Genetic diversity of Hucul horse, based on microsatellite data in Slovak Republic.

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Abstract:

The goal of the Hucul International Federation (H.I.F.) is to protect Hucul horse through the Hucul's breeding area. The Hucul is an independent and unique breed of horses in Slovakia. In our study, a total of 48 animals were tested for genetic variability. The microsatellite loci showed the polymorphism with allele numbers ranging 4 to 9 and polymorphism information content in the range 0,47 to 0,827, observed heterozygosity 0,583 to 0,833, expected heterozygosity 0,552 to 0,837 and F_{it} , F_{is} -0,189 to 0,2. According to dendrogram and another biodiversity data, the population of Slovak Hucul Horse is not affected by loss of genetic diversity.

Introduction:

This past century has been a rapid decline in numbers within many breeds of horses. Hucul breed belongs to typical representatives of small mountain horses and it is classified as small numbered breed. Since 1979, Hucul belongs to protected gene fund of original and primitive animal breeds of FAO (**Mason**, 1996). Origin is not uniform and also the opinions about the origin and breed formation differ a lot. Hucul horse is indigenous breed of Carpathian Mountains. Origin is possible to find in original mountain horse, old polish, old moldavian horse, consequently in different modifications of mountain tarpan type. Type variability was caused by breeding with impact of blood of arabian horses, lipican, hafling, norik breed and in second half of past century with the effort to enlarge the breed also the crossing with fjord and other breeds occurred (**Kováč**, 2006).

First founders of the breed lines in controlled breed were breeding stallions Hroby and Goral, later in 40ties Slovak line Gurgul was developed. Except that the lines Oušol and Prislop are in use in the Slovak Republic (**Koubek *et al*.**, 1958, **Gabriš and Brauner**, 1987).

Hucul breed reaches 350–450 kg of body weight and 125–145 cm as height at the withers. Hard constitution, solid body and limbs frame, good health and resistance belong to characteristic trait and properties of the breed. Hucul is strong, skillful horse for endurance in mountain terrain. Most common colors are bay and chestnut, often with dorsal stripe on the back, mouse-colored and sometimes solid black (**Hörman *et al*.**, 1957). In the Slovak Republic the National Stud in Topoľčianky is responsible for the breed development, stud-book administration and gene reserves protection guarantee. Except the original area of breeding in Carpathian region of Ukraine, Romania, Slovakia and Poland the breeding was extended to the Czech Republic, Hungary and Austria (**Kadlečík** *et al*., 2004, **Kováč**, 2006).

In our study, we are using DNA markers–microsatellites to identify genetic variation in Hucul breed in Slovakia.

Microsatellites have been identified in all eukaryotic species studied so far. They were utilised for genetic mapping (**Weissenbach *et al.***, 1992) and have been extensively used for linkage analyses in the association with disease susceptibility genes (**Robinson *et al.***, 1996). In addition they have proven useful for analysis the paternity and identification (**Queller *et al.***, 1993). Comparison of levels of variation between species and populations have proven suitable in the assessment overall genetic variation. They can be used to estimate effective population size and to gain insight into the degree of population substructure genetic relationship among the various subpopulations. The genetic variation was quantified by conventional statistic parameters as frequency of alleles, heterozygosity, PIC value, F_{IT}, F_{ST}.

Material and methods:

DNA was isolated from peripheral blood from a total of 48 Hucul horses. For blood samples used in this study, DNA extraction basically followed the protocol of Promega (Wizard Genomic DNA Purification Kit). A set of 12 microsatellites–AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HMS7, HTG4,

HTG6, HTG7, HTG10, VHL 20 was analysed in this study. Twelve microsatellite DNA markers included in the set of the ABI StockMark have been chosen for the analysis. PCR products have been tested automatically by an ABI 310 Genetic Analyzer and their fragment size by Genescan software. PIC value, heterozygosities, frequencies of alleles, F_{IT}, F_{ST} were calculated using Powermarker 3.25 software.

