

Population Epigenetic Diversity versus Subspecies Detachment of the Forest Dormouse, *Dryomys nitedula* (Pallas, 1778) (Rodentia: Gliridae), in a Long Distance Transect in Eurasia: Implication for its Conservation

Georgi G. Markov¹, Ercument Colak², Nuri Yigit², Maria A. Kocheva¹, Milena K. Gospodinova¹ & Hristo Dimitrov³

¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd, 1000 Sofia, Bulgaria; E-mail: georgimar@gmail.com

²Biology Department, Faculty of Science, Ankara University, 06100 Besevler, Ankara, Turkey

³Department of Zoology, Faculty of Biology, Plovdiv University, Plovdiv, Bulgaria

Abstract: Geographical variation and intraspecific taxonomy of forest dormouse (*Dryomys nitedula*) has not yet been investigated adequately, and its population epigenetic uniqueness and the status of many subspecies require clarification. To advance knowledge on the interspecies variety of forest dormouse, we studied the epigenetic cranial polymorphism in eight forest dormouse populations located along a transect line from Central Anatolia region to Central Southeast Europe and inhabiting an area of suspected different subspecies taxa. Our results indicate that the mapping of the population epigenetic diversity of forest dormouse within the area of the classically described subspecies manifested some overlaps of their epigenetic distances with the geographic remoteness between them. The revealed pattern of population epigenetic diversity in the investigated territories of the classically described subspecies of the forest dormouse expands the knowledge of the species diversity in its range. These highlights of the intraspecific taxonomy of *D. nitedula* need to be examined in a future large-scale study of its population differentiation and uniqueness of several subspecies.

Key words: Forest dormouse, *Dryomys nitedula*, non-metric characters, epigenetic distance, population uniqueness, subspecies detachment

Introduction

The forest dormouse, *Dryomys nitedula* (Pallas, 1778) occurs from Western Europe across Asia Minor and Caucasus and reaches to Central Russia and Central Asia. In this extensive area, the species is rare in many parts of its native range, especially in Western Europe and Russia (BATSAIKHAN *et al.* 2008). However, due to its ecological adaptability (AIRAPETYANTZ 1983), the species is locally common in some south-eastern areas and its population seems to have stable abundance in South-eastern part of Balkan Peninsula (KRYŠTUFEK 1999). Geographical variation and intraspecific taxonomy of the forest dormouse has not yet been investigated adequately,

and the status of many subspecies requires clarification. This taxonomic uncertainty in the majority of the populations is determined by the typically high degree of colour variations of the upper side of the body and the tail, by the presence of spots on the forehead, throat, chest, behind the ears, and by the colouration of the lower side of the body (OGNEV 1947), external marks that are too often used to describe subspecies within *D. nitedula* in its extensive Palaearctic range.

Epigenetic modifications are considered important for understanding the uniqueness of the populations and are crucial for their phenotypic diversification.

tion. The morphological investigations of non-metric skeletal characters offer an opportunity to use mammal skulls kept in museum collections for epigenetic studies (ANSORGE 2001, ANSORGE *et al.* 2009). The assessment of non-metric skeleton characters as a morphological tool has been applied for the analysis of population differentiation in a large number of mammal species, including humans, rodents, mustelids, pinnipeds and small cetaceans (for details, see BAUCHAU 1988, TRIMBLE & PRADERI 2008).

A study revealing the polymorphism expressed through nonmetric characters (i.e., coded by discrete states and also known as qualitative traits) may contribute to advance the knowledge on the interspecies variety of forest dormouse based on classically described by colorimetric exterior signs subspecies (OGNEV 1947) and to establish the population epigenetic structure of the species. Therefore, these characters are suitable to assess genetic variability and divergence among populations because they are exposed to minimum selection pressure (SJVOLD 1977).

In the study of forest dormouse populations from geographic regions covering a significant part of the species geographical range, we have adopted the classical definition of the taxonomic category "subspecies" as a particular type within a species, the members of which are different in some clear ways from those of other types of the species. The establishment of epigenetic characterisation of different geographical forest dormouse populations would assist in decision-making for the presence of distinguishable subspecies taxa in the research area.

