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CHARACTERISTICS OF MICROSATELLITE LOCI OF ORDABASY AND KARAKUL SHEEP

Abstract. In order to accumulate data on the genetic structure of coarse wool breed sheep population, 100 heads were genotyped with respect to 7 microsatellite DNA. Biological materials (histological tissues) were brought from basic farms. DNA analysis and PCR were performed according to existing recommendations. Studies on 7 microsatellite loci are given. 81 alleles were found, of which 43 are alleles in microsatellite loci in Ordabasy sheep and 38 alleles in Karakul sheep. On average, the locus was 6.14±0.65 and 5.43±0.63 alleles, respectively. 45 informative alleles were identified. The average number of informative alleles per locus in the sheep group of Ordabasy and Karakul breeds was 3.29±0.17 and 3.14±0.13, respectively. On average, the number of effective alleles per locus in the populations was 3.11±0.13 and 2.90±0.09. 55 private alleles were identified in the studied populations. Of these, 31 alleles in Ordabasy and 24 alleles in Karakul sheep breed. The average number of private alleles per locus in the populations was 4.44±0.45 and 3.43±0.34, respectively.

Key words: Ordabasy sheep, Karakul sheep, DNA sample, microsatellite, allele, heterozygosity, population.

The most important factor in accelerating scientific and technological progress in animal breeding is the widespread introduction into production of modern advances in biotechnology. This is explained by the fact that the complex processes of interaction of genes and the whole genotype with the environment cause a high phenotypic variability of characters in populations of small sheep breeds, which makes it extremely difficult to analyze the study of quantitative characters [1].

This problem can be largely solved by studying the animal genome using methods based on DNA polymorphism analysis.

The study of the nucleotide genome sequence allows not only to assess the true genetic potential for productivity, but also to obtain highly valuable information for selection, which increase the acceleration of their rates in a given direction of productivity.

Identification of gene variants reflecting the genetic animal potential in terms of productivity allows breed out at the DNA level, i.e. according to the genotype.

Microsatellites are widely used to create genetic maps [2]. Due to their high specificity, microsatellites are the initial marker for identifying individuals [3], according to DNA control in forensic examination, in a biological/evolutionary context they are useful as markers for analyzing the origin [4-6]. The probability of a mismatch is one in a million.

Analysis of microsatellite loci was proposed by Weber et al. [7]. The authors showed the possibility of using microsatellites as genetic markers and proposed an effective method for analyzing these markers – their amplification using PCR with the subsequent separation of the reaction products in polyacrylamide gel, which allowed to sharply increase the sensitivity immunity and speed of analysis compared to traditional methods based on hybridization of genomic blots.

It should be noted that modern methods allow separate fragments that differ by only one pair of nitrogenous bases [8].

The first studies concerning the possibility of using the method of determining the microsatellite polymorphism in controlling the reliability of the origin of farm animals began in the early 90s of the last century, and almost immediately microsatellite markers showed high efficiency in controlling the origin of farm animals [8]. When using a common panel of genetic blood systems (7 blood groups, 16 protein loci and enzymes, and 8 microsatellite loci), the effectiveness in controlling the origin increases to 99.9% [10].

Currently, the success of breeding work depends on the correct identification and certification of animals. Therefore, it is relevant to study the genetic diversity of existing breeds of farm animals and carry out their certification with the use of DNA technologies.

In general, the PCR analysis showed the presence of a low polymorphism of the allelic profile in the studied sheep breeds on the studied microsatellite loci. This indicates that selection for certain signs of productivity in the studied populations is close to the biological boundaries of development. In this regard, it is necessary to assess the degree of genetic diversity of these sheep breeds using genetic markers, which more thoroughly assess the inherited abilities of animals and identify the systems of genes responsible for the development of specific signs of productivity.

Research materials and methods. Sheep of Ordabasy (50 heads, "Seraly" farm) and Karakul breeds (50 heads, "Zhomart" farm) of Arys-Turkestan region were investigated in the experiment.

The material for the study was samples of tissue (earmark) of sheep.

The research work was carried out in the laboratory of LLP "South Western Research Institute of cattle and plant breeding" and Republican state enterprise on the right of economic management "South Kazakhstan State Pharmaceutical Academy".

Sampling for DNA extraction and DNA extraction were carried out according to the Methodological recommendations for the molecular-genetic analysis of sheep using microsatellite markers, 2004 [11].

Statistical data processing was performed by standard methods.

