ISSN 1022-7954, Russian Journal of Genetics, 2007, Vol. 43, No. 8, pp. 899–904. © Pleiades Publishing, Inc., 2007.
Original Russian Text © L.V. Yakimenko, K.V. Korobitsyna, 2007, published in Genetika, 2007, Vol. 43, No. 8, pp. 1084–1090.

ANIMAL GENETICS

Variability of Chromosome 1 with HSR Insertions in Natural and Synanthropous Populations of House Mouse *Mus musculus* L. 1758

L. V. Yakimenko[a] and K. V. Korobitsyna[b]

[a] *Vladivostok State University of Economics and Service, Vladivostok, 690990 Russia; e-mail: lyudmila.yakimenko@vvsu.ru; fax: (4232) 404-154*
[b] *Institute of Biology and Soil Science, Russian Academy of Sciences, Vladivostok, 690022 Russia*

Received August 22, 2006

Abstract—House mice carrying aberrant chromosome 1 with an insertion of homogeneously stained regions (HSR) have been studied. The mice were collected in the North Caucasus, Chita and Amur oblasts, Spitzbergen and Kunashir Islands, Altai krai, Khabarovsk krai, Primorye, Sakhalin, Kamchatka, Turkmenistan, and Kazakhstan. In these mice, the aberrant chromosomes were assigned to the "Asian" type, i.e. they carried two HSR insertions. The aberrant chromosome 1 in house mice from different geographic regions was shown to differ in size of HSR, staining intensity, and some other features of Q-H, C, and G-banding, which suggests independent origin of this aberration in house mouse populations from different taxa and regions. A novel variant of chromosome 1 in mice of the subspecies *M. m. wagneri* was found.

DOI: 10.1134/S102279540708011X

INTRODUCTION

Cytogenetic investigations of natural populations of the house mouse *Mus musculus* L. have refuted the view on karyotype similarity of these rodents. It is currently known that the house mouse is characterized by Robertsonian polymorphism, a wide range of pericentric heterochromatin variability, and, finally, the phenomenon of aberrant chromosome 1 carrying HSR insertions. In the house mouse, chromosome 1, which is larger in size then the normal homolog due to one or two interstitial insertions, is referred to as aberrant, or abnormal, chromosome 1. As the insertions look homogeneous after G-banding, they were termed HSR (homogeneously staining regions). HSR is a cytogenetic manifestation of DNA amplification, namely a high-copy state of gene *Sp100*, which is a relatively recent evolutionary acquisition characteristic only of *M. musculus.* The so-called European variant of the aberrant chromosome 1 contains one HSR region resulting from *Sp100* duplication-amplification. The European variant of aberrant chromosome 1 is associated with *M. m. domesticus*. In members of the subspecies group *M. m. musculus*, aberrant chromosome 1 carries two HSR insertions. This is the so-called Asian variant of this chromosome. It is thought that the two-insertion variant has appeared via paracentric inversion in an aberrant chromosome 1 having one HSR insertion [1–4].

MATERIALS AND METHODS

Based on cytogenetic analysis of more than 700 house mice from samples taken on the territory from Moldavia to Kazakhstan (Fig. 1), we have obtained original data on the incidence of chromosome 1. The aberrant chromosome 1 was found in 34 animals.

Chromosome preparations were prepared from bone marrow cells following a standard protocol. Heterochromatin staining was conducted as in Samner [5]. Fluorescence Q-H-banding was done as in Ioshida et al. [6]. Differential G-banding was conducted according to Seabright [7]. The karyotypes of two mice carrying aberrant chromosome 1 were examined only by routine staining. The remaining animals were studied using C, Q–H, or G-banding (table).

RESULTS AND DISCUSSION

Using the results of our long-term cytogenetic studies and literature data, we have constructed a distribution map of the carriers of chromosome 1 with insertions HSR (Fig. 1). In all of the mice examined, the chromosomes 1 were assigned to the Asian type, i.e., they have two HSR insertions. The abnormality of this type, first found and described by us in the Primorye population [8], was also observed in *M. musculus* from Sweden, Yakutia, and Novosibirsk [9–11], Sverdlovsk

