tion[1,10-12]. The exothermic reaction of MIC gas with cellular water is thought to cause molecular[11-15], cellular[1,5], cytogenetic[5,16,17] and morphological damage[4,8]. Most of the abnormalities could not be discarded by self-eliminating processes in successive generations of plants; this indicates that MIC has long-term effects on biological systems.

1 Picot, A., The World Scientist *10* (1986) 46.
1 Jayaraman, K. S., Nature *337* (1989) 679.
3 Agarwal, A., New Scientist *38* (1985) 1484.
4 Singh, I. P., and Roy, S. K., Curr. Sci. *56* (1987) 679.
5 Kumar, G., Tripathi, D. S., and Roy, S. K., Envir. exp. Bot. *29* (1989) 261.
6 Gupta, B. N., Rastogi, S. K., Chandra, H., Mathur, A. K., Mathur, N., Mahendra, P. N., Pangetey, B. S., Kumar, S., Kumar, P., Seth, R. K., Dwivedi, R. S., and Ray, P. K., Ind. J. exp. Biol. *26* (1988) 149.
7 Caspary, W. J., and Myhr, B., Mutat. Res. *174* (1986) 285.
8 Singh, I. P., and Roy, S. K., Curr. Sci. *58* (1989) 381.
9 Gustafsson, A., K. Fysiogr S allsk. Lund. Forh. *36* (1940) 1.
10 Gruzdyev, G. S., Zinchenko, V. A., Kalinn, V. A., and Slovtsov, R. I., in: Chemical protection of plants, p. 227. Ed. G. S. Gruzdyev. MIR Publisher, Moscow 1983.
11 Mericle, L. W., and Mericle, R. P., Envir. Mutat. Soc. News-sheet *5* (1971) 28.
12 Lindgreen, D., Hereditas *70* (1972) 165.
13 Stark, G. R., Biochemistry *4* (1965) 1030.
14 Coutino, R. R., Envir. Health Perspect. *31* (1979) 131.
15 Kihlman, B. A., in: Action of chemical on dividing cells, p. 96. Prentice Hall Press, New York 1966.
16 Vogel, E., and Natarajan, A. T., Mutat. Res. *62* (1979) 101.
17 Wade, D. R., Airy, S. C., and Singheimer, J. E., Mutat. Res. *58* (1978) 217.

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Cytogenetic and biochemical comparison of *Mus musculus* and *Mus hortulanus*[1]

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Summary. Two chromosome markers of *Mus hortulanus* are described: a dotted Y chromosome exceeding half of the length of autosome 19, and the 'domesticus' type of C-banding in the X chromosome. In *Mus musculus* from distant regions of the USSR (more than 200 specimens of various subspecies), the Y chromosome is equal to autosome 19, and the X chromosome is of the 'molossinus' type. Specific biochemical characteristics of house mice of the USSR are shown.
Key words. Mus musculus; *Mus hortulanus*; karyotype; X chromosome; Y chromosome; protein electrophoresis.

The relative uniformity of all chromosomes of the house mouse complement hinders cytogenetic studies of these animals. Beginning from 1970 the newest techniques have been used for analysis of chromosome structure in *Mus musculus*. Nevertheless, the problem of chromosome markers for differentiation of laboratory strains, and as subspecific and specific diagnostic criteria for various forms of house mice, has not been solved.

Comparative electrophoretic studies[2-4] provided the framework for the recognition of five genetic units in Europe: *Mus domesticus, M. musculus, M. spretus, M. hortulanus* and *M. abbotti. M. domesticus* and *M. musculus* hybridize in Central and South Europe[5], and from there *M. musculus* extends all over the USSR. *M. hortulanus* inhabits southern Europe, but the eastern limit of its range has not been determined. *M. abbotti's* range extends at least close to the Caucasus, but remains to be clarified. Also, the relationships between these species and Asian house mice are not known[6,7].

