

Genetic Features and the Putative Sources of Formation of Isolated Populations of the Striped Field Mouse *Apodemus agrarius* Pallas, 1771 in Magadan Oblast

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Abstract—The striped field mouse *Apodemus agrarius* is an invasive species new for Magadan oblast; however, the adaptation of the animals to the conditions of the habitats north of the Sea of Okhotsk has been successful. The full nucleotide sequence of the cytochrome *b* (*cytb*) gene from the mtDNA has been determined for mice from four local populations in the region (settlements of Snezhnaya Dolina, Snezhnyi, Solnechnyi, and Talon), and five *cytb*-haplotypes have been detected. Phylogenetic analysis revealed the similarity of *cytb* nucleotide sequences in the mice from the habitats north of the Sea of Okhotsk and the conspecifics from the Far East-Chinese part of the range. The invasion of *A. agrarius* into Talon is likely to have started from Primorsky krai, whereas the animals captured in Snezhnaya Dolina had ancestors from both Primorsky krai and from China, and the animals captured in Snezhnyi and Solnechnyi were exclusively of Chinese ancestry. The striped field mice from Snezhnyi and Solnechnyi were of a single monophyletic origin. The origin of mice captured in Snezhnaya Dolina was apparently polyphyletic, and the origin of the animals from Talon was monophyletic and different from the origin of other populations of the enclave located north of the Sea of Okhotsk. Investigation of 16 allozyme loci revealed highly significant differences between the samples of striped field mice of Snezhnyi, Solnechnyi, and Talon. The variability parameters in the set of biochemical gene markers used for the analysis showed a trend to a decrease in striped field mouse samples from the habitats north of the Sea of Okhotsk. Genetic analysis revealed that the local settlements of *A. agrarius* in Magadan oblast are currently represented by small isolated populations.

Keywords: striped field mouse, *Apodemus agrarius*, invasive species, cytochrome *b* (*cytb*) gene, allozyme variability, genetic diversity, phylogenetic analysis

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INTRODUCTION

The striped field mouse *Apodemus agrarius* Pallas, 1771 is a agrophylic rodent that belongs to the group of alien invasive species in certain regions of Russia (Tupikova et al., 2000; Bobrov et al., 2008; Khlyap and Varshavskii, 2010; Khlyap et al., 2011). This species is an important component of the ecosystem, since it serves as prey for some predators, participates in soil formation, causes certain damage to crops, and represents a reservoir of various feral herd infections (Karaseva, 1979). The transmission of hemorrhagic fever with renal syndrome from striped field mice to

humans is common in the Far East (Lee, 2003). The range of the striped field mouse is subdivided into two isolated areas, the European-Siberian-Kazakhstan area and the Far East-Chinese area. The expansion and formation of the range is largely dependent on the economic activity of humans (Volkov et al., 1979; Karaseva et al., 1992; Tikhonova et al., 1992; Kostenko, 2000; Tupikova et al., 2000; Neronov et al., 2001; Bobrov et al., 2008; Bazhenov et al., 2015). The species was not sighted in Magadan oblast prior to the mid-1990s (Yudin et al., 1976; Chernyavskii, 1984; *Pozvonochnye...*, 1996). The first instances of capture of

the striped field mouse in the settlements of Talon and Snezhnaya Dolina date back to the year 1995 (Dokuchaev et al., 2001; Andreev et al., 2006). The animals were regularly captured at certain sites in Magadan oblast (near the town of Magadan and in Snezhnaya Dolina and Talon) in the following years. Striped field mouse populations became rather large in certain areas. For instance, N.E. Dokuchaev captured 63 individuals in less than a day at the beginning of October 2003; the capture sites were located at the periphery of a postharvest potato plot with an area of approximately 1600 m² in Talon. The density of the rodent population was thus close to 400 individuals per 1 ha (Primak et al., 2004). The sites populated by the striped field mouse in Magadan oblast are mostly in agricultural use (fields, vegetable gardens, backyards, and allotment gardens). Some of these lands are abandoned and overgrown with tall weeds, the same as at the boundaries of the patches. Some representatives of the species were captured in the natural biotopes as well (larch forests with dwarf pine and birch thicket, willow bushes, cereals, and mixed herbs in the floodplain of the Dukcha River), at a distance of hundreds of meters from the nearest vegetable gardens. Monitoring of striped field mouse populations in Magadan oblast for 20 years revealed successful adaptation of the species to the habitat characterized by an extremely short vegetation period, prolonged persistence of snow cover, and night frosts and snowfalls observed until May (and sometimes even in June). The females who survive the winter give birth to three litters during the warm season, and the young females give birth to one or two litters. The average fertility inferred from the number of embryos and placental spots in 23 sexually mature females was 7.1 ± 0.29 pups per litters (N.E. Dokuchaev, personal communication). Thus, the fertility of the animals was even higher than in the south of the Far East region of Russia, where the average number of embryos per female was 6 (Kostenko, 2000).

The striped field mouse apparently arrived in Magadan oblast located at a distance of more than 1300 km of the native range of the species together with animal feed or other agricultural products transported by sea, since there is no railroad transport into Magadan oblast and transport by sea accounts for most of the cargo turnover in the region (*Government ...*, 2016). Regular maritime transport was established at the beginning of the 1930s. The major direction of cargo transportation from the commercial seaport of Magadan oblast corresponds to the ports of the south of the Far East region of Russia (Vladivostok, Vostochnyi (Nakhodka), and Vanino). The cargo that arrives from Primorsky krai, Khabarovskiy krai, Siberia, China, Kazakhstan, and Korea is packed into containers in the ports named above and then transported to Magadan oblast. Agricultural enterprises (sovkhozes) that were under economic and administrative control of Magadan and provided the area with vegetables,

potatoes, and hay have existed in the south of Primorsky krai (on the Khankaiskaya Plain) for many years (the population of the striped field mice near these agricultural facilities was very high) (Kostenko, 2000). Regular supply of agricultural products to Magadan oblast continued until the 1990s (*Priozernoe ...*, 2007; *Priozernoe (Primorsky krai)...*, 2016). The transport of these rodents to Magadan oblast by cars or airplanes can be decisively ruled out. The R504 Kolyma federal highway that connects Magadan and Yakutsk does not enter the territory inhabited by the striped field mouse, and the cargo transported by airplanes is limited to small quantities of vegetables and fruit.

