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ANIMAL GENETICS

Variability in Size of the Nuclear Genome in Pygmy Wood Mouse *Sylvaemus uralensis* (Rodentia, Muridae)

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Abstract—Earlier, in an integral genetic study, the Asian and European races were distinguished within the species *Sylvaemus uralensis* (pygmy wood mouse) and the European race was divided into the East European and South European forms. Each of these groups differed from the others, in particular, in the quantity of the centromeric heterochromatin in karyotypes of the animals. To establish the pattern of its changes in *S. uralensis,* in the present study the DNA content in splenocyte nuclei in all races and forms of pygmy wood mice was assessed using DNA flow cytometry. The heterochromatin amount in karyotypes and genome size were shown to be correlated. The East European chromosomal race of *S. uralensis* (Central Chernozem and Non-Chernozem regions of Russia, Crimea Peninsula, Middle Volga region, and Southern Ural) and the Asian race of this species (East Kazakhstan, Uzbekistan, and East Turkmenistan), which have respectively the highest and the lowest amounts of centromeric heterochromatin in the karyotype, exhibit the greatest difference in the DNA content in the genome. On average, the difference is approximately 8% in males and 6.7% in females; in both cases, the ranges of variability were distinctly different. Against the general background of the trait variation, the Asian race, whose members have the smallest DNA amount in their cells, looks homogeneous. The genome of the South European chromosomal form of *S. uralensis* (Caucasus, Transcaucasia, Carpathians, and Balkan Peninsula), which exhibits an intermediate content of the centromeric heterochromatin in the karyotype, is smaller that the genome of the East European race (by 3.2% in the group of males and by 1.9%, in the group of females), but larger than that of the Asian race (by 5% in either sex). Thus, the variability of size of centromeric C-blocks in pygmy wood mouse is likely to be associated with elimination (or, conversely, an increase in the amount) of the genetically inert chromatin. It is suggested that a significant contribution to the variability of genome size in *S. uralensis* is made by heterochromosomes, or, more precisely, their variable regions, which seem to be largely heterochromatic.

INTRODUCTION

The species *Sylvaemus uralensis* (syn.—*S microps,* pygmy wood mouse) is genetically heterogeneous. In particular, the heterochromatic part of its genome displays wide variability, while the number of chromosomes and their morphology are stable ($2n$ = 48, all chromosomes have a single arm).

Analysis of heterochromatin variation in *S. uralensis* is complicated by irregularity of its manifestation, which is observed even in different metaphase plates of one individual with the use of standard methods of preparing suspensions and slides [1] and C-banding [2]. Apparently, its low repeatability is explained by some specific chemical and structural features of heterochromatic segments and (or) their different functional state in different bone marrow cells. This complication can be overcome by selecting on each slide several metaphase plates with best manifestation and the maximum number of C-blocks [3].

The views on the character of *S. uralensis* differentiation in the heterochromatin amount in animal genomes are controversial. The disputable issues concern subdivision of the C-band variability range in this species, namely, how many forms should be distinguished and what traits should be taken into account or ignored because of their high population variability [3–7]. The taxonomic significance of differences in this character has also been under debate: Orlov *et al.* [4, 5] have suggested that the chromosomal forms of pygmy wood mouse be regarded as independent species, but this view was not supported by other researchers [3, 6–9].

One of the authors of the present work has earlier described three chromosomal forms of *S. uralensis* on the basis of analysis of his own and literature data on variation in size and the number of centromeric C-blocks in the pygmy wood mouse karyotypes [3]. Owing to a wide range and relatively low precision of the C-banding technique, the insignificant fluctuations of the character, which "disappeared" against the general variation background when increasing the sample size or adding material from related populations, was not though to have a diagnostic value. The identified

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chromosomal forms were named in approximate accordance to their geographic distribution.

