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SYMPATRIC AREA OF *MYODES GLAREOLUS* AND *M. RUTILUS* (RODENTIA, CRICETIDAE): HISTORIC AND RECENT HYBRIDIZATION

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ABSTRACT

The bank vole (*Myodes glareolus*) and the northern red-backed vole (*M. rutilus*) are two phylogenetically close sylvatic species with a widely sympatric range (European part of Russia, Western Siberia). A significant number of *M. glareolus* with mitochondrial genome of *M. rutilus* was detected in this sympatry zone earlier and only one of the first generation hybrid (F1) was discovered. The aim of the present study is to assess the extent of modern hybridization and to analyze the possible conditions of interspecies hybridization between the voles. The cytochrome *b* gene sequences of *M. glareolus* (164) and *M. rutilus* (108) sampled in the sympatric area were studied. In order to identify the modern hybrids, 841 individuals of *M. glareolus* were analyzed with cytochrome *b* PCR-typing, two microsatellite loci and one nuclear gene (LCAT). The detected unique case of the hybridization between *M. glareolus* and *M. rutilus* in nature is evidence that it is a possible at present but rare event. According to findings in the Urals *M. glareolus* populations, the chances of modern hybridization in the depression phases were higher than those regardless of cycle phase. Interspecific hybridization between these vole species in the historical past may have occurred in the southern Urals refuge during the Last Glacial Maximum, at a low density of both species. A mass independent hybridization during the formation of the sympatry seems less likely.

Key words: hybridization, mitochondrial DNA introgression, *Myodes*, Rodentia, sympatric range

ЗОНА СИМПАТРИИ *MYODES GLAREOLUS* И *M. RUTILUS* (RODENTIA, CRICETIDAE): ДРЕВНЯЯ И СОВРЕМЕННАЯ ГИБРИДИЗАЦИЯ

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РЕЗЮМЕ

Европейская рыжая полевка (*Myodes glareolus*) и сибирская красная полевка (*M. rutilus*) два филогенетически близких лесных вида с широкой зоной симпатрии (Европейская часть России, Западная Сибирь). Значительное количество *M. glareolus* с митохондриальным геномом *M. rutilus* было обнаружено в зоне симпатрии ранее, но при этом был выявлен только один гибрид первого поколения (F1). Целью данного исследования является оценка масштабов современной гибридизации и анализ возможных условий межвидовой

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гибридизации между лесными полевками. Исследовались последовательности цитохрома b от 164 экземпляров *M. glareolus* и 108 экземпляров *M. rutilus* из зоны симпатрии. С помощью ПЦР-типирования цитохрома b, двух микросателлитных локусов и одного ядерного гена (LCAT) исследовано 841 экземпляров *M. glareolus*. На изученной территории F1 гибридов в дальнейшем обнаружено не было. Единственный выявленный случай гибридизации между *M. glareolus* и *M. rutilus* в природе свидетельствует о том, что гибридизация возможна в настоящее время, но крайне редка. Согласно результатам, полученным по уральским популяциям *M. glareolus*, шансы современной гибридизации выше в фазу депрессии численности, чем вне зависимости от фазы популяционного цикла. Межвидовая гибридизация между этими видами полевок в историческом прошлом, вероятно, произошла в рефугиуме на южном Урале во время последнего ледникового максимума, при низкой плотности обоих видов. Массовая независимая гибридизация при формировании симпатрии представляется менее вероятной.

Ключевые слова: гибридизация, интрогрессия митохондриальной ДНК, *Myodes*, Rodentia, симпатрический ареал

INTRODUCTION

The bank vole (*Myodes glareolus* Schreber, 1780) and the northern red-backed vole (*M. rutilus* Pallas, 1779) are closely related species of small forest rodents. The ranges of these species are highly overlapping and forming a wide sympatric zone in the European part of Russia and Western Siberia (Shenbrot and Krasnov 2005). In this zone, *M. glareolus* specimens with a mitochondrial (mt) genome of *M. rutilus* were detected and it was supposed that the appearance of such individuals could be explained as the consequence of an ancient hybridization (Tegelström 1987; Dekonenko et al. 2003; Deffontaine et al. 2005; Potapov et al. 2007; Abramson et al. 2009b). Potapov et al. (2007) suggested that the hybridization possibly occurred in the late Pleistocene, during Holocene together with the formation of the sympatric zone, and possibly in the Recent. However, this assumption was based on a limited material from both vole species with only cytochrome *b* gene (cyt *b*) being analyzed. In the previous study (Abramson et al. 2009b), the mtDNA PCR-typing of *M. glareolus* was used for detecting *M. glareolus* with mt genome of *M. rutilus*. The distribution of the mtDNA of *M. rutilus* in the bank vole populations may be traced from the southern Urals along the mountain range to the northern populations at the Kola Peninsula. However, the cited paper touched the issue of the hybridization superficially. The extension of the research material allowed us to consider the borders of the ancient hybridization zone in details and suggest several possible scenarios of interspecies hybrids appearance.

In the previous study (Abramson et al. 2009c), the technique of F1 hybrids detection was elaborated and one F1 hybrid was found in Visim State Biosphere Natural Reserve (VSBNR, the middle Urals). The hybrid had mt genome of *M. rutilus* (cyt *b*) and was heterozygous at one microsatellite locus and nuclear gene (LCAT, lecithin-cholesterol acyltransferase). The molar patterns of this hybrid displayed intermediate morphological features between *M. rutilus* and *M. glareolus* (Borodin et al. 2011). The specimen was caught in the trough phase of the population cycle.

In the present study we notably enlarged *M. glareolus* and *M. rutilus* samples and used some improved methods of hybrids detection in order to find out how often interspecies hybridization occur in nature at present and to analyze the possible conditions, which may lead to interspecies hybridization.

MATERIAL AND METHODS

The polymorphism of the cyt *b* gene fragment was analyzed in 164 bank voles and in 108 northern red-backed voles sampled throughout the sympatric range of these species (Fig. 1, Table 1). Ninety-three sequences of *M. glareolus* and 18 sequences of *M. rutilus* were taken from the previous study (Abramson et al. 2009b, Table 1). Thus, the 71 new sequences of *M. glareolus* from 17 sites and 90 new *M. rutilus* sequences from 26 sites were used here. The total genomic DNA was extracted from ethanol-preserved tissues following a salt extraction method (Miller et al. 1988). The cyt *b* was amplified with the primers (UCBU and LM) and protocols as was described earlier (Abramson et al. 2009b). The sequencing was carried out on ABI 3130 automated DNA analyzer (Applied Biosystems) using the manufacturer's protocols in both directions. Sequences were aligned using the Clustal W algorithm (Thompson et al. 1994) in BIOEDIT 7.0.5.3 (Hall 1999) and checked manually.

