ISSN 2079-0864, Biology Bulletin Reviews, 2020, Vol. 10, No. 5, pp. 383–393. © Pleiades Publishing, Ltd., 2020.
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Differentiation of Continental Isolates of the Striped Field Mouse (*Apodemus agrarius* Pallas, 1771) by Microsatellite Loci

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Received September 6, 2018; revised March 3, 2019; accepted April 29, 2019

Abstract—Microsatellite analysis was used to examine intraspecific polymorphism in two extensive continental isolates of the striped field mouse (*Apodemus agrarius* Pallas, 1771) separated by the Baikal disjunction. Striped field mice from the western isolate (from the European and Kazakh-Siberian parts of the range) and from the eastern isolate (from the territory of the Middle Amur Region and Coastal Territory of Far East) were tested. The analysis used 180 specimens collected from 33 localities and five microsatellite loci developed earlier for the genus *Apodemus*. The work was carried out based on the summation of local samples in each of the aforementioned geographical regions. It was shown that allelic diversity and the number of specific alleles were higher in the eastern isolate that may be the result of the longer habitation of the striped field mouse in the eastern part of the range. The limited number of specific alleles in the western isolate as compared to the eastern one can be determined by the founder effect and may reflect the direction of the historical migration of the species from east to west. Our results demonstrate no more than a population level of differentiation within the continental isolates of the striped field mouse and indicate no more than a subspecies level of differences between these isolated forms, i.e., the relatively recent penetration of *A. agrarius* to western Eurasia.

DOI: 10.1134/S2079086420050035

INTRODUCTION

The striped field mouse (*Apodemus agrarius* Pallas, 1771) inhabits an extensive area from Central Europe to the Pacific coast of Asia. The species inhabits also some islands of the Pacific Ocean. The mainland part of the range of *A. agrarius* is subdivided into two large isolated massifs. The break in the range occurs in the arid Transbaikalia that is not surprising for the species ecologically associated with moist forest-meadow landscapes and a forest–steppe penetrated by watercourses (Karaseva et al., 1992; Gromov and Erbaeva, 1995). The western isolate of the striped field mouse includes European, Kazakh, and Siberian populations, and the eastern isolate includes populations of the south of the Russian Far East, the Korean peninsula, and eastern China. Due to the large extent of the range of *A. agrarius* and its disjunction in Transbaikalia, researchers are permanently interested in the study of this species with respect to the level of its genetic differentiation, the taxonomic status of isolated population groups, analysis of the direction of their distribution and the time of separation. The performed molecular genetic studies, allozyme analysis, Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD–PCR) analysis, and sequencing of the mitochondrial and nuclear DNA, demonstrated that the isolated population groups of *A. agrarius* belong to the same species (Pavlenko and Vorontsov, 1990; Mezhzherin and Zykov, 1991; Atopkin et al.,

2007; Suzuki et al., 2008; Sakka et al., 2010) or even the same subspecies (Koh et al., 2014). The higher polymorphism of populations within the eastern isolate indicated its proximity to the center of origin of the species, as well as an east-to-west path of past migration of the striped field mouse. Based on molecular clocks, Suzuki et al. (2008) estimated the time of penetration of the striped field mouse from the eastern to the western Palaearctic to be 200000 years ago. According to the assumptions of Atopkin et al. (2007), this event could have occurred at the temperature optimum of the Holocene, i.e., 4500–7000 years ago. Based on paleontological data, both the appearance of the striped field mouse in Europe in the Early Holocene (Kowalski, 2001) and several expansions from Asia to Europe during the Pleistocene (Popov, 2017) were proposed. Some authors indicate the presence of numerous fossils of striped field mice in the territory of Moldova and the Trans-Urals only in Holocene deposits (David and Chemyrtan, 1976; Ivakina et al., 1997). Even if we accept a minimal estimate of the time of distribution of the striped field mouse across the Palaearctic and the subsequent disjunction of the range, it can be expected that the isolation period could have led to differences in the population structure in the western and eastern Palaearctic.

One of the most sensitive methods of population research is analysis of the polymorphism of microsatellites, codominantly inherited and neutral markers. They are characterized by a high mutation rate, uneven amplification, and rapid propagation along the genome that leads to the homogenization of certain repeat families (Bannikova, 2004). The listed features determine the prospects for the use of microsatellites in the study of intraspecific variability. For the striped field mouse, there are data on the polymorphism of microsatellite loci in populations of local regions of both the western (Makova et al., 1998, Gortat et al., 2013) and eastern (Jo et al., 2016) isolates. The degree of differentiation of microsatellites between the western and eastern isolates and the populations of these isolates is not yet clear.

The goal of the study was to assess the allelic diversity and level of differentiation by microsatellite loci within and between mainland isolates of the striped field mouse. Microsatellite loci, as a rule, are characterized by a large number of alleles, which implies the use of local representative samples and, if material from individual populations is not sufficient, the use of the pooled samples from separate geographical areas was made. The latter approach was used in this study.

