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# MOLECULAR GENETIC PROFILE
# OF KAZAKHSTAN POPULATIONS OF CATTLE BREEDS

**Abstract.** Modern methods of cattle breeding provide for active use of new breeding programs. The introduction of molecular-genetic studies predetermines the prospects for the use of DNA microsatellites (STR-loci). These microsatellites are widely used for studying the allele pool of farm animals. Based on the above-mentioned, the aim of the research was to study the genetic structure and analysis of the degree of genetic differentiation of cattle breeds in the Republic of Kazakhstan, based on molecular genetic information.As a biological material for research, the semen of bulls was used. The paper presents research materials of DNA profiles on 11 microsatellite loci of dairy (Holstein, Black-and-white, Aulie-Ata), combined (Alatau) and meat (Auliekol, Kazakh whiteheaded) breeds.

The microsatellite profile of animals was represented by the following loci: BM1824, ETH225, INRA23, BM2113, SPS115, ETH10, TGLA122, TGLA126, TGLA227, ETH3, TGLA53, included in the recommended panel ICAR and ISAG. The genetic pattern of populations was analyzed according to F-statistics. Genetic identity indicators were calculated according to Nei. Populations' heterozygosity was determined according to the Wright's fixation index. The differences in breeds in the direction of productivity were analyzed by the share of variations of microsatellite loci. As a result of research, it was found that the implementation of molecular genetic methods in the selection of farm animals will significantly increase the development potential of cattle breeding resources available in the Republic of Kazakhstan.

**Keywords:** genetic potential, genetic progress, breeding value, genomic assessment, microsatellites, DNA profile.

**Introduction.** At breeding farm animals, it is important not only to obtain high productive livestock, but also continuous advance, improvement of the genetic potential of their productive qualities and acceleration of genetic progress in breeds [1]. It is known that cattle breeding methods provide for active use of modern breeding programs [2].

Currently, animal identification methods are based on the analysis of two main types of genetic markers - single nucleotide polymorphism (SNP) and microsatellites (Short tandem repeat, STR) [3, 4].

Undoubtedly, the analysis of a large number of genetic markers (SNP and STR) allows to solve a number of acute issues regarding the population of animals, as well as the "purity" of the genotype of the analyzed individual, which is important from the point of view of conservation of animal genetic diversity.

It is known that microsatellite DNA loci, consisted of STR - Short Tandem Repeats, are widely used in animal husbandry as genetic markers. The use of such highly polymorphic loci gives information about the genome of animals, determines their individuality and genetic uniqueness, which should be taken into account in breeding programs when improving livestock [5-7].

According to research by a number of scientists such as Gautier et al., 2007, Li et al., 2007, Zhang et al., 2007, Flury et al., 2009, Sodhi et al., 2011, microsatellite markers are the most common tool for characterizing and differentiating population structures. Over the past 15 years, the relevance of using microsatellite markers in assessing the genetic diversity of cattle breeds has been documented in numerous studies [8-12].

Single nucleotide polymorphisms (SNPs) play an important role in programs for genetic assessment of livestock, as they can help improve the accuracy of animal genome predictions and genome selection of economically important traits. Additionally, SNP markers can help identify genes affecting economic traits [13].

The results of full genomic research by a team of scientists: Hayes B., Goddard M., Meuwissen T. proved that the effect of individual quantitative trait loci (QTL) on the productivity of animals is small. The marker-assisted selection (MAS) takes into account a small number of DNA markers, therefore, it is more difficult to explain the genetic variability of traits. In this regard, there was a developed technology using information of single nucleotide polymorphisms (SNP), resulting in genomic assessment of animal. The development of genomic assessment methods significantly intensifies the breeding process of the entire population [14, 15]. High information value of SNP - genetic markers associated with the desired combination of manifestations of economic traits was proved.

Currently, genomic selection is widely used in dairy cattle breeding. According to numerous studies, in theory, the inclusion of information about the markers increases the effectiveness of breeding programs compared to traditional selection. However, previously only a few genes were reported associated with changes in dairy productivity, and they explained only a small part of the hereditary variation of cattle [16-20].

