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ANIMAL GENETICS

Genetic and Taxonomic Diversity of the House Mouse *Mus musculus* from the Asian Part of the Former Soviet Union

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Abstract—Genetic diversity of the house mouse *Mus musculus* from 12 local populations ($n = 65$) of the central and eastern parts of the former Soviet Union was examined using RAPD–PCR. About 400 loci were identified, encompassing approximately 500 kb of the mouse genome. Genetic diversity was assessed using NTSYS, POPGENE, TFPGA, and TREECON software programs. In general, the house mouse sample from the regions examined was characterized by moderate genetic variation: polymorphism $P = 95.6\%$, $P_{99} = 60.7\%$, $P_{95} = 24.2\%$; heterozygosity $H = 0.089$; the mean observed number of alleles $n_a = 1.97$; effective number of alleles $n_e = 1.13$; intrapopulation differentiation $\delta_S = 0.387$; gene diversity $h = 0.09$. Individual local populations displayed different levels of genetic isolation: the genetic subdivision index G_{st} varied from 0.086 to 0.324 at gene flow *Nm* varying from 5.3 to 1.05, while the interpopulation genetic distance D_N ranged from 0.059 to 0.186. Most of the genetic diversity of the total sample resided within the local populations: $H_S = 0.06$, total gene diversity $H_T = 0.09$. The exact test for differentiation, however, did not confirm the affiliation of all the mice examined to one population: $\chi^2 = 1446$, *d.f.* = 724, $P = 0.000$. Molecular markers specific to four subspecies (*musculus*, *castaneus*, *gansuensis*, and *wagneri*) were identified. Moreover, in some cases the populations and individual animals exhibited traits of different subspecies, suggesting their introgressive hybridization. It was demonstrated that the house mouse fauna on the territories investigated was characterized by the prevalence of *musculus*-specific markers, while *gansuensis*-specific markers ranked second. The *castaneus*-specific markers were highly frequent in the Far East, but almost absent in Central Asia, where *wagneri*-specific markers were detected. It was suggested that house mice from Turkmenistan could belong to one of the southern subspecies, which had not deeply penetrated into the Asian fauna of the former Soviet Union. In phenogenetic (UPGMA) and phylogenetic (NJ) reconstructions this form with the high bootstrap support was placed at the tree base, while the isolation of other clusters was not statistically significant. It is thus likely that the house mice from Turkmenistan are closest to the ancestral form of the genus *Mus* on the territory of the former Soviet Union.

INTRODUCTION

The house mouse (*Mus musculus* Linnaeus, 1758) is a cosmopolitan species commensal with humans during many centuries. Nevertheless, the intraspecific systematics of *Mus musculus* is still not completely elaborated. The problem of the house mouse taxonomy has been attracting attention of researchers during the whole period of development of the murine systematics [1–3]. A great variety of the house mouse forms described (up to 133 subspecies) has made its subspecific systematics cumbersome and unclear. Use of genetic and biochemical methods to study *Mus musculus* has demonstrated that it is a complex species consisting of several genetically discrete forms [4–6]. Genetic analysis resulted in the isolation of the four major house mouse subspecies, including *domesticus* (North and South America, Africa, Western Europe, and Near East), *musculus* (East Europe, Central Asia, and the Far East), *castaneus* (from Ceylon to Southeast Asia, including Indo-Malasian Archipelago), and *bactrianus* (Middle East) [7]. Some authors consider *Mus musculus* to be an overspecific complex, regarding *domesticus*, *musculus*, *castaneus* and *bactrianus* as independent species.

Utilization of a number of genetic methods mediated revising the house mouse systematics throughout the vast range of this species. The molecular genetic approaches seem most promising in this respect. They enable revealing heterogeneity at the population level, evaluating genetic diversity in the contact zones between different forms, and estimating the relationships between these forms. This provides more precise characterization of the population structure and the processes taking place in the hybrid zones. The mice from Europe, Transcaucasia, and Japan, where the hybrid zones between the different forms have been described, are most thoroughly studied [8–14, and others]. The *Mus musculus* from the eastern part of the species range in Russia has been extensively investigated using biochemical, chromosomal, and mitochondrial markers

[11, 12, 14–17, and others]. These data, however, are not always mutually consistent.

In the present study, the degree of molecular differentiation and the level of genetic polymorphism within and between the populations were evaluated using RAPD–PCR markers. The RAPD–PCR approach has shown its effectiveness in our previous studies of the house mouse [18, 19].

This study was focused on analysis of genetic and taxonomic diversity of *M. musculus* from the Asian part of the former Soviet Union, where overlap zones between several forms, differing by hybridization intensity and hybrid group composition were found. The tasks of the study included: (1) identification of the subspecies- and population-specific molecular markers; (2) quantitative evaluation of genetic variation in the local populations, as well as the degree of their differentiation; (3) estimation of the total level of genetic diversity in the total sample; and (4) reconstruction of phenogenetic and phylogenetic relationships among the house mice.

