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Phylogeography of the forest dormouse *Dryomys nitedula* (Gliridae, Rodentia) in Russian Plain and the Caucasus

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Abstract. The genetic variation of the forest dormouse *Dryomys nitedula* (Pallas, 1779) from isolated populations of Russian Plain and the Caucasus was investigated using cytochrome *b* gene (cyt*b*). The genetic distance calculated between these populations of forest dormouse was 9.94 %, which corresponds to the typical distance between biological species of mammals. The genetic distance of cyt*b* between Western and Central Caucasus forest dormouse populations is also significant, 6.0 %. Probably, there was a long-term isolation of European and Caucasian areas of *D. nitedula* during the whole Pleistocene.

Key words: mitochondrial phylogeography, mitochondrial DNA, cytochrome *b*, haplogroups, taxonomy

Introduction

Modern ranges of forest species common for the Russian Plain and the North Caucasus have a break in the steppe region between the Lower Don and Kuban rivers. In this region, distribution areas of arboreal species that are closely associated with deciduous forests are disruptive. This gap existed since the late Pleistocene. Prolonged range separation of forest dormouse of Russian Plain and Caucasus had little impact on morphological differences between isolated populations (Ognev 1947, Rossolimo 1971). Genetic differences between forest dormouse populations in Russian Plain and the Caucasus has not yet been investigated. In this report, we present the results of a phylogeographic study of *D. nitedula* in Russian Plain and the Caucasus to clarify the Pleistocene-Holocene history of colonization and the taxonomic status of isolated populations.

Material and Methods

We obtained 51 samples of *D. nitedula* from 12 localities (Table 1, Fig. 1). For comparison, we included the sequence AJ225116. This sequence is derived from a sample of 0-22 from ZMMU collection (Montgelard et al. 2003), which was erroneously considered as originating from Georgia. The actual collecting site is Stavropol region, Arhyz, 43°33′57″ N, 41°16′44″ E, which is confirmed by the records in a field diary of Baskevich M. I. We used *Eliomys quercinus* (AJ225030) as an outgroup.

For the DNA analysis, we used ear and wool samples fixed in 96 % ethanol. Total DNA was isolated by standard procedure of liver tissue lysis with proteinase K in the presence of SDS followed by deproteinization with phenol-chloroform. A fragment of cyt*b* gene of the *D. nitedula* was amplified with primers L14725 (Pääbo et al. 1988) and H15915 (Irwin et al. 1991) or with F_ Dr.n_cyt (5′-TGACAAACATCCGTAAAACT-3′) and R_Dr.n_cyt (5′-CTGAATATGGGGAAGAGGA-3′). Amplification was performed using Tercik Thermal Cycler (DNA Technologies) with 30 cycles (30 s at 94 °C, 30 s at 50 °C, and 60 s at 72 °C) after an initial step at 94 °C for 3 min and a final extension step at 72 °C for 5 min. Samples were subsequently sequenced for both strands on an ABI PRISM