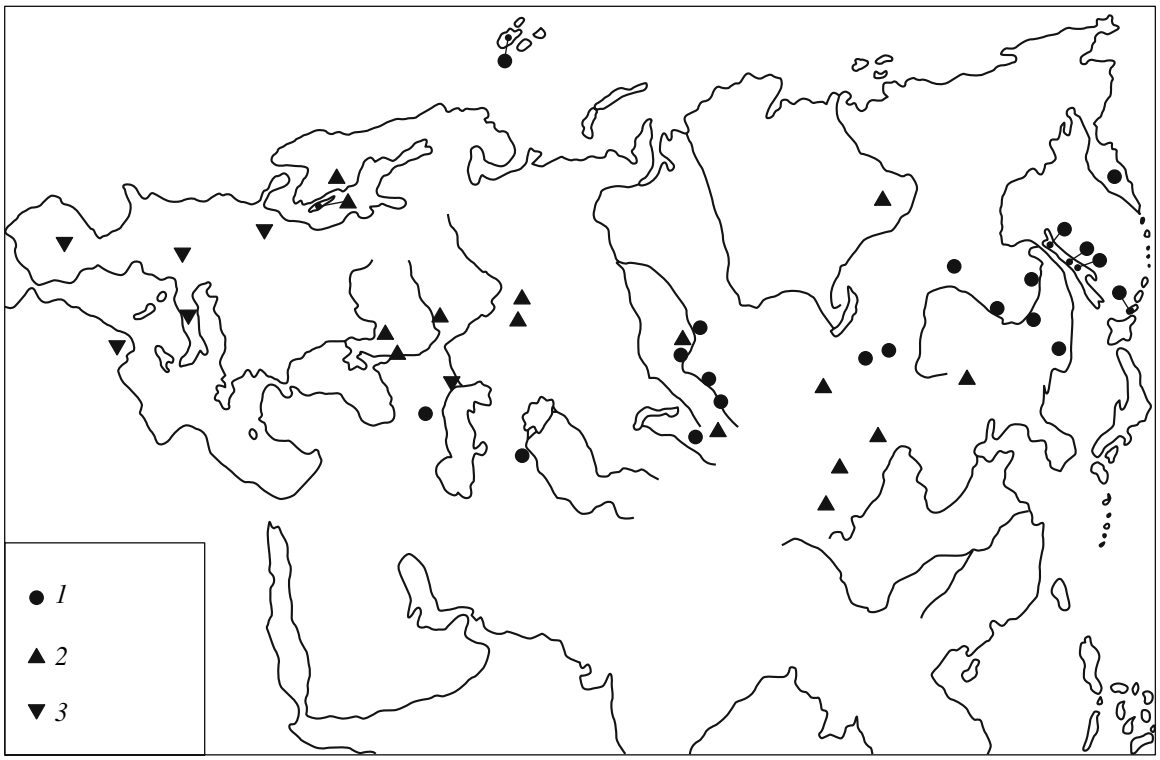

Fig. 1. Geographic distribution of aberrant chromosome 1 with HSR in natural populations of the house mouse: *1*, Asian variant, our data; *2*, Asian variant, literature data; *3,* European variant, literature data.

and Chelyabinsk oblasts [12], Kursk, Voronezh, Rostov, Mongolia [13], and many localities in China [4].

Abnormal chromosome 1 of the European type (with one HSR insertion) was detected in mice from Switzerland, Germany, Italy, and Spain; in Russia, it was found only in Astrakhan'. Weichenhan et al. [14] have shown that chromosome 1 is normal when the number of the *Sp100* gene copies is 60 to 200. If this number exceeds 200, chromosome 1 is considered aberrant. One HSR insertion can make chromosome 1 6-30% larger than the normal homolog [1]. According to our visual estimation, two insertions increase the chromosome by 30-70%. In some regions, the size of the aberrant chromosome exceeds the norm only by 30–40%, whereas its maximum length, being 60–70% of the norm, was observed by us in animals from many regions (Fig. 2). According to our data, abnormal chromosomes 1 of the house mouse from different regions have different HSR region size and some features of Q-H, C, and G-banding, which suggests the independent appearance and existence of several insertion types in the house mouse populations.