MATERIALS AND METHODS

A total of 65 mice of the species *M. musculus* from 12 localities were examined. The mice were captured both in human dwellings and in natural habitats. The sampling localities were Primorskii krai, the settlement of Pogranichnyi (n = 8); the settlement of Chuguevka (n = 7), and the village of Bulyga-Fadeevo (n = 6); Kamchatka Peninsula, the settlement of Elizovo (n = 4); Yakutia, the settlement of Khatassy (n = 4); Novosibirsk (n = 8); the south of Chita oblast, the settlement of Tsasuchei (n = 9); Altai krai, the settlement of Solton (n = 2); Turkmenistan, Balkan velayat, Kara-Kala raion, Syunt-Khasardag Preserve, the foot of the Isak Mountain (n = 3); Kazakhstan, Semipalatinsk oblast, Aksuat raion, about 60 km northwest of the settlement of Aksuat, the bank of Zaisan Lake (n = 5), Semipalatinsk oblast, Aksuat raion, 25 km southwest of the settlement of Aksuat, Tarbagatai mountain range (n = 2), Taldy-Kurgan oblast, Karatal raion, 28 km northwest of the city of Ushtobe, the flood-plain of the Karatal River (n = 5); Chimkent oblast, outskirts of the settlement Kyzyl Oktyabr', the flood-plain of the Arys' River (n = 2). The house mice from Kazakhstan and Turkmenistan were captured in the wild and, probably, represented the aboriginal forms. The samples from the settlements of Solton and Kyzyl Oktyabr' were examined only for the genetic similarity to the mice from other localities and were not scored in the population analysis. Two other species of the subgenus *Mus*, *M. abbotti* and *M. spicilegus* were taken as the outgroups.

DNA was extracted from the fresh liver samples according to a standard method using proteinase K, phenol–chloroform treatment, and precipitation with isopropanol and ethanol [20]. RAPD analysis was performed using five primers (OPC-02, OPC-05, OPC-12, OPC-16, and OPD-05, Operon Technologies Inc., United States), generating well-distinguishable and reproducible amplification products. PCR reaction was performed in a final volume of 25 μl, containing 30 ng of total DNA; 1 × buffer (67 mM Tris–HCl, pH 8.8; 2 mM $MgCl_2$; 0.01% Tween-20; 0.01 M 2-mercaptoethanol), 0.2 mM of each dNTP; 0.5 μl primer; 1 Unit *Taq* polymerase. The initial denaturation at 94°C for 2 min was followed by 41 cycles of denaturation at 94°C for 1 min; annealing at 37°C for 30 s, 45°C for 15 s; elongation at 72°C for 2 min; and the final elongation at 72°C for 6 min. RAPD–PCR products were analyzed by use of electrophoresis in 2% agarose gel in the presence of 0.5 μg/ml ethidium bromide in the 1 × TBE buffer and photographed in the UV light. The phage λ DNA digested with the *Pst*I restriction endonuclease was used as a molecular size marker. Electreophoregrams were used for the construction of binary matrices, where the presence of a band was designated as 1, and its absence, as 0. For calculation of the fragment number, all visually detected fragments were scored.

The genetic variation and population differentiation parameters were estimated using the NTSYS-pc ver. 1.7 [21], POPGENE [22], and TFPGA-ver. 1.3 [23] software packages. Genetic variation within the populations was quantified using the proportion of polymorphic loci at the 95% significance level (P_{95}) for the total sample, average (n_a) and effective (n_e) number of alleles per locus, mean expected heterozygosity (H), genetic diversity (h) and intrapopulation differentiation, as well as the similarity/dissimilarity index, S/D [21]. Genetic differences between the populations and their isolation were estimated by interpopulation differentiation index, G_{ST}, and the effective number of migrants per generation, Nm, between local populations, as well as the total genetic diversity between the samples, D_{ST}, calculated using the estimates of total genetic variation, H_T, and average genetic variation H_S, as described by Gregorius [24]. In addition, interpopulation genetic distances D_N were calculated [22], and the exact test for the population differentiation was performed [25]. Phylo- and phenohenetic trees were constructed using the TREECON-ver. 1.3 software package [26].

RESULTS

Diversity of RAPD–PCR profiles. Each of the primers initiated synthesis of a specific set of DNA fragments, differing in molecular weight and expression. The band number in the RAPD profiles varied from 12 to 20, while the fragment sizes varied from 0.26 to 2.5 kb, constituting on average 1.38 kb. About 400 characters were identified (in total encompassing the region of about 500 kb, which corresponds to approximately 30 structural genes). RAPD profiles of the house mice from different localities were highly similar. However, in addition to the fragments shared by most of the individuals, unique fragments occurring in individual samples or in the samples from several