MATERIALS AND METHODS

The study involved 180 *A. agrarius* specimens captured in 33 points on the mainland. The available material from the territory of the western isolate allowed the formation of two pooled samples of 30 specimens: the first ("Eastern Europe") included striped field mice from the European part of the range, and the second ("Siberia + Kazakhstan") included mice from the Asian part of the range west of Lake Baikal. Our material from the territory of the eastern isolate, which is collected in the populations of two regions separated by an insulating barrier (Amur River), allowed the formation of four pooled samples of the same size (30 specimens each): two in the Middle Amur region ("Middle Amur Region–West" and "Middle Amur Region–Center") and two in Coastal Territory of Far East, so-called Primorye ("Western Primorye" and "Southern Primorye"). Table 1 and Fig. 1 show the material-collection points and the grouping into pooled samples.

DNA was isolated with the standard salt extraction procedure (Aldjianabi and Martinez, 1997) from tissues fixed in 96% ethanol. Primer sequences and amplification modes from a previously published study were used (Makova et al., 1998). Primers were synthesized by BioBeagle (St. Petersburg). Fluorescent label 6-FAM (fluorescein) was used. Fragment lengths were analyzed with an AB-3500 eight-channel genetic analyzer (Applied Biosystems, United States) in a POP-7 gel polymer in the presence of a molecular weight marker, LIZ 600. Five loci differing in both the length of the repeating motif and the number of known alleles were analyzed for each DNA sample: GTTDS8, GATAE10A, CAA2A, GTTF9A, and GSADT7S (Makova et al., 1998). The loci were identified with the Gene Mapper program, version 4.1 (Applied Biosystems, United States). For each locus, alleles differing only in the number of motif repeats were taken into account.

The results of the study of polymorphism of microsatellites were assessed based on the analysis of genotypic and allelic frequencies. The allele frequencies in pooled geographic samples were calculated with the formula (Kuznetsov, 2014)

$$\hat{p}_i = \left(n_{i,i} + 0.5\sum_{j=1}^{r} n_{i,j} \right) \Big/ N ,$$

where $\hat{p}_i$ is the frequency of allele i, r is the number of alleles in the polymorphic locus, $n_{i,j}$ is the number of specimens with a genotype corresponding to the combination of alleles i and j in the locus, and N is the sample size.

Their standard error was calculated with the formula

$$\mathrm{SE}(\hat{p}_i) = \sqrt{\mathrm{Var}(\hat{p}_i)},$$

where $\mathrm{Var}(\hat{p}_i)$ is the sample binomial variance (σ^2) in the equilibrium state of the population according to Hardy–Weinberg:

$$\mathrm{Var}(\hat{p}_i) = \hat{p}_i(1 - \hat{p}_i)/2N .$$

Table 1. Collection points and pooled sample structure of the striped field mouse

No.	Geographic collection points	Amount of animals
	Southern Primorye	
1	Russia, Primorskii krai, Khasansky district, vicinity of Karasik River	15
2	Russia, Primorskii krai, Ussurisk district, the village of Knevichi	15
	Western Primorye	
3	Russia, Primorskii krai, Khankaisky district, eastern shore of Khanka Lake, Vostochny cordon, Rechnoy section, Khankaisky State Nature Biosphere Reserve	22
4	Russia, Khabarovsk krai, vicinity of the village of Orenburgskoe, right bank of Bikin River	8
	Middle Amur Region–Center	
5	Russia, Jewish Autonomous oblast, Birobidzhan district, 54th km of the Dubovoe-Nadezhdinskoye road	3
6	Russia, Jewish Autonomous oblast, Birobidzhan district, vicinity of the village of Kazanka	4
7	Russia, Jewish Autonomous oblast, Obluchinsky district, vicinity of the village of Izvestkovyi	23
	Middle Amur Region–West	
8	Russia, Jewish Autonomous oblast, Obluchinsky district, vicinity of the city of Obluch'e	4
9	Russia, Amur oblast, 2 km south of the city of Zeya, near the village of Sosnovyi	26
	Siberia + Kazakhstan	
10	Russia, the city of Novosibirsk, Akademgorodok	4
11	Russia, Altai krai, Solton district, 9–10 km north-west of the village of Nizhnyaya Neninka	4
12	Russia, Altai Republic, north-western outskirts of the city of Gorno-Altaysk	4
13	Russia, Altai Republic, Shebalinsky district, the village of Cherga, floodplain of Sema River	1
14	Kazakhstan, East Kazakhstan oblast, Zharminsky district, 6 km east of the village of Karatube, Kalbinsky Range	5
15	Kazakhstan, Almaty oblast, Alakolsky district, 1.5–2 km north of the village of Bibakan (Uspenovka), floodplain of Orta-Tentek River	4
16	Russia, Omsk oblast, Novovarshavsky district, 1 km along the railway from the station 136th km towards the city of Omsk (134–135th km of the railway Omsk–Irtyshskoe–Karasuk)	2
17	Russia, Omsk oblast, Novovarshavsky district, 6 km along the railway from the station Irtyshskoe towards the city of Omsk	1
18	Russia, Omsk oblast, Novovarshavsky district, 8.5 km east of the village of Bogdanovka, thickets of the floodplain of Irtysh River and nearby steppe plots	2
19	Russia, Omsk oblast, Okoneshnikovsky district, vicinity of the village of Leninsk	2
20	Russia, Omsk oblast, Tavrichesky district, ~1 km from the Amre railway station towards Omsk (73rd km of the railway Omsk–Irtyshskoe–Karasuk)	2
21	Russia, Omsk oblast, Lubinsky district, vicinity of the village of Stepanovka	1
	Eastern Europe	
22	Russia, Samara oblast, Stavropol'sky district, Samarskaya Luka, the territory of the Zhiguli Nature Reserve, Razorennaya Polyana	3
23	Russia, Ryazan oblast, Saraevsky district, vicinity of the village of Romanovka	2
24	Russia, Ryazan oblast, Saraevsky district, 1.8 km east of the village of Alekseevka	2
25	Russia, Kursk oblast, the territory of the Central Chernozem Nature Reserve, Streletsky site, Dubroshina tract	2
26	Russia, Belgorod oblast, Borisovsky district, vicinity of the village of Borisovka, Belogorye Nature Reserve	1
27	Russia, Belgorod oblast, Borisovsky district, vicinity of the village of Borisovka, Ostrasievy Yary	1
28	Russia, Belgorod oblast, vicinity of the village of Konshino, 30 km southwest of the Yamskaya steppe	2
29	Russia, Belgorod oblast, Yamskaya steppe, Sura Log	2