M. hortulanus differs from *M. musculus* in several ecological and ethological traits, even though the two species are very similar in morphology[7,8]. The hitherto-described karyotype of *M. hortulanus*, when compared to that of the house mouse, reveals no peculiarities by conventional staining, or in C-banding[7,9].

The paper presents the results of comparative studies of *M. musculus* s. str. and *M. hortulanus*, thus completing the data of Mezhzherin[10]. The species assignment of animals was determined according to Bonhomme et al.[11].

Materials and methods

More than 200 mice from 35 localities in the USSR were studied (table). Specimens from Kalmykia, Tuva and Transbaikalia were obtained both indoors and outdoors in summer. Animals from Moldavia were obtained in winter.

The chromosomes were prepared from cells of the bone marrow using standard procedures. C-banding for revealing the heterochromatin regions of chromosomes was performed according to Sumner[12]. The horizontal electrophoresis of proteins was carried out in starch gel with the buffer systems described by Selander et al.[13].

Allelic variation in diagnostic loci

Samples	Idh-1				Sod-1		Aat-1		Mbb			Alb		Est-1				Est-2			
	a	b	c	d	a	b	a	b	s	d	p	a	f*	l**	a	x***	b	b	c	x****	10f
1. Moldavia, obtained in mounds, n = 8	+				+		+			+			+				+				+
2. Moldavia, obtained indoors and in haystacks, n = 10		+				+	+		+	+	+	+			+			+	+		
3. Podmoskovye, Apsheron, Kalmykia, Siberia, Central part of Tuva and Transbaikalia, n = 29		+				+					+ 0.95	+		+	+	+		+	+		
4. Extreme south of Tuva and Transbaikalia, n = 40	+	+		+	+	+	+	+	+ 0.03	+	+	+			+	+	+	+	+		
5. Primorye, n = 123	+	+	+	+	+	+	+	+	+	+	+	+ 0.95	+	+	+	+	+	+	+	+	+

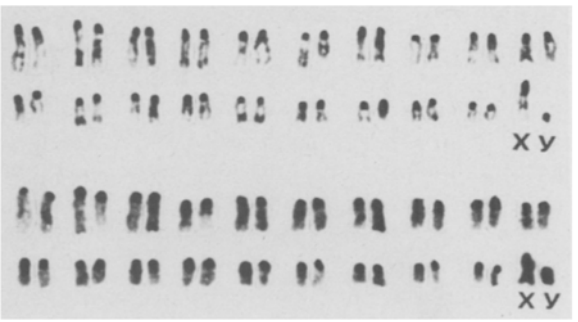
Alb-1-f* - a little faster than Alb-I-a
Est-1-1** - a little faster than Est-1-a
Est-1-x*** - a little slower than Est-1-a
Est-2-x**** - a little slower than Est-2-c

Results and discussion

Electrophoretic analysis of proteins. An electrophoretic study of the following 26 loci was conducted: Ldh-1, Ldh-2, Ldr, Mod-1, Mod-2, Mor 1, Idh-1, Sod-1, Sod-2, Pgd, Sdh, α-Gpd, Aat-1, Aat-2, Hbb, esterases and common muscle protein. The table summarizes data on the occurrence of alleles of the marker proteins. The result, as compared with the profiles published in Bonhomme et al.[11], allows the Moldavian mice obtained in mounds to be referred to Mus-4B (= *M. hortulanus*). Mice of samples 2,3 belong to the form Mus-2A (= *M. musculus*). For the mice of the extreme south of Tuva and Transbaikalia (wagneri-raddei?), there is evidence for a slight difference. Thus: Idh-1-a was found here along with Idh-1-b, and Aat-1-a and Sod-1-a are represented here together with Aat-b and Sod-1-b. The difference is also confirmed by a karyotypical peculiarity of these mice. They are characterized by very large marker C-blocks in chromosomes 17 and 18[8,14]. At the same time, an analysis of 26 loci does not allow us to assign to them any specific status other than *M. musculus*.