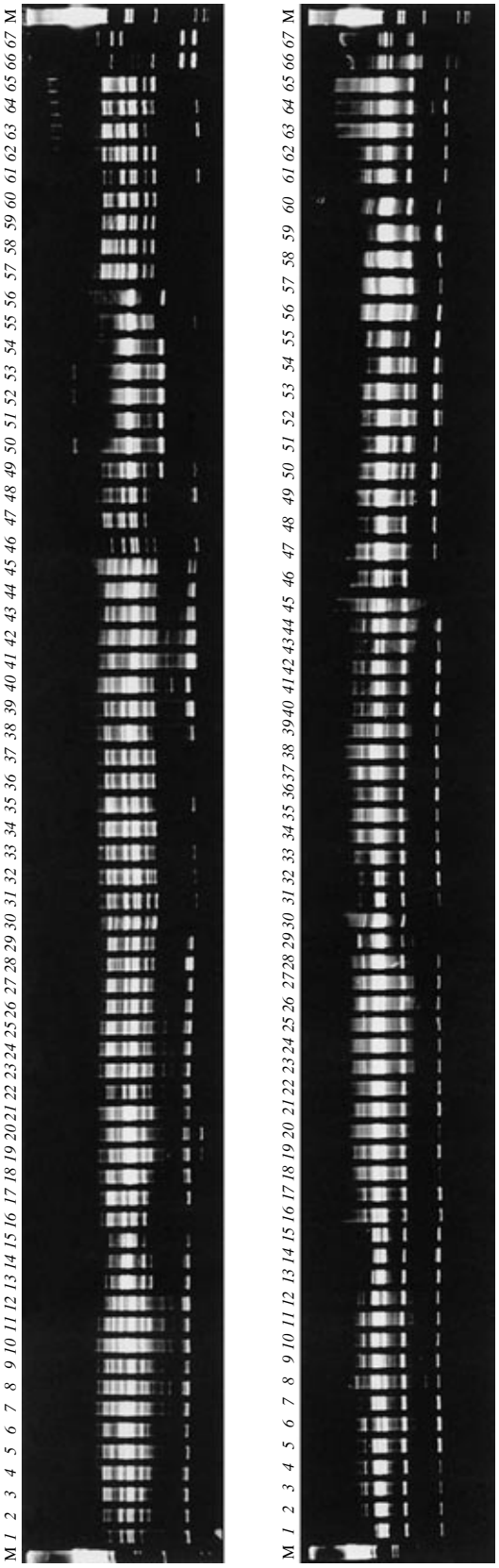

Fig. 1. RAPD profiles of the *M. musculus* DNA (no. *1–65*) obtained by use of primer OPC-05 (top) and OPC-02 (bottom): *1* to *4*, Khatassy; 5 to *11*, Chuguevka; *12* to *17*, Bulyga-Fadeevo; *18* to *21*, Elizovo; 22 to 29, Novosibirsk; *30* to *38*, Tsasuchei; *39* to *46*, Pogranichnyi; *47* and *48*, Solton; *49* to *55*, Semipalatinsk oblast; *56* to *60*, Taldy-Kurgan oblast; *61* and *62*, Chimkent oblast; *63* to *65*, Kara-Kala raion; *66*, *M. spicilegus*; *67*, *M. abbotti*; M, phage λ DNA digested with the *Pst*I restriction endonuclease.

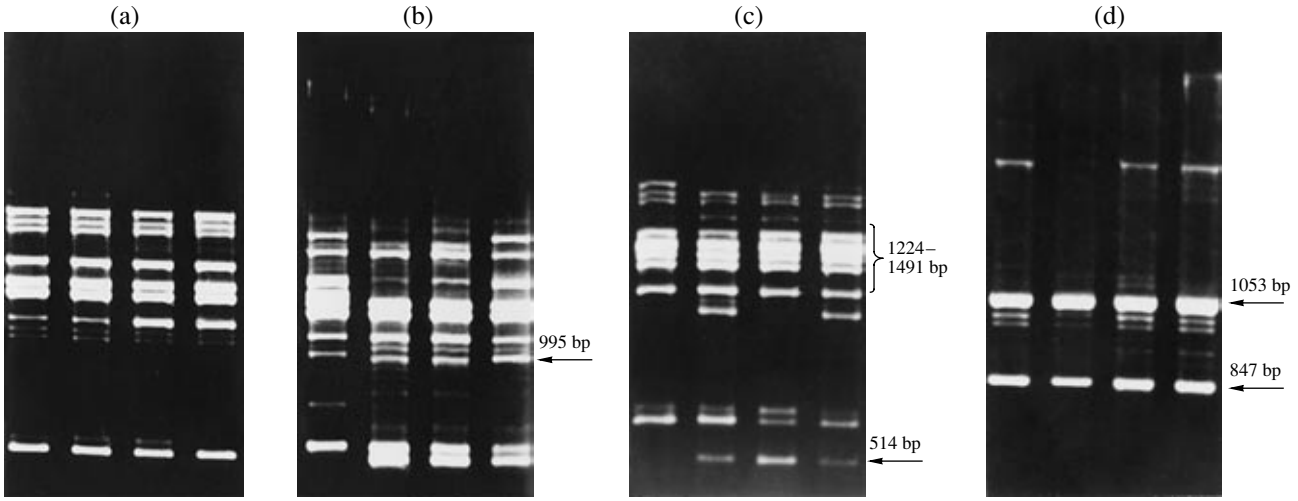

Fig. 2. RAPD profiles of the *M. musculus* total DNA samples obtained using primer OPC-05. (a) Type *musculus*; (b) type *gansuensis*; (c) type *castaneus*; (d) type *wagneri*.

localities were reported. With regard to the fragment sets generated by use of different primers, almost each animal had a specific RAPD profile.

