

# The Phylogeography and Demographic History of the Common Shrew *Sorex araneus* L., 1758 (Eulipotyphla, Mammalia)<sup>1</sup>

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**Abstract**—The phylogeographic structure of *Sorex araneus* was assessed on the basis of the sequences of mitochondrial *cytb* gene sampled across the most of the species range. Two major haplogroups were revealed: a European group, which is distributed westwards from Western Siberia, and a Siberian group, which prevails in the eastern part of the range. The Siberian haplotypes are also present in southern Fennoscandia, but are absent in the northeast of Europe and in the Northern Urals. The demographic analysis indicates a rapid species-wide expansion from a single western source at the Pleistocene/Holocene boundary and a later expansion in the Siberian part of the range.

**Keywords:** Historical demography, phylogeography, *Sorex araneus*, Pleistocene, chromosomal races

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## INTRODUCTION

The history of the common shrew population range and the time of the formation of its unique chromosomal polymorphism in particular (as of this day, more than 70 chromosomal races of *Sorex araneus* have been described) has been the subject of many studies. A number of authors suggest that the most of the separation of chromosomal races [1–4] was preglacial (pre-LGM), implying that, during the last glacial maximum, different chromosome variants were fixed in multiple separate refugia. According to another hypothesis, various karyogroups correspond to independent refugia [5, 6]. This scenario implies that most of the chromosomal races within the groups arose during or after the recolonization of the range [7] or, following the stasipatric model [8, 9], after the range formed. For North European, West European, and East European groups of races, it was shown that the structure of mitochondrial variation does not support the subdivision into chromosomal races or groups of races [7, 10].

Although this pattern corresponds better to the single refugium hypothesis, the estimated age of the *S. araneus* last common ancestor and the expansion time for the populations of Central Europe was placed in the pre-LGM time [7]. The alternative hypothesis proposed by Bannikova et al. [11] suggests that the species range was recolonized from a single refugium

and most of the contemporary chromosomal races emerged in the Holocene.

This study attempts to describe the phylogeographic structure and the history of the *S. araneus* range on the basis of a large sample that includes specimens from northeastern Europe, Fennoscandia, and Siberia. Using the molecular clock method to assess the age of chromosomal races is problematic, firstly, because of the absence of a clear correlation between chromosomal and molecular structures and, secondly, because of the lack of calibration data that would allow one to assess the rate of molecular evolution in this species.

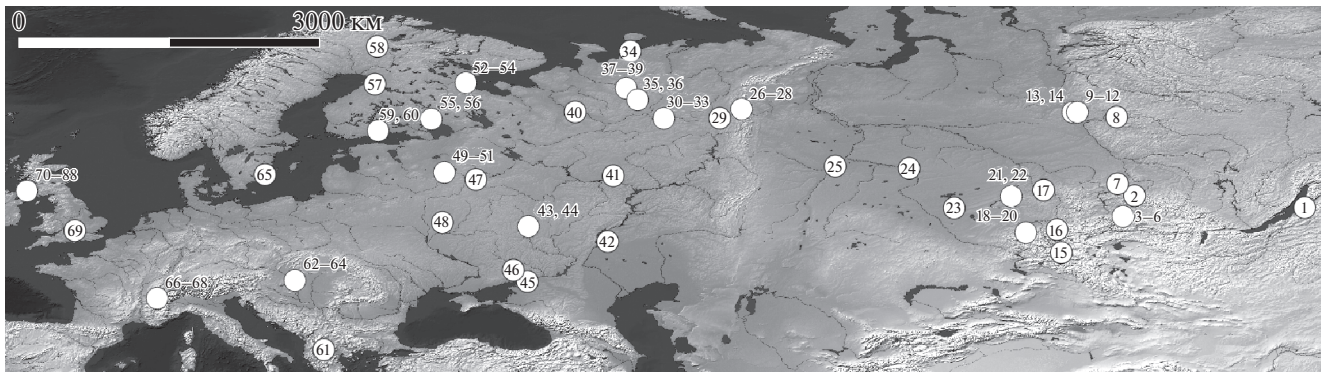
Taking into account the rate decay phenomenon, caused primarily by the effect of purifying selection on effectively neutral substitutions [12, 13], recent events should be dated with the use of calibration information that correlates with the age.

We believe that the postglacial expansion of some boreal species associated with forest belt restoration in Eurasia after the last deglaciation can be used for molecular clock calibration.

## MATERIALS AND METHODS

The sample includes 214 original mitochondrial the cytochrome *b* gene sequences of *S. araneus* from 45 localities. In addition, the sequences deposited in GenBank were used (accession numbers are shown in Fig. 1). Locations, chromosome races, and sample sizes are shown in Fig. 1. A total of 362 sequences of

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**Fig. 1.** Sampling localities (Sample number/Locality/GB accession number/Chromosome race/Sample size).