Primorye is populated obligately by the synantropic form of house mouse, which externally is mostly similar to *castaneus*. According to the set of variants of marker proteins, a mixing of gene pools of two forms at least takes place over the territory given[15]. The biochemical form 2C-*castaneus* has moved here from the south. The second partner may be either 2A-*musculus* or most probably some other Central-Asian form.

The karyotype analysis. The karyotypes analysed in this study contain exclusively acrocentric chromosomes (2n = NF = 40). The chromosomes form a series continuously decreasing in size. An exception is the Y chromosome of *M. hortulanus*, which has a dotted appearance and a length which is no more than half of that of autosome 19 (fig., a). As far as this can be judged from a pattern of metaphase chromosomes in squash preparations, the same dotted Y chromosome is typical for another aboriginal species, *M. spretus*[16]. We also found the reduced size of the Y chromosome in *M. abbotti* from Transcaucasus[17].



C-banded karyotype of male mice. a) *M. hortulanus*; b) *M. musculus*. Note the dotted appearance of the Y chromosome of *M. hortulanus* and the difference in C-band patterns of the X chromosomes of *M. musculus* and *M. hortulanus*.

Minor variations of the relative length of the Y chromosome in house mice are known[18,19]. This chromosome may be as long as autosome 18, or may be somewhat shorter than autosome 19. The Y chromosome of examples of *M. musculus* studied is comparable in size to the autosomes 18 and 19 (fig., b). Our studies, based on literature data and a large number of specimens of our

own, show that the frequency of occurrence of the dotted Y chromosome in the genus *Mus* is known today only in the aboriginal species *M. spretus*, *M. abbotti* and *M. hortulanus*.

Peculiarities of C-banding of the chromosomes of some forms of house mice may serve as a systematic character. The heterochromatin material of *M. hortulanus* is also concentrated in the pericentromeric regions of its chromosomes and distributed rather regularly in chromosomes. The C-band pattern did not show any characters marking this form, except in the X chromosome. This sign deserves special attention when cytogenetic analyses of house mice are being carried out.

Today, two variants of C-band pattern of the X chromosome are known for house mice. The C-band pattern in *M. domesticus* from various laboratory strains[19–21] is characterized by a bright pericentromeric block; the intensity of euchromatin staining is similar to that of autosomes. Another variant of the X-chromosome was found in *M. m. molossinus* from Japan[21] and later in *M. musculus* from the USSR[14]. The C-band in this variant is very small and stains dimly; after mild treatment with barium hydroxide, the staining of euchromatin is more intensive than in autosomes. The slight C-positive band is observed in the proximal part (fig., b). These variants of the X chromosome we have tentatively called the 'domesticus' and 'molossinus' types, respectively[14]. We have found the 'domesticus' type of X chromosome in *M. hortulanus* (fig., a) and *M. abbotti*[17].

According to our studies, the range of variation in size and distribution of the C-heterochromatin in the karyotype of *M. musculus* of the USSR fauna is very great, and obviously superior to such indices in *M. domesticus* from the natural population and laboratory strains. In this case the character of C-banding of the X chromosome in our samples turned out to be stable and of the 'molossinus' type. The uniform variant of the X chromosome observed by us in all specimens of *M. musculus* may indicate that these mice constitute a monophyletic group. These data agree well with the results of molecular investigation[22,23], according to which four subspecies of *M. domesticus* have the same variant of the Y chromosome, whereas *M. m. molossinus*, *M. m. castaneus* and *M. m. musculus* possess the other one. According to mtDNA results[24] *molossinus* and *musculus* are remarkably similar. It is noteworthy that the 'molossinus' type of the X chromosome was found by us both in synantropic and in supposedly wild forms of mice (wagneri and raddei from the south of Siberia), thus making it possible to assume that they are closer than is currently accepted. We assume that the 'domesticus' type may be a plesiomorphic variant of the X chromosome.

