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ANIMAL GENETICS

Chromosome Variation in the Striped Field Mouse *Apodemus agrarius* (Rodentia, Muridae)

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Abstract—Chromosome sets of 114 *Apodemus agrarius* mice from 29 localities in Moldova, Ukraine, Siberia, and the Far East were studied by means of G-, C-, and NOR-banding. In all populations studied, the Y chromosome was shown to be a medium-size acrocentric chromosome consisting of heterochromatin. Chromosome polymorphism observed in populations from Primorskii krai concerned (1) the morphology of the first two autosome pairs (variants A/A, A/ST, and ST/ST), (2) the number of metacentric chromosomes (from 6 to 8), and (3) heterochromatin localization in the pericentromeric regions of two metacentric chromosome pairs. A karyotype with an additional heterochromatic microchromosome found in all the metaphases studied was described in one mouse from a locality of western Primorye that has not been studied previously. In the karyotype of 15 mice from four populations of Primorye, the pool of nucleolus organizer regions is distributed over three autosome pairs rather than over four, as is the case in *A. agrarius* from Europe. Based on the analysis of literature sources and our own data, the problem of chromosome polymorphism in the field mouse is discussed.

INTRODUCTION

The striped field mouse *Apodemus agrarius* Pall., 1778, is a wide-range Paleoarctic species that is spread throughout the territory from Central Europe to Far Eastern regions of Asia. The range consists of two isolated tracts of land: European–Siberian and Far Eastern–Chinese (Fig. 1). The subspecies system of this species is far from perfect and calls for revision [1].

Immunobiological studies on field mice from different parts of the species range allowed their differentiation into four immunological races: European, Altai, Khabarovsk, and Primorye, and raised the problem of their taxonomic status [2]. In the same work, an attempt was made to discover the karyological heterogeneity in the populations studied, using the methods of differential chromosome staining (G- and C-banding). However, no differences between immunological races of mice was found. An interesting fact is that three pairs of small metacentric chromosomes (*NF* = 54) were detected in the karyotypes of all mice, in contrast to the four pairs (*NF* = 56) described in the majority of papers (see Table 1). However, this species was not classified as promising for karyological studies. An analysis of literature sources and our own data indicates that this conclusion was erroneous.

In the material from China, the diploid set $2n = 50$ was described in the *A. agrarius* mice [3]. Subsequently, a more complete and accurate description of the karyotype was made by R. Matthey in 1936, based on material from eastern Europe [4], and by S. Makino, who used material from Taiwan ($2n = 48$, *NF* = 56) [5]. The karyotype of this species was studied in mice from various populations of Yugoslavia [6–10], Poland, Czechoslovakia [9], Romania [12, 13], the Caucasus [14], eastern Siberia [15], the Far East–Primorye [15, 16], China [5, 17], and Korea [5, 18]. As a rule, a stable number and morphology of chromosomes were recorded in all papers (Table 1). However, some mice from the populations of Yugoslavia [7] and Romanian Moldova [12] were characterized by six metacentric chromosomes, with a corresponding decrease in the number of arms (to 54). A total of ten metacentric chromosomes were described in the populations from Azerbaijan [19] and China [20]. The data on the size of the Y chromosome in *A. agrarius* are contradictory. In different papers, the Y chromosome is described as the smallest [2, 15, 16, 21], a medium [11, 22], and the largest [17] acrocentric chromosome in the chromosome set.

The objective of our study was to detect the cytogenetic heterogeneity of karyotypes of field mice from different parts of the species range: Europe, the Caucasus, Siberia, and the southern Far East. The goals of our study were the following: (1) to study the chromosomes of field mice from various geographic localities using the method of C-banding, which allows positive identification of sex chromosomes; (2) to study variation in the karyotype using differential G-, C-, and NOR-banding on chromosomes; and (3) to compare our results with published data.

MATERIALS AND METHODS

Chromosome sets of 114 *A. agrarius* mice from 29 localities of Moldova, Ukraine, Siberia, and the Far East were studied (Fig. 1; Table 2).

