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VARIABILITY OF MICROSATELLITES IN SHEEP BREEDS RACED IN RUSSIA

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Abstract

At the current stage of biological development is impossible to establish conservation programs and to monitor genetic resources of sheep without a preliminary study by DNA markers. The Russian sheep breeding is represented by wide variety of breeds, including all productivity and wool types. However, until recently only some sheep breeds, which belong to the same breeding zone or productivity type, were investigated by DNA markers including microsatellites. We studied 25 Russian sheep breeds ($n = 751$), including fine-fleeced — Dagestan Mountain (DAG), Grozny (GRZ), Kulunda (KUL), Manych Merino (MNM), Salskaya (SAL), Stavropol (STA), Soviet Merino (SVM), Volgograd (VOL), Baikal's fine-fleeced (ZBL); semi fine-fleeced — Altay Mountain (ALT), Kuibyshev (KUI), North Caucasian (NC), Russian long-haired (RLH), Tsigai (TSIG); coarse-wooled — Andean (AND), Buubey (BUB), Edilbai (EDL), Karachaev (KAR), Kuchugur (KCH), Kalmyk (KLM), Karakul (KRK), Lezgin (LEZ), Romanov (ROM), Tushin (TSH), Tuvan short fat-tailed (TUV). The research was conducted using 11 microsatellite loci (OarCP49, INRA063, HSC, OarAE129, MAF214, OarFCB11, INRA005, SPS113, INRA23, MAF65 и McM527). The data were processed using GenAIEx 6.5 and PAST software. In general, the studied breeds were characterized by moderately high allelic diversity. The average number of alleles per locus is varied from 7.20 ± 0.98 in KUL and 10.30 ± 0.99 in TSIG. The values of N_a 10.0 were found in TSIG, TUV, BUB and KRK, values of N_a 8.0 were identified in KUL, RLH and SVM. The effective allele number was the highest in the KRK and TUV (N_e 5.7) and the minimum was detected in KCH, ALT, RLH and NC (N_e 4.3). The level of the observed heterozygosity in 21 of the 25 studied breeds ranged from 0.489 ± 0.095 in TUV to 0.651 ± 0.050 in ROM and 0.651 ± 0.060 in SVM, and four other breeds (BUB, TSIG, ZBL and TUV) it varied from 0.798 ± 0.023 in BUB up 0.977 ± 0.017 in TUV. There was a substantial deficit of heterozygotes in 21 of the 25 studied breeds (F_{IS} values ranged from 0.13 in ROM to 0.36 in KAR and SAL), in the other four (BUB, TSIG, ZBL and TUV) an excess of heterozygotes (F_{IS} values ranged from -0.04 to -0.22) was detected. The analysis of molecular variance (AMOVA) showed that 5.02 % of genetic variation is composed of differences among breeds and 94.98 % is explained by within breeds' component. Analysis of the structure of the UMPGA phylogenetic tree, based on the matrix of pairwise genetic distances by M. Nei (1972), showed that the nature of the identified relationships is mainly related with the wool type, productivity type and breeding region. Thus, the identified polymorphism of eleven microsatellite loci is quite powerful for differentiating sheep of various breeds. For a better understanding population structure and obtaining new information on the genetic diversity at the genomic level the application of DNA microarrays, based on the multiple SNPs-markers, is required.

Keywords: sheep breeds, microsatellites, genetic diversity

Thanks to advancing DNA-based technology, various genetic markers have been detected and applied, including microsatellites, which became an important source of information about the current status of animal genetic resources [1, 2]. Choosing microsatellites as genetic markers is determined by their

unique features, such as the widespread and uniform distribution in the genome, allelic diversity, great informative value, codominant Mendelian-type inheritance, high reproducibility, and ease of analysis automation [3-5]. In the global and domestic practice of sheep rearing, microsatellite analysis is used to assess the genetic diversity of breeds and study phylogenetic relationships between them [6-8], proof of origin [9] and pedigree of animals [10], to investigate the population structure and genetic drift [11, 12], and establish the level of inbreeding in specific groups and herds [13].