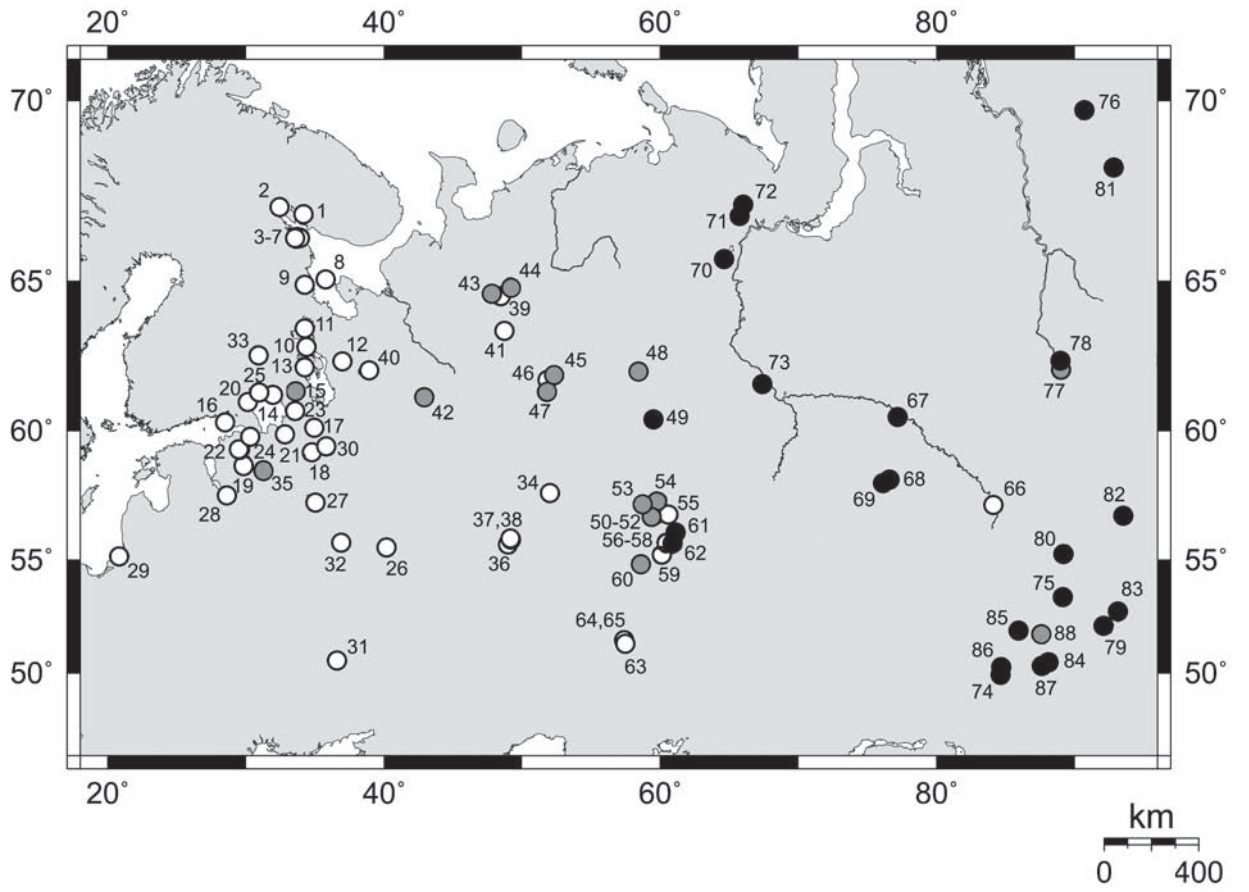

Fig. 1. Geographic distribution of *Myodes glareolus* (white circles), *M. rutilus* (black circles), both vole individuals from one location (grey circles) in the sympatric zone based on previously published results (Abramson et al. 2009b) and present study. The locality numbers correspond to those in Table 1. Sites no. 10, 11, 20, 21, 33, 34, 36–38, 56–60, 64, 65, 77 for *M. glareolus* and no. 35, 49, 60–62, 68– 88 for *M. rutilus* refer to new data (see Table 1).

In order to identify the bank voles with the alien mitotypes the PCR-typing method (fragments of the mtDNA cyt *b*) was employed, as designed earlier (Abramson et al. 2009b). The 316 *M. glareolus* samples were studied previously with primers combination (UCBU, 793 and 339) (Abramson et al. 2009b). Here we used new primers combination: the direct primer UCBU (working on both species) and two new reverse primers, each of them is specific only for one of these species: 544R (5' TTG ATT GTG TAG TAG GGG TGA AAG G 3') and 409G (5' GGA ATG CGA AGA ATC GTG TGA GA 3'). This mix produces PCR products, which are different in length in two species: 544 base pairs (bp) for *M. rutilus* (or *M. glareolus* with alien mtDNA) and 409 bp for *M. glareolus*. In total, 841 bank voles, 555 of which were sampled in 11 localities at the middle and southern Urals, were analyzed (Table 1). Logistic regression was used to calculate the dependence of the proportion of *M. glareolus* with mitotype of *M. rutilus* within southern and middle Urals populations on the latitude with standard modules of STATISTICA 6.0.

Additionally, we employed microsatellite loci in order to check the species identification and to detect F1 hybrid individuals among the bank voles with alien mt genome. The two of microsatellite loci (dinucleotide repeats, MsCg9 used earlier (Abramson et al. 2009c) and new locus LIST3-001 from Barker et al. 2005), among 14 tested (Gockel et al. 1997; Barker et al. 2005), were species-specific and were used in this study. The microsatellite loci were amplified individually in accordance with recommendations (Gocker et al. 1997). The microsatellite analysis was carried out on ALFexpress II DNA analyzer (Amersham Biosci-

Table 1. Map references (see details in Fig. 1), geographical locations, number of haplotypes, the total number of individuals used PCR-type method and GenBank Accession Numbers of the studied specimens of *Myodes rutilus* and *M. glareolus*. Published data (Abramson et al. 2009b) are labeled by an asterisk.

(Map ref.) Coordinates	*M. glareolus*	N samples of PCR-typing	*M. rutilus*
Murmansk Region			
(1) 66°59′N 34°11′E	h26 (EU035681*)	6	
(2) 67°11′N 32°25′E	h26 (EU035682*) h7 (EU035693*; EU035706*)	10	
Karelia Republic			
(3) 66°18′N 33°53′E	h9 (EU035643*)	1	
(4) 66°16′N 33°39′E	h9 (EU232164*)	3	
(5) 66°19′N 33°40′E	h19 (EU035646*)	9	
(6) 66°17′N 33°54′E	h20 (EU035642*)	4	
(7) 66°17′N 33°33′E	h9 (EU035645*)	3	
(8) 65°03′N 35°45′E	h16 (EU232139*; EU232146*)	8	
(9) 64°54′N 34°14′E	h21 (EU232156*)	1	
(10) 62°54′N 34°22′E	h110 (JF714780; JF714781; JF714782) h147 (JF714779) h61 (JF714739; JF714741; JF714742; JF714747; JF714749) h62 (JF714740; JF714745) h16 (JF714743; JF714748)	14	
(11) 63°30′N 34°15′E	h63 (JF714744) h56 (JF714746)	2	
(12) 62°26′N 36°59′E	h18 (EU232169*; EU232171*) h107 (EU232170*)	6	
(13) 62°14′N 34°14′E	h108 (EU232157*) h109 (EU232158*; EU232159*) h110 (EU232160*; EU232162*) h111 (EU232161*)	6	
(14) 61°17′N 31°56′E	h112 (EU035710*)	21	
(15) 61°00′N 33°00′E	h112 (EU035707*) h113 (EU035709*) h114 (EU232164*)	16	h60 (EU232163*; EU035708*)
Leningrad Region			
(16) 60°19′N 28°29′E	h16 (EU035692*) h97 (EU232140*; EU232141*)	25	
(17) 60°07′N 34°57′E	h99 (EU035704*) h100 (EU035703*) h117 (EU035688*)	3	
(18) 59°13′N 34°47′E	h111 (EU035651*)	1	
(19) 58°44′N 29°51′E	h115 (EU035654*)	4	
(20) 61°02′N 30°07′E	h116 (JF714750)	1	
(21) 59°52′N 32°49′E	h117 (JF714751) h118 (JF714752)	3	

