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Original scientific paper

INVESTIGATION OF GENE POOL AND GENEALOGICAL LINKS BETWEEN SHEEP BREEDS OF SOUTHERN RUSSIA BY BLOOD GROUPS AND DNA MICROSATELLITES

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Zinovieva, N..A., M..I.Selionova, E..A.Gladyr, M.P. Petrovic, V. Caro Petrovic, D. Ruzic Muslic, M..M.Petrovic (2015): *Investigation of gene pool and genealogical links between sheep breeds of southern Russia by blood groups and DNA microsatellites*.- Genetika, Vol 47, No. 2, 395 - 404.

To study the gene pool and the establishment of genealogical relationships between breeds of sheep of different directions productivity bred in Russia, were used two classes of genetic markers - blood and DNA microsatellites. The included sample sheep are fine-wool Merino breeds: Grozny (GR), Caucasian (CA), Manychskij merino (MM), the Soviet Merino (SM), Stavropol (ST) and coarse wool breeds: Edilbaevskaya (ED), Karakul (CR) and Romanov (RO). For the study of erythrocyte, were selected antigens (blood group) in 1159 samples from 11 breeding farms. For microsatellite DNA study - 598 from 10 breeding farms. Microsatellite analysis revealed that the most polymorphic were Stavropol breed sheep that have identified an average of 18.27 alleles per locus were relatively conservative Romanov breed sheep - 9.7 alleles per locus. The minimum genetic distances established between Grozny and Soviet Merino - 0.0569 (for microsatellites) and 0.0741 (blood groups - later in the same sequence). The rocks of the Stavropol – Grozny were 0.0861 and 0, 0810. Whereas Stavropol and Soviet Merino 0.0861 and 0.1094. Also relatively close between Grozny – Edilbaevskoy , Grozny - Karakul, Edilbaevskoy - Karakul: 0.1364 and 0.0851, respectively; 0.1620 and 0.1208; 0.1875 and 0.1192. The highest genetic distances were between Stavropol and Karakul - 0.2664 and 0.1804, as well as between the Romanov and all studied species - 0.2491 ... 0.3211 and 0.1734 ... 0.2235.

Key words: blood group, DNA microsatellites, genetic markers, genealogical analysis, **p**olymorphism, sheep

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INTRODUCTION

Genetic improvement of sheep is the basis for sustainable development of this branch of animal husbandry. Phenotypic characteristics of sheep are influenced by various genetic and environmental factors (CARO PETROVIC *et al*., 2013). Different breeds have a special genetic structure and interaction with the environment (SNOWDER and VIECK, 2003; PETROVIC *et al*., 2011). Since opening at the turn of 19-20 centuries biochemical markers, based on which it became possible to study the gene pool, genetic polymorphism and genealogical relationships of farm animal breeds interest in this problem continues unabated to the present time (BUDAK YILDIRAN *et al.,* 2012; CHEN *et al*., 2009; HODA *et al*., 2009). The polymorphic system of proteins and enzymes, blood groups were widely used in the assessment of the genetic structure, differentiation interbreed different species of domestic animals (GORELOV *et al*., 2011; TAPIO *et al*., 2003). However, with the development of molecular genetics greater recognition and use to investigate the origin, identify the genetic relationships between different breeds of farm animals received a new class of genetic markers - DNA microsatellites (DIEZ –TASCÓN *et al*., 2000; PETROVIC, 2000; TAPIO *et al*., 2005; GLADYR *et al*., 2005; GLADYR *et al*., 2011; ZINOVIEVA *et al*., 2011; SELIONOVA *et al*., 2012). Microsatellites - is anonymous (not carrying the coding functions) repetitive genomic DNA fragments that are uniformly distributed throughout the genome, and which accounted for 30% of the entire genome of farm animals. Microsatellites is now often used in the analysis of the genome of sheep around the world (CANON *et al.,* 2001; ARRANZ *et al.,* 2001; RENDO *et al.,* 2004; BAUMUNG *et al.,* 2006; PETER *et al.,* 2007; DORJI *et al.,* 2010).

High polymorph character (in ruminants by an average of one locus, there are about eight alleles) and Mendelian pattern of inheritance makes them extremely informative to assess the genetic diversity of populations (NEI *et al.,* 2000).

The aim of this work was to study the gene pool and genealogical links between breeds of sheep in Russia, which have the greatest distribution, based on different genetic markers - blood groups and microsatellites, and comparative mapping of the data.