The results obtained allow us to conclude that not only biochemical characters but peculiarities of sex chromosomes as well are convenient markers for the diagnosis of some taxa in the subgenus *Mus*. The pattern of C-segmentation of the X chromosome may serve as a diagnostic criterion for elucidating the question of involvement of *M. domesticus* (e.g. *M. d. bactrianus*) in the formation of the fauna of house mice in the USSR. It may also help to solve the problem of the origin of some laboratory strains as well as to determine the boundaries of distribution of the forms *domesticus* and *musculus*. The described peculiarities of the Y chromosome in *M. spretus*, *M. hortulanus* and *M. abbotti* will help to outline distinctly the limits of their distribution and to differentiate between aboriginal and synantropic house mice.

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2 Sage, R. D., in: The Mouse in Biomedical Research, p. 39. Eds H. L. Foster, J. D. Small and J. G. Fox. Academic Press, New York 1981.
3 Thaler, L., Bonhomme, F., and Britton-Davidian, J., Symp. zool. Soc. Lond. *47* (1981) 27.
4 Bonhomme, F., Catalan, J., Gerasimov, S., Orsini, Ph., and Thaler, L., Z. Säugetierkunde *48* (1983) 78.
5 Hunt, W., and Selander, R., Heredity *51* (1973) 11.
6 Argiropulo, A. I., Fam. Muridae, Mice. Acad. Nauk SSSR, Moscow-Leningrad 1940, in Russian.
7 Marshall, J. T., and Sage, R. D., Symp. zool. Soc. Lond. *47* (1981) 15.
8 Sokolov, V. E., Kotenkova, E. V., and Lyalyhina, S. I., Dokl. Akad. Nauk SSSR *275* (1984) 1264, in Russian.
9 Bulatova, N. Sh., Orlov, V. N., Kotenkova, E. V., Dokl. Akad. Nauk SSSR *275* (1984) 758, in Russian.
10 Mezhzherin, S. V., Genetica *24* (1988) 2197, in Russian.
11 Bonhomme, F., Catalan, J., Britton-Davidian, J., Chapman, V. N., Moriwaki, K., Nevo, E., and Thaler, L., Biochem. Genet. *22* (1984) 275.
12 Sumner, A. T., Exp. Cell Res. *75* (1982) 304.
13 Selander, R. K., Smith, M. H., Jang, S. J., Johnson, W. E., and Gentry J. B., Studies in Genetics. Univ. Texas Publ. 7103 (1971) 49.
14 Yakimenko, L. V., in: Evolutionary Studies. Vavilov's Readings, p. 74. Ed. V. A. Krassilov. Vladivostok 1988, in Russian.
15 Frisman, L. V., in: Evolutionary Studies. Vavilov's Readings p. 94. Ed. V. A. Krassilov. Vladivostok 1988, in Russian.
16 Matthey, R., Rev. suisse Zool. *62* (1955) 163.
17 Yakimenko, L. V., Korobitsyna, K. V., Frisman, L. V., Vorontsov, N. N., in: Evolutionary and Genetical Investigations of Mammals. In Russian, in press.
18 Sofuni, T., and Makino, S., Chrom. Inf. Serv. *7* (1966) 15.
19 Yoshida, M. C., and Kodama, Y., Cytogenet. Cell Genet. *35* (1983) 51.
20 Dev, V. G., Miller, D. A., and Miller, O. J., Genetics *75* (1973) 663.
21 Dev, V. G., Miller, D. A., Tantravahi, R., Scheck, R. R., Roderick, T. H., Erlanger, B. F., and Miller, O. J., Chromosoma *53* (1975) 335.
22 Nishioka, Y., and Lamothe, E., Genetics *113* (1976) 417.
23 Tucker, P. K., Lee, B. K., and Eicher, E. M., Genetics *122* (1989) 169.
24 Ferris, S. D., Sage, R. D., Prager, E. M., Ritte, U., and Wilson, A. C., Genetics *105* (1983) 681.

0014-4754/90/101075-03$1.50 + 0.20/0