Inter-population genetic diversity of cattle of the Kazakhstan population of Santa Gertrude Breed of Zhetsu type by microsatellite DNA

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Abstract. Analysis of the studied parameters of the population and genetic structure the cattle of Zhetsu type of Santa Gertrude breed has confirmed the presence of poorly differentiated subpopulations of animals in the southern and southeastern regions of Kazakhstan. The population and genetic structure of Zhetsu type of Santa Gertrude breed shows the differentiation of the population as a whole. The total number of alleles found in 11 microsatellite loci made up 135 including 121 – informative alleles, 90.72 – effective, and 14 – private ones. The average expected heterozygosity levels range from 0.7997 to 0.9117. According to Fis coefficient (individual fixation index), an excess of heterozygotes was found at TGLA53, ETH10, TGLA122, INRA23 and BM1824 loci. In other loci, a deficiency of heterozygotes is observed. The inbreeding coefficient Fst (subpopulation fixation index) made up 0.0094 which indicates the subdivision of the population ($\chi^2 = 34.30, df = 1$, significance level $\alpha<0.001$).

1. Introduction
The scientists of the Kazakh Scientific Research Institute of Animal Husbandry and Feed Production, together with specialists from agricultural enterprises, have created a new intra-breed (zonal) cattle type “Zhetsu” (zhetsu) of Santa Gertrude breed. In the process of creating this inbreed type, the methods of crossing and breeding “in oneself” of 7/8 and 13/16 blood hybrids of Santa Gertrude breed with local Kazakh cattle were used.

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 [1].
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) [2-4].

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 [5-7].

The Creole populations display a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity, whereas the British breeds displayed reduced levels of genetic diversity. The analysis of molecular variance indicated that 7.8% of variance can be explained by differences among taurine and zebu breeds. Consistent with these results, the first principal component (PC), which comprised the 40% of the total variance, clearly distinguishes these two groups. In addition, all constructed phylogenetic trees cluster together Nelore and Brahman breeds with robust bootstrap values. Only 1% of variance was due to difference between American Creole and European taurine cattle. Although this secondary split was supported by the classical genetic distance and the second PC (15%), the topology of trees is not particularly robust. The presence of zebu-specific alleles in Creole cattle allowed estimating a moderate degree of zebu admixture. When these data were compared with mitochondrial and Y chromosomal studies, a clear pattern of male-mediated introgression was revealed. The results presented here contribute to the understanding of origin and history of the American Creole cattle [8].

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 [1].

The purpose of the study is to evaluate the interpopulation genetic differentiation of Zhetysu type of Santa Gertrude cattle breed in Kazakhstan by the polymorphism of microsatellite DNA loci.

2. Materials and methods
The biological samples (hair follicles) of 426 animals from seven households served as material. The Kazakhstan animal population was divided into two subpopulations depending on their geographical location. The first subpopulation (SGT_1) includes 1 household with a sample size of 100 animals located in the south (Zhambyl district, Almaty region); the second subpopulation (SGT_2) – 6 households – in the southeast of the Republic of Kazakhstan (Panfilovskiy district, Almaty region) with a sample size of 326 animals.

DNA isolation was carried out in accordance with the protocol of the reagent manufacturer (Invitrogen, Applied Biosystems, USA). Then, 10 μL of 0.5 ng/μL DNA preparations were placed in standard 1.5 mL microcentrifuge tubes. 300 μl of PrepFiler™ Lysis buffer and 3 μL of 1.0 MDTT were added. Stirred on a vortex at low rpm for 5 seconds, then briefly centrifuged. The samples were incubated in a heat shaker at 70 °C and 900 rpm for 40 min. The microcentrifuge tubes were centrifuged at 12000 g for 2 min. The lysate was transferred to a clean 1.5 mL tube. 15 μL of magnetic particles were added to tubes containing lysate. Stirred on a vortex at low rpm for 5 sec, then briefly centrifuged. 180 μL of isopropanol (Binding buffer) was added. Stirred on a vortex at low rpm for 5 sec, then briefly centrifuged. The tubes were placed in a shaker and stirred for 10 min at 1000 rpm at room temperature. Samples were mixed
on a vortex. The tubes were placed in a magnetic tripod and held until the precipitate stopped accumulating (2 min). By holding the tubes in a magnetic tripod, the supernatants were collected and removed. Added on 300 μL PrepFiler™ Wash buffer to test tubes. It was mixed on the vortex at maximum speed so that no magnetic particles were visible on the walls of the tubes, then briefly centrifuged. The tubes were placed in a magnetic tripod for 30-60 sec. By holding the tubes in a magnetic tripod, the supernatants were collected and removed. This washing step was repeated three times. While holding the tubes in a magnetic tripod, the magnetic particles were dried for 7 min on a table. Added on 50 μL PrepFiler™ Elution Buffer to test tubes. It was mixed on the vortex at a maximum speed of 5 seconds so that no magnetic particles were visible on the walls of the tubes, then briefly centrifuged. The tubes were placed in a magnetic tripod for 7 min. The supernatant (which should contain genomic DNA) was transferred to a new 1.5 mL storage tube.