The present-day populations of the striped field mouse in Magadan oblast are isolated and associated with inhabited localities or the environs thereof; the distances between the ranges of the populations range from 5 to 130 km (Fig. 1). The species behaves as an herbivorous rodent species with a clear preference for synanthropy; similar behavior was observed in other regions of Russia (Kucheruk and Karaseva, 1992; Khlyap and Varshavskii, 2010; Tikhonova et al., 2012). The population of Talon is the most isolated, whereas the local character of the three populations that inhabit the environs of Magadan is somewhat arbitrary. All these populations are located in river floodplains, and roads flanked by kitchen gardens run through all three sites. Thus, the expansion of the striped field mouse population is not hindered; however, the populations are separate and all animals captured in the area were from the above-named settlements, rather than from the areas between the settlements.

The Tauiskii sovkhoz (agricultural enterprise), which specialized in cattle breeding and potato cultivation, existed in Talon until the mid-1990s. Cows and pigs were reared in Snezhnyi (where the Snezhnyi sovkhoz existed between 1968 and 1997). The Severnaya poultry farm functioned in Solnechnyi between the early 1980s and the mid-1990s. Recreation facilities are located in Snezhnaya Dolina. Private farms and subsidiary farms, allotment gardens, and kitchen gardens are currently found in the area. Agricultural products and animal feed (including grains and compacted hay) were transported into all the above-named areas through the Magadan commercial seaport.

Several attempts at elucidating the area of origin of the rodents were undertaken. The comparison of striped field mouse samples from Magadan oblast, Primorsky krai, and the south of Khabarovskiy krai involved several genetic experiments, such as the investigation of allozyme variability (Primak et al., 2004, 2005; Zasyupkin et al., 2007) and RAPD-PCR analysis (Dokuchaev et al., 2008). The results of the above-mentioned studies allowed for the assumption of multiple instances of introduction of the striped field mouse to the territory of Magadan oblast. Molecular genetic methods were recently validated for the

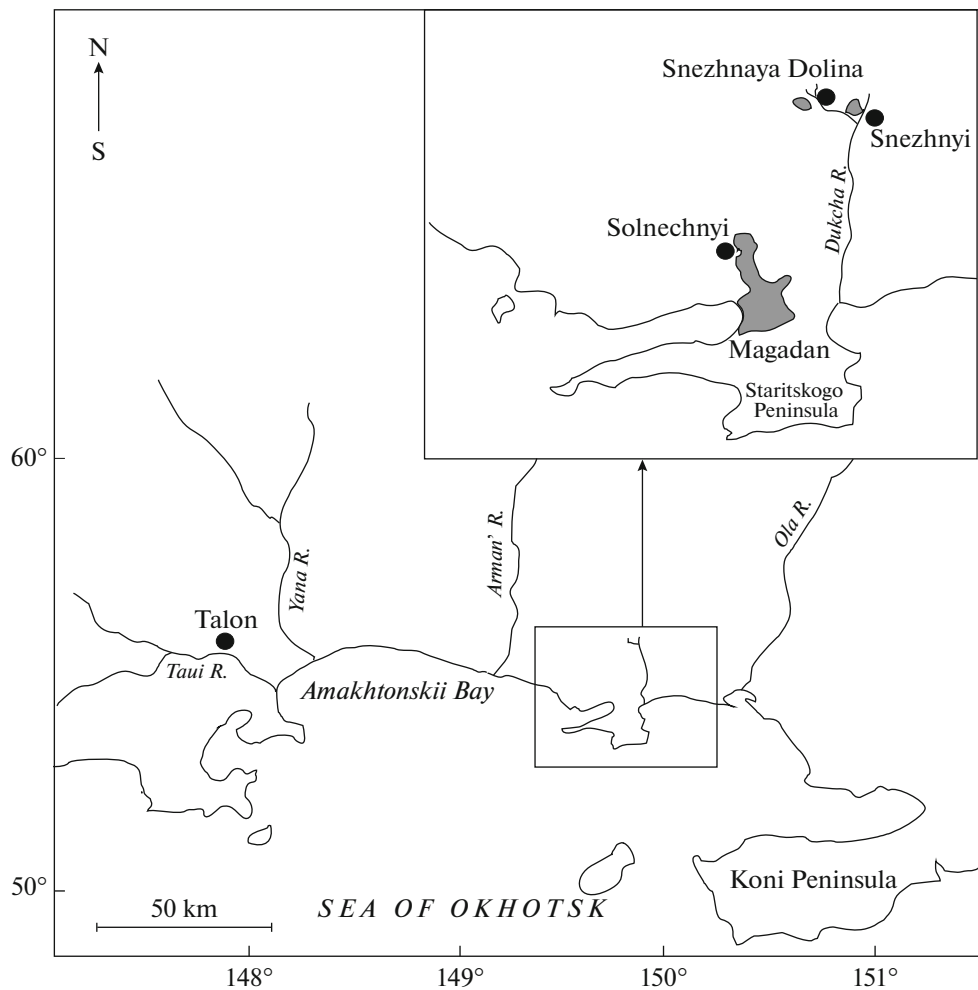


Fig. 1. The area of striped field mouse invasion north of the Sea of Okhotsk. Striped field mouse sighting points and capture sites are shown by dots.

precise elucidation of the phylogenetic relationships within this invasive species (Primak, 2013; Primak and Pereverzeva, 2015). The considerable polymorphism of the nucleotide sequence of the cytochrome *b* (*cytb*) mtDNA gene selected for the analysis is worth mentioning, since it allows for the successful use of molecular genetic analysis of the *cytb* haplotypes in phylogenetic research on *A. agrarius* (Serizawa et al., 2000; Reutter et al., 2003; Liu et al., 2004; Suzuki et al., 2008; Dubey et al., 2009; Sakka et al., 2010; Oh et al., 2013; Pereverzeva and Pavlenko, 2014; Koh et al., 2014; Kim and Park, 2015).

The genetic structure of the striped field mouse populations in the areas north of the Sea of Okhotsk is presumably in the formative stage, since the habitat is still new for the species. The gene pool of *A. agrarius* individuals found in Magadan oblast is apparently shaped by founder effects and gene drift to the greatest extent. A small-scale but regular supply of new genes with the striped field mouse individuals transported together with animal feed and agricultural produce

from the southern part of the Far East of Russia cannot be ruled out.

The aim of the present work consisted in the identification of donor populations of the striped field mouse of Magadan oblast and assessment of the genetic variability level of this species in the populations located to the north of the Sea of Okhotsk.

