ISSN 1022-7954, Russian Journal of Genetics, 2006, Vol. 42, No. 7, pp. 802–809. © Pleiades Publishing, Inc., 2006.
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ANIMAL GENETICS

Genetic Evidence of Extensive Introgression of Short-Tailed Ground Squirrel Genes in a Hybridization Zone of *Spermophilus major* and *S. erythrogenys*, Inferred from Sequencing of the mtDNA Cytochrome *b* Gene

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Received December 30, 2004; in final form, February 20, 2006

Abstract—We have completely sequenced the mtDNA cytochrome *b* gene of ground squirrels from the zone of overlapping ranges of *Spermophilus major* and *S. erythrogenys* in the Tobol–Ishim interfluve, which is a putative hybridization zone of these species. The results of the sequencing showed extensive introgression of mtDNA genes of the short-tailed ground squirrel *S. e. brevicauda*, whose haplotype had fully replaced the *S. major* haplotype. All of the ground squirrels from the Tobol–Ishim interfluve had a variant of the *S. e. brevicauda* mtDNA haplotype that was specific for this zone. On average, 119 substitutions (10.44%) were found between *S. major* from Ul'yanovsk oblast and *S. e. brevicauda* from the northern Kazakhstan, the mean genetic distance (*D*) between them being 0.115, which conforms to the corresponding parameters for the *S. e. brevicauda*–*S. pygmaeus* pair (122 substitutions, $D = 0.118$). Insignificant differences (seven substitutions, $D = 0.043$) were found between the *S. major* and *S. pygmaeus* haplotypes, which suggest that these species have similar mitochondrial haplotypes. Five to ten nucleotide substitutions (0.44–0.88%) were detected between the animals from the Tobol–Ishim interfluve and *S. e. brevicauda*. The mtDNA haplotype divergence *D* within the genus *Spermophilus* (ten species) for all codon positions ranged from 0.035 to 0.158. Phylogenetic reconstructions (MP, ML, and NJ trees) showed two well-differentiated clusters with high bootstrap support. However, there was different branching topology within the cluster and their species composition varied. The maximum likelihood tree, ML, differentiating the species into two subgenera, *Citellus* and *Colobotis*, most reliably reflected taxonomic relationships of the species from the genus *Spermophilus*, inferred from morphological and genetic biochemical data. The morphologically pure *S. major* (subgenus *Colobotis*) animals, used in the analysis, proved to carry the haplotype of another species, *S. pygmaeus* (subgenus *Citellus*). This poses a question on the existence of the specific haplotype of *S. major*, the reason of its replacement by haplotype of other species, and possible consequences of this phenomenon for survival of the species.

DOI: 10.1134/S1022795406070167

INTRODUCTION

In recent years, the problem of hybridization of species with overlapping ranges, particularly rodents, has been intensely studied at different levels of their organization. Morphology, physiology, social behavior, and ecology of ground squirrels have been thoroughly studied, but the evidence obtained has low resolving power, while the results of these studies are different and even controversial [1]. In the present work, we have adopted the views of Gromov [2, 3] on the taxonomic positions of the ground squirrel species examined and the boundaries of their ranges, based on bioacoustic, craniometric [4–6], cytological, and biochemical [7] data, which assign ground squirrels from the left bank of the Irtysh River (form *heptneri* = *ungae*) to the species *S. erythrogenys.* In our previous study [8], we have shown similarity between the subspecies *S. e. brevicauda, S. e. heptneri,* and *S. e. erythrogenys*; the former two subspecies were more similar genetically than either of them and *S. e. erythrogenys.*