Multiplex cattle genotyping was performed using Stock Marks Cattle kit (Applied Biosystems, USA) at 11 loci recommended by the International Society for Animal Genetics (ISAG). The technology is based on the analysis of the polymorphism of STR loci (short tandem repeats) - microsatellites that are evenly distributed throughout the genome.

Amplification products were identified on a genetic analyzer ABI Prism 310 (Applied Biosystems, USA).

Identification of amplification products was executed using the genetic ABI Seq Studio analyzer (Applied Biosystems, USA) with a capillary electrophoresis. Interpretation of the received graphic results was carried out in the Gene Mapper 5.0 program.

For describing polymorphism, the following indicators were used: allele frequency, the average observed and expected heterozygosity as well as the average heterozygosity on loci, number of alleles in a locus, number of informative alleles (frequent, with more than 1% frequency), number private alleles (rare, with less than 1% frequency) in a locus, number of effective alleles and the individual index of fixing Fis.

Biometric processing of population genetic parameters was performed using the statistical package and Fortran Power Stationv 2.0 software package [1].

Alleles frequency of occurrence, a minimum, a maximum and an average number of alleles, alleles frequencies, a number of informative alleles, a number of effective alleles, private alleles number and frequencies of occurrence were determined.

Allele frequencies were calculated separately for each locus according to the equation (1):

\[ P_i = \frac{N_p}{2N} \]  

where \( P_i \) – the i-th allele frequency of occurrence, \( N_p \) – quantity of the i-th allele, in sampling, \( 2N \) – number of animals in sampling.

The number of informative alleles was calculated as number of alleles in population with a frequency of occurrence more than 1%.

The number of effective alleles, i.e. number of the alleles meeting with equal frequency in ideal population which is necessary for receiving the same degree of homozygosity or a genetic variety in real population, were calculated by a formula (2):

\[ Ne = \frac{1}{1 - He} \]  

where \( Ne \) – a number of effective alleles in population, \( He \) – an average expected heterozygosity degree.

The number of private alleles was calculated as number of alleles in population with a
frequency of occurrence no more than 1%.

Average observed degree of heterozygosity (Ho) was calculated for each locus as the ratio of number of heterozygotes to total number of the studied animals. For calculation of Ho of an individual it was found an arithmetic average Ho value on all studied 11 loci.

The average expected degree of heterozygosity (He) was calculated for each locus, using the equation (2):

$$H_e = 1 - \sum_i p_i^2,$$

(3)

where $p_i$ – the frequency of occurrence of the $i$-th allele. For calculation of He of an individual it was found an arithmetic average He value on all studied 11 loci.

The individual index of fixing (Fis) is a coefficient at individuals in relation to subpopulation, it serves as a measure of decrease in level of heterozygosity of an individual owing to nonrandom pairing in each subpopulation. For calculation a formula was used:

The total expected heterozygosity ($H_t$) for each locus was calculated:

$$H_t = 1 - \sum_i p_{si}^2,$$

(4)

where $p_{si}$ is the frequency of occurrence of $i$-allele in subpopulations.

The individual index of fixing (Fis) is a coefficient at individuals in relation to subpopulation, it serves as a measure of decrease in level of heterozygosity of an individual owing to nonrandom pairing in each subpopulation. For calculation a formula was used:

$$F_{is} = \frac{H_e - Ho}{He},$$

(5)

The population fixation index (Fit) was calculated – the coefficient in individuals with respect to the population as a whole:

$$Fit = \frac{H_t - Ho}{H_t},$$

(6)

where $H_t$ is the total expected degree of heterozygosity of the population as a whole.