Currently, high-performance genotyping methods are increasingly used, such as genome-wide SNP (single nucleotide polymorphism), scanning using DNA microarrays with different densities [14-17] and genotyping-by-sequencing (GBS) [18]. Despite this, microsatellites remain relevant as highly informative DNA markers in conducting population genetic studies [5]. A microsatellite-based analysis is still an indispensable tool for the routine animal testing on the origin authenticity and the breed. This is primarily due to the fact that the panels for origin diagnostics based on highly informative SNP markers are rather expensive, and blood groups are much inferior to the microsatellites in the testing accuracy [19].

In Russia, there are 37 sheep breeds, including 13 fine-fleeced, 10 semi-fine-fleeced, 12 coarse-wool and 2 medium-wool breeds [20]. They are also represented by all known types based on productivity (wool, mutton-wool, wool and meat, fur, fat-tailed, mutton-wool and dairy, hair and meat). This diversity is due to the peculiarities of climate, feed and social factors in the regions where the breeds were developed.

In recent years, genetic studies have been performed on some Russian breeds of sheep using the microsatellite markers [21-23]. Within the framework of the genetic diversity study program and the establishment of the population structure of sheep breeds in North Eurasia, the Russian breeds were compared with breeds from Norway, Denmark, Sweden and Estonia [24]. For the genetic classification of semifine-fleeced breeds, the Kuibyshev, Russian long-haired and North Caucasian mutton-wool sheep breeds were involved [25]. However, until now genetic studies of sheep based on the microsatellite markers in Russia have involved only several breed groups, combined by the productivity type or a breeding region.

In this paper, we first studied and compared 25 Russian sheep breeds representing all known types by their microsatellite markers. In general, polymorphism in 11 microsatellite loci was quite informative for the interbreed differentiation. Our findings most completely reflect the state of the allele pool and genetic diversity of most of the major sheep breeds, commonly spread in Russia.

The purpose of the research was the study of variability of microsatellites, the assessment of diversity and degree of genetic differentiation among different sheep breeds, raised in Russia.

Technique. Tissue samples were taken from sheep of 25 Russian breeds, such as fine-fleece, semifine-fleece, coarse-wool, with different types of meat, wool and dairy productivity ($n = 751$).

To isolate the DNA the Nexttec columns (Nexttec Biotechnologie GmbH, Germany) were used, as well as the DNA Extran test kit (CJSC Syntol, Russia) and the extraction method using sodium perchlorate [26]. For the genetic research, we selected 11 microsatellite loci grouped into two multiplex panels including the following loci: OarCP49, INRA063, HSC, OarAE129, MAF214, OarFCB11, INRA005, and SPS113, INRA23, MAF65 and McM527 loci. The reactions were performed in a final volume of 10 μ l in the PCR buffer containing 2 mM dNTPs, 1.0 mM of $MgCl_2$, 0.5 mM of the primer mixture, 1 unit of

Taq-polymerase (Dialat Ltd., Russia), and 50-100 ng of genomic DNA. The composition of the PCR buffer was as follows: 16.6 mM (NH₄)₂SO₄, 67.7 mM of Tris-HCl (pH = 8.8), 0.1 vol of Tween 20. After the initial denaturation (95 °C, 4 min) we performed 41 (Panel 1) and 35 (Panel 2) amplification cycles in the following temperature-time regime: 95 °C, 20 sec; 63 °C (Panel 1) and 55 °C (Panel 2), 30 sec; 72 °C, 1 min. Fragments were examined using the ABI3130xl genetic analyzer (Applied Biosystems, USA) and GeneMapper 4 software (Applied Biosystems, USA).