(1) Rep. of Buryatia, Lake Baikal, RF/AJ312029/Ba?/1; (2) Krasnoyarsk krai, Kuturchinsky Belogorye, Sobolinaya Mts., RF/To-Sr?/1; (3) Krasnoyarsk krai, Ergaki Ridge, RF/To/1; (4) Krasnoyarsk krai, Bagazul R., RF/To/13; (5) Krasnoyarsk krai, Tanzybey, RF/To/3; (6) Krasnoyarsk krai, Oya R., RF/To-Ye?/2; (7) Krasnoyarsk krai, Stolby National State Reserve, RF/Sr/1; (8) Evenk Autonomous Okrug, Podkamennaya Tunguska R., RF/Sr/7; (9) Krasnoyarsk krai, Suhaya Bakhta R., RF/To/6; (10) Krasnoyarsk krai, Lebed, RF/Sr/7; (11) Krasnoyarsk krai, Malaya Varlamovka R., RF/To/6; (12) Krasnoyarsk krai, Mirny, right bank of the Yenisei R., RF/To/8; (13) Krasnoyarsk krai, Mirny, left bank of the Yenisei R., RF/No/7; (14) Krasnoyarsk krai, Sarchikha R., RF/No/7; (15) Altai Rep., Gorno-Altaysk, RF/To/1; (16) Altai Rep., Lake Teletskoye, RF/To/4; (17) Kemerovo oblast, near Kemerovo, RF/To/5; (18) Altai Rep., Kyrlyk, RF/To/1; (19) Altai krai, Topolnoe v., RF/To/1; (20) Altai krai, Soloneshnoe v., RF/To/7; (21) Novosibirsk oblast, near Novosibirsk, RF/No/3; (22) Novosibirsk oblast, Berdsk, RF/No/5; (23) Novosibirsk oblast, Karasuk, RF/No/1; (24) Omsk oblast, Omsk, RF/No/2; (25) Tyumen oblast, Tyumen, RF/No/1; (26) Komi Rep., Pechoro-Ilych National Reserve, Janyupuner Ridge, RF/Se/6; (27) Komi Rep., Pechoro-Ilych National Reserve, Kybla-Kyrta, RF/Se/4; (28) Komi Rep., Pechoro-Ilych National Reserve, Garevka, RF/Se/4; (29) Komi Rep., Pechoro-Ilych National Reserve, Yaksha, RF/Se/7; (30) Komi Rep., Storozhevsk, RF/So/7; (31) Komi Rep., Priozerny, Biological Station of Syktyvkar State University, RF/So/3; (32) Komi Rep., Dan, the left bank of the Lokchim R., RF/Ma/1; (33) Komi Rep., Ezhva, RF/So/2; (34) Komi Rep., Glotovo, RF/Ma/1; (35) Nenets Autonomous Okrug, Lake Janemdeito, RF/Kr-Pt?/9; (36) Komi Rep., Koslan, RF/Ma/2; (37) Arkhangelsk oblast, Voshgora, right bank of the Mezen R., RF/Pt/2; (38) Arkhangelsk oblast, Leshukonsky distr., Kobylja, RF/Pt/Ma?/6; (39) Arkhangelsk oblast, Ust-Nizemye, RF/Kr/4; (40) Komi Rep., Malaya Sluda, RF/So/4; (41) Mari El Rep., Bolshaya Kokshaga National State Reserve, RF/So/9; (42) Saratov oblast, Dyakovka, RF/So/13; (43) Lipetsk, RF/Ne/3; (44) Voronezh oblast, near Voronezh, RF/Ne/1; (45) Rostov oblast, Kagalnik, RF (JN984114, 119)/Ne/3; (46) Dnepropetrovsk oblast, Zhdanovka, Ukraine/Ne?/3; (47) Tver oblast, Kru-titsy, RF/Mo/18; (48) Bryansk oblast, Savichki, RF/Ne/10; (49) Tver oblast, Belkovo, RF (JN984060)/Wd/1; (50) Tver oblast, Rogovo, RF (JN984089)/Mo/1; (51) Tver oblast, Lake Seliger, RF/Mo-Sl?/7; (52) Murmansk oblast, Moscow State University White Sea Biological Station, RF/Py/5; (53) Karelia rep., Kem, RF/Am/15; (54) Karelia Rep., Segezha, RF/Am/11; (55) Karelia Rep., Sortavala, RF/Il/8; (56) Leningrad oblast, Priozersk, RF/Lm/6; (57) Oulu, Finland (GQ374429-435)/Ku/7; (58) Lappe, Finland (GQ374425)/Sa/1; (59) Etela-Suomi, Finland (GQ374426-428)/Ka/3; (60) Uusima, Finland (AJ000416)/Ka/1; (61) Pelister, Macedonia (AJ312031)/Pe/1; (62) Velence, Hungary (AJ312028)/Ul/1; (63) Gyor-Moson-Sopron, Hungary (GQ374416-421)/Ul/6; (64) Zala, Hungary (GQ374412, 437, 436, HQ008361)/Ul/4; (65) Madesjo, near Nybro, Lan Kalmar, Sweden (AJ245993)/Ha/1; (66) Bassins, Vaud, Switzerland (AJ000415)/Vd/1; (67) Cordon, Haute-Savoie, France (AJ312032)/Co/1; (68) Champéry, Valais, Switzerland (AJ312033)/Br/1; (69–88) United Kingdom (AJ312030, EU121233-EU121281)/Ab, Ox/96.

the cytochrome *b* (*cytb*) gene from 800 to 1140 bp from 88 localities were analyzed. To estimate the rate of molecular evolution of the *Sorex* genus, we used the *cytb* sequences deposited in Genbank: *S. antinorii* (HQ901808, 822–839, 841, 842, 851–854, 856–858, 860, 861, 872–882, 884–889, 905–910), *S. asper* (AJ000425, 426, HM992658–677), *S. minutus* (AF445038–041, AJ535399–419, 425–446, 450–454, D85358, FJ623774–802, 805, 808–837, 840–845, 850–854, 856–861, 867–878, 883, 884, 887–892, GQ272502, 503, GQ494305–350, GU827394, JF318995, JF510322–358), *S. minutissimus* (AB028584–587, GU223625, 629, 631, 632, 636, 637, HM002678, 680, 682, 683, 687, 694, 702, 704, 705), and *S. yukonicus* (GU223639–665). Additionally, sequences were obtained for *S. caecutiens* (N = 31) and *S. isodon* (N = 23) from Siberia.

#### DNA Isolation, Amplification, and Sequencing

The DNA was extracted from ethanol preserved tissues, using standard protocol of proteinase K digestion, phenol-chloroform deproteinization, and isopropanol precipitation. The entire cytochrome *b* gene (1140 bp) for *S. araneus*, *S. isodon*, and *S. caecutiens* was amplified as described by Bannikova and Lebedev [15]. In addition, one internal *S. araneus* specific primer was developed: 5'-AGTTGTCTGG-GTCTCCTAATAAGTC-3' (SarH760). PCR products were visualized in 1% agarose gel and purified by precipitation with NH<sub>4</sub>EtOH or the Diatom DNA PCR Clean-Up kit.

The sequencing was performed with internal primers L395, H589st [15], and SarH760 and with ABI PRISM® BigDye™ Terminator v. 3.1; the products

were subsequently analyzed with an Applied Biosystems 3730 DNA Analyzer automatic sequencer.

### *Phylogeographic Analysis*

Genetic distances between the samples ( $F_{st}$ , Net distances), the genetic diversity metrics (the nucleotide and haplotype diversity for the samples with  $N \geq 6$ ), mismatch distribution parameters, the expansion time ( $\tau$ ) and its confidence interval [16], and Tajima's [17] and Fu's [18] neutrality tests were calculated in Arlequin 3.1 [19].

We also conducted a principal coordinate analysis based on Net distances between large geographic samples [20, 21] (the samples were combined according to the regional pattern). Initially, the number of principal coordinates was equal to the number of samples. However, henceforward, we performed this analysis in two-dimensional space since 99.7% of the variance was described by the first two coordinates.

The geographical structuring of genetic variation was examined with the use of the spatial analysis of molecular variance SAMOVA [22] with the number of a priori defined groups ( $K$ ) varying in the range from 2 to 15, with 1000 steps in the simulated annealing process, with 1000 initial conditions, and with K2P as genetic distance. Samples with  $N < 3$  and island samples from Great Britain were excluded from the analysis.

The median-joining haplotype network was reconstructed in the Network 5.0 program, using the star contraction algorithm as recommended in the program user's guide. This algorithm identifies clusters of nodes resulting from local expansion and reduces the complexity of the network by shrinking these nodes back toward the founder nodes. We used only complete sequences of *cytb* (1140 bp, 336 sequences) for this analysis.

The spatial interpolation of the nucleotide and haplotype diversity was performed with the use of the inverse distance method [27] implemented by Qgis software [26] for the space of  $300 \times 300$  cells with cell size of  $0.4 \times 0.2$  and distance coefficient = 4.

The procedure of genetic landscape interpolation was performed with the use of the AIS program [28]. This method produces a three-dimensional graphical representation of genetic distance patterns across the landscape analyzed in the study and is helpful for the visualization of genetic heterogeneity. We used the "residual genetic distances" derived from the linear regression of all pairwise genetic distances on geographical distances as recommended by the software developers. The inverse distance weighted procedure with power of 0.1 and  $50 \times 50$  grid was used for the interpolation. The coordinates of localities were transformed using the principal coordinates analysis to change the coordinate space to Euclidean. The isolation by distance was tested using the Mantel test [29]

for matrices of genetic and geographic distances (1000 iterations).