Primers OPC-05 and OPC-02 appeared to be most informative for identification of the population subdivision and detection of individual polymorphism (Fig. 1). A typical RAPD profile is presented in Fig. 2a. The sizes of its fragments varied from 0.586 to 2.192 kb. This fragment set was most frequent (up to 100%) in the local samples from Yakutia and Novosibirsk. Karyotypic analysis of the mice from the second collection locality, harboring all of the markers mentioned, showed the prevalence of the *musculus* form [16]. For this reason, this type of the RAPD profile was designated as *musculus*.

In addition to the *musculus* type, RAPD profiles in most of the localities were characterized by the presence of the OPC-05_{995} fragment (Fig. 2b). This fragment was highly prevalent in two local samples, Tsasuchei (78%) and Pogranichnyi (100%). The Tsasuchei sample was represented by morphologically typical *M. m. gansuensis* individuals [16], i.e., the animals had white bellies and short tails. Karyotypic analysis demonstrated the presence of the chromosomal markers typical of *M. m. gansuensis* in this sample. Thus, fragment OPC-05_{995}, which was probably the marker of this subspecies, was arbitrarily designated as type *gansuensis*.

Comparison of the OPC-05 profiles for all animals showed that only the mice from Elizovo (all samples) along with the two animals from Bulyga-Fadeevo were

Table 1. Intrapopulation genetic diversity of the house mouse

Locality (sample size)	n_a	n_e	h	H (unbiased)	δ_S	S/D	N	P, %
Khatassy (n = 4)	1.14 ± 0.35	1.07 ± 0.21	0.04	0.042	0.462 ± 0.05	0.93/0.31	40	11.83
Chuguevka (n = 7)	1.31 ± 0.46	1.12 ± 0.24	0.08	0.088	0.347 ± 0.03	0.88/0.59	103	30.47
Pogranichnyi (n = 8)	1.34 ± 0.47	1.12 ± 0.24	0.07	0.080	0.361 ± 0.05	0.88/0.51	107	31.66
Bulyga-Fadeevo (n = 6)	1.28 ± 0.45	1.11 ± 0.22	0.07	0.078	0.432 ± 0.04	0.88/0.69	95	28.11
Elizovo (n = 4)	1.24 ± 0.43	1.11 ± 0.23	0.07	0.069	0.614 ± 0.04	0.87/0.58	72	21.30
Novosibirsk (n = 8)	1.29 ± 0.46	1.11 ± 0.22	0.07	0.062	0.283 ± 0.04	0.89/0.62	90	26.63
Tsasuchei (n = 9)	1.24 ± 0.43	1.10 ± 0.23	0.05	0.056	0.266 ± 0.03	0.92/0.38	77	22.78
Semipalatinsk oblast (n = 7)	1.29 ± 0.46	1.27 ± 0.25	0.08	0.080	0.329 ± 0.04	0.87/0.63	88	26.04
Taldy-Kurgan oblast (n = 5)	1.35 ± 0.48	1.13 ± 0.23	0.08	0.089	0.287 ± 0.03	0.86/0.67	112	33.14
Kara-Kala raion (n = 3)	1.16 ± 0.37	1.11 ± 0.27	0.06	0.084	0.405 ± 0.04	0.89/0.45	50	14.79
Total (n = 61)	1.97 ± 0.18	1.13 ± 0.20	0.09	0.089	0.387 ± 0.01	0.87/0.61	338	95.58

Note: H (unbiased), heterogeneity measure with the correction for the sample size; N, the number of polymorphic loci.