MATERIALS AND METHODS

Striped field mice used in the study were captured during the years 1997–2015 by N.E. Dokuchaev, E.A. Dubinin, and A.A. Primak (IBPN FEB RAS) in the settlements listed above. The samples for molecular genetic investigation (skeletal muscle) were fixed in 96% ethanol, and those used for the allozyme analysis were frozen and stored at -20°C . The isolation of total DNA from ethanol-fixed muscle tissue, as well as DNA purification, followed the procedure described by Fleming and Cook (2002) with some modifications. The full nucleotide sequence of the *cytb* gene

		111223344	4566677789	11
		3346378928	8428846847	00
		9275434660	9346718002	36
M1	KR338983	CGACTTCGGG	ACATTGCCCC	CA
M2	KR338982	.A.....	...C.A....	.C
M3	KR338984	.A.T.C.C..	..G...T...	.C
M4	KR338985	T.....	G.....T...	..
M5	KR338986	TAG.A.T.AA	.T..C..TTT	TC

Fig. 2. Haplotypes of the cytochrome *b* gene in striped field mice from Magadan oblast. Nucleotide substitutions relative to the sequence variant M1 are shown. The numbering of the substitution sites starts from the beginning of the cytochrome *b* gene of the mitochondrial genome of *A. agrarius*, GenBank no. HM034866 (Oh et al., 2011).

from the mitochondrial genome was determined using the primers ApoL14061 (cta cac agc att caa ctg tga cta atg aca tg) and ApoR15351 (cct tct tct tga tgc cct gag aag aga agt tct tcg) designed at the laboratory of population genetics (IBPN FEB RAS). The conditions of the amplification of the nucleotide sequence of the *cytb* gene were as described by Balakirev et al. (2007). The amplified mtDNA fragment was purified and prepared for sequencing according to the conventional procedure that employed the Diatom™ DNA Clean-Up kit (Laboratoriya Izogen, Russia). The nucleotide sequences of the *cytb* mtDNA gene were obtained according to the standard procedure that employed the DNA Big Dye Terminator reagent kit (Applied Biosystems, v. 3.1) and the gene analyzer ABI Prism 3130 (Applied Biosystems, United States). The *cytb* gene was mapped to the full nucleotide sequence of *A. agrarius* mtDNA (GenBank no. HM034866 (Oh et al., 2011)).

Phylogenetic analysis was performed with the data obtained in the present study and the sequences retrieved from GenBank; the latter were represented by full or partial (880 bp) nucleotide sequences of the *cytb* gene in 191 individuals of the striped field mouse from different localities (Serizawa et al., 2000; Liu et al., 2004; Sakka et al., 2010; Oh et al., 2013; Per-everzeva and Pavlenko, 2014; Koh et al., 2014; Kim and Park, 2015). All animals selected for the genetic analysis originated from Primorsky krai, Khabarovsk krai, Siberia, China, Kazakhstan, and Korea, since the probability of introduction of the striped field mouse into Magadan oblast was the highest for these areas. The nucleotide sequence of the *cytb* gene of *Apodemus chevrieri* (Milne-Edwards, 1868), GenBank no. AB096818, was used as the outgroup. The MEGA 6.0.2.74 (Tamura et al., 2013), ARLEQUIN ver. 3.5 (Excoffier et al., 2005), and Network 4.5.1.0 (Bandelt et al., 1999) software packages were used for statistical processing and analysis of the genetic data.

The allozymes were separated by vertical electrophoresis in 6.5–7.5% polyacrylamide gel in a modified electrophoretic cell (Zasytkin, 1983, 1986); the buffer

systems used were Tris-borate-EDTA (Peacock et al., 1965) or Tris-glycine (Davis and Ornstein, 1959). The enzyme activity assays were carried out according to standard procedures (Manchenko, 2003). Fifteen enzyme systems presumably encoded by 16 interpretational loci were analyzed. The symbols of the loci correspond to the abbreviated names of enzymes suggested by Manchenko (2003). CHIHW and CHIRXC software (Zaykin and Pudovkin, 1993) was used to test the correspondence of the relative abundances of the genotypes to the Hardy–Weinberg distribution for the allele frequencies identified and to assess the genetic heterogeneity of the samples. The total value of the $\Sigma\chi^2$ parameter based on the additivity of the χ^2 distribution was calculated as described in (Zhivotovskii, 1991).

RESULTS AND DISCUSSION

The full nucleotide sequence of the *cytb* mtDNA gene was determined in striped field mice captured in four local communities in Magadan oblast (Fig. 1). The *cytb* gene of this species consists of 1143 base pairs (bp) and is located between the bases 14127 and 15270 of the mitochondrial genome. The total number of single-nucleotide polymorphisms in the *cytb* sequence of the animals analyzed was 22, and combinations of these polymorphisms corresponded to five different haplotypes of the *cytb* gene (Fig. 2). The M1–M5 nucleotide sequences obtained were submitted to GenBank (nos. KR338982–KR338986).

The single-nucleotide polymorphisms detected in the *cytb* gene sequence of the striped field mouse were mostly synonymous, with 18 transitions and three transversions observed in position 3 of the codon. The results obtained are in agreement with the reports on the highest variability of the third nucleotide in most codons of the translated fragments of genes; this feature is due to the degenerate character of the genetic code (Zardoya and Meyer, 1996). The replacement of thymine by cytosine in the M2 haplotypes affected the second nucleotide of a triplet (at 686 bp from the gene start site) and led to the replacement of isoleucine by threonine in position 229 of the polypeptide chain of cytochrome *b*. This amino acid residue is located in the VI transmembrane domain of the enzyme and is characterized by an intermediate degree of conservation (Howell, 1989). The modified physicochemical Grantham distance for this substitution is 59, this being higher than the threshold value of 57.9 for single substitutions and thus being indicative of the conservative character of the substitution (Butvilovskii et al., 2009). The amino acid substitution observed is apparently neutral from the evolutionary point of view.

The distribution of the frequencies of the *cytb* gene haplotypes in the striped field mouse samples investigated is shown in Table 1.

Striped field mice captured near Snezhnaya Dolina constituted the only group characterized by a poly-

Table 1. Haplotype frequencies of the cytochrome *b* gene in striped field mice captured at different sites in Magadan oblast

Sampling site	Sample size	Haplotypes of the cytochrome <i>b</i> gene				
		M1	M2	M3	M4	M5
		haplotype abundance in the sample				
Snezhnaya Dolina	6	0.1667	0.1667	0.1667	0.0000	0.5000
Snezhnyi	17	0.0000	0.0000	0.0000	0.0000	1.0000
Solnechnyi	34	0.0000	0.0000	0.0000	0.0000	1.0000
Talon	23	0.0000	0.0000	0.0000	1.0000	0.0000

morphic structure of the *cytb* gene. Since the number of animals investigated was relatively small ($n = 6$), the presence of other *cytb* haplotypes in the striped field mice from this area cannot be ruled out. The population of this species in Snezhnaya Dolina was presumably formed as a result of several “waves” of invasion by small numbers of animals from different donor populations or the same donor population that gave rise to several instances of invasion. The invader animals apparently had different mtDNA haplotypes in the case of invasion that corresponded to the latter model. A single instance of introduction of a large group of unrelated animals appears less probable.