Genomic selection is based on the genomic estimated breeding value (GEBV) of animals. GEBV is calculated as the sum of the effects of genetic markers or haplotypes of these markers over the entire genome, thus potentially capturing all QTLs that contribute to the variability of the trait. The reliability of GEBV has been proven more than once in the world. For example, scientists from the United States, New Zealand, Australia and the Netherlands used in their research the population of Holstein-Friesian bulls, tested for the quality of their offspring, having offspring from 650-4500 animals.The studied bulls have been genotyped by 50,000 full-genome markers. As a result, it was found that the reliability of GEBV of the population ranged from 20 to 67%, without taking into account the producers' estimation of the quality of the offspring. However, one should not lose sight of the fact that the reliability of the assessment also depends on the studied trait [16]. For example, in the works of many scientists, it was found that for milk productivity traits with heritability $h2=0.281-0.401$, the accuracy of GEBV for young bulls was in the range from $r2=0.180$ to $r2=0.347$, and for fertility rates with $h2=0.035-0.068$ GEBV reliability was higher and amounted to $r2=0.428-0.515$, the genomic assessment allowed to increase the accuracy of the prediction of the genotype by an average of 30.5%, which is equivalent to the presence of indicators of $\approx 10$ daughters [21].

Studies by a number of scientists such as N. Zinovyeva, N. Strekozov, I. Yanchukov, A. Ermilov, G. Eskin proved that the genomic assessment system plays an important role in ensuring the competitiveness of breeding material, its creation is one of the priorities of the development of livestock breeding [22].

In the opinion of scientists Guarini A.R., Lourenco D.A.L., Brito L.F., Sargolzaei M., Baes C.F., Miglior F., Misztal I., Schenkel F.S., the success and sustainability of a breeding program that includes genomic information, depends largely on the prediction accuracy. To achieve high accuracy of GEBV, large training populations with low heritability traits are required. By means of simultaneously including genotyped and non-genotyped animals in the assessment, the BLUP's step-by-step genomic approach (ssGBLUP) can provide more accurate and less biased genomic estimates [23].

After obtaining the results of the genomic assessment in the United States, some countries, such as Canada, Australia, New Zealand, France, the Netherlands, Germany, Denmark, Israel, Poland, China, also began to use genomic assessment in practical selection and today they have a lot of positive results [24]. In this connection, a need arises to study the prospects of using the results of molecular-genetic research in Kazakhstan.

Within the Republic of Kazakhstan, there are over 20 cattle breeds of different directions of productivity. To date, the gene pool of these animals at the molecular genetic level is not fully explored. In this regard, the use of the DNA information of the animal genotype will make it possible to introduce into the selection practice a number of advantages over traditional methods of selection.DNA diagnostics of animal genotypes can be performed at an early age. It should be noted that pre-selection of animals is a prospective source of bias in international animal assessments, if not properly taken into account in

national assessments. However, pre-selection does not create bias in the traditional assessment of breeding value, if it includes data from all animals.

The country has accumulated a lot of data that allow to conduct an effective selective and breeding work with animals of dairy cattle breeds. Based on modern conditions for the breeding of highly productive herds, a new methodological basis is essential, which takes into account genetic factors. The application of genetic markers is especially important for the assessment of traits, the phenotypic manifestation of which occurs relatively late or is limited by sex, also for traits that are strongly influenced by non-genetic factors (e.g., environmental factors).Currently, the only effective way to control the reliability of the origin and identification of livestock is genetic testing based on the use of the phenomenon of genetic polymorphism [25].

Unfortunately, in our country, genomic evaluation is practically not used to assess the breeding value of animals at pedigree levels of management. However, it is applicable solely to assess the breeding values of servicing bulls.

