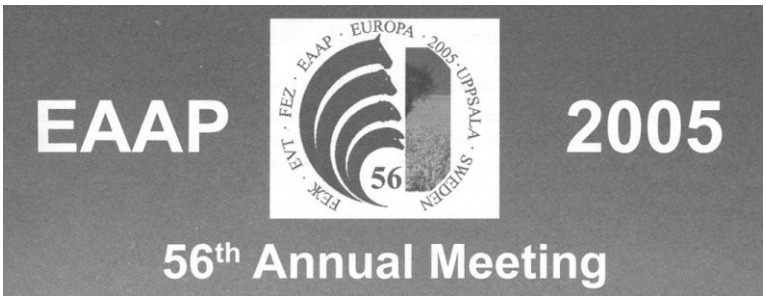

DNA MICROSATELLITE TEST OF HUTSUL HORSES IN HUNGARY

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SUMMARY

The name of the "**Hucul**" breed, unlike other breeds, does not originate from one or two outstanding strain founders or a breeing site, but from a race of people. This primitive breed with a past of several centuries was developed in the hands of the Hucul people, who lived in the Carpathians, in the source regions of the Tisza, Prut and Brodina, and in Bukovina, Gallicia and the old border regions of Hungary. In the mid-1990's Poland, Slovakia, the Czech Republic, Hungary and Austria established the breed's international organisation, under the name of **HUZUL INTERNATIONAL FEDERATION**.

The 12 microsatellite markers (**VHL20, HTG4, AHT4, HMS7, AHT5, HMS6, HTG6, ASB2, HTG10, HTG7, HMS3, HMS2**) were analysed for Hutsul horses (N=81). The samples were analysed by ABI PRISM™ 310 Genetic Analyzer and Genotyper Software (Applied Biosystems, USA) was used for the evaluation of analyses.

According to our results remarcable differences were found in the number of alleles (**VHL20, ASB2, HMS2**) and allelfrequences (**HTG4, HMS7, HTG7**) of some microsatellites in Hutsul compared to Thoroughbred.

On the basis of the examination of genetic markers based on microsatellite investigation, numerous calculations can be executed and in addition to the establishment of their frequency, additional information may be obtained on the breed and in the evaluation carried out within the herd (e.g. heterozygosity, inbreeding, presence of alleles within each microsatellite). Consequently they can be used in comparison of herds and the estimation of genetic distance (similarity).

INTRODUCTION

The DNA molecule, as carrier of hereditary traits, is the nucleus of microsatellite tests. In terms of our examination, and from the polymorphisms, the so called variable number of tandem repeats (VNTR) is remarkable. The rather strong polymorphism of VNTRs is due to the different number of tandem repeating DNA sequences, which can be found in the *eucariota* genome.

Microsatellites (STR: Short Tandem Repeat) are efficient tools for genome mapping. By their application, animals can be identified easily and accurately.

The relatively equal distribution of microsatellites in the genome makes the above mentioned gene mapping possible, while the considerable marker activity could be effectively utilized both in the individual breed identification and the genetic comparisons (*Takahashi at al. ,1998*; *Zhou et al., 1999*; *Romanov and Weigend, 2001*). Their typifying is based on the numerical variability of the repetition of some base pairs, that can be found between two sequence sections typical of only a given genome section (*Weber, May, 1989*).

The aim of the present study was to characterize the genetic structure of the Hutsul horses in Hungary, basing on microsatellite DNA sequences.

MATERIALS AND METHODS

Sampling of animals

The tests covered **Hutsul horses** (N=81) in Hungary. Blood samples from animals sent to the Immune-Genetic Laboratory of the National Institute for Agricultural Quality Control for routine parentage testing were selected for the present analysis. Tubes containing EDTA were used to take 10 ml blood samples from the jugular vein for DNA microsatellite determination. Blood samples were stored at –20°C until the analyses.