The GenAIEx 6.5 software [27] was used to calculate the following statistical parameters: mean number of alleles per locus (N_a), the effective number of alleles (N_e), the number of informative alleles or alleles with a frequency of > 5 % (N_a 5 %), the expected (H_e) and observed (H_o) heterozygosity, and inbreeding coefficient (F_{IS}). The degree of genetic differentiation of breeds was assessed by the F_{ST} parameter [28] and genetic distances by M. Nei (D_N) [29] for paired comparison. The F_{ST} values were visualized by analyzing the principal coordinates (Principal Coordinates Analysis, PCoA) using GenAIEx 6.5 software. Based on the genetic distance matrix by M. Nei [29] in the PAST software [30], a phylogenetic tree was built by the unweighted pair-group method using arithmetic averages (UPGMA).

Results. Table 1 provides a summary of sheep population involved in the study.

1. The characteristics of 25 breeds of sheep (*Ovis aries*), most common in Russia and assessed based on 11 microsatellite loci (n = 751)

Breed	Code	n	Region
Fine-fleece breeds			
Wool			
Grozny	GRZ	30	Republic of Kalmykia
Stavropol	STA	32	Stavropol Krai, Kalmykia
Manych Merino	MNM	30	Stavropol Krai
Soviet Merino	SVM	23	Stavropol Krai
Salskaya	SAL	30	Rostov Province
Mutton-Wool			
Volgograd	VLG	30	Volgograd Province
Dagestan Mountain	DAG	30	Republic of Dagestan
Wool and meat			
Transbaikalian fine-fleece	ZBL	30	Republic of Sakha (Yakutia)
Kulunda	KUL	30	Altai Krai
Semifine-fleece breeds			
Mutton-Wool			
Russian long-haired	RLH	30	Voronezh Province
Kuibyshev	KUI	30	Samara Province
North Caucasian Mutton-Wool	NC	30	Stavropol Krai
Wool and meat			
Tsigai	TSIG	30	Saratov Province, Rostov Province
Altay Mountain semifine-fleece	ALT	30	Altai Krai
Coarse-wool breeds			
Hair and meat			
Romanov	ROM	30	Yaroslavl Province, Ryazan Province, Moscow Province.
Fur-bearing			
Karakul	KRK	32	Republic of Kalmykia, Astrakhan Province
Fat-tailed			
Edilbai	EDL	30	Volgograd Province
Kalmyk fat-rumped	KLM	30	Republic of Kalmykia
Mutton-Wool			
Bubey	BUB	30	Republic of Sakha (Yakutia)
Tuvan short fat-tailed	TUV	30	Republic of Tyva
Kuchugur	KCH	34	Voronezh Province
Dual-purpose and dairy			
Karachayev	KAR	30	Karachay-Cherkessia
Lezgin	LEZ	30	Republic of Dagestan
Andean	AND	30	Republic of Dagestan
Tushin	TSH	30	Republic of Dagestan

The average number of alleles per locus ranged from 7.20±0.98 in KUL

to 10.30 ± 0.99 in TSIG (Table 2). The highest values of this parameter were characteristic of four breeds (TSIG, TUV, BUB, KRK with Na 10.0), the lowest — of three breeds (KUL, RLH, SVM with Na 8.0). The effective number of alleles was highest in KRK and TUV breeds (Ne 5.7), and lowest — in KCH, ALT, RLH and NC breeds (Ne 4.3). The range of variability in terms of the number of informative alleles per locus (Na 5 %) varied from 4.70 in STA to 6.40 in TSIG.

2. The characteristics of the allele pool and genetic diversity parameters in the Russian breeds of sheep (*Ovis aries*) by 11 microsatellite loci

