Monitoring of genetic variability in different horse populations from the Czech Republic and the Netherlands

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Introduction

In this study we have been focused in genotyping of the genomic DNA sequences for microsatellite repeats. The aim of study is to characterize and monitor the extent of genetic diversity in selected horse populations from the Czech Republic and the Netherlands. Genetic research is aimed at the Czech gene reserves of Old Kladruby Horse, Silesian Noric, Hucul, Czech-Moravian Belgik, and Dutch Fjord Horse.

Material and Methods

Samples of 757 horses from the following populations were analysed: Old Kladruby Horse (n=159), Silesian Noric (n=105), Hucul (n=294), Czech-Moravian Belgik (n=66), and Dutch Fjord Horse (n=133).

The genotyping of microsatellite markers was performed on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by fluorescent fragment analysis and detected by software GeneScan® 3.7 NT, Genotyper® 3.7 NT. Reference samples distributed by ISAG were used to standardize all allele sizes.

The allele frequencies, observed, and expected heterozygosity, have been tested for deviations from Hardy-Weinberg equilibrium and polymorphism information content according to Green (1999). Exclusion probabilities (EP) and combined exclusion probabilities (CEPs) were calculated according to Jamieson and Taylor (1997).

Results and Discussions

In total, 757 animals have been genotyped for 17 microsatellites markers (AHT4, AHT5, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20, ASB2, ASB17, ASB23, CA425 and LEX3) recommended by ISAG. The number of allele per each locus ranged from 4 (HTG7, HTG6, HMS1) to 12 (ASB17, LEX3) with a mean of 7.26 alleles (Tab 1.).

The probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion were in Old Kladruby Horse 99.88% / 99.67% / 99.99%, Silesian Noric 99.99% / 99.98% / 99.99%, Hucul 99.99% / 99.99% / 99.99%, Czech-Moravian Belgik 99.99% / 99.97% / 99.99% and Fjord Horse 99.99% / 99.93% / 99.99%, respectively. The assay provides high CEPs in all tested breeds. The research concerns the variability of microsatellite DNA markers in genotypes of horses. The results have been revealed that the Hucul has quite high genetic variability (Tab 2.).

Tab. 1.: Number of alleles (NA), theoretical heterozygosity (tH)

	Old Kladruby Horse		Silesian Noric		Hucul		Czech-Moravian Belgik		Fjord Horse	
Loocus	NA	tH	NA	tH	NA	tH	NA	tH	NA	tH
AHT4	8	0.72	7	0.80	11	0.85	8	0.80	7	0.77
AHT5	6	0.70	8	0.75	7	0.81	7	0.76	6	0.66
HMS1	6	0.55	7	0.66	7	0.66	4	0.46	7	0.79
HMS2	6	0.67	9	0.73	8	0.80	8	0.72	6	0.69
HMS3	7	0.66	7	0.77	8	0.83	7	0.78	7	0.79
HMS6	6	0.68	6	0.69	6	0.70	5	0.67	6	0.76
HMS7	6	0.77	6	0.81	7	0.63	5	0.73	8	0.55
HTG4	5	0.78	6	0.67	6	0.75	5	0.74	5	0.70
HTG6	6	0.61	4	0.50	7	0.55	4	0.29	5	0.20
HTG7	4	0.24	4	0.71	5	0.63	4	0.70	4	0.61
HTG10	9	0.70	10	0.72	10	0.80	10	0.83	8	0.81
VHL20	7	0.79	9	0.80	10	0.87	8	0.83	9	0.70
ASB2	7	0.79	8	0.77	10	0.78	9	0.66	10	0.81
ASB17	10	0.70	12	0.89	12	0.85	11	0.89	11	0.83
ASB23	6	0.78	6	0.83	10	0.86	5	0.80	7	0.80
CA425	6	0.60	6	0.75	11	0.83	8	0.74	6	0.71
LEX3	5	0.59	12	0.87	9	0.83	9	0.86	7	0.84

