GENETIC STRUCTURE OF HUCUL HORSES USING MICROSATELLITE LOCI OF DNA

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Actuality of problem of conservation of local and indigenous breeds of farm animals does not cause a doubt at majority of scientists. The high resistance of these breeds of horses to disease and strict local climatic conditions is a valuable genetic resource. These breeds have a rare gene pool and enormous reserve of genetic changeability, which play a basic role in microevolution process. Hucul breed is one of unique and original breeds of horse of Ukraine and neighboring Carpathian countries region. Original location of this breed is the Hucul region in East Carpathians, which now belongs to modern Romania.

The morphology of Hucul horses make them similar to the Tarpan. They characterized by a strong and lean constitution, lively temperament and longevity, the easy with which they find their food, high resistant to disease, high vitality and fertility. They are well adapted for use in harsh mountain conditions.

Since 1979, the Hucul horses according to the Food and Agricultural Organization (FAO) recommendations on the Preservation of Genetic Resources of Farm Animals is classified as the local and small breed and they were included in the conservative breeding program (**Mason I.L.**, 1997.). For this purpose, in 1994, the Hucul International Federation (HIF) was established mainly to create breeding goals in Hucul breeder countries. In Ukraine, the issue of preservation of Hucul breed is included in the State Program of breeding farm animals.

Currently the Hucul horse is bred mainly in Ukraine, Romania, Hungary, Czech and Slovak Republics, Poland and Austria. In recent years the popularity of Hucul horses reached even England. Despite this, the state of the breed in Ukraine leaves much to be desired. In comparison with other countries, in Ukraine, livestock of Hucul horses since 1.01.2011 (according to data HIF) was only 299 goals (including 159 females). With the reduction in the number of Hucul livestock their gene pool could be in the jeopardy reducing of the genetic diversity.

In order to effective breed local horse breeds are widely used different DNA markers, among which preference is given directly to microsatellite loci of DNA which are characterized by highly variable, codominant inheritance, high level of polymorphism, known localization in the genome etc.. The horse microsatellites were characterized first by Ellegren et al. (**Ellegren H.,** 1992, a, b). Most laboratories parentage testing of horses are performed using microsatellite DNA loci. In accordance with the recommendations of the International Society of Animal Genetics (ISAG) and the International Committee of the Stud Book (ISBC) the genotyping of Thoroughbred horses genetic laboratories are required to conduct this type of genetic markers. The design and number of microsatellites that should be used in parentage testing depends on the characteristics of each locus and on the variability of the studied breed (**Double M.C.**, 1997).

The aim of this study was to conduct a genetic analysis of 38 Ukrainian Hucul horses by 12 microsatellite DNA loci. Using microsatellite data, we analyzed the genetic structure of investigated population.

MATERIAL AND METHODS

The research was conducted in Ukrainian Laboratory of Quality and Safety of Agricultural Products. We collected fresh peripheral blood samples from 38 horses belonging to farm «Polonynske gospodarstvo» in tubes coated with EDTA. The isolation of genomic DNA from fresh blood was performed with the DNA Extraction kit «DNA-sorb-B» («Amplisens», Russia) according to manufacturer's protocol. The evaluation of the genetic structure was investigated using 12 microsatellites (AHT04, AHT05, ASB17, ASB23, CA425, HMS03, HMS06, HMS07, HTG04, HTG06, HTG07, VHL20) recommended by ISAG.

PCR was performed under standard conditions on a thermocycler Veriti 96-Well (Applied Biosystems, USA) (**Dimsoski P.,** 2003). After the amplification process, PCR products were denaturized by the formamide (Sigma, USA) and separated by capillary electrophoresis using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). Allele sizes were scored against the size standard Genescan-LIZ 500 (Applied Biosystems, USA) using «Gene Mapper 3.7» software (Applied Biosystem, USA).