MATERIALS AND METHODS

For the immunogenetic and molecular genetic studies have collected sampled blood or tissue, respectively, from the fine-wool Merino sheep breeds Russian selection: Grozny (GT), Caucasian (CA), Manychskij merino (MM), the Soviet Merino (SM), Stavropol (ST) and coarse wool sheep breeds: Edilbaevskaya (ED), Karakul (CR) and Romanov (RO). For the study of erythrocyte, antigens (blood group) had selected in 1159 samples from 10 breeding farms while for microsatellite DNA study have taken 598 samples from 11 breeding farms. The study samples were typical for each species of animals from different breeding plants (populations) (Table. 1).

Genotyping of sheep blood groups in the reactions of agglutination and hemolysis in five systems, including 11 of erythrocyte factors using monospecific standard sera bank immunodiagnostics was carried out at All-Russian Research Institute of sheep and goat. Microsatellite analysis was performed on 11 loci identified in sheep (MsM42, OarFCB20, OarFCB11, MAF65, MsM527, OarCP49, OarAE119, HSC, MAF214) and cattle (TGLA53, INRA49). Set of loci for analysis had chosen in accordance with the recommendations of the International Society of Genetics (ISAG) comparative testing of sheep and goats. Used two multiplex PCR incorporating labeled with fluorescent colorants (FAM, HEX or TET) primers, respectively, to 5 and 6 loci. Separation amplificates performed using capillary electrophoresis with laser analyzer MegaBACE 1000 (Amersham Bioscience). Processing of the data had

performed by WEIR (1995) with the help of programs MSA_WINv 2.65. Genetic distances between breeds calculated by M.Nei (NEI, 1972). Calculations had done using the statistical language processing R. Visualization of results in the form of a phylogenetic tree had obtained in a medium Rstudio

Table 1 The studied population of sheep of different breeds

Breed	By blood group		DNA microsatellites	
	Population (breeding farms)	n	Population (breeding farms)	n
Grozny (GR)	" Chervlennaya Burunov " Republic of Dagestan	124	"Chernozemelsky" Republic of Kalmykia	34
	"Chernozemelsky" Republic of Kalmykia	76	"Erdnievsky" Republic of Kalmykia	56
	"Roshinsky", Stavropol area	97		
Stavropol (ST)	"Soviet Fleece" Stavropol area	104	Experimental farm KNIISKH Republic of Kalmykia	69
	"Second Five-Year Plan" Stavropol area	70		
	"Lenin's Path" Stavropol area	138		
Soviet Merino (SM)	"Niva" Stavropol area	146	"Novoselivskyi" Rostov region	36
	"Red October" Stavropol area	121	"Kirov" Kalmykia	66
Edilbaevskaya (ED)	"Artesian" Stavropol area	67	"Tabun-Aral" Astrakhan region.	30
			Farming Kazakhstan	41
Karakul (KR)	"Erdnievsky" Republic of Kalmykia Kalmykia	114	"Erdnievsky" Republic of Kalmykia	106
			Farm-cooperative "Privolzsky" Astrakhan region	110
Romanov (RO)	"Polar Star" Stavropol area	102	"Russia" Moscow region	50
Total		**1159**		**598**

RESULTS AND DISSCUSSION

Analysis of the results revealed the genetic characteristics of the studied species polymorphism of erythrocyte antigens (Table. 1). Sheep Grozny fine wool breed characterized by a high prevalence of Aa and Da - antigens and the average frequency of occurrence of R - factor and blood groups in the system. The Stavropol Sheep breed had the highest frequency of gene Aa, Bc and Be - antigens, blood groups Bd, Bi, Bg, Ca, Da while R - average and Ab - the lowest. Among the animals, the Soviet merino with high frequency identified individuals with Aa, Be, Ca, and R - antigens, less carriers Bc, Bd, Bg - factors and very rarely - Ab, Bi, Da. Edilbaevskoy breed of sheep with the greatest frequency in comparison with the other species investigated, the animals were observed with Aa - antigen and lowest Bd and R. For karakulskih sheep population was characteristic widespread individuals with Bb, Ca, and R groups and low blood Bc and Bg antigens. Romanov breed sheep differed relatively low spread across the investigated range of erythrocyte antigens, with the lowest frequency of detected individual carriers Aa, Ab, Bb, Bc, Be and Da factors (Table. 2).