Fjord Horse

Tab. 2. Alleles identified in Old Kladruby Horse, Silesian Noric, Hucul, Czech-Moravian Belgik and Fjord Horse

Locus	Old Kladruby Horse	Silesian Noric	Hucul	Czech-Moravian Belgik	Fjord Horse
AHT4	H, I, J, K, M, N, O, P	H, I, J, K, L, N, O	**F**, H, I, J, K, L, M, N, O, P, R	H, I, J, K, L, M, O, P	H, J, K, N, O, P, R
AHT5	J, K, L, M, N, O	I, J, K, L, M, N, O, P	**H**, J, K, L, M, N, O	I, J, K, L, M, N, O	J, K, L, M, N, O
HMS1	I, J, L, M, N, Q	I, J, K, L, M, N, Q	I, J, K, L, M, N, Q	J, K, L, M	I, J, K, L, M, N, Q
HMS2	H, I, J, K, L, M	H, I, J, K, L, M, P, R, S	H, I, J, K, L, M, P, R	H, I, J, K, L, O, R, S	H, I, J, K, L, M
HMS3	I, M, N, O, P, Q, R	I, M, N, O, P, Q, R	I, M, N, O, P, Q, R, **S**	I, M, N, O, P, Q, R	I, M, N, O, P, Q, R
HMS6	K, L, M, N, O, P	K, L, M, N, O, P	K, L, M, N, O, P	L, M, N, O, P	K, L, M, N, O, P
HMS7	J, K, L, M, N, O	J, K, L, M, N, O	J, K, L, M, N, O, Q	J, L, M, N, O	**I**, J, K, L, M, N, O, Q
HTG4	K, L, M, N, O	K, L, M, O, P, **Q**	K, L, M, N, O, P	K, L, M, O, P	K, L, M, O, P
HTG6	G, I, J, M, O, R	G, I, J, O	G, I, J, M, N, O, P	G, I, J, O	G, J, N, O, P
HTG7	K, M, N, O	K, M, N, O	**I**, K, M, N, O	K, M, N, O	K, M, N, O
HTG10	I, K, L, M, O, P, R, S, **T**	I, K, L, M, N, O, P, Q, R, S	I, K, L, M, N, O, P, Q, R, S	I, K, L, M, N, O, P, Q, R, S	I, K, L, M, N, O, P, R
VHL20	I, L, M, N, P, Q, R	I, J, L, M, N, O, P, Q, R	I, J, **K**, L, M, N, O, P, Q, R	I, J, M, N, O, P, Q, R	I, J, L, M, N, O, P, Q, R
ASB2	I, K, M, N, O, P, Q	B, I, K, M, N, O, P, Q	B, I, J, K, M, N, O, P, Q, R	B, I, K, M, N, O, P, Q, R	B, **C**, I, J, K, M, N, O, P, Q
ASB17	F, G, **H**, I, K, M, N, O, Q, R	I, J, K, **L**, M, N, O, P, Q, R, S, **T**	G, I, J, K, M, N, O, P, Q, R, S, **W**	F, G, I, K, M, N, O, P, Q, R, S	F, G, I, K, M, N, O, P, Q, R, S
ASB23	I, J, K, L, S, U	I, J, K, L, S, U	**H**, I, J, K, L, Q, R, S, T, U	I, K, L, S, U	J, K, Q, R, S, T, U
CA425	I, J, L, M, N, O	J, K, L, M, N, O	**F**, G, **H**, I, J, K, L, M, N, O, **P**	G, I, J, K, L, M, N, O	J, K, L, M, N, O
LEX3	H, L, M, N, P	**E**, F, **G**, H, I, **J**, K, L, M, N, O, P	F, H, I, K, L, M, N, O, P	F, H, I, K, L, M, N, O, P	F, H, K, L, M, N, P

bold - alleles found only in one breed; underlined - alleles with the highest occurrence

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