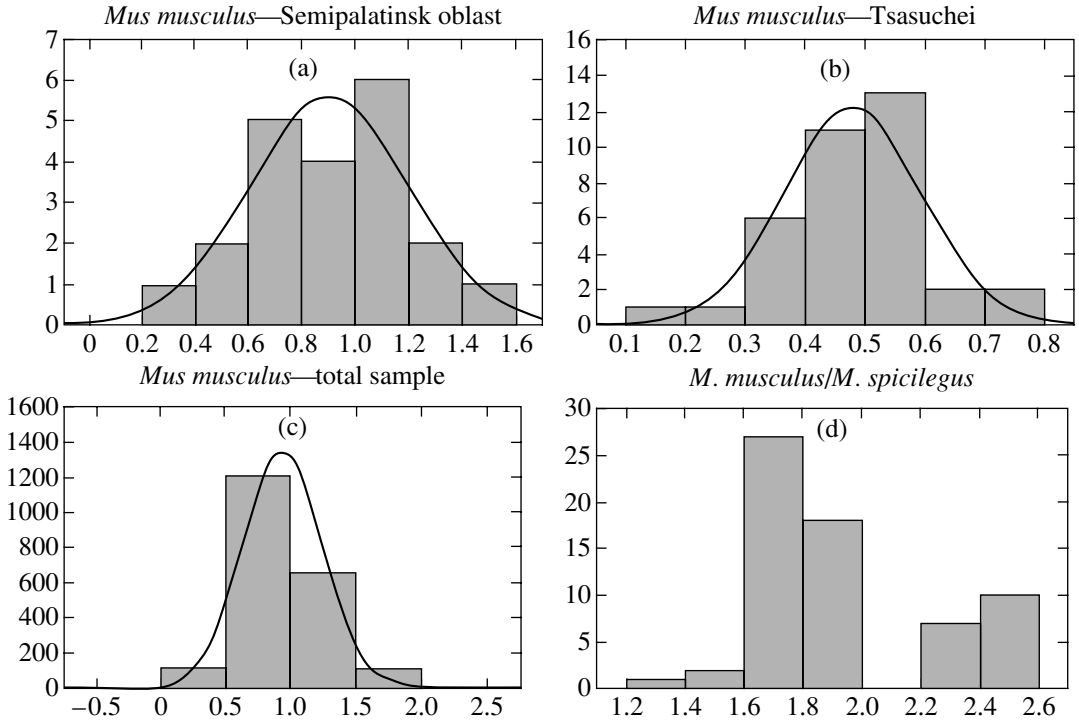

Fig. 3. Histograms of the distribution of intraspecific (a–c) and interspecific (d) pairwise genetic distances. On abscissa, genetic distance values; on ordinate, the numbers of animal pairs compared at the calculation of the genetic distances. The normal distribution curves are imposed over the histograms (a–c).

characterized by the presence of a marker zone, consisting of a fragment block of 1.224 to 1.491 kb. In addition, in the Elizovo sample, a unique OPC-05 fragment of 514 kb, specific for this population, was identified (Fig. 2c). Exactly in this population karyotypic markers of subspecies *M. m. castaneus* were detected [16]. Hence, the marker fragment block identified in the present study can be defined as the *castaneus* type. Interestingly, the RAPD DNA profiles of some animals from Elizovo in addition to the *castaneus* fragment block also contained the OPC-05_{995} fragment typical of *M. m. gansuensis* (see below).

RAPD profiles for all house mice from Semipalatinsk oblast (Tarbagatai mountain range and the Zaisan Lake region) and for one mouse from Taldy-Kurgan oblast, generated by use of primer OPC-05, displayed the presence of a specific fragment set, which was detected nowhere else (Fig. 2d). In addition, a unique fragment OPC-02_{847} was revealed in these animals. It was suggested that the molecular markers described could belong to subspecies *M. m. wagneri*, since this subspecies is aboriginal for the Central Asia region examined. It should be noted that the animals from Semipalatinsk oblast, Taldy-Kurgan oblast, Chimkent oblast, and Tsasuchei were characterized by weak expression of the OPC-05 fragments ranging in size from 0.605 to 0.633 kb.

Population genetic variation. RAPD–PCR analysis has demonstrated a very low level of genetic diversity of the mice from the collecting localities examined (h = 0.04 to 0.08) (Table 1). The average number of alleles per locus (n_a) in the samples from different populations was rather low and varied from 1.14 (Khatassy) to 1.35 (Taldy-Kurgan oblast). Similarly to the previous estimate, the effective number of alleles, n_e, in local populations of the house mouse was characterized by low values (1.07 to 1.27). However, the n_a value for the total sample increased up to 1.97, while the value of n_e remained low (1.13).

The next criterion used for evaluating genetic variation was heterozygosity H, which showed how many loci, on average, are in the heterozygous state in each individual, population, or species. The value of mean expected heterozygosity for the total sample was low (H = 0.089). The minimum H value of 0.042 was observed in the house mice from Khatassy, and the maximum value was detected in the mice from Chuguevka and Taldy-Kurgan oblast, H = 0.088 and 0.089, respectively (Table 1). Despite the small sample sizes, the mean expected heterozygosities obtained seem to be close to the real values, since they were in good agreement with the figures obtained in a study of protein variation in *M. musculus* from Central and Eastern Europe, and Central Asia, carried out using representative samples [27]. In particular, the mean expected heterozygosities obtained for the mice samples from Kara-Kala raion using RAPD and protein variation analyses were identical (H = 0.084 and 0.083, respectively).

Table 2. Parameters of interspecific genetic differentiation between some local populations of *M. musculus*