Laboratory analysis

The collected samples were exposed to PCR and microsatellite analysis. Genomic DNA was isolated from lymphocytes on the basis of the method described by *Marklund et al. (1994).* After isolation DNA, the product was multiplied (*Panaccio, et al., 1993*). To carry out PCR, the kit of ***Applied Biosystems StockMarks***® developed for horses was used. Reproduction of DNA sequences included in the samples was executed by means of

GeneAmp PCR System 9700 (Applied Biosystems, USA) apparatus. Then samples were analysed by means of ***ABI PRISM^{TM} 310 Genetic Analyzer***. Genotyper Software (Applied Biosystems, USA) was used for the evaluation of analyses.

Statistical analysis

Statistical testing of the degree of differences and similarities was made by means of homogeneity examination, $\chi 2$-tests used for case number tables (*Pirchner, 1983; Andersson, 1985*). Test was performed for some alleles / factors, as well as distributions between lines of differences). The $\chi 2$-tests indicate the significance grade of the genetic "distance" of breeds.

RESULTS

Comparising the two breeds (Hutsul, Thoroughbred) tested significant differences were obtained in the allele frequency of microsatellites (e.g.**VHL20, HMS7, ASB2, HTG7**; $P<0.001$). We found essential differences in number of alleles (e.g. **VHL20, HMS2**) and allelfrequences (e.g. **HMS7, HTG7**) of some microsatellites in Hutsul compared to Thoroughbred.

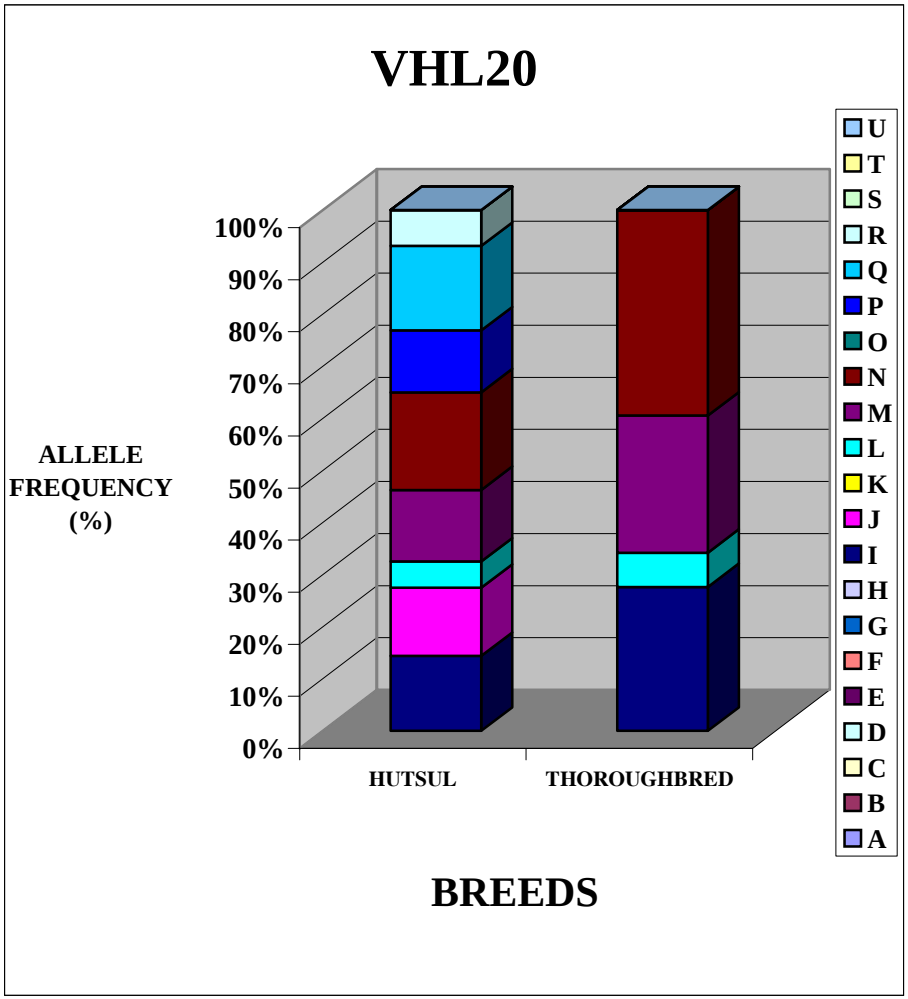
Out of the factors of microsatellite **VHL20**, the alleles **I, L, M** and **N** were present in Thoroughbred while alleles **I, J, L, M, N, P, Q** and **R** in **Hutsul** horses. Some alleles (**J, P, Q, R**) found in Hutsul were not present in Thoroughbred (Diagram 1.).