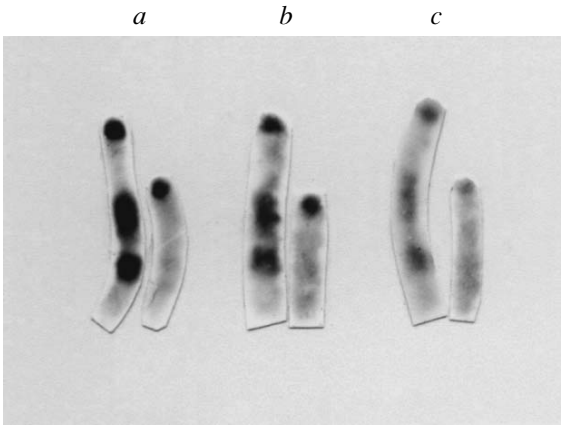

Fig. 2. Comparative size of the aberrant and normal homologs of chromosome 1 in the house mouse and variation in HSR C-banding intensity in the aberrant chromosome of the Asian type. (*a*) bright banding (Mil'kovo settlement, Kamchatka); (*b*) moderate banding (Mariinskoe settlement, Khabarovsk krai); (*c*) pale banding (Tashauz, Turkmenistan).

In our view, there are several variants of the aberrant chromosome 1 of the Asian type. This view is supported by Winking et al. [9], who have found different variants of this chromosome differing in size of the distal and proximal HSR region in the populations of the Eland Island (Sweden). Not only deletion-duplication amplification, but also unequal crossing over occurs in HSR [4]. Trout et al. [15] have also shown that gene *Sp100* is a chimeric structure that had appeared via fusion of the *sp100* gene proper with receptor gene *Csprs*. Analysis of the above data indicates that mutations leading to new variations of the aberration may occur in the initial aberrant chromosome 1 variants.

Occurrence of house mice carrying aberrant chromosome 1, banding methods, and HSR characteristics

Sampling locality	Zool. no.	Banding method	Genotype	HSR C-banding, intensity	Number of subblocks in proximal/distal HSR blocks
Spitzbergen Island	358	Routine banding	HSR/+	?	?/?
North Caucasus					
Groznyi	314	C	HSR/+	Bright	2/1
	317	C	HSR/+	Bright	2/1
	318	C	HSR/+	Medium	2/1
	319	C	HSR/+	Medium	2/1
Novosibirsk	593	C	HSR/HSR	Bright	2/2
	595	C	HSR/+	Bright	2/2
	597	C	HSR/HSR	Medium	2/2
Altai krai					
Andreevka village (100 km to southwest from Barnaul)	702	C	HSR/+	Pale	1/3
Gorno-Altaisk	906	C, Q–H	HSR/+	Bright	2/2
Chita region					
Tsasuchei settlement	877	C, Q–H	HSR/+	Bright	3/2
	879	Q–H	HSR/+	?	?/?
	881	C, Q–H	HSR/+	Bright	2/1
	883	C, Q–H	HSR/HSR	Bright	2/1
Amur region					
Blagoveshchensk	524	Q–H	HSR/+	?	?/?
Tynda	895	C, Q–H	HSR/+	Medium	2/1
Khabarovsk krai					
Khabarovsk	915	C, Q–H	HSR/+	Medium	2/2
Mariinskoe settlement	722	C	HSR/+	Bright	2/1
Primorye					
Arsen’ev	159	C, G	HSR/+	Bright	2/1
Sakhalin region					
Okha	934	C, Q–H	HSR/+	Medium	2/1
	936	C, Q–H	HSR/+	Medium	2/1
	959	C, Q–H	HSR/+	Medium	2/1
Aleksandrovsk-Sakhal.	925	C	HSR/+	Medium	2/1
Tymovsk	588	C	HSR/+	Bright	2/2
Kamchatka					
Myl’kovo settlement	759	C, Q–H	HSR/+	Bright	2/1
	760	C, Q–H	HSR/+	Bright	2/1
	761	C, Q–H	HSR/+	Bright	3/2
	763	C, Q–H	HSR/HSR	Bright	2/2
Kunashir Island	359	Routine banding	HSR/+	?	?/?
Turkmenistan					
Tashauz	258	C	HSR/+	Pale	1/2
Kazakhstan					
Ayaguz R. valley	313	C, G	HSR/+	Bright	2/1
	314	C, G	HSR/+	Bright	2/1
	325	C, G	HSR/+	Bright	2/1
	326	C, G	HSR/+	Bright	2/1