At the moment, about 60% of countries participating in international assessments of servicing bulls have already adopted genomic selection in their animal breeding programs. Thus, the data sent for multiple international assessments can be quite diversified, and to ensure a fair comparison of the estimates of animals included in international genetic assessments, an appropriate test method is required for all countries [26].

Thus, molecular-genetic methods enable to select among animals of very early ages, which significantly increases the efficiency. From this, it follows that the introduction of molecular genetic methods in the selection of animals is crucial.

Based on the above-mentioned, the aim of the research was to study the genetic structure and analysis of the degree of genetic differentiation of cattle breeds in Kazakhstan based on molecular genetic information.

The novelty of the research lies in the fact that for the first time the study of allelic polymorphism on 11 microsatellites of dairy, combined and meat productivity breeds related to the breeding resources of Kazakhstan was conducted. Reliably determined the importance of the use of molecular genetic markers in breeding work.

**Methods of research.** Biological material for research was semen of bulls. Samples of biological material (sperm) of servicing bulls were used to create a database of reference samples. The studies were carried out in the Laboratory of Molecular Bases of Breeding of the Department of Biotechnology and Molecular Diagnostics of Animals at the Federal Science Center for Animal Husbandry named after L.K. Ernst.

DNA extraction from semen samples was performed using the DNA-EXTRAN-2 reagent kit (SYNTHOL EX-511-100, Russia). DNA extraction was carried out using the protocol in accordance with the recommendations of the manufacturers.

In the course of the research, DNA profiles on 11 microsatellite loci of bulls of dairy (Holstein breed - 34 animals, Black-and-white - 18 animals, Aulie-Ata - 5 animals), combined (Alatau - 18 animals) and meat breeds (Auliekol - 5 animals, Kazakh whiteheaded - 14 animals), belonging to the Asyltulik JSC. The microsatellite profile of animals was determined by DNA analyzer with a laser detector ABI3130xl by the following loci: *BM1824, ETH225, INRA23, BM2113, SPS115, ETH10, TGLA122, TGLA126, TGLA227, ETH3, TGLA53,* included in the recommended panelICARandISAG.*GenAlEx 6.501*and *structure 2.3*programs were used for the analysis of the results, *MicrosoftExcel 2013*software was used for data visualization.

**Research results and their discussion**. In order to define the community of populations origin, the $F_{st}$ (fixation index) coefficients were calculated. In the course of the work, pairwise analysis of the genetic structure of the studied populations was made. The obtained $F_{st}$ data allowed to establish the degree of divergence between populations according to the direction of their productivity. The research results are summarized in table 1.

According to the data given in table 1, above the diagonal, the smallest genetic distances were observed between the Black-and-white and Holstein (0.016) breeds that indicates a high degree of divergence. However, one should not forget that this coefficient also testifies to the general origin of the above-mentioned breeds. The genetic distances between the dairy breeds of the European and Kazakh breeding were: on the one hand, in the Black-and-white, Aulie-Ata and Alatau - 0.064 and 0.107 respectively, on

the other hand, in Holstein - 0.078 and 0.119, respectively.The coefficients between the Black-and-white and Aulie-Ata, Black-and-white and Alatau, Holstein and Aulie-Ata breeds show the average degree of divergence, i.e. they confirm the common origin and direction of productivity. Between the Holstein and Alatau breeds, a large degree of divergence was observed. For meat breeds, the $F_{st}$ index between Auliekol and Kazakh whiteheaded breeds was less than 0.058, which indicates a weak degree of divergence. This fact confirms the history of the creation of the Auliekol breed, as it is known, this breed was created using the Kazakh whiteheaded cows and the Charolais and Aberdeen Angus bulls.