Using the craniological non-metric characters, we expect to: (i) explore the geographic populations of the forest dormouse in a long distance transect in Eurasia in view of the evaluation of their epigenetic characteristics versus the previously described subspecies taxa and (ii) to establish their epigenetic uniqueness for clarifying the guidelines for its population conservation in the investigated area.

Materials and Methods

The epigenetic cranial polymorphism was studied in eight forest dormouse populations located at transect from Central Anatolia region to Central Southeast Europe. Seven populations inhabit typical for species forest habitat in hilly and mountainous areas and one population inhabits artificial forest shelterbelts in plain agroecosystems. One is located in Central Anatolia region in Turkey (POP 1, N=43), six population are located in South-eastern part of Balkan Peninsula: POP 2 unites the individuals in-

habiting South-eastern Trace in European Turkey (N=25); POP 3 inhabits wooded hills in South-eastern Bulgaria (N=21); POP 4 inhabits wooded hills in North-eastern Bulgaria (N=11); POP 5 inhabits artificial forest shelterbelts in plain agroecosystems (N=78); POP 6 inhabits mountain forest areas in Stara Planina (Central Balkan) (N=23); POP 7 inhabits mountain forest areas in Vitosha Mountain (N=20), and one population (POP 8) is located in Central Southeastern Europe and consists of individuals inhabiting wooded hills near to the town Godolo, Hungary (N=23) (Fig. 1).

The specimens of POP 1 and POP 2 were stored in the collection of the Department of Biology, Faculty of Science, University of Ankara, Turkey; the specimens of POP 3–7 were kept in the work collection in the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, and the skulls used for epigenetic characterization of forest dormouse of POP 8 were from the collection of the National Museum of Natural History in Budapest.

Along the studied transect, the variability of non-metric traits was studied on the skulls of 244 specimens of the forest dormouse. The occurrence of 10 cranial traits, representing orifices of nerves and blood vessels, was scored on the skull left side as shown in Table 1. These traits were selected from those proposed by BERRY (1963, 1968), BERRY & SEARLE (1963) and HEDGES (1969) for description of the cranial epigenetic polymorphism in rodents.

Results

In spite of the absence of sexual and age dimorphism in the studied non-metric cranial characters in rodents, the independent appearance of the features studied in males and females as well as in juvenile and adult individuals from the same locality was examined by applying the χ^2 -test at a significance level of $p = 0.05$ to determine whether there are significant differences among sexes and age groups. The proved absence of sexual and age dependence in the studied characters permitted to pool the specimens of both sexes and age groups into one sample. These pooled population samples were subject of the study of the intraspecific epigenetic polymorphism of the forest dormouse in Central and South-eastern Europe and Anatolian Peninsula.

The epigenetic variability (V_i) (after SMITH 1981) and the mean measure of divergence (MMD) (after SJVOLD 1973) were computed based on the observed frequency distribution of the studied 10 epigenetic characters. The Measure of uniqueness (MU) was calculated after BERRY (1963) for each

sample as the sum of its epigenetic distances from the other samples. The interpopulation epigenetic detachment was graphically presented using the cluster (UPGMA) analysis of the statistical package STATISTICA FOR WINDOWS (1993).

Epigenetic variability of the pooled sample of all the forest dormouse populations was $V_i = 0.0962$. The established epigenetic variability of the studied populations varied from $V_i = 0.051$ realized in the POP 1 to $V_i = 0.199$ implemented in POP 3 (Fig. 2a).

Populations POP 1, POP 2, POP 3, POP 7 and POP 8 have lower epigenetic variability than the average value established of the pooled sample of all forest dormouse populations in the region, while in populations POP 4, POP 5 and POP 6 it was higher.



Fig. 1. Geographical location of the studied populations of forest dormouse (*D. nitedula*) inhabiting Central Anatolia region in Turkey (1 – POP 1), Southeastern part of Balkan Peninsula: (2 – POP 2; 3 – POP 3; 4 – POP 4; 5 – POP 5; 6 – POP 6; 7 – POP 7) and Central South-eastern Europe (8 – POP 8)

Statistical analysis of the calculated inter-population epigenetic distances between all the compared populations established some populations with statistically insignificant epigenetic distances to the others. At the same time, the values of the epigenetic distances among the remaining populations were of various magnitudes and were statistically significant (Table 2).