The monomorphic structure of the *cytb* nucleotide sequence in the striped field mice from all other samples is apparently due to synergy of the founder effect and the gene drift effect. All individuals from Snezhnyi had the M5 haplotype, which was common for the animals from Snezhnaya Dolina as well. These settlements are located on different banks of the Dukcha River, and the distance between them is less than 2 km. The striped field mice of Magadan oblast inhabit areas populated by humans and vegetable gardens adjacent to these areas, and therefore the existence of limitations on the flow of genes between the local communities of this species in Snezhnyi and Snezhnaya Dolina can be expected. The individuals with the M5 haplotype were apparently transported into both neighboring settlements with the same shipment of agricultural produce (or hay), whereas the invasion of individuals with other *cytb* haplotypes might not have occurred in Snezhnyi. Another putative scenario implies the introduction of striped field

mice carrying other variants of the *cytb* gene into Snezhnyi followed by stochastic elimination of haplotypes other than M5 from the gene pool of mice in this population.

All striped field mice from Solnechnyi were carriers of the M5 variant. This settlement is connected to Snezhnyi by an 18-km road flanked by isolated vegetable gardens, but no striped field mice were captured in the area between the populated territories. This may be due to the small size of striped field mouse populations in these settlements and the related low migratory activity of the animals. All striped field mice of these populations carried the M5 haplotype, this apparently being due to the invasion of rodents from the same area.

All mice that inhabited the environs of Talon were carriers of the M4 haplotype, and this may be due to the combined action of the founder effect and gene drift as well. The neighbor-joining (NJ) method implemented in the MEGA6 software package was used to construct a tree that reflected the phylogenetic relationships between striped field mouse populations of Magadan oblast (Fig. 3).

The NJ dendrogram included two clusters. The groups of M5 haplotype carriers from Snezhnyi and Solnechnyi constituted the first clade. The mouse population from Snezhnaya Dolina characterized by polymorphism of the *cytb* nucleotide sequence of the gene and the M4 haplotype carriers from Talon constituted the second clade. These populations could be combined owing to the structural similarity of the M1–M3 variants (Snezhnaya Dolina), on one hand, and the M4 variant (Talon) of the *cytb* haplotype of the

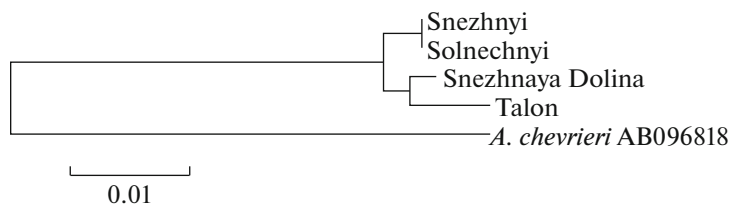


Fig. 3. NJ phylogenetic tree that shows relationships between the striped field mouse colonies in Magadan oblast. The tree is based on the data on the variability of the cytochrome *b* gene of mtDNA. The scale bar shows the number of nucleotide substitutions per site.

striped field mouse, on the other hand. The branch that corresponded to the animals captured in Talon was longer, since the M4 haplotype carriers occurred only at this site. Striped field mice were apparently introduced into this settlement with a single lot of agricultural produce that was transported exclusively to Talon. Carriers of other *cytb* haplotypes either did not arrive in Talon or were eliminated from the population owing to stochastic processes.

Farm animal feed and agricultural produce that could be the source of striped field mice introduced to Magadan oblast were mostly brought from Primorsky krai and Khabarovsk krai, and some lots of these goods were from Siberia, Kazakhstan, China, and Korea. Therefore, sequences of the *cytb* gene (or its fragments of not less than 880 bp in length) of striped field mice from this region were retrieved from GenBank.

Initial analysis revealed the similarity of the haplotypes M1–M4 and the nucleotide sequences (742 bp) of *cytb* gene variants B1, B2, B3, B5, B7, and B8 (GenBank nos. FJ906759, FJ906756, FJ906761, FJ906760, FJ906762, and FJ906764, respectively) detected in striped field mice captured in the south of Primorsky krai (Pereverzeva and Pavlenko, 2014). More detailed analysis involved the registration of full nucleotide sequences of the *cytb* gene corresponding to every single haplotype of the individuals captured in Primorsky krai. The sequences obtained were deposited in GenBank. The GenBank sequence KU859999 corresponds to the B1 haplotype; KU860001, to B2; KU860002, to B3; KU860000, to B5; KU860003, to B7; and KU860006, to B8. Importantly, exactly these haplotypes were detected in striped field mice captured near the village of Priluki in Khorol'skii raion (Pereverzeva and Pavlenko, 2014), the area adjacent to the farmlands of the village of Priozernoie that were the source of agricultural produce supplies to Magadan oblast until the late 1990s. The neighbor-joining (NJ) method implemented in the MEGA6 software package was used to construct a tree that illustrated the phylogenetic relationships between the nucleotide sequences characteristic of the haplotypes M1–M5 and the variants of the *cytb* gene of striped field mouse individuals captured at the above-mentioned sites (Fig. 4). The results obtained point to the high degree of polymorphism of the nucleotide sequence of the *cytb* gene in *A. agrarius*, as evident from the existence of subclusters with high bootstrap index values. The M5 variant is located on a branch close to the root of the tree with a 70% bootstrap support. The M5 haplotype apparently belongs to a more ancient and isolated genetic subline.

The nucleotide sequences selected for further phylogenetic analysis were located in the same subclusters as M1–M5 on the NJ tree; the median network that obeyed the principle of minimal number of nucleotide substitutions was constructed for these sequences (Fig. 5).

The median network (Fig. 5) was used for a more detailed elucidation of the phylogenetic relationships between the M1–M5 haplotypes. The M1 variant (within the 880-bp fragment) was identical to the nucleotide sequences of the AM495857 sample (animal captured in the zone of range disjunction, in the Onon River valley, near the village of Karaksar in Zabaykalsky krai close to the border of the Far East okrug) (Pavlenko et al., 2007; Sakka et al., 2010) and the KU860002 sample (animal captured in Primorsky krai). It is reasonable to assume that the carriers of the M1 haplotype of this species were transported into Magadan oblast from Primorsky krai, since the economic ties between Primorsky krai and the Magadan area are better developed than those between the latter and Zabaykalsky krai. The nucleotide sequence of the *cytb* gene of the KJ082007 and KJ082011 individuals (Kim and Park, 2015) captured in China was almost identical to M1, with only one nucleotide substitution (Fig. 5). China borders on Primorsky krai, and therefore the flow of genes between *A. agrarius* populations of these areas is entirely possible.