Localities	H_T (±)	H_S (±)	D_{ST} (±)	G_{ST}	Nm	D_N	Ext (χ^2/*d.f.*/*p*)
Khatassy/Pogranichnyi	0.07 (0.02)	0.06 (0.01)	0.01 (0.01)	0.166	2.511	0.083	165.59/724/1.000
Khatassy/Elizovo	0.08 (0.02)	0.05 (0.01)	0.03 (0.01)	0.231	1.661	0.102	81.29/724/1.000
Khatassy/Tsasuchei	0.05 (0.01)	0.04 (0.01)	0.00 (0.00)	0.175	2.351	0.059	140.67/724/1.000
Khatassy/Kara-Kala raion	0.07 (0.02)	0.05 (0.01)	0.02 (0.01)	0.324	1.045	0.154	222.01/724/1.000
Khatassy/Novosibirsk	0.06 (0.02)	0.05 (0.01)	0.01 (0.01)	0.194	2.073	0.070	177.60/724/1.000
Pogranichnyi/Elizovo	0.09 (0.02)	0.07 (0.01)	0.02 (0.01)	0.193	2.081	0.104	245.98/724/1.000
Pogranichnyi/Novosibirsk	0.08 (0.02)	0.07 (0.01)	0.01 (0.01)	0.134	3.235	0.068	305.57/724/1.000
Pogranichnyi/Tsasuchei	0.07 (0.02)	0.06 (0.01)	0.01 (0.01)	0.146	2.930	0.066	373.47/724/1.000
Pogranichnyi/Kara-Kala raion	0.09 (0.01)	0.07 (0.01)	0.02 (0.01)	0.235	1.631	0.186	440.50/724/1.000
Pogranichnyi/Semipalatinsk oblast	0.09 (0.01)	0.07 (0.01)	0.02 (0.01)	0.165	2.535	0.075	250/724/1.000
Tsasuchei/Kara-Kala raion	0.08 (0.02)	0.05 (0.01)	0.03 (0.01)	0.301	1.150	0.164	364.74/724/1.000
Tsasuchei/Semipalatinsk oblast	0.07 (0.02)	0.06 (0.01)	0.01 (0.01)	0.167	2.488	0.061	361.47/724/1.000
Semipalatinsk oblast/Kara-Kala raion	0.09 (0.02)	0.06 (0.01)	0.03 (0.01)	0.286	1.248	0.181	311.36/724/1.000
Taldy-Kurgan oblast/ Kara-Kala raion	0.08 (0.02)	0.06 (0.01)	0.02 (0.01)	0.213	1.851	0.148	259.13/724/1.000
Chuguevka/Pogranichnyi	0.09 (0.02)	0.08 (0.01)	0.01 (0.01)	0.113	3.940	0.088	317/724/1.000
Chuguevka/Bulyga-Fadeevo	0.08 (0.02)	0.08 (0.01)	0.00 (0.01)	0.086	5.296	0.072	188.21/724/1.000
Bulyga-Fadeevo/Pogranichnyi	0.09 (0.02)	0.08 (0.01)	0.01 (0.01)	0.129	3.365	0.095	342.46/724/1.000
Total sample	0.09 (0.14)	0.06 (0.01)	0.03(0.01)	0.314	1.09	0.105	1446.74/724/0.000

Local population samples of the house mouse displayed rather high interpopulation differentiation δ_S, i.e., the proportion of individuals with different genetic information in the population [28], which varied from 0.283 in the population of Novosibirsk to 0.614 in Elizovo.

Genetic polymorphism was estimated as the proportion of the polymorphic RAPD loci in the total number of such loci. In the total sample, this parameter was 95.58%. At the 99 and 95% significance levels, the genetic polymorphism values in the total sample decreased to 60.65 and 24.26%, respectively, which testified to the high content of rare alleles. The highest levels of polymorphism were detected in the populations of Primorskii krai and in one population from Kazakhstan (Taldy-Kurgan oblast) (Table 1).

The animals within each sample were characterized by high genetic similarity *S*, which constituted 0.87 for the total sample (Table 1). The distribution of the pairwise genetic distances in the samples from a number of geographical localities of the *M. musculus* range is presented in Figs. 3a and 3b. As far as it is possible to judge from the small sample sizes, the presented histograms demonstrate the differences in the sample genetic heterogeneity, which seems likely to be associated with the number of subspecies having participated in the hybridization events on the territories. The mouse sample from Tsasuchei was the most genetically homogenous (Fig. 3b), which was confirmed by the lowest intrapopulation differentiation shown for this sample: δ_S = 0.266 (Table 1). The histogram for the total sample has one peak, confirming that all mice examined belong to one species (Fig. 3c). The histogram in Fig. 3d demonstrates interspecific relationships between *M. musculus* and *M. spicilegus*, displaying two peaks corresponding to intra- and interpopulation differences.

Population genetic differentiation. The values of genetic differentiation between the samples from different localities are presented in Table 2. In pairwise comparisons, the values of mean within-sample (H_S = 0.05 to 0.07) and total (H_T = 0.05 to 0.1) genetic diversity indices were low. For the species as a whole, total genetic diversity, H_T was 0.094, and within-sample diversity H_S was 0.06. Total genetic diversity, D_{ST}, between the local samples was also low varying from 0 to 0.04. This means that most of the genetic diversity resided within each local population.

Analysis of the local samples for interpopulation differentiation did not reveal dramatic differences in any case. However, analysis over all localities statistically significantly demonstrated that the samples examined did not belong to one population (χ^2 = 1446, *d.f.* = 724; P = 0.000).

The degree of the population genetic subdivision, calculated using gene fixation coefficient G_{ST} varied from 0.086 (between the house mice from Chuguevka and Bulyga-Fadeevo) to 0.324 (between the individuals from Khatassy and Kara-Kala raion), which corresponded to the gene flow Nm = 5.30 for the first population pair, and Nm = 1.05 for the second pair of populations.