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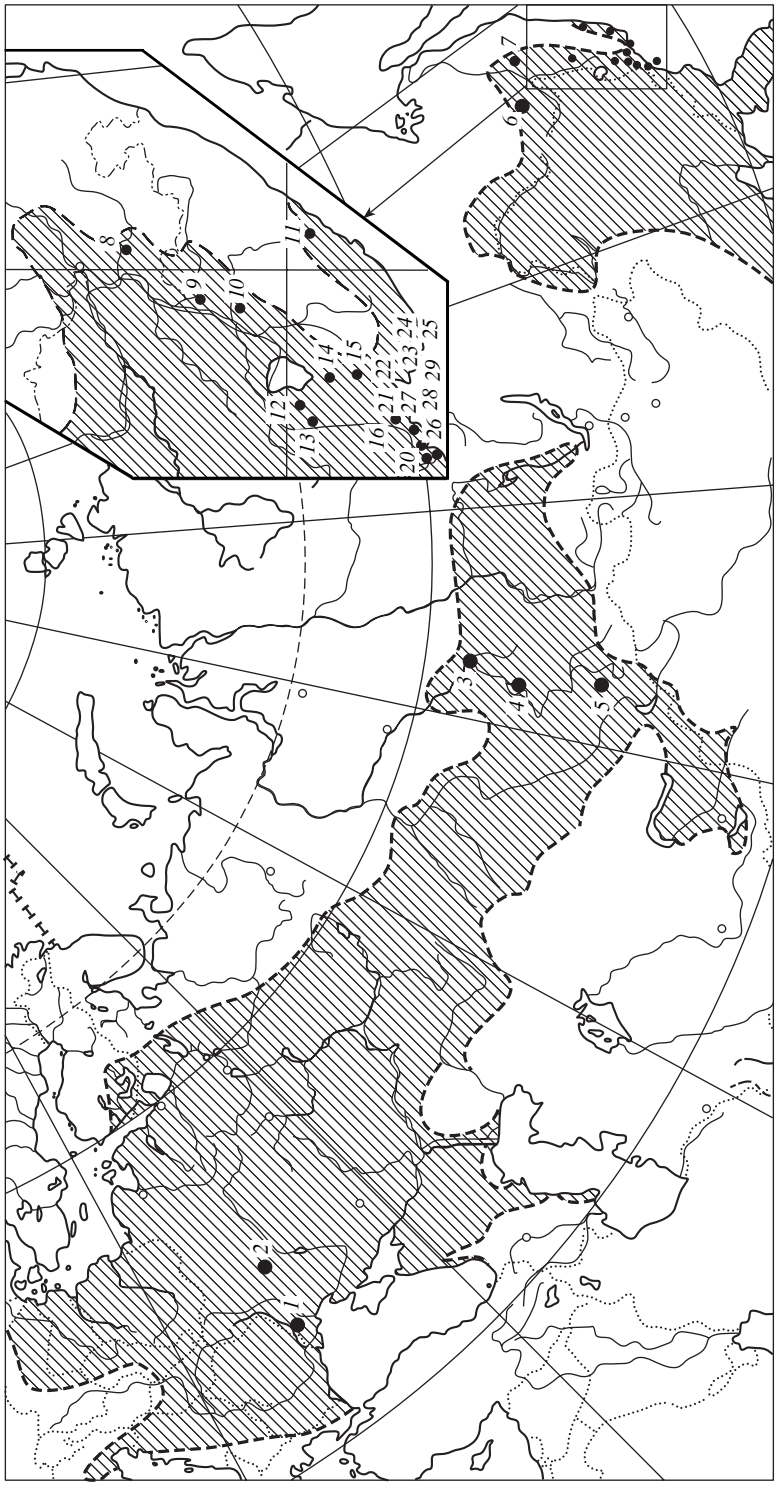

Fig. 1. The range of *A. agrarius* and location of sampling sites. Numbers of the latter correspond to those in Table 2.

Table 1. Characteristics of Karyotype in field mice *A. agrarius* from different localities

Subspecies and the locality of karyotype description	2*n*	*NF*	Number of M autosomes	Differential staining	Source
A. a. agrarius					
Western Europe	48	–	–	–	[4]
Hungary	48	56	8	–	[37]
Czechoslovakia and Poland	48	56	8	–	[8]
Moscow oblast	48	54	6	C, G	[2]
The same	48	56	8	NOR	[33]
Estonia	48	56	8	NOR	[33]
Ukraine	48	56	8	NOR	[33]
The same	48	56	8	C, G	Our data
Moldova	48	56	8	C, G	″
Romania	48	56–54	6–8	–	[12]
	48	56	8	C, G	[40]
A. a. kahmanni					
Yugoslavia	48	56	8	–	[6, 7, 15]
The same	48	56–54	8–6	–	[8, 9]
″	48	56	8	G	[11]
″	48	56	8	G	[21]
Greece	48	56	8	G	[41]
Balkan Peninsula	48	56	8	G	[42]
A. a. caucasicus					
Dagestan	48	56	8	NOR	[33]
North Ossetia	48	56	8	NOR	[33]
Azerbaijan	48	58	10	–	[19]
The same	48	56	8	C, NOR	[14]
″	48	56–55	8–7	C, NOR	[29]
Chechen-Ingush Republic	48	56	6	C, G	[2]
A. a. ognevi					
Altai	48	54	6	C, G	[2]
The same	48	56	8	C, G	Our data
Western Siberia:					
Tomsk, Novosibirsk	48	56	8	–	[15]
The same	48	56	8	G	[16, our data]
A. a. mantchuricus					
Amur oblast	48	56	8	C, G	Our data
Khabarovsk krai:					
Khabarovsk	48	54	6	C, G	[2]
Malyshevo	48 + 1B	56	8	C, G	[22]
Primorskii krai					
Ussuriisk	48	56	8	–	[15]
Ussuriisk, Vladivostok, Khasan raion, and Shkotovo raion	48	56	8	C, G	[16]
Slavyanka	48	54	6	C, G	[2]
	48	56	8	NOR	[33]
Vladivostok	48 + 1B	56	8	C	[22]
Primorskii krai, 22 localities*	48	56–54	8–6	C, G, NOR	Our data
A. a. pallidior					
China	48	56	8	G, C	[17]
A. a. ningpoensis	48	56	8	–	[3]
China	48	56	8	–	[5]
The same	48	56–58	8–10	–	[20]
Taiwan	48	56	56	–	[5]
A. a. coreae					
Korea	48	56	8	C, G	[18, 27, 28]

* See Tables 2 and 3 for a detailed description of sampling sites and characteristics of chromosomes.