The facts of hybridization cast some doubt on the taxonomic status of the hybridizing forms. This requires, on the one hand, to strictly define the species, and, on the other hand, to revise its significance for the hybridizing taxa. Some authors believe that the effects of hybridization are small, while others assign to it greater role, stating that it enhances genetic variability and promote accumulation of novel adaptive traits. If natural hybridization occurs between taxa that were earlier believed to be species (according to Mayr [9], natural hybridization is possible and occurs only between closely related taxa, such as subspecies and

intraspecific forms), it results in mixing of characters and loss (homogenization) of genetic information (or characters) that differentiates and isolates these characters from one another. Consequently, the species lose their genetic discreteness and degrade, while a reduction in the number of individuals leads to hybridization of similar closely related taxa. However, in his later works, Mayr accepted the possibility of interspecific hybridization, in which hybrids are always less viable and even sterile [10]. According to Panov [11], hybridization enhances genetic variability and genetic diversity and thus, together with the processes of hereditary variation (recombination, mutation, etc.) should be regarded as a source of evolutionary material. Recently, hybridization was increasingly often considered as a source of new forms [12, 13].

To date, the recently reported facts of hybridization between some *Spermophilus* species, having zones of overlapping ranges in the Volga region and in the Tobol–Ishim interfluve, have been extensively studied [4, 5, 8, 14–17]. Using molecular genetic methods, cases of hybridization were found and its traces have been detected even in animals that had been thought "pure" on the basis of morphological, ecological and bioacoustic traits [8]. For instance, Ermakov et al. [14] have sequenced the mtDNA C-region of four ground squirrel species from their sympatry zone in the Volga region. These authors have found that the russet ground squirrel, in addition to its own haplotype, possessed two "alien" mitotypes, those of *S. fulvus* and *S. pygmaeus*, while the yellow and the minor ground squirrels had only one haplotype each.

Our previous studies based on RAPD markers have shown that, first, some phenotypically pure ground squirrels from the Tobol–Ishim interfluve have a hybrid origin; second, the russet ground squirrel hybridize with the subspecies *S. e. brevicauda* rather than with *S. e. erythrogenys*; and, third, strong selection seems to act at the nuclear genome level, which preserved the nuclear characters of *S. major* and *S. e. brevicauda* respectively in the north and in the south of the interfluvial zone [8]. To analyze interspecies hybridization of ground squirrels from this zone in more depth, mitochondrial markers should be used, because their mode of inheritance and mutation accumulation rate differ from those of RAPD markers.

The aim of the present work was to assess the scale of hybridization between *S. major* and *S. erythrogenys* on the territory of the Tobol–Isim interfluve. To do this, we established the level of differences between *S. major* and *S. e. brevicauda*, using mitochondrial markers, and determined the level of their genetic variation; detected mtDNA haplotypes of animals inhabiting the Tobol–Ishim interfluvial zone and find the divergence between them and their parental forms; reconstructed the phylogenetic relationships of the ground squirrel forms studied with other *Spermophilus* species (data from GenBank).

MATERIALS AND METHODS

We examined 18 *S. major* × *S. erythrogenys* individuals from the zone of their overlapping ranges in the Tobol–Ishim interfluve,and four animals from the village of Andreevka, which were assigned to *S. e. brevicauda* based on the results of RAPD analysis (Table 1, Fig. 1) [8]. The ranges of the ground squirrels analyzed are given as in Ognev [18] with supplements by Nikol'skii [4] and Korablev et al. [7]; these ranges have been used by zoologists until the present time. However, on the long-term scale, the ground squirrel ranges have expanded and narrowed, up to the total extinction of ground squirrels on certain territories. On the other hand, the territories between these ranges were not studied, forming a gap of approximately 800–1000 km^2 on the map (Nikol'skii, personal communication). The absence of literature data does not exclude the existence of *S. e. brevicauda* on these territories and its contact with *S. major.* Hence, our results may motivate zoologists to investigate these territories. The pure parental species were *S. major* (n = 3): Ul'yanovsk oblast, near the village of Malovka; *S. erythrogenys* was represented by the subspecies *S. e. brevicauda* (n = 2): Kazakhstan, 149 km along the Almaty–Narynkol highway. For comparison, mtDNA cytochrome *b* gene sequences of 19 closely related ground squirrel species were retrieved from GenBank (see Fig. 1, Table 1) [1].

