Genetic diversity of Kemerovo pig breed based on microsatellite markers

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Abstract. Today, the urgent task is to preserve the gene pool of local and regional breeds with their special and useful traits. These features allow them to represent an additional reservoir of genetic diversity. Analysis of the genetic diversity of three pig populations (Kemerovo pig breed, Kemerovo × Landrace crossbreds and Kemerovo × Landrace × Pietrain crossbreds) by microsatellites showed that significant allelic diversity is observed for some loci, while for others it is low. The highest number of alleles was in the purebred pigs in comparison with crossbreds. The greatest range of alleles per locus was noted in the three-breed cross pigs. The most polymorphic locus in all populations was SO005, and the least polymorphic was SW72. It was noted that for most loci the observed heterozygosity corresponds to the expected one, while for others a shortage of heterozygotes is detected.

1. Introduction
Effective management of farm animal genetic resources requires a comprehensive analysis covering structure, population size, geographic distribution, productivity and reproduction levels, as well as genetic diversity within and between breeds.

Molecular markers based on the detection of DNA polymorphism have gained widespread acceptance for prioritizing the conservation of the gene pool of local breeds [1, 2, 3, 4]. It is assumed that microsatellites are neutral for selection, and the observed genetic diversity in a population is a effect of genetic drift and mutations [5, 6].

Local breeds are well adapted to specific environmental conditions, which allows them to be used in cross-breeding programs. However, it is quite difficult or almost impossible to protect all local breeds of farm animals, since modern commercial breeds, synthetic pig lines are widely used all over the world, which leads to a decrease in genetic diversity and monobreeding.

Kemerovo pig breed is adapted to the harsh Siberian climate. Animals are hardy, have good viability, high reproductive characteristics, good taste of pork and high content of intramuscular fat [7].

The aim of the study was to investigate the genetic diversity of the Kemerovo pig breed and its hybrids with commercial breeds based on microsatellite markers.
2. Materials and methods

2.1. Sample collection and DNA extraction
Biological samples (ear clip) were taken at animal slaughter. A total of 65 pigs (gilts and castrates) were examined: Kemerovo pig breed (K, n=35), Kemerovo × Landrace crossbreds (K×L, n=12) and Kemerovo × Landrace × Pietrain crossbreds (K×L×P, n=18). All animals at the time of study reached the live weight of 100±10 kg.

Isolation, extraction and purification of DNA and microsatellite analysis were carried out on the basis of L. K. Ernst Federal Research Center. Genomic DNA was isolated by using DNA extraction kit DIAtom™ DNA Prep 100. The kit is based on the properties of the lysis solution with guanidine thiocyanate, which lyses cells, solubilizes cellular debris, and denatures cellular nucleases.

2.2. Microsatellite data analyses
The set of 9 microsatellite markers (S0155, S0355, S0386, SW72, SW951, S0101, SW240, SW857 and SO005) recommended for genetic diversity studies by the ISAG/FAO [8] was used for genotyping. The polymorphism of STR-loci was determined according to the method developed [9] in ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, USA).

2.3. Statistical Data Analysis
Statistical data is processed by the PopGenReport software package (version 3.0). Fragment sizes were determined by GeneMapper® Software v4 («Thermo Fisher Scientific, Inc.», USA). Total number of alleles, average number of alleles per locus (Na), observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content (PIC) population were calculated using the GenAlEx v. 6.5.1. [10]. Chi-square test is usually applied to a large number of samples, so in this case used the Bonferroni correction.

3. Results and discussion
9 microsatellite loci recommended by FAO/ISAG were selected to determine the genetic heterogeneity of Kemerovo breed pigs, their consolidation and uniqueness compared to their hybrids with commercial breeds. All of the loci were polymorphic. The total number of alleles sampled across all groups was 55. The highest number of alleles was in the Kemerovo subpopulation (50), followed by K×L×P crossbreds (46) and after them K×L crossbreds (42).