Comparative evaluation of the population epigenetic uniqueness (MU), based on the found population epigenetic polymorphism of forest dormouse from investigated area revealed the well-expressed cranial epigenetic uniqueness of the studied populations (Fig 2b).

The average epigenetic uniqueness of pooled sample of all the investigated forest dormouse populations was $MU = 3.451$. The values of the epigenetic uniqueness of individual populations were within the range from $MU = 4.694$ realized in POP 1 to $MU = 2.105$ realized in POP 6. Populations POP 1, POP 2, POP 5 and POP 7 have epigenetic uniqueness higher than the average for the species in the region, while populations POP 3, POP 4 and POP 8 have lower than average value established for the species in the research area.

The average epigenetic variation of the European and Asiatic populations was $V_i = 0.1024$ and $V_i = 0.053$, respectively, differing by 52%. The Asiatic population demonstrates also a high value of the population uniqueness, $MU = 4.695$. The highest difference of epigenetic variability of forest dormice among the European populations – 52% was found between POP 5, the population with the highest epigenetic uniqueness among the European populations, and POP 2 – two populations, which are not very distant geographically.

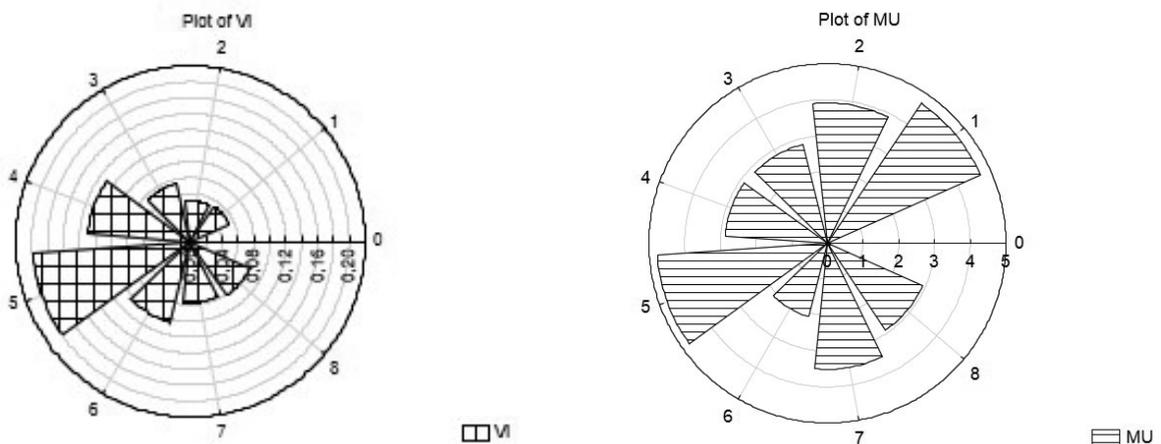


Fig. 2. Cranial epigenetic variability (2a) and uniqueness (2b) of forest dormouse (*D. nitedula*) populations inhabiting Central Anatolia region in Turkey (1 – POP 1), South-eastern part of Balkan Peninsula: (2 – POP 2; 3 – POP 3; 4 – POP 4; 5 - POP 5; 6 – POP6; 7 – POP7) and Central South-east Europe (8 – POP 8)

Table 1. The observed skull traits, their evaluated status and frequency distribution in the forest dormouse (*Dryomys nitedula*) within populations inhabiting in Central part of Anatolian Peninsula (POP 1), South-eastern part of Balkan Peninsula (POP 2-7) and Central South-eastern Europe (POP 8)