Individuals of the East European chromosomal race of *S. uralensis*, inhabiting Central Chernozem and Non-Chernozem regions of Russia, Crimea Peninsula, Middle Volga region, and South Ural show the highest heterochromatin content in karyotypes: the great majority of chromosomes in these animals have large centromeric C-blocks. In pygmy wood mice of the South European form, which occurs in the Caucasus, Transcaucasia, the Carpathians, and Balkan Peninsula and was erroneously attributed to a separate species *S. ciscaucasicus* [4, 5, 10–12], the heterochromatin content in karyotypes is lower: almost all average-sized and small chromosomes lack large centromeric C-bands, which, nevertheless, are regularly found in seven to nine longest chromosome pairs ([4–6, 10, 12], unpublished data of the authors). This chromosome form is very similar to the previous one. In animals of the Asian form of *S. uralensis*, described on the basis of the material from eastern Kazakhstan regions, Uzbekistan, and East Turkmenistan, heterochromatic segments of all chromosomes are dim, diffused, have medium size, and very irregularly distributed. By contrast, the number of chromosomes with telomeric C-blocks, which are also characterized by inconsistent staining, and their properties lack substantial intraspecific variability [3]. The distribution boundaries of each of the *S. uralensis* chromosome forms are still unclear.

The Asian chromosome forms was shown to be somewhat different from the other forms in specific localization of nucleolar-organizing regions in animal karyotypes [3] and in the allele frequencies of several polymorphic proteins [9]. On the other hand, the differences between the East European and Asian chromosome forms in the RAPD–PCR DNA patterns are similar to the interspecific ones [13]. The patterns of *S. uralensis* differentiation in all characters were the same. Nevertheless, according to a comprehensive allozyme study, genetic distances among the chromosome forms of *S. uralensis* are about an order of magnitude lower than the distances between "good" *Sylvaemus* species. Thus, to date chromosome forms of *S. uralensis* cannot be regarded as isolated species. Since the Asian chromosome form differs from the two other forms in several genetic (and not only karyological) features, it was suggested to term it as race. The "alternative," European race included the East European and South European forms, which are similar to each other [9].

The character of changes that have occurred in the centromeric heterochromatin of *S. uralensis* is still unknown. Theoretically, two explanations are possible. On the one hand, the quantitative variation in heterochromatin may be explained by a change in its state and partial or complete decondensation (or, conversely, condensation), which is supported by irregular appearance of C-blocks in the same animal; on the other hand, it can be explained by elimination (or, conversely, accumulation) of genetically inert chromosomal material. If the second hypothesis is true, then an increase or a reduction of C-segments must be accompanied by a corresponding change in the total DNA amount in the genome, which can be easily checked by cytometry. In this case, heterochromatin variations can be precisely assessed quantitatively, which is virtually impossible by karyological analysis.

To choose between these two hypotheses, we have estimated the DNA content in cell nuclei in all races and forms of pygmy wood mice, using DNA flow cytometry. The same chromosome sets and their morphological similarity in all *S. uralensis* individuals, as well as a lack of drastic fluctuations of the number and size of nucleolar-organizing regions, suggest that the genome size changes in this species primarily result from variation of polymorphic heterochromatic regions.