Allele frequencies, number alleles per locus (Na), the ef-

Table 1. **Allele frequency of 12 microsatellite loci in Hucul horses**

Allele \ Locus	AHT04	AHT05	ASB17	ASB23	CA425	HMS03	HMS06	HMS07	HTG04	HTG06	HTG07	VHL20
F	-	-	-	-	-	-	-	-	-	-	-	-
G	0.0526	0.0132	0.0263	-	-	-	-	-	-	0.0132	-	-
H	0.1974	0.0263	-	-	-	-	-	-	-	0.0921	-	0,0921
I	0.0132	0.0132	-	0.1447	0.0132	0.0790	0.0132	0.0263	-	-	-	0,2105
J	0.0132	0.1316	-	0.0658	0.0921	-	0.0395	0.0921	0.0526	-	-	-
K	0.0395	0.0263	-	0.1711	0.2105	-	0.1579	0.0921	0.0658	0.1447	0.1711	-
L	0.0658	0.0658	-	0.0790	0.0658	0.0132	0.2368	0.5132	0.2500	0.1447	0.0132	0,2500
M	-	0.2500	0.3026	-	0.1316	0.3290	0.1842	-	0.2368	0.0263	0.0790	0,1316
N	0.1974	0.3553	0.1184	-	0.3684	0.0790	0.0790	0.2237	0.1711	0.0263	0.1579	0,0921
O	0.2763	0.0395	0.0263	-	0.1184	0.1184	0.0921	0.0526	0.0526	0.0526	0.4079	0,0658
P	0.1447	0.0790	-	-	-	0.2895	0.1711	-	0.1316	0.4868	0.1184	0,1184
Q	-	-	0.0790	-	-	0.0790	0.0263	-	0.0395	0.0132	0.0526	0,0395
R	-	-	0.4474	-	-	0.0132	-	-	-	-	-	-
S	-	-	-	0.1447	-	-	-	-	-	-	-	-
T	-	-	-	0.0395	-	-	-	-	-	-	-	-
U	-	-	-	0.3026	-	-	-	-	-	-	-	-
V	-	-	-	0.0526	-	-	-	-	-	-	-	-

fective number of alleles (Ne), observed (Ho) and expected heterozygosity (He), polymorphic information content (PIC), fixation index (F) and exclusion probabilities (PE) were obtained using Cervus 3.0.3, GENALEX 6 (**Peakall R. at al.,** 2006) and PowerStats (Promega) software.

RESULTS AND DISCUSSION

The results showed that the total number of detected alleles in 38 horses across 12 microsatellite markers was 95. Allele frequency was calculated for each locus separately (Table 1). We distinguished a number of alleles with higher frequencies (AHT04 – allele O, AHT05 – allele N, ASB17 – allele R, ASB23 – allele U, CA425 – allele N, HMS03 – allele M, HMS06 – allele L, HMS07 – allele L, HTG04 – allele L, HTG06 – allele P, HTG07 – allele O, VHL20 – allele I).

Population genetic structure of investigated population is presented in Table 2. The number of alleles per locus (Na) varied from 6 (ASB17 and HMS07) to 10 (AHT05). The average allele number per locus was 7.917, higher than the average of 6,667 and 6,883 alleles obtained by **Georgescu et al.** (2008) and Trandžík J. et al. (2009) and slightly higher than the average of 7.583 alleles obtained by **Kusza et al.** (2013).

The effective allele number (Ne) ranged from 2.996 (HMS07) to 6.211 (VHL20). We found that the average value of observed heterozygosity (Ho) at this study was 0.757. The highest Ho was for HMS06 (0.947). A similarly high heterozygosity, above average, was ascertained for loci AHT04, AHT05, ASB17, ASB23, CA425, HTG04 and VHL20. HTG07 has shown the minimum of expected heterozygosity – 0.526.

The mean value of expected heterozygosity was 0,784 and ranged from 0.675 for HMS07 to 0.850 for HMS06 and VHL20. Higher Ho than He was obtained for six microsatellite loci. All loci deviated from Hardy-Weinberg equilibrium (HWE). The deviation observed value of the expected may be due to a variety of causes including population subdivision and genetic drift (**Lawson R.**, 1989).

The larger value of polymorphic information content (PIC) can provide the more genetic information. According to **Botstein et al.** (1980), 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. In this study, the entire batch of 12 microsatellite loci were highly informative. The mean PIC was 0.745. The highest value of PIC was 0.820 (VHL20) and the least polymorphic was HMS07 (0.627). Using 12 microsatellite loci, Trandžík J. et al. (2009) reported the highest value of PIC in Hucul horses was VHL20 (0.827) and the least value of PIC were HTG06 (0.638), HMS06, HMS07 (0.640).