DNA was extracted by means of phenol–chloroform procedure, following the standard protocol [19]. Direct sequencing of the mitochondrial cytochrome *b* gene of 27 ground squirrel samples was carried out in the Laboratory of Animal Sciences of the Metropolitan Institute of Medical Sciences, Japan. The following primers were used for sequencing:

L14724: (5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'),
L14841: (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'),
L15162: (5'-GCAAGCTTCTACCATGAGGACAAATATC-3'),
H15149: (5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'),
H15915: (5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3').

The concentration of total DNA in the probe was 10 ng/μl. Amplification was run in 50 μl of a reaction mixture (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM dNTP mixture, 0.2 mM of

Table 1. Samples examined in the present study

Species	Sampling locality	Number of specimens	Locality no.
S. major	Ul'yanovsk oblast, village of Malovka	3	1
S.e. brevicauda	Kazakhstan, 149 km along Almaty–Narynkol highway (GenBank)	2	5
S.e. brevicauda	Kazakhstan, Kokchetav oblast, Rusaevskii raion, village of Andreevka	4	15
S.e. brevicauda	Kazakhstan, Chilikskii raion, settlement of Tolkin (GenBank)	1	3
S.e. brevicauda	Kazakhstan, Chilikskii raion, settlelent of Teskensi (GenBank)	3	4
S.e. erythrogenys	Novosibirsk oblast, Karasukskii raion, settlement of Karasuk (GenBank)	2	2
S. alashanicus	Mongolia, 15–20 km west of Dalandzadgad (GenBank)	1	17
S. pallidicauda	Mongolia, 50 km southwest of Mandalgobi (GenBank)	2	16
S. parryi leucosticus	Yakutia, Magadanskii raion, village of Atka (GenBank)	1	–
S. fulvus	Kazakhstan, Uralskaya oblast, village of Tarlykova (GenBank)	2	–
S. musicus	Kabardino-Balkaria, Priel'brus''e, 5 km of village of El'brus (GenBank)	2	–
S. pygmaeus	Saratov oblast, Dergachevskii raion, village of Stepanovka (GenBank)	1	19
S. pygmaeus × *S. major*	Saratov oblast, Dergachevskii raion, village of Stepanovka (GenBank)	1	19
S. pygmaeus	Saratov oblast, Piterskii raion, village of Kozlovka (GenBank)	1	18
S. citellus	Moldova, Kamenskaya oblast, village of Zhabka, west of Dnestr R. (GenBank)	2	–
	Animals from Tobol–Ishim interfluve		
Morphotype "*major*"	Kurgan oblast, Pritobol'nyi raion, village of Zaborskoe	1	9
	Kurgan oblast, Pritobol'nyi raion, village of Obukhovo	2	10
	Kurgan oblast, Ketovskii raion, village of Temlyakovo	2	8
	Kurgan oblast, Pritobol'nyi raion, village of Utyatskoe	2	7
	Kurgan oblast, Kurtamyshskii raion, village of Stepnoe	1	20
Hybrid with *major* characters	Kurgan oblast, Vargashinskii raion, village of Shastovo	1	11
Morphotype "*erythrogenys*"	Kurgan oblast, Polovinskii raion, village of Privol'noe	7	13
	Kurgan oblast, Polovinskii raion, village of Sukhmen'	2	14

each primer, and 0.05 u Ampli-*Taq* Golg DNA polymerase) in 40 cycles under the following conditions: denaturing for 30 s at 94°C, annealing for 30 s at 55–60°C, and elongation for 1 min at 72°C. Sequencing was carried out using a Big-Dye terminator cycle sequencing kit (Applied Biosystems). 373A Sequencer was used for automated sequencing.

The percentage of interpopulation and interspecies differences was measured by the number of transversions and transitions separately in each codon position. The optimal model for constructing the phylogenetic trees was selected using the Akaike information criterion (AIC) [20] and the Modeltest ver. 3.06 software program [21]. Pairwise genetic distances *D* between cytochrome *b* gene haplotypes were estimated using the two-parameter Kimura model [23]. The ML and MJ trees were constructed using the PAUP ver. 4.0b10 software package [23]. The reliability of each branching was assessed by bootstrap [24] with 100 replications.

