ordu; 13, Gürgentepe-Ordu; 14, Ulubey-Ordu;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