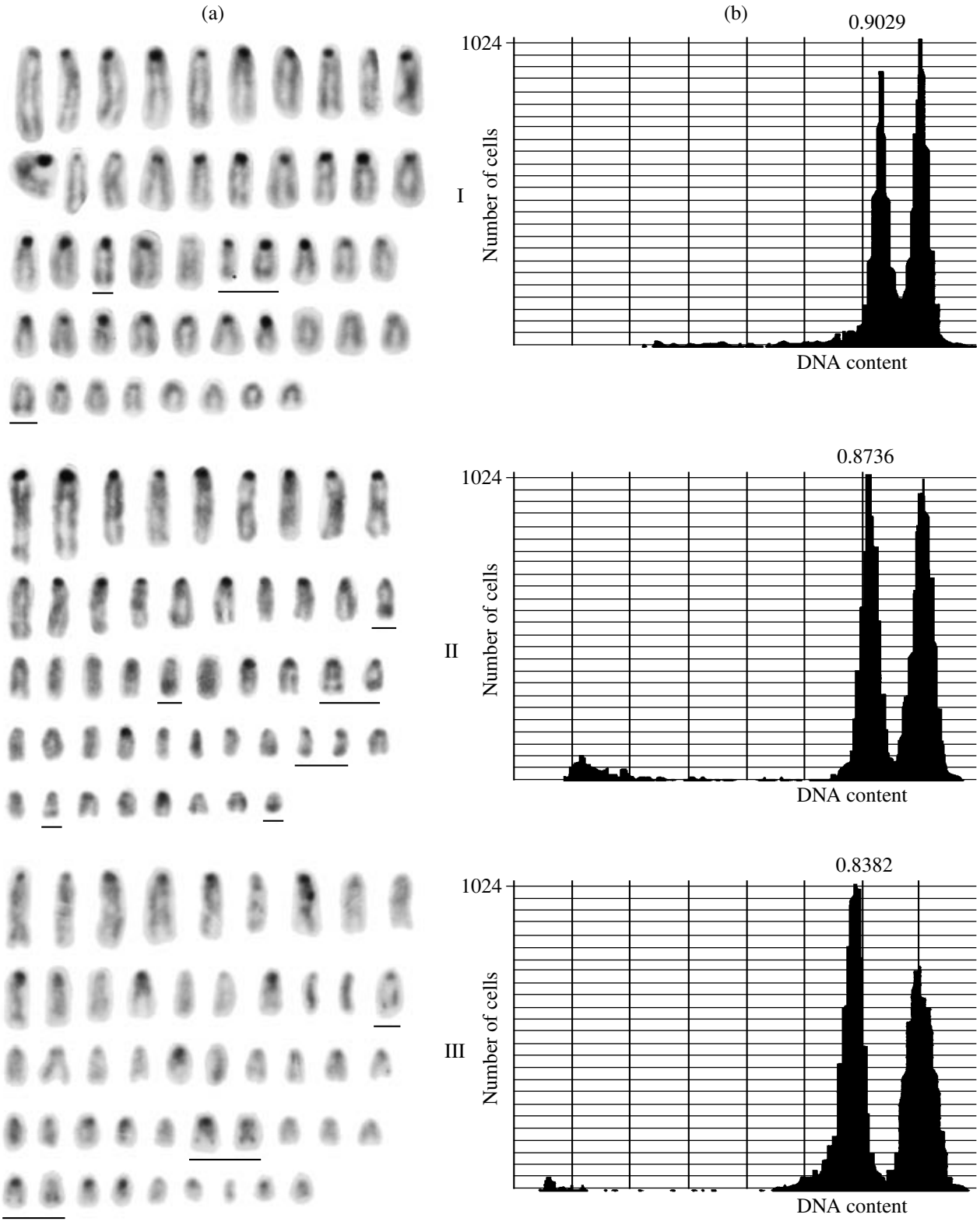
MATERIALS AND METHODS

Below are given the sampling localities of pygmy wood mice, the number of mice examined, and their chromosome form or race attribution, preliminarily done by C-banding.

European Race of S. uralensis

East European chromosome form. 1. Kostroma oblast, about 15 km north of the city of Kostroma (2♀♀). 2. Ivanovo oblast, Pestyakovskii district, near the settlement of Demidovo (1♂, 1♀). Moscow oblast, Zaraiskii district, the town of Zaraisk (1♂, 1♀). 4. Ryazan' oblast, Saraevskii district, near the settlement of Alekseevka (3♂♂, 3♀♀). 5. Tambov oblast, Mordovskii district, near the settlement of Mordovo (1♂, 1♀). 6. The city of Cheboksary (2♂♂, 3♀♀). 7. Near the city of Kursk, the Central Chernozem Natural Reserve (1♀). 8. Belgorod oblast, Borisovskii district, near the settle-