Table 2. Sampling sites and characteristics of *A. agrarius* chromosomes

Sampling site and number*	Zoological number	Morphology of chromosome pair 1	Morphology of chromosome pair 2	Number of metacentric chromosomes
Moldova				
1. Lozovo	43-90	A/A	A/A	6
	41-90	A/A	A/A	6
	42-90	A/A	A/A	8
Ukraine				
2. Kiev	18-91	A/A	A/A	8
Siberia				
3. Tomsk	32-90	ST/ST	A/A	8
	34-90	A/A	A/A	8
4. Novosibirsk	19-90	A/A	A/A	8
	20-90	A/A	A/A	8
	73-94	A/A	A/A	8
	74-94	A/A	A/A	7
Altai				
5. Cherga	22-90	ST/ST	A/A	8
Jewish Autonomous Region				
6. Birakan	32-91	A/A	A/A	6
Khabarovsk krai				
7. Malyshevo	67-91	A/A	A/A	7
	70-91	A/A	A/A	8
	72-91	A/A	A/A	8 + 1B (micro)
Primorskii krai				
8. Krasnyi Yar	39-96	A/A	A/A	6
	41-96	A/A	A/A	7
	42-96	A/A	A/A	7
	43-96	A/A	A/A	8
9. Iman	71-91	A/A	A/A	8
10. Gornye Klyuchi	38-90	A/A	A/ST	8
	52-89	A/A	A/ST	8
11. Dal'negorsk	2-2	A/A	A/A	8
	11-3	A/ST	A/A	8
	13-2	ST/ST	A/A	8
	14-2	A/ST	A/A	8
	15-2	ST/ST	A/A	8
	16-2	A/A	A/A	6
	17-2	A/ST	A/A	8
	18-2	ST/ST	A/A	8
	20-2	ST/ST	A/A	8 + 2SM
	26-2	ST/ST	A/A	6
	44-2	ST/ST	A/A	8
	46-2	A/ST	A/A	8
	17-93	ST/ST	A/A	8
	18-93	ST/ST	A/A	7
	7-95	A/ST	A/A	8

Table 2. (Contd.)

Sampling site and number*	Zoological number	Morphology of chromosome pair 1	Morphology of chromosome pair 2	Number of metacentric chromosomes
12. Barabash-Levada	9-95	ST/ST	A/A	8
	7-98	A/A	A/A	8
	8-98	A/A	A/A	8
	9-98	A/A	A/A	8
	10-98	A/A	A/A	8
	12-98	A/A	A/A	8
	13-98	A/A	A/A	7
	14-98	A/ST	A/A	8
	20-98	A/A	A/A	8
	26-98	ST/ST	A/A	8
	30-98	A/ST	A/A	7
	33-98	A/A	A/A	6
13. Pogranichnyi	139-98	A/A	A/A	7
	140-98	A/A	A/A	8 + 1B (micro)
	141-98	A/ST	A/A	6
	142-98	A/A	A/A	8
	143-98	A/A	A/A	8
	144-98	ST/ST	A/A	6
14. Khorol’	7-95	A/ST	A/ST	8
	8-95	A/ST	A/ST	8
	10-95	A/ST	A/ST	8
15. Ussuriiskii Reserve	43-89	A/A	A/A	8
	5-92	A/A	A/A	6
	15-95	A/A	A/A	8
	16-95	A/A	A/A	8
	17-95	A/A	A/A	8
	28-95	A/A	A/A	6
	32-95	A/A	A/A	6
	38-95	A/A	A/A	8
	39-95	A/ST	A/A	6
	47-95	A/ST	A/A	8
	52-95	A/ST	A/A	8
	53-95	A/ST	A/A	8
	57-95	A/A	A/A	7
	26-96	A/A	A/A	8
	30-96	A/A	A/A	8
	41-96	A/A	A/A	8
16. Ryazanovka	49-90	A/ST	A/A	7
	48-90	A/ST	A/A	8
	47-90	ST/ST	A/A	8
	40-90	ST/ST	A/A	7

Table 2. (Contd.)

Sampling site and number*	Zoological number	Morphology of chromosome pair 1	Morphology of chromosome pair 2	Number of metacentric chromosomes
17. Kedrovaya Pad' Reserve	29-95	A/A	A/A	6
	30-95	A/A	A/A	6
	37-95	A/A	A/A	7
	40-95	A/A	A/A	6
	192-96	A/A	A/A	8
18. Zarubino	35-91	A/A	A/A	8
	26-91	A/A	A/A	8
	18-97	ST/ST	A/A	8
19. Kraskino	202	A/ST	A/A	8
	203	A/A	A/A	8
20. Khasan railway station	45-88	ST/ST	A/ST	8
21. Vladivostok	43-89	A/A	A/A	8 + 1B (acro)
	15-90	A/A	A/A	8
	14-90	A/ST	A/A	8
	17-90	A/A	A/A	8
	36-90	A/A	A/ST	8
22. Shkotovo	37-90	A/A	A/A	8
	39-90	A/A	A/ST	8
	19-91	A/A	ST/ST	8
23. Lazo	45-89	A/A	A/A	6
24. Novolitovsk	142-96	A/A	A/A	8
	148-96	A/A	A/A	7
	151-96	A/A	A/A	7
	152-96	A/A	A/A	8
	153-96	A/A	A/A	8
25. Nakhodka	45-90	A/A	A/A	6
	46-90	A/ST	A/ST	6
	44-90	A/ST	A/A	8
	24-90	A/ST	A/ST	7
26. Furuhelm Island	23-91	ST/ST	ST/ST	8
	24-91	ST/ST	A/A	8
27. Russkii Island	93-98	A/A	A/A	8
	94-98	A/A	A/A	8
	128-98	ST/ST	A/A	8
28. Popov Island	41-95	ST/ST	A/ST	8
29. Putyatin Island	25-92	A/ST	A/A	8

* Sampling sites are numbered as in Fig. 1.