Table 1 – Above the diagonal - the degree of divergence ($F_{st}$) in populations, below the diagonal - analysis of genetic identity according to Nei

| | Black-and-white | Holstein | Aulie-Ata | Alatau | Auliekol | Kazakh whiteheaded |
|---|---|---|---|---|---|---|
| Black-and-white | – | 0.016 | 0.064 | 0.107 | 0.122 | 0.098 |
| Holstein | 0.910 | – | 0.078 | 0.119 | 0.133 | 0.104 |
| Aulie-Ata | 0.556 | 0.500 | – | 0.054 | 0.074 | 0.061 |
| Alatau | 0.214 | 0.207 | 0.600 | – | 0.052 | 0.043 |
| Auliekol | 0.203 | 0.195 | 0.512 | 0.667 | – | 0.058 |
| Kazakh whiteheaded | 0.295 | 0.323 | 0.555 | 0.678 | 0.627 | – |

According to the results of the analysis of genetic distances, indicators of genetic identity (n = 94) were calculated according to Nei [7]. Calculations of the analysis of genetic identity are shown in Table 1 below the diagonal.

The calculation of the genetic distances between the studied breeds, carried out according to Nei, showed that the Black-and-white and Holstein breeds were characterized by the greatest affinity in the genetic structure due to the common origin - 91%. For Black-and-white and Aulie-Ata cattle, affinity was 56%. For the Kazakh whiteheaded and Auliekol breeds, the identity coefficient was 63%. The greatest differences, as one would expect, were between the populations of dairy, combined and meat breeds from 20.3% to 32.3%, i.e. populations are characterized by a high degree of differentiation. This fact explains the breeding pressure by the traits of breed productivity.

Polymorphism of loci, estimated by the number of alleles per locus, diversity of alleles, average value of the total number of alleles, heterozygosity, and the informational content of polymorphism are described in table 2.

For 11 loci of three populations, 253 alleles were found in our study. The number of alleles per locus for dairy breeds ranged from 4 (TGLA126A) to 8 (TGLA122A, TGLA53A) with an average value of 6, for mixed breeds - from 3 (TGLA126A) to 14 alleles (BM2113A, TGLA227A) with an average value of 9, and for meat breeds - from 4 (BM1824A, TGLA126A) to 11 alleles (BM2113A, TGLA227A) with an average value 8.

The observed heterozygosity (Ho) in the dairy cattle population varied from 0.563 (SPS115A) to 0.867 (BM2113A), in the combined cattle population from 0.667 (BM1824A) to 1.000 (TGLA227A), in beef cattle - from 0.684 (BM1824A) to 0.902 (BM1824A).

The expected heterozygosity (He) in the dairy cattle population varied from 0.627 (SPS115A) to 0.810 (TGLA122A), in the combined cattle population from 0.554 (TGLA126A) to 0.898 (TGLA127A), and for meat cattle from 0.601 (TGLA126A) to 0.859 (BM2113A).

Unbiased expected heterozygosity (uHe) for dairy cattle ranged between 0.663 (SPS115A) and 0.852 (BM2113A), for cattle of the combined productivity direction - from 0.570 (TGLA126A) to 0.924, (TGLA227A), for beef direction - from 0.626 (TGLA126A) to 0.894 (BM2113A).

Of 11 loci, 7 loci of the dairy population had negative inbreeding coefficients (Fis). Negative Fis in the population of the combined productivity direction was in 6 loci, and in the beef cattle population - 7 loci.

The calculation of the analysis of the genetic diversity of the studied breeds (Fit) at the molecular level showed that the varianceamong populations is 13%, among breeds - 2%, and among individuals (or intra-breed variations) - 85%. The results of the analysis are shown in figure 1.