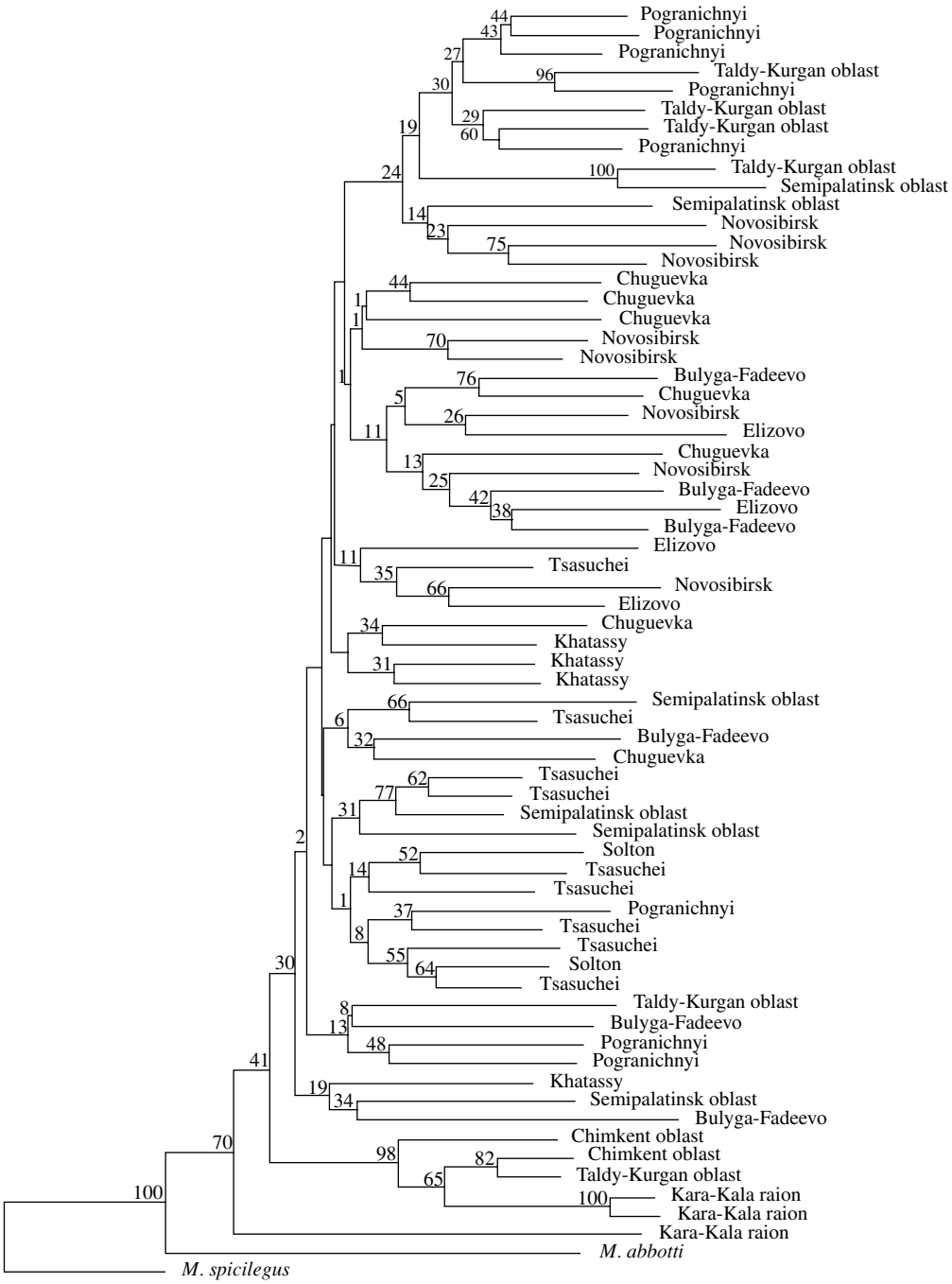

Fig. 4. Phylogenetic relationships among the *M. musculus* individuals from different populations (NJ tree).

Interpopulation genetic distances D_N in *M. musculus* were strongly different, varying from 0.059 to 0.186. Interestingly, the mice from Tsasuchei were close to the house mice from both Semipalatinsk oblast ($D_N = 0.061$) and Khatassy ($D_N = 0.059$), which carried different genetic markers, but belonged to the short-tailed forms of the house mouse. The longest genetic distances were revealed between the sample from Kara-Kala raion and all other samples, from 0.148 to 0.186. If this sample is excluded from the total sample, the genetic distances within the species *M. musculus* dramatically decrease (0.07 to 0.09) and the hiatus between the average intra- and interspecific values is formed. Genetic divergence for all populations of *M. musculus* was on average equal to 0.105. For comparison, the genetic distances between *M. musculus* and *M. abbotti*, and between *M. musculus* and *M. spicilegus* constituted 0.147 and 0.181, respectively. Thus, the divergence between these two species was higher than

the mean interspecific differentiation for *M. musculus*, but did not exceed its upper limit.

Phenogenetic and phylogenetic reconstructions. To examine pheno- and phylogenetic relationships among the house mice, UPGMA and NJ trees were constructed. Since the reconstructions had similar topology, only the NJ tree is presented (Fig. 4). In general, the analysis did not reveal distinct geographic differentiation of the house mice. The values of the bootstrap support were low for almost all clusters. The exceptions were branching of the *M. abbotti* and *M. spicilegus* outgroups (70 and 100%, respectively) and the cluster that was closest to the NJ tree base and was formed by the mice from Chimkent oblast, Taldy-Kurgan oblast, and Kara-Kala raion (98%).