The nucleotide sequence of this gene fragment was identical in mice from Khabarovsk krai (AM495865) and Primorsky krai (KU860000) (Fig. 5). This *cytb* haplotype was almost identical to M1, except for a single nucleotide substitution. It is the ancestor haplotype for a small subcluster that includes the *cytb* gene variants detected in the individuals captured in Primorsky krai (KU859999, KU860001, and KU860003) and for the M2 haplotype (Fig. 5). This may be indicative of the single origin of these haplotypes. One may assume that the carriers of the M2 haplotype originate from Primorsky krai as well (or from Khabarovsk krai).

The gene variants M3 and M4 have similar nucleotide sequences and form a small subcluster that originates from M1 (Fig. 5). Very extensive polymorphism of the nucleotide sequence of the *cytb* gene is characteristic of *A. agrarius*. For instance, the number of variants of the 742-bp fragment of the *cytb* gene in a striped field mouse sample from the south of Primorsky krai was 36 (Pereverzeva and Pavlenko, 2014). One may assume that the number of variants of the *cytb* gene will increase as the full nucleotide sequence (1143 bp) is analyzed in the representatives of the population of this species in Primorsky krai. The presence of *cytb* haplotypes identical to the hypothetical mv1 or more closely related to M3 and M4 in the gene pool of the striped field mouse is highly probable. However, mice that carry these genotypes have not been captured yet, and the respective nucleotide sequences of the *cytb* gene have not been registered in GenBank. The genetic relatedness of the M1 nucleotide sequence and the sequences M3 and M4 is apparent, and this points to the putative invasion of striped field mice with M3 and M4 haplotypes from Primorsky krai into Magadan oblast.

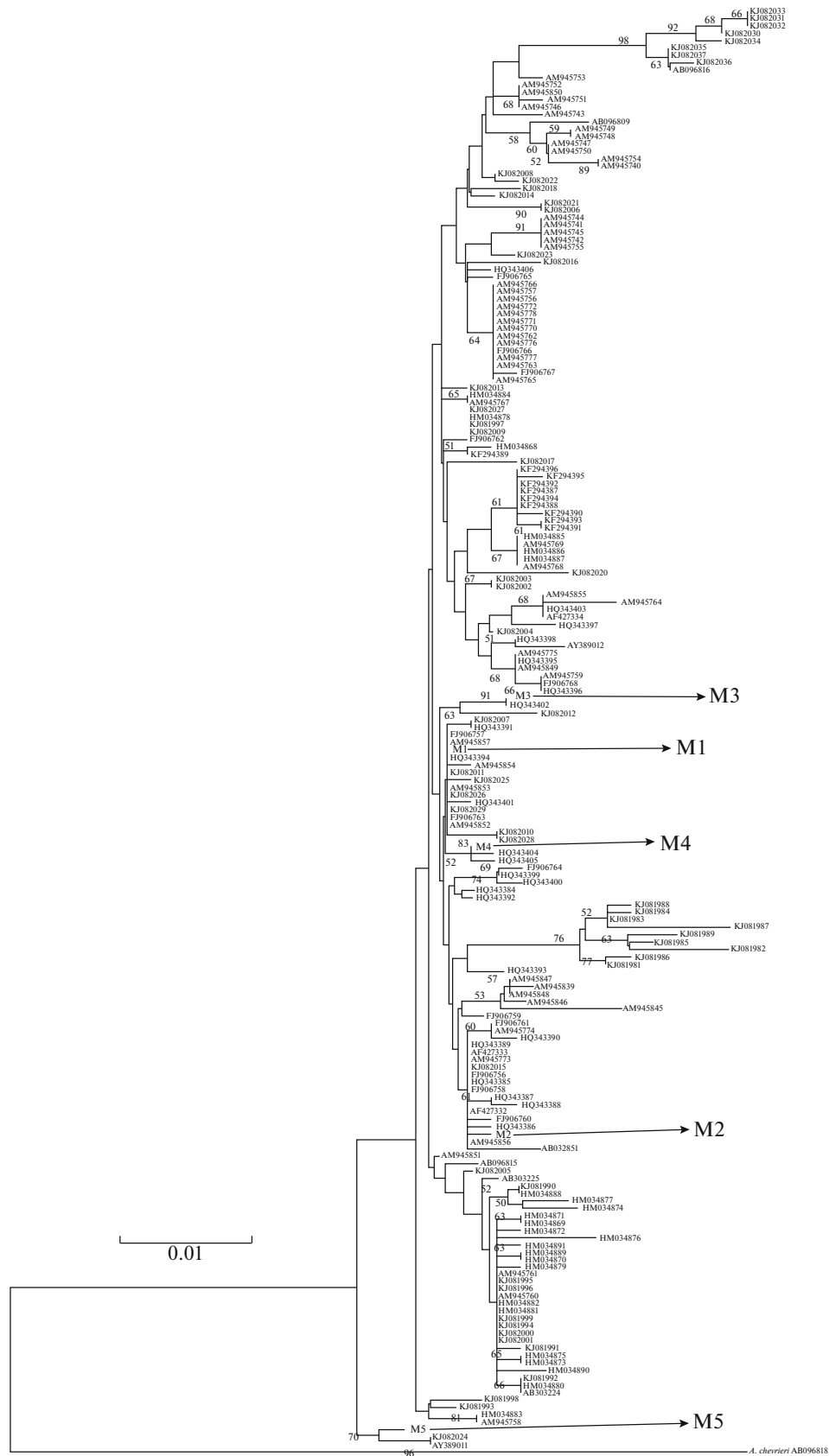


Fig. 4. NJ phylogenetic tree based on the data on the variability of a 880-bp fragment of the cytochrome *b* gene of mtDNA of striped field mouse from Magadan oblast and from the putative donor populations. The bootstrap indices >50% are shown on the branches of the tree. The scale bar corresponds to the genetic distance between the haplotypes.