The mean Fis for all loci was 0.037 and ranged from -0.172 (ASB17) to 0.313 (HTG07). Positive Fis value suggested inbreeding to be one of the main causes for the shortage of heterozygotes in this population. Fis values were signifcant for excess homozygotes in all breeds, after a Bonfer-

Table 2. **Observed (Na) and effective (Ne) number of alleles, observed (Ho) and expected (He) heterozygosity, polymorphic information content (PIC), fixation index (F) and exclusion probability (PE) of Hucul horses using 12 microsatellite loci**

Locus	Na	Ne	Ho	He	PIC	F_{is}	PE
AHT04	9	5.429	0.868	0.827	0.791	-0.051	0.731
AHT05	10	4.548	0.816	0.791	0.752	-0.032	0.629
ASB17	6	3.191	0.816	0.696	0.637	-0.172	0.629
ASB23	8	5.630	0.816	0.833	0.801	0.021	0.629
CA425	7	4.457	0.789	0.786	0.746	-0.004	0.580
HMS03	8	4.443	0.553	0.785	0.743	0.296	0.238
HMS06	9	6.197	0.947	0.850	0.818	-0.115	0.893
HMS07	6	2.996	0.553	0.675	0.627	0.181	0.238
HTG04	8	5.663	0.842	0.834	0.800	-0.009	0.769
HTG06	9	3.426	0.711	0.718	0.680	0.010	0.445
HTG07	7	4.102	0.526	0.766	0.726	0.313	0.212
VHL20	8	6.211	0.842	0.850	0.820	0.009	0.679
Mean	7,917	4.691	0.757	0.784	0.745	0.037	0.556
CPE							0,999988

roni's correction for multiple tests ($P< 0.05$). These results may refect the effect of the use a small number of stallions in each generation or mating among closely related animals, especially in populations where there is no control over reproduction. The heterozygote deficiency could also be explained as a Wahlund effect if population subdivision is occurring, linkage with loci under selection (genetic hitchhiking), population heterogeneity, null alleles (non-amplifying alleles), or inbreeding (**Silva A.C.M.,** 2012).

Parentage testing should rely on exclusion based on the incompatibility of two or more markers, because an exclusion based on a single marker may involve an element of uncertainty. The total PE for 12 microsatellite loci ranged from 0,238 (HMS03 and HMS07) to 0.893 (HMS06). Our results shows that The CPE was higher than 99.99%, that shows that our selected microsatellites have greater power of exclusion, given the fact we could reach a very high level of exclusion with selected 12 microsatellites loci of DNA.

CONCLUSION

The conducted analysis showed the 12 microsatellite marker system has high efficiency for genetic evaluation of Hucul horses. The most informative locus was VHL20 and the least informative was HMS07.

Positive Fis value (3.7%) suggested inbreeding to be one of the main causes for the shortage of heterozygotes in this population. Our results have been demonstrated a losses of the genetic variability in Hucul breed, as shown by the allele number and heterozygosity level. To prevent these losses of valuable genetic material of Hucul horses is necessary to monitor the genetic processes that occur in this breed.

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SUMMARY

The aim of the present work was to conduct a genetic analysis of 38 Hucul horses by microsatellite DNA. In the study the genetic structure of Hucul horses was investigated using 12 microsatellite markers (AHT04, AHT05, ASB17, ASB23, CA425, HMS03, HMS06, HMS07, HTG04, HTG06, HTG07, VHL20) recommended by the International Society of Animal Genetics (ISAG). The results showed that the mean number of allele across the 12 loci was 7.917 and ranged from 6 (ASB17, HMS07) to 10 (AHT05). The mean value of observed heterozygosity (0.757) was lower than expected (0.784), indicating the prevalence in the studied group of animals homozygous genotypes. Despite the fact that all analyzed loci were polymorphic, the most polymorphic locus was VHL20 (0.820). In turn, the locus HMS07 (0.627) had the lowest value of PIC (0.589). Combined exclusion probability was 99.99%, that allow us to show the efficiency of selected microsatellite loci of DNA for genetic analysis of Ukrainian population of Hucul horses.

Key words: *Hucul horses, microsatellites, genetic analysis, polymorphism.*
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