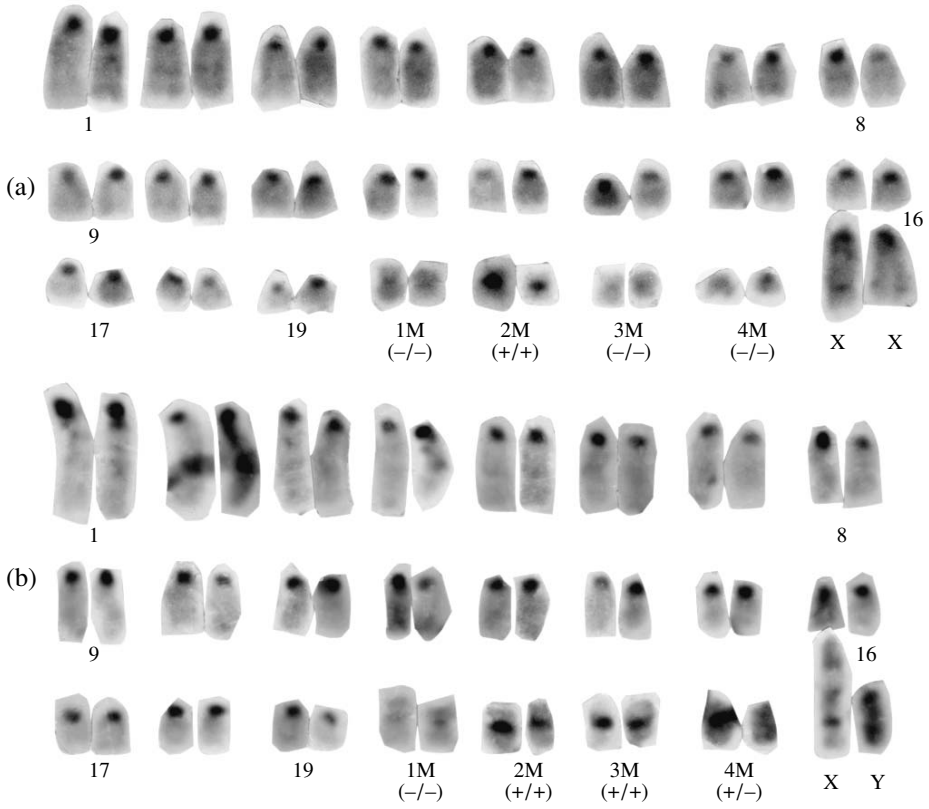

Fig. 2. Chromosomes of *A. agrarius* stained for structural heterochromatin: (a) a female from Khorol', Primorskii krai; and (b) a male from Malyshevo, Khabarovsk krai; (+/–) chromosome pair heteromorphic in the presence/absence of the C-band.

Preparations of chromosomes from bone marrow cells were made using a conventional technique [23], and differential G-, C-, and NOR-banding was performed by standard methods [24, 25, 26]. NOR-banding was only performed in chromosome preparations of 15 mice from four populations of Primorskii krai ($N = 12$, 13, 24, and 27). No less than 20 metaphases were analyzed for each mouse. Only preparations with similar degrees of chromosome coiling and a well-defined morphology of small biarmed autosomes were included in the analysis. In calculating *NF*, the number of small arms of subtelocentric chromosomes was not taken into consideration.

RESULTS

The karyotype of all the mice studied is represented by 48 acrocentric chromosomes that can be arranged in descending order according to size. In 3 of 114 mice studied, an additional chromosome (the B-chromosome) was found ($2n = 48 + 1B$). Karyotypes of two mice, each having an additional chromosome, were described earlier in the populations of Khabarovsk and Primorskii krais [22]. The third mouse, from the previously unstudied region of western Primorye (Table 2), is first reported in this work. The karyotype of this mouse, as well as that of the mouse from the Khabarovsk population, is characterized by one additional microchromosome in all metaphases studied.

Autosomes. Nineteen pairs of acrocentric (A) autosomes can be arranged in an order of decreasing size. In some populations, one to four large autosomes can be substituted by a corresponding number of subtelocentric structures (ST) with well-defined short arms (Figs. 2, 3). For example, in the populations of Primorskii krai, the first pair of chromosomes has two morphological variants: subtelocentric and acrocentric (Table 2). Variants A and ST were found in a homozygous state (A/A and ST/ST, respectively) with the frequencies of 0.576 and 0.192, respectively. The frequency of the heterozygous state was 0.232. As seen from Table 3, the peak frequency was detected in variant A (0.692). Variant ST prevailed in three localities: Dal'negorsk, Ryazanovka, and Furuhelm Island; moreover, this variant was found in a heterozygous state in single animals from two additional localities (Tables 2, 3). Both these variants (A and ST) have been detected in the second pair of chromosomes. The frequencies of variants A and ST in the homozygous and heterozygous states (A/A, ST/ST, and A/ST) were 0.869, 0.02, and 0.111, respectively. As seen from the frequency distribution of variants, the subtelocentric second chromosome has been found rarely (with a frequency of 0.075) and in only 8 out of 22 studied localities of Primorskii krai (Tables 2, 3).