Table 1. (Contd.)

No.	Geographic collection points	Amount of animals
30	Ukraine, Kharkov oblast, Velikoburluksky district, vicinity of the farm of Nesterovka	1
31	Russia, Krasnodar krai, territory subordinate to the city of Sochi, outskirts of the city of Khosta	4
32	Russia, Krasnodar krai, territory subordinate to the city of Sochi, vicinity of the village of Monastyr	3
33	Russia, Kabardino-Balkaria Republic, northwestern outskirts of the city of Nalchik	7

If the equilibrium state was impaired in the sample, then

$$\mathrm{Var}\left(\hat{p}_i\right) = \left(\hat{p}_i + n_{i,i}/N - 2\hat{p}_i^2\right)/2N .$$

The null allele frequency and its effect on the frequencies of the remaining microsatellite alleles were determined with the Micro-Checker 2.2.3 program (van Oosterhout et al., 2004). The genetic linkage disequilibrium, the observed and expected heterozygosity, the correspondence to the Hardy–Weinberg distribution, and the F statistics were estimated with the Arlequin program (Excoffier et al., 2005). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram based on pairwise Fst values was constructed with the PHYLIP program, version 3.6 (Felsenstein, 2004).

RESULTS

Table 2 shows the data on the fragment size and the number of alleles found in mainland populations of the striped field mouse, as well as the characteristics of