Breed	Na	Ne	Na 5 %	H _o	H _e	F _{IS}
EDL	9.30±1.21	4.66±0.71	5.30±0.72	0.557±0.073	0.730±0.043	0.27±0.07
VLG	8.90±1.22	5.08±0.70	5.80±0.63	0.525±0.082	0.751±0.047	0.33±0.08
SAL	8.50±0.92	5.05±0.63	5.90±0.64	0.512±0.089	0.764±0.036	0.36±0.09
KAR	9.20±1.10	5.25±0.72	5.70±0.63	0.516±0.087	0.764±0.040	0.36±0.09
KLM	9.50±0.96	5.07±0.63	5.90±0.50	0.577±0.071	0.771±0.030	0.27±0.08
GRZ	9.00±1.14	4.92±0.62	5.40±0.67	0.540±0.089	0.761±0.033	0.33±0.09
DAG	9.00±1.07	5.45±0.82	5.40±0.86	0.560±0.079	0.774±0.032	0.30±0.08
TSH	9.60±1.13	5.02±0.79	5.70±0.60	0.507±0.081	0.748±0.042	0.35±0.08
AND	8.70±0.98	4.80±0.58	5.40±0.65	0.550±0.074	0.757±0.033	0.29±0.08
LEZ	8.60±0.72	4.74±0.74	5.80±0.71	0.510±0.070	0.730±0.044	0.33±0.06
ALT	8.50±0.99	4.28±0.70	4.80±0.61	0.509±0.084	0.678±0.065	0.26±0.09
KUL	7.20±0.98	4.42±0.72	5.30±0.70	0.489±0.095	0.701±0.053	0.33±0.12
KRK	10.00±0.91	5.75±0.86	5.40±0.76	0.634±0.047	0.785±0.033	0.20±0.04
KUI	8.50±1.06	5.32±0.80	5.90±0.69	0.646±0.052	0.767±0.036	0.16±0.05
KCH	9.20±1.15	4.24±0.53	4.80±0.39	0.574±0.059	0.729±0.032	0.22±0.08
RLH	8.00±0.79	4.28±0.51	4.90±0.41	0.555±0.066	0.726±0.041	0.26±0.07
NC	8.50±0.90	4.32±0.59	5.00±0.42	0.586±0.053	0.726±0.041	0.20±0.04
STA	9.20±0.92	4.88±0.63	4.70±0.45	0.575±0.061	0.765±0.027	0.26±0.06
MNM	8.20±0.90	4.54±0.51	5.00±0.45	0.647±0.055	0.752±0.029	0.15±0.05
TSIG	10.30±0.99	5.53±0.42	6.40±0.48	0.873±0.014	0.807±0.019	-0.09±0.03
TUV	10.10±1.16	5.74±0.58	6.30±0.52	0.977±0.017	0.808±0.020	-0.22±0.04
BUB	10.00±1.13	5.11±0.69	6.30±0.76	0.798±0.023	0.774±0.026	-0.04±0.04
ZBL	8.90±0.77	5.32±0.53	6.00±0.45	0.891±0.018	0.794±0.021	-0.13±0.03
ROM	9.80±1.04	5.27±0.81	5.20±0.57	0.651±0.050	0.758±0.043	0.13±0.06
SVM	8.00±0.75	4.95±0.44	5.20±0.42	0.651±0.060	0.782±0.020	0.17±0.07
Mean	8.99±0.20	4.96±0.13	5.50±0.59	0.616±0.015	0.756±0.007	0.20±0.02

Note. Na — mean number of alleles per locus; Ne — the effective number of alleles; Na 5 % — the number of informative alleles per locus (with a frequency of > 5 %); H_o — the observed heterozygosity; H_e — the expected heterozygosity; F_{IS} — coefficient of inbreeding. See Table 1 for the explanation of the abbreviations for the sheep breeds.

The observed heterozygosity in 21 of 25 studied breeds varied from 0.489 ± 0.095 in TUV to 0.651 ± 0.050 in ROM and 0.651 ± 0.060 in SVM. In four breeds (BUB, TSIG, ZBL, TUV), heterozygosity was significantly higher, such as from 0.798 ± 0.023 in BUB to 0.977 ± 0.017 in TUV. A comparison of the observed and expected heterozygosity revealed a significant deficit of heterozygotes in 21 of 25 studied breeds which was from 10.5 % (ROM) to 24.9 and 25.1 % (in KAR and SAL, respectively). The deficit of heterozygotes was confirmed by the positive values of the coefficient of inbreeding F_{IS}, which ranged from 0.13 in ROM to 0.36 in KAR and SAL. A slight (2.5 to 16.8 %) excess of heterozygotes was detected in BUB, TSIG, ZBL and TUV. The F_{IS} values for these four breeds ranged from -0.04 to -0.22.

