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Evaluation of changes in the genetic structure of Hucul horses based on the analysis of I type of genetic markers

Ocena zmian w strukturze genetycznej koni huculskich na podstawie analizy markerów genetycznych klasy I

Summary. The objective of the study was to determine genetic differentiation of the current Hucul horse population and to analyse gene frequency changes during the course of the breeding programme for this breed. A total of 5000 Hucul horses raised in Poland were investigated. Based on the identified erythrocyte antigens and electrophoretic variants of blood proteins and enzymes in 14 loci, the allele frequency of the analysed markers was calculated in two groups of horses born before 2001 and during 2001–2010. Statistically significant differences were found in allele frequencies in both groups of animals. The investigated population is in Hardy-Weinberg genetic equilibrium except for esterase. The average degree of heterozygosity in horses increased to 0.413, while the average inbreeding coefficient in the herd, calculated from allele frequencies at 7 protein loci, decreased to 0.0118.

Key words: Hucul horses, antigens, polymorphic proteins, genetic structure

INTRODUCTION

Genetic variation is of crucial importance to all species of animals because it determines the adaptability of populations to diverse environmental conditions. Long-term selection, especially in small populations, reduces the gene pool and may negatively affect breeding. The assessment of genetic variation enables the optimal selection strategy and animals for reproduction to be chosen. The results of breeding work can be monitored in a population by studying the frequencies of genetic marker alleles. For this purpose, I type and II type of markers (polymorphic plasma proteins, erythrocyte enzymes, blood groups and microsatellite DNA sequences) are most often used [Nogaj and Nogaj 2001, Tozaki *et al*. 2001, Ząbek *et al*. 2006].

Huculs, the primitive mountain horses, are one of the oldest Polish breeds with a fixed genotype [PZHK 1999]. They have been protected as an endangered relict species since 1979, and involved in a special conservation breeding scheme since the year 2000. The horses of this breed, raised in Poland during the period 1989–1990, have been studied for genetic structure based on differences in erythrocyte antigens and blood proteins [Nogaj 1995, Nogaj and Nogaj 2001]. At that time, the small population had fewer than 300 animals. The modern Hucul horse is raised strictly according to the accepted breeding programme, which for 10 years has prescribed avoiding excess relatedness among the animals.

The objective of the present study was to analyse frequency changes in the I type genetic marker alleles that occurred in the Hucul horse population during 1990–2010 as a result of selective breeding and mating selection, as well as to determine the genetic structure of the current Hucul population in Poland.

MATERIAL AND METHODS

Subjects were Hucul horses which pedigrees were tested at the Horse Blood Typing Laboratory of the Experimental Station of the National Research Institute of Animal Production in Chorzelów, Poland. All animals with incorrect parentage information were eliminated from further breeding and disregarded in the study. The analysis included Hucul horses raised all over Poland, but most of them originated from southern and south-eastern Poland. Allele frequency was determined in two groups of horses:

– group I – 1139 Hucul horses born before 2001,

– group II – 4041 Hucul horses born between 2001 and 2010.

Erythrocyte blood group antigens were identified at seven loci following the method described by Stormont and Suzuki [1964) and Stormont *et al*. [1964]. A panel of 35 test reagents, standardized through comparison tests under the auspices of the International Society for Animal Genetics (ISAG) was used. The polymorphism of erythrocyte enzymes: 6-phosphogluconate dehydrogenase (PGD) and phosphoglucose isomerase (PGI) was determined by agarose gel electrophoresis according to Gahne and Juneja [1985], and plasma proteins were identified using polyacrylamide gel electrophoresis according to Juneja *et al.* [1978].

The inheritance of the identified antigens was analysed in family data by determining phenogroups in individual animals based on traits passed from parents to offspring. The frequencies of alleles at the EAC and EAK loci were calculated by the square root method, and those at the EAA, EAD, EAP, EAQ and EAU loci were estimated based on the number of animals of a specific genotype. The frequencies of protein and erythrocyte enzyme alleles were calculated based on the identified phenotypes. Significant differences in allele frequency in both horse groups were calculated by chi-square test. The average coefficient of heterozygosity for animals in the herd was estimated using the formula by Nei and Roychoudhury [1974]. The average inbreeding coefficient for the Hucul breed was calculated using a formula provided by Kidd *et al*. [1980], and polymorphic information content (PIC) according to the formula of Botstein *et al.* [1980].

RESULTS AND DISCUSSION

As a result of the present study, conducted in a population of 5180 Hucul horses, the polymorphism of type I of genetic markers was determined at 14 loci. Differences in the genetic variants of proteins and blood enzymes were analysed at 7 loci (AL, GC, ES, A1B, TF, PGD and GPI), in which 52 phenotypes were found. No significant differences were observed between the expected and observed frequency of phenotypes at particular loci, except for esterase (Table 1).