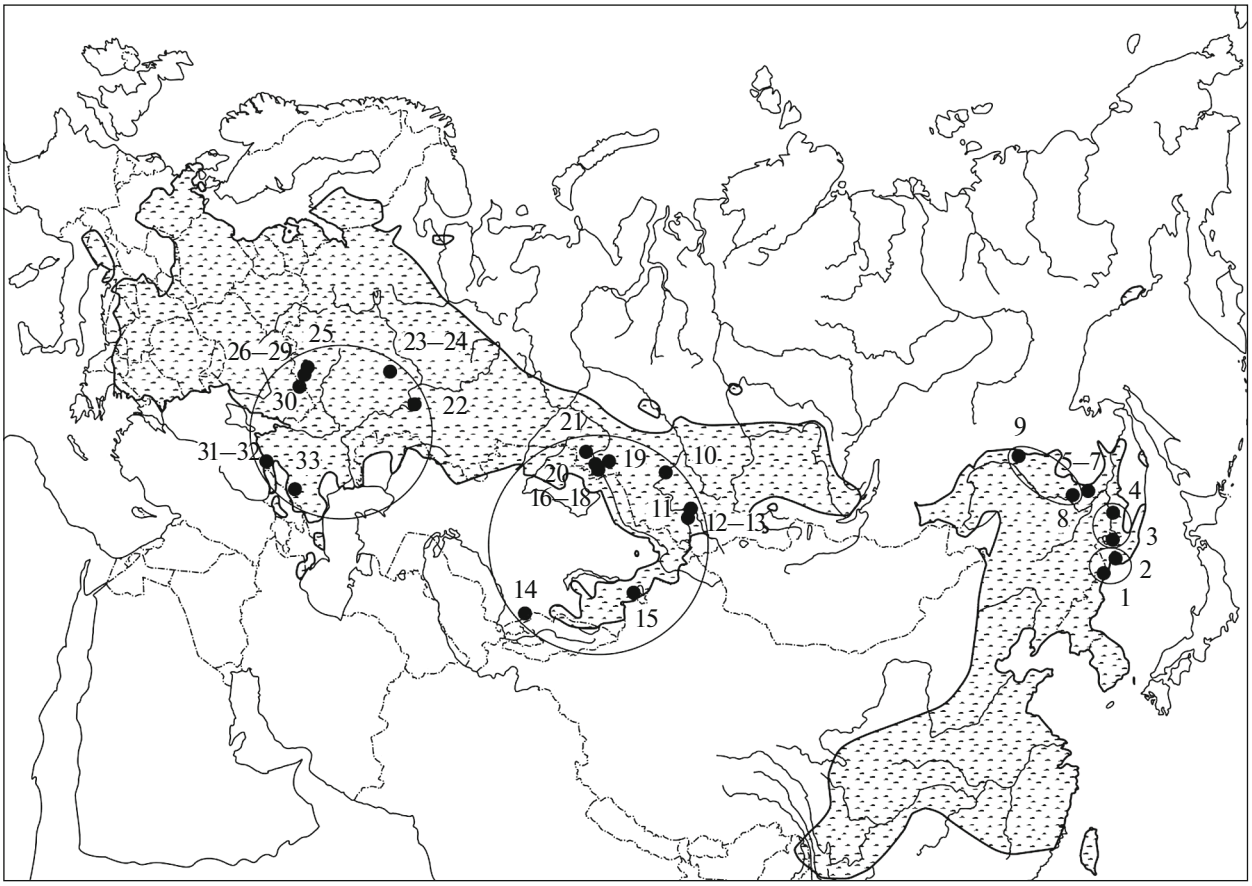

Fig. 1. Geographic points of the collection of the striped field mouse material and the structure of the pooled samples. The construction was based on the map of the striped field mouse range from the Top-100 Web portal (2016). Combined samples: Southern Primorye, points 1, 2; Western Primorye, 3, 4; Middle Amur Region–Center, 5–7; Middle Amur Region–West, 8–9; Siberia + Kazakhstan, 10–21; Eastern Europe, 22–33.

Table 2. Characterization of the studied microsatellite loci of the striped field mouse

According to Makova et al. (Makova et al., 1998)				Our data for *A. agrarius*		
locus	cloned repeat	fragment size	number of alleles	fragment size	number of alleles	number of common alleles for isolates
CAA2A	$(CA)_{21}$	94–118	6	84–116	17	11
GTTDS8	$(GTT)_9$	99	1	93–99	2	1
GTTF9A	$(GTT)_{14}$	95–120	3	86–120	12	8
GATAE10A	$(GATA)_9$	212–242	4	214–254	11	7
GSADT7S	$(CA)_{6...}$ $(GCAT)_3$ $(GCAC)_3$	199–296	2	186–234	21	10

For GSADT7S, the cloned repeat is $(CA)_{6...}GCAT_3\ (GCAC)_3$.

Table 3. Genetic characteristics of pooled samples of the stripe field mouse

Locus	Characteristics	Southern Primorye	Western Primorye	Middle Amur Region–Center	Middle Amur Region–West	Siberia + Kazakhstan	Eastern Europe
CAA2A	*n*	13	12	11	8	11	9
	Ho	0.567	0.467	0.567	0.333	0.5	0.433
	He	0.904	0.859	0.811	0.859	0.903	0.853
	HWE	<0.05	<0.05		<0.05	<0.05	<0.05
GSADT7S	*n*	13	13	10	10	7	11
	Ho	0.533	0.5	0.633	0.567	0.3	0.333
	He	0.827	0.667	0.853	0.825	0.432	0.604
	HWE	<0.05	<0.05		<0.05	<0.05	<0.05
GTTF9A	*n*	12	7	8	8	7	8
	Ho	0.633	0.633	0.6	0.667	0.6	0.533
	He	0.827	0.808	0.802	0.856	0.669	0.657
	HWE	<0.05					<0.05
GATAE10A	*n*	9	8	8	8	7	6
	Ho	0.367	0.467	0.7	0.667	0.567	0.8
	He	0.825	0.818	0.842	0.825	0.74	0.78
	HWE	<0.05	<0.05	<0.05			

n, the number of alleles; *Ho*, observed heterozygosity; *He*, expected heterozygosity; HWE < 0.05, result of exact test of deviation from Hardy–Weinberg equilibrium.

the analyzed loci (according to Makova et al., 1998) in a local Chelyabinsk sample of eight animals.

Table 3 shows the genetic characteristics of the six pooled samples for each locus: the number of alleles (*n*), the observed (*Ho*) and expected (*He*) heterozygosities, and the Hardy–Weinberg equilibrium estimate (probability HWE < 0.05). Figure 2 shows the distribution of detected allele frequencies.

The GTTDS8 locus, which is monomorphic in a small Chelyabinsk sample according to Makova et al., 1998 turned out to be practically monomorphic throughout the studied part of the range of the striped field mouse. In all pooled samples, with the exception of Southern Primorye, the same GTTDS8-99 allele was detected. Another GTTDS8-93 allele with a frequency of 0.03 was detected in the Khasan local sample, which is a part of the Southern Primorye group. The remaining four loci were highly polymorphic and were represented by 11–21 alleles. Polymorphism was expressed at these loci in both the western and eastern isolates; moreover, half or more of the alleles of each locus were common to them.