Results and discussion:

Genetic analyzing was performed using 12 microsatellite loci (table 1). Allele frequency and standard deviation were calculated for each locus separately. The number of allele per each locus ranged from 4 (HTG7) to 9 (VHL20, HTG10, ASB2). In selected population, we are able to observe absence of several alleles (HTG6 allele H, L, N, P, R), as well as increase of specific allele frequency (HMS2 allele H; HMS6 allele P; HTG7 allele K and O).

Table 1: Allele presents in Slovak Hucul horse population

Marker	Allele	bp	Freq.	SD	Marker	Allele	bp	Freq.	SD	Marker	Allele	bp	Freq.	SD
AHT4	H	148	0.162	0.043	HMS3	I	152	0.281	0.049	HTG6	G	84	0.162	0.043
	I	150	0.135	0.037		M	160	0.104	0.033		I	88	0.068	0.028
	J	152	0.149	0.042		N	162	0.167	0.034		J	90	0.162	0.043
	K	154	0.095	0.032		O	164	0.135	0.038		M	96	0.068	0.034
	N	160	0.054	0.026		P	166	0.198	0.041		O	100	0.514	0.059
	O	162	0.338	0.058		Q	168	0.104	0.036		Q	104	0.027	0.027
	P	164	0.068	0.028		S	172	0.010	0.010	HTG7	K	120	0.417	0.054
AHT5	H	128	0.014	0.013	HMS61	K	159	0.010	0.010		M	124	0.028	0.019
	J	132	0.176	0.044		L	161	0.115	0.030		N	126	0.042	0.023
	K	134	0.243	0.053		M	163	0.240	0.042		O	128	0.514	0.060
	L	136	0.027	0.019		N	165	0.021	0.014	HTG10	K	97	0.074	0.030
	M	137	0.068	0.028		O	167	0.125	0.031		L	99	0.059	0.028
	N	140	0.176	0.039		P	169	0.479	0.042		M	101	0.191	0.059
	O	142	0.297	0.049		Q	171	0.010	0.010		N	103	0.015	0.014
ASB2	I	236	0.178	0.042	HMS7	J	173	0.043	0.030		O	105	0.132	0.043
	K	240	0.244	0.040		K	175	0.043	0.021		P	107	0.074	0.037
	L	242	0.011	0.011		L	177	0.424	0.055		Q	109	0.015	0.014
	M	244	0.056	0.023		M	179	0.098	0.029		R	111	0.397	0.065
	N	246	0.156	0.038		N	181	0.337	0.051		S	113	0.044	0.024
	O	248	0.200	0.043		O	183	0.054	0.023	VHL20	I	89	0.135	0.035
	P	250	0.044	0.021	HTG4	L	131	0.240	0.042		J	91	0.083	0.031
	Q	252	0.100	0.034		M	133	0.354	0.062		L	95	0.021	0.014
	R	254	0.011	0.011		N	135	0.021	0.014		M	97	0.177	0.040
HMS2	H	218	0.392	0.051		O	137	0.135	0.035		N	99	0.208	0.041
	I	220	0.041	0.029		P	139	0.250	0.044		O	101	0.021	0.014
	K	224	0.297	0.045							P	103	0.156	0.037
	L	226	0.162	0.038							Q	105	0.021	0.014
	P	234	0.108	0.039							R	107	0.167	0.037

Population genetic structure of observed group of animals is presented in Table 2. The average values of expected and observed heterozygosity indicate sufficient heterogenity between animals. The average value of expected heterozygozity (He) was 0.734. The higher value was detected in VHL20 (0.837) and lowest in HTG7 (0.552) system. HTG7 system was simultaneously the system with lowest number of alleles. The average value of observed heterozygosity was 0.378 and ranged from 0.844 (ASB2) to 0.583 (HTG7). According to average value of polymorphic information content (PIC), we can validate the high polymorphism of chosen markers. The inbreeding coefficient based on observed allele frequencies (from 0 to 0.129) show similar results about very low genetic similarity of observed animals. Computed values of Fis and Fit (0,004) for each microsatellite system as well as for entire group and average value of χ^2 (35.59) support our result.