Table 1. *Continued.*

(Map ref.) Coordinates	*M. glareolus*	N samples of PCR-typing	*M. rutilus*
(22) 59°18′N 29°32′E	h119 (EU035687*) h117 (EU035668*; EU035652*)	8	
(23) 60°43′N 33°33′E	h120 (EU232144*) h121 (EU232151*) h122 (EU232152*)	3	
(24) 59°47′N 30°19′E	h119 (EU035650*)	3	
(25) 61°22′N 30°57′E	h133 (EU035683*)	2	
Vladimir Region (26) 55°31′N 40°11′E	h124 (EU035648*)	2	
Tver′ Region (27) 57°19′N 35°03′E	h125 (EU035649*)	5	
Pskov Region (28) 57°36′N 28°36′E	h111 (EU035656*; EU035669*)	15	
Kaliningrad Region (29) 55°09′N 20°50′E	h126 (EU035658*)	12	
Vologda Region (30) 59°26′N 35°48′E	h117 (EU035686*)	2	
Belgorod Region (31) 50°36′N 36°35′E	h134 (EU232148*)	6	
Moscow Region (32) 55°43′N 36°53′E	h124 (EU232150*)	7	
Finland (33) 62°38′N 30°56′E	h97 (JF714758; JF714759; JF714761; JF714763) h136 (JF714760) h137 (JF714762) h139 (JF714765) h138 (JF714764; JF714766; JF714767)	13	
Udmurtia Republic (34) 57°42′N 52°02′E	h140 (JF714768) h97 (JF714769; JF714770) h141 (JF714771) h142 (JF714772) h143 (JF714773) h127 (JF714774) h144 (JF714775)	11	
Novgorod Region (35) 58°32′N 31°15′E	h97 (EU035657*)	4	h38 (JF714801)
Tatarstan Republic			
(36) 55°38′N 49°00′E	h148 (JF714783)	1	
(37) 55°51′N 49°12′E	h149 (JF714784)	1	
(38) 55°53′N 49°09′E	h111 (JF714785) h97 (JF714786)	2	
Arkhangel′sk Region			
(39) 64°32′N 48°27′E	h7 (EU035641*; JF714716; JF714717; JF714720)	5	
(40) 62°07′N 38°56′E	h15 (EU232166*) h16 (EU232167*) h17 (EU232168*)	12	

Table 1. *Continued.*

(Map ref.) Coordinates	*M. glareolus*	N samples of PCR-typing	*M. rutilus*
(41) 63°26′N 48°44′E	h96 (EU035671) h97 (EU035672*; EU077269*) h98 (EU035699*)	8	
(42) 61°12′N 42°56′E	h106 (EU035691*) h105 (EU232136*)	5	h8 (EU035661*)
(43) 64°36′N 47°47′E	h1 (EU035639*) h3 (EU232165*) h6 (JF714715) h5 (JF714714) h4 (JF714713)	6	h2 (EU035664*)
(44) 64°48′N 49°13′E	h10 (JF714718) h7 (JF714719; EU035640*) h97 (EU232137*)	6	h11 (EU035694*; EU035696*; EU035697*) h12 (EU035695*) h13 (EU035677*) h14 (EU035698*)
Komi Republic			
(45) 61°57′N 52°20′E	h101 (EU035700*) h102 (EU035701*) h103 (EU035702*) h96 (EU035673*; EU035675*)	10	h11 (EU035676*)
(46) 61°47′N 51°49′E	h96 (EU035659*)	4	
(47) 61°23′N 51°48′E	h123 (EU035670*)	2	h22 (EU035663*; EU232145*)
(48) 62°06′N 58°26′E	h104 (EU035705*) h97 (EU035674*)	2	h11 (EU232143) h23 (EU232138*; JF714788) h24 (JF714787) h25 (JF714789)
(49) 60°25′N 59°30′E			h37 (JF714800)
Sverdlovsk Region			
(50) 56°50′N 59°52′E	h130 (EU035689*) h31 (EU035685*) h51 (JF714726)	24	
(51) 56°49′N 59°34′E	h131 (EU232142*) h31 (EU232147*)	179	
(52) 56°51′N 59°48′E	h31 (EU035680*)	55	h30 (EU035679*; JF714793)
(53) 57°15′N 58°44′E	h129 (EU035666*)	65	h32 (JF714794; EU232149*)
(54) 57°22′N 59°46′E	h11 (EU035684*) h127 (EU035667*; JF714754) h128 (JF714753)	128	h11 (EU035678*) h29 (JF714792) h28 (JF714791) h27 (JF714790)
(55) 56°51′N 60°36′E	h132 (EU232153*; EU232154*; EU232155*)	15	
Chelyabinsk Region			
(56) 55°42′N 60°28′E	h31 (JF714735) h145 (JF714776; JF714777) h146 (JF714778)	2	

Table 1. *Continued.*

(Map ref.) Coordinates	*M. glareolus*	N samples of PCR-typing	*M. rutilus*
(57) 55°31′N 60°20′E	h31 (JF714736)	14	
(58) 55°35′N 60°24′E	h44 (JF714737)	14	
(59) 55°13′N 60°07′E	h59 (JF714738)	14	
(60) 54°49′N 58°37′E	h31 (JF714721) h43 (JF714722; JF714725) h39 (JF714807) h44 (JF714723) h45 (JF714724)	24	h39 (JF714802)
(61) 56°08′N 61°08′E			h40 (JF714803)
(62) 55°41′N 60°55′E			h11 (JF714804) h41 (JF714805) h42 (JF714806)
Orenburg Region			
(63) 51°20′N 57°27′E	h16 (EU035665*)	1	
(64) 51°30′N 57°23′E	h57 (JF714727; JF714729) h58 (JF714728)	8	
(65) 51°21′N 57°28′E	h57 (JF714730; JF714731; JF714732; JF714734) h58 (JF714733)	12	
Tomsk Region			
(66) 57°13′N 84°07′E	h97 (EU523549*; EU523550*; EU523551*)	5	
(67) 60°29′N 77°11′E			h33 (EU035662*) h36 (JF714798)
(68) 58°09′N 76°56′E			h34 (JF714795) h35 (JF714796)
(69) 58°09′N 76°15′E			h16 (JF714797; JF714799)
Yamalo-Nenetsk National Area			
(70) 65°40′N 64°38′E			h54 (JF714819) h55 (JF714820; JF714822) h56 (JF714823)
(71) 66°55′N 65°44′E			h11 (JF714821) h94 (JF714873)
(72) 67°15′N 66°00′E			h95 (JF714874)
Khanty-Mansi National Area (73) 61°39′N 67°25′E			h11 (JF714824) h64 (JF714825) h51 (JF714826) h65 (JF714827) h29 (JF714828) h66 (JF714829)
Kazakhstan Republic (74) 49°55′N 84°40′E			h56 (JF714844; JF714846) h78 (JF714845)