№	Skull traits		Frequency distribution							
	Trait	Evaluated status	POP 1	POP 2	POP 3	POP 4	POP 5	POP 6	POP 7	POP 8
1	Fenestra flocculi	present	0.0233	0.0833	0.0833	0.6250	0.1935	0.4783	0.5715	0.7273
2	Preorbital foramen	double	0.0000	0.0435	0.0435	0.5000	0.2254	0.1304	0.2857	0.1818
3	Anterior frontal foramen	present	0.0698	0.0417	0.0417	0.1250	0.2821	0.0435	0.1429	0.0909
4	Posterior frontal foramen	present	0.3023	0.6800	0.6800	0.1250	0.2692	0.0870	0.0714	0.0455
5	Maxillary foramen I	present	0.0732	0.0800	0.0800	0.2500	0.1538	0.2174	0.0714	0.0455
6	Maxillary foramen II	present	0.0238	0.0000	0.0000	0.1250	0.4102	0.0870	0	0.0455
7	For. sphenoidale medium	present	0.0714	0.0417	0.0417	0	0.3438	0	0	0.0000
8	Foramen hypoglossi	double	0.0270	0.0000	0.0000	0.7500	0.5645	0.6522	0.9286	0.0909
9	Foramen basioccipitale	present	0.0000	0.0000	0.0000	0.1250	0	0.0870	0	0.0000
10	Foramen mentale	double	0.0244	0.0435	0.0435	0	0.4524	0	0	0.2273

The established degree of dissimilarity between epigenetically studied geographic populations of Forest dormouse, determined through comparison of epigenetic distances between them, show the picture of their epigenetic differentiation based on the Mean measure of divergence (MMD) (Fig.3).

The computed unweighted pair-group average dendrogram (UPGMA) of the MMD distances specifies that the population POP 5 from the central part of the species' area in Bulgaria differ distinctly from the other Bulgarian populations. The population POP 8 from Central South-eastern Europe form one cluster with the remaining populations of the Balkan Peninsula, situated on the territory of Bulgaria. A pronounced correlation was found between the geographic remoteness and the epigenetic distances of the neighbouring Bulgarian populations POP 3, POP 4, POP 6 and POP 7, which did not show significant differences in MMD (Fig. 3).

Discussion

The results of this study demonstrate that the characterization of the skull of the forest dormouse by nonmetric cranial characters gives an opportunity to assess genetic variability and divergence among its populations. They did agree with the conclusion of SJØVOLD (1977) and ANZORGE (2001) that qualitative traits coded by discrete states are suitable to assess genetic variability and divergence among mammalian populations.

The results obtained from the comparative

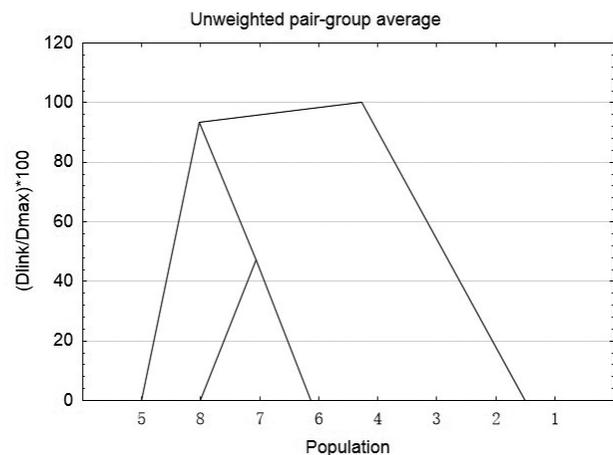


Fig. 3. Relative cranial epigenetic degree of dissimilarity between populations of the forest dormouse (*D. nitedula*) inhabiting Central Anatolia region in Turkey (1- POP_1), South-eastern part of Balkan Peninsula: (2 – POP 2; 3 – POP_3; 4 – POP 4; 5 – POP 5; 6 – POP 6; 7 – POP 7) and Central Southeast Europe (8 – POP 8) based on their mean measure of divergence (MMD). The topographical population's locations in the study area are shown in Fig. 1

Table 2. Mean measures of divergence (MMD) between the forest dormouse populations inhabiting Central Anatolia region in Turkey (POP 1), South-eastern part of Balkan Peninsula: (POP 2-7) and Central South-eastern Europe (POP 8). The asterisks indicate statistical significance and *n. s. s.* Indicates that the value is not statistically significant at the level of $p = 0.05$

POP No	POP_2	POP_3	POP_4	POP_5	POP_6	POP_7	POP_8
POP_1	<i>n. s. s.</i>	0.708*	0.911*	0.870*	0.669*	1.005*	0.488*
POP_2		0.489*	0.829*	0.719*	0.511*	0.860*	0.452*
POP_3			<i>n. s. s.</i>	0.757*	<i>n. s. s.</i>	<i>n. s. s.</i>	0.254*
POP_4				0.488*	<i>n. s. s.</i>	<i>n. s. s.</i>	0.364*
POP_5					0.516*	0.791*	0.637*
POP_6						<i>n. s. s.</i>	0.276*
POP_7							0.441*

analysis of epigenetic variation and uniqueness of forest dormouse did not indicate a strong geographical trend in their expression in the research area. They rather reflect the mosaic manifestation of the uniqueness of the studied populations located in research geographic transect from the Central part of Anatolian Peninsula, across South-eastern part of Balkan Peninsula to Central South-eastern Europe.