Table 1. Frequency of phenotypes of electrophoretic protein and enzyme variants in the Hucul population
Tabela 1. Frekwencja fenotypów elektroforetycznych wariantów białek i enzymów w populacji koni rasy huculskiej

Loci Loci	Phenotypes Fenotypy	Number of animals Liczba zwierząt		Frequencies of phenotypes Frekwencja fenotypów	Significance of differences Istotność różnic
		observed obserwowana	expected oczekiwana		
1	2	3	4	5	6
AL N = 5178	A	1766	1802.0	0.341	n.s.
	AB	2574	2505.1	0.497	
	B	838	870.4	0.162	
GC N = 5169	F	5153	5148.3	0.997	n.s.
	FS	16	20.6	0.003	
	S	0	0.02	0.000	
ES N = 5171	F	564	539.5	0.109	19.15***
	FI	1813	1813.9	0.351	
	FS	400	447.6	0.077	
	I	1486	1524.7	0.287	
	IS	833	752.5	0.161	
	S	75	92.8	0.015	
A1B N = 5180	F	0	0.0	0.000	n.s.
	FK	4	2.0	0.001	
	FS	0	0.0	0.000	
	K	4975	4974.9	0.960	
	KS	200	203.0	0.039	
	S	1	2.1	0.000	
TF N = 5153	D	216	195.9	0.042	n.s.
	DF1	35	30.1	0.007	
	DF2	613	631.0	0.119	
	DF3	1	0.4	0.000	
	DH1	10	8.0	0.002	
	DH2	405	438.1	0.078	
	DO	112	140.7	0.022	
	DR	400	369.8	0.078	

cd. tabeli 1
table 1 cont.

1	2	3	4	5	6
TF N = 5153	F1	3	1.1	0.001	n.s.
	F1F2	44	48.5	0.009	
	F1F3	0	0.0	0.000	
	F1H1	0	0.6	0.000	
	F1H2	26	33.7	0.005	
	F1O	7	10.8	0.001	
	F1R	35	28.4	0.007	
	F2	498	508	0.097	
	F2F3	1	0.6	0.000	
	F2H1	10	12.9	0.002	
	F2H2	723	705.5	0.141	
	F2O	265	226.5	0.052	
	F2R	579	595.4	0.113	
	F3	0	0.0	0.000	
	F3H1	0	0.0	0.000	
	F3H2	0	0.5	0.000	
	F3O	0	0.1	0.000	
	F3R	0	0.4	0.000	
	H1	0	0.1	0.000	
	H1H2	15	9.0	0.003	
	H1O	1	2.8	0.000	
	H1R	10	7.6	0.002	
	H2	258	244.9	0.050	
	H2O	170	157.3	0.033	
	H2R	395	413.4	0.077	
	O	20	25.2	0.004	
	OR	120	132.7	0.023	
	R	181	174.5	0.035	
PGD n=3058	D	0	0.0	0.000	n.s.
	DF	11	10.6	0.004	
	DS	0	0.3	0.000	
	F	2858	2858.9	0.934	
	FS	187	184.5	0.061	
	S	2	3.0	0.001	
GPI n=3062	F	4	6.1	0.0013	n.s.
	FI	265	261.4	0.0865	
	FS	1	0.1	0.0003	
	I	2790	2791.5	0.9112	

n.s. – not significant/nieistotne.
*** $P \leq 0.001$ – highly significant differences/różnice wysoko istotne.

Table 2. Frequencies of alleles at blood group, protein and blood enzyme loci in two age groups of the Hucul horses
Tabela 2. Frekwencja alleli grup krwi, białek i enzymów krwi u koni rasy huculskiej w dwóch grupach wiekowych

Locus Locus	Alleles Allele	Frequency Frekwencja		Chi^2
		N = 1139	N = 4041	
1	2	3	4	5
EAA	adf	0.471	0.440	**
	adg	0.061	0,060	
	b	0.190	0.198	
	bc	0.003	0.001	
	c	0.201	0.236	***
	-	0.074	0.065	
EAC	a	0.390	0.345	***
	-	0.610	0.655	***
EAD	adlnr	0.011	0.001	
	adlr	0.028	0.049	*
	bcmq	0.091	0.079	
	cegimnq	0.003	0.001	
	cgm	0.005	0.002	
	cgmq	0.120	0.108	
	cgmqr	0.001	0.001	
	deloq	0.383	0.424	***
	delq	0.048	0.032	***
	dghm	0.002	0.000	
	dghmq	0.000	0.000	
	dghmqr28	0.220	0.195	**
	dkl28	0.097	0.108	
	dlnq	0.001	0.000	
	dlqr28	0.000	0.000	
EAK	a	0.000	0.000(5)	
	-	1.000	0.999(5)	
EAP	a	0.273	0.288	
	ad	0.046	0.037	
	b	0.001	0.003	
	bd	0.001	0.001	
	d	0.006	0.001	
	-	0.674	0.668	
EAQ	ac	0.037	0.038	
	b	0.259	0.239	*
	bc	0.000	0.000	
	c	0.381	0.350	**
	-	0.323	0.372	***

cd. tabeli 2
table 2 cont.