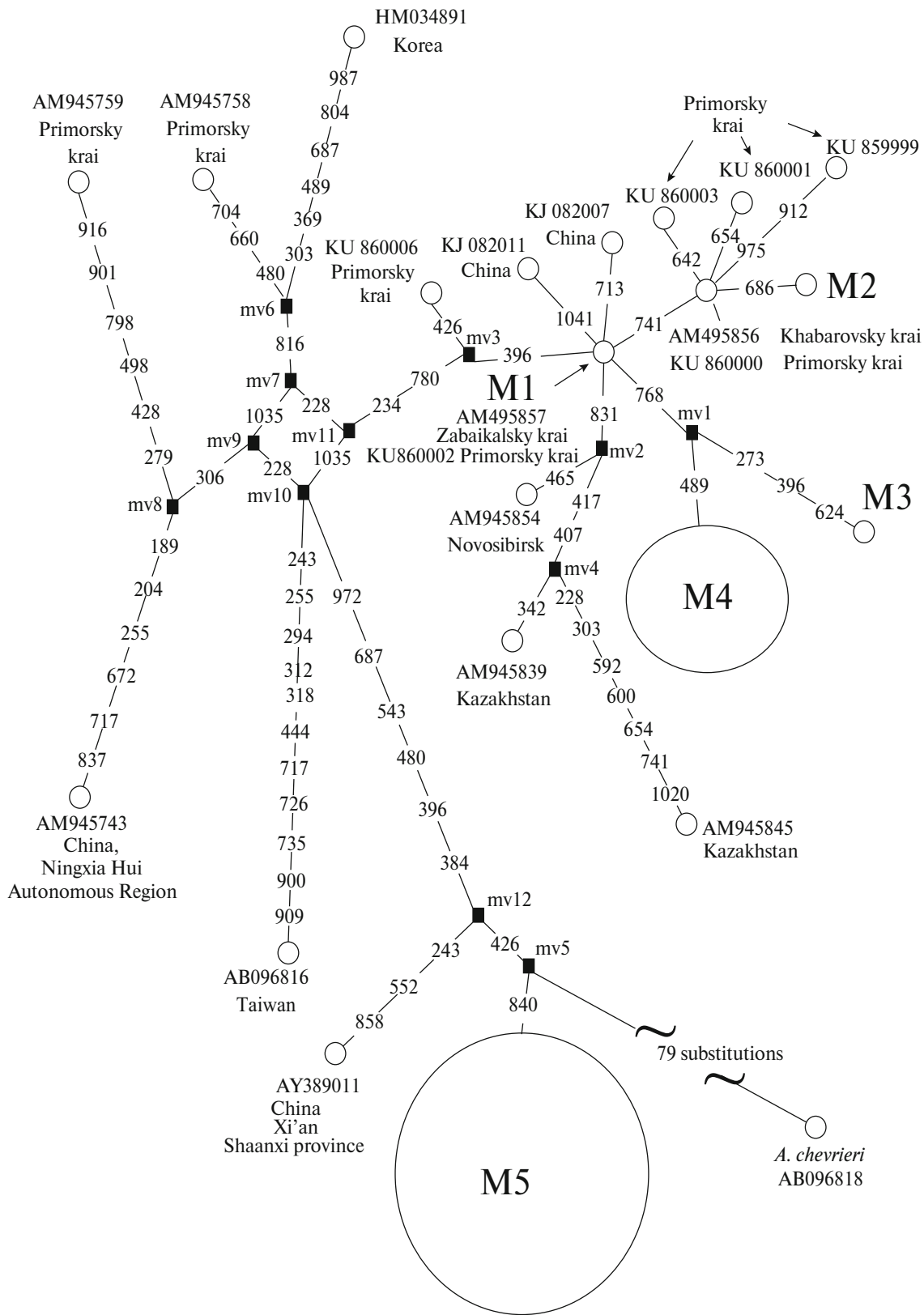


Fig. 5. The median network of *A. agrarius* mtDNA haplotypes registered in Magadan oblast and the putative donor populations. The size of the circles is proportional to the frequency of occurrence of the mtDNA variant in striped field mouse samples from the colonies of Magadan oblast. The numbers correspond to the location of the mutation sites relative to the start of the cytochrome *b* gene of the mitochondrial genome of *A. agrarius*, GenBank no. HM034866 (Oh et al., 2011). The hypothetical haplotypes mv 1–12 are marked by black squares.

Thus, Primorsky krai is the most likely source of invasion of striped field mice with the haplotypes M1–M4 into Magadan oblast. This assumption is supported by the highest similarity of the nucleotide sequences of mtDNA variants of striped field mice captured near the village of Priluki in Primorsky krai (near the farm that regularly supplied agricultural produce to Magadan oblast) to the M1–M4 haplotypes. It is also important to note that the haplotypes of striped field mice from Primorsky krai most similar to the *cytb* variants of the animals from Magadan oblast included the variants B2 (KU860001), B3 (KU860002), and B7 (KU860003), which we previously assumed to be of adaptive value (Pereverzeva and Pavlenko, 2014).

The M1 variant is ancestral both for M2–M4 detected in the animals from Magadan oblast and for other haplotypes from the eastern part of Asia, including those from Primorsky krai, Khabarovsk krai, China (the Far East-Chinese part of the range), and Eastern Siberia and Kazakhstan (the European-Siberian-Kazakhstan block) (Fig. 5). Therefore one may assume that the M1 variant is relatively common for striped field mouse populations of the eastern part of Asia. This variant is apparently characteristic of *A. agrarius* individuals of the Far East-Chinese block. However, M1 may also be the ancestor variant of the nucleotide sequences from certain individuals captured in the European-Siberian-Kazakhstan part of the range (AM945839, AM945845, and AM945854). Notwithstanding the isolation of the two parts of *A. agrarius* range, accidental transport of striped field mice from one area to the other cannot be ruled out. Such transport would most likely be related to economic activity of people, namely, to the railroad transportation of agricultural produce or farm animal feed. Therefore, isolation of the mice of the European-Siberian-Kazakhstan and Far East-Chinese parts of the range might not be complete (Sakka et al., 2010; Frisman et al., 2015; Kim and Park, 2015), but the gene flow between the *A. agrarius* populations in these two blocks is apparently rather limited.

The M5 haplotype occupies an isolated position in the median network (Fig. 5). M5 is closer to the *cytb* gene from *A. chevrieri* than any other variant of this part of the mitochondrial genome of *A. agrarius*. This is evident of the archaic character of this nucleotide sequence. The M5 variant belongs to a genetically isolated (bootstrap index of 70%) subcluster that also includes the AY389011 sample from an animal captured in China (Central China, Shaanxi province, Xi'an (Liu et al., 2004)) (Fig. 4). The similarity of the nucleotide sequences of M5 and AY389011 points to the possible origin of these haplotypes from a single genetic subline of *A. agrarius*. The nucleotide sequences of these samples contain 16 (M5) and 17 (AY389011) single-nucleotide substitutions relative to the sample AM945743 (closest to these sequences with regard to structure and retrieved from an animal captured in the neighboring area of China, the Ningxia-Hui autonomo-

ous region (Sakka et al., 2010)). The number of substitutions observed upon the comparison with M1 is 11 and 12, respectively (Fig. 5). However, if one takes the topology of the NJ tree (Fig. 4) into account, the invasion of carriers of the M5 haplotype from the central provinces of China appears highly probable. The trade ties between Magadan oblast and China are currently very well developed. Goods are transported through the ports of the Far East of Russia, and therefore direct transportation of striped field mouse individuals from China into Magadan oblast is possible.

