

Yu. V. Guzeiev, O. V. Melnyk, E. A. Gladyr, N. A. Zinovieva. The polymorphism of five microsatellite DNA loci in the study of Ukrainian Grey and Bulgarian Grey cattle breeds

The problem of preserving genetic diversity as a component of the environment, has recently become global. Among the cattle breeds that require special attention in terms of preserving genetic diversity is grey steppe cattle. Grey steppe cattle are very ancient livestock, representatives of grey steppe cattle in Ukraine is Ukrainian Grey, in Bulgaria – Bulgarian Grey breed. The purpose of this study was to conduct a comparative analysis of allele diversity of Ukrainian Grey and Bulgarian Grey cattle breeds using microsatellite DNA loci.

This analysis was performed on 32 animals of Ukrainian Grey breed bred in the LLC "Holosiyevo", Brovary district, Kyiv region. Their genetic studies were carried out in the Laboratory of Molecular Genetics and Cytogenetics of Animals in the center of biotechnology and molecular diagnostics of the All-Russian Research Institute of Animal Husbandry (Dubrovitsy vil., Moscow reg.). Genomic DNA was isolated from the biological material obtained from the earmark, according to the method described by N. A. Zinovieva and co-authors.

Genetic analysis of Ukrainian Grey and Bulgarian Grey breeds was conducted at five microsatellite DNA loci: BM1824, BM2113, ETH225, SPS115, TGLA126, which are included in the list recommended by the ISAG-FAO for genotyping of cattle. The data on Bulgarian Grey breed were used after Teneva A. et.al. (2005).

Electrophoretic separation of DNA fragments by capillary electrophoresis was performed on the device MegaBace 500. For identification of alleles of studied loci MS Genetic Profiler 2.0 software was used. These alleles of each animal were summed to a Microsoft Excel spreadsheet. The resulting matrix of genotypes served as the basis for the statistical processing of the results.

For statistical data processing software Cervus 3.0.3, PowerStatsV12 (Promega), GENALEX 6 was used.

The studies carried out in 5 microsatellite DNA loci identified 26 alleles in Ukrainian Grey breed and 30 alleles in Bulgarian Grey breed. The SPS115 locus in both breeds identified 7 alleles, with the highest frequency of allele 248 bp.

At Ukrainian Grey breed in BM2113 locus the highest frequency was discovered at the alleles 135 and 139 bp, while in micropopulation of Bulgarian Grey breed the highest frequency was demonstrated by the allele 133 bp.

In BM1824 locus allele 188 bp has the highest frequency. Alleles 188 and 192 are present only in the micropopulation of Ukrainian Grey breed. In the micropopulation of Bulgarian Grey breed allele 184 is identified with frequency of occurrence 0.386.

In ETH225 locus in the micropopulations of Ukrainian Grey and Bulgarian Grey breeds 6 loci were revealed. Allele 152 was present only in the group of Ukrainian Grey breed, and allele 158 with frequency 0.043 and allele 146 with frequency 0.129 were identified only in the micropopulation of Bulgarian Grey breed. The highest frequency of alleles in the studied Bulgarian Grey breed was at allele 140

with frequency 0.371, and in the micropopulation of Ukrainian Grey breed it was at allele 148.

In TGLA126 locus 7 alleles were identified: 109, 115, 117, 119, 121, 123, 125. Allele 115 was detected only in the micropopulation of Ukrainian Grey breed, and allele 109 with frequency 0.014 and allele 121 with frequency 0.014 were detected only in the micropopulation of Bulgarian Grey breed.

Furthermore, the quantity of the informative value of the used markers (PIC) was calculated. The larger the value for the PIC locus is, the more informative the locus is as a marker. According to Botstein et al. the locus with $PIC > 0.500$ value is very informative (high polymorphic); with $0.5 > PIC > 0.25$ is informative enough (moderately polymorphic); and with $PIC < 0.250$ is slightly informative.

In the micropopulation of Ukrainian Grey breed the highest value was observed at loci BM1824 $PIC = 0.710$, and ETH225 $PIC = 0.710$. In the micropopulation of Bulgarian Grey breed most polymorphic loci were BM2113 $PIC = 0.790$ and ETH225 $PIC = 0.740$.

The average value of N_a at Ukrainian Grey breed was 5.2 alleles in five loci; in the micropopulation of Bulgarian Grey breed the average value of N_a was 6.0 alleles; the average value of the observed degree of heterozygosity H_o in micropopulations of Ukrainian Grey breed was 0.656, at Bulgarian Grey breed it was 0.783. The expected degree of heterozygosity H_e at Ukrainian Grey breed was 0.704, at Bulgarian Grey breed it was 0.813, that indicates a greater genetic diversity in the micropopulation of Bulgarian Grey breed. The total average value of F_{is} in the micropopulation of Ukrainian Grey breed was 0.074, at Bulgarian Grey breed it was 0.030. The excess of heterozygotes was detected in the micropopulation of Ukrainian Grey breed on loci BM2113 and BM1824 (18.2 and 2.5%, respectively), in the micropopulation of Bulgarian Grey breed it was detected on BM1824 locus (15.1%).

Heterozygosity deficit was identified on all the loci, with the exception of the loci BM2113 (-0.182) and BM1824 (-0.025) in the micropopulations of Ukrainian Grey breed and BM1824 locus (-0.151) in Bulgarian Grey breed. The highest value F_{is} was found in SPS115 locus (0.444) of Ukrainian Grey breed. Precisely this can explain the high deficit of heterozygosity in the micropopulation of Ukrainian Grey breed.

This study confirms the effectiveness of the use of microsatellite DNA loci to characterize the genetic diversity of populations of grey steppe cattle bred in many countries around the world. Ukrainian Grey and Bulgarian Grey breeds are genetically very similar to each other. The genetic analysis shows that they have a low genetic variability, although in both micropopulations deficit of heterozygotes was detected, but it was higher in micropopulation of Ukrainian Grey breed. The results may be useful in breeding grey cattle breeds, to monitor them in order to preserve their genetic diversity.

Keywords: genetic analysis, grey steppe cattle, breed, microsatellite DNA loci, alleles, polymorphism, micropopulation