DISCUSSION

RAPD–PCR technique is successfully used for the analysis of the population structure and the species genetic variation in both plants [28, 29] and animals [30, 31, and others]. In recent years, the house mouse has been extensively studied across its vast range using allozymic and cytogenetic analysis [12, 16, 27, 32]. Moreover, mtDNA regions [14, 33] and the rRNA genes [34] were sequenced.

RAPD DNA analysis of the house mice inhabiting the vast territories of the Asian part of the former Soviet Union revealed substantial genetic heterogeneity of the samples examined at the individual and population levels. Molecular markers for both individual local populations and the subspecies of *M. musculus* were identified. The data on the variation of some genetic characters, however, point not only to substantial differentiation, but in some cases to hybridization between different intraspecific groups of *M. musculus*.

For example, as inferred from the RAPD data, all mice from the Khatassy and Novosibirsk samples were characterized by the type *musculus* RAPD profile detected with primer OPC-05. Sequencing of the mtDNA D-loop region, however, showed that the mice from the first sampling locality carried two types of nucleotide substitutions (*musculus* and *castaneus*), while the animals from the second locality had only one nucleotide substitution type (*musculus*) [35]. In addition, karyotypic analysis of the Khatassy sample revealed the presence of chromosomal markers atypical of the *musculus* group. At the same time, in Novosibirsk the presence of two cytogenetic forms of *M. m. musculus* was demonstrated [16].

Some authors assign *M. m. wagneri* along with the morphologically and karyotypically close subspecies *M. m. gansuensis* and *M. m. manchu* (mice with short tails and white bellies) to an individual intraspecific complex, or even to an independent species [16, 36, 37]. The results of the present study are consistent with the karyotypic data, supporting genetic closeness of *M. m. gansuensis* and *M. m. wagneri* along with their distinct remoteness from other subspecific groups (Fig. 4). At the same time, RAPD markers effectively differentiate *M. m. gansuensis* from *M. m. wagneri*. It is important, since these forms cannot be distinguished using mtDNA D-loop sequencing and allozyme analysis.

Fragment OPC-05_{995}, typical of *M. m. gansuensis*, is distributed both in the Far East and Central Asia (Taldy-Kurgan oblast), where it occurs at low frequency. Hence, *M. m. gansuensis* could be one of the forms that actively participated in the development of the house mouse fauna in these regions.

As already mentioned, DNA fragment OPC-05_{995} was found in the genomes of some house mice from Elizovo (despite the fact that RAPD profiles of all animals from that sampling locality were highly specific and belonged to the type *castaneus*), which, according to the data of karyotypic analysis, carried *gansuensis*-specific chromosomal markers [16]. Sequencing of the mtDNA D-loop region from the Elizovo mice demonstrated the presence of the *musculus*- and *castaneus*-specific nucleotide substitutions [35]. Thus, the mouse sample from Elizovo represents a mixture of the genetic characters of different *M. musculus* subspecies. Maximal value of the intrapopulation differentiation index confirms this finding (Table 1).

The highest genetic variation was found in Primorskii krai, where a vast hybrid zone of different house mouse subspecies had been recorded. The lowest genetic variation was observed in Chimkent oblast and in Solton (Table 1). However, the data for the two latter collecting localities can be considered only as preliminary, due to the small sample sizes. The marked differences in the levels of genetic polymorphism and the proportion of rare alleles described may be explained by the presence of well-differentiated genetic forms in some of the samples.

The low G_{ST} value points to the intense gene exchange between the geographically close local populations of Chuguevka and Bulyga-Fadeevo (Table 2), which are located within the vast hybrid zone of several *M. musculus* subspecies. In particular, according to karyotypic data, short-tailed *gansuensis*-like forms had a substantial influence on the formation of the murine fauna of Western Primor'e [16]. By contrast, the gene flow between the populations of Kara-Kala raion and the settlement of Khatassy, which were maximally distant from each other, was almost absent ($Nm = 1.045$). Apparently, the development of these populations involved the participation of different subspecific forms. In the first case the prevalence of the aboriginal long-tailed *bactrianus* form is suggested, while the second population was probably formed with the participation of the *castaneus* form [16]. Interestingly, local house mouse populations from Kazakhstan are characterized by the lowest gene exchange with other populations, compared to the other regions. On the contrary, the values of the gene flow indices between the populations of Trans-Baikal region, Western Siberia, and the

Far East point to the high exchange of the gene pools. It seems likely that this effect was caused by the intensive trade and transportation links between these territories, which permitted house mice to move across large distances.

Thus, RAPD analysis has identified molecular markers specific to the subspecific forms of *M. musculus*, including *musculus*, *castaneus*, *gansuensis*, and *wagneri*. The populations of the Russian Far East, Siberia, and Kazakhstan examined differed in genetic variation and differentiation. On the one hand, this observation, probably, reflects substantial genetic diversity of the house mice of these regions. On the other hand, this seems to result from the hybridization between different subspecies. It can be affirmed that most of the animals examined possessed the characters of more than one form of *M. mussculus*, which were differently expressed at morphological, karyotypic, and molecular levels.

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