Table 2 – Polymorphism of 11 loci in three populations

| Pop | | BM 1824A | ETH 225A | INRA 023A | BM 2113A | SPS 115A | ETH 10A | TGLA 122A | TGLA 126A | TGLA 227A | ETH 3A | TGLA 53A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dairy | N | 19 | 19 | 18 | 19 | 19 | 19 | 19 | 19 | 14 | 19 | 19 |
| | Na | 5 | 6 | 7 | 7 | 5 | 7 | 8 | 4 | 7 | 5 | 8 |
| | Ne | 4 | 4 | 4 | 6 | 3 | 5 | 5 | 3 | 5 | 4 | 5 |
| | Ho | 0.701 | 0.793 | 0.840 | 0.867 | 0.563 | 0.829 | 0.688 | 0.564 | 0.804 | 0.829 | 0.809 |
| | He | 0.707 | 0.743 | 0.771 | 0.808 | 0.627 | 0.803 | 0.810 | 0.648 | 0.797 | 0.738 | 0.805 |
| | uHe | 0.743 | 0.784 | 0.811 | 0.852 | 0.663 | 0.846 | 0.851 | 0.681 | 0.841 | 0.778 | 0.847 |
| | Fis | 0.021 | -0.062 | -0.091 | -0.075 | 0.059 | -0.029 | 0.153 | 0.128 | -0.009 | -0.122 | -0.006 |
| Combined | N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | Na | 4 | 10 | 10 | 14 | 8 | 8 | 14 | 3 | 14 | 5 | 9 |
| | Ne | 3 | 7 | 6 | 11 | 4 | 6 | 10 | 2 | 10 | 2 | 5 |
| | Ho | 0.667 | 0.833 | 0.833 | 0.778 | 0.833 | 0.722 | 0.889 | 0.889 | 1.000 | 0.889 | 0.833 |
| | He | 0.693 | 0.856 | 0.824 | 0.910 | 0.750 | 0.836 | 0.897 | 0.554 | 0.898 | 0.591 | 0.793 |
| | uHe | 0.713 | 0.881 | 0.848 | 0.937 | 0.771 | 0.860 | 0.922 | 0.570 | 0.924 | 0.608 | 0.816 |
| | Fis | 0.038 | 0.027 | -0.011 | 0.146 | -0.111 | 0.137 | 0.009 | -0.604 | -0.113 | -0.504 | -0.051 |
| Meat | N | 19 | 19 | 18 | 19 | 19 | 19 | 19 | 19 | 16 | 19 | 19 |
| | Na | 4 | 8 | 8 | 11 | 7 | 8 | 11 | 4 | 11 | 5 | 9 |
| | Ne | 3 | 6 | 5 | 9 | 3 | 6 | 7 | 3 | 7 | 3 | 5 |
| | Ho | 0.684 | 0.813 | 0.837 | 0.822 | 0.698 | 0.776 | 0.788 | 0.726 | 0.902 | 0.859 | 0.821 |
| | He | 0.700 | 0.800 | 0.798 | 0.859 | 0.688 | 0.820 | 0.853 | 0.601 | 0.847 | 0.665 | 0.799 |
| | uHe | 0.728 | 0.833 | 0.829 | 0.894 | 0.717 | 0.853 | 0.887 | 0.626 | 0.883 | 0.693 | 0.831 |
| | Fis | 0.029 | -0.017 | -0.051 | 0.036 | -0.026 | 0.054 | 0.081 | -0.238 | -0.061 | -0.313 | -0.028 |