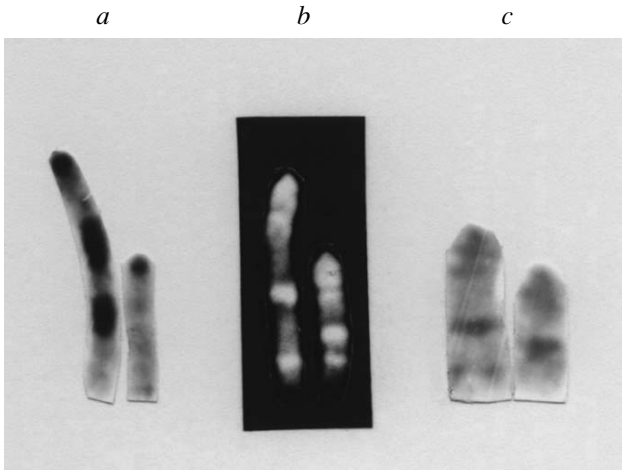

Fig. 3. Asian type aberrant chromosome 1 and its normal homolog in the house mouse after different banding procedures: (*a*) C-banding; (*b*) Q–H-banding; (*c*) G-banding.

Analysis of our data demonstrated the following pattern of aberrant chromosome 1 variation. According to the HSR staining intensity, we classified all of the mice examined into three groups: animals with bright, intermediate, and pale staining (see the table and Fig. 2). Earlier, we have noted that each HSR can consist of two small, closely located subblocks [8]. Now we can conclusively state that this character displays variation even within a population (see the table). Apparently, variation in relative size of the proximal and distal HSR is associated, among other factors, with the number of HSR subblocks.

Only 4 out of 34 HSR carriers examined proved to be homozygous for the aberration (see the table). Gileva [12] has found a similar ratio of homo- and heterozygous HSR carriers (5 out of 33) in the South Urals population. However, Traut et al. [1] recorded a very different ratio of homo- and heterozygous European-type HSR carriers in a population from South Germany: 28 homozygotes and 11 heterozygotes out of 39 carriers. Moreover, very high frequency of HSR carriers in this population is striking (39 of 44 examined). As was later stated by Agulnik et al. [11], viability of the carriers of the European type aberration was not lower than that of the normal animals. In addition, homozygous state of the aberration of this type had no effect on viability of its carriers [11]. Comparison of these and our results (34 carriers of the Asian aberration variant of 700 animals examined and only 4 homozygotes versus 30 heterozygotes) suggests that the Asian chromosome 1 type reduces viability of its carriers. This may have the following explanation: in essence, HSR is a visual cytological expression of instable tandem repeats, similar to expansion of repetitive DNA sequences, which in human lead to more than 50 diseases [16]. These mutations were termed dynamic because of rapid and drastic changes in repeat size, which are characteristic of expansions. A decrease in viability in HSR carriers may be related to the phenomenon of anticipation (exacerbation of a disease with increasing length of the repeats over generations), which was documented for humans [17].

Pericentric autosome regions in the house mouse are typically bright upon C- and Q-H-banding. Bright fluorescence upon Q- or Q-H-banding is thought to reflect enrichment of the chromosome region by AT base pairs. C-banding of HSR is also positive, with intensity comparable for HSR and pericentric regions. Q-H-banding of HSR, in contrast to pericentric regions, in the house mouse is always pale and homogeneous, as well as G-banding (Fig. 3), which indicates the total lack of AT base pairs in this DNA region.

An unusual variant of abnormal chromosome 1 was found in East Kazakhstan (Ayaguz River valley, animal nos. 313, 314, 325, and 326). It is noteworthy that these animals were collected in an open station, and morphologically and karyologically represent the typical wild-living aboriginal Central Asian form *M. w. wagneri*. Upon C-banding, two bright subblocks are seen in the abnormal homologs of their chromosomes 1, which is characteristic of most Asian type aberrant chromosomes (Fig. 4a). However, C-banding of abnormal chromosomes of these mice was absolutely not typical for HSR-containing autosomes. The HSR regions were stained much darker than normal for them and showed marked striation (Fig. 4b). Insertions that are bright upon C-banding correspond to other, unevenly stained regions of G-banded chromosomes. Based only on these G-banding patterns of these and typical for HSR (Fig. 3c) chromosomes, we can state that the chromosomes of Kazakhstan mice did not contain traditional HSR.