Karyotypes, C-banding (a), and DNA histograms for splenocytes (b) of pygmy wood mice of the East European (I), South European (II) chromosome forms and the Asian race (III). For each chromosome form/race, a karyogram and a histogram of the same animal are presented. East European chromosome form: ♂, Orenburg oblast, Kuvandykskii district, near the settlement of Kancherovo; South European chromosome form: ♂, Kabardino-Balkaria, outskirts of the city of Nal'chik; Asian race: ♂. Kazakhstan, Taldykurgan oblast, Gvardeiskii district, 10 km northeast of the settlement of Rudnichnyi. The metaphase plates with the best manifestation of the chromatin were selected for the demonstration. Chromosomes are presented singly, in order of decreasing size, since the fairly homogeneous pattern of C-banding did not allow us to identify homologs and heterochromosomes. Occasionally appearing chromosomes with telomeric C-block are underlined. The right peak on all histograms is formed by signals from guinea pig cell nuclei; the left peak, by signals from pygmy wood mice cell nuclei. The genome size in arbitrary units, corresponding to the given *S. uralensis* specimen is given above the left peak (relative to the DNA content in the guinea pig splenocyte nuclei).


(a)
(b)
0.9029
1024
I
Number of cells
DNA content
0.8736
1024
II
Number of cells
DNA content
0.8382
1024
III
Number of cells
DNA content

DNA content in splenocyte nuclei of pygmy wood mice (relative to the DNA content in splenocyte nuclei of guinea pigs)

Race	Chromosome form	Sampling locality	DNA content in splenocyte nuclei			
			of individual animals (♂♂)	on average in males of a chromosome form/race	of individual animals (♀♀)	on average in females of a chromosome form/race
E	EE	1			0.9147	
					0.9124	
		2	0.9001		0.9078	
		3	0.8915		0.9027	
		4	0.9113		0.9057	
			0.9098		0.9006	
			0.9006		0.8994	
		5	0.9064		0.9154	
		6	0.9142		0.9237	
			0.9080	0.9023 ± 0.00248	0.9005	0.9097 ± 0.00193
					0.9003	
		7		C_v = 1.17%	0.9267	C_v = 0.87%
		8	0.8787			
		9	0.9090			
		10	0.9245			
			0.9023		0.9123	
			0.8975			
		11	0.8897			
		12	0.9036		0.9120	
			0.9029		0.9119	
			0.9018		0.9115	
			0.8898		0.9068	
	SE	13	0.8808			
		14			0.9118	
				0.8736 ± 0.00271	0.9034	0.8928 ± 0.00602
		15	0.8776		0.8991	
			0.8749	C_v = 0.76%	0.8893	C_v = 1.65%
			0.8736		0.8811	
			0.8734		0.8722	
			0.8613			
A		16	0.8342			
		17	0.8455			
		18	0.8345		0.8611	
		19	0.8371		0.8575	
			0.8288	0.8301 ± 0.00308	0.8405	0.8486 ± 0.00339
		20	0.8382		0.8624	
				C_v = 1.23%	0.8545	C_v = 1.20%
					0.8425	
		21	0.8367			
		22	0.8144			
		23	0.8264		0.8428	
			0.8217		0.8401	
			0.8134		0.8361	

Table (Contd.)