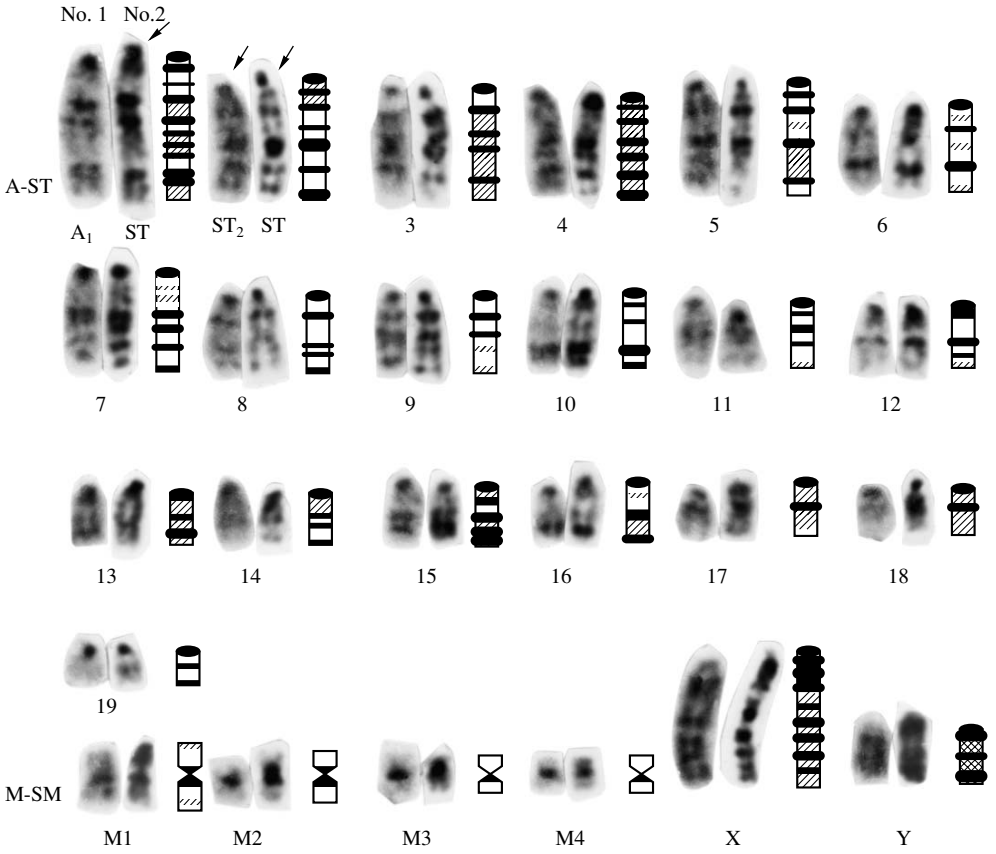

Fig. 3. Chromosome G-banding in two male *A. agrarius*. The first and second homeologs of each pair (nos. 1 and 2) belong to mice from southern Primorye (Ussuriiskii Reserve) and northern Primorye (the city of Dal'negorsk), respectively. A, ST, and M are acrocentric, subtelocentric, and metacentric chromosomes, respectively. Arrows indicate subtelocentric chromosomes.

As a rule, biarmed autosomes were represented by eight and, occasionally, six small metacentric (M) chromosomes. The karyotype with eight M chromosomes was found in all populations studied; mice with six M chromosomes were found in a Moldovan population and in two populations of Primorye (Table 2, 3). We found that 69, 16.5, and 14.5% of mice from the populations of Primorye had 8, 6, and 7 M chromosomes, respectively. Thus, eight metacentrics were found in the majority of mice studied. Variation in the number of M chromosomes is likely caused by pericentric inversion in the fourth M-chromosome pair. Moreover, this inversion may result in the occurrence of both subtelocentric and acrocentric chromosomes. As a rule, the fourth chromosome pair was metacentric and was found in a homozygous state (M/M) with the frequency of 0.707 in the *A. agrarius* populations of Primorye. Other variants (M/ST, ST/ST, A/A, and M/A) were rarer, and their frequencies were 0.081, 0.111, 0.081, and 0.020, respectively (Table 2). Thus, 8, 6, and 7 M chromosomes (variants M/M; ST/ST and A/A; and M/ST and M/A) were found in 70.7, 19.2 and 10.1% of mice, respectively. The maximum frequency was detected for the M variant (0.758). Furthermore, we studied three sibling mice from the Nakhodka population, Primorskii krai (no. 25). These three mice differed from one another in the number of metacentric chromosomes (6, 7, and 8; variants A/A, ST/M, and M/M, respectively). Unfortunately, the karyotypes of the parents have not been studied.

Differential Staining of Chromosomes

C-Banding. Staining for structural heterochromatin demonstrated that all acrocentric chromosomes had pericentromeric C-bands. In all cases, the short arms of subtelocentric elements consisted of brightly stained heterochromatin. In 3 out of the 15 mice studied in the Dal'negorsk population of Primorskii krai, short heterochromatin arms were detected in two small autosomes, in addition to those on large autosomes, and the number of small biarmed chromosomes increased to ten (Table 2).

The study of structural heterochromatin of M chromosomes revealed that the pattern of C-banding on the first two metacentric pairs was similar in all the mice studied, whereas this pattern on two other pairs was variable. Thus, the first pair of M chromosomes demonstrated a weak diffuse banding throughout its length, with a slightly stained pericentromeric region; the sec-

Table 3. Frequency of variants of three autosome pairs in *A. agrarius* populations from Primorskii krai