The general pattern of demographic history was described with the use of the Bayesian Skyline Plot (BSP) method [30], which allows one to estimate the change in the effective population size over time. The analysis was performed in Beast 1.8.1 software [31] with the MCMC chain length of up to  $10^7$  steps and 10 groups. In accordance with the results of the model choice procedure performed in Treefinder software [32] we used the following models of the nucleotide sequence evolution: K80+I for the first, HKY+I for the second, and TrN+G for the third codon positions. The molecular clock hypothesis (constant substitutions rate in *S. araneus*) was tested using the baseML module of the Paml program [33] and was rejected for our data (the likelihood ratio test [34],  $p < 0.05$ ). All analyses were carried out at least twice; chains were considered to reach convergence when ESS was higher than 200. The visualization of BSP analysis was performed in Tracer 1.6 software [35].

### *Estimate of Molecular Evolution Rate*

To assess the molecular evolution rate, we used the following events in *Sorex* evolution:

*S. antinorii* expansion in the western and north-western Alps.

The species is believed to have formed allopatrically as a result of isolation in the Apennine Peninsula during the Late Pleistocene glaciation [36–39]. The colonization of the western Alps valleys presumably occurred at the Pleistocene–Holocene boundary (15000–18000 years ago) [40]. Later, *S. antinorii* appeared in the northwestern Alps—this area could have been colonized through the Simplon and Grand St Bernard passes [40–42], for which the minimum time of deglaciation is 12 850 years ago [43, 45]. This time we assume to be the hard upper limit of the expansion of the northwestern population of *S. antinorii*.

### *Expansion of Several Sorex minutus Populations*

Several phylogroups identified in the *S. minutus* range correspond to the LGM refugia of this species [45]. The north-central phylogroup dispersed from the hypothetical northern refugium in the postglacial period, i.e., not earlier than 11.5 ka, after the end of the Younger Dryas [46]. The expansion of the central clade in mainland England seemingly occurred at the same time [47]. The upper limit of this event is about 8 ka—the time of the Pas de Calais land bridge inundation. The Inner Hebrides were colonized around the same time. The colonization of Ireland by the Pyrenean phylogroup of *S. minutus* probably occurred simultaneously with the colonization of the island by humans about 6 ka [48, 49].

**Table 1.** The molecular evolution rate for several species of the genus *Sorex* estimated using mismatch distribution parameters

Species	Group	<i>N</i>	$\tau$ (95% CI)	Rate (95% CI)
<i>S. antinorii</i>	Northwestern Alps	64	2.6 (2.0–3.3)	10.11 (7.55–12.70)
	Western Alps	47	4.4 (1.7–6.4)	13.18 (5.05–19.20)
<i>S. minutus</i>	North clade	90	5.9 (4.9–8.8)	23.00 (19.03–34.28)
	Eastern European clade	20	4.9 (3.1–6.4)	20.04 (12.72–26.41)
	Central Great Britain	88	6.0 (5.2–7.3)	26.42 (22.91–32.16)
	Ireland	57	2.9 (2.1–3.6)	21.58 (15.60–26.69)
	Inner Hebrides	38	5.7 (2.3–12.4)	22.25 (9.01–48.50)
<i>S. minutissimus</i>		29	1.9 (0.3–3.8)	7.18 (1.07–14.07)
<i>S. yukonicus</i>		20	2.3 (1.0–3.6)	8.02 (3.64–12.37)
<i>S. caecutiens</i>		31	5.8 (4.0–7.2)	30.40 (21.02–37.49)
<i>S. isodon</i>		23	3.8 (2.3–6.8)	19.79 (12.10–35.63)
<i>S. asper</i>		22	5.3 (2.2–11.9)	20.65 (8.45–46.09)

*The Expansion of the Siberian Group  
of S. minutissimus and S. yukonicus in Alaska*

The expansion of *S. yukonicus* in Alaska occurred in the Late Pleistocene–Holocene as a result of the dispersal of the eastern group of *S. minutissimus* [50]. The lower limit of this event is 12.9 ka—a time when high-shrub tundra and deciduous forests started to emerge in the central part of Beringia [51]. The time of the flooding of the Bering land bridge, which occurred no later than 11 ka, can be seen as the upper limit of this event. The expansion of the Siberian group of *S. minutissimus* took place between the end of the Sarmatian glaciation (15 ka) and the expansion of *S. yukonicus* (12.9 ka).

*The Expansion of S. caecutiens  
and S. isodon Mainland Populations*

According to our hypothesis, the colonization of the contemporary range by these species occurred after the last glaciation and was caused by the expansion of the taiga zone in Siberia [52, 53].

The mainland group of *S. caecutiens* increased in number not earlier than the start of the boreal period (10–9 ka) and the more thermophilic *S. isodon* expanded its range not earlier than 8 ka.

*The Expansion of S. asper*

With regard to known range dynamics of species co-occurring with *S. asper* [54, 55] (contraction in LGM and expansion in the postglacial period), we can assume that the Tien Shan shrew experienced population growth in the Early Holocene.

We accepted the median of estimates for the six species as an estimate of the evolution rate for the genus *Sorex*. In the cases where several species estimates were obtained (*S. minutus*, *S. antinorii*, *S. minu-*

*tissimus/yukonicus*), the final rate was also calculated as their median.

To assess the error of the rate estimate, we used a procedure that combined parametric and nonparametric bootstrap. To account for the error of  $\tau$ , we modeled it as a lognormally distributed random variable with the mean and variance as estimated in Arlequin 3.1 [19] for each species. On the basis of these distributions, we generated pseudoreplicates using nonparametric bootstrap, which were used to calculate the error of the final estimate of the rate in *Sorex*.

The errors of time estimates for individual events in the history of *S. araneus* were assessed on the basis of the errors of rate and  $\tau$  using the delta method [56].

The time of divergence among *S. araneus* populations was assessed on the basis of the IM (isolation+migration) model [57] with IMa2 software [58]. Considering the fact that the diversity in the ancestral population is difficult to estimate on the basis of data only on mtDNA, we had to simplify the model by excluding the migration parameter.

## RESULTS

*Estimate of the Rate of Molecular Evolution in Sorex*

In all examined populations of *Sorex* species, there is a signature of recent expansion; Tajima's *D* and Fu's *F* statistics are significantly negative, the mismatch distribution is unimodal and fits the expectation of the sudden expansion model.

The estimates of the expansions times ( $\tau$ ) and the substitution rate are given in Table 1.

Our estimate of the substitution rate applicable for recent events in the evolution of *Sorex* is equal to  $18.4 \pm 4.6\%$  per My. This value is comparable to the population-level estimate calculated by Bannikova et al [59] (substitution rate = 13.9%).



### *Phylogeography of the Common Shrew*

The results of the principle coordinate analysis based on the net distance matrix indicate the isolated position of the Hungarian sample, while the Siberian samples clustered separately from most of the European samples (Fig. 2a). The only exception is the sample from southern Karelia, which appears to be closer to Siberian shrews, while the sample from the vicinity of Novosibirsk occupies an intermediate position between the European and Siberian clusters. The topology of the median-joining network (Fig. 3) is structurally close to the starlike one; however, it differs in having not only the main central node but also an additional node, which includes the majority of haplotypes occurring in Siberia (Fig. 3—S). This “Siberian” node also includes the haplotypes originating from eastern Fennoscandia (samples 54–57, 59, 60). The proportion of “Siberian” haplotypes in Karelian samples decreases northwards (Fig. 4b; Spearman rank correlation,  $r = -0.83$ ,  $p < 0.05$ ). The central node contains haplotypes from most of the range: Western and Eastern Europe and Fennoscandia, and it includes also a small fraction of haplotypes from Siberia. Only one sample from western Fennoscandia (sample no. 65) has a “European” haplotype. Western Siberia is dominated by haplotypes of the “European” group, their proportion decreasing eastwards (Fig. 4a).