In microsatellite **HMS2** alleles **H, J, K, L** and **M** were present in Thoroughbred and **H, I, K, L, P** and **R** in **Hutsul** (Diagram 1.). The allele frequency of **HMS2-I, HMS2-P** and **HMS2-R** proved to be **0.0268**, **0.3571** and **0.0268** respectively, in the Hutsul (Table 1.). However, these alleles could not be detected in the Thoroughbred.

In microsatellite **HMS7** five alleles (**J, L, M, N, O**) were present in Thoroughbred and similarly five (**K, L, M, N, O**) in **Hutsul** (Diagram 2.).

The frequency values of all alleles in microsatellite **HTG7** differed between the two breeds (Diagram 2.). Of all the alleles present in Thoroughbreds allele **HTG7-O** (0.4868) had the highest frequency value. However, in **Hutsuls HTG7-K** (**0.7938**) was the dominant allele (Table 1.).

Diagram 1.: Allele frequency distribution (%) of microsatellite **VHL20** and **HMS2** between the two (hutsul, thoroubred) breeds



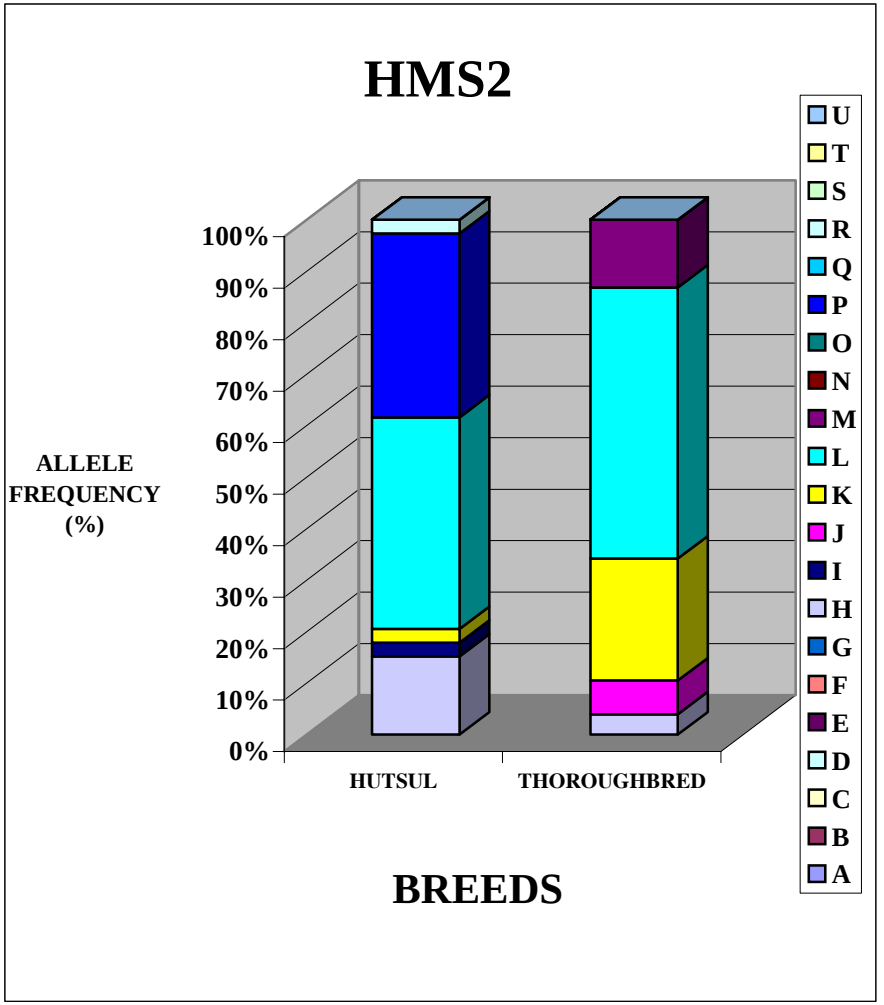
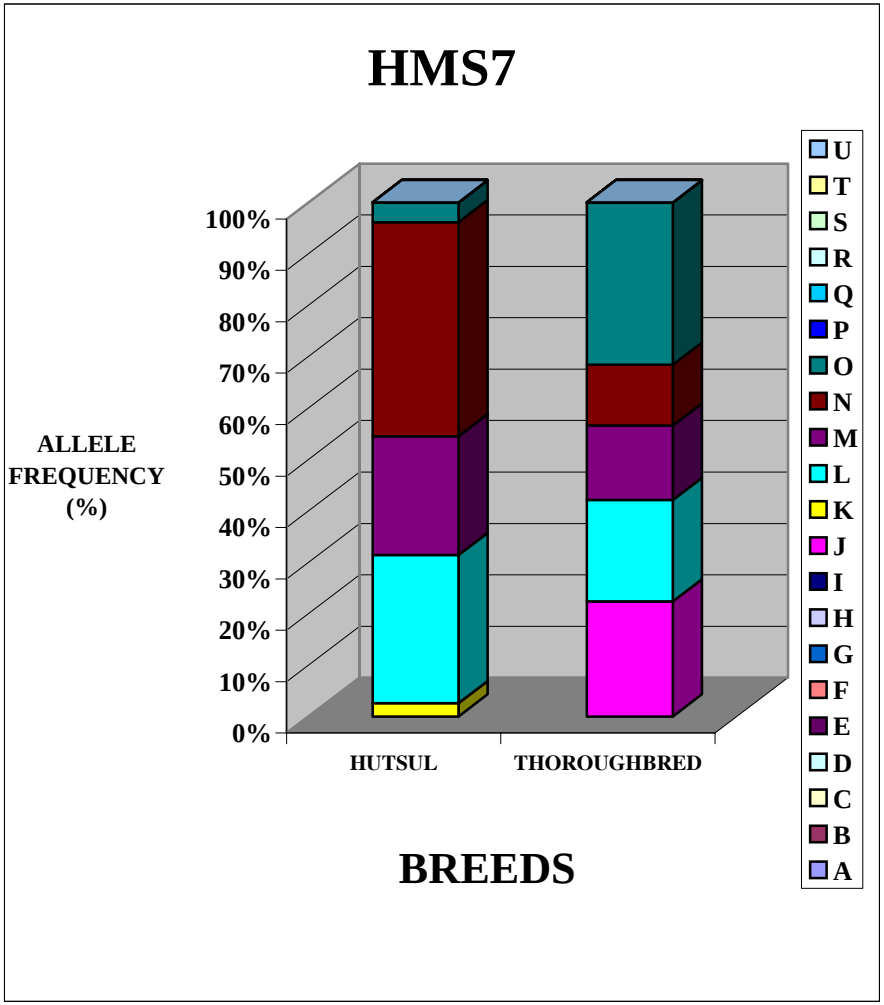


Diagram 2.: Allele frequency distribution (%) of microsatellite **HMS7** and **HTG7** between the two (hutsul, thoroughbred) breeds