Research results. The analysis of theoretical and practical aspects of methods for assessment and selection of farm animals using DNA technology based on existing genodiagnostics methods (perchlorate, Kawasaki and salt extraction) for sheep genotyping was carried out. An effective variant was chosen DNA extraction from the tissue by the perchlorate method.

In the base specialized farms for breeding sheep of Ordabasy ("Seraly" farm) and Karakul ("Zhomart' farm) breeding sheep, 50 samples of biomaterial were selected (histological tissue from the earmark) for DNA extraction.

Microsatellites are characterized by an unusually high level of polymorphism and Mendeleyev type of research.

In this regard, the authors carried out PCR analysis on 7 loci of microsatellites (tables 1 and 2).

Tables 1 and 2 present the DNA microsatellites used to analyze the coarse wool genotype of Ordabasy and Karakul breeds.

Table 1 – The frequency of occurrence of alleles in the microsatellite loci in sheep of Ordabasy breed

Loci	Number of alleles (unit)	Numerical code of alleles
MAF-214	9	196, 202, 205, 208, 217, 225, 230, 233, 252
DYMS-1	7	145, 148, 156, 160, 166, 174, 233
HSC	6	211, 22, 228, 241, 246, 256
TGLA-53	3	154, 158, 164
ILTS-005	5	181, 188, 192, 199, 214
INRA-23	7	199, 201, 203, 205, 215, 219, 221, 225
INRA-063	6	169, 173, 177, 185, 189, 191
Total	43	

The data in table 1 show the considerable effectiveness of the MAF-214 microsatellite locus when analyzing the coarse wool breed genotype, since this locus differed in the number of occurrence of alleles compared with other loci. A total of 9 alleles were found, with a high occurrence of alleles 196 (37.4%)

and 225 (36.0%). In the DYMS-1 locus, the number of alleles was 7, and among the identified alleles, according to the frequency of occurrence, the allele 174 was different, and made up 34.8% of the total number of alleles in the locus. In the HSC locus, 6 alleles were identified. At the same time, in this locus, the frequency of occurrence of alleles 228 and 246 was high and amounted to 37.8% and 36.6%, respectively. The TGLA-53 microsatellite locus was distinguished by a low number of alleles (only 3 units) and a high frequency of occurrence of allele 154 (40.3%). The use of the ILTS-005 locus identified 5 alleles with a high frequency of alleles 181 (37.5%) and 192 (40.9%). In the INRA-23 and INRA-063 loci, 7 and 6 alleles were found, respectively. Alleles 199 (38.7%) and 205 (44%), as well as alleles 177 (39.7%) and 185 (42.5%) are characterized by high frequency of occurrence. In total, 43 alleles were found in the studied microsatellite loci.

A similar tendency in the frequency of occurrence of alleles in different loci in Karakul sheep can be seen from the data in table 2.

Table 2 – The frequency of occurrence of alleles in various microsatellite loci in sheep of Karakul breed

Loci	Number of alleles (unit)	Rank of alleles
MAF-214	7	184, 192, 202, 210, 215, 220, 230
DYMS-1	6	145, 147, 149, 155, 158, 162
HSC	5	211, 218, 226, 234, 262
TGLA-53	3	143, 150, 165
ILTS-005	3	188, 190, 200
INRA-23	7	203, 205, 209, 211, 223, 225, 229
INRA-063	7	167, 171, 177, 179, 187, 189, 195
Total	38	

For example, the MAF-214, INRA-23 and INRA-063 loci were characterized by a higher genetic diversity. In these microsatellite loci, 7 alleles were identified in each locus. It should be noted that alleles 215 (0.424%), 220 (30.3%), 209 (39.0%), 223 (39.0%), 167 (37.5%) and 179 (38.9%) in the studied loci were encountered with relatively high frequency. The number of alleles in the DYMS-1 and HSC loci is varied within 5-6. The studies showed that the frequency of occurrence of alleles 149, 158, 218, 226 were considerably higher and ranged from 32.3 to 43.5%.

The TGLA-53 and ILTS-005 loci were characterized with a lower frequency of occurrence of alleles, i.e. only 3 alleles were identified per each locus. Such a number of fragments in the microsatellite markers indicate homozygosity of the studied genotypes by this locus.

In general, the number of identified alleles in Karakul sheep population was 38 and varied from 3 to 7 alleles in loci.

When studying the allelic profiles of various genotypes of Ordabasy and Karakul sheep breeds, the following indexes were determined: minimum, maximum and average number of alleles and frequency of occurrence of informative, effective and private alleles.

The results of the analysis of Ordabasy and Karakul sheep breeds' genetic diversity according to the number of total allele and types of alleles per locus of microsatellites are presented in table 3.