Table 2. Frequency of blood groups in different breeds of sheep

Breed	Groups of blood, system, erythrocyte factors										
	A				B				C	D	R
	Aa	Ab	Bb	Bc	Bd	Be	Bi	Bg	Ca	Da	R
GR	0,428	0,289	0,383	0,333	0,125	0,294	0,192	0,213	0,343	0,492	0,298
ST	0,473	0,143	0,326	0,544	0,292	0,473	0,369	0,292	0,215	0,319	0,351
SM	0,401	0,207	0,487	0,288	0,336	0,456	0,156	0,288	0,525	0,176	0,198
ED	0,601	0,122	0,418	0,214	0,081	0,361	0,301	0,162	0,253	0,281	0,112
KR	0,253	0,315	0,627	0,178	0,433	0,251	0,271	0,125	0,432	0,297	0,498
RO	0,225	0,108	0,117	0,068	0,205	0,088	0,343	0,205	0,294	0,167	0,127

Analysis of microsatellite markers showed a high level of polymorphism at each of the eleven loci studied. On average, the study of rocks number of alleles per locus was 15,00 ± 0,99, with the largest number mentioned in the locus OarCP49 - 17,3 ± 1,52, with a range from 11 alleles in Grozny to 21 in breeds of Stavropol and Karakul. The minimum number of locus found in OarFCB11 with variability from 6 to 12 in the Romanov breed in Stavropol.

Number of alleles in a certain measure characterizes interbreed genetic diversity and the probability of its preservation in subsequent generations. Scientists today, disagree about the effect of selection pressure on the degree of polymorphism and heterozygosity of populations. In some cases, due to the long unidirectional selection has marked by the consolidation of certain genetic parameters, which leads to a reduction of genetic diversity and the loss of individual alleles in the rocks, then in the high-stage, the increase in the number of heterozygotes and polymorphism in comparison with the original version (NEI *et al.,* 2000). Analysis of the data revealed microsatellite alleles showed that the most polymorphic were Stavropol breed sheep that have identified an average of 18.27 alleles per locus, while in two markers (MAF214 and OarAE119) revealed the largest number - 27 and 25. This number is not mentioned none of the examined loci and in any formation. A relatively large number of alleles detected in animals' karakul breed and Soviet Merino (respectively 16.09 and 15.00). Relatively conservative based on individuals were Romanov breed, in the studied microsatellites that recorded an average of only 9.7 alleles (Table. 3).

The observed degree of heterozygosity ranged from 56.7% in sheep Stavropol breed to 72.4% in karakul sheep. The degree of expected heterozygosity was higher than that observed in all the studied species and ranged from 77.9 to 88.1%.

Fixation index F_{is}, gives an indication of the degree of inbreeding populations (NEI *et al*., 2000), and indicates a lack of heterozygotes in the studied rocks. Deficit averaged 21.18% and ranged from 5.95 in Edilbaevskoy breed to 35.2% - in the Stavropol. Four of the six species studied, the difference between the observed and expected heterozygosity degree was significant (Table. 4).

Table 3. The number of alleles of microsatellite loci in different breeds of sheep

Breed	The number of alleles at loci microsatellites											
	HSC	Oar AE119	Oar CP49	Oar FCB11	MAF 214	McM 42	TGLA 53	MAF 65	McM 527	INRA 49	Oar FCB20	Mean N_{cp}±M
GR	14	16	11	10	13	13	12	10	13	11	14	12,46 ±0,56
ST	17	25	21	12	27	16	18	13	18	17	17	18,27 ±1,37
SM	20	22	18	11	19	11	12	12	14	12	14	15,00 ±1,21
ED	14	11	17	9	8	11	14	17	16	15	14	13,27 ±0,94
KR	19	13	21	12	17	14	17	16	19	12	17	16,09 ±0,91
RO	10	7	16	6	8	7	12	13	13	7	8	9,73 ±0,99
Mean	15,67	15,67	17,33	10,0	15,33	12,0	14,17	13,50	15,5	12,33	14,00	
N_{cp}±M	±1,52	±2,78	±1,52	±0,93	±2,97	±1,26	±1,11	±1,05	±1,06	±1,41	±1,34	

Table 4. Observed and expected levels of heterozygosity (at microsatellite loci)