Agulnick et al. [11] argued that aberrant chromosome 1 occurs only in *M. m. domesticus* and *M. m. musculus*, as well as in their hybridization zones with other house mouse subspecies. Our results demonstrate that this chromosome also occurs in subspecies *M. m. gansuensis* (semi-synanthropous population from southern Chita oblast) and *M. m. wagneri* (the Ayaguz River valley, a wild-living subspecies that was not subject to hybridization!). Similarly to the fact that the European and Asian variants of aberrant chromosome 1 are associated with subspecies *M. m. domesticus* and *M. m. musculus*, respectively, the novel rare variant that we have found is associated with subspecies *M. m. wagneri.* The appearance of this new and rare variant could be promoted by the geographical vicinity of the Ayaguz River valley to the Semipalatinsk nuclear testing grounds. This novel variant is of obvious interest for further research.

According to Trout et al. [15], in cell lines resistance to chemicals and in tumor cells frequently harboring HSR, the latter are derived from more than one region of the mammalian genome. Thus, although, as currently known, the rearrangement in the aberrant chromosome 1 carriers involves only gene *Sp100*, we can-

not exclude the possibility of amplification of other genes in the house mouse populations where unusual HSR variants have been recorded.

Since it was shown that both European and Asian aberrant chromosome 1 variants originated through amplification of the *Sp100* gene, the visual differences in the abnormality manifestation are still to be explained. It may well be that different variants of aberrant chromosome 1 differ not only in repeat copy number. Of course, our proposal does not mean that each mouse population, in which the abnormality carriers are found, carries the unique HSR variant. To date it is clear that there are two major variants of aberrant chromosome 1, which appeared in populations by two possible pathways: de novo resulting from *Sp100* gene amplification and through migration of HSR carriers into the population.

Thus, it has been documented that aberrant chromosomes 1 can differ in the HSR number (one or two), the copy number of the *sp100* gene amplified in HSR (60–200 and higher), the ratio of the proximal and the distal HSR regions, intensity of C-banding of HSR, and the effect on viability of their carriers.

Can we consider high aberrant chromosome 1 frequency as an ecological indicator of adverse environment? This was suggested by Gileva [12] in her study of the frequencies of HSR-carrying house mice in the Urals region, on areas characterized by different environmental pollution. We believe that this hypothesis could be true in the following cases. The aberration arises de novo fairly easily and often under the impact of external factors, e.g., mutation load. Then, mutation drive (which was earlier shown for the Asian type aberrant chromosome 1 [18–20]) is switched on. Meiotic drive promotes the accumulation of aberrant chromosome 1 in the population and its further spreading. However, in this case, various aberrant chromosome 1 variants should be present on the same, relatively small territory. This assumption needs testing. According to another scenario, heterozygous aberration carriers, colonizing polluted territories via migration, prove to be more resistant to ecotoxicants and radiation than normal animals, like cultured cells carrying HSR chromosomes exhibit higher resistance to chemical preparations [21, 22]. However, in her study of areas with different levels of pollution, Gileva [12] has shown that the total frequency of aneuploid and polyploid cells was 2.5-fold higher in HSR-carrying mice. Note that we did not observe HSR carriers in the Primorye zone of ecological crisis—the Dal'negorsk region, where the level of genetic abnormalities in domestic mice is sever times higher than normal [23]. Therefore, there may be no direct association between environmental pollution and the aberration.

House mice from the territory of the former Soviet Union are highly heterogeneous taxonomically [24, 25]. The regions with high frequency of the aberration may be inhabited by mice of the taxa that have genetic determination for the appearance and long-term preservation of the mutation in the population gene pool. This system, including distorter and responder genes, has been described for Siberian populations of the house mouse [18–20]. These authors have shown that this system resulted from an insertion of the distal HSR region. The European variant with one non-inverted HSR region does not have such system that would ensure meiotic drive. Using a mathematical model, Sabantsev et al. [18] have demonstrated that this mutation, conferring very low fertility and viability of its carriers in homozygous state, can be maintained in a population and spread over the area only upon high levels of meiotic drive in favor of aberrant chromosome 1. The nature of the mechanisms underlying the preservation and spreading of the European type aberrant chromosome 1 is unclear.