Site no.*	Sample size	Morphology of chromosomes																	
		pair 1					pair 2					metacentric pair 4							
		A/A	ST/ST	A/ST	frequency of variants		A/A	ST/ST	A/ST	frequency of variants		A/A	ST/ST	M/M	M/A	M/ST	frequency of variants		
					A	ST				A	ST						A	ST	M
8	4	4	0	0	1.000	0.000	4	0	0	1.000	0.000	0	1	1	0	2	0.000	0.500	0.500
9	1	1	0	0	1.000	0.000	1	0	0	1.000	0.000	0	0	1	0	0	0.000	0.000	1.000
10	2	2	0	0	1.000	0.000	0	0	2	0.500	0.500	0	0	2	0	0	0.000	0.000	1.000
11	15	2	8	5	0.300	0.700	15	0	0	1.000	0.000	0	2	12	0	1	0.000	0.167	0.833
12	12	8	2	2	0.750	0.250	12	0	0	1.000	0.000	1	0	9	1	1	0.125	0.042	0.833
13	6	4	1	1	0.750	0.250	6	0	0	1.000	0.000	1	1	3	0	1	0.167	0.250	0.583
14	3	0	0	3	0.500	0.500	0	0	3	0.500	0.500	0	0	3	0	0	0.000	0.000	1.000
15	16	12	0	4	0.875	0.125	16	0	0	1.000	0.000	2	2	11	0	1	0.124	0.156	0.720
16	4	0	2	2	0.250	0.750	4	0	0	1.000	0.000	1	0	2	1	0	0.375	0.000	0.625
17	5	5	0	0	1.000	0.000	5	0	0	1.000	0.000	0	3	1	0	1	0.000	0.700	0.300
18	3	2	1	0	0.667	0.333	3	0	0	1.000	0.000	0	0	3	0	0	0.000	0.000	1.000
19	2	1	0	1	0.750	0.250	2	0	0	1.000	0.000	0	0	2	0	0	0.000	0.000	1.000
20	1	0	1	0	0.000	1.000	0	0	1	0.500	0.500	0	0	1	0	0	0.000	0.000	1.000
21	5	4	0	1	0.900	0.100	4	0	1	0.900	0.100	0	0	5	0	0	0.000	0.000	1.000
22	3	3	0	0	1.000	0.000	1	1	1	0.500	0.500	0	0	3	0	0	0.000	0.000	1.000
23	1	1	0	0	1.000	0.000	1	0	0	1.000	0.000	1	0	0	0	0	1.000	0.000	0.000
24	5	5	0	0	1.000	0.000	5	0	0	1.000	0.000	2	0	3	0	1	0.333	0.084	0.583
25	4	1	0	3	0.625	0.375	2	0	2	0.750	0.250	0	2	1	0	0	0.000	0.667	0.333
26	2	0	2	0	0.000	1.000	1	1	0	0.500	0.500	0	0	2	0	0	0.000	0.000	1.000
27	3	2	1	0	0.667	0.333	3	0	0	1.000	0.000	0	0	3	0	0	0.000	0.000	1.000
28	1	0	1	0	0.000	1.000	0	0	1	0.500	0.500	0	0	1	0	0	0.000	0.000	1.000
29	1	0	0	1	0.500	0.500	1	0	0	1.000	0.000	0	0	1	0	0	0.000	0.000	1.000
$N = 22$	99	57	19	23	0.692	0.308	86	2	11	0.925	0.075	8	11	70	2	8	0.090	0.152	0.758

* For names of sampling sites, see Table 2.

ond pair demonstrated a bright C-band in the pericentromeric region (Fig. 2a). The remaining two pairs were variable with respect to the content and localization of C-bands in the pericentromeric regions (Figs. 2a, 2b). The third pair of M chromosomes with a bright C-band was found in mice from Moldova, Khabarovsk krai, and Primorskii krai; this chromosome was without the C-band in mice from Siberia and Altai. The fourth pair of M chromosomes with a bright C-band was only detected in the karyotype of mice from Malyshevo (Khabarovsk krai). In one of these two mice, however, this pair was heteromorphic both for the morphology (M/ST) and localization of heterochromatin (+/–) (Fig. 2b). The subtelocentric chromosome contained no C-band. Probably, the pericentric inversion was followed by the loss of heterochromatin.

G-Banding. Differential staining revealed that all chromosomes of field mice from Europe, Siberia, Altai, and the Far East have a clear pattern of G-banding and can be well distinguished and classified into pairs. G-banded chromosomes of mice from two population of Primorskii krai (Ussuriisk and Dal'negorsk) are shown in Fig. 3. Undoubtedly, the pattern of G-banding of one mouse coincides with that of another in such a manner, as the chromosomes were of the same metaphase plate. However, the chromosomes of pairs 1 and 18 are subtelocentric. The banding pattern of autosomes agrees with the data on European [11, 21] and Far Eastern [16] populations that have been published earlier.

Nucleolus Organizer Regions (NORs). In 15 field mice from three populations of Primorye, NORs were localized on two large acrocentric autosome pairs (within a telomeric region) and on a small acrocentric autosome (within its centromeric region). Moreover, NORs may be found in a single homolog of a pair; i.e., they may be detected in five or four chromosomes. The