*N = number of alleles; Na = number of alleles per locus; Ne= number of effective alleles = 1 / (Sumpi^2); Ho = observed heterozygosity = No. ofHets / N; He = expected heterozygosity = 1 - Sumpi^2; uHe = Unbiased expected heterozygosity = (2N / (2N-1)) \* He; Fis= inbreeding coefficient = (Mean He - Mean Ho) / Mean He.*
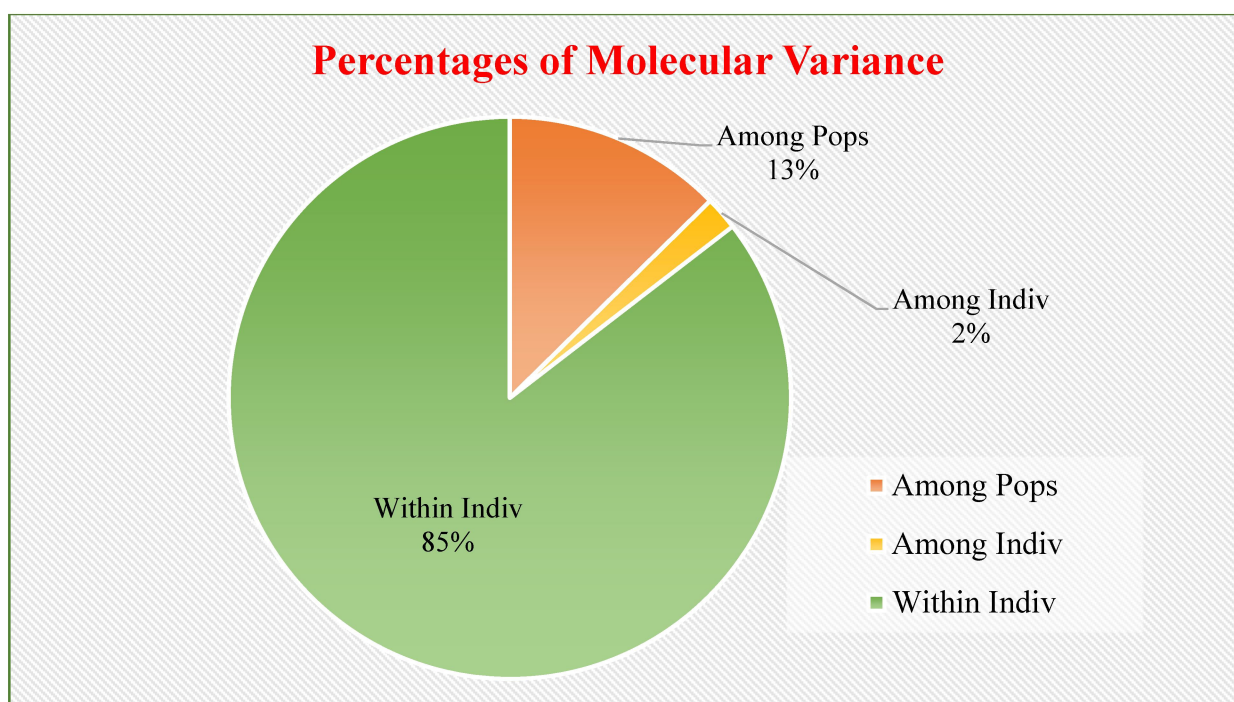