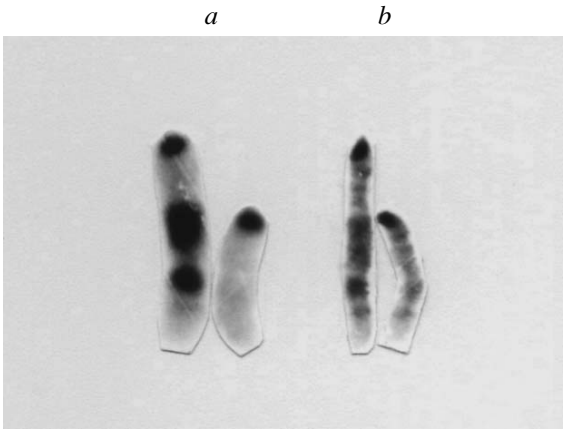

Fig. 4. Rare variant of aberrant chromosome 1 and its normal homolog in house mice from East Kazakhstan (Ayaguz River valley): (*a*) C-banding; (*b*) G-banding.

Our results indicate that aberrant chromosome 1 occurs with different frequencies in different regions. In Primorye, the territory well studied karyologically, we have found only one individual of house mouse carrying this abnormality. Judging from our material, abnormal chromosome 1 occurs at high frequency in Sachalin (found in three out of four samples). Literature data indicate that this chromosome occurs at high frequency in Novosibirsk region and the Urals. It may well be that this abnormality is common in southern European Russia [13], in West Siberia in general and in southern Chita region (our data). The remaining cases of finding mice with HSR can be interpreted as the presence of the abnormality in the given region.

We believe that, on the basis of known geographical distribution of the abnormality and territorial assignment of different variants, one can obtain in natural and synanthropous house mouse populations new material for studying this phenomenon and using them as a promising model for analysis of diseases associated with dynamic mutations.

ACKNOWLEDGMENTS

We thank the staff of the Laboratory of Evolutionary Zoology and Genetics, Institute of Biology and Soil Sciences, Far Easter Division of the Russian Academy of Sciences L. Frisman, V. Korablev, M. Pavlenko, A. Kryukov, I. Kartavtseva nd Moscow zoologists F. Bokstein and V. Lapshov for their many-year assistance in collecting the material studied in this work.