Too often, the features used to describe a new subspecies in forest dormouse were not bound with the characteristics reflecting the actual biological diversity of species that has led to description of too many not well-defined subspecies taxa. The slight colour variations over the distribution range of *D. nitedula* in Anatolian Peninsula and adjacent territories led to assignation of a number of subspecies, e.g.: from Eastern Turkey – *D. n. pictus* (BLANFORD 1875), from Greece – *D. n. wingei* (NEHRING 1902), from Western Turkey – *D. n. phrygius* (THOMAS 1907), from Bulgaria – *D. n. robustus* (MILLER 1910) and from Armenia – *D. n. tichormirovi* (SATUNIN 1920) (for details see YIGIT *et al.* 2003, KRYŠTUFEK & VOHRALIK 1994, KRYŠTUFEK & VOHRALIK 2005).

The mapping of the population epigenetic diversity of the forest dormouse within the area of the classically described subspecies manifested some overlapping of their epigenetic distances with geographic remoteness between them.

This degree of overlapping is strongly expressed for the Bulgarian populations, which except for population POP_5, showed a pronounced epigenetic similarity between them. Strongly expressed epigenetic differentiation of the population POP_5 is probably bound to its origin and its subsequent development in an artificially created habitat. Population POP_5 inhabits a man-made shelter forest belt and loses its connection with the natural habitats of the species in the region due to degradation of forest plantations that connect the forest belt with surrounding forests. Population POP_5 remains isolated in an atypical

habitat representing an intensely cultivated agricultural ecosystem.

Unlike well expressed degree of similarity between epigenetic and geographic distances in Bulgarian populations, the populations from Central Anatolia and European part of Turkey, which according to the classical notions of interspecies taxonomic differentiation of forest dormouse belong to different subspecies, did not manifest any reliable statistical difference in their craniological epigenetic determination.

The population of Central South eastern Europe POP_8, inhabiting forested hills, located in agricultural region, showed a specific epigenetic determination but by its epigenetic characteristics it remains more strongly associated with populations of South-eastern part of Balkan Peninsula than the population of Anatolian Peninsula.

Established picture of the population epigenetic diversity in the territories of the classically described subspecies taxa of forest dormouse expands the knowledge on the realization of the species diversity in its geographical range. It highlights the need to conduct future large-scale study of its population differentiation and uniqueness of several subspecies. Conducting future taxonomic revisions of the described subspecies diversity would probably lead to changes in the intra-species structure of the forest dormouse that could lead to a reduction in actual subspecies taxa (GROMOV & ERBAEVA 1995).

In addition to strictly taxonomic and biogeographic consequences, determining the population uniqueness of the forest dormouse contributes to conservation of its biological diversity. In the same time, the epigenetic modifications are considered important to advance the understanding of the population uniqueness and are crucial for the phenotypic diversification. An important step in managing the conservation of the species biodiversity is to define its unique populations, because each one of them re-

quires evaluation and treatment as a separate unit. Our study is the first to explore population biodiversity of the forest dormouse over large distances. The future studies on population genetic diversity of the forest dormouse in Palearctic should cover even more extended area and should be complemented with an assessment of genetic diversity using DNA molecular markers and an examination of the filogeographic relations between subspecies taxa. This would make the picture of the actual genetic and

taxonomic diversity of the species clearer.

A better understanding of biodiversity of forest dormouse in Palearctic scale would provide an important tool for the development of management plans aimed at preserving its biodiversity and population genetic differentiation.

Acknowledgments: This study was supported by the bilateral project between Bulgarian academy of sciences, Bulgaria and TUBiTAK, Turkey.