Subpopulation fixation index – inbreeding coefficient in subpopulations with respect to the whole population was determined by the formula:

$$F_{st} = \frac{Var(p_{si})}{p_{si}^{ps} \left(1 - p_{si}^{ps}\right)},$$

(7)

where $Var(p_{si})$ is the average dispersion of allele frequencies in $i$-locus, $p_{si}$ – the frequency of alleles in the subpopulations, $s$ – the number of subpopulations, and $p_{si}^{ps}$ – the average frequency of the given allele between subpopulations.

The calculation of the gene flow rate is according to [1, 2].

The gene flow between the subpopulations was determined:

$$M = 0.25 \left(1 - F_{st}\right),$$

(8)

where $F_{st}$ is the subpopulation fixation index (random inbreeding).

The divergence duration indicator between subpopulations was calculated by the formula:
\[ t = \frac{l_n \left( 1 - F_{st} \right)}{l_n \left( 1 - \frac{1}{2n_e} \right)}, \tag{9} \]

where \( F_{st} \) is the subpopulation fixation index (random inbreeding), \( n_e \) – the effective population size.

Biometric processing was carried out according to the program of Fortran Power Station 2.0 and the method of D.A. Baimukanov and others [9].

In this table the following is specified: \( N \) - the number of alleles, \( Na \) – the number of informative alleles \( (Na \geq 1\%) \), \( Npr \) – the number of private alleles \( (Npr < 0.1\%) \), \( Ne \) – the number of effective alleles, \( He \) – the average expected heterozygosity, \( Ho \) – the average observed heterozygosity and \( Fis \) – an individual fixation index.

3. Results

3.1. All-Breed (population) Differentiation

A characteristic of Zhetysu type of Santa Gertrude cattle breed in the context of population genetic differentiation is presented. We have proposed the results of genotyping of 11 microsatellite loci (table 1). In this table the following is specified: \( N \) – the number of alleles, \( Na \) – the number of informative alleles \( (Na \geq 1\%) \), \( Npr \) – the number of private alleles \( (Npr < 0.1\%) \), \( Ne \) – the number of effective alleles, \( He \) – the average expected heterozygosity, \( Ho \) – the average observed heterozygosity and \( Fis \) – an individual fixation index.

Loci TGLA227, BM2113, TGLA53, TGLA122, INRA23 with 15, 14, 19, 24 and 13 alleles, respectively, are the most polymorphic for this Zhetysu population of Santa Gertrude cattle, loci ETH225 and BM1824 are the least polymorphic (7 alleles). Genetic inbreeding polymorphism is shown by the presence of informative, effective alleles and the presence of rare (private) alleles. A total of 135 alleles were identified, of which 121 were informative, 90.72 – effective, and 14 – private. The average number of alleles for all loci made up 12.27, for all informative alleles – 11, for effective – 8.25 and for private – 1.27 (figure 1).

Table 1. Identified allelic variants of microsatellite loci in the cattle population of Zhetysu type of Santa Gertrude breed (sample size 426 animals).

| Microsatellite Locus | N  | Na  | Npr | Ne  | He  | Ho  | Fis  |
|---------------------|----|-----|-----|-----|-----|-----|------|
| TGLA 227            | 15 | 15  | 0   | 10.12 | 0.9012 | 0.8991 | 0.0024 |
| BM 2113             | 14 | 12  | 2   | 9.93  | 0.8993 | 0.7981 | 0.1125 |
| TGLA 53             | 19 | 16  | 3   | 8.87  | 0.8873 | 0.8897 | -0.0027 |
| ETH 10              | 8  | 8   | 0   | 6.46  | 0.8352 | 0.8709 | -0.0427 |
| SPS 115             | 8  | 8   | 0   | 7.26  | 0.8622 | 0.8527 | 0.0110 |
| TGLA 126            | 9  | 9   | 0   | 8.21  | 0.8682 | 0.8380 | 0.0348 |
| TGLA 122            | 24 | 15  | 9   | 12.77 | 0.9117 | 0.9507 | -0.0428 |
| INRA 23             | 13 | 13  | 0   | 9.30  | 0.8925 | 0.8944 | -0.0021 |
| ETH 3               | 11 | 11  | 0   | 4.99  | 0.7997 | 0.7513 | 0.0606 |
| ETH 225             | 7  | 7   | 0   | 6.33  | 0.8420 | 0.8292 | 0.0151 |
| BM1 824             | 7  | 7   | 0   | 6.47  | 0.8454 | 0.8856 | -0.0475 |
| \( \Sigma \)        | 135| 121 | 14  | 90.72 | 9.5447 | 9.4597 | 0.0985 |
| Average             | 12.27 | 11.00 | 1.27 | 8.25  | 0.8677 | 0.8600 | 0.0090 |
Figure 1. Proportion of informative and private alleles in 11 MC loci of Zhetysu type of Santa Gertrude breed. The dark tone indicates the proportion of informative alleles; the light one – the proportion of private alleles (horizontal axis – Microsatellite Locus, along the vertical axis – Number of alleles).