Table 1. *Continued.*

(Map ref.) Coordinates	*M. glareolus*	N samples of PCR-typing	*M. rutilus*
Khakassia Republic (75) 53°26′N 89°10′E			h67 (JF714830) h68 (JF714831)
Krasnoyarsk Territory			
(76) 69°45′N 90°43′E			h82 (JF714854,JF714855) h83 (JF714856) h84 (JF714857)
(77) 62°09′N 89°01′E	h135 (JF714755; JF714756; JF714757)	3	h46 (JF714808) h16 (JF714809; JF714817; JF714849; JF714875) h47 (JF714810) h48 (JF714811) h53 (JF714818) h80 (JF714848) h52(JF714816)
(78) 62°27′N 89°00′E			h85 (JF714858; JF714859) h86 (JF714860) h87 (JF714861) h88 (JF714862) h16 (JF714863) h89 (JF714864) h90 (JF714865)
(79) 52°09′N 92°04′E			h49 (JF714812)
(80) 55°15′N 89°11′E			h50 (JF714813) h16 (JF714814) h51 (JF714815)
(81) 68°15′N 92°48′E			h79 (JF714847)
(82) 56°48′N 93°31′E			h81 (JF714850) h16 (JF714851; JF714852; JF714853)
(83) 52°48′N 93°08′E			h91 (JF714866; JF714867) h92 (JF714868; JF714872) h93 (JF714869) h50 (JF714870) h16 (JF714871)
Altai Republic			
(84) 50°30′N 88°08′E			h69 (JF714832)
(85) 51°57′N 85°56′E			h77 (JF714843) h70 (JF714833)
(86) 50°16′N 84°41′E			h71 (JF714834) h72 (JF714835) h50 (JF714836)
(87) 50°19′N 87°39′E			h73 (JF714837) h74 (JF714838)
(88) 51°37′N 87°41′E	h97 (EU523552*; EU523553*)	2	h75 (JF714839; JF714840; JF714841) h76 (JF714842)

ence), the size of alleles was determined using ALFwin Fragment Analyser 1.03.01 software package.

Finally, all individuals that according to the results of the microsatellite analysis carried the alleles of both species were tested for the presence of heterozygosity by a fragment (590 bp after alignment) of nuclear LCAT gene with species-specific single-nucleotide polymorphism (SNP). Heterozygotic individuals are characterized by double peaks in all cases of species-specific variable sites. Amplification conditions of LCAT gene were used as in Abramson et al. (2009a).

The obtained sequences were deposited in GenBank: cyt *b* – JF714713–JF714875, LCAT gene – JF807915–JF807942 (12 new sequences: JF807920, JF807922– JF807925, JF807936– JF807942, others used for comparison of Abramson et al., 2009c).

In order to test the independence of the ongoing hybridization chance from the population cycle phase, population dynamics of *M. glareolus* was studied in the permanent plot in native forest of VSBNR, where F1 hybrid was caught (locality 54). The data were gathered during 1995–2009 (10,500 trap-nights). The 438 individuals of *M. glareolus* and 42 *M. rutilus* from middle Urals populations were analyzed with molecular methods to test the chance of ongoing hybridization. The analyses of contingency table "species(3)-phase(3)" after its collapsing to 2x2 table "hybrid (yes/no) – trough (yes/no)" and Fisher test were used in STATISTICA 6.0. Special attention was paid to the populations of VSBNR, where 128 individuals were collected during years 2005–2009 (the trough – 2005, 2009; the increase in 2006–2007 and the peak in 2004, 2008).

Relationships between haplotypes of *M. glareolus* and *M. rutilus* were estimated using the median-joining (MJ) approach in NETWORK 4.6.1.0 (Bandelt et al. 1999). Haplotype (h) and nucleotide (π) diversities (Nei 1987) and their standard deviations (±SD) (Tajima 1993) were estimated using DNASP 5.10 (Rozas et al. 2003). The demographic history of the voles' lineages was inferred employing a pairwise mismatch distribution analysis in ARLEQUIN 3.11 (Excoffier et al. 2005). Multimodal distributions would be consistent with demographic stability, while sudden expansion would generate a unimodal pattern (Slatkin and Hudson 1991). A parametric bootstrapping approach (Schneider and Excoffier 1999) was used to obtain the probability that the observed data conform to the model using the sum of square deviations (SSD) between the observed and the expected mismatch distribution as a test statistic. The confidence intervals (CI) for τ were calculated with 1,000 bootstrap replicates for the alpha level of 0.050. In order to detect the population demographic expansion, several neutrality tests were calculated (Tajima's *D* and Fu's *Fs*). For neutral markers, significant negative *D* and *Fs* values can be expected in cases of population expansion (Tajima 1989; Fu 1997). These statistical tests and their significance were evaluated by 1,000 random permutations in ARLEQUIN.

RESULTS

The identification of introgressant forms and F1 hybrids. Results of the PCR-typing showed that among of 841 individuals of *M. glareolus*, 227 bear mitotype of *M. rutilus* (Fig. 2). Accumulation of descendants of the ancient hybrids, where *M. glareolus* with own mt genome were not detected at all, were found at the Kola Peninsula, in the areas adjacent to the White Sea (Abramson et al. 2009b) and the extreme southern Urals. The two transitional zones were revealed, one from the south to the north in the southern and middle Urals (Fig. 3) and another one from the north to the south in the north of European part of Russia. Within both zones there is a transition between the populations of *M. glareolus* with *M. rutilus* mitotype and the bank vole with its own mt genome. For the Ural populations the chance of *M. glareolus* to carry foreign mitotype is decreasing by 1.04 (95% confidence interval (CI) 1.02–1.07) times from south to north for one minute. The expected 50:50 ratio is located around 56°N – in the southern Urals.