Figure 1 – Genetic diversity of the population

The spatial distribution of breeds in the coordinates of genetic variability is shown in figure 2. The Black-and-white, Holstein and Aulie-Ata breeds are grouped distinctly from the other three breeds. In turn, the Kazakh whiteheaded, Auliekol, and Alatau breeds formed their own separate cluster.
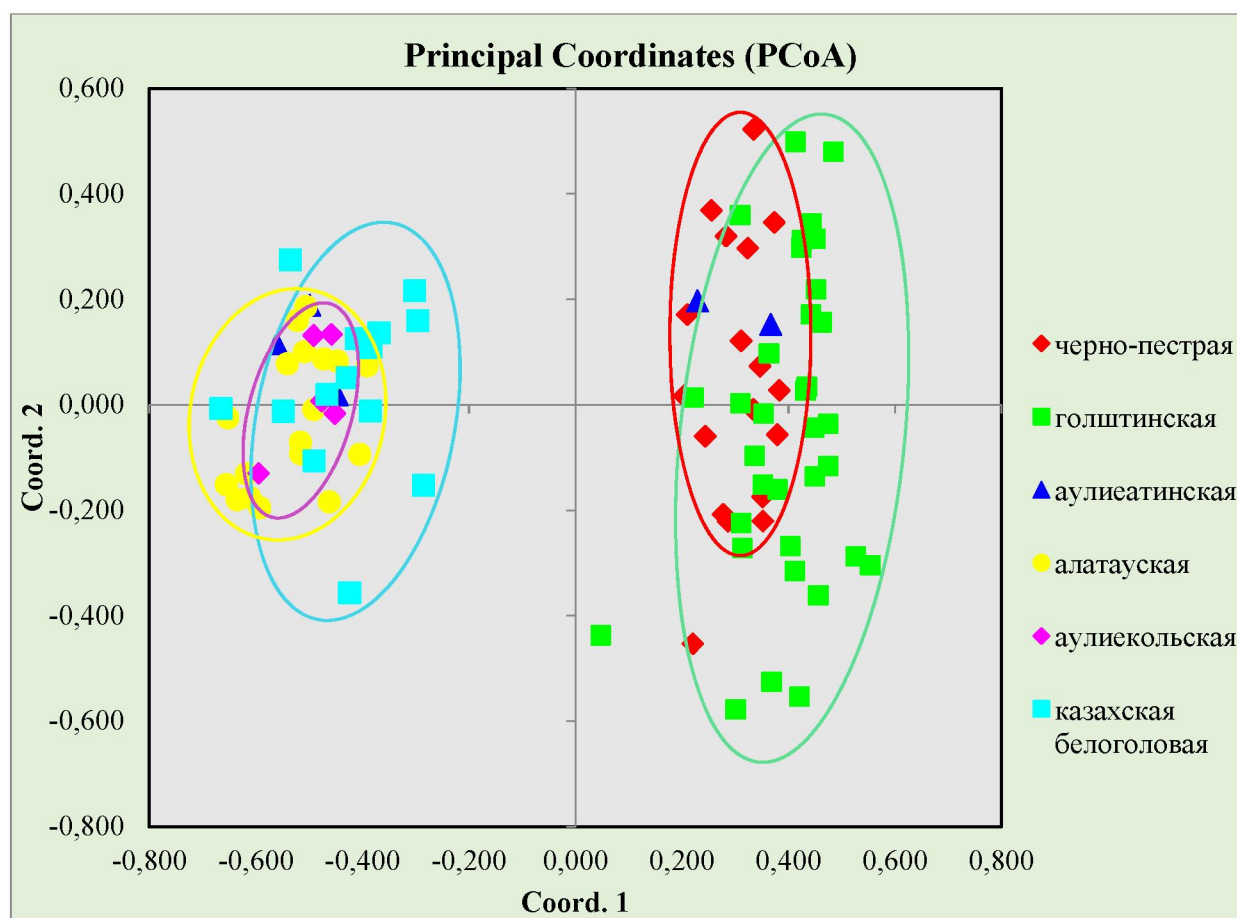


Figure 2 – Spatial distribution of breeds in the coordinates of genetic variability

The analysis based on microsatellite markers seems to indicate that the breeds within the clusters have a similar gene pool. This underscores the need for additional microsatellite markers to more accurately identify the aforementioned clusters.

The share of variation in microsatellite loci explaining the diversity of breeds in the direction of productivity for the first component (PC1) reached 17.4%, for the second (PC2) - 4.9%.

Thus, studies based on microsatellite markers, as well as single nucleotide polymorphisms (SNPs), have shown genetic variation between related breeds, as a result of which there is a fairly clear division into clusters.

**Conclusions.** The introduction of molecular genetic methods in breeding will significantly increase the development potential of breeding resources available on the territory of the Republic of Kazakhstan due to own reproduction of livestock of various breeds of different productivity direction [29]. These methods will create a basis for the implementation of such approaches as genomic selection, which in turn will provide an increase in the intensity of the breeding process.

Overall, the progress in the field of applied research and the active implementation of their results in practice will further deepen research and empower genetic investigations in Kazakhstan.

Concluding the above-mentioned data, it is important to note that the present study provides valuable information on the genetic diversity of cattle in Kazakhstan and lays the foundation for future more in-depth research.