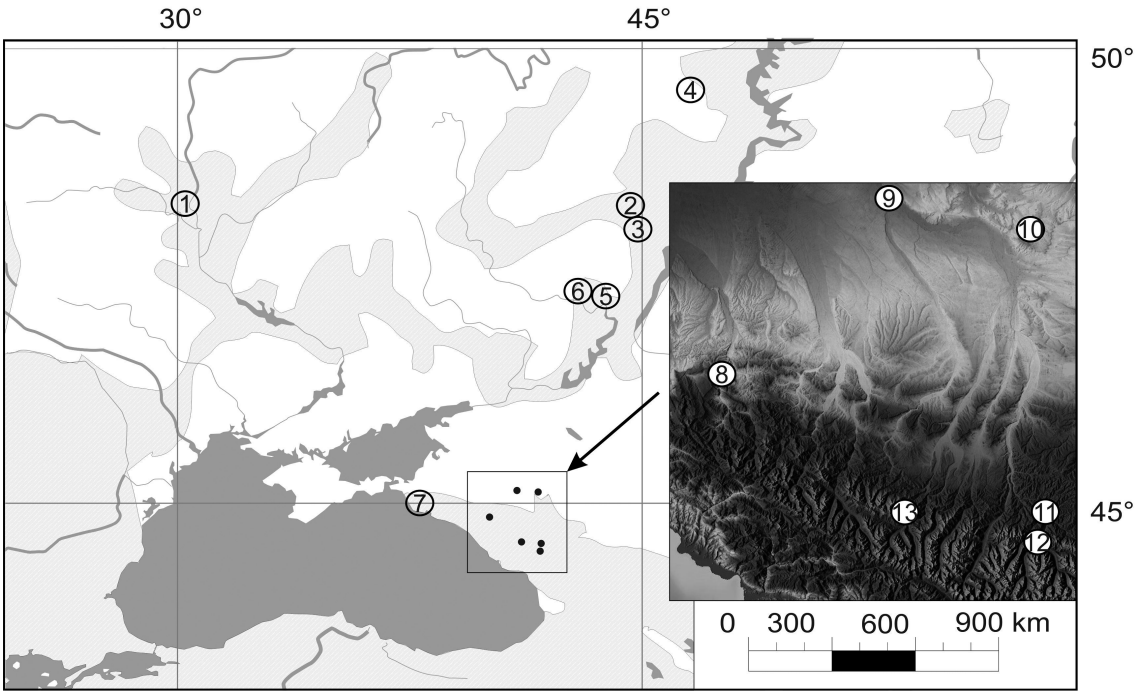

Fig. 1. Geographic distribution of the studied samples. The numeration as in Table 1.

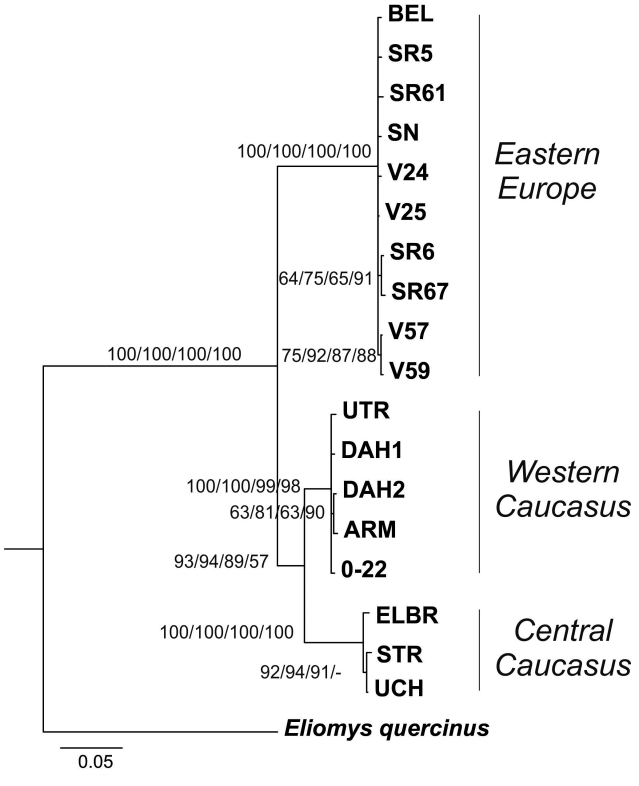

Fig. 2. ML-tree for cyt*b* genes of *D. nitedula*. Numbers indicated on the branches correspond to bootstrap support obtained in the NJ, MP, ML analysis (left) and Bayesian probabilities (right).

3100-Avant with internal primers F_Dr.n_int (5′-ATAGCAACCGCATTCATAGG-3′) and R_Dr.n_int (5′-AAAAGCGGGTTAGTGTTGC-3′).

Phylogenetic analysis was carried out using Neighbor-Joining (NJ), Maximum Parsimony (MP), Maximum-Likelihood (ML) and Bayesian (BI) methods. Phylogenetic distances were calculated on the basis of 3-parametric Tamura algorithm with 1000 bootstrap replicas. MP analysis included TBR method by replacing branches (10000 random additions, 1000 bootstrap). These two types of analysis were performed using MEGA v.6.06. ML analysis was conducted using PHYML v.3.0 (1000 bootstrap) after the definition of an appropriate model of nucleotide substitution, selected on the basis of the Bayesian (BIC) criterion and the Akaike Information Criterion (AIC), using JMODELTEST v.2.1 (the model was GTR + I). Bayesian analysis was performed using MRBAYES 3.1, with 4 Markov Chain Monte Carlo chains run for 2000000 generations and sampled every 100 generations, and with the first 500000 generations discarded as burn-in (Huelsenbeck et al. 2001). For visualization, we used FIGTREE v.1.4.

Results

ML-tree topology corresponds to topologies of MP-, NJ- and BI-trees (Fig. 2). Samples of *D. nitedula* from Russian Plain are clustered into the Eastern European haplogroup. Intragroup genetic-distances (T3P) are low, d = 0.3 ± 0.1 %. This haplogroup forms an independent monophyletic lineage in relation to the Caucasian haplogroups (d = 9.3 ± 1.3 %, *p*-value < 0.001 and 10.4 ± 1.4 %, *p*-value < 0.001). These distances are

Table 1. Numbers of specimens (N), geographic origin, sample and haplotype names of *D. nitedula*.

