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SHORT COMMUNICATIONS

The First Genetic Evidence of Hybridization between West European and Northern White-breasted Hedgehogs (*Erinaceus europaeus* and *E. roumanicus*) in Moscow Region

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Abstract—The complex genetic examination of hedgehogs from the vicinity of the village of Nikolina Gora (Moscow region, Odintsovskii district) showed both *Erinaceus europaeus* and *E. roumanicus* in the sample. One of the hedgehogs was designated as *E. roumanicus* by the nucleotide sequence of 1 TTR intron but possessed mitochondrial DNA of *E. europeus*. Only one of the chromosomal pairs that differ in *E. europeus* and *E. roumanicus* was heteromorphic in this specimen. Its hybridous origin as the offspring of one or several backcrosses between F_1 hybrid and *E. roumanicus* was suggested.

Key words: *E. europaeus*, *E. roumanicus*, natural hybridization, karyological, and DNA analyses.

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INTRODUCTION

According to modern ideas, the genus of European hedgehogs Erinaceus L., 1758 includes four species: *E. europaeus* L., 1758; *E. amurensis* Schrenk, 1859; *E. concolor* Martin, 1838, and *E. roumanicus* Barrer-Hamilton, 1900 (Hutterer, 2005). Until recently *E. roumanicus* was considered to consist of polytypic species *E. concolor* s. lato. However, by means of morphological (Peshev, Hussein, 1990; Tembotova, 1999; Krystufek, 2002) and molecular genetic tests (Filippucci, Simson, 1996; Santucci et al., 1998; Seddon et al., 2001; Bannikova et al., 2003; Bannikova, Lebedev, 2007), it was shown that *E. roumanicus* is an independent species.

E. europaeus is spread in western and central Europe, South Scandinavia, Estonia, and in the northern and central regions of European Russia. *E. roumanicus* inhabits central and eastern Europe and is limited to Transcaucasia and Asia Minor. It was found in Australia, Poland, Slovakia, Balkan Peninsula, Ukraine, and central and south regions of European Russia and the northern Caucasus. Thus, *E. europaeus* and *E. roumanicus* are parapatric species. The central European contact zone of these species is well known (Ruprecht, 1973; Markov, Dobrijanov, 1974; Kratochvil, 1975; Bauer, 1976; Holz, 1978; Krystufek, 1983), but the eastern one is less known. Study of *E. europaeus* and *E. roumanicus* is complicated due to their great morphological variability, which is transgressive for separate features. Because of that, some specimens can be identified by means of craniological and exterior features only (Zaytsev, 1982, 1984), and also using genetic methods. Caryotypes of *E. europaeus* and *E. roumanicus* include the same number of chromosomes ($2n = 48$), but several chromosome pairs differ in heterochromatin localization (Gropp et al., 1969; Mandahl, 1978; Grafodatski et al., 1991; Sokolov et al., 1991). Investigations of polymorphism of nuclear and mitochondrial DNA (Bannikova et al., 1995, 2003; Surin et al., 1997; Santucci et al., 1998; Seddon et al., 2001; Bannikova, Lebedev, 2007) showed that *E. europaeus* and *E. roumanicus* are isolated genetically.

For the first time, cohabitation of *E. europaeus* and *E. roumanicus* on the territory of eastern Europe was mentioned by Ognev (1928) in Surazhskii district of Chernigov province (V.A. Filatova's collection). There is no doubt that both species occur in Moscow region. A collection of both species sampled in Zvenigorodskaya biostation of Moscow State University (vicinity of Lutsino village, Odintsovskii district) by V.V. Kucheruk (May 1941) is stored in the Zoological Museum of Moscow University. Later, the use of cytogenetic methods showed cohabitation both of *E. europaeus* and *E. roumanicus* among specimens sampled near Chernogolovka (Noginskii district) (Sokolov et al., 1991). Our observations also confirm cohabitation of both species in the vicinities of Zvenigorod, Krasnogorsk, and Chernogolovka.