Breed	F_{is}	Degree of heterozygosity		p
		observed	expected	
GR	0,2495	0,619±0,039	0,830±0,017	p<0,001
ST	0,3520	0,567±0,031	0,881±0,012	p<0,001
SM	0,2702	0,614±0,030	0,850±0,021	p<0,001
ED	0,2776	0,595±0,032	0,853±0,023	p<0,001
KR	0,0627	0,724±0,035	0,808±0,036	p>0,05
RO	0,0591	0,707±0,029	0,779±0,031	p>0,05
Average	0,2118	0,638±0,033	0,833±0,023	-

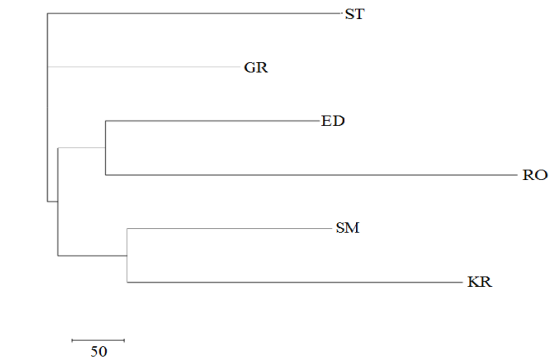
Table 5. Genetic distances between breeds (1 for microsatellites, 2 - blood groups)

Breed	GR		ST		SM		ED		KR		RO
	1	2	1	2	1	2	1	2	1	2	
GR	-										
ST	0,0861	0,0810	-								
SM	0,0569	0,0741	0,0861	0,1094	-						
ED	0,1364	0,0851	0,2360	0,1432	0,2024	0,1143	-				
KR	0,1620	0,1208	0,2664	0,1804	0,1863	0,1017	0,1875	0,1192	-		
RO	0,2491	0,1925	0,3211	0,2235	0,2547	0,1734	0,2670	0,2007	0,2527	0,1946	-

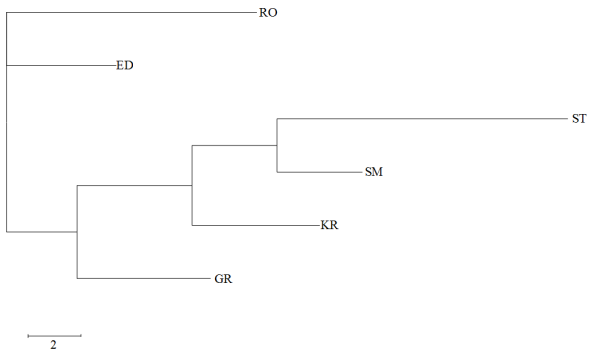
Based on the frequency of occurrence of blood group alleles and loci microsatellite genetic distances were calculated (Table. 5). It should been emphasized that the samples of

biological material for the study of blood groups and microsatellite loci had taken from the sheep of the same species, but in different breeding plants (populations). However, analysis of the data shows that at a certain difference between the numerical values in the patterns of genetic differentiation of the studied rocks, obtained using different genetic markers were similar in many ways.

Thus, the minimum genetic distance was between the rocks Grozny and Soviet Merino - 0.0569 (for microsatellites) and 0.0741 (blood groups - later in the same sequence). While the genetic distances between the rocks of the Stavropol – Grozny; Stavropol and Soviet Merino - 0.0861 and 0.0810; 0.0861 and 0.1094. Relatively close is between rocks Grozny – Edilbaevskoy; Grozny – Karakul; Edilbaevskoy - Karakul: 0.1364 and 0.0851, respectively; 0.1620 and 0.1208; 0.1875 and 0.1192, the highest - between Stavropol and Karakul - 0.2664 and 0.1804, as well as between the Romanov and all studied species - 0.2491 ... 0.3211 and 0.1734 ... 0.2235.



a)



b)

Fig. 1 Dendrograms of genetic distances sheep breeds in Russia, built on M.Nei based on the matrix of genetic distances by: a) blood groups, b) microsatellite loci

Because of the distance matrix had constructed in Euclidean space, which was the basis of the cluster analysis by the method of the nearest neighbor. In the study of other authors (TAPIO *et al.,* 2003), comparison of microsatellite and blood protein diversity in Finnsheep, Romanov, Oxford Down and three local breeds from Finland or northwestern Russia showed that genetic distances were relatively low for protein variation compared with microsatellites. Microsatellite variation correlated positively with protein variation, but for the local Viena sheep, protein variation was comparatively low. Populations had significant differences in allelic richness, but not in genetic diversity.