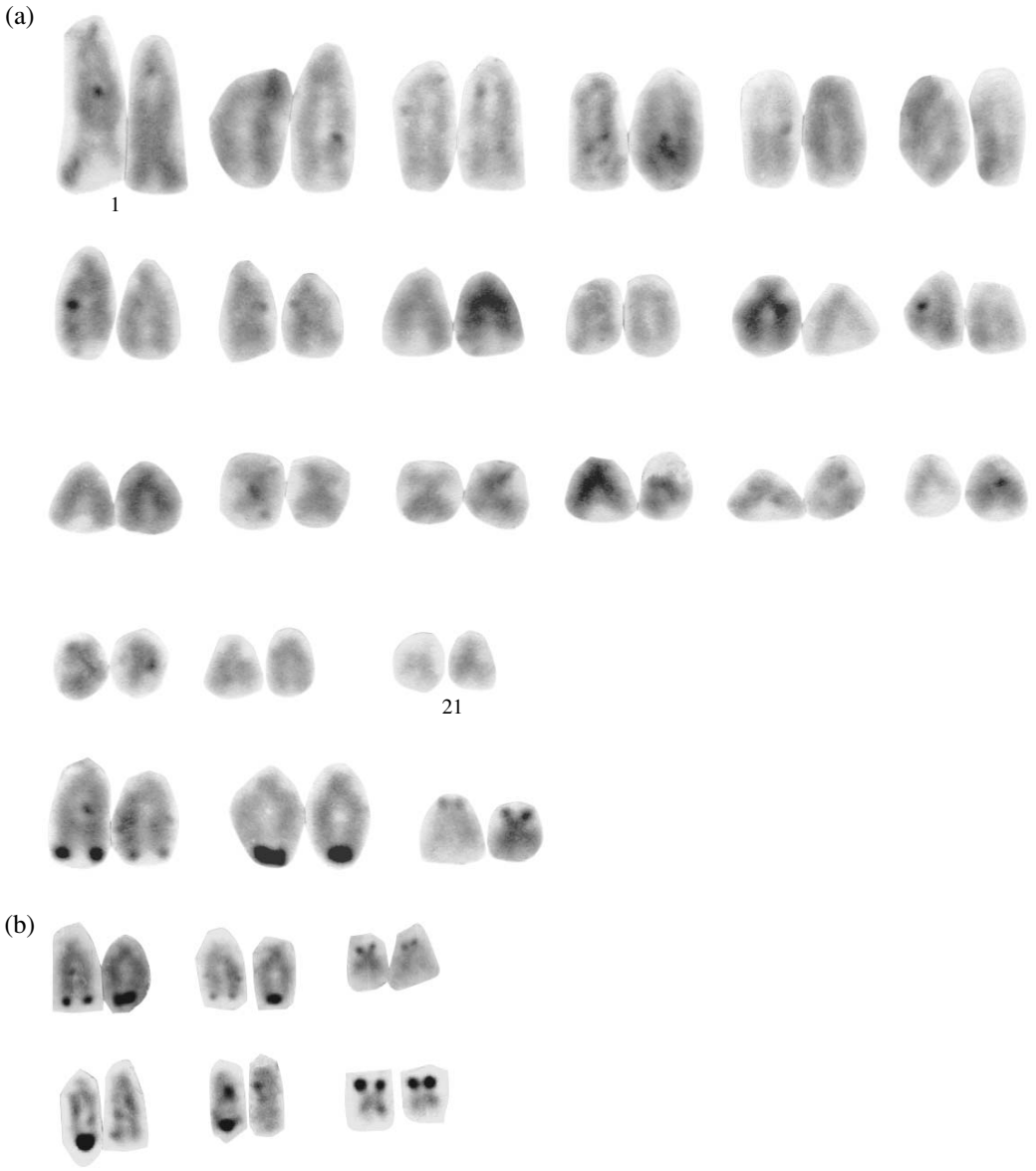

Fig. 4. Chromosome NOR-banding in *A. agrarius*: (a) karyotype of a female (no. 151-96, Novolitovsk, Primorskii krai) and (b) different NOR-banding patterns of homologous chromosomes (no. 94-98, Russkii Island).

density of Ag-banding proved to be variable (Figs. 4a, 4b), which may be explained by the differential activity of ribosomal gene clusters in homologs.

Sex Chromosomes. Sex chromosomes have been precisely identified by staining for structural heterochromatin (Fig. 3).

The Y chromosome is a medium-size acrocentric characterized by a dense C-banding over its entire length with two bright bands, one localized within the pericentromeric region and the other interstitial.

The size of the acrocentric X chromosome is comparable to that of the first autosome pair in all populations studied. By staining for structural heterochromatin, two C-bands were detected: a large pericentromeric band that occupies almost one-fourth of the chromosome and a bright interstitial band that is localized within a lower part of the arm (Fig. 2). Such a banding pattern of the X chromosome permits its use as a marker. In G-banding, the X chromosome is characterized by three bright bands located at the center of the chromosome with an approximately equal space between them and two bright bands located close to one another in the upper part of the arm (Fig. 3).

DISCUSSION

In the genus *Apodemus*, additional chromosomes (B chromosomes) have been described in five species: *A. flavicollis* [7, 13, 37, 38], *A. sylvaticus* [38], *A. argenteus* [39], *A. agrarius* [22], and *A. peninsulae* (= *A. gi-*

liacius [34] = *A. speciosus* [15]) [35, 36]. As a rule, these structures are represented by acrocentric chromosomes and, in the latter species, metacentric ones. The B chromosomes found by us in the field mice from the Far Eastern populations were classified as microchromosomes in two out of three cases. Interestingly, the additional microchromosomes have previously been described only in *A. peninsulae*. In spite of the fact that a large number of mice from various localities were studied in the European part of the *A. agrarius* range (Table 1), B chromosomes have been found in Far Eastern populations only. The frequency of mice with B chromosomes was 2.59. This result is close to the frequency of 2.4 obtained for the European species of wood mice (*A. sylvaticus*) [40].

