



Genetic diversity in the genetic resource of Old Kladruber Horse using microsatellite DNA markers

Putnová L.^{1*}, Vrtková I.¹, Hořín P.², Říha J.¹, Dvořák J.¹

¹Mendel University of Agriculture and Forestry Brno, Department of Animal Morphology, Physiology and Genetics, Zemedelska, 613 00 Brno, Czech Republic, putnova@mendelu.cz

²University of Veterinary and Pharmaceutical Sciences Brno, Palackeho, 612 42 Brno, Czech Republic

Introduction:

The Old Kladruber horse is the most important genetic resource in the Czech Republic. Seventeen microsatellites were genotyped in this breed using a panel of microsatellites recommended for routine parentage testing by the ISAG Equine Genetics Standing Committee.

Results:

The analysis of all loci in the population revealed 105 alleles. The mean number of alleles per locus was 6.18, ranging from 4 (HTG7) to 10 (ASB17). Allele frequencies for the most frequently allele were as follows: AHT4 (H=0.37), HMS7 (O=0.31), HTG4 (M=0.27), VHL20 (Q=0.38), AHT5 (N=0.47), ASB2 (M=0.35), ASB23 (J=0.30), HMS6 (L=0.47), HTG6 (O=0.59), ASB17 (R=0.44), CA425 (N=0.58), HMS1 (M=0.60), LEX3 (L=0.61), HMS2 (K=0.46), HMS3 (P=0.45), HTG10 (O=0.48), HTG7 (O=0.87).

The highest heterozygosity (above 70%) was observed for HTG4 (78.47), ASB2 (77.95), VHL20 (77.77), ASB23 (77.05), HMS7 (76.93) and AHT4 (71.68). The lowest heterozygosity (below 50%) was found for HTG7 (22.47). The value of inbreeding coefficient was zero (Tab. I). In the Hardy-Weinberg equilibrium conformity test only HMS3, LEX3 and HTG7 were not in equilibrium ($P < 0.01$). The average probabilities of paternity exclusion/one parental genotype unavailable/parentage exclusion (CEPI-3) estimated for this panel were 99.81 % / 99.59 % / 99.99 %.

Material and methods:

- ✂ Genomic DNA was extracted from blood using JETquick kit (Genomed). Multiplex polymerase chain reactions were performed in a 9700 GeneAmp PCR system (Applied Biosystems, Foster City, CA). The PCR conditions were those described in the StockMarks for Horses equine genotyping kit (Applied Biosystems).
- ✂ PCR products were detected by capillary electrophoresis using an ABI PRISM 310 DNA genetic analyzer with GeneScan (Fig. 1) and Genotyper analysis software (Applied Biosystems). The LIZ 500 bp internal standard (Applied Biosystems) was used for sizing alleles. The set of 153 animals was genotyped at each loci.

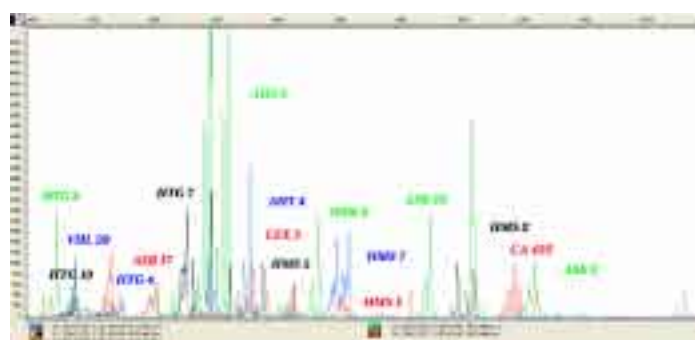


Figure 1: GeneScan analysis of multiplex data – one sample

Table I: Genetic variability – General information of the analysis

Marker	Major allele frequency	Observed Alleles	A llele No	Genotype No	N	Availability	G ene Diversity	Heterozygosity	PIC	f
AHT4	0.3725	H, I, J, K, M, N, O, P	8	18	153	I	0.7185	0.7386	0.6732	-0.0246
AHT5	0.4800	J, K, L, M, N, O	6	15	153	0.9804	0.6781	0.6733	0.6316	0.0104
ASB17	0.4412	F, G, H, I, K, M, N, O, Q, R	10	19	153	I	0.6967	0.7190	0.6510	-0.0286
ASB2	0.3454	I, K, M, N, O, Q	6	20	153	0.9935	0.7791	0.7829	0.7479	-0.0016
ASB23	0.2972	I, J, K, L, S, U	6	15	153	0.9346	0.7567	0.7692	0.7140	-0.0131
CA425	0.5980	I, J, L, M, N, O	6	10	153	0.9673	0.5646	0.6419	0.5069	-0.1337
HMS1	0.5980	I, J, M, N, Q	5	9	153	I	0.5528	0.5752	0.4871	-0.0371
HMS2	0.4561	H, I, K, L, M	5	10	153	0.9673	0.6496	0.7162	0.5812	-0.0991
HMS3	0.4342	I, M, O, P, Q, R	6	7	153	0.2484	0.7109	I	0.6681	-0.3955
HMS6	0.4706	K, L, M, N, O, P	6	12	153	I	0.6698	0.6863	0.6162	-0.0214
HMS7	0.3170	J, K, L, M, N, O	6	18	153	I	0.7688	0.8105	0.7336	-0.0510
HTG10	0.4852	I, K, L, M, O, P, R, S, T	9	17	153	0.8824	0.6768	0.7630	0.6328	-0.1236
HTG4	0.2712	K, L, M, N, O	5	15	153	I	0.7843	0.7516	0.7492	0.0450
HTG6	0.5980	G, I, J, M, O	5	10	153	I	0.5868	0.6340	0.5454	-0.0771
HTG7	0.8693	K, M, N, O	4	4	153	I	0.2297	0.2549	0.2075	-0.1066
LEX3	0.6144	H, L, M, N, P	5	11	153	I	0.5742	0.4118	0.5363	0.2860
VHL20	0.3758	I, L, M, N, P, Q, R	7	23	153	I	0.7810	0.7974	0.7543	-0.0178
Mean	0.4720		6.1765	13.7059	153	0.9396	0.6576	0.6897	0.6139	-0.0448