1	2	3	4	5
EAU	a1	0.000	0.001	
	a2	0.117	0.049	***
	a3	0.259	0.220	***
	a4	0.170	0.245	***
	-	0.454	0.485	*
AL	Al^A	0.595	0.588	
	Al^B	0.405	0.412	
GC	GC^F	0.998	0.998	
	GC^S	0.002	0.002	
ES	ES^F	0.291	0.332	**
	ES^I	0.566	0.537	*
	ES^S	0.143	0.131	
A1B	$A1B^F$	0.000	0.0005	
	$A1B^K$	0.978	0.9806	
	$A1B^S$	0.022	0.0189	
TF	TF^{D1}	0.160	0.141	*
	TF^{D2}	0.032	0.053	***
	TF^{D3}	0.000	0.001	
	TF^{F1}	0.013	0.015	
	TF^{F2}	0.332	0.308	
	TF^{F3}	0.000	0.000	
	TF^{H1}	0.003	0.005	
	TF^{H2}	0.230	0.215	
	TF^O	0.065	0.071	
	TF^R	0.165	0.191	**
PGD	PGD^D	0.004	0.001	
	PGD^F	0.956	0.973	
	PGD^S	0.040	0.026	
GPI	GPI^F	0.049	0.042	
	GPI^I	0.951	0.957	
	GPI^S	0.000	0.001	

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Analysis of the 35 erythrocyte antigens identified at 7 loci (EAA, EAC, EAD, EAK, EAP, EAQ and EAU) revealed the presence in the Hucul breed of 41 phenogroups (alleles), most of which (15) occurred at the EAD locus (Table 2).

Significant differences in allele frequencies between the grup of horses born before 2001 and the second group born during 2001-2010, are shown in Table 2.

The average coefficient of inbreeding, calculated from allele frequencies at 7 selected protein and enzyme loci in the investigated Hucul population is low at (0.0118, Table 3), while the average degree of heterozygosity in animals is 0.413 (Table 4). Polymorphic information content (PIC), which shows differentiation of alleles at the 14 loci of identified markers, is presented in Table 4.

Table 3. Average inbreeding coefficient in the Hucul horses
Tabela 3. Średni współczynnik inbredu u koni huculskich

Loci Loci	Inbreeding coefficient Współczynnik inbredu	
	at locus i w locus i	average średni
AL	- 0.0270	
GC	+ 0.2250	
ES	- 0.0113	
A1B	+ 0.0150	0.0118
TF	+ 0.0054	
PGD	- 0.0156	
GPI	- 0.0162	

Table 4. Average heterozygosity coefficient in the Hucul population and polymorphic information content (PIC)
Tabela 4. Średni współczynnik heterozygotyczności osobników w populacji koni rasy huculskiej oraz średni stopień polimorfizmu (PIC) w badanych loci

Locus Locus	Heterozygosity coefficient Współczynnik heterozygotyczności		Polymorphic information content at locus Stopień polimorfizmu PIC
	in locus w locus	total ogółem	
EAA	0.7017		0.6587
EAC	0.4580		0.3532
EAD	0.7568		0.7346
EAK	0.0008		0.0008
EAP	0.4684		0.3971
EAQ	0.6824		0.6168
EAU	0.6631	0.413	0.5964
AL	0.4838		0.3774
GC	0.0060		0.0060
ES	0.5830		0.5076
A1B	0.0392		0.0388
TF	0.7917		0.7683
PGD	0.0643		0.0620
GPI	0.0875		0.0828

The population of more than 5000 Hucul horses was analysed for genetic structure based on the polymorphism of I type of genetic markers: erythrocyte antigens, and blood proteins and enzymes. The investigations included most of the Hucul animals currently raised in Poland. The determination of allele frequencies in both investigated groups of horses made it possible to analyse gene frequency changes that occurred over more than ten years as a result of mating selection and selective breeding. In total, 67 alleles of the analysed markers were identified in the population. For 20 of these, significant and highly significant differences in frequency were found between the groups of earlier and later born horses.

The erythrocyte antigens were identified using an extensive panel of test reagents standardized through international comparison tests organized by ISAG [Nogaj *et al*. 1999], which enabled determining blood group polymorphism for 35 antigenic characters located in 41 phenogroups (alleles). Most phenogroups (15) were detected in the most polymorphic EAD locus.