The level of the average expected heterozygosity of cattle at loci varies from 0.7997 (at ETH3 locus) to 0.9117 (TGLA122), the average indicator for all loci makes up 0.8677. This pattern is also observed for the levels of the average observed heterozygosity as indicated in figure 2.

Figure 2. Heterozygosity of 11 Microsatellite loci of Zhetysu type of Santa Gertrude breed (the dark color indicates the average expected heterozygosity $He$, the light color – the average observed heterozygosity $Ho$) (horizontal axis – Microsatellite Locus, along the vertical axis – the average expected heterozygosity).

It can be clearly seen from the figure 2 that the most polymorphic for this population of the Zhetysu type of the santa gertrude breed of cattle from 11MC loci are the loci TGLA227, BM2113, TGLA53, TGLA122, INRA23 with 15, 14, 19, 24 and 13 alleles, respectively, the loci ETH225 and BM1824 are least polymorphic (7 alleles each).

According to one of the indicators of population differentiation, $Fis$ coefficient (individual fixation index), an excess of heterozygotes was found at loci TGLA53, ETH10, TGLA122, INRA23 and BM1824 (figure 3).
Based on the analysis of the figure 3, it should be concluded that on one of the indicators of population differentiation, the Fis coefficient (individual fixation index), an excess of heterozygotes was found at loci TGLA53, ETH10, TGLA122, INRA23 and BM1824, and on other loci a deficiency of heterozygotes was detected.

3.2. Intra-Breed (subpopulation) Differentiation

For the genetic characterization of Zhetysu type of Santa Gertrude cattle in the context of subpopulation breed differentiation, the Kazakhstan population was divided into 2 subpopulations (SGT_1, SGT_2) for each of which the generally accepted population and genetic parameters were calculated the data of which are shown in table 2. The results are good consistent with the studies [6, 7]. So, as a result of complex studies, we found that the Fst inbreeding coefficient (subpopulation fixation index) was 0.0094, which indicates the subdivision of the population ($\chi^2=34.30, \text{df}=1, \text{significance level } \alpha<0.001$); the inbreeding coefficient Fis (individual fixation index) made up 0.0090 which indicates the relative “youth” of the breed ($\chi^2=0.1038, \text{df}=1, \text{significance level } \alpha=0.7472$); the inbreeding coefficient Fit (population fixation index) made up 0.0169; the total expected heterozygosity $H_t=0.8694$; the expected heterozygosity $H_e=0.8677$; the observed heterozygosity $H_o=0.8600$. Our findings are consistent with the research of the scientists cited in the above sources.

Based on the obtained population genetic data, an analysis of the genes flow of and divergence was carried out. The gene flow index between subpopulations was $M=26.49$. Gene exchange between 2 subpopulations took place at an intensity of $m=33.36\%$ per generation.

Currently, since its creation, the Zhetysu population of Santa Gertrude breed has been divided into a number of subpopulations, and for a final subpopulation with an effective number $N_e$ and panmixis, the coefficient of random inbreeding is equal to the coefficient of community by origin: $F=F_{ST}$ [10].

With an average population size of 258 animals (=426/2) and a sex ratio of 1:15, 26.49 (assumption), the effective size $n_e$ made up 79.38. The gene flow between the subpopulations was $M=26.49$ according to formula (8). Gene exchange between two subpopulations took place with an intensity of $n=33.36\%$ per generation (table 2).

The data obtained allow performing targeted breeding in the subsequent research to increase the meat productivity of cattle.