RESULTS

Examination of the mtDNA cytochrome *b* gene in 46 ground squirrels showed that 566 out of 1140 nucleotide positions are variable and 356 are parsimon informative. Out of these, for the animals from the Tobol–Ishim intefluve the numbers of variable and parsimonially informative nucleotide positions were respectively 161 and 126. The nucleotide composition of the cytochrome *b* gene in the examined representatives of the genus *Spermophilus* is presented in Table 2.

The highest number of substitutions at the cytochrome *b* gene in the genus *Spermophilus* was found between *S. parryi* (Magadan oblast) and the remaining species analyzed (Table 3). This species demonstrated the maximum differences with *S. erythrogenys*, and the minimum differences, with *S. pygmaeus* (D = 0.176 and 0.146, respectively). On average, 119 substitutions (10.44%) were found between *S. major* and *S. e. brevicauda*; the mean genetic distance between these species was 0.115, which corresponds to these parameters

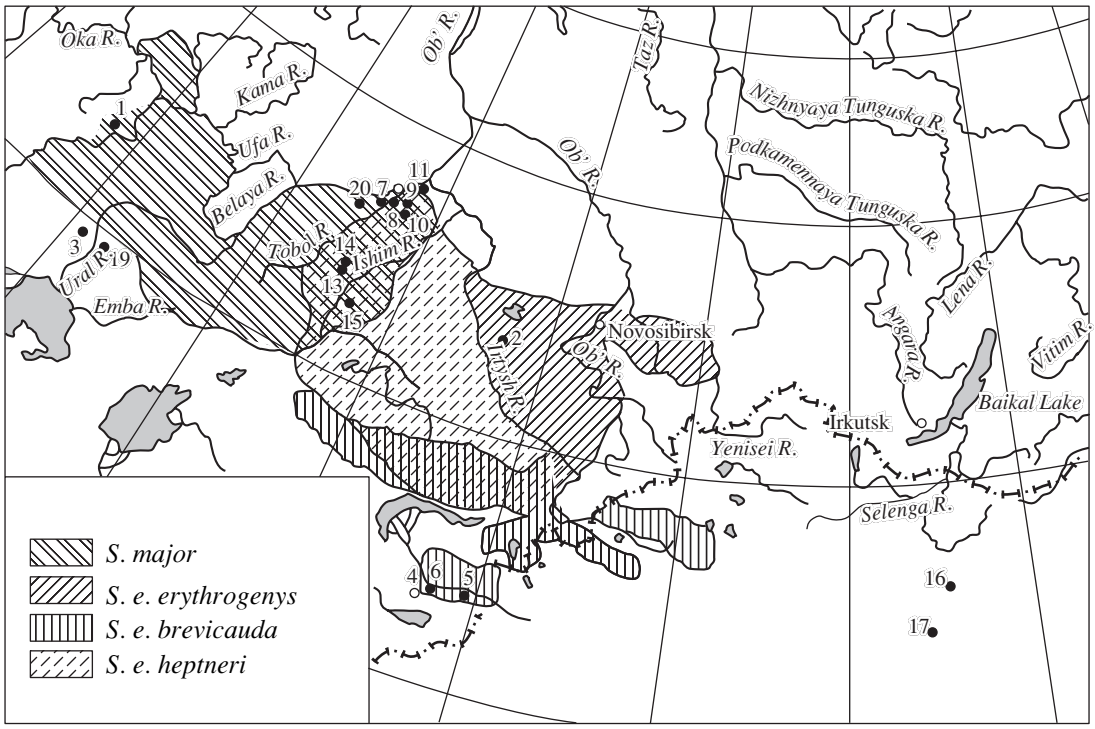

Fig. 1. Ranges and sampling localities of two species of the genus *Spermophilus*. The numbers of localities are as in Table 1. The range map is as in [18].