An accurrence of linkage disequilibrium was found between the CAA2A and GTTF9A loci in the pooled Eastern Europe sample. The same pair of loci shows linkage disequilibrium in the Western Primorye

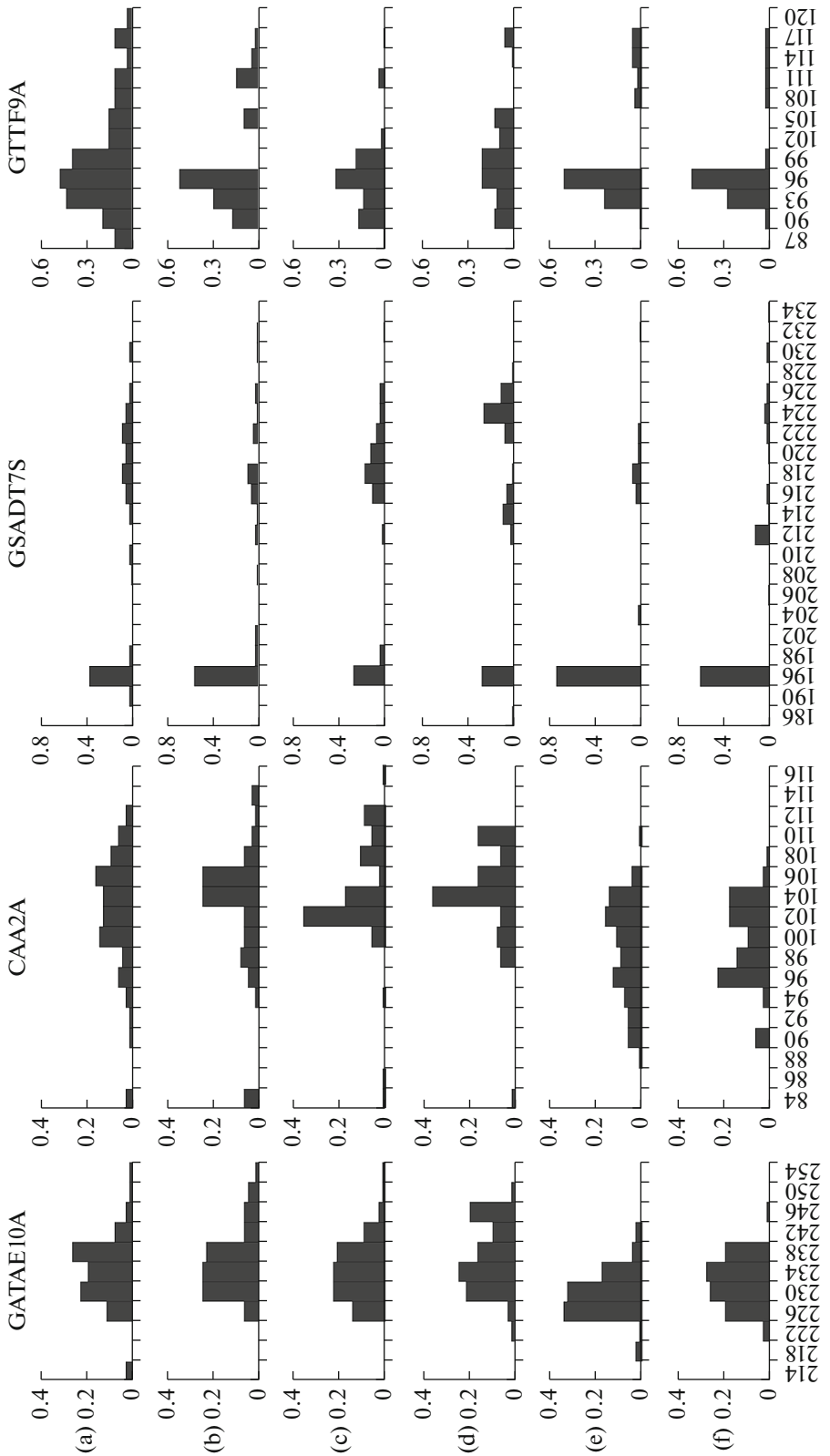

Fig. 2. Histograms of the distribution of allele frequencies in the studied pooled samples of the striped field mouse. The abscissa is the allele length. The allele frequency in fractions is shown along the ordinate axis. Designations: (a) Southern Primorye; (b) Western Primorye; (c) Middle Amur Region–Center; (d) Middle Amur Region–West, (e) Siberia + Kazakhstan, (f) Eastern Europe.