Haplotypes originating from European populations are found mainly in the central part of the network. Most of the haplotypes from Great Britain cluster in a separate group (Fig. 3—UK), which is close to the central haplotype. The exception is the set of haplotypes from the islands of Skye and Raassay (SK, RS), which form a separate branch. Most of the specimens from Hungary have haplotypes that are connected by relatively long branches (5–7 substitutions from the central haplotype). However, one of these haplotypes was found in the Nenets Autonomous Okrug (Fig. 3—HN).

The spatial analysis of molecular variance (SAMOVA, Fig. 2b) demonstrated that the among-group  $F$  statistics ( $F_{ct}$ ) and among-group variance component ( $V_a$ ) reached a maximum at  $K$  (number of groups) = 2. The subdivision into two groups resulted in separation of one of the Hungarian samples (no. 64). Since we were interested in the elucidation of the range-wide structure, we repeated the analysis with the Hungarian samples (nos. 63, 64) excluded. In this case  $V_a$  is at a maximum for  $K = 3$ , while  $F_{ct}$  reaches a plateau at  $K = 4$ , showing negligible changes for  $K = 5, 6, 7$ . The maximum increase in both indicators corresponds to  $K = 3$ . At this value of  $K$ , the Siberian and European samples cluster into separate groups; the third group includes one of the samples from southeastern Fennoscandia (no. 55), in which both “Siberian” and “European” haplotypes are found. The sample from Berdsk (no. 22), which contains “European” haplotypes, clusters with European samples. If  $K$  is set equal to 4, the fourth

group corresponds to the sample from the vicinity of Novosibirsk (no. 21).

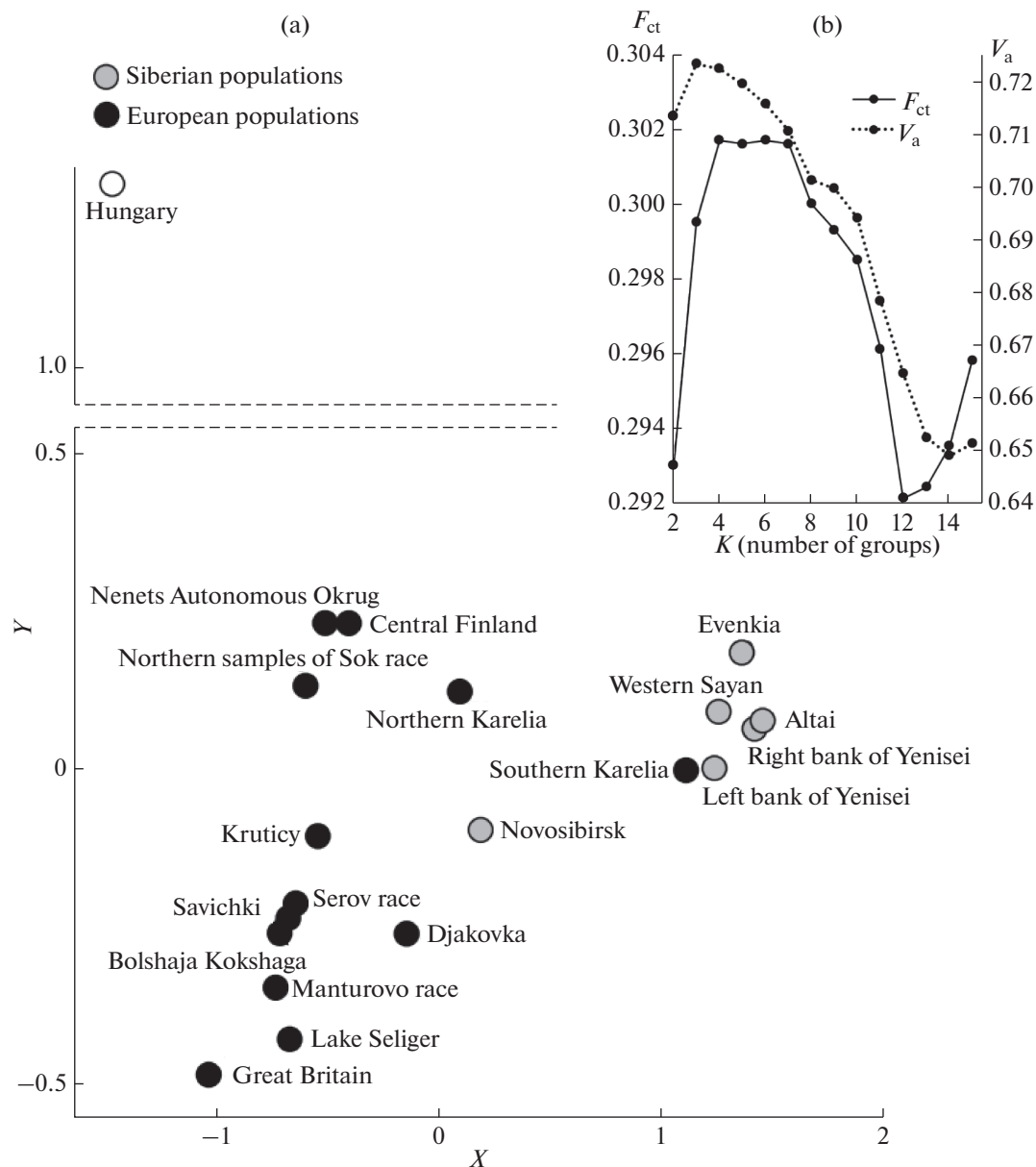
The values of diversity indices (number of haplotypes, gene diversity, nucleotide diversity) for individual samples and geographic regions are given in Table 2. The results of the genetic landscape interpolation are presented in Figs. 5a and 5b. The Siberian populations are shown to be less variable than European ones in both measures. Nucleotide diversity demonstrates a tendency to decrease eastwards (Spearman rank correlation,  $r = -0.52$ ,  $p < 0.05$ ); the highest level of variation is observed in the southwestern part of the range. For haplotype diversity, this tendency is nonsignificant ( $r = -0.33$ ,  $p = 0.12$ ). The lowest values of both indices are found in the population of the right bank of the middle Yenisei.

In the European part of the range, the lowest values of haplotype and nucleotide diversity was revealed in the samples of eastern Fennoscandia. In Siberia, the highest haplotype diversity is found in the Sayan range, while the nucleotide diversity was expectedly higher in the population where haplotypes of the European group co-occur with the Siberian ones. A significant decrease in genetic diversity in Siberia is also evident from the genetic landscape reconstructions. In the European part of the range, the highest genetic heterogeneity is observed in the southwestern part, while being lower in the northern regions (Fig. 5c). The correlation between the genetic and geographic distances, which is indicative of isolation by distance, is low, albeit significant (Mantel test,  $r = 0.19$ ,  $p < 0.001$ ).