Hybrids between *E. europaeus* and *E. roumanicus* were described on the basis of morphology in their central Europe contact zone (Ruprecht, 1973; Kratochvil, 1975) and were hatched due to crossing in the laboratory (W. Poduschka and Ch. Poduschka, 1983). However, due to the absence of confirmations with genetic markers, there are no direct proofs of hybridization both of these species in natural conditions. Due to the poor accuracy of the morphological methods, complex genetic analysis can have a decisive importance in identifying the parents and hybrids, including distant hybrids. The purpose of this study is to investigate one of the heteromorphic samples of hedgehogs from Moscow region by means of some genetic methods.

MATERIAL AND METHODS

On the left bank of the Moscow River, 12 km downstream from Zvenigorod near the village of Nikolina Gora, five hedgehogs were captured. The Carcasses and craniums of these specimens were transmitted to the Zoological Museum of Moscow University (numbers 1–5 used in work conform to numbers 168662–168666 in the museum). Karyotypes of all hedgehogs were analyzed. In two specimens (no. 3 and no. 5), we determined the full nucleotide sequence (1140 n. p.) of the mitochondrial gene of cytochrome *b* (cyt *b*) and partial sequence (1500 n. p.) of nuclear intron 1 TTR, which were compared with the same of the earlier determined specimens of *E. europaeus* and *E. roumanicus* from different populations of Russia, and also with the data from the GenBank database.

Chromosome preparations were extracted by means of the standardized procedure from marrow cells of animals (Ford, Hamerton, 1956). Heterochromatin staining was carried out by the Sumner method (Sumner, 1972), and detection of nucleolus organizer regions (NOR's) was by means of Howell and Black's method (Howell, Black, 1980).

Genomic DNA was extracted from muscle and the liver and preserved with ethanol by means of phenol chloroform method after treatment of tissue homogenate with proteinase K (Sambrook et al., 1989). For sequencing and reading of the full sequence of the cyt *b* gene, we used modified primers (Ohdachi et al., 2001), but for sequencing and reading of 1 TTR intron, we used a combination of special primers A625eri_L and B1628_H (Bannikova, Lebedev, 2007). Amplification was carried out by means of equipment from MJ Research Inc. (United States) and TERTSIK (Russia). Purification of amplification products was carried out with a Whatman DEAE (Whatman, United States) or by means of sedimentation with a mixture of ammonium acetate and 70% ethanol. Automatic sequencing was realized in the laboratory CCU Genome by means of the sequenator ABI 3100-Avant with the kit ABI PRISM®BigDye™ Terminator v. 3.1 (ABI, United States). Phylogenetic analysis and plotting were carried out by means of the computer program PAUP 4.0.

RESULTS

Cytogenetic analysis showed that the sample from near the village of Nikolina Gora consists of two *E. roumanicus* specimens (no. 2, no. 4) and two *E. europaeus* specimens (no. 1, no. 5). The data on the morphology of chromosomes and distribution of heterochromatin and NOR's in karyotypes of these specimens conform well to data published earlier (Geisler, Gropp, 1967; Kral, 1967; Gropp et al., 1969; Mandahl, 1978, 1979; Grafodatskii et al., 1991; Sokolov et al., 1991). The karyotype of another specimen (no. 3, ♀) was unique according to some peculiarities. One pair of chromosomes (no. 20) is sharply heteromorphic: one homolog (metacentric) is lacking in the heterochromatin and so is less in size than the subtelocentric chromosome, which is paired to it, and whose heterochromatic segment fills more than half of the long limb (Fig. 1a). It is necessary to note that the pair of chromosomes both in *E. europaeus* and *E. roumanicus* is noted by a large heterochromatin block and size of itself respectively (C-blocks are in *E. roumanicus* and lacking in *E. europaeus*). Moreover, the submetacentric chromosomes of the 19th pair differ from one another by NOR's: in one homolog NOR's was stained by silver practically in all metaphase plates, but in the other, it was revealed extremely rarely as small blocks (Fig. 1b).