References

- AIRAPETYANTZ A. 1983. Dormice. Leningrad, Leningrad University Publishing House, 188 pp. (In Russian)
- ANSORGE H. 2001. Assessing non-metric skeleton characters as a morphological tool. *Zoology* **104**: 268–277.
- ANSORGE H., RANYUK M., KAUALA K., KOWALCZYK R. & STIER N. 2009. Raccoon dog *Nyctereutes procyonoides* populations in the area of origin and in colonised regions – epigenetic variability of an immigrant. *Annales Zoologici Fennici* **46**: 51–62.
- BATSAIKHAN N., KRYŠTUFEK B., AMORI G. & YIGIT N. 2008. *Dryomys nitedula*. The IUCN Red List of Threatened Species 2008: e.T6858A12810747. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T6858A12810747.en>.
- BAUCHAU V. 1988. Non-metrical variation in wild mammals: a bibliography. *Mammal Revue*, **18** (4): 195–200.
- BERRY R. J. 1963. Epigenetic polymorphism in wild populations of *Mus musculus*. *Genetics Research* **4**: 193–220.
- BERRY R. J. 1968. The biology of non-metrical variation in mice and men. In: BROTHWELL D. R. (ed.): The skeletal biology of earlier human populations. London, Pergamon Press, 103–113.
- BERRY R. J. & SEARLE A. G. 1963. Epigenetic polymorphism in rodents. *Proceedings of Zoological Society, London*, **140**: 577–615.
- GROMOV I. M. & ERBAEVA M. A. 1995. Mammals of Russia and adjacent countries. Lagomorphs and Rodents. Sankt Petersburg, Nauka, 520 pp. (In Russian)
- HEDGES S. R. 1969. Epigenetic polymorphism in populations of *Apodemus sylvaticus* and *Apodemus flavicollis* (Rodentia, Muridae). *Journal of Zoology*, **159**: 425–442.
- KRYŠTUFEK B. 1999. *Dryomys nitedula*. In: MITCHELL-JONES A. J., AMORI G., BOGDANOWICZ W., KRYŠTUFEK B., REIJNDERS P. J. H., SPITZENBERGER F., STUBBE M., THISSEN J. B. M., VOHRALÍK V. & ZIMA J. (eds): The Atlas of European Mammals, pp. 300–301. Academic Press, London, UK.
- KRYŠTUFEK B. & VOHRALÍK V. 1994. Distribution of the forest dormouse *Dryomys nitedula* (PALLAS, 1779) (Rodentia, Myoxidae) in Europe. *Mammal Review* **24**: 161–177.
- KRYŠTUFEK B. & VOHRALÍK V. 2005. Mammals of Turkey and Cyprus. Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. – Zgodovinsko društvo za južno Primorsko, Koper, Slovenia, 292 pp. ISBN 961-6033-60-3.
- OGNEV S. I. 1947. Beasts of the USSR and adjacent countries, Volume 5, Moscow-Leningrad, Publishing House of Academy of Sciences of USSR, 809 pp. (In Russian)
- SJØVOLD T. 1973. The occurrence of minor, non-metrical variation in the skeleton and their quantitative treatment for population comparison. *Homo* **24**: 204–233.
- SJØVOLD T. 1977. Non-metrical divergence between skeletal populations. *Ossa* **4**, Suppl: 1–133.
- SMITH M. F. 1981. Relationships between genetic variability and niche dimensions among coexisting species of *Peromyscus*. *Journal of Mammalogy*, **62**(2): 273–285.
- STATSOFT 1993. Statistic for Windows. Release 4.3. Tulsa.
- TRIMBLE M. & PRADERI R. 2008. Assessment of nonmetric skull characters of the franciscana (*Pontoporia blainvillei*) in determining population differences. *Aquatic Mammals*, **34** (3): 338–348.
- YIGIT N., ÇOLAK E., ÇOLAK R., ÖZKAN B. & ÖZKURT Ş. 2003. On the Turkish population of *Dryomys nitedula* (PALLAS, 1779) and *Dryomys laniger* (FELTEN and STORCH, 1968) (Mammalia: Rodentia). – *Acta Zoologica Academiae Scientiarum Hungaricae*, **49** (Suppl.1): 147–158.