group, where the GSADT7S–GTTF9A and CAA2A–GSADT7S loci were also defined as linked pairs. The phenomenon of linkage disequilibrium may due to several causes. One of them is the localization of linked loci in the same chromosome. In this case, it is logical to assume that the linkage should be represented in the species as a whole, i.e., throughout the range; however, we observed it only in two regions. Linkage disequilibrium can be caused by impairment of panmixia. As it can be seen from Table 3, the ratio of observed and expected heterozygosities in the pooled samples of striped field mouse in most cases indicates a deficiency of heterozygotes, i.e., impairment of panmixia. A significant deficiency of heterozygotes (21–44% of specimens in pooled samples) was also indicated by the obtained values of the individual Wright's fixation index (Fis) (Table 4). This may be a consequence of the internal heterogeneity of the pooled samples due to the spatial separation of their local populations. However, there are other possible causes of impaired panmixia. Among these reasons are null alleles, i.e., a lack of amplification during PCR due to mutations in the DNA sequences, which flank on microsatellites and hybridize with primers. A microsatellite allele linked to such mutation is not amplified in a specimen, homozygous for the null allele, and only one microsatellite is amplified in heterozygous specimens; in the latter case, false homozygosity is observed (Kordicheva et al., 2010). False homozygosity results in a shift in population genetic characteristics, par example, it is responsible for an excess of homozygotes. Of the 180 studied animals, only one locus (CAA2A) in one specimen (from Altai Mountains) did not yield a PCR product; it indicates that the animal is homozygous for the null allele of this microsatellite. The estimation of null allele frequency in heterozygous specimens implies knowledge of the proportion of the observed heterozygote deficiency due to the presence of a null allele. However, even if we consider the entire heterozygote deficiency observed in each of the pooled samples as a result of the influence of only null alleles, the correction of microsatellite allele frequencies falls within the statistical error of the values, which was determined without allowance for the influence of the null allele (Fig. 2). The results make it possible to assess the variability and differentiation of the striped field mouse populations from the mainland area based on the revealed allelic diversity.

Allelic diversity. In a total pool of 180 specimens, 63 allelic variants were found at five loci. Of these variants, 58 were found in the eastern isolate, and 42, in the western isolate. The predominance of the number of alleles in the eastern isolate could be the result of a mismatch in the number of animals in each of the isolates (60 in the western isolate and 120 in the eastern isolate). However, a comparison of pooled samples of the same size showed that higher observed allelic diversity in the eastern isolate was real: only 42 alleles were found in the western isolate, while 45 alleles, in the Amur Region (60 specimens in total), and 51 alleles, in Primorye (60 specimens). The total number of alleles in pooled samples decreased from east to west; in the eastern isolate, it also decreased from south to north. The total number of alleles was 48 in the Southern Primorye sample, 40 in the Western Primorye sample, 39 in the Middle Amur Region-Center sample, and 33 alleles were found in the Middle Amur Region–West sample and in the pooled samples from the western isolate (Siberia + Kazakhstan and Eastern Europe). Thus, on average, 44 alleles were detected in the pooled sample in Primorye, 36 alleles were found in the Amur Region, 40 alleles, in the entire eastern isolate, and 33, in the western isolate. It is interesting that the western-most sample of the eastern isolate (Middle Amur Region–West) in allelic diversity was comparable to the pooled samples of the western isolate. More than half of the detected alleles (37 of 63) were represented in populations of both western and eastern isolates. Twenty-six alleles were specific, i.e., present in populations of only one of the isolates: 21 of them marked the eastern isolate, and five, the western isolate. The distribution of specific alleles within the isolates was also nonuniform. Nine (CAA2A-84, CAA2A-112, GSADT7S-198, GTTDS8-93, GTTF9A-87, GTTF9A-102, GTTF9A-105, GATAE10A-250, and GATAE10A-254) of the 21 alleles marking the eastern isolate were also common in the Primorye and Amur Region populations. The other alleles were found in striped field mice in one of the considered Far Eastern regions. The GSADT7S-208 allele was found in both samples from Primorye. Six alleles, CAA2A-114, GSADT7S-190, GSADT7S-210, GTTF9A-120, GATAE10A-214, and GTTDS8-93, were found only in the pooled Southern Primorye sample. Two specific alleles were detected in the Western Primorye (CAA2A-114 and GSADT7S-202), Middle Amur Region–Center (CAA2A-86 and CAA2-116), and Middle Amur Region–West (GSADT7S-186 and GSADT7S-228) samples. Of five specific alleles of the western isolate, three (CAA2A-88, GSADT7S-204, GATAE10A-218) were present in the Siberia + Kazakhstan sample and two (GSADT7S-206, GSADT7S-234) were found in the Eastern Europe sample. Figure 3 shows the total pattern of the ratio of common and specific allele frequencies in the considered pooled samples. Despite the high proportion of

Table 4. Value of Wright's F-statistics for four microsatellite loci of the striped field mouse

Locus	Fis	Fit	Fst
CAA2A	0.4425	0.4590	0.0313
GSADT7S	0.3217	0.3558	0.0543
GTTF9A	0.2112	0.2383	0.0281
GATAE10A	0.2560	0.2688	0.0140
On average	0.3078	0.3305	0.0320