By means of molecular genetic markers, as of chromosomes only, hedgehog no. 5 was identified as *E. europaeus*. Specimen no. 3 has only one variant of the sequence of 1 TTR intron, which is identical to the same of *E. roumanicus* from Stavropol (Fig. 2). According to genomic DNA analysis by means of inter-SINE-PCR (Bannikova et al., 2003), hedgehog no. 3 fell within the cluster group with *E. roumanicus* from Bryansk, Ryazan, Kaluga, Stavropol, Nalchik, and Dagestan, though it was closer to Caucasian populations. However, the cyt *b* gene sequencing gave the opposite result: the haplotype of specimen no. 3 was very close to the same of no. 5 and the specimen differs from it by only one alternate (site 796 n). Parsimonius phylogenetic trees showed that, according to nuclear DNA analysis, specimen no. 3 was identified as *E. roumanicus*, and fell within group together with *E. europaeus* (Fig. 2).

Analysis of specimens from near Nikolina Gora by means of the craniological features used by Zaitsev (1984) gave interesting results. In this case specimens no. 1 and no. 5 were identified as *E. europaeus*, but no. 2 and no. 4 were identified as *E. roumanicus*. Specimen no. 3 has the one foramen mentale on the right branch of lower jaw that is typical for most of *E. europaeus*, and two foramens on the left side that is typical for most of *E. roumanicus*. The form of the maxillary-premaxillary suture on the right side of head is typical for that in *E. europaeus* and does not occur among *E. roumanicus* specimens from the northern part of the area. The form of the maxillary-premaxillary suture on the left side of the head is typical for *E. roman-*