The microsatellite results confirmed that all individuals of the bank vole with alien mt genome belong to *M. glareolus*. The wavelength of allele MsCg9 locus in *M. glareolus* is 160–180 and in *M. rutilus* is 145–167. The allele length range in locus LIST3-01 in *M. glareolus* is 125–139 whereas in *M. rutilus* is 88–108. Alleles of locus MsCg9 differed by parity in the two species and alleles lengths of locus LIST3-001 of *M. glareolus* and *M. rutilus* do not overlap. Since the locus MsCg9 has the allele length range of coverage and the possible single-nucleotide mutations, it is not reliable in detecting F1 hybrids. Some individuals with *M. rutilus* mitotypes (N=6) carried the alleles of the both species in MsCg9 locus, while in LIST3-01 they have only alleles of *M. glareolus*. We analyzed them with the nuclear LCAT gene. The alignment showed that all individuals of

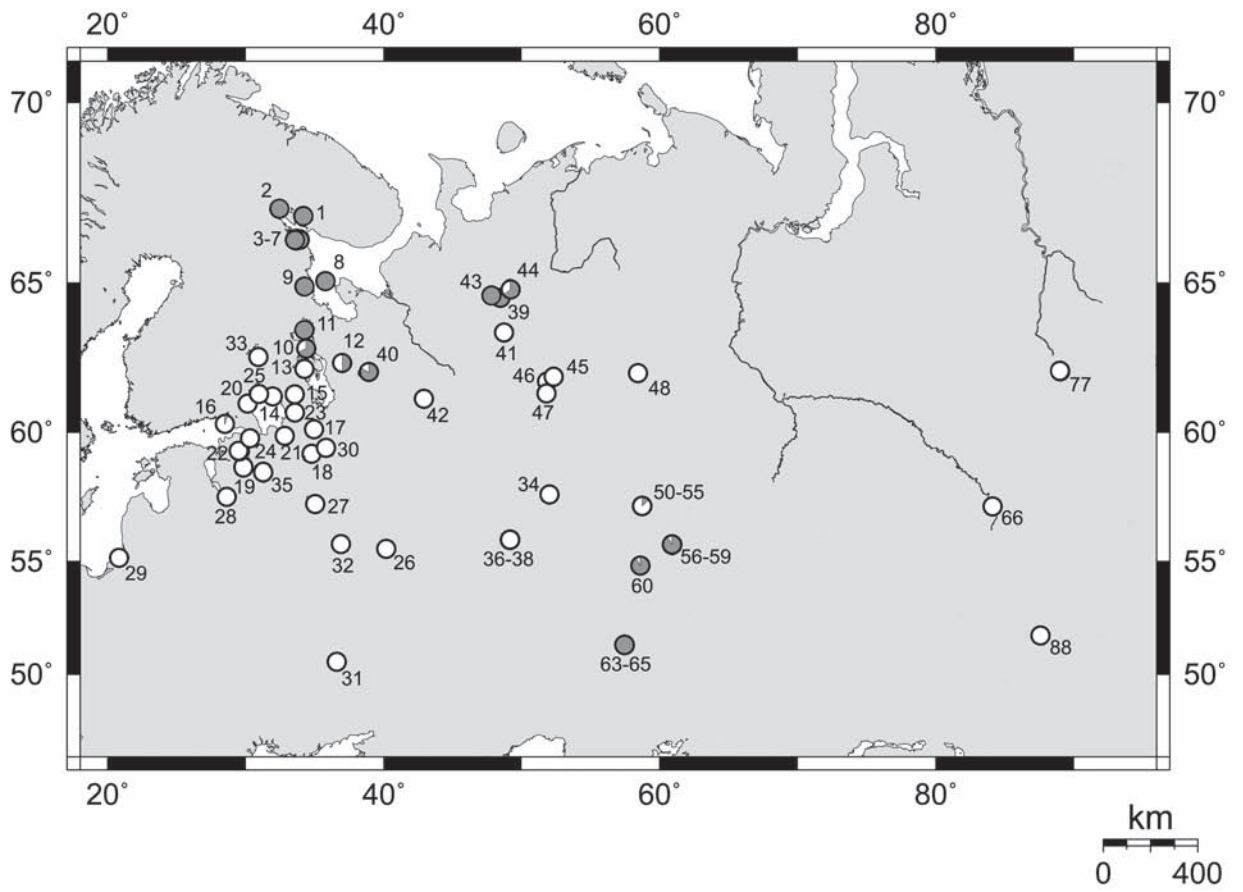

Fig. 2. The quantitative ratio of *M. glareolus* close to *M. rutilus* in the bank vole populations based on previously published results (Abramson et al. 2009b, 2009c) and present study. The fraction of the *M. glareolus* individuals with foreign mitotypes is shown black and the rate of pure *M. glareolus*, white. The localities correspond to those in Table 1 and Fig. 1. The total number of the bank vole from each location is given in Table 1.

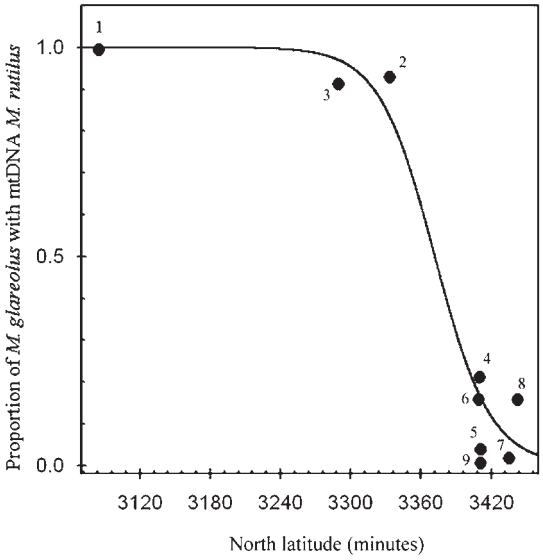

Fig. 3. The dependence of probability of *M. glareolus* with mtDNA of *M. rutilus* on the north latitude (in minutes) for the southern (1–3) and middle Urals (4–9) populations; the populations correspond to the following number locations (for sites details see Table 1): 1 – 63–65 (samples total number (n) = 21); 2 – 56–59 (n = 44); 3 – 60 (n = 24); 4 – 50(n = 24); 5 – 52 (n = 55); 6 – 51 (n = 179); 7 – 53 (n = 65); 8 – 54 (n = 128); 9 – 55 (n = 15).

Table 2. Genetic diversity assessed for *M. glareolus* and *M. rutilus* samples using the cytochrome *b* gene. Number of individuals examined (N), number of haplotypes (H) and number of segregating sites (S), Tajima's *D*, Fu's *Fs*, for the voles. Estimates of time since expansion in mutational units (τ) are calculated from mismatch distribution with percentile confidence intervals based on 1,000 simulated samples. Statistical significance for Tajima's *D* and Fu's *Fs* statistics: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Groups	N	H	S	h±SD	π±SD (%)	Tajima's *D*	Fu's *Fs*	τ (95% CI)
M. rutilus	108	70	87	0.98±0.01	0.45±0.02	−2.478***	−25.907***	4.01 (2.46–4.81)
M. glareolus	100	54	61	0.95±0.01	0.45±0.04	−2.148***	−25.931***	2.06 (1.60–2.63)
M. glareolus with mtDNA of *M. rutilus*	64	29	36	0.95±0.01	0.35±0.03	−1.959**	−22.053***	3.01 (2.22–3.69)

the bank vole with *M. rutilus* mt genome have typical nuclear SNP of *M. glareolus*. Only specimen in VSBNR (JF807935 in GenBank, Abramson et al. 2009c) has double peaks in species-specific variable sites in LCAT and has the alleles of the both species with both microsatellite loci.