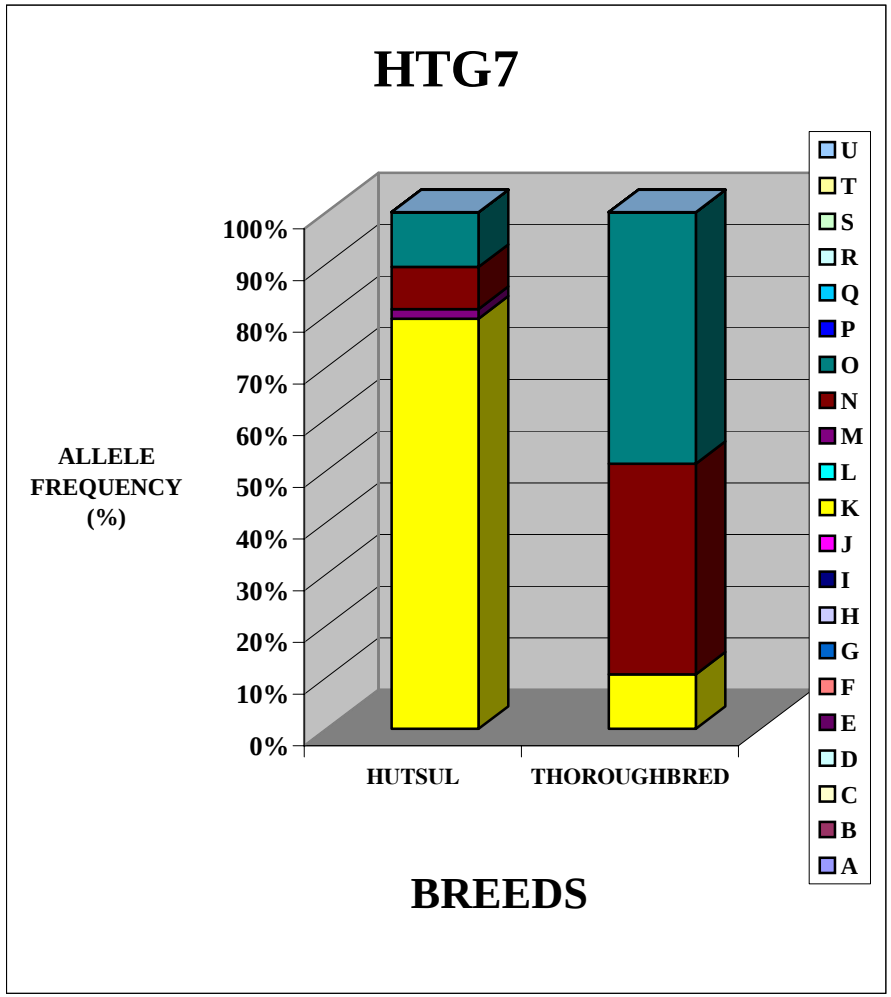


Table 1.: Alleles and allelfrequences of 12 microsatellites in Hutsul and Thoroughbred horses