Race	Chromosome form	Sampling locality	Character of differences in DNA content in splenocyte nuclei		
			between males and females of a certain chromosome form/race	between the East European, the South European chromosome forms and the Asian race of *S. uralensis*; the calculations were made separately for	
				males	females
E	EE	1–12	0.8% The transgression area covers half of the variation range in two given samples (Tr = 52.3%). Between-sample differences are statistically significant: $t_f = 2.35$; $t_{st\ 5\%} < t_f < t_{st\ 1\%}$; $0.01 < P < 0.05$	3.2% Tr = 3.3% Between-sample differences are statistically significant: $t_f = 7.8$; $t_f > t_{st\ 0.1\%}$, $P < 0.001$ 8.0% No transgression. Between-sample differences are statistically significant: $W_f = 66$; $W_f < W_{l\ 1\%}$, $P < 0.01$	1.9% Tr = 22.8% Between-sample differences are statistically significant: $W_f = 35$; $W_f < W_{l\ 1\%}$, $P < 0.01$ 6.7% No transgression. Between-sample differences are statistically significant: $W_f = 45$; $W_f < W_{l\ 1\%}$, $P < 0.01$
	SE	13–15	2.2% Tr = 17.0%. Between-sample differences are statistically significant: $W_f = 26$; $W_{l\ 1\%} < W_f < W_{l\ 5\%}$, $0.01 < P < 0.05$		
A		16–23	2.2% Tr = 19.2%. Between-sample differences are statistically significant: $W_f = 136$; $W_f > W_{u\ 1\%}$, $P < 0.01$	5.0% No transgression. Between-sample differences are statistically significant: $W_f = 87$; $W_f > W_{u\ 1\%}$, $P < 0.01$	5.0% No transgression. Between-sample differences are statistically significant: $W_f = 75$; $W_f > W_{u\ 1\%}$, $P < 0.01$

Note: See Materials and Methods for numerical designation of the sampling localities. Differences (%) between the character means in the samples examined are set out in larger type. Abbreviations and designations: E, European; A, Asian races of *S. uralensis*; EE, East European; SE, South European chromosome forms within the European race; C_v, coefficient of variation; Tr, approximate transgression of variation ranges (%), calculated as $(x_{max} - y_{min})/(y_{max} - x_{min}) \times 100\%$, where x and y are the maximal and the minimal variants of different samples, $x_{min} < y_{min} < x_{max} < y_{max}$; t_f, the calculated (factual) value of Student's t test; t_{st}, the tabulated ("standard") value of Student's t test, limiting the area of acceptance of the null hypothesis at a certain significance level; W_f, the calculated value of Wilcoxon–Mann–Whitney W-test; W_l and W_u, tabulated critical (lower and upper) values of Wilcoxon–Mann–Whitney W-test, limiting the area of acceptance of the null hypothesis at a certain significance level.

ment of of Borisovka, Belogorye Reserve (1♂). 9. Samara oblast, Stavropol'skii district, near the settlement of Bakhilova Polyana, Zhigulevskii reserve (1♂). 10. Samara oblast, Borskii district, near the settlements of Gerasimovka and Gostevka (3♂♂, 1♀). 11. Saratov oblast, Ozinskii district, near the settlement of Modin (1♂). 12. Orenburg oblast, Kuvandykskii district, near the settlements of Burangulovo, Mukhamed'yarovo, and Kancherovo (4♂♂, 4♀♀).

South European chromosome form. 13. Krasnodar krai, the territory subordinate to the city of Sochi, the suburbs of the town of Khosta (1♂). 14. Krasnodar krai, the territory subordinate to Sochi, 5 km to the northeast of the settlement of Krasnaya Polyana, the Caucasian Reserve (2♀♀). 15. Kabardino-Balkaria, suburbs of the city of Nal'chik (5♂♂, 4♀♀).