Number on the map and geographic origin	N	Voucher no.	Haplo-types	GenBank assession no.
1 Belorussia, Krasnoye 51°30′40″ N, 30°30′10″ E	2	Bel114, Bel115	BEL	KJ739693
2 Russia, Saratov 51°41′00″ N, 44°53′51″ E	2	C5 C6	SR5 SR6	KF699220 KF699221
3 Russia, Saratov 51°17′37″ N, 44°59′40″ E	1 3	C60-63, C69, C80, C83 C67-68, C78-79, C81-82	SR61 SR67	KF699223 KF699226
4 Russia, Saransk 54°10′30″ N, 46°9′59″ E	5	S1, S2, S4, S5, S7	SN	KJ739694
5 Russia, Serafimovich 49°33′26″ N, 42°39′48″ E	7	LD24, LD33 LD25-LD27, LD37, LD39	V24 V25	KJ739695 KJ739696
6 Russia, Serafimovich 49°42′15″ N, 42°47′52″ E	7	RD58, RD60, RD62, RD65, RD66 RD57 RD59	V25 V57 V59	KJ739696 KJ739697 KJ739698
7 Russia, Utrish 44°42′19″ N, 37°28′16″ E	3	UTR1-3	UTR	KJ739699
8 Russia, Dahovskaya 44°10′58″ N, 40°08′48″ E	2	DAH1 DAH2	DAH1 DAH2	KJ739700 KJ739701
9 Russia, Armavir 44°57′21″ N, 41°10′07″ E	2	ARM1-2	ARM	KJ739702
10 Russia, Strizhament 44°48′33″ N, 42°02′01″ E	1	STR	STR	KJ739703
11 Russia, Elbrus 43°33′17″ N, 42°07′56″ E	1	ELBR	ELBR	KJ739704
12 Russia, Uchkulan 43°27′15″ N, 42°05′26″ E	6	UCH1-6	UCH	KJ739705
13 Russia, Arhyz 43°33′57″ N, 41°16′44″ E	1	0-22	0-22	AJ225116

half as much as *D. nitedula – E. quercinus* distance (20.4 ± 0.4 %, *p*-value < 0.001). For the greater reliability, we calculated *p*-distances between groups, based on the amino acids data (d_{AA}). They were in agreement with T3P-distances: Eastern European – Caucasian haplogroups distances were 25.4 ± 2.9 % and 26.6 ± 2.9 %, half as much *D. nitedula – E. quercinus p*-distance (d_{AA} = 45.5 ± 1.0 %).

The Western Caucasian (Dahovskaya, Arhyz, Utrish, Armavir) and the Central Caucasian (Strizhament, Uchkulan, Elbrus) haplogroups can be regarded as sibling. The genetic distance between them is significant, d = 6.0 ± 0.9 % (*p*-value < 0.001), d_{AA} = 16.1 ± 2.4 %.

The nearest collecting points of the Western and Central Caucasian haplogroups are at a distance of 60 km with no signs of hybridization on cyt*b* gene. Probably the gene flow between these haplogroups is interrupted by geographic or reproductive isolation.

Discussion

Modern intraspecific taxonomy of *D. nitedula* is solely based on colouration features. In the latest revision of geographic variation and taxonomy of *D. nitedula*, dormice of Russian Plain and the Western Caucasus distributed to the East to approximately 42° E belong to the nominative subspecies (Rossolimo 1971). Presumably, at this longitude in the Caucasus the border of the nominative subspecies and *Dryomys nitedula ognevi* Heptner & Formosov, 1928 (subspecies from southern Dagestan, syn. *caucasicus*) passes.

The presumptive border of the Central and Western Caucasian haplogroups coincides with that one of the nominative subspecies and *D. n. ognevi* and with the Western and Eastern Caucasian landscapes (Sokolov & Tembotov 1989).

The observed level of genetic divergence between Russian Plain and Caucasian populations of *D. nitedula* is high enough. This allows us to assume that there was a long-term isolation of the European and Caucasian areas of *D. nitedula* during the whole Pleistocene.

The three discovered haplogroups of *D. nitedula* differ at high nucleotide and amino acid levels of cytochrome *b* gene. Sister species of mammals, defined on morphological differences, typically have more than 5 % values of cyt*b* genetic distances and the genetic distance close to 10 % is typical for "good" species (Bradley & Baker 2001). In any case, we should be more cautious with the species status confirmation. That is why it is very important to investigate other populations and other genetic markers to confirm their specific status as it was performed by Grigoryeva et al. (2015).

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