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GENETIC VARIATION WITHIN AND RELATIONSHIPS AMONG FIVE SUBPOPULATIONS OF SLOVAK THOROUGHBRED

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Genetic variation at six microsatellite loci was analysed for five Thoroughbred subpopulations to determine the magnitude of genetic differentiation and the genetic relationships among the subpopulations. Significant deviations from Hardy–Weinberg equilibrium were shown for a number of locus–population combinations, with all subpopulations. The genetic diversities and relationships of five Thoroughbred subpopulations were evaluated using six microsatellites recommended by the International Society of Animal Genetics (ISAG). The allele frequencies, the effective numbers of alleles, and the observed and expected heterozygosities were calculated. POPGENE v. 1.31 (Yeh et al., 1997) was used to test for deviations from the Hardy–Weinberg (H–W) equilibrium and to assign F_{IS} estimates (Weir, 1990). The utility of microsatellites for evaluating genetic diversity of horses is discussed.

Key words: Thoroughbred, horse, genetic diversity, microsatellites, subpopulations

In the last century, there was a rapid decline in numbers within many breeds of horses in Slovakia. Breeds such as the Thoroughbred have few breeding animals (only 20 studs in Slovakia) and might suffer a loss in the amount of genetic variations present within the breed. This means that less genetic variation exists among animals in the breed. Therefore, evaluation and conservation (if reasonable) are essential tasks for animal breeders and geneticists.

The genetic variability of horse populations was examined using a number of different types of polymorphic gene loci. The commonest among these are the

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red blood cell group loci, the genes of major histocompatibility complex, the biochemical genetic loci, the mitochondrion DNA and the VNTR markers (microsatellites and minisatellites).

Microsatellites are promising genetic markers for studying the demographic structure and phylogenetic history of populations. They are a class of tandem repeat loci, where alleles can be distinguished by their size (Tautz, 1993). The range of allele sizes found at microsatellite loci is typically limited (Garza et al., 1995).

Microsatellites are useful for a number of analyses. They were originally utilised for genetic mapping (Weissenbach et al., 1992) and have been extensively used for linkage analyses in the association with disease susceptibility genes. In addition, they were proven useful in paternity and kinship analysis (Queller et al., 1993) and also in the probability of sample identity at both individual (Edwards et al., 1992) and population levels (Paetkau and Strobeck, 1995). Microsatellites can be used to estimate effective population size (Allen et al., 1995) and to gain insight into the degree of population substructure including both the amount of migration between subpopulations (Allen et al., 1995) and genetic relationships among various subpopulations (Bowcock et al., 1994; Estoup et al., 1996; Lade et al., 1996).

Evidence from several sources indicates that microsatellites mutate via a mechanism which favours small changes (usually one repeat unit) in array length (Shriver et al., 1993; Weber and Wong, 1993). The mutation rate is exceptionally high (Jeffreys et al., 1988; Kelly et al., 1991), implying a high degree of polymorphism.

In our study the population structures within and among five subpopulations of Slovakian Thoroughbred breeds was characterised.

Materials and methods

Blood samples were collected from 133 Thoroughbred horses in different subpopulations of Bratislava (Quo Vadis, n = 16), Kobylany (n = 34), Motešice (n = 24), Kuchyňa (Gestut Guthler, n = 47) and Šurany (n = 12).

DNA was obtained following the protocol of Promega (Wizard Genomic DNA Purification Kit).

Polymerase chain reaction (PCR) was performed on a PTC 200 (MJ Research). Microsatellites (ASB2, HMS3, HMS6, HMS7, HTG4, VHL20) were amplified in a single PCR. For each PCR reaction, one primer was 5′-end labelled with Texas Red. Primer sequences and details on the PCR amplifications of the six microsatellites is shown in Table 1 (Ellegren et al., 1992; Guérin et al., 1994; Van Haeringen et al., 1994).