Variation in the number of small metacentric chromosomes (from six to eight) has been described in Yugoslavia and Romania, as we mentioned above (Table 1). Eight metacentrics were described as characteristic of the species, and six, as a rare variant. However, in one paper, six M chromosomes were described in mice from different populations inhabiting the territory of the former Soviet Union, while the karyotype presented by the authors contained eight M chromosomes [2]. This suggests that the authors studied both mice with six and mice with eight M chromosomes. Our studies revealed variation in the number of metacentrics in the *A. agrarius* karyotype. As a rule, the chromosome set includes four pairs of M chromosomes. A decrease in the number of M chromosomes to seven or six is apparently accompanied by pericentric inversion. Alternatively, an increase in the number of small biarmed chromosomes up to ten, as observed in a population from Primorskii krai, is probably caused by duplication of heterochromatin localized within the centromeric regions of acrocentric chromosomes, which formed a second arm. The same number of M chromosomes was described earlier in field mice from China [20] and Azerbaijan [19]. The study of three sibling mice from the population of Primorskii krai (no. 25), where three variants of the fourth metacentric chromosome pair (A/A, ST/M, and M/M) were found, suggests that variation in the morphology of this chromosome cannot serve as a differentiating character.

Variation in the number of large subtelocentric chromosomes (the first and second chromosome pairs, variant ST/A) was detected earlier in field mice from the Korean population [27, 28] and in some individual mice from Azerbaijan [29]. The latter authors explain the occurrence of short arms by an increase in the amount of heterochromatin within the pericentromeric region of a large acrocentric chromosome. Probably, such structures occurred in mice from other populations studied earlier but were, for some reason, classified as acrocentric chromosomes. Our studies revealed that variation in the morphology of the first two chromosomes pairs is typical of field mice from the Far East. The first pair is mainly subtelocentric; the second, acrocentric. In all studied mice, short arms of subtelocentric chromosomes are formed from brightly stained heterochromatin, as in mice from Azerbaijan. Although the variation of heteromatin in mice of the genus *Apodemus* is regarded as important for intra- and interspecies diagnosis [4], we are inclined to explain variation in the amount of heterochromatin in the short arms of *A. agrarius* by the functional state of the organism, as has been done earlier in other animal species [42, 43].

Remarkably, such a trait as the number and localization of NORs is a differentiating character in 11 species of the genus *Apodemus* [33]. In field mice from the populations of Estonia, Russia, Ukraine, Dagestan, North Ossetia [33], and Azerbaijan [29], NORs are localized on eight chromosomes: telomeric NORs are localized on four large acrocentric autosomes, and pericentromeric NORs, on four small acrocentric autosomes. Only mice from Saaremaa Island (Estonia) lack two of the aforementioned NORs. The authors [33] believe that this probably indicates the ancient geographical isolation of this population and the initial stages of its karyological divergence from continental populations. In all studied mice from both Primorskii krai and Saaremaa Island, NORs are localized on six chromosomes, which sets them apart from European and Caucasian populations. This case requires us to recall that the range of this species consists of two separate parts differing in the distribution of NORs: European–Siberian and Far-Eastern–Chinese.

The staining of chromosome preparations for structural heterochromatin allowed us to distinguish sex chromosomes. The morphology and size of the X chromosome, as a rule, are unquestioned. The obtained pattern of structural heterochromatin proved to differ from the published data on X chromosome C-banding. Thus, the pericentromeric C-band of the X chromosome was detected by all authors who had conducted differential staining, whereas interstitial C-bands have only been detected in mice from China [17] and Azerbaijan [29]. We do not exclude the possibility of polymorphism for the number and localization of heterochromatin within an interstitial region of the X chromosome in mice from different localities. Nevertheless, we found that differences in the treatment of chromosome preparations in an alkaline solution may result in the loss of C-banding in the X chromosome, whereas other chromosomes retain their C-banding pattern. The localization of the C-band within an interstitial region of the X chromosome may depend even on slight changes in the technique of staining.

The Y chromosome was a medium-size acrocentric in all studied males from both European and Far Eastern populations. The description of the Y chromosome as the smallest acrocentric chromosome of the chromosome set has been presented by authors who did not perform staining for structural heterochromatin. Thus, such a description of the Y chromosome of field mice from Primorskii krai is likely to be erroneous, because the Y chromosome has a definite medium size when

examined using C-banding [16]. Probably, a similar situation occurred in the work on identification of sex chromosomes in Chinese mice [17]: if one reverses the positions of the X and Y chromosomes, the situation will be similar to our description. An erroneous identification of the Y chromosome cannot be excluded if chromosome banding is not performed. We have already experienced such difficulties in the identification of the Y chromosome. For instance, variation in the morphology of the Y chromosome was found in the populations of the midday gerbil *Meriones meridianus* from the right and left banks of the Volga River. These populations differ from one another by a factor of 1000 in their resistance to the plague pathogen [30]. After staining for structural heterochromatin, it became clear that karyotypic variation is caused by the occurrence of a heterochromatin arm on the 11th autosome pair, rather than by the variation of the Y chromosome [31]. However, we are not sure that the size of the Y chromosome in *A. agrarius* undergoes no variation, because polymorphism for the size of sex chromosomes is typical of many rodent species (see [32] and other references).

Thus, we can conclude that the populations of field mice from Europe, Siberia, and the Far East (the populations of Khabarovsk and Primorye) differ in the number of biarmed chromosomes as well as in the number and localization of heterochromatin within pericentromeric regions of biarmed chromosomes. Populations from the European part of the species range differ from Asian populations in the number of NORs. The variation of heterochromatin in the short arms of acrocentric chromosomes, which has only been detected in the populations of Transcaucasia and Primorskii krai, is highly heterogeneous in the populations of Primorye; therefore, we do not classify this trait as differentiating.

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