Given the use of complex biological products, it is quite possible to produce high-quality beef for all social categories of the population of the Republic of Kazakhstan.
Table 2. Identified population and genetic parameters in cattle populations of Zhetysu type of Santa Gertrude breed according to two subpopulations.

| Indicators | Value of Indicator by 2 subpopulations |
|------------|----------------------------------------|
|            | SGT_1 | SGT_2 |
| N          | 11.91  | 12.27 |
| N_pr       | 0.55   | 1.00  |
| Ne         | 7.90   | 8.13  |
| He         | 0.8667 | 0.8684|
| Ho         | 0.8745 | 0.8483|
| Fis        | -0.0091| 0.0232|
| Fstav      | 0.0094 (χ²=34.30, df=1, significance level α<0.001) |
| Fisav      | 0.0075 (χ²=0.1038, df=1, significance level α=0.7472) |
| Htav       | 0.8694 |
| Fitav      | 0.0169 |

4. Discussion

We have presented a characterization of Zhetysu type cattle of Santa Gertrude breed in the context of population and genetic differentiation and proposed the results of genotyping of 11 microsatellite loci. In general, the performed analysis of the allele pool of this sample of cattle has revealed a range of values characteristic of Zhetysu type of Santa Gertrude breed. The most polymorphic for this population of 11 Microsatellite loci are TGLA227, BM2113, TGLA53, TGLA122, INRA23 loci with 15, 14, 19, 24 and 13 alleles, respectively, the least polymorphic are ETH225 and BM1824 (7 alleles each). The genetic intrabreed diversity (polymorphism) reflects the presence of informative and effective alleles as well as the presence of rare (private) alleles. A total of 135 alleles have been identified, of which 121 were informative, 90.72 – effective, and 14 – private. The average number of alleles for all loci made up 12.27, for all informative alleles – 11, for effective – 8.25 and for private – 1.27. The level of average expected heterozygosity of cattle at the loci varies from 0.7997 (at ETH3 locus) to 0.9117 (TGLA122), the average indicator for all loci is 0.8677. This pattern is also observed for the average observed heterozygosity [10].

According to one of the indicators of population differentiation, Fis coefficient (individual fixation index), an excess of heterozygotes was found at TGLA53, ETH10, TGLA122, INRA23 and BM1824 loci. In other loci, a deficiency of heterozygotes was observed. For the genetic characterization of the cattle of Zhetysu type of Santa Gertrude breed in the context of subpopulation breed differentiation, the Kazakhstan population was divided into 2 subpopulations (SGT_1, SGT_2), for each of which the generally accepted population and genetic parameters were calculated. Based on the obtained population and genetic data, an analysis of the flow of genes and divergence was made.

With an average population size of 258 animals (426/2) and a sex ratio of 1:15, 26.49 (assumption), the effective size ne made up 79.38. The gene flow between subpopulations was M=26.49. Gene exchange between 2 subpopulations took place with an intensity of m=33.36% per generation.

The data obtained allow performing targeted breeding in subsequent research to increase the meat productivity of cattle. Given the use of complex biological products, it is quite possible to produce high-quality beef for all social categories of the population of the Republic of Kazakhstan.

5. Conclusion

The population and genetic structure of Zhetysu type of Santa Gertrude breed shows the differentiation of the population as a whole. The total number of alleles found in 11
microsatellite loci made up 135, 121 of which were informative alleles, 90.72 – effective and 14 – private ones. The average expected heterozygosity levels range from 0.7997 to 0.9117. According to Fis coefficient (individual fixation index), an excess of heterozygotes was found at TGLA53, ETH10, TGLA122, INRA23 and BM1824 loci. In other loci, a deficiency of heterozygotes is observed. The inbreeding coefficient Fst (subpopulation fixation index) made up 0.0094 which indicates the subdivision of the population ($\chi^2=34.30$, df=1, significance level $\alpha<0.001$). The inbreeding coefficient Fis (individual fixation index) made up 0.0090 which indicates the relative “youth” of the breed ($\chi^2=0.1038$, df=1, significance level $\alpha=0.7472$). The inbreeding coefficient Fit (population fixation index) made up 0.0169. The total expected heterozygosity $H_t=0.8694$. The expected heterozygosity $H_e=0.8677$. The observed heterozygosity $H_o=0.8600$.

Analysis of the studied parameters of the population and genetic structure the cattle of Zhetyusu type of Santa Gertrude breed has confirmed the presence of poorly differentiated subpopulations of animals in the southern and southeastern regions of Kazakhstan.

The results of the evaluation of the interpopulation genetic differentiation of the Zhetyusu type of the Santa Gertrude breed of cattle by polymorphism of DNA microsatellite loci make it possible to carry out targeted selection work to realize the bioresource potential of adaptive, productive and reproductive qualities of cattle, which is important in terms of ensuring food security of any state.

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