between *S. e. brevicauda* and *S. pygmaeus* (122 substitutions, $\overline{D} = 0.118$). Haplotypes of *S. major* and *S. pygmaeus* showed insignificant differences (seven substitutions, $\overline{D} = 0.043$), which suggests that these species have a common mitochondrial haplotype. Five to ten nucleotide substitutions (0.44–0.88%) were detected between animals from the Tobol–Ishim interfluve and *S. e. brevicauda.* It is of interest that one animal from the sympatry zone of the russet ground squirrel and the little ground squirrel, taken from the GenBank database, which was a *S. major–S. pygmaeus* hybrid, according to the results of the cytochrome *b* gene sequencing had a low number of substitutions (0.5%) and low genetic distances (0.005) with the animals from the Tobol–Ishim interfluve, which possess the haplotype similar to that of *S. e. brevicauda.* However, five to ten nucleotide substitutions (0.44–0.88%) were found between the animals from the Tobol–Ishim interfluve and *S. e. brevicauda*. The overwhelming majority of nucleotide substitutions (up to 134) in all ground squirrels were in the third codon position. In the second and the first codon positions, up to 11 and 30 substitutions respectively were found. Table 3 presents the data on genetic distances (*D*) and substitutions in the cytochrome *b* gene sequences in ground squirrel species, including other species taken from GenBank.

In all, the Ts/Tv ratio was 2.631 for all animals ($\kappa = 5.281$). Since the *S. major* individuals examined, which were initially thought to be a "pure" species, proved to carry the mitochondrial haplotype of *S. pygmaeus*, all genetic comparisons of them with other animals should be attributed to *S. pygmaeus.* The Ts/Tv values in the pair hybrids–*S. e. erythrogenys* varied from 12.45 to 20.76; in the pair hybrids–*S. e. brevicauda*, from 1 to 10.1; while the divergence values or the former and the latter species pair were respectively $\overline{D} = 0.005$ and 0.006. The Ts/Tv values for the animals from the Tobol–Ishim interfluve ranged from 0 to 8. The proportions of transitions and transversions between *S. e. erythrogenys* and *S. e. brevicauda* were respectively Ts = 5.7% and Tv = 0.35%. Between hybrids and *S. e. erythrogenys*, Ts = 5.53% and Tv = 0.35%; between hybrids and *S. e. brevicauda*, Ts = 0.6–1.1% and Tv = 0.09%.

Analysis of interspecies differences in ground squirrels dwelling outside the interfluvial zone showed that, based on the cytochrome *b* gene sequences, the closest

Table 2. Nucleotide composition of the cytochrome *b* gene in species of the genus *Spermophilus*

Codon position	A	C	G	T
All	0.291	0.257	0.126	0.326
First	0.297	0.253	0.201	0.249
Second	0.199	0.235	0.134	0.431
Third	0.382	0.283	0.025	0.310

Table 3. Mean genetic distances between some species of the genus *Spermophilus*, obtained from the two-parameter Kimura's model (below diagonal) and mean number of substitutions (Ts + Tv) (above diagonal)

	Subgenus *Colobotis*							Subgenus *Citellus*			
Species	Animals from the interfluve	*S. e. brevicauda*	*S. pygmaeus* × *S. major*	*S. e. erythrogenys*	*S. pallidicauda*	*S. fulvus*	*S. musicus*	*S. pygmaeus*	*S. cittelus*	*S. alashanicus*	*S. parryi*
Animals from the interfluve	–	8	6	63	40	35	130	121	110	37	159
S. e. brevicauda	0.006	–	9	65	42	38	129	122	112	39	158
S. pygmaeus × *S. major*	0.005	0.008	–	65	42	38	130	121	113	39	158
S. e. erythrogenys	0.058	0.066	0.060	–	64	60	136	133	127	63	172
S. pallidicauda	0.036	0.046	0.038	0.058	–	45	129	122	118	3	159
S. fulvus	0.031	0.035	0.034	0.054	0.041	–	128	117	121	45	128
S. musicus	0.127	0.119	0.127	0.134	0.126	0.125	–	61	130	129	151
S. pygmaeus	0.118	0.118	0.117	0.130	0.118	0.112	0.060	–	123	121	147
S. cittelus	0.106	0.108	0.109	0.124	0.115	0.117	0.128	0.128	–	115	159
S. alashanicus	0.033	0.046	0.035	0.058	0.003	0.040	0.125	0.117	0.111	–	162
S. *parryi*	0.160	0.157	0.159	0.175	0.160	0.165	0.151	0.146	0.160	0.163	–