The analysis of molecular variance (AMOVA) showed that 5.02 % of the genetic variability of breeds accounted for the differences between breeds and 94.98 % — for the intrabreed component.

The genetic relationship between the studied breeds based on the F_{ST} index is presented in Figure 1 as the PCoA plot. The lowest values of the index were observed for the following pairs: GRZ-STA (F_{ST} = 0.013), MNM-SVM (F_{ST} = 0.014), STA-SVM (F_{ST} = 0.014), ZBL-SVM (F_{ST} = 0.014), and the highest for KUL-EDL (F_{ST} = 0.071) and ALT-ROM (F_{ST} = 0.070) pairs.

Fig. 1. Genetic differentiation of 25 Russian breeds of sheep (*Ovis aries*), belonging to different classes according to the wool and productivity types, in the space of the first two principal coordinates, calculated by the F_{ST} index based on PCoA in paired comparison for 11 microsatellite loci: ● — coarse-wool, ■ — semifine-fleece, ◇ — fine-fleece sheep breeds. See Table 1 for the explanation of the abbreviations for the sheep breeds.

sistent with our findings: the F_{ST} values between TSIG-KUI and TSIG-NC pairs were 0.026 and 0.031, respectively, and only for a NC-KUI pair this value was lower ($F_{ST} = 0.015$).

The analysis of genetic distances [29] showed the greatest remoteness of KCH-BUB, ROM-TUV, KCH-TUV, ALT-ROM and KUL-TUV breeds, for which the D_N values were 0.490, 0.457, 0.448, 0.431 and 0.430, respectively. This could be due to the geographical remoteness of breeding regions, which prevented the exchange of genetic material. The minimum genetic distances were detected mainly between breeds belonging to the same productivity type: GRZ-STA ($D_N = 0.087$), MNM-SVM ($D_N = 0.099$), STA-SVM ($D_N = 0.102$). In general, it can be concluded that the genetic relationships between the studied breeds, assessed by the D_N and F_{ST} parameters were similar.

The phylogenetic tree (Fig. 2) built on the basis of the genetic distances (D_N) demonstrated marked independent branches formed by Kuchugur and Romanov breeds, which probably was the result of their genetic uniqueness. The remaining breeds formed two clusters, the first of which was presented exclusively with fine-fleece and semifine-fleece breeds, and the second — with all coarse-wool breeds as well as Tsigai and Transbaikal fine-fleece breeds.

Four subclusters were distinguished in the first cluster, each of which included several breeds. Thus, a general subcluster (1-1) was formed by fine-fleeced breeds, such as Grozny, Stavropol, Manych merino and Soviet merino, which could be explained by one productivity type, breeding habitat, and the long-term (over 25 years) use of the gene pool of Australian merino sheep for their improvement [32]. Semifine-fleece breeds, such as Kuibyshev, North Caucasian mutton-wool and Russian long-haired, formed a separate subcluster (1-2). It is known that rams of the Romney Marsh breed originated in England were used to improve the first two breeds [33, 34], while Lincoln sheep were involved in deriving the Russian long-haired breed (at the initial stage) and North Caucasian mutton-wool breed (at the final stage of breed development) [35]. Combining breeds of the Mutton-Wool productivity type (Dagestan Mountain and Volgograd) in a separate cluster (1-3) could be due to the use of local inbred coarse-wool fat-rumped and fat-tailed sheep (as a maternal base) at the initial stage of their breeding, and Caucasian and Grozny merino breeds at the final