Hybridization and the phase of population cycle. We studied the population of voles and found that the ratio of *M. rutilus* to *M. glareolus* in the area where the hybrid was caught is about 1:20. The long-term trapping data and materials from the middle Urals (438 specimens of *M. glareolus* tested by molecular methods) allowed to find out that the odds of capturing F1 hybrid or pure species is 1/79 (p 0.013, 95% CI 0.0003-0.068) in the trough phase of the vole population cycle. Similarly, regardless of the phase, the chances are 1/824 (p 0.0012, 95% CI 0.00003-0.0067). The hypothesis of independence of the ongoing hybridization chance on the population cycle phase may be rejected at marginally significant level p (exact Fisher test) = 0.088.

Genetic diversity and demographic history of both vole species in sympatry. As was previously observed (Deffontaine et al. 2005; Potapov et al. 2007; Abramson et al. 2009b), the studied voles show two haplotype groups: group A unites *M. glareolus* haplotypes with their own mitotype (100 specimens) and group B with some part of *M. glareolus* (64 individuals) and *M. rutilus* haplotypes (108 specimens). Group A (Fig. 4) has a star-like pattern with a dominant haplotype h97, widespread in populations from the south part of Kola Peninsula to Western Siberia. Haplotypes from the north of European Russia and from the population of the south Urals differ from all others. Group B (Fig. 5) also has a star-like structure with several central haplotypes. Widespread haplotype h16 was found in the northern red-backed vole from the Eastern Siberia and the bank vole in the European part of Russia. Haplotype h11 is widely distributed in *M. rutilus* populations from the east and west of the Ural Mountains. In the bank vole, this haplotype was found (Abramson et al. 2009c) in the only F1 hybrid (locality 54). Common haplotypes (h11, h16, h51, h56) were found in *M. glareolus* and *M. rutilus* in the geographically distant populations.

The nucleotide diversity was similar for *M. rutilus* and *M. glareolus* (Table 2), however, the haplotype diversity was higher in *M. rutilus*. The greatest genetic diversity (π=0.65±0.06%) was found in the populations of *M. rutilus* from south of Siberia (localities 84–88). The minimum nucleotide diversity (π=0.06±0.01%) is shown in *M. glareolus* from Western Siberia. The introgressant forms display high nucleotide diversity and low haplotype diversity.

The mismatch distributions were unimodal for *M. rutilus*, *M. glareolus* and *M. glareolus* with *M. rutilus* mitotype, indicating the populations' sudden expansion. This result is similar with our previous data (Abramson et al., 2009b). The tests of the datasets by two models of sudden (Rogers and Harpending 1992) and spatial (Excoffier 2004) expansion have supported both these models. Fu's Fs and Tajima D tests results showed high and negative values for all groups (Table 2) supporting the hypothesis of a recent population expansion and thus in favor of the model of sudden expansion.

M. glareolus with mtDNA of *M. rutilus* experienced expansion somewhat earlier (τ=3.01, 95% CI 2.22–3.69), than the bank vole (τ=2.06, 95% CI 1.60–2.63). The τ value of *M. rutilus* is 4.01 (95% CI

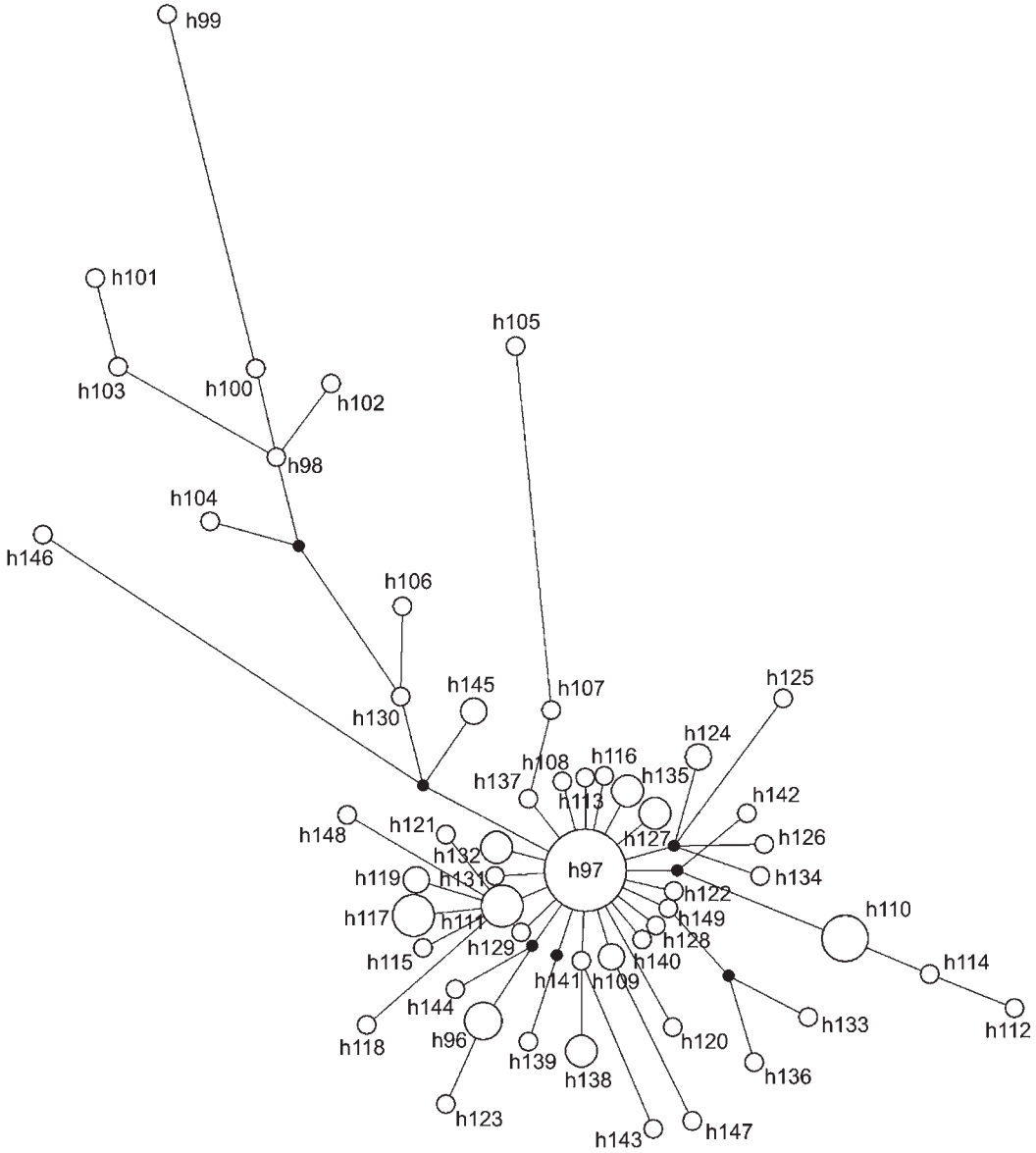

Fig. 4. Median-joining tree of mtDNA haplotypes in *Myodes glareolus* with own mitotypes (Clade A). The size of the circles corresponds to the haplotype frequencies. Number of mutational steps is proportional to the length branches. See Table 1 for the haplotype designations.