In general, the striped field mice of Magadan oblast are likely to have originated from the inhabitants of the Far East-Chinese part of the range of this species, that is, from striped field mice of Primorsky krai and China.

The M5 haplotype was detected in mice from the colonies of Snezhnyi, Snezhnaya Dolina, and Solnechnyi (Table 1). This may be indicative of the introduction of the representatives of this species into Magadan oblast from a single donor population.

The similarity of the gene pools of *A. agrarius* from Snezhnyi and Solnechnyi was characterized in more detail using genetic analysis of the biochemical markers of nuclear genes. Sixteen allozyme loci were detected and interpreted in the samples of striped field mice from Magadan oblast. The loci LDH-1, LDH-2, IDH-2, PGD, GR, SOD, HK, AK, and EST-D were monomorphic in all groups investigated. Seven polymorphic enzyme systems were detected in the mouse colonies investigated. Characteristics of the polymorphic loci, parameters of allozyme variability of the samples, and estimates of intersample heterogeneity for these gene markers are listed in Table 2.

The frequency of the major allele in the polymorphic systems ranged from 0.556 to 0.98. Most loci included two alleles, with the exception of the EST-M locus, which included three and four alleles, respectively, in the animals from Talon and Solnechnyi. A heterozygous individual from the latter area carried two rare alleles.

The study revealed polymorphic gene markers that were restricted to a single group of mice, identified allozyme loci polymorphic between two samples of individuals, and pointed to markers polymorphic in all groups investigated.

Significant deviation of the genotype abundances observed from those expected for a Hardy–Weinberg distribution was observed in three cases. Firstly, one of the individuals captured near Solnechnyi had the 0.90/1.30 genotype in the EST-M locus and was the only carrier of two rare alleles, this being the reason for the genetic disequilibrium observed. Secondly, a significant excess of individuals heterozygous in the ACP locus was observed in the same sample. Several putative reasons for this deviation from the equilibrium state can be stated. The animals were captured at the end of August on a relatively small part of a large field;

Table 2. Allele frequencies for the polymorphic loci, assessment of the genetic heterogeneity level, and allozyme variability parameters for the striped field mouse samples from Magadan oblast

Locus	<i>F</i>	Capture sites			χ^2 value
		Talon <i>N</i> = 62	Solnechnyi <i>N</i> = 42	Snezhnyi <i>N</i> = 18	
		Allele frequencies			
G-3-PD	1.00	1.00	1.00	0.972	$\chi^2 = 5.83$ d.f. = 2
	1.10			0.028	
	H_{obs}			0.056	
ME	1.00	0.911	1.00	1.00	$\chi^2 = 11.70^{**}$ $P < 0.01$ d.f. = 2
	1.10	0.089			
	H_{obs}	0.177			
GOT	1.00	1.00	0.369	0.861	$\chi^2 = 88.69^{***}$ $P < 0.001$ d.f. = 4
	1.10		0.631	0.139	
	H_{obs}		0.500	0.278	
EST-M	0.90	0.113	0.012	1.00	$\chi^2 = 37.42^{***}$ $P < 0.001$ d.f. = 12
	1.00	0.556	0.750		
	1.20	0.331	0.226		
	1.30	0.000	0.012		
	H_{obs}	0.548	0.381		
ACP	$L(\chi^2)$		84.03 ^{***} $P < 0.001$		$\chi^2 = 14.99^{**}$ $P < 0.01$ d.f. = 4
	d.f.		6		
	1.00	0.847	0.702	0.806	
	1.10	0.153	0.298	0.194	
	H_{obs}	0.274	0.548	0.167	
GLO	$L(\chi^2)$		4.03* $P < 0.05$	3.94* $P < 0.05$	$\chi^2 = 30.12^{***}$ $P < 0.001$ d.f. = 4
	d.f.		1	1	
	1.00	1.00	1.00	0.833	
PGM	1.10			0.167	$\chi^2 = 1.97$ d.f. = 2
	H_{obs}			0.222	
	1.00	0.984	1.00	1.00	
Allozyme variability parameters in the samples					$\Sigma \chi^2 = 190.72^{***}$ $P < 0.001$ d.f. = 30
H_{obs}	0.064	0.089	0.045		
$N_{A/L}$	1.3	1.3	1.1		

F—relative mobility of the allele, *N*—the number of individuals investigated, $L(\chi^2)$ —value of the χ^2 parameter for the Hardy–Weinberg estimate in the locus, d.f.—number of degrees of freedom, *P*—confidence level, H_{obs} —average heterozygosity observed, $N_{A/L}$ —average number of alleles per locus, $\Sigma \chi^2$ —integrated value of the χ^2 parameter.

therefore, the predomination of related animals of less than one year of age and the same heterozygous genotype was possible. Thirdly, a significant deviation from the genotype distribution was observed in case of the ACP locus in striped field mice of Snezhnyi. The disequilibrium observed for this sample was related to the presence of two rare homozygote individuals. The number of animals captured at the same site was relatively small (Table 2), and therefore the carriers of these genotypes may belong to the same litter. The fre-

quencies of the genotypes for the rest of polymorphic allozyme loci in the striped field mouse samples obeyed the Hardy–Weinberg law.

The level of heterogeneity was assessed in order to reveal the putative isolation of specific groups of striped field mice in Magadan oblast (Table 3).

The distribution of the alleles of biochemical gene markers in every sample of mice was significantly different from that in the other samples. This is indicative of complete isolation of the Magadan oblast colonies

Table 3. Total genetic heterogeneity of striped field mouse samples from Magadan oblast as inferred from the analysis of polymorphic allozyme loci

Capture site	Talon	Solnechnyi	Snezhnyi
Talon		$\Sigma\chi^2 = 117.90^{***}$ d.f. = 12	$\Sigma\chi^2 = 75.52^{***}$ d.f. = 13
Solnechnyi	$P < 0.001$		$\Sigma\chi^2 = 53.68^{***}$ d.f. = 10
Snezhnyi	$P < 0.001$	$P < 0.001$	

$\Sigma\chi^2$ —the total value of the χ^2 parameter for all loci, P —confidence level, d.f.—number of degrees of freedom.

of *A. agrarius* analyzed in the present study, and therefore the colonies can be regarded as isolated populations. The gene pool of isolated colonies of the striped field mouse is shaped by the synergy of the founder effect, gene drift, and selection. The number of striped field mouse individuals transported into different settlements of Magadan oblast could have been small, and thus the original gene pool could have contained a limited number of alleles. Importantly, animals that ended up in different settlements carried different allele combinations. These relatively small sets of alleles unique for each colony of striped field mice laid the foundations for the formation of the unique gene pools of these populations. Smaller sizes of the local colonies and more pronounced size fluctuations are predisposed to higher intensity of stochastic changes in the allele frequency of various genes that are not directly related to selection (gene drift). The striped field mouse is a species characterized by rapid reproduction and strong fluctuations of the population size. This is confirmed by the results of annual test captures of animals in the farmlands near Solnechnyi during the years 2005–2013. Striped field mice were numerous in this area in the years 2005 and 2008, whereas the number of animals captured in the other years was either very low or zero (no animals captured) (A.A. Primak, personal communication). A dramatic decrease in population size results in the survival of a small number of individuals with a very restricted allele pool. The gene pool of the colony undergoes a “bottleneck” stage that leads to the decrease in polymorphism.