### *Demographic History of the Common Shrew*

The reconstruction of the demographic history based on the total sample with the use of the BSP method (Fig. 6a) indicates rapid population growth at 7–11 ka. A similar time frame of demographic expansion was inferred for European populations (Fig. 6a); in contrast to that, the growth of Siberian populations occurred significantly later (5–2 ka; Fig. 6c). The expansion of the “European” haplotypic lineage in Europe happened simultaneously with the expansion of the entire species (5–10 ka), while the expansion of the “Siberian” haplotypic lineage in Siberia started not earlier than 3 ka (Figs. 6d, 6e). The results of Tajima’s and Fu’s neutrality tests, the mismatch distribution parameters, the values of  $\tau$  (relative time since expansion in substitutions per gene), and the absolute times of expansion and their standard errors calculated with and without correction for the molecular rate error are given in Table 3.

Significantly negative values of Tajima’s  $D$  and Fu’s  $S$  statistics and the unimodal mismatch distributions also suggest a recent rapid expansion of the common shrew across its entire range, including Europe and Siberia. The hypothesis of the population size

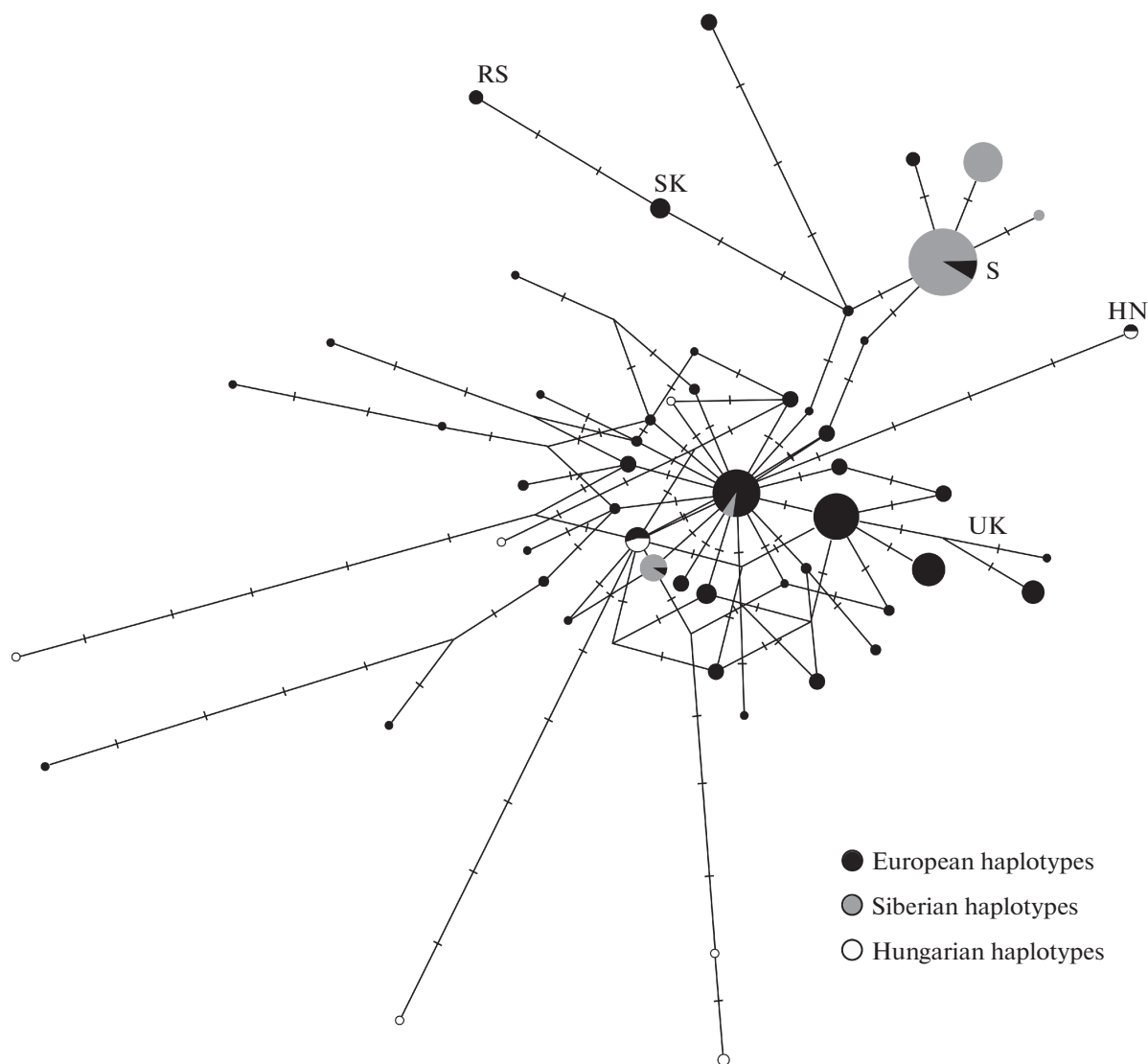


**Fig. 2.** (a) Results of principal coordinate analysis based on Net distances between samples (PC1 versus PC2); (b) Plot of among-group F statistics ( $F_{ct}$ ) and among-group variance component ( $V_a$ ) against the number of groups ( $K$ ) (SAMOVA).

constancy is rejected by all neutrality tests for all of the large regional samples such as those from eastern Fennoscandia, Great Britain, and Western Sayan and for the sample of the Serov chromosomal race. For smaller samples, the results of the neutrality tests are more ambiguous.

Calculating the time of expansion in separate populations with high precision is impossible because of the large error of the molecular rate estimate. However, the obtained confidence ranges are incompatible with the pre-LGM expansion. Population growth began not earlier than 20 ka; the mean estimate corresponds to Bolling–Allerød warming (12.8 (20.5–7.5) ka).

A similar estimate is inferred for the European part of the range (13.4 (20.8–7.8) ka). The mean estimate for Siberia corresponds to the Boreal period of the Holocene (8.8 (28.4–1.6) ka). The wide confidence range can be related to the co-occurrence of the “European” and “Siberian” haplotypic lineages in Siberia. The expansion of the haplotypes that belong to the “Siberian” lineage could have taken place in the Early to Middle Holocene, which is significantly younger than the Late Pleistocene expansion of the “European” lineage in Europe (6.5 (21.5–1.1) ka and 12.1 (19.1–7.1) ka, respectively). In individual European populations, the expansion took place within the interval between 13 ka



**Fig. 3.** Median-joining haplotypes network for *cytb* of *S. araneus* (S—"Siberian" node, UK—haplotypes from Great Britain, SK—haplotypes from Skye Island, RS—haplotypes from Raassay Island, HN—the haplotype shared between Hungary and Nenets Autonomous Okrug).