The data in table 3 show that, in the above sheep breeds, the average total allele per locus is 6.14±0.65 and 5.43±0.63 alleles.

The total number of informative alleles in sheep of Ordabasy and Karakul breeds on average per locus was 3.29±0.17 and 3.14±0.13, respectively. At the same time, the number of highly informative alleles in Ordabasy breed populations was 30.2±9.5%, and in Karakul breed populations was 34.2±10.1%.

The number of average informative and low informative alleles in the studied populations ranged from 23.3±8.8% and 21.1±8.7% to 46.5±10.4% and 44.7±10.6%.

On average, in populations, the number of effective alleles per locus was 3.11±0.13 and 2.90±0.09%.

In all studied populations, 55 private alleles were found in 7 microsatellite loci. Of these, 31 private alleles were diagnosed in Ordabasy breed and 24 alleles were found in Karakul breed.

The average number of private alleles per locus in the studied populations was 4.45±0.45 and 3.43±0.34, respectively.

Table 3 – The frequency of occurrence of different types of alleles in the studied sheep populations

Types of alleles	Sheep breed	
	Ordabasy	Karakul
Number of alleles, total	43	38
per locus	6.14±0.65	5.43±0.63
Types of alleles: informative, total	23	22
per locus	3.29±0.17	3.14±0.13
including: highly informative, %	30.2±9.5	34.2±10.1
average informative, %	23.3±8.8	21.1±8.7
low informative, %	46.5±10.4	44.7±10.6
effective, total	21.79	20.3
per locus	3.11±0.13	2.90±0.09
private, total	31	24
per locus	4.45±0.45	3.43±0.34

The studies showed that in the populations of Ordabasy sheep breed, the allele 154 of the TGLA-53 locus (40.3%) and the allele 185 of the INRA-063 locus (42.5%) are distinguished by the maximum frequency of occurrence.

In the populations of Karakul sheep breed, 5 alleles have the maximum frequency of occurrence. This is the allele 149 (41.7%) of the DYMS-1 locus, the allele 150 (45.2%) of the TGLA-53 locus, the allele 200 (50.0%) in the ILTS-005 locus, and also the alleles 209 (42.2%) and 179 (41.7%) in the INRA-23 and INRA-063 loci.

The remaining private alleles are found with average and low frequency.

In general, the PCR analysis showed the presence of a low polymorphism of the allelic profile in the studied sheep breeds on the studied microsatellite loci. This indicates that selection for certain signs of productivity in the studied populations is close to the biological boundaries of development. In this regard, it is necessary to assess the degree of genetic diversity of these sheep breeds using genetic markers, which more thoroughly assess the inherited abilities of animals and identify the systems of genes responsible for the development of specific signs of productivity.

Data on the genetic heterogeneity of a population is extremely important for an objective assessment of the genetic diversity and correction of breeding programs. As an index of heterogeneity is a reflection of mutational processes occurring in populations.

As a criterion for assessing the genetic variability in the studied sheep breeds, heterogeneity of allelic variants identified in various microsatellite loci was used.

Table 4 – Level of heterogeneity of various populations

Indexes	Sheep breed	
	Ordabasy	Karakul
Actual degree of heterozygosity, %	0.405±0.029	0.380±0.032
Expected degree of heterozygosity, %	0.675±0.028	0.652±0.031
Lack of heterozygosity, %	-0.270	-0.272

As follows from the data in Table 4, sheep of Ordabasy and Karakul breeds are characterized by a low actual degree of heterozygosity (0.405±0.029 and 0.380±0.032%), which is a consequence of less genetic diversity in these populations.

Heterozygosity does not fully reflect the degree of genetic variability in populations, especially where inbred mating often occurs. Therefore, for an accurate assessment of population variability, the expected heterozygosity index is used, which reflects the level of allelic diversity. And the expected heterozygosity itself is determined on the basis of the allele frequencies obtained from random crossing in a population.

As a result of the study, it was established that the level of the actual degree of heterozygosity in the tested sheep breeds considerably differs from the level of the expected heterozygosity. For example, the expected degree of heterozygosity in the populations of Ordabasy sheep breeds was 0.675±0.028%, and in the populations of Karakul sheep breeds was 0.652±0.031%.

At the same time, the lack of heterozygotes in the studied sheep breeds was 0.270 and 0.272%, which indicates the static reliability of these indexes.

This suggests that in the tested populations, the process of homogenization of allelic variants intensifies and indicates the need to maintain the polymorphism of their allele pool, taking into account various selective values of the genotypes encountered.