species were *S. fulvus* and *S. e. brevicauda,* whose haplotypes differ by 38 substitutions (3.33%). The highest number of substitutions in the genus *Spermophilus* was found between *S. parryi* and *S. e. erythrogenys* (see Table 3).

To construct phylogenetic trees, we used the GTR model selected by means of the Modeltest ver. 3.06 software program [21]. It was shown that in all samples examined, synonymous substitutions (C–T transitions) prevailed in all positions (48.7). However, in the third codon position, the A–G substitutions prevailed over the C–T ones (19.56 versus 7.23), which seems to be characteristic for this group of animals. This prevalence of one substitution type over another in certain codon position is known as codon usage [25]. Marais and Duret [25] have shown for *Caenorhabditis elegans* that codon usage may affect accuracy of protein synthesis in multicellular animals. The low value of Γ (0.218) suggests a nonuniform distribution of substitutions along the cytochrome *b* gene.

Based on the GTR phylogenetic model, we have constructed the MP, ML, and NJ trees. All reconstructions showed two clusters with high bootstrap support, but these clusters had different topologies and species composition. The ML (maximum likelihood) tree was the one that most reliably reflected the relationships among the species (Fig. 2). In the ML reconstructions, the species are grouped into two clusters, corresponding to the subgenera *Citellus* and *Colobotis,* which are distinguished in ground squirrels by many zoologists on the basis of morphological characteristics [2, 3]. The *Colobotis* cluster contained species *S. erythrogenys, S. pallicauda, S. fulvus*, form *brevicauda, S. alashanicus*, and all animals from the Tobol–Ishim interfluve. Each species was characterized by a specific haplotype, supported by high bootstrap values. This cluster also included an animal from Shastovo that was identified as hybrid according to two different criteria: bioacoustic signal and RAPD analysis. In the cluster formed by the animals from the Tobol–Ishim interfluve and short-tailed ground squirrels, several haplotype variants were identified with high bootstrap support: two in *S. e. brevicauda* from Kazakhstan; two in the animals from the village of Andreevka, one in the settlement of Zaborskoe; and the widely spread haplotype occurring in most animals from the Tobol–Ishim interfluve. In addition, this cluster included the *S. pygmaeus–S. major* hybrid animal from the GenBank. As, according to the karyological data, the *S. alashanicus* individual examined was a first-generation hybrid with *S. pallicauda* (Korablev, personal communication), its clustering with *S. pallidicauda* (subgenus *Colobotis*) seems quite reasonable, although by its systematic position, it should be assigned to the subgenus *Citellus.*

The second cluster includes species *S. citellus, S. musicus*, and *S. pygmaeus,* which is in good agreement with the systematics of the subgenus *Citellus,* accepted by many zoologists. It was surprising that the *S. major* individuals, attributed by zoologists to the subgenus *Colobotis*, fell into this cluster together with *S. pygmaeus.* Consequently, the *S. major* individuals, collected outside the sympatry zone with other species and previously thought to be "genetically pure," have the haplotype of a sympatric species, *S. pygmaeus*, and