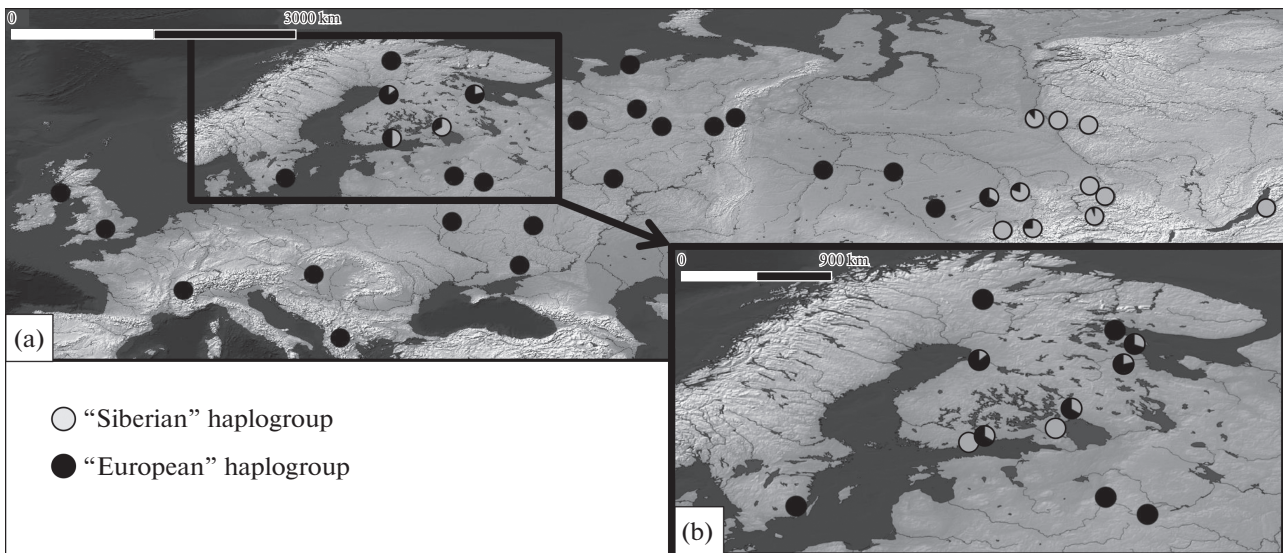
(southern part of Eastern Europe) and 9 ka (Fennoscandia). In Siberia, the earliest expansion is inferred for the populations of Western Sayan and the west bank of the Yenisei (around 8.0–8.5 ka). Our data suggest that the populations of the northeast of the Siberian range expanded at 2 ka at the earliest.

All ages of divergence among individual populations as estimated by IMA fall within postglacial times. The earliest divergence among the examined population pairs was found between the populations of Poland and Hungary: 13.5 (7–23) ka. The splits between the populations of Poland and northeastern Europe as well as between northeastern Europe and Siberia correspond to the Pleistocene–Holocene boundary (10.4 (5.7–17.6) ka and 9.4 (5.1–15.9) Kya, respectively. The population of Fennoscandia cer-

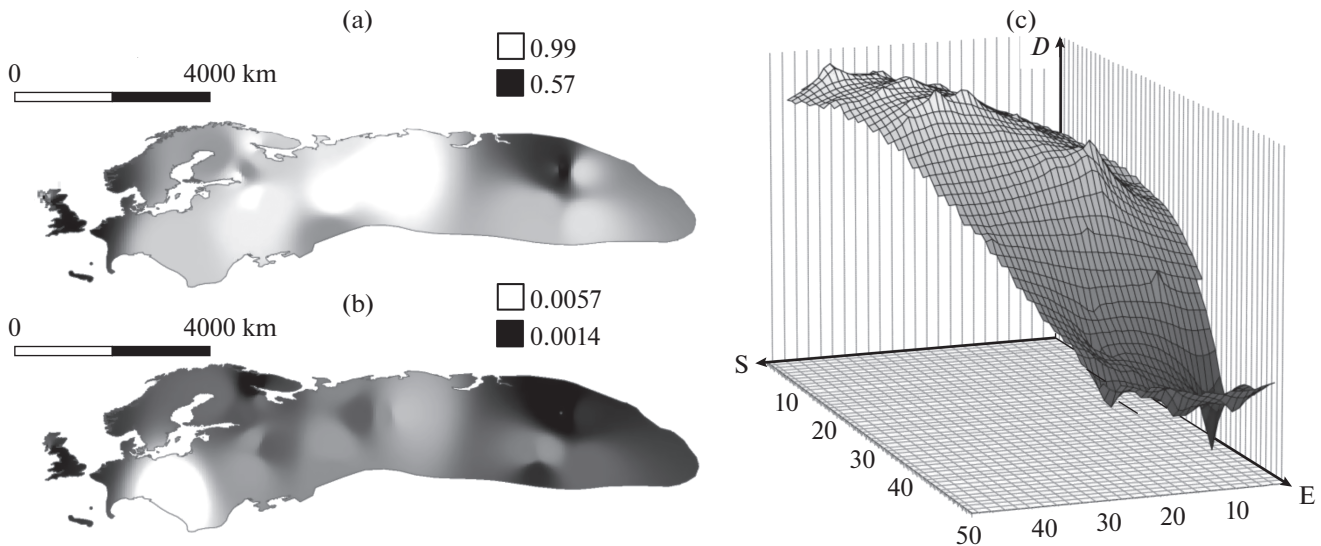
tainly separated from the populations of northeastern Europe and Siberia after the Bølling–Allerød interval (6.9 (3.7–11.9) ka and 7.2 (3.9–12.1) ka, respectively). The populations of the Moscow chromosomal race diverged from the southern populations of the Sok race and northeastern Europe at approximately the same time (6.4 (3.3–11.2) ka and 7.8 (4.1–13.4) ka, respectively). The ages of splits of populations of Poland from those of the Alps and Great Britain are estimated at 9.4 (5.1–15.9) ka and 8.0 (4.4–13.4) ka, respectively.

## DISCUSSION

The pattern of mtDNA diversity indicates rapid and relatively recent expansion across the entire range



**Fig. 4.** (a) Distribution of “Siberian” and “European” haplotypes across the *S. araneus* range. (b) Distribution of “Siberian” and “European” haplotypes in Fennoscandia.



**Fig. 5.** The results of spatial interpolation of the nucleotide (a) and haplotype (b) diversity. The genetic landscape—a three-dimensional graphical representation of the genetic distance patterns across the landscape ( $D$ — $p$ -distance).

of the species. This is also confirmed by the starlike topology of the haplotype network with its central haplotype occurring in numerous localities, as well as high and significant values of the tests for selective neutrality and unimodal mismatch distribution.

The molecular dating of *S. araneus* expansion based on BSP and the sudden expansion model attributes this event to postglacial time. In this case, the lack of structuring in the mtDNA diversity, which has been repeatedly demonstrated [10, 7, etc.], is not surprising.