Table 1

Primer sequences used for the amplification of the microsatellite loci

Locus	Primer sequences (5`–3`)	References
HMS3	P1: CCA ACT CTT TGT CAC ATA ACA AGA P2: CCA TCC TCA CTT TTT CAC TTT GTT	Guérin et al. (1994)
HMS6	P1: GAA GCT GCC AGT ATT CAA CCA TTG P2: CTC CAT CTT GTG AAG TGT AAC TCA	Guérin et al. (1994)
HMS7	P1: CAG GAA ACT CAT GTT GAT ACC ATC P2: TGT TGT TGA AAC ATA CCT TGA CTG T	Guérin et al. (1994)
HTG4	P1: CTA TCT CAG TCT TGA TTG CAG GAC P2: CTC CCT CCC TCC CTC TGT TCT C	Ellegren et al. (1992)
VHL20	P1: CAA GTC CTC TTA CTT GAA GAC TAG P2: AAC TCA GGG AGA ATC TTC CTC AG	Van Haeringen et al. (1994)
ASB2	P1: CCT TCC TGT AGT TTA AGC TTC TG P2: CAC AAC TGA GTT CTC TGA TAG G	Breen et al. (1997)

Electrophoresis and analysis were performed using an Automated Laser Fluorescent DNA sequencer (A.L.F. DNA sequencer, Pharmacia). Sizing of PCR products was accomplished both by external standards (50–500 bp size marker-ladder, Pharmacia) and by standard samples specific for each microsatellite locus. Alleles at each locus were assigned letter codes, and POPGENE v.1.31 (Yeh et al., 1997) was used to calculate allele frequencies and estimates of genetic variation as follows: expected heterozygosity (H_e) (Nei's unbiased estimate, Nei, 1973) for each locus, population and observed heterozygosity (H_o). We also calculated an average number of alleles, effective number of alleles (number of alleles that would result in that level of heterozygosity if all alleles were in equal frequencies; Hartl and Clark, 1989), deficiency of heterozygotes relative to Hardy–Weinberg expectation-F_{IS} (Hartl and Clark, 1989; Weir, 1990).

Table 2

Allele frequency of five Thoroughbred subpopulations

Locus	Bratislava	Kobylany	Motešice	Kuchyňa	Šurany
ASB2	0.032–0.312	0.014–0.206	0.042–0.354	0.032–0.436	0.042–0.25
HMS3	0.063–0.656	0.031–0.563	0.021–0.542	0.031–0.511	0.080–0.625
HMS6	0.031–0.625	0.015–0.529	0.021–0.354	0.022–0.359	0.042–0.458
HMS7	0.031–0.281	0.031–0.318	0.042–0.229	0.011–0.309	0.042–0.458
HTG4	0.031–0.531	0.031–0.406	0.104–0.5	0.425–0.575	0.292–0.708
VHL20	0.033–0.367	0.033–0.367	0.021–0.354	0.011–0.351	0.083–0.458

Table 3

Observed number and effective number of alleles (Kimura and Crow, 1964)

Locus	Bratislava			Kobylany			Motešice			Kuchyňa			Šurany		
	n	na*	ne*	n	na*	ne*	n	na*	ne*	n	na*	ne*	n	na*	ne*
ASB2	32	8	5.51	68	9	5.53	48	9	5.17	94	8	3.72	24	8	6.00
HMS3	32	5	2.14	64	5	2.55	48	6	2.92	94	6	3.11	24	3	2.07
HMS6	32	5	2.30	68	6	2.58	48	6	4.11	92	5	3.70	24	5	3.13
HMS7	32	7	5.45	66	8	5.07	48	7	5.17	94	7	4.74	24	5	2.97
HTG4	32	4	2.23	68	3	1.84	48	3	2.40	94	2	1.96	24	2	1.70
VHL20	30	6	4.25	68	4	3.28	48	6	4.14	94	5	3.78	24	4	2.55
Mean	30	5.83	3.64	67	5.83	3.47	48	6.17	3.98	94	5.50	3.50	24	4.00	3.07
St. D.		1.47	1.62		2.32	1.49		1.94	1.14		2.07	0.92		2.07	1.53

n = sample size; na = Observed number of alleles; ne = Effective number of alleles; St. D. = standard deviation

Table 4

Number of alleles (n), observed heterozygosities (Ho) and expected heterozygosities (He) for six microsatellite loci across the investigated Thoroughbred subpopulations