According to D.L. Hartl and A.G. Clark [31], the F_{ST} values < 0.05 indicate a negligible genetic differentiation, 0.05 to 0.15 — a moderate, and 0.15 to 0.25 — a significant genetic differentiation. Most of the studied Russian breeds were characterized by negligible to moderate genetic differentiation. It is noteworthy that KUL and ALT breeds were at some genetic distance from most others. This was confirmed by $F_{ST} = 0.05$. M.Yu. Ozerov et al. [25] found that the F_{ST} values between Russian semifine-fleece sheep breeds averaged to 0.03. In general, this is con-

stage [36]. The fourth subcluster (1-4) included Salskaya, Kulunda and Altay Mountain semifine-fleece breeds.

Fig. 2. The phylogenetic tree built based on the matrix of pairwise genetic distances between the studied Russian breeds of sheep (*Ovis aries*) by M. Nei [29]: 1-1, 1-2, 1-3 и 1-4 — subclusters of the cluster 1; 2-1, 2-2 and 2-3 — subclusters of the cluster 2. One asterisk denotes coarse-wool, and two asterisks — semifine-fleece breeds; all the remaining are fine-fleece breeds. The UMPGA method was used to construct a dendrogram.

Three subclusters stood out in the cluster 2. The first one (2-1) was formed by sheep of Karachaev, Andean and Lezgin breeds developed in different mountainous areas of the North Caucasus through the long-term sustained selection of local coarse-wool sheep by local inhabitants. A distinctive feature of these breeds is an equally weighted ratio of meat, wool and dairy productivities, as well as an exceptional adaptability to keeping on the mountainous and lowland pastures [37, 38], which apparently determined their genetic proximity.

The second subcluster (2-2) included Edilbai, Kalmyk fat-rumped, Tushin and Karakul breeds, characterized by increased deposits of fat in the tail area. The first two breeds are of meat-fat productivity type, well adapted to grazing over large distances in semi-deserts and deserts, and have the fat tail. Furthermore, it is known that Edilbai breed was the improver at the initial stage of Kalmyk breed development [36]. The Tushin and Karakul breeds belong to fat-tailed sheep by the morphological classification, and also tend to fat deposition. Given the zoological classification, the productivity type, the area of spreading, the history of breeding and phenotypic similarity of breeds, an isolation of the two subclusters within the second cluster can be considered reasonable.

The third subcluster included Tsigai, Transbaikalian fine-fleece, Buubey and Tuvan short fat-tailed breeds of different productivity types and historical origins. The presence of the Transbaikalian fine-fleece, Buubey and Tuvan breeds in one subcluster is possibly due to the fact that indigenous coarse-wool Buryat-Mongolian sheep are found in their genealogy. Thus, Buubey breed was developed through a long-term improvement of indigenous Buryat sheep, Transbaikalian fine-fleece breed — through a long transformation of inbred coarse-wool Buryat-Mongolian sheep using the gene pool of the North Caucasian fine-fleece breeds [39]. Previously, the genetic similarity of the Tuvan indigenous breed of sheep, raised by the local tribes in the Republic of Tuva, and the Mongolian sheep has been already pointed out [40]. The explanation for the clustering of these breeds may be in the fact that all of them were derived and are still being raised in the extreme climatic conditions of Transbaikalia and Western Siberia. Probably, adaptive mechanisms, developed over hundreds

of years, affected many loci, including microsatellite ones, and led to the similarity of the genetic profile. However, this assumption requires further investigation and validation.

Therefore, our findings give the most complete presentation of the state of the allele pool and genetic diversity of the majority of sheep breeds, the most commonly spread in Russia. In general, polymorphism in 11 microsatellite loci was quite informative for the differentiation sheep belonging to different breeds, while the nature of the identified relationships were mainly due to the wool type, productivity type and a breeding region. For a more profound research of the population structure and additional information on the genetic diversity at the genomic level, the investigation of Russian sheep breeds is anticipated with the application of DNA microarrays based on the multiple SNP markers.

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