The presence of common haplotypes in such geographically removed and disjunct localities as Hungary and the Nenets Autonomous Okrug, the Scottish Islands, and the Northern Urals indicates a significant degree of retention of ancestral polymorphism and genetic homogeneity in the entire range of the species, as a consequence of rapid and recent expansion from a single source. The lack of clearly defined haplogroups in the median haplotype network also points to a weak phylogeographical structure in *S. araneus*, which does not mean it is completely absent. The subdivision of *S. araneus* into “Siberian” and “European” hap-



**Table 2.** Genetic diversity in *Sorex araneus* samples

Sample (no.)	<i>N</i>	nH	<i>H</i> ± sd	$\pi$ ± sd ( $10^{-2}$ )
Krasnoyarsk krai, Bagazul R. (4)	13	10	0.96 ± 0.04	0.30 ± 0.19
Left bank of the Yenisei R. (13, 14)	11	7	0.87 ± 0.09	0.24 ± 0.17
Right bank of the Yenisei R. (9–12)	21	10	0.81 ± 0.08	0.08 ± 0.06
Novosibirsk oblast (21, 22)	6	5	0.93 ± 0.12	0.40 ± 0.26
Altai (15, 16, 18–20)	14	9	0.92 ± 0.05	0.22 ± 0.14
Western Sayan (3–6)	19	12	0.90 ± 0.06	0.28 ± 0.17
European haplotypes in Siberia	12	7	0.86 ± 0.08	0.18 ± 0.12
Siberian haplotypes in Siberia	76	28	0.82 ± 0.04	0.19 ± 0.12
Siberia (1–25)	88	35	0.87 ± 0.03	0.27 ± 0.16
NAO (35)	9	8	0.97 ± 0.06	0.38 ± 0.24
Northern Urals (26,27,28)	20	20	1.00 ± 0.02	0.45 ± 0.25
NE Europe, Sok race (30, 31, 33)	7	7	1.00 ± 0.08	0.39 ± 0.25
NE Europe (26–40)	54	45	0.99 ± 0.01	0.42 ± 0.23
Mari El Rep., Bolshaya Kokshaga National Reserve (41)	9	9	1.00 ± 0.05	0.44 ± 0.27
Tver oblast, Krutitsy village (47)	10	9	0.98 ± 0.05	0.27 ± 0.18
Bryansk oblast, Savichki village (48)	10	9	0.98 ± 0.05	0.45 ± 0.27
Center of Eastern Europe (41, 47–51)	32	32	1.00 ± 0.01	0.42 ± 0.24
North Karelia (53, 54)	26	24	0.99 ± 0.013	0.26 ± 0.16
South Karelia (55, 56)	14	11	0.96 ± 0.045	0.30 ± 0.19
Eastern Fennoscandia (52–60)	47	29	0.96 ± 0.02	0.33 ± 0.19
European haplotypes in Eastern Fennoscandia	29	16	0.93 ± 0.03	0.23 ± 0.14
Saratov oblast, Dyakovka village (42)	6	4	0.80 ± 0.17	0.40 ± 0.27
South of Eastern Europe (42, 45, 46)	12	9	0.94 ± 0.06	0.51 ± 0.30
Hungary (62–64)	13	9	0.94 ± 0.05	0.63 ± 0.35
Europe (26–68)	166	126	0.99 ± 0.003	0.44 ± 0.24
European haplotypes in Europe	148	113	0.99 ± 0.004	0.44 ± 0.24
Siberian haplotypes in Europe	18	13	0.90 ± 0.07	0.17 ± 0.11
Siberian haplotypes (all)	94	40	0.84 ± 0.04	0.20 ± 0.12
European haplotypes (all)	160	119	0.99 ± 0.003	0.43 ± 0.23
The whole sample	254	161	0.98 ± 0.004	0.46 ± 0.25

logroups observed in the haplotype network is also supported by the analysis of intergroup genetic distances by PCA and SAMOVA.

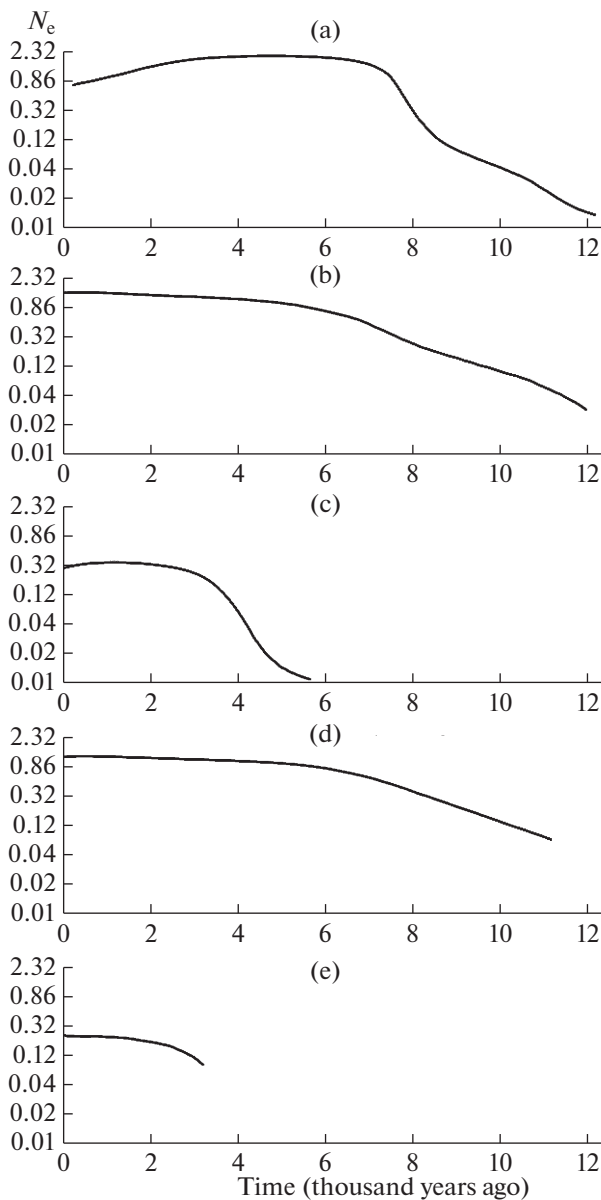
The European population of *S. araneus* shows a strong signature of recent and rapid population growth. According to our results, this event occurred at the end of Pleistocene, concurrently with the expansion across the entire range. The mismatch distribution and the BSP data for the Siberian samples suggest that the expansion in the Siberian part of the range occurred later, probably in the Holocene.

The British Isles are a special case within the “European” haplogroup. According to our dating, the time of the expansion of the common shrew in the north of Great Britain is 9.8 (5.1–17.4) ka. According to the paleogeographic and paleoclimatic reconstructions,

the expansion of *S. araneus* in Great Britain could have occurred between the end of Younger Dryas (11 ka) and the time of flooding of the land bridge between Great Britain and Europe (Doggerland, 8 ka) [60, 61], which is consistent with our estimate of the molecular evolution rate.

High nucleotide diversity, high intragroup distances, and relatively longer branches in the haplotype network in the south of the European part of the range indicate the proximity of this area to the refugium or at least point to the fact that it was colonized from a source with a high effective number.

In particular, among the samples studied, only the Hungarian samples demonstrate a significant rejection of the sudden expansion model.



**Fig. 6.** Demographic history reconstruction using Bayesian Skyline plot (BSP) for (a) the whole sample, (b) European samples, (c) Siberian samples, (d) European haplotypes in Europe, and (e) Siberian haplotypes in Siberia.

Combined with the insignificant values of Tajima's and Fu's tests, this indicates that the Hungarian population stays stable and does not grow for long periods of time. A relatively old age of the Hungarian population of *S. araneus* is also supported by the acrocentric karyotype of Ulm race [38], to which Hungarian populations belong. Many authors believe that populations that subsequently colonized the greater part of Europe could have been preserved during the LGM in southeastern refugia—the Balkans or in the area between the Black Sea coast and the Carpathian Mountains [5, 9, 36, 37, 62]. In our view, the hypoth-

esis according to which *S. araneus* survived during the LGM in a single refugium located in the Balkans or the Carpathian Mountains is realistic and justifies a dedicated study.