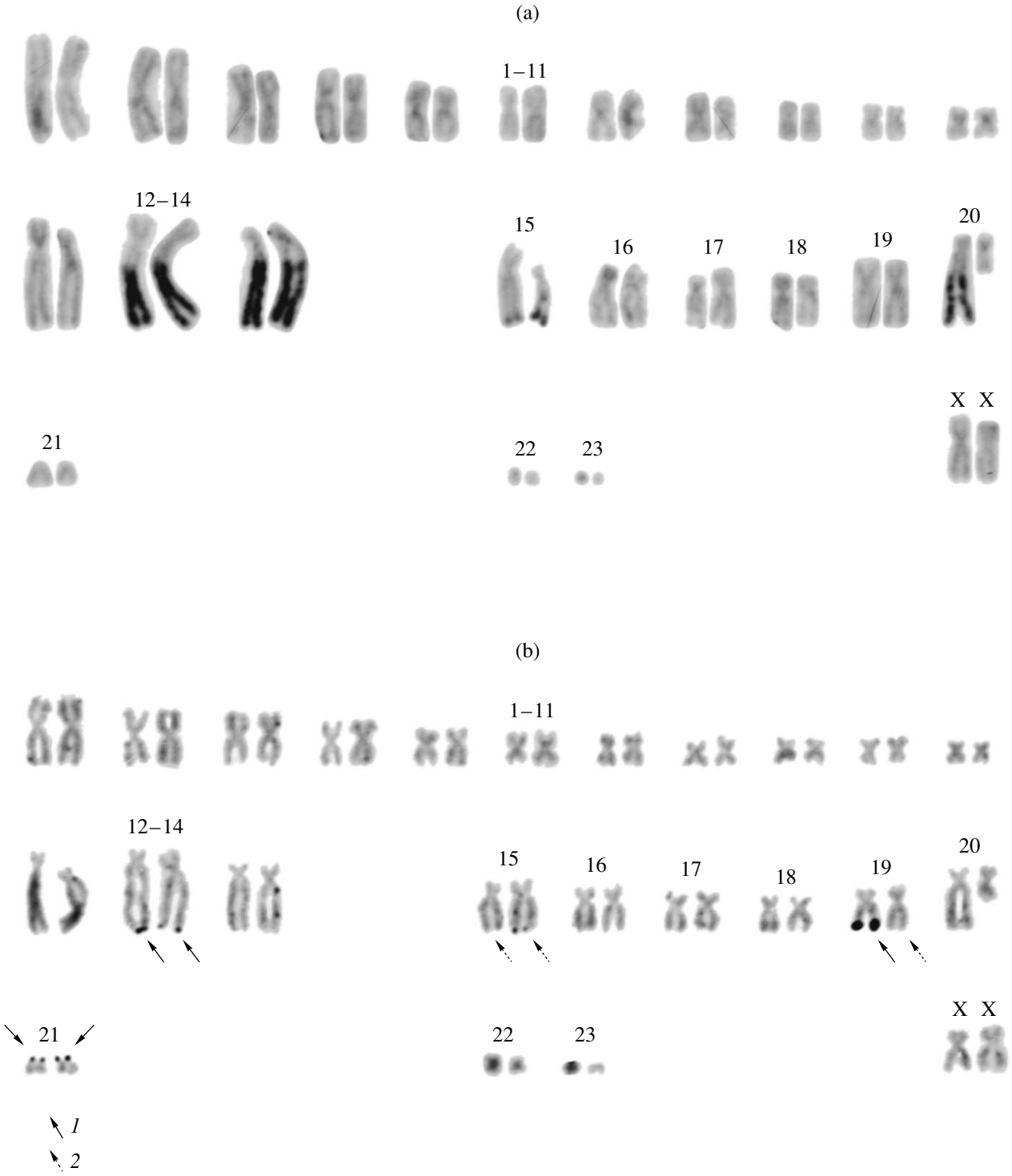

Fig. 1. Localization of heterochromatic segments (a) and NOR's (b) in the hedgehog karyotype (no. 3) from around the village of Nikolina Gora. Chromosomal pairs are placed and numbered according to the Mandahl scheme (Mandahl, 1978, 1979). Chromosomes with NOR's are marked with arrows: (*1*) chromosomes with stable NOR's appearance, (*2*) chromosomes with weak NOR's staining.