Asian Race of S. uralensis

16. Kazakhstan, Semipalatinsk oblast, Zharminskii district, near the settlement of Karatube, Kalbinskii Ridge (1♂). 17. Kazakhstan, Taldykurgan oblast, Karatal'skii district, 28 km northwest of the town of Ushtobe, floodlands of the Karatal River (1♂). 18. Kazakhstan, Taldykurgan oblast, Borlitobinskii district, 33 km west of the settlement of Matai, floodlands of the Aksu River (1♂, 1♀). 19. Kazakhstan, Taldykurgan oblast, Uigentasskii district, near the settlement of Bibakan, Dzhungarskii Alatau (2♂♂, 2♀♀). 20. Kazakhstan, Taldykurgan oblast, Gvardeiskii district, 10 km northeast of the settlement of Rudnichnyi, Dzhungarskii Alatau (1♂, 3♀♀). 21. Kazakhstan, Taldykurgan oblast, Kerbulakskii district, Altynemel' ridge, Altynemel pass (1♂). 22. Kazakhstan, Almaty oblast, Kaskelenskii district, 20 km south of the town of Kaskelen, Zailiiskii Alatau (1♂). 23. Turkmenistan, Lebapskii velayat, Kugitang ridge, Airybaba Mountain and the adjacent area: Darai-Dara canyon, near the settlement of Svintsovyi Rudnik; Kyrkkyz Canyon, near the settlement of Khodzhaipil' (3♂♂, 3♀♀).

We used splenocytes isolated from spleens stored at –70°C. Unfortunately, these storage conditions lead to partial destruction of cells and nuclei, which results in reduced peaks on histograms and in a "tail" formed by fragmented DNA; nevertheless, this "noise" can be readily identified and separated from the major peaks.

DNA staining and estimation of its content in cell nuclei followed the protocols described in [14]. Splenocytes of a female guinea pig were used as standard.

According to the results of analysis of G-stained karyotypes, the X chromosomes of pygmy wood mice from different localities was 2 to 2.5-fold larger in size than the Y chromosome [7, 15]. Because of the expected sex variation in genome size, the data on males and females were analyzed separately.

Statistical estimation of differences between samples, each of which had a normal distribution of variants (males and females of the East European chromosome form, as well as males of the South European chromosome form), was performed using parametric Student's *t*-test [16]; in all other cases the rank Wilcoxon–Mann–Whitney test was employed [17]. The percent difference between the means was calculated dividing their difference by the greater of the compared values.

RESULTS AND DISCUSSION

The amount of DNA in splenocyte nuclei of all pygmy wood mice examined is presented in the table. The figure presents C-banded karyotypes and histograms, typical of animals of the East European, South European chromosome forms and the Asian race of *S. uralensis.*

A distinct relationship between the karyotype heterochromatin content and genome size was recorded in both males and females. The East European chromosome form and the Asian race of *S. uralensis* were characterized by respectively the highest and the lowest amounts of centromeric heterochromatin in karyotypes. They also displayed maximum difference in the DNA content in cell nuclei: on average, the difference was approximately 8% in the group of males and 6.7% in the group of females; the variability ranges in both cases are separated by a substantial gap. Against the general variation background of the character, the Asian race, which has the smallest genome, seems to be homogeneous. A comparison of the South European chromosome form, which is intermediate in the heterochromatin content, with the Asian race revealed no transgression, although the difference between them, as expected, were lower (about 5% in both males and females). The East European and South European chromosome forms are most similar in the cell DNA content: the former exceeds the latter only by 3.2% in males and by 1.9% in females, where noticeable transgression of the values was found. Thus, variability of C-blocks in pygmy wood mouse seems to be associated with elimination (or, conversely, increment) of genetically inert chromatin.

Interestingly, on average the differences between sexes in the DNA content in cell nuclei of the East European chromosome form (0.8%) are far lower than in the South European chromosome form (2.2%) and the Asian race (2.2%). This suggests a substantial contribution of heterochromosomes to the genome size variability of the pygmy wood mouse genome. More specifically, this contribution is made by variable regions of these chromosomes, which are probably largely heterochromatic.

Thus, our results support the hypothesis on homogeneity of the Asian race of *S. uralensis* and considerable genetic isolation of this race. The method of DNA flow cytometry can be reliably used in diagnostics of pygmy wood mice in the zone of possible contact of the East

European chromosome form and the Asian race (North Kazakhstan, South Zaural'e).

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