As is known, as a result of mutations in all populations, there is a hereditary heterogeneity that creates genetic prerequisites for variability. The law put forward by Hardy-Weinberg, according to which the frequencies of genotypes in a population can be predicted from the frequencies of genes, provided that they are randomly crossed.

The results of the analysis of Ordabasy and Karakul sheep populations' genetic structure by seven microsatellite loci are shown in table 5.

Table 5 – Genetic structure of Ordabasy and Karakul sheep breeds

Indexes	Sheep population	
	Ordabasy	Karakul
Heterozygosity test	-0.321	-0.381
Homozygosity ratio	0.518	0.529
The degree of realization of possible variability,%	56.2	54.9
The level of polymorphism of alleles	1.93	1.89
The average homozygosity of the population, %	59.5	62.0
x^2	40.7	38.5
df	6	6
Reliability	P>0.001	P>0.001
Genetic equilibrium	no	no

Analysis of the genetic structure of various populations showed that the index in the heterozygosity test, reflecting the state of the population in relation to heterozygous genotypes, is characterized by a negative value and indicates a lack of heterozygotes. This index in the populations of Ordabasy breed was -0.321, and Karakul breed was -0.381.

The homozygosity coefficient of Ordabasy sheep population was 0.518 and of Karakul sheep population was 0.529, and the degree of realization of possible variability was 56.2 and 54.9%, respectively.

The level of polymorphism of active effective alleles in the studied microsatellite loci is 1.93 and 1.89, and this value indicates that the number of active alleles in the population for loci is less than the possible one.

The average homozygosity index in the studied populations was 59.5% and 62.0%, respectively.

The gene state was studied by seven microsatellite loci and a significant disruption of gene equilibrium due to the saturation of homozygotes was found and a considerable lack of heterozygotes was observed in the studied populations.

For example, the chi-square (x^2) in the populations of sheep was: in Ordabasy breed -40.7 (P>0.001) and in Karakul breed -38.5 (P>0.001).

In general, the obtained data indicate that the choice and selection carried out has a considerable impact on the genetic state of the population, increasing its "homozygosis".

In this regard, it is necessary to use microsatellites as genetic markers when adjusting the crossing pattern of the genotypes of these sheep breeds.

Assessment of the part of intra-breed and inter-population variability in the total genetic diversity of the studied sheep breeds was carried out on the basis of calculation of the index of the total inbreeding coefficient (Fis) and weighted average Fis and Fit by the studied microsatellite loci.

Table 6 – F-statistics index

F-statistics index	Sheep breed	
	Ordabasy	Karakul
Fis index	0.401±0.131	0.417±0.132
Fit index	0.409±0.058	
Fst index	0.084±0.033	

The Fis index serves as a measure of decrease in the level of heterozygosity of an animal unit caused by non-random mating within each group of genotypes in populations.

This fixation index in the populations of Ordabasy and Karakul sheep breeds was 0.401±0.131 and 0.417±0.132, respectively, and indicates a lack of heterozygotes in the studied breeds.

The Fst index shows a decrease in the level of heterozygosity in a breed, caused by random genetic drift of genes.

The Fst index was calculated as the weighted average by the populations, and it was 0.084±0.033. This indicates that 91.6% of all variability is due to intra-breed diversity and only 8.4% is due to inter-breed diversity.

The index of the total inbreeding coefficient Fit was calculated as the average by the studied populations. Its average value was 0.409±0.058%, which indicates a considerably large lack of heterozygotes (40.9%) in the studied sheep in relation to the total population.

Thus, the indicators of F-statistics indicate a high probability of inbreeding in the studied sheep breeds by microsatellites.

This indicates that the genetic diversity in the studied populations is far from optimal. Selection in such small populations of sheep in the future will not give a perceived effect. Therefore, for the effectiveness of the breeding process in the studied populations of sheep, the choice and selection of highly productive species should be carried out in compliance with strict genetic monitoring. Therefore, the control of the genetic diversity in Karakul and Ordabasy sheep populations will be a considerable factor increasing the expected effect in their selection.

Conclusion. The studies on 7 microsatellite loci were carried out. 81 alleles were found, of which 43 are alleles in the microsatellite loci in Ordabasy sheep and 38 alleles in Karakul sheep. On average, the locus was 6.14±0.65 and 5.43±0.63 alleles, respectively.