According to our estimate, the expansion of *S. araneus* in the south of Russia occurred in the Allerød warm period simultaneously to the increase in the entire species population, while the populations of the center and the north of European Russia increased in the Holocene. The high haplotype diversity together with relatively low nucleotide diversity in the central and the northern populations of European Russia points to the rapid expansion in these areas from a source with low effective number [63].

According to the hypothesis based on the karyotype distribution data, the refugium from which Eastern Europe, Fennoscandia, and Western Siberia were colonized was located in the Southern Urals. In addition, the Serov race distributed in the Urals is sometimes considered as the ancestral of the West Siberian and Scandinavian races [6, 64]. A relatively high diversity of the Serov race samples from the Northern Urals may account for a long period of stability of the effective number in populations of this region. At the same time, the demographic analysis shows a rapid expansion at the Pleistocene–Holocene boundary (about 12 ka).

This does not contradict the hypothesis of the Southern Ural refugium, but neither does it support the assumption that the refugium existed during the LGM. Potentially, the populations of *S. araneus* persisted in the Southern Urals during the Younger Dryas cold episode. According to paleontological data, the fossils of *S. araneus* disappeared from the Southern Ural sediments in the Late Holocene (in LGM). *S. tundrensis* became a dominant species in the *Sorex* community during that time and it remained such until the end of the Preboreal period of the Holocene. The remains of *S. araneus* reappeared in the Allerød warming and became dominant in the Atlantic period of the Holocene at the earliest [65].

We do not have a clear explanation of the distribution of genetic diversity in Fennoscandia. The study of the control region of mtDNA diversity in that area shows [66] that *S. araneus* could have colonized deglaciated Fennoscandia from Utland and through Western Europe, and this is supported by the genetic uniqueness of South Finland populations. The pattern of *cytb* diversity in Karelia follows the same trend since a large proportion of *S. araneus* from South Karelia have haplotypes of the Siberian group. However, we did not find any evidence that the source of the Fennoscandian population is located in the northeastern Europe. The Siberian haplotypes were found neither in the northeastern European, nor in the Urals, nor in other European samples. This pattern can be explained by the genetic drift and an accidental fixation of one of the ancestral haplotypes as a result of the rapid advance of the expansion wave. Siberian haplotypes

**Table 3.** *Sorex araneus* expansion time (in thousand years and *cytb* substitution numbers), Tajima and Fu neutrality tests values, mismatch distribution parameters for the whole sample and several regional samples

	$\tau$ (95% CI)	Time (95% CI) ka	Tajima's D	Fu's F	SSD	Ragg. index
The whole sample	5.37(4.31–5.77)	12.81 (7.48–20.53)	–2.59***	–24.85***	0.001	0.014
Europe (26–88)	5.51(4.97–5.94)	13.14 (7.78–20.83)	–2.61***	–25.32***	0.004**	0.013
South of E. Europe (42–46)	5.49(2.35–7.58)	13.10 (5.77–25.74)	–1.12	–1.82***	0.011	0.045
Center of E. Europe (47–51)	4.99(2.70–6.02)	11.91 (6.08–21.07)	–2.42***	–25.63***	0.01	0.032
North of E. Europe (30–40)	4.12(2.60–5.31)	9.83 (5.17–17.03)	–2.43***	–25.70***	0.003	0.026
European hapl. in Europe	5.16(4.50–5.70)	12.31 (7.25–19.62)	–2.57***	–25.39***	0.002	0.02
Siberian hapl. in Europe	1.97(0.96–3.05)	4.69 (2.08–9.17)	–2.21***	–10.88***	0.004	0.073
Fennoscandia (52–60)	3.73(1.49–6.98)	8.89 (3.28–19.56)	–1.90***	–21.43***	0.002	0.013
European hapl. in Fennoscandia	2.85(0.95–4.39)	6.79 (2.52–14.89)	–1.88**	–8.03***	0	0.022
Northern Urals (26–28)	4.91(3.02–6.46)	11.72 (6.07–20.55)	–1.76**	–14.54***	0.003	0.023
Great Britain (69–88)	4.14(3.08–6.68)	9.88 (5.10–17.36)	–2.14***	–25.40***	0.001	0.011
Hungary (62–64)			–1.43	–1.71	0.060***	0.145***
Siberia (1–25)	3.69(0.42–6.84)	8.80 (1.59–28.36)	–2.33***	–26.25***	0.002	0.009
Left bank of the Yenisei R. (13, 14)	3.41(0.90–6.51)	8.15 (2.39–20.55)	–1.22	–0.58	0.014	0.043
Right bank of the Yenisei R. (9–12)	0.15 (0.11–5.35)	0.35 (0.03–1.51)	–2.25***	–5.62***	0.019	0.108
European hapl. in Siberia	2.68(0.85–4.85)	6.40 (2.14–14.99)	–1.57	–2.49	0.028	0.121
Siberian hapl. in Siberia	2.72(0.30–5.10)	6.49 (1.13–21.25)	–2.45***	–24.71***	0.001	0.016
Western Sayan (3–6)	3.57(1.20–5.95)	8.52 (3.05–19.12)	–1.96**	–5.35***	0.006	0.032
Altai (15, 16, 18–20)	2.52(0.88–3.92)	6.00 (2.28–12.99)	–1.60	–3.93**	0.002	0.038
Siberian haplotypes	2.27(0.64–5.26)	5.41 (1.47–14.23)	–2.61***	–27.12***	0.003	0.022
European haplotypes	4.05(3.72–6.59)	9.66 (5.31–16.21)	–2.62***	–25.39***	0	0.013

could have been fixed during the colonization of the eastern part of the range independently.

Some authors suggested earlier that the eastern part of the *S. araneus* range was colonized by the Siberian karyogroup from an LGM refugium in the Altai-Sayan region [6].

Paleoclimatic reconstructions and the ecological niches modeling support the possibility of *S. araneus* preservation in refugia of the Altai-Sayan region during the LGM [67, 68]. However, the interpretation of paleontological data is not as straightforward.

A number of large-scale studies of Pleistocene deposits in the Altai region did not identify reliable *S. araneus* remains in the layers dated to MIS3 [69–71], the only exception being certain deposits of the Denisova cave, where this species was found in all horizons [69].

Our results do not support the Siberian refugium hypothesis [6] and do not allow us to suggest the Siberian origin of the “Siberian” group of haplotypes. Even the highest genetic diversity among the Siberian samples is slightly lower than the diversity in Central and Northern Europe. According to our data, the expansion of the Altai populations occurred at the end of LGM at the earliest, and the average of this estimate falls into the Subboreal period of the Holocene. The

expansion in the Western Sayan population occurred earlier, but even the rigorous estimate of the confidence interval places it after the LGM. According to the IMA results, the time of divergence of the Siberian and the European populations is definitely postglacial.

## CONCLUSIONS

The phylogeographic structure of *S. araneus* is defined by the presence of two haplogroups: the “European” (distributed from the western border of the species range to Western Siberia) and the “Siberian” (Central Siberia), which was also found in southern Fennoscandia, but was absent in northeastern Europe and the Northern Urals. The demographic analysis shows a rapid expansion of *S. araneus* and its European populations at the Holocene–Pleistocene boundary. In the Siberian part of the range, the expansion took place at a later time.

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of interests.** The authors declare that they have no conflict of interest.

**Statement on the welfare of animals.** This article does not contain any studies involving animals performed by any of the authors.

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SPELL: 1. deglaciation