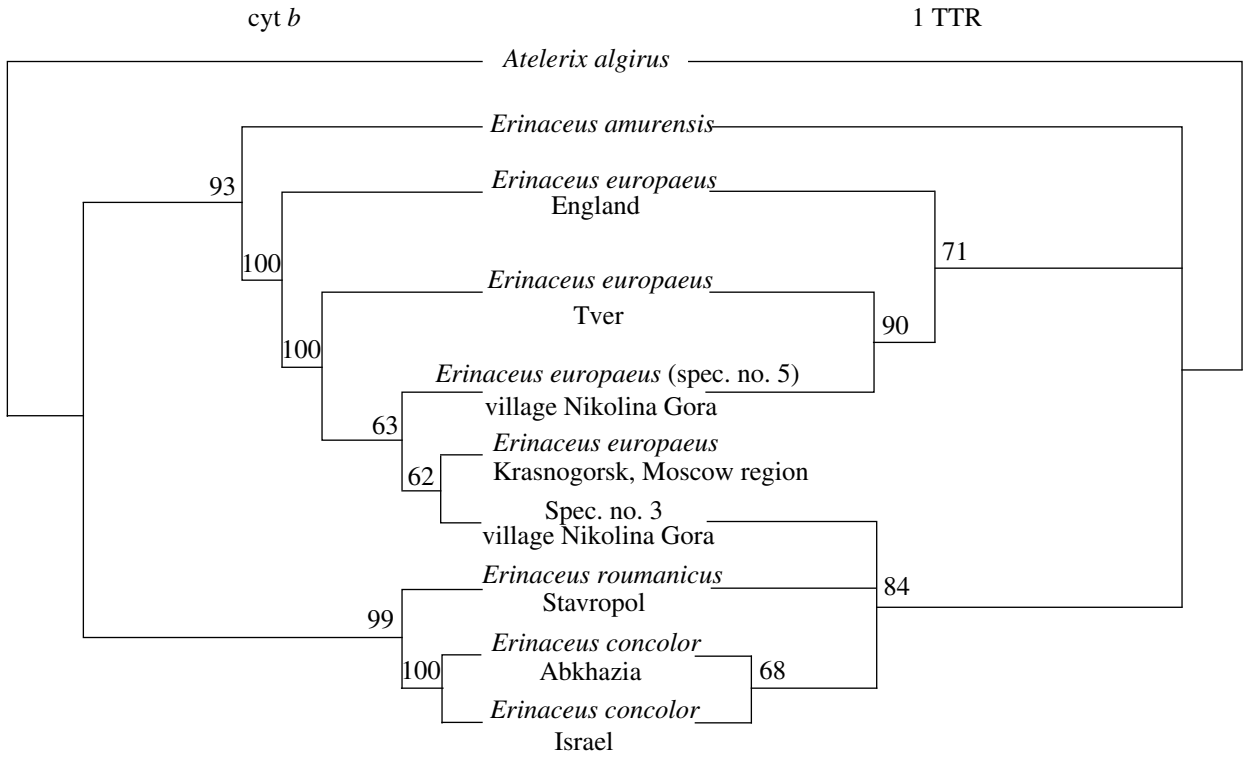

Fig. 2. Parsimonious trees, indicating genetic similarity of hedgehog no. 3 (environs of the village of Nikolina Gora) with *E. europaeus* and *E. roumanicus* by the nucleotide sequences of the cyt *b* gene and 1 TTR intron. The numbers in bifurcation points of dendrograms are the values of the bootstrap-index (%), calculated for 1000 replications.

icus. Thus, accurate identification of specimen no. 3 according to morphological features is impossible.

DISCUSSION

The inconsistency between data received due to mitochondrial and nuclear molecular genetic markers proves that hedgehog no. 3 is a hybrid. Moreover, the homozygosis of this specimen by the nucleotide sequence of 1 TTR intron, which is identical to the same of one *E. roumanicus* specimen, indicates that specimen no. 3 is an offspring from one or several back crossings between F_1 hybrids and *E. roumanicus*. It conforms to hybridization experiments between *E. europaeus* and *E. roumanicus*; F_2 hybrids were hatched solely by the back crossing between F_1 hybrids and *E. roumanicus*, whereas crossing between F_1 hybrids and *E. europaeus* was abortive (Poduschka W., Poduschka Ch., 1983).

The results of chromosome analysis, regardless of molecular genetic data, lead to the same conclusions. Hedgehog no. 3 has only one heteromorphic chromosome pair, which can be used to distinguish the *E. europaeus* karyotype from that of *E. roumanicus*. All other marker chromosome pairs (no. 15, no. 21) of this specimen were not heteromorphic that naturally for F1 hybrids, and had chromosomes appropriate for *E. roumanicus* (equal in size, morphology, localization, and the size of C-blocks).

It is difficult to explain the presence of NOR's in one chromosome of specimen no. 3. Such staining was not recorded in any *Erinaceus species*. Judging by similar size of the chromosome of the 19th pair, the forming of a large NOR's-block does not connect with significant translocations and duplications, which sharply increase the number of gene copies of ribosomal RNA. Possible, an increase of gene activity happened.

One should not exclude the possibility that cytogenetic anomalies of specimen no. 3 were caused by chromosome mutation, deletion of the heterochromatic segment, but connection of these anomalies with hybridization between two species seems more probable.

The data received are the first genetic proof of hybridization between *E. europaeus* and *E. roumanicus* in the vicinity of Moscow. Further, we plan to determine the hybridization tendency, intensity, and the boundaries of the hybrid zone.

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