LOKUS	BREEDS	ALLELES AND ALLÉLFREQUENCES OF MICROSATELLITES																				
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
VHL20	THOROUGHBRED									0,2763			0,0658	0,2632	0,3947							
	HUTSUL									0,1437	0,1313		0,05	0,1375	0,1875		0,1187	0,1625	0,0688			
HTG4	THOROUGHBRED											0,4474	0,2368	0,2368	0,0264		0,0526					
	HUTSUL											0,0063	0,1687	0,4875	0,0375		0,2937	0,0063				
AHT4	THOROUGHBRED								0,171	0,0131	0,2895	0,079				0,4474						
	HUTSUL								0,60	0,10	0,0563		0,0125		0,0186	0,1063	0,1063					
HMS7	THOROUGHBRED										0,2237		0,1974	0,1447	0,1184	0,3158						
	HUTSUL											0,0256	0,2884	0,2308	0,4167	0,0385						
AHT5	THOROUGHBRED										0,2368	0,25		0,1842	0,2632	0,0658						
	HUTSUL										0,3526	0,3141	0,0449	0,0641	0,0961	0,1282						
HMS6	THOROUGHBRED											0,1184	0,1054	0,3026		0,2368	0,2368					
	HUTSUL												0,0769	0,3013	0,0385	0,1154	0,4679					
HTG6	THOROUGHBRED							0,4342			0,3816			0,0526		0,1053			0,0263			
	HUTSUL							0,0625		0,0313	0,1062			0,1875		0,5938			0,0187			
ASB2	THOROUGHBRED		0,1316	0,0526							0,0264	0,3553		0,0921	0,0789	0,0526		0,1184	0,0921			
	HUTSUL		0,04	0,04		0,04		0,04	0,06	0,12		0,24	0,12	0,02	0,16	0,08			0,04			
HTG10	THOROUGHBRED									0,4079		0,1579	0,1053	0,2368		0,0526			0,0131	0,0264		
	HUTSUL											0,0667	0,0066		0,12	0,0868	0,0066	0,12	0,5933			
HTG7	THOROUGHBRED											0,1053			0,4079	0,4868						
	HUTSUL											0,7938		0,0187	0,0813	0,1062						
HMS3	THOROUGHBRED									0,5263				0,0921	0,0526	0,1842	0,1448					
	HUTSUL									0,3687				0,15	0,0063	0,0125	0,2438	0,1375	0,0812			
HMS2	THOROUGHBRED								0,0395		0,0658	0,2368	0,5263	0,1316								
	HUTSUL								0,1518	0,0268		0,0268	0,4107				0,3571		0,0268			

CONCLUSION

Many researchers have expressed doubts regarding the usefulness of DNA marker research over the past years (Breen et al., 1994).

DNA markers, due to their extremely large number and the more definite polymorphism of some loci, can be used on higher level and more efficiently in numerous fields of animal breeding, e.g. examination of genetic structure of populations, test of homozygosity, estimation of the degree of inbreeding of populations, maintenance of autochthonous populations (gene-reserve), parentage control, estimation of genetic distance between populations and breeds, planning of crossing programs (heterosis breeding).

Based upon the microsatellite investigations the conclusion can be drawn that the alleles of Thoroughbred horses could be observed in Hutsul breed. But the number of alleles were higher compared to the Hutsul breed. Some alleles found in Hutsul were not present in Thoroughbred.

REFERENCE

Andersson, L. (1985): The estimation of blood group gene frequencies: a note on the allocation methods. *Anim. Blood Grps. Biochem. Genet*. 16. 1-7.

Breen, M., Downs, P., Irvin, Z. and Bell, K. (1994): Intrageneric amplification of horse microsatellite markers with emphasis on the Przewalski's horse (E. przewalskii). *Animal Genetics* 25. 401-405.

Marklund, S.; Ellegren, H.; Eriksson, S.; Sandberg, K.; Andersson, L. (1994): Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. *Animal Genetics*, 25. 19-23.

Panaccio, M.; Georgesz, M.; Lew, A. M. (1993): FoLT PCR: a simple PCR protocol for amplifying DNA from whole blood. *Biotechniques*, 14. 238-243.

Prichner, F. (1983): *Population genetics in animal breeding*. Freeman Company, San Fransisco.

Romanov, M. N.; Weigend, S. (2001): Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. *Poultry Sci*., 80. 1057-1063.

Takahashi, H. K.; Nirasawa, K.; Nagamine, Y.; Tsudzuki, M.; Yamamoto, Y. (1998): Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphism, *J. Hered*. 89. 543-546.

Weber, J. L.; May, P. E. (1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics*, 44. 388-396.

Zhou, H. J.; Lamont, S. J. (1999): Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Animal Genetics*, 30. 256-264.