Locus	Bratislava			Kobylany			Motešice			Kuchyňa			Šurany		
	n	H_o	H_e	n	H_o	H_e	n	H_o	H_e	n	H_o	H_e	n	H_o	H_e
ASB2	8	0.81	0.84	9	0.82	0.83	9	0.88	0.82	8	0.70	0.74	8	0.92	0.87
HMS3	5	0.50	0.55	5	0.59	0.62	6	0.63	0.67	6	0.63	0.69	3	0.42	0.54
HMS6	5	0.75	0.58	6	0.74	0.62	6	0.83	0.77	5	0.59	0.74	5	0.92	0.71
HMS7	7	1.00	0.84	8	0.91	0.81	7	0.96	0.82	7	0.91	0.80	5	0.83	0.69
HTG4	4	0.63	0.57	3	0.62	0.46	3	0.83	0.59	2	0.51	0.49	2	0.42	0.43
VHL20	6	0.87	0.79	4	0.82	0.71	6	0.92	0.77	5	0.79	0.74	4	0.58	0.63

Table 5

Summary Chi-squared test for Hardy–Weinberg equilibrium for five Thoroughbred subpopulations

Population locus	Bratislava		Kobylany		Motešice		Kuchyňa		Šurany	
	df	P	df	P	df	P	df	P	df	P
ASB2	28	0.8132	36	0.4866	36	0.9797	28	0.6462	28	0.8925
HMS3	10	0.3176	10	0.5254	15	0.6314	15	0.0274*	3	0.4081
HMS6	10	0.8767	15	0.00019**	15	0.1841	10	0.3244	10	0.1116
HMS7	21	0.2271	28	0.000001**	21	0.000005**	21	0.1635	10	0.7688
HTG4	6	0.8771	3	0.203	3	0.0131	1	0.8172	1	0.9012
VHL20	15	0.0007**	6	0.4478	15	0.67902	10	0.0702	6	0.6914

*$P < 0.05$; **$P < 0.0001$

Table 6

Fixation index values average across loci in five Thoroughbred subpopulations (statistically highly significant deviation given in bold)

Locus	Bratislava	Kobylany	Motešice	Kuchyňa	Šurany
ASB2	0.0072	–0.0053	–0.085	0.0396	–0.1
HMS3	0.0623	0.0225	0.0489	0.028	0.1946
HMS6	–0.3287	–0.2006	–0.1009	0.1953	–0.3469
HMS7	–0.2249	–0.1327	–0.1884	–0.1598	–0.2565
HTG4	–0.1348	**–0.351**	**–0.4307**	–0.0444	–0.0084
VHL20	–0.1337	–0.1848	–0.2082	–0.0708	0.04

Results and discussion

A total of 49 alleles were detected across the six loci analysed. Allele distributions and frequencies (Table 2) and the effective numbers of alleles (Table 3) varied greatly between the subpopulations.

For six microsatellites, the observed mean heterozygosity (H_o) ranged from 0.4167 (Šurany) to 1 (Bratislava), whereas the expected mean heterozygosities (H_e) varied between 0.4312 (Šurany) and 0.8696 (Šurany), with 2–9 alleles segregating at each locus within the subpopulations (Table 4). From 30 instances (5 subpopulations, 6 loci), four deviations proved significant at 0.1% level and one at 5%, when the H–W equilibrium was calculated. Observed H–W equilibrium deviations were not consistent over loci, but generally occurred with different microsatellites in different subpopulations (see Table 5). Deviations from expected values may occur for a variety of reasons such as selection, inbreeding and the Wahlund effect.

F-statistics estimated individually for the six loci and combined for the set are presented in Table 6. Sample size and significance levels (P values) are also given. At individual loci, F_{IS} estimates ranged between –0.6667 (Motešice, locus HTG4) and 1.0 (Motešice, locus HMS7). Heterozygote deficiency (F_{IS} = 1.0, locus HMS7, Motešice) can be caused by inbreeding, the Wahlund effect or selection including micro-scale differentiation.

Two total F_{IS} were estimated to be significant at 5% level (Table 6). Microsatellite loci had an observed heterozygosity similar to that expected under H–W expectation (total F_{IS} ranged from –0.347 to 0.0623). Locus HTG4 showed a deficiency of homozygotes (F_{IS} = –0.351 Kobylany and –0.431 Motešice).

Finally, we noted that the relationship between the sampling units and the population breeding structure affected the expectation of F_{IS}. The F_{IS} values reflected the pattern of mate exchanges within and between subpopulations. Our values of total F_{IS} showed intensive gene flow and migration.

The comparisons of the allele numbers and observed H–W equilibrium deviations from five Thoroughbred subpopulations imply that recent artificial selection has affected the within-breed genetic composition of the studied Thoroughbred subpopulations.

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