Comparison of the levels of protein polymorphism in striped field mice from different populations of Magadan oblast and the Far East-Chinese part of the range was based on data on allozyme variability of *A. agrarius* samples from Primorsky krai and Khabarovsk krai. The integrated variability parameters of the set of allozyme loci analyzed showed a trend to a decrease in the groups of striped field mice that inhabited the territories north of the Sea of Okhotsk. The number of polymorphic loci among the 16 loci investigated ranged from three to four for all populations of *A. agrarius* investigated, whereas the number of polymorphic loci in the above-mentioned set of allozyme markers ranged from four to six in the native popula-

tions of this species in Primorsky krai and Khabarovsk krai. The EST-M locus was the only one with four alleles in the animals from the colonies of Magadan oblast, whereas the presence of 3–4 alleles was registered for several loci (G-3-PD, ME, EST-M, and GLO) in striped field mice from the south of the Far East of Russia (Zasytkin et al., 2007).

CONCLUSIONS

Comparative analysis of the data of molecular genetic and allozyme studies can be used to reconstruct the history of formation of the gene pool of new *A. agrarius* populations that emerged in Magadan oblast owing to multiple invasions. The monomorphic structure of the nucleotide sequence of the *cytb* gene in the individuals from three colonies of the four investigated is worth mentioning. Considerable polymorphism of this mtDNA region is characteristic of the native populations of this species. This is evident of intense microevolutionary processes in the isolated *A. agrarius* colonies investigated. The information on the phylogenetic relationships of the haplotypes of the *cytb* mtDNA gene in striped field mouse captured north of the Sea of Okhotsk pointed to Primorsky krai as the putative source of invasion of this species into Snezhnaya Dolina and Talon, whereas the ancestors of certain animals captured in Snezhnaya Dolina, Snezhnyi, and Solnechnyi were apparently imported from China. These territories belong to the Far East-Chinese part of the range of *A. agrarius*. The detection of M5 *cytb* haplotypes in the samples from Snezhnyi and Solnechnyi is evident of the single monophyletic origin of these colonies. Transportation of a single lot of agricultural produce into these settlements apparently resulted in the invasion of matrilineally related striped field mice with the same mitotype. The invasion of individuals with different *cytb* haplotypes into Snezhnyi and Solnechnyi cannot be ruled out, but in this case, the invasion was apparently followed by the elimination of the carriers of these haplotypes from the colonies in question owing to stochastic processes. Several instances of invasion by this species due to repeated transportation of agricultural produce into the settlements investigated cannot be ruled out. The frequency of *cytb* haplotypes introduced during the

repeated invasions was apparently low, and gene drift led to the elimination of these mitotypes from the gene pool of striped field mice of Snezhnyi and Solnechnyi. Highly significant differences between the sets of biochemical genetic markers of these samples are apparently due to the synergy of the founder effect and gene drift. The level of allozyme polymorphism in the donor population of *A. agrarius* was apparently higher, but only part of the gene pool of this population was transported to the colonies of this species formed in Magadan oblast, since the number of invading individuals was low. The polymorphism in the EST-M locus was conserved only in the striped field mouse colony of Solnechnyi, whereas the polymorphism in the loci G-3-PD and GLO was conserved in Snezhnyi. Stochastic processes could have contributed to the loss of certain alternative alleles of these loci as well, since the striped field mouse groups investigated were small and isolated.

The only *A. agrarius* colony of Magadan oblast that showed polymorphism in the structure of the nucleotide sequence of the *cytb* gene inhabited Snezhnaya Dolina. The formation of this population was apparently due to repeated invasion of small groups of animals from the same donor population in China (carriers of the M5 mitotype) and from several donor populations of Primorsky krai (carriers of the haplotypes M1–M3). An alternative scenario of the formation of the gene pool of the striped field mouse in Snezhnaya Dolina is possible as well. This scenario involves a single instance of invasion by striped field mice with the M5 haplotype (from a single Chinese population) and several instances of invasion by the carriers of the M1–M3 *cytb* gene variants from the same population of Primorsky krai.

Talon is situated at a distance of 130 km from Magadan. The M4 haplotype was detected only in the rodents from this settlement, and this may be indicative of a unique monophyletic origin of the rodent community from Talon. The range of polymorphic allozyme markers of this sample had certain distinctive features as well. The ME and PGM loci were polymorphic in the mice captured in Talon, whereas these loci were monomorphic in the mouse samples from Snezhnyi and Solnechnyi. Polymorphism in the GOT locus was detected in the DNA of mice from Snezhnyi and Solnechnyi; however, this marker was monomorphic in the mice from Talon (Table 2). Striped field mice were presumably transported to Talon with a single lot of agricultural produce supplied from Primorsky krai.

The results of molecular genetic and allozyme analysis are evident of the existence of small isolated *A. agrarius* colonies in Magadan oblast. The single monophyletic origin of striped field mice from Snezhnyi and Solnechnyi was revealed. The origin of the colony of this species in Snezhnaya Dolina was apparently polyphyletic. The origin of the striped field mouse population of Talon was monophyletic and dif-

ferent from that of all other colonies of this species in the area north of the Sea of Okhotsk. Importantly, the degree of geographical isolation was the highest for this population. Highly significant genetic differences between the striped field mouse samples collected in Magadan oblast point to the isolated character of the colonies identified and show that these colonies can be regarded as independent populations. These *A. agrarius* colonies were probably formed at different times, and several instances of invasion by the striped field mouse contributed to their formation. The density of these populations was generally low, with local peaks of population size observed during certain years (the year 2003 in the case of Talon and the years 2005 and 2008 in the case of Solnechnyi). The small population size apparently is the reason for low migration activity of the rodents and the existence of isolated local colonies of the striped field mouse in Magadan oblast.

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