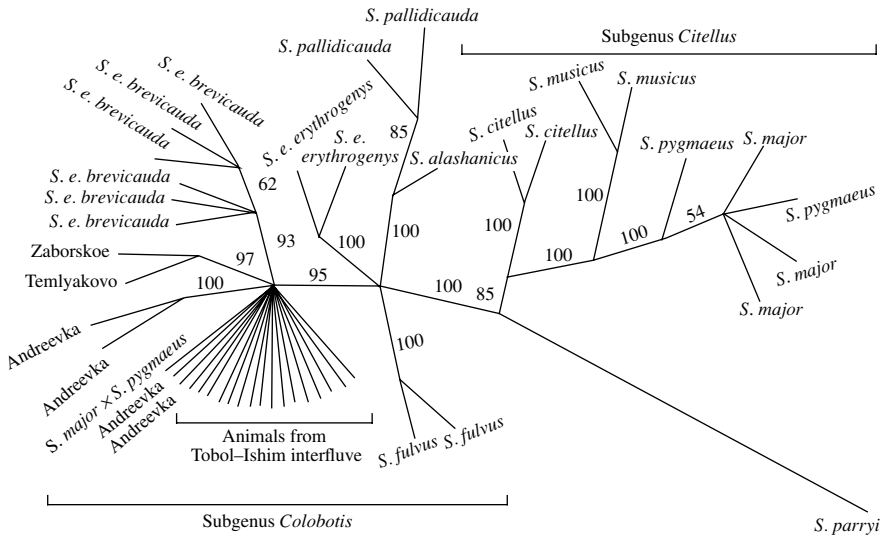

Fig. 2. Phylogenetic relationships between ground squirrels from the Tobol–Ishim interfluve and some other *Spermophilus* species, inferred from sequencing of the mtDNA cytochrome *b* gene and constructed using the maximum likelihood (ML) method and the PAUP program.

thus are genetic hybrids. The Arctic ground squirrel *S. parryi*, belonging to the subgenus *Urocitellus*, in the phylogenetic reconstructions proved to be most distant from the above two subgenera; the *Colobotis* cluster, branching from it, had 100% bootstrap support, while the *Citellus* cluster, only 85% support.

DISCUSSION

Hybridization and backcrosses with one or both parental species may result in the introgression of alleles of one taxon into the gene pool of another. To describe this phenomenon, Anderson and Hubricht [26] have coined the term *introgressive hybridization.* During hybridization, marker genes may show a different degree of introgression. Moreover, in some cases introgression is fundamentally asymmetric, which may reflect a recent geographic shift of the hybrid zone.

The mean genetic distances in the pairs *S. major–S. e. brevicauda*, *S. major–S. e. erythrogenys*, and *S. e. brevicauda–S. e. erythrogenys*, inferred from RAPD–PCR data and mitochondrial haplotype sequencing, correlate with one another and show a general trend of the impact of the *S. e. brevicauda* genome on the species hybridizing with it. Thus, the animals examined carry both nuclear and (to even greater extent) mitochondrial characters of *S. e. brevicauda*. We could also conjecture hybridization between *S. major* and *S. e. heptneri*, whose range directly overlaps that of *S. major.* However, the gene flows, measured by the number of migrants per generation (*Nm*) on the basis of the RAPD data, are nearly threefold higher in the animals from the Tobol–Ishim interfluve with *S. e. brevicauda*, than with other forms [8].

Successful use of mitochondrial markers seems to depend on the level of coordination in the interaction of nuclear and mitochondrial DNA in the given taxon [22]. The results of the RAPD–PCR analysis and mtDNA cytochrome *b* gene sequencing suggest that the nuclear and mitochondrial DNA of the ground squirrels from the Tobol–Ishim interfluve interact in a somewhat coordinated fashion. This interaction implies that the genome of each species has a unique specific mtDNA haplotype. However, the sequencing showed that *S. e. brevicauda* had several variants of the mtDNA haplotype. One of these variants, found in two animals from Andreevka, exhibited traits of the *S. e. brevicauda* nuclear genome and differed from the haplotype of Northern Kazakhstan *S. e. brevicauda* by 12 positions, but in essence was intermediate between *S. e. brevicauda* and the animals from the Tobol–Ishim interfluve. Another variant, detected in the animals from settlements of Temlyakovo and Zaborskoe, differed from the above haplotype by nine positions. All individuals from the Tobol–Ishim interfluve, identified as *S. major* and *S. e. brevicauda* on the basis of nuclear markers, shared a common mtDNA haplotype close to that of *S. e. brevicauda*. The widely presented mtDNA variant differed from the standard *S. e. brevicauda* haplotype only by seven positions. Since these forms fell into the same cluster, which included animals attributed either to morphotype *major* or to morphotype *erythrogenys* on the basis of morphological characters and nuclear DNA, we supposed that all these animals are mainly of hybrid origin. Thus, it is likely that all individuals from the Tobol–Ishim interfluve (100%) are hidden genetic hybrids, which testifies to extensive introgressive hybridization in the interfluvial area. In contrast to our results, the data on sequencing of the mtDNA C region in three ground squirrel species from a sympatry zone in the Volga region revealed only sporadic hybridization [14].