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## ІРІ ҚАРА МАЛ ТҰҚЫМДАРЫНЫҢ ҚАЗАҚСТАНДЫҚ ПОПУЛЯЦИЯСЫНЫҢ МОЛЕКУЛЯРЛЫ-ГЕНЕТИКАЛЫҚ ПРОФИЛІ

**Аннотация.** Ірі қара мал өсірудің заманауи әдістері селекциялық бағдарламалардың жаңа тәсілдерін кеңінен пайдалануға негізделген. Молекулярлы-генетикалық зерттеулерді енгізу – келешекте ДНК микросателлиттерін (STR-локустар) пайдалануды көздейді. Қазіргі таңда аталмыш микросателлиттер ауыл шаруашылығы малдарының аллелофондын зерттеу барысында кеңінен пайдаланылуда. Осы орайда, молекулярлы-генетикалық ақпарат негізінде Қазақстан республикасында өсірілетін ірі қара мал тұқымдарының генетикалық құрылымы мен генетикалық дифференциясының дәрежесін талдау – зерттеу жұмыстарының мақсаты болып табылды. Биологиялық материал ретінде асыл тұқымды бұқалардың ұрығы алынды. Аталмыш жұмыста сүт бағытындағы (голштин, қара-ала, әулеата), қос бағыттағы (алатау) және ет бағытындағы (әулиекөл, қазақтың ақбас) тұқымдардың 11 микросателлитті локустары бойынша ДНК-профильдерін зерттеу мәліметтері келтірілген.

Жануарлардың микросателлитті профилі, ICAR және ISAG мекемелерімен ұсынылған панельге кіретін, BM1824, ETH225, INRA23, BM2113, SPS115, ETH10, TGLA122, TGLA126, TGLA227, ETH3, TGLA53 локустарымен көрсетілді. Популяциялардың генетикалық құрылымы F-статистикасына сәйкес талданды. Генетикалық ұқсастық көрсеткіштері Ней бойынша есептелінді. Популяциялардың гетерозиготалығы Райттың фиксация индексіне сәйкес анықталынды. Тұқымдардың өнімділік бағыты бойынша ажырауы микросателлитті локустардың вариация үлесінің негізінде талданды. Зерттеу жұмыстарының нәтижесінде ауыл шаруашылығы малының селекциясына молекулярлы-генетикалық әдістерді енгізу – Қазақстан Республикасындағы асыл тұқымды мал қорларының даму әлеуетін едәуір жоғарылататыны айқындалды.

**Түйін сөздер:** генетикалық әлеует, генетикалық прогресс, асыл тұқымдық құндылық, геномдық бағалау, микросателлиттер, ДНК-пішін.

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## МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ ПРОФИЛЬ КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ ПОРОД КРУПНОГО РОГАТОГО СКОТА

**Аннотация.** Современные методы разведения крупного рогатого скота предусматривают активное использование новых селекционных программ. Внедрение молекулярно-генетических исследований предопределяет перспективы использования микросателлитов ДНК (STR-локусов). Данные микросателлиты получили широкое применение для изучения аллелофонда сельскохозяйственных животных. Исходя из вышесказанного, целью исследований явилось изучение генетической структуры и анализ степени генетической дифференциации пород крупного рогатого скота, разводимых в Республике Казахстан, на основе молекулярно-генетической информации. В качестве биологического материала для исследований использовалось семя быков-производителей. В работе приведены материалы исследований ДНК-профилей по 11 микросателлитным локусам молочных (голштинская, черно-пестрая, аулиеатинская), комбинированных (алатауская) и мясных (аулиекольская, казахская белоголовая) пород.

Микросателлитный профиль животных был представлен локусами: BM1824, ETH225, INRA23, BM2113, SPS115, ETH10, TGLA122, TGLA126, TGLA227, ETH3, TGLA53, входящими в рекомендованную панель ICAR и ISAG. Генетическую структуру популяций анализировали согласноF-статистики. Показатели генетической идентичности вычислены по Нею. Герерозиготность популяций определена согласно индексу фиксации Райта. Расхождение пород по направлению продуктивности было проанализировано по доле вариаций микросателлитных локусов. В результате исследований было установлено, что внедрение молекулярно-генетических методов в селекцию сельскохозяйственных животных существенно повысит имеющийся на территории Республики Казахстан потенциал развития племенных ресурсов крупного рогатого скота.

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