Like in the previously investigated population [Nogaj 1995], the currently used Hucul horses are in Hardy-Weinberg genetic equilibrium (Table 1) except for esterase, in which a statistically significant difference between the observed and expected phenotype frequency was already observed at that time.

The analysis of allele frequencies in the group of older and younger horses revealed significant differences at 7 loci: EAA, EAC, EAD, EAQ, EAU, ES and TF (Table 2). In the A group system (EAA locus), a highly significant increase in A^c allele frequency was observed. High antigenic diversity at this locus and high A^c allele frequency, not found in any other horse breed except for Biłgoraj horses (0.178), remains a characteristic trait of the Hucul horses [Nogaj *et al*. 2003]. It is known that this allele has low frequency (less than 1%) in Pure Arabian horses and Thoroughbred horses [Trommershausen-Bowling and Clark 1985].

The frequency of the D^{deloq} allele is still high despite a small decrease in older horses from group I (0.383). In horses from the second group , a highly significant increase in the frequency of this allele is observed (0.424). An analogous situation was reported in a previous study of horses born between 1990 and 1992 [Nogaj 1994], when this allele was identified in more than half of the foals. This process was stopped in later years, which is noticeable in the first group of the currently studied horses (0.383), but in the second group, D^{deloq} is again found in almost half of the animals.

The frequency of the C^a allele at the EAC locus consistently declines. In the population of horses bred during 1989-1990 it had a frequency of 0.462 [Nogaj 1995], whereas in the currently studied horses its frequency is 0.390 (first group) and 0.345 (second group). Allele diversity at the EAQ and EAU loci is decreasing. Highly significant differences were observed in the frequency of the Q^c and Q^- alleles. Compared to the horse population from the 1980s, the Q^c allele increased and the Q^- allele decreased in frequency. An increase in the frequency of the U^- allele is noticeable. In Polish horse breeds, the low frequency of the C^a (0.300) and U^- alleles (0.333) was only found in Biłgoraj horses [Nogaj *et al*. 2002, 2003].

Compared to the population of the oldest horses [Nogaj 1995], a downward tendency is seen for the frequency of the AL^A gene, the high frequency of which was characteristic of the Huculs (0.602, 0.595 and 0.588, respectively). Meanwhile, the frequency of TF^R (0.124, 0.164, 0.191) and D^{dkl28} (0.076, 0.097, 0.108) consistently increases. It should be noted that the D^{dkl28} allele is characteristic of Małopolski, Wielkopolski and Polish Warmblood horses [Nogaj et al. 2001], and TF^R is typical of coldblood horses (0.220 – our unpublished data.

The highly significant differences in allele frequencies between the groups of older and younger horses may suggest that preference is given to selecting animals with traits found in warmblood horses while in Hucul horses of relatively high diversity of alleles at the EAA (PIC-0.6587) and EAU loci (PIC-0.5964), the low frequency of the C^a allele and the high frequency of the D^{deloq} allele is preserved.

In the currently studied Hucul K^a and $D^{cegimnq}$ phenogroups, as well as GC^S, PGD^D and GPI^S alleles were identified such phenogroups and alleles did not occur in the previously analysed population of this breed [Nogaj 1995). The appearance of "new" alleles is probably the result of introducing more than ten new horses bought in Romania, which had both $D^{cegimnq}$ and K^a phenogroups and the GC^S, PGD^D and GPI^S variants.

CONCLUSION

The analysis of the I type of markers in Hucul horses shows that during the 20 years of breeding work, the average coefficient of heterozygosity in the population slightly increased from 0.403 to 0.413 and the degree of inbreeding decreased from 0.042 to 0.0118. The changes may be due to the breeding programme which prevents an excess of relatedness among animals of the breed.

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Streszczenie. Badania miały na celu określenie zróżnicowania genetycznego obecnie hodowanej populacji koni huculskich oraz analizę zmian frekwencji genów podczas realizowanego w tej rasie programu hodowlanego. Ogółem badaniami objęto ponad 5000 koni huculskich hodowanych w Polsce. Na podstawie zidentyfikowanych antygenów erytrocytów i elektroforetycznych wariantów białek i enzymów krwi w 14 loci obliczono częstość występowania alleli badanych markerów w dwu grupach koni, urodzonych przed 2001 rokiem i urodzonych w latach 2001–2010. Stwierdzono statystycznie istotne różnice frekwencji alleli w obu grupach zwierząt. Badana populacja znajduje się w równowadze genetycznej H-W, z wyjątkiem esterazy. Zwiększył się średni stopień heterozygotyczności osobników i wynosi 0.413, natomiast zmniejszył się średni współczynnik inbredu w stadzie, wyliczony na podstawie frekwencji alleli w 7 loci białek i wynosi 0.0118.

Słowa kluczowe: konie rasy huculskiej, antygen, polimorficzne białka, struktura genetyczna