45 informative alleles were identified. The average number of informative alleles per locus in the sheep group of Ordabasy and Karakul breeds was 3.29±0.17 and 3.14±0.13, respectively. On average, the number of effective alleles per locus in the populations was 3.11±0.13 and 2.90±0.09. 55 private alleles were identified in the studied populations. Of these, 31 alleles in Ordabasy and 24 alleles in Karakul sheep breed. The average number of private alleles per locus in the populations was 4.44±0.45 and 3.43±0.34, respectively.

The level of observed heterozygosity in each studied sheep breeds was statistically considerably different from the level of expected heterozygosity, which indicates a high probability of inbreeding in the populations by microsatellites.

The inbreeding coefficients Fis and Fit indicate a lack of heterozygotes in the studied breeds. The Fst index indicates a high intra-breed diversity (91.6%) and a low interbreed diversity (8.4%).

A considerable disturbance in the gene equilibrium due to the saturation of homozygotes in the studied populations (x^2=40.7 and 38.5) was found, which require correcting the crossing patterns and selection methods that contribute to the stabilization of the gene frequencies in their allele pool.

The created “Animal Database” system stores data on genotyped animals. Currently, this system stores information about identified and certified 100 animal heads, including 50 heads of Ordabasy breed and 50 heads of Karakul breed.

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ОРДАБАСЫ ЖӘНЕ ҚАРАКӨЛ ҚОЙ ТҰҚЫМДАРЫНЫҢ МИКРОСАТЕЛЛИТТІ ЛОКУСТАРЫНЫҢ СИПАТТАМАСЫ

Аннотация. Асыл тұқымды қойлардың популяциясының генетикалық құрылымы туралы мәліметтерді жинау мақсатында 7 микросателлиттік локус бойынша 100 бас қой генотиптелді. Биологиялық материалдар (гистологиялық ұлпалар) шаруашылықтардан алынды. Ордабасы және қаракөл қой тұқымдарының құлақ шеміршегінен бөлініп алынған ДНК фрагментін электрофорез тәсілі арқылы жіктелінді. 81 аллелдің ішінде 43 аллелдер ордабасы қойлардың микросателлиттік локусында және 38 аллелдер қаракөл қойлар популяциясында анықталған. Орта есеппен локусқа 6,14±0,65 және 5,43±0,63 аллелдер тиісінше құрады.

45 ақпараттық аллельдер анықталынды. Ақпараттық аллельдер локусқа шаққанда орта есеппен ордабасы мен қаракөл қойларында 3,29±0,17 және 3,14±0,13 болды. Популяциялар бойынша тиімді аллельдер көрсеткіші орта есеппен 3,11±0,13 және 2,90±0,09 құрады. Зерттелген популяцияларда 55 өзіне тән аллельдер анықталынды. Олардың ішінде 31 аллельдер ордабасы қойларында және 24 аллельдер қаракөл қойларында болды. Популяцияларда бір локусқа шаққанда өзіне тән аллельдер саны тиісінше 4,44±0,45 және 3,43±0,34 көрсетті.

Түйін сөздер: Ордабасы қой тұқымы, қаракөл қой тұқымы, ДНК үлгі, микросателлит, аллель, гетерозигота, популяция.

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ХАРАКТЕРИСТИКА МИКРОСАТЕЛЛИТНЫХ ЛОКУСОВ ОРДАБАСИНСКОЙ И КАРАКУЛЬСКОЙ ПОРОД ОВЕЦ

Аннотация. С целью накопления данных о генетической структуре популяции грубошерстной породы овец были генотипированы 100 голов по 7 микросателлитным ДНК. Биологические материалы (гистологические ткани) были привезены из базовых хозяйств. Анализ ДНК и постановку ПЦР выполняли согласно существующим рекомендациям. Приведены исследования по 7 локусам микросателлитов. Обнаружено 81 аллелей, из них 43 аллелей в микросателлитных локусах у овец ордабасинской породы и 38 аллелей у популяции каракульских овец. В среднем на локус составил 6,14±0,65 и 5,43±0,63 аллелей соответственно. Выявлено 45 информативных аллелей. Среднее число информативных аллелей на локус в группе овец ордабасинской и каракульской породы составило 3,29±0,17 и 3,14±0,13 соответственно. В среднем по популяцием число эффективных аллелей на локус составил 3,11±0,13 и 2,90±0,09. В исследованных популяциях выявлено 55 приватных аллелей. Из них 31 аллелей у ордабасинской и 24 аллелей у каракульской породы овец. Среднее число приватных аллелей на локус в популяциях составило 4,44±0,45 и 3,43±0,34 соответственно.

Ключевые слова: Ордабасинская порода овец, каракульская порода овец, образец ДНК, микросателлит, аллель, гетерозиготность, популяция.

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