2.46–4.81), which also points to earlier expansion than *M. glareolus*.

DISCUSSION

According to the "scarcity-of-conspecifics hypothesis", low population density may promote the breakdown of isolating mechanisms (Selander 1971). In species with a low population density or in species on the verge of extinction hybridization may occur because of the lack of a conspecific mate (Newton 2003). The hybridization of *M. rutilus* and *M. glareolus* in laboratory (Osipova and Soktin 2008) demonstrated that it possible only in the absence of conspecifics but the number of females giving offspring in a pair *M. rutilus* female – *M. glareolus* male was higher than in a

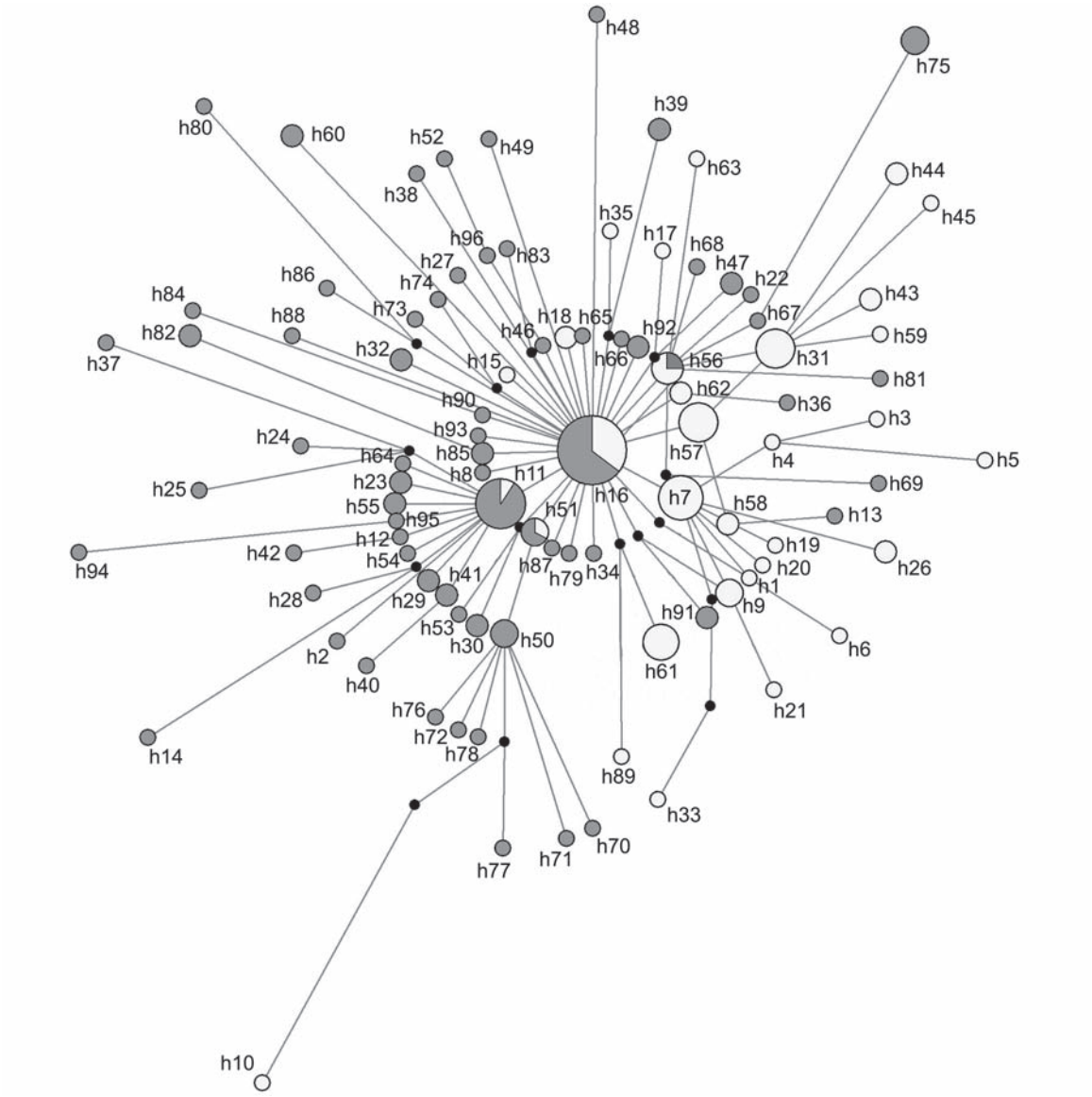

Fig. 5. Median-joining tree of mtDNA haplotypes in *Myodes rutilus* (grey circles) and some of *M. glareolus* (white circles) with foreign mitotypes (Clade B). The size of the circles corresponds to the haplotype frequencies. Haplotypes shared between the two species are represented by circles with mixed colours, in which the relative frequency is indicated by proportion of grey and white. Number of mutational steps is proportional to the length branches. See Table 1 for the haplotype designations.

pair of *M. glareolus* female – *M. rutilus* male. Probably, some predominance of the bank vole individuals may contribute to a pair of *M. rutilus* female – *M. glareolus* male and resulted in unidirectional introgression. Usually, the bank vole population dynamics is characterized by 3–5 year cycle, however, amplitude and periodicity of population fluctuation are not stable within the bank vole range (Bashenina 1981). The population dynamics at the middle Urals native forests is characterized by clear 3-year cycles (Kshnyasev et al. 2011). The analysis of *M. glareolus* with *M. rutilus* mitotype with mt and nuclear markers showed lack of F1 hybrids in the studied area. Up to now only one F1 hybrid was detected in VSBNR (Abramson

et al. 2009c), which was caught in the trough phase of the population cycle. According to findings in the Urals *M. glareolus* populations, the chances of modern hybridization in the depression phases were higher than those regardless of cycle phase. The data obtained support the hypothesis on the dependence of hybridization on the population cycle phases. We studied *M. glareolus* specimens in VSBNR in trough phase (in 2005 and 2009) and can assume that the hybridization most likely takes place in low numbers of both species, with some predominance of the bank vole, although we found no more natural hybrids. The extremely low density in the trough phase of population cycle and special species ratio has lead to ongoing hybridization and these observations can be used as a contemporary model of ancient hybridization.