Table 2: Population genetic structure of Slovak Hucul horse population

Marker	Počet alel	He	Ho	PIC	F	Fis	Fit	χ^2
AHT4	7	0.791	0.811	0.779	0.000	-0.010	-0.010	14.347
AHT5	7	0.775	0.838	0.752	0.000	-0.067	-0.067	27.809
ASB2	9	0.820	0.844	0.807	0.000	-0.019	-0.019	28.156
HMS2	5	0.710	0.811	0.671	0.027	-0.129	-0.129	26.131
HMS3	7	0.805	0.750	0.788	0.090	0.078	0.078	17.226
HMS6	7	0.678	0.813	0.640	0.000	-0.189	-0.189	64.063
HMS7	6	0.682	0.609	0.640	0.140	0.118	0.118	66.772
HTG4	6	0.735	0.729	0.699	0.002	0.009	0.009	11.107
HTG6	6	0.664	0.622	0.638	0.129	0.078	0.078	48.874
HTG7	4	0.552	0.583	0.466	0.000	-0.042	-0.042	4.497
HTG10	9	0.758	0.618	0.746	0.179	0.200	0.200	44.430
VHL20	9	0.837	0.833	0.827	0.016	0.015	0.015	73.733
Priemer	6.833	0.734	0.738	0.704	0.049	0.004	0.004	35.595

In observed population, dendrogram shows several clusters which represented relationships in observed population (Picture 1). The genetic distance was calculated by **Nei** (1973), and had ranging from 0 to 1. Number 0 represent two control identical animals (10104 and 4904). The average value based on individual distances between all used animals combinations was 0,617. In our study dendrogram shows relatively high distances between individuals. The levels of genetic diversity observed in the Slovak Hucul horse is higher to the other horse breeds in comparing studies of horse populations (**Achmann *et al.***, 2004, **Hertner *et al.***, 2000, **Cothran *et al.***, 2000, **Solis *et al.***, 2000). According to dendrogram and another biodiversity data, we can resume the population of Slovak Hucul horse is not affected by loss of genetic diversity.

References

Achman, R. *et al.* (2004): Microsatellite diversity, population subdivision and gene flow in the Lipizzan horse. Anim. Genet. 35, p. 285–292

Cothran, E. G. *et al.* (2000): Genetic variation in seven native horse breeds from Greece. Comparative Genomics, A026, 27th International Society of Animal Genetics, Minneapolis, USA, p. 9

Gabriš, J., Brauner, P. (1987): Zootechnika pre veterinárnych medikov, Príroda, Bratislava, 593 pp.

Hertner, U. *et al.*, (2000): Phylogenetic analysis and temporal changes in genetic variation of 18 horse breeds. A038, 27th International Society of Animal Genetics, Minneapolis, USA, p. 12

Hörman, Š., Žikavský, P., Landau, L., Gabriš, J., Šulgan, E., Svoboda, Z. (1957): Chov koní na Slovensku, Slovenské vydavateľstvo pôdohospodárskej literatúry, Bratislava, 449 pp.

Kadlečík, O. *et al.* (2004): Ohrozené plemená zvierat na Slovensku, 1. ed., SPU Nitra, 100 pp., ISBN 80-8069-459-1

Koubek, K. *et al.* (1958): Speciální zootechnika, Chov koní, (3. ed.), SZN Praha, 1031 pp.

Kováč, **P.** (2006): Huculský kôň včera a dnes, Slovenský chov, 9, 4, p. 30–32

Mason, I. L. (1996): A world dictionary of livestock breeds, types and varieties. 4th ed., CAB Int., 273 pp.

Queller, D. C. *et al.*, (1993): Microsatellites and Kinship. Tree 8, p. 285–288

Solis, B. *et al.*, (2000): Genetic diversity and genetic distance in threatened horse breeds from western Pyrenees by microsatellites. A043, 27th International Society of Animal Genetics, Minneapolis, USA, p. 13

Weissenbach, J. *et al.*, (1992): A second generation linkage map of the human genome. Nature 359, p. 794–801

Figure 1: Dendrogram based on genetic distances