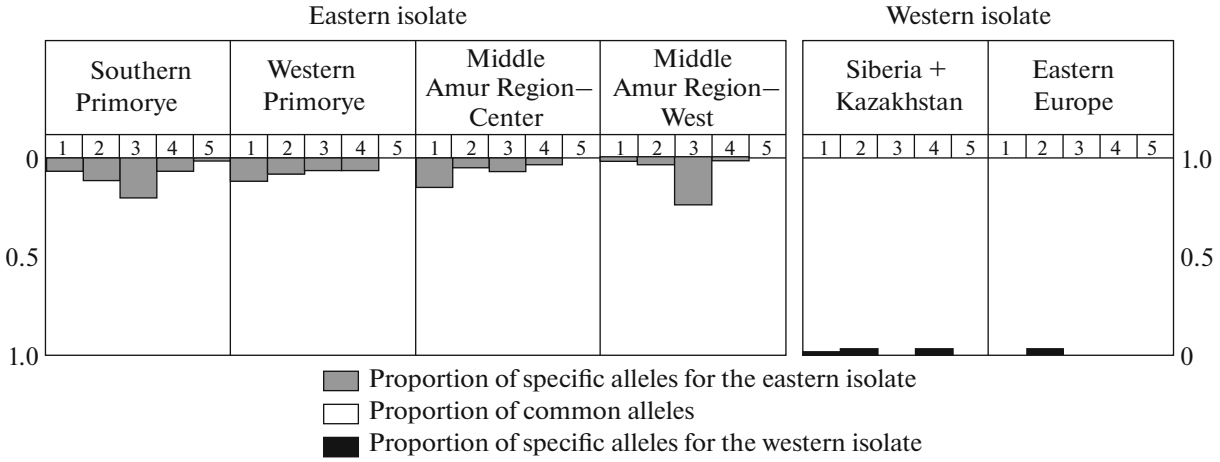

Fig. 3. Distribution of common and specific alleles in the striped field mouse isolates. Designations: 1, CAA2A; 2, GSADT7S; 3, GTTF9A; 4, GATAE10A; 5, GTTDS8.

common alleles, the genetic peculiarity of isolates can be traced. The allelic diversity and the number of specific alleles were higher in the eastern isolate that may be the result of a longer habitat of the striped field mouse in the Far Eastern part of the range. The limited number of specific alleles in the western isolate as compared to the eastern isolate may be determined by the founder effect and may reflect the direction of the historical migration of the species from east to west. The obtained results generally coincide with the results of preliminary studies that we conducted earlier (Frisman and Bogdanov, 2014; Frisman et al., 2015).

Genetic differentiation. The F-statistics coefficients proposed by Wright (Wright, 1978) were used to determine the level of differentiation of the studied population groups within isolates and between them. Table 4 presents the values of the inbreeding coefficients calculated for each of the polymorphic loci relative to the population (Fis), the species (Fit), and the degree of subdivision of the populations (Fst).

The F-statistics coefficients indicate a significant deficiency of heterozygotes, reaching 44.2% at the level of individual populations (Fis) and up to 45.9% at the level of the species as a whole (Fit). The Fst-subpopulation fixation index reflects a weak level of differentiation at each locus. Judging by the average value of this parameter, only 3.2% of the variability is localized between the combined samples, and 96.8% is localized within them.

The quantitative assessment of differentiation was based on the Fst coefficient, which, in a pair wise comparison of samples, serves as a measure of genetic distances. Of the four intraspecific differentiation levels proposed by Wright (weak but deserves attention, $Fst < 0.05$; intermediate, $0.05 < Fst < 0.15$; large, $0.15 < Fst < 0.25$; and very large, $Fst > 0.25$), only two lower levels were found in the mainland range of the striped filed mouse (Table 5). The level of differences between the samples was weak ($Fst < 0.05$) or intermediate ($0.05 < Fst \le 0.109$). The smallest Fst values were obtained in the comparison of pairs of Eastern Europe/Siberia + Kazakhstan ($Fst = 0.008$) samples, samples from Primorye ($Fst = 0.004$), and Western Primorye/Middle Amur Region–Center samples, which are separated by Amur River ($Fst = 0.009$). In general, the pooled samples within isolates were characterized by a weak differentiation level ($Fst = 0.004$–0.031). Comparison of the pooled samples of different isolates showed higher Fst values (from 0.022 to 0.109) reflecting a weak or intermediate level of differentiation. Nevertheless, the UPGMA dendrogram demonstrates a clear separation of clusters, including combined samples from the western isolate, on the one hand, and from the eastern isolate, on the other (Fig. 4).

DISCUSSION

It is known that different groups of animals show a range disjunction, which separates the European-Siberian and Far Eastern complexes of populations and can be traced at several taxonomic levels (from generic to intraspecific, up to the absence of distinct subspecies). It has long attracted the attention of researchers (Matyushkin, 1976). The striped field mouse belongs to species that have accumulated weak differences as a result of disjunction of the range. Although our study of five microsatellite loci showed some differences in the allelic composition in the western and eastern isolates, the level of their genetic differentiation should be defined only as moderate. This result is consistent with data obtained in other genetic and classical morphological studies (Pavlenko and Vorontsov, 1990; Mezhzherin and Zykov, 1991; Koh, 1991; Koh et al., 1998; Atopkin et al., 2007; Suzuki et al., 2008; Sakka et al., 2010). The allelic diversity and the number of specific alleles were higher in the eastern isolate that may be the result of the lon-