REFERENCES

1. Traut, W., Winking, H., and Adolph, S., An Extra Segment in Chromosome 1 of Wild *Mus musculus*: A C-Band Positive Homogeneously Staining Regions, *Cytogenet. Cell Genet.*, 1984, vol. 38, pp. 290–297.
2. Traut, W., Rahn, I.M., Winking, H., et al., Evolution of a 6-200 Mb Long-Range Repeat Cluster in the Genus *Mus*, *Chromosoma*, 2001, vol. 110, pp. 247–252.
3. Agulnik, S.I., Borodin, P.M., Gorlov, I.P., et al., The Origin of a Double Insertion of Homogeneously Staining Regions in the House Mouse (*Mus musculus musculus*), *Heredity*, 1990, vol. 65, no. 2, pp. 265–267.
4. Winking, H., Weith, A., Boldyreff, B., et al., Polymorphic HSRs in Chromosome 1 of the Two Semispecies *Mus musculus musculus* and *M. musculus domesticus* Have a Common Origin in an Ancestral Population, *Chromosoma*, 1991, vol. 100, no. 3, pp. 147–151.
5. Sumner, A.T., A Simple Technique for Demonstrating Centromeric Heterochromatin, *Exp. Cell Res.*, 1972, vol. 75, pp. 304–306.
6. Yoshida, M., Ikeuchi, T., and Sasaki, M., Differential Staining of Parental Chromosomes in Intraspecific Cell Hybrids with a Combined Quinacrine and 33258 Hoechst Technique, *Proc. Jpn. Acad.,* 1975, vol. 51, pp. 184–187.
7. Seabright, M., A Rapid Banding Technique for Human Chromosomes, *Lancet*, 1971, vol. 11, pp. 971–972.
8. Yakimenko, L.V. and Korobitsyna, K.V., Rare Variant of Chromosome 1 in the House Mouse: the Presence of Tow Extra Heterochromatin Segments, *Genetika* (Moscow) 1988, vol. 24, no. 2, pp. 376–378.
9. Winking, H., Bostelmann H., and Fredga K., Incidence of Double-Band HSRs in Chromosome 1 of the House Mouse, *Mus musculus musculus*, from Oland (Sweden): A Population Study, *Hereditas*, 1991, vol. 114, no. 2, pp. 111–116.
10. Agulnik, S.I., Gorlov, I.P., and Agulnik, A.I., Mouse Chromosome 1 with Two Insertions Is (HSR; IC5) I eq and Is (HSR; IE3) 2feq, *Mouse News Letts.*, 1987, vol. 78, p. 80.
11. Agulnik, S., Adolph, S., Winking, H., and Traut, W., Zoogeography of the Chromosome 1 HSR in Natural Populations of the House Mouse (*Mus musculus*), *Hereditas*, 1993, vol. 119, no. 1, pp. 35–46.
12. Gileva, E.A., *Ekologo-geneticheskii monitoring s pomoshch'yu gryzunov (ural'skii opyt)* (Ecological Genetic Monitoring with the Help of Rodents (Ural Experience)), Yekaterinburg: Ural Univ., 1997.
13. Nadzhafova, R., Kovalskaya, Yu., Fedorovich, E., et al., A Geographic Area of Distribution of the HSR 1 Chromosome Marker in House Mice of European Russia, *Evolution, Genetics, Ecology and Biodiversity*, (Proc. International Conference), Vladivostok, 2001, p. 63.
14. Weichenhan, D., Kunze, B., Winking, H., et al., Source and Component Genes of a 6-200 Mb Gene Cluster in the House Mouse, *Mammalian Genome*, 2001, vol. 12, pp. 590–594.
15. Traut, W.I., Weichenhan, D., Eickhoff, U., and Winking, H., Origin of the Chromosome 1 HSR of the House Mouse Detected by CGH, *Chromosome Res.*, 1999, vol. 7, no. 8, pp. 649–653.
16. Mandel, J.-L., Trinucleotide Diseases on the Rise, *Nature Genet.*, 1994, vol. 7, pp. 453–455.
17. Illarioshkin, S.N., Ivanova-Smolenskaya, I.A., and Markova, E.D., Novel Mutational Mechanism in Man: Expansion of Trinucleotide Repeats, *Russ. J. Genet.*, 1995, vol. 31, no. 11, pp. 1254–1263.
18. Sabantsev, I.D., Spitsyn, O.A., Agulnik, S.I., and Ruvinski, A.O., Population Dynamics of Chromosome 1 with the Insertion in Mice, *Genetika* (Moscow), 1992, vol. 28, no. 1, pp. 163–173.
19. Agulnik, S.I., Sabantsev, I.D., Orlova, G.V., and Ruvinski, A.O., Meiotic Drive on Aberrant Chromosome 1 in the Mouse Is Determined by a Linked Distorter, *Genet. Res.*, 1993, vol. 61, no. 1, pp. 91–96.
20. Agulnik, S.I., Sabantsev, I.D., and Ruvinski, A.O., Effect of Sperm Genotype on Chromatid Segregation in Female Mice Heterozygous for Aberrant Chromosome 1, *Genet. Res.,* 1993, vol. 61, no. 1, pp. 97–100.
21. Pogosyants, E.E., New Decisions in Cancer Cytogenetics, *Genetika* (Moscow), 1981, vol. 17, no. 12, p. 2087.
22. Biedler, J.L. and Spengler, B.A., Metaphase Chromosome Anomaly: Association with Drug Resistance and Cell-Specific Products, *Science*, 1976, vol. 191, p. 185.
23. Yakimenko, L.V., Kartavtseva, I.V., and Korobitsyna, K.V., Ecological Genetic Monitoring in Dalnegorsk Zone of Ecological Crisis (Primorsky Kray), in *Aktual'nye problemy ekologicheskoi fiziologii i genetiki zhivotnykh* (Current Problems of Animal Ecological Physiology and Genetics), (Proc. International Scientific Conference), Saransk: Izd. Mordvinia Uni., 2005, pp. 284–286.
24. Yakimenko, L.V., Korobitsyna, K.V., Frisman, L.V., et al., Tsitogenetika i sistematika domovykh myshei Rossii i prilezhashchikh stran, in *Problemy evolyutsii* (Cytogenetics and Systematics of House Mice of Russia and Adjacent Territories), Vladivostok: Dal'nauka, 2003, vol. 5, pp. 60–87.
25. Korobitsyna, K.V. and Yakimenko, L.V., The Role and Place of *wagneri*-Like Morphs of House Mouse (Rodentia: Muridae) in the Fauna of Russia and Adjacent Countries, *Zool. Zh.*, 2004, vol. 83, no. 8, pp. 1018–1030.