V. V. Dzitsiuk, S. G. Kruhlyk, V. G. Spyrydonov. Genetic analysis of German Shepherd dogs using microsatellite DNA markers

Modern methods of breeding dogs are based on getting of stable phenotypic uniformity by using close inbreeding or breeding by one line, but such strategy leads to a loss of genetic diversity, and as a result there are genetic defects in breeds which have no external manifestations or manifest in adulthood of dogs and are transmitted from generation to generation. Therefore, to prevent use of dogs with genetic abnormalities in breeding, and to develop standards for a breed and make an accurate pedigree, must carry out the genetic evaluation of animals.

One of the modern tools for dogs' genetic evaluation is DNA-testing using microsatellite loci permitting to match the parental couple effectively, identify (to certify) animals, undertake a comprehensive assessment for heterozygous and homozygous genotypes in populations, permitted for use in the selection process, and illustrate clearly the impact of artificial selection on the genetic characteristics of breeds.

The study was conducted in Ukrainian Laboratory of Quality and Safety of Agricultural Products in Department of Molecular Biology Research. For the genetic analysis 42 German Shepherd dogs, used for breeding in kennels of Ukrainian Kennel Union (UKU), were selected. The material for the research was DNA isolated from dogs' buccal epithelium cells and blood. Genomic DNA was extracted using a standard set of reagents for DNA isolation.

Level of theoretically expected heterozygosity (H_{exp}) varied between 0.385 (PEZ1) to 0.835 (PEZ8). On average theoretically expected heterozygosity with coefficient of 0.657 had not significant advantage over value of actual heterozygosity (0.629), it also shows that the status of the sample of dogs is close to balance. The same is observed in actual and expected heterozygosity for PEZ 6 (0.629) and PEZ 8 (0.657) loci, which also shows the balance.

For FHC2010 loci actual heterozygosity is higher than expected, indicating increasing the number of heterozygous individuals. For FHC2054 locus, by contrast, theoretically expected heterozygosity (0.670) dominates the actual (0.429), indicating the lack of heterozygous genotypes in this micropopulation.

The value of PIC (polymorphism information content) of the analysed loci ranged from 0.325 to 0.740 with average value 0.574. PEZ6, PEZ8, FHC 2010 and FHC 2054 loci optimally meets their suitability for genetic certification of genotypes because their frequency varies from 0.587 to 0.740. The reduced average index of polymorphism for PEZ1 locus with coefficient of 0.325 confirmed the insufficient level of its polymorphism for full genetic evaluation of the micropopulation of German Shepherd dogs (PIC < 0.500), as confirmed by Chinese researcher J.-H. Ye, according to his data PIC value for PEZ1 locus was 0,320, which correlates with our results. And PIC value for PEZ8 locus was 0.740 in our studies, whereas according to J.-H. Ye – 0,720, which, by contrast, indicates high polymorphism and confirms the effectiveness of its use in genotyping of dogs.

Probability of exclusion of accidental allele coincidence (PE), which is 0.675 on average, indicates a lack of the number and informativeness of the selected microsatellite markers for German Shepherd as in this case a combined probability (CPE) of accidental allele coincidence is 0.933886 or 93.3%.

The chosen microsatellite loci to study the genetic structure of the German Shepherd dog population, show a sufficiently high informativeness of chosen system of molecular genetic DNA markers. However, there is the need for using additional microsatellite markers which will increase the combined probability of accidental allele coincidence (CPE) from 93.3% to 99.9%.

The analysis of heterozygosity is important in studying the dynamics of genetic processes in populations, because heterozygosity has an effect on many factors, including mutations, selection, non-random mating, genetic drift, etc., so continuous monitoring of genetic diversity is required for their timely identification and development of measures to improve breeding work on biodiversity in different dog breeds.

Keywords: German Shepherd, microsatellites, DNA, polymorphism, genotype, homozygosity, heterozygosity