Combining the overall cluster of fine-wool breeds - Stavropol and Grozny (dendrogram on blood groups, Fig. 1a), Stavropol, Grozny and Soviet Merino (dendrogram on microsatellites Fig. 1b) seems logical and is largely due to the history of the origin of these rocks and the direction of productivity. So, all these fine-fleeced breeds of sheep pronounced wool productive direction were created based on mazaevskih and novokavkazskih merino with the use of the early stages of the gene pool of the breed American Rambouillet, in the future for improvement - Australian merino sheep. These species were traditionally bred animals in the dry steppe areas of the Southern region of Russia, which apparently, also has an impact on the distribution of similar genotypes capable in this ecological niche producing thin merino wool quality. Combining Romanov and Edilbaevskoy rocks in a single cluster, as blood groups, and microsatellites (see Fig. 1a, b) because both belong to the same breed towards productivity by type of wool, ie coarse wool breeds. It should be noted that the use of different genetic markers greatest genetic divergence from the studied species exhibits a unique prolific Romanov breed, bred in the 17th century in the Yaroslavl region and originating from the northern short-tailed sheep offspring, it is possible to determine the characteristics of its by genetic-ray structure.

Somewhat unexpected is the genetic closeness of karakul sheep and Soviet Merino breed, which can be traced as blood groups, and on the microsatellite loci (see Fig. 1a, b). Karakul breed is one of the oldest in the world and bred for karakul astrakhans, the type of wool refers to the coarse wool breeds. Soviet Merino, as noted above, fine wool breeds wool productive direction. Perhaps the close distribution of frequencies of alleles and groups CROI microsatellites, influenced by the fact that both breeds are bred in the steppe zones with a sharp continental climate. Adaptive response is likely to have affected part of similar genetic loci. However, this assumption needs further investigation and validation.

Thus, the comparative analysis of phylogenetic relationships between species, prepared using as genetic markers of different systems - the blood groups and microsatellite DNA showed similar patterns, which can be seen in favor high resolution of the used markers on the one hand and "genetic stability and immutability "gene pool breeds of sheep. The results obtained can serve as an informative base for research, you-fill-in the other breeds of sheep for comparison and phylogenetic analysis. The data presented may be useful to scientists and practitioners in the justification of the choice of species to develop options for mating and production of new original forms of sheep for breeding purposes and the development of new species.

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ISPITIVANJA GENSKOG PULA I GENEOLOŠKIH VEZA IZMEĐU GENOTIPOVA OVACA JUŽNE RUSIJE KORIŠĆENJEM KRVNIH GRUPA I DNK MIKROSATELITA

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Izvod

U istraživanjima su korišćene dve klase genetičkih markera – krvne grupe i DNK mikrosateliti. Uključeni su uzorci ovaca merino rasa fine vune: grozni (GR), kavkaska (CA), manički merino (MM), sovjetski merino (SM), stavropoljski (ST) i gruborune rase: edilbaevska (ED), karakul (CR) i romanovska (RO). Za proučavanje eritrocita, izabrani su antigeni (krvne grupe) u 1159 uzoraka sa 11 fari. Za ispitivanje DNK mikrosatelita uzeto je 598 uzoraka sa 10 farmi ovaca. Mikrosatelitska analiza pokazala je da najveću polimorfnost ima stavropoljska rasa ovaca gde je identifikovano u proseku 18,27 alela po lokusu. Najmanje vrednosti su utvrđene kod romanovske rasa ovaca - 9.7 alela po lokusu. Minimalna genetska distanca ustanovljena je između rasa grozni i sovjetski merinos - 0.0569 (za mikrosatelite) i 0.0741 (krvne grupa - kasnije u istom redosledu). Između rasa stavropoljska - grozni 0.0861 i 0, 0810; stavropoljska i sovjetski merino 0.0861 i 0.1094. Takođe, relativno bliske su populacije grozni - edilbaevska, grozni - karakul, edilbaevska - karakul: 0.1364 i 0.0851; 0,1620 i 0.1208; 0.1875 i 0,1192. Najveća genetička distanca bila je između Stavropoljske rase i Karakula - 0,2664 i 0.1804, kao i između Romanovske i svih ispitivanih populacija - 0.2491 ... 0.3211 i 0.1734 ... 0.2235.

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