As a result of the microsatellite analysis of the Kemerovo pig breed, it was found that the number of alleles per locus varies from 3 to 8 (Fig. 1). The smallest number of alleles was found at locus SW72, which is observed in the studies of other authors [2, 12], as well as at locus SW951. The number of alleles ranked from 2 to 8 in the K×L group. The greatest range of alleles per locus (2-10) was noted in the group of K×L×P crossbreds. The same number of alleles in the three groups of animals was recorded for loci SW951 (3), S0355 (4), and SW240 (6).
The most polymorphic locus in all populations was SO005, which is comparable to the results of Slovenian autochthonous pig breeds [10]. Locus SW857 (6.75) has the highest allele frequency in the Kemerovo pig breed, and the lowest one - locus SW72 (2.31). The SO005 locus was the most polymorphic in crossbred pigs.

The number of loci not in Hardy-Weinberg equilibrium in subpopulations ranged between 1 (S0101) in two-breed animals, 2 (SO005 and S0386) in three-breed cross pigs and 4 (SO005, S0386, S0355 and S0101) in purebred pigs, probably because we previously found that the observed heterozygosity for these loci in the Kemerovo breed was lower than expected [10].

Mean allelic diversity shows the informativeness of the analysis and demonstrates the genetic diversity in the pig subpopulations [13, 14, 15]. The mean allele richness per locus was found in Kemerovo breed (4.51±0.52) then K×L×P crossbred animals (4.48±0.56) and in K×L crossbred (4.35±0.61).

The average observed heterozygosity in the studied subpopulations was 0.59 (table 1). There is a deficiency of heterozygotes (Fit>0) at 8 loci out of 9, which indicates the presence of inbreeding in the studied groups. On the other hand, the Fit for these loci is far from 1 and the highest indicator for the S0101 locus was 0.42. It can be noted that there is a sufficient reserve of heterozygotes among animals, especially at loci S0155, SW857, SW951. At the same time, at the locus SW72 Fit=-0.1 that characterizes the studied individuals at this locus as the most genetically differentiated, despite the fact that this locus has the lowest level of polymorphism.

Table 1. The population wide expected and observed heterozygosity, PIC, Fis, Fit and Fst values for each locus.

| Locus  | He   | Ho   | PIC   | Fit  | Fis  | Fst  |
|--------|------|------|-------|------|------|------|
| S0155  | 0.716| 0.677| 0.672 | 0.07 | 0.05 | 0.02 |
| SO005  | 0.812| 0.631| 0.768 | 0.25 | 0.20 | 0.06 |
| SW72   | 0.507| 0.569| 0.489 | -0.10| -0.14| 0.03 |
| SW951  | 0.376| 0.369| 0.377 | 0.02 | 0.03 | -0.01|
| S0386  | 0.764| 0.662| 0.719 | 0.15 | 0.13 | 0.03 |
| S0355  | 0.697| 0.569| 0.633 | 0.21 | 0.16 | 0.06 |
| SW240  | 0.694| 0.578| 0.692 | 0.19 | 0.16 | 0.04 |
| SW857  | 0.823| 0.815| 0.807 | 0.02 | 0.01 | 0.01 |
| S0101  | 0.680| 0.415| 0.591 | 0.42 | 0.36 | 0.09 |
The greatest allelic diversity in pigs of the Kemerovo breed was observed for loci S0155, S0386, SW857. It is interesting to note that 4 private alleles for these loci were detected in this group of animals. Two-breed cross pigs had 1 specific allele (SW857159) and three-breed cross pigs had 2 specific alleles (SO005205, SW951126). The presence of a larger number of private alleles indicates the originality of the allelic pool of the Kemerovo pig breed.

4. Conclusion
Microsatellite analysis showed that the Kemerovo pig breed has a reasonable level of genetic diversity. The tendency of the advantage of purebred animals over crossbred animals in allelic diversity is noted. The observed heterozygosity for all loci except SW72 was lower than expected, which according to [16] may indicate heterogeneity of animals within populations. Fst values showed that the general genetic variability is formed mainly by individual differences.

The results of our study indicate that local pig breeds are a source of allelic diversity for widely used commercial breeds. The establishment of genetic diversity will be useful for programs to conserve and further improve the local pig breed.

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