All demographic parameters indicate a recent population expansion of *M. glareolus* with own mt genome – Eastern lineage (phylogroup) (Deffontaine et al. 2005; Abramson et al. 2009b; Wojcik et al. 2010). The genetic diversity of the bank vole studied across the north of the European part of Russia to Western Siberia was found to be higher in the western part than in the eastern, suggesting that the expansion of the bank vole occurred from the west to the east. The demographic expansion of *M. glareolus* possibly took place with forest advance following the Last Glacial Maximum (LGM) 24,000–15,000 BP (Markova et al. 1995; Wojcik et al. 2010). Some authors reported (Deffontaine et al. 2005; Wojcik et al. 2010) that the Eastern lineage most likely derived from a refugium close to the Ural Mountains. The southern Urals during the Pleistocene was a refuge suitable both for the steppe and forest species (Markova et al. 1995; Jaarola and Searle 2002; Brunhoff et al. 2003). However, our results showed that the Urals populations did not possess a high value of nucleotide diversity ($\pi = 0.40 \pm 0.07\%$), which would be logically expected in the case of refuge favorable for forest species. The remains of *M. glareolus* at the glacial maximum (24,000–10,000 BP) are known from the sites at the Ural Mountains (Markova et al. 1995). The Eastern lineage of *M. glareolus* in the north of the European part of Russia is notable for a very high genetic diversity ($\pi = 0.61 \pm 0.08\%$). Such high genetic diversity can be explained by the fragments of forests (up to 56° N), in which populations could have survived (Valiranta et al. 2011). The distribution of *M. glareolus* fossils (the Pechora basin and the northern Urals, at 15,000–10,000 BP) supports the assumption that this forest-dwelling species was constantly present in the periglacial faunas in the immediate vicinity of the ice sheet, even under conditions of the most severe climates of the Valdai glaciation (Markova et al. 1995).

The obtained results confirm the previous assumption based on a smaller data set (Abramson et al. 2009b) that the Western lineage of the northern red-backed vole experienced a rapid demographic expansion from the ancestral population (Table 2, Fig. 5). The tau (τ) for *M. rutilus* showed that the expansion of this species likely was earlier than those of *M. glareolus* (Table 2). The colonization of the Western lineage of *M. rutilus* to the north-west regions could have started from the refuge in the south of Altai Mountains, where high nucleotide diversity is observed. The boreal forests and parklands were common in the LGM in the mountains of southern Siberia (Velichko 2009), where remains of *M. rutilus* were found (Markova et al. 1995). The phylogeographic study of larch species (*Larix*) also pointed on the existence of refuge in the south of Altai (Semerikov and Lascoux 2003). Remains of *M. rutilus* at the LGM are recorded from the Ural Mountains (Markova et al. 1995), where a forest-steppe refuge was supposed (Markova and Kolfschoten 2008; Velichko 2009), but populations of *M. rutilus* within this region are characterized by low level of nucleotide diversity.

Combining the data obtained earlier (Abramson and Bodrov 2008, Abramson et al. 2009b, 2009c) and in the current study, we can propose several possible scenarios of interspecies hybrids appearance.

The known reforestation direction of the deglaciated area, the mismatch distribution and the absence of a genetic hiatus between the populations from the Kola Peninsula and the south Urals supports the idea that observed distribution of introgressant forms (Fig. 2) resulted from the dispersal of ancient hybrids from a single source. If hybridization occurred only once, then at the haplotype network (Fig. 5) haplotypes of *M. glareolus* would have formed a separate cluster among the haplotypes of *M. rutilus*, but we do not find that. Since descendants of the ancient hybrids differ from *M. rutilus*, it is possible to assume that either hybridization cases were separated in time, or there were few hybrid founders, or hybridization may have occurred rather long ago and *M. rutilus* had sufficient time to accumulate differences, as well as did ancient hybrids. The genetic diversity of introgressive forms is lower than that of *M. rutilus,* which is consistent with the founder effect.

Several scenarios could be proposed for the observed pattern of distribution of introgressive forms based on climatic and biotic preferences of these sylvatic voles. The hybridization could occur either during the formation of the sympatry between the species or in a refuge with subsequent dispersal of the hybrid forms. Potapov et al. (2007) advocate the hypothesis of the post-glacial northwards model by *M. rutilus* and *M. glareolus*. After the retreat of the glacier, *M. rutilus* occupied the territory of the northern Europe covered by coniferous forests. The extension of warming led to deciduous forests reaching the far north, which led to decrease of *M. rutilus* range and expanding of the bank vole habitat in the middle Holocene. The advance of *M. glareolus* to north-east could lead to possible depression of *M. rutilus* population and reduction in their habitat (as we can see in most of contemporary sympatric areas at least in the European part of Russia and middle Urals (Kshnyasev and Marin 2012)) that would result in hybridization. This scenario does not explain the fixation of the hybrid individuals in populations on such a wide territory and the accumulation of the descendants of hybrids in the southern Urals and in the Kola Peninsula (Fig. 2). If hybridization occurred in different area independently, the hybrids would meet frequently throughout the area of species sympatry (in the central regions of the European part of Russia) but we do not observe such a pattern. The similarity of the introgressant populations from the southern Urals and the Kola Peninsula, and the difference between the northern red-backed vole haplotypes and the haplotypes of the bank vole close to *M. rutilus* can be attributed to extinction of some *M. rutilus* haplotypes. It is possible that populations of *M. rutilus* previously had higher levels of diversity, and this may explain, why we observe a large number of unique haplotypes in the introgressant forms.

Another scenario is that the hybridization may have occurred in the refuge during the LGM at low density of conspecifics, and then after the retreat of the glacier descendants of hybrid populations have spread with the forests in the north-west direction along the Urals through the north of the European Russia to the Kola Peninsula. There was a colonization route along the west side of the Ural Mountains from refuge in the southern Urals in the post Pleistocene period (Velichko 2009). The north-east colonization route of the Fennoscandia was opened up with the onset of the deglaciation of south-west Finland about 10 Ka (Jaarola et al. 1999) and the bank vole with *M. rutilus* mitotype could have colonized this region only in the Holocene. If hybridization occurred as a result of invasion of the bank vole with the spread of mixed forests to the territory yet occupied by *M. rutilus* in the middle Holocene (first scenario, see above), then the bank vole with own mtDNA would have also colonized the Fennoscandia, but we do not observe this. The rarity of modern hybrids, the distribution pattern of ancient hybrids and the lack of differences between populations of the ancient hybrids within the Urals and the northern territories of the European part of Russia speak in favor of this scenario. The modern distribution of the northern red-backed vole shows that this species expands far to the north and rises higher into the mountains than the bank vole. Within sympatric areas, it is found in the zone of strong anthropogenic disturbance, where the bank vole is apparently absent (Stenseth and Gustafsson 1985; Mukhacheva et al. 2010). Based on current range of *M. glareolus* and the asymmetrical character of the introgression, the bank vole could be more vulnerable in terms of landscape and climate changes during the climatic oscillations of the Pleistocene.

At present, we found one population in the middle Urals where hybridization occurred. Such a low frequency of contemporary hybridization as well as experimental hybridization in the laboratory indicates the difficulty of this process. Among factors that lead to a break in species reproductive isolation barrier may be disturbed habitats, dramatically uneven number of species and sex ratio. The present sympatry of *M. glareolus* and *M. rutilus* most likely was formed in the late Pleistocene–Holocene and the borders of the sympatry probably were changing alongside with the forest expansion during this time. Interspecific hybridization between these species in the historical past and subsequent introgression led to the formation of mixed populations in part of the sympatric area. This may have taken place in the southern Urals refuge during the LGM, at a low density of both species. A mass independent hybridization during the formation of the sympatry seems less likely.

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