Table 5. Matrix of Fst values for pairwise comparison of pooled samples from western and eastern isolates of the striped field mouse

Pooled samples	1	2	3	4	5
1. Southern Primorye	0				
2. Western Primorye	0.004	0			
3. Middle Amur region–Center	0.031	0.009	0		
4. Middle Amur region–West	0.029	0.018	0.038	0	
5. Siberia + Kazakhstan	0.04	0.056	0.073	0.109	0
6. Eastern Europe	0.022	0.037	0.053	0.077	0.008

ger habitat of the striped field mouse in the Far Eastern part of the range. The limited number of specific alleles in the western isolate as compared to the eastern one may be due to the founder effect and may reflect the direction of the historical migration of the species from east to west that also agrees with the findings of previous studies. Our data, as well as the results of an earlier allozyme analysis (Mezhzherin and Zykov, 1991), indicate a relatively recent penetration of the striped field mouse into western Eurasia. However, repeated contacts between the western and eastern isolates, i.e., the periodic appearance of a bridge between them during favorable periods and its subsequent disappearance, might take place. It is known that the expansion of east isolate to the west occurs at present (Bazhenov et al., 2014).

Our results demonstrate that there is no more than a population level of differentiation within the mainland striped field mouse isolates (Fst from 0.004 to 0.031). Similar values of this parameter (Fst from 0.008 to 0.046) were obtained in the analysis of five microsatellite loci in striped field mice from the Korean Peninsula belonging to a single subspecies (Jo et al., 2016). When comparing the mainland (Korean Peninsula) and island (Jeju Island) subspecies, Jo et al. obtained somewhat higher Fst values (from 0.015 to 0.080). The Fst values determined by us (from 0.022 to 0.109) showed that the differentiation of the continental western and eastern isolates also reached only a moderate level and corresponds to no more than subspecies differences.

CONCLUSIONS

Thus, the study results allowed us to determine the level of genetic diversity and differentiation of the striped field mouse, the range of which is divided into two huge continental isolates. The analysis of only five microsatellite loci and the use of pooled samples is only the beginning of the study of the effect of isolation on the genetic structure of populations. A more realistic reconstruction of the evolutionary history and determination of the taxonomic status of isolated population groups of the striped field mouse require detailed estimations. In the next stage of the study, we intend to analyze the genetic differentiation of *A. agrarius* in the mainland and island parts of its range based on representative samples. The analysis of representative local samples will describe both the intraspecific differentiation and the intrapopulation genetic heterogeneity.

ACKNOWLEDGMENTS

We express our sincere gratitude to O.V. Brandler (Institute of Developmental Biology, Russian Academy of Sciences), A.Yu. Puzachenko (Institute of Geography, Russian Academy of Sciences), S.B. Pole (Kazakh Scientific Center of Quarantine and Zoonotic Diseases), P.A. Tilba and B.S. Tuniev (Sochi National Park), R.I. Dzuev (Kabardino-Balkarian State University), A.A. Vlasov (Central Chernozem Nature Reserve), V.P. Vekhnik (Zhiguli Nature Reserve), V.V. Yakimenko (Omsk Research Institute of Natural Focal Infections), E.I. Zholnerovskaya (Institute of

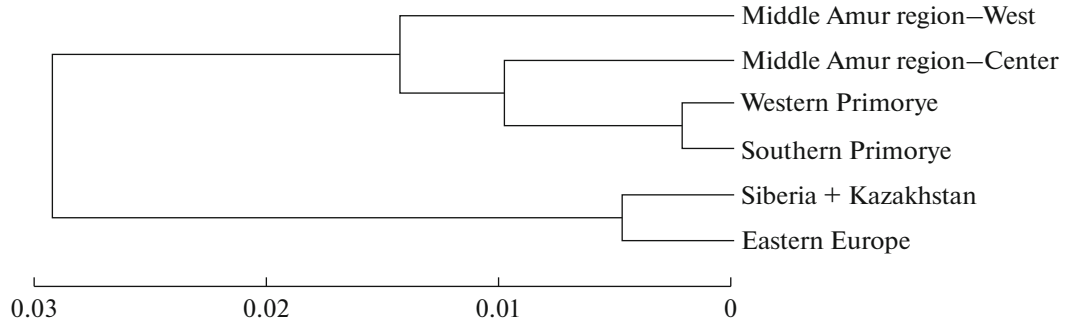

Fig. 4. UPGMA dendrogram of genetic relationships of pooled samples of the striped field mouse (based on pairwise Fst values).

Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences), V.I. Ermolaev (Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences), and L.N. Kapitonova (Institute for Complex Analysis of Regional Problems, Far Eastern Branch of the Russian Academy of Sciences) for the assistance with mouse trapping and laboratory processing of the material. The authors are grateful to E.A. Erofeeva for the creation of the material collection map.

FUNDING

The study was partially supported by the Russian Foundation for Basic Research, project no. 15-04-03871, and the Far East Branch of the Russian Academy of Sciences, grant no. 18-4-031.

COMPLIANCE WITH ETHICAL STANDARDS

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

Statement on animal welfare. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Translated by V. Mittova