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GENETIC ANALYSIS OF SOME HORSE BREEDS BY MEANS OF MICROSATELLITE DNA LOCI

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By means of 12 microsatellite DNA loci, wich are recommended by the International Society of Animals Genetics, genetic analysis of three horse breeds was performed. The high level of polymorphism and a similar number of alleles by locus in studied populations were revealed. For all populations heterozygotes deficiency, confirming index fixation was found. Determination of genetic similarity between the studied species confirms an active influence Thoroughbred horse breed on the formation of Ukrainian Rider breed. The future research of greater number horses of these breeds is perspective.

Keywords: microsatellite loci, genetic polymorphism, heterozygosity, genetic similarity, Thoroughbred horse breed, Ukrainian Rider horse breed, Hucul breed.

Recently Marker Assisted Selection is being promoted in animal breeding including horse breeding. It allows to conduct the efficient breeding work based on the genetic potential of animals. Due to constant genetic changes as a result of mutation and evolution a regular genetic testing allows to observe genetic processes that take place within populations and species, and to plan the further work with them. From the genetic point of view the special attention deserve native breeds, which are breeding in local areas, and thoroughbred breeds, which are breeding only purebred.

Among the wide variety of genetic markers especially popular in horse breeding are microsatellite sequences of DNA. Microsatellites, which

characterized by high variability, codominant feature, high polymorphism level, known localization in genome, are widely used to determine the genetic structure of breeds and populations, study of breeds origin and evolution, genetic monitoring of breeds to preserve the pool of alleles of local breeds and etc. [3]. International Society for Animal Genetics (ISAG) contains a list of microsatellite DNA loci, which are recommended for parentage verification of horses. In accordance with the recommendations of ISAG and ISBC genetic analysis of Thoroughbred horses must to be conduct using this type of genetic markers. In Ukraine the study of horses by means of microsatellite markers started not so long ago [1, 2], and many breeds have not been investigated of this type of genetic markers.

The aim of our work was to conduct genetic between-breeds differentiation of populations of Thoroughbred, Ukrainian Rider and Hucul horses.

Materials and methods. The research was conducted in Ukrainian Laboratory of Quality and Safety of Agricultural Products. The materials of research were 123 horses of different breeds (51 Thoroughbred, 34 Ukrainian Rider and 38 Hucul horses). Peripheral blood was collected in tubes coated with EDTA. The isolation of genomic DNA was performed with the DNA Extraction kit «DNA-sorb-B» («Amplisens», Russia) according to manufacturer instruction. Genotyping was carried out by the analysis of 12 horse microsatellites loci of DNA (AHT04, AHT05, ASB17, ASB23, CA425, HMS03, HMS06, HMS07, HTG04, HTG06, HTG07, VHL20), which are recommended by ISAG. The polymerase chain reaction was conducted in standard conditions [5]. The products of amplification were denaturized by the formamide (Sigma, USA) and electrophoresis was performed on 4-capillary genetic analyzer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) according to manufacturer instruction. The sizes of alleles were determined by using the size standard Genescan-LIZ 500 (Applied Biosystems, USA), «Gene Mapper software 3.7» (Applied Biosystem, USA) and internal control.

Number alleles per locus (Na), observed (Ho) and expected heterozygosity (He), polymorphic information content (PIC), fixation index (F) were determined

using Cervus 3.0.3 [6] and PowerStats (Promega). Based on allele frequencies genetic distances and genetic similarity by Nei were obtained [7].

Results and discussion. As a result of the research it was found that each horse breed characterized by a certain range of allele frequencies and has its own characteristics (Table. 1).

1. Genetic characterization of studied horse breeds by means of microsatellite loci

Index	Breed		
	Thoroughbred	Ukrainian Rider	Hucul
Total number of alleles, Na	94	98	95
Mean number of alleles per locus	7,833	8,167	7,917
Polymorphic information content, PIC	0,675	0,736	0,745
Observed heterozygosity, Ho	0,642	0,708	0,757
Expected heterozygosity, He	0,679	0,777	0,784
Fixation index, F	0,094	0,077	0,037

The number of detected alleles serves as an indicator of population genetic diversity. For all studied breeds total number of alleles was approximately the same, although was highest it was for Ukrainian Rider horses (98 alleles). The average number of alleles per locus for all breeds ranged from 7,833 to 8,167.

According to Botstein et al. [4], PIC > 0,50 indicates a highly informative locus, 0,25 < PIC < 0,50 indicates a reasonable informative locus, and PIC < 0,25 indicates a slightly informative locus. PIC showed that all studied populations are highly polymorphic. The most polymorphic, despite the small size and local nature dilution, was Hucul horses, gene pool of which since 1979 is protected.

Analysis of heterozygosity levels of three breeds showes deficiency of heterozygous genotypes, which also shows positive F. The highest value of F was Thoroughbred population. Despite the fact that heterozygote deficit in Hucul horses was the lowest (3,7%), due to the limited number of breed and to prevent

loss of valuable genetic material, the genetic control of processes in population deserves special attention.

Using the allele frequencies genetic distance and genetic similarity between studied populations were estimated (Table 2).

2. Genetic distance (below diagonal) and genetic similarity (above diagonal) between studied horse breeds by means of 12 microsatellite loci

	Thoroughbred	Ukrainian Rider	Hucul
Thoroughbred	-	0,816	0,625
Ukrainian Rider	0,203	-	0,732
Hucul	0,471	0,313	-

The highest level of genetic similarity was determined for Ukrainian Rider and Thoroughbred horses. It indicates that Thoroughbred horses have significant part of in the formation of Ukrainian Rider horses. Hucul horses was more remote from the studied rider breeds, that confirms its uniqueness and formation features.

Conclusions and perspectives for further researches. As a result of conducted researches of three horse breeds using 12 microsatellite DNA loci between-breeds genetic differentiation of studied horse populations was conducted. In all populations there was a deficit of heterozygous genotypes, indicating that their consolidation and possible further reduction of genetic diversity. Depending on the breeder aim it can lead to positive and negative consequences. Analysis of genetic similarity proved a significant impact Thoroughbred horse breed horses for the formation of Ukrainian Rider horses. However the native Hucul horses was more remote from rider breeds. Further perspective studies are the study of greater number horses using informative microsatellite DNA loci.

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