The hybrid nature of *S. major* individuals, which were earlier believed to be "pure," is in good agreement with the presence in *S. major*, in addition to its "own" haplotype, haplotypes of *S. pygmaeus* and *S. fulvus,* which was established by Ermakov et al. [14]. The question of the specific *S. major* haplotype remains open, because, as seen from our data, its haplotype reported by Ermakov et al. [14], may prove to belong to, e.g., *S. e. brevicauda*.

The unexpected fact of the *S. pygmaeus–S. major* hybrid possessing the *S. e. brevicauda* haplotype indicates broad introgression of the latter into other closely related species, which may not be involved in direct hybridization with *S. e. brevicauda*. To assess the extent of the introgression of *S. e. brevicauda* mtDNA into other species, further research involving more extended material is needed.

The early stages of form development are accompanied mainly by the accumulation of transitions [27]. The prevalence of transitions over transversions, as well as low genetic distances in ground squirrels from the Tobol–Ishim interfluve, seem to suggest that they are not highly diverged forms. Hence, the presence of a specific haplotype in this group maybe explained by the fact that either his haplotype has been newly arisen (which is unlikely, although interspecies hybridization can enhance mutation) or it rarely occurs in the parental forms in the natural populations, being selectively advantageous for the hybrid genomes, or else a third form is involved in hybridization.

Thus, our results indicate a hybrid origin not only of the phenotypic hybrids, found in studies of bioacoustic signals of *S. major* and *S. erythrogenys* from the Tobol–Ishim interfluve, but also of hidden genetic hybrids, even on a larger scale than demonstrated by RAPD. Moreover, analysis of the hybrid zone revealed introgression of *S. e. brevicauda* genes, evidenced by stronger similarity of hybrid haplotypes exactly with this form.

Complex molecular genetic analysis has shown, on the one hand, that all animals from the Tobol–Ishim interfluve belong to the form *S. e. brevicauda* on the basis of their mitochondrial haplotype, and to *S. e. brevicauda* and *S. major* on the basis of nuclear characters. On the other hand, this analysis has revealed significant genetic differences between forms *S. e. brevicauda* and *S. e. erythrogenys*. Thus, we confirmed the earlier suggestion that *S. major* hybridizes with *S. e. brevicauda*, while in the Tobol–Ishim interfluvial area, extensive introgression of mtDNA haplotype of the latter species is observed. This evidence, inferred from both RAPD analysis and cytochrome *b* gene sequencing, indicates past sympatry between *S. e. brevicauda* and *S. major*, which promoted extensive genetic exchange between these forms. Notwithstanding extensive hybridization, stable morphotypes are maintained, with the prevalence of the nuclear genotype *major* in the north, and *brevicauda*, in the south of the zone of range overlapping. At the mtDNA level, a different mode of selection is observed: on the one hand, ground squirrels from the Tobol–Ishim interfluve generally exhibit higher genetic diversity; on the other, most animals from that area have a unique haplotype.

ACKNOWLEDGMENTS

We thank A.A. Nikol'skii for discussion of the manuscript and critical comments, and A.A. Goncharov for help in computer processing of the results.

This work was supported by the Program of the Presidium of the Russian Academy of Sciences "Dynamics of Plant, Animal, and Human Gene Pools."

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