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CLASS I MARKER POLYMORPHISM IN POLISH MOUNTAIN SHEEP OF COLOURED AND WHITE VARIETIES*

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Abstract

The aim of the study was to determine the polymorphism of erythrocyte antigens in 6 blood group systems (A, B, C, D, M, R) as well as the polymorphism of serum (transferrin) and erythrocyte (haemoglobin) proteins in 110 Polish Mountain Sheep (POG) of white variety and in 108 POG sheep of coloured variety. In the analysed sheep population, the mean effective number of alleles and the mean degree of heterozygosity ($\overline{H}$) were determined based on the frequency of selected genetic markers. In the sheep of coloured variety, these values (3.5 for $\overline{E}$ and 0.5440 for $\overline{H}$) were similar to those of sheep of white variety (3.6 and 0.5541, respectively). The high degree of genetic marker polymorphism at some loci is indicative of considerable genetic variation in both varieties of Polish Mountain Sheep. The results obtained can also serve as an important source of information for development of breeding programmes aimed at preserving genetic variation in small populations of animals.

Key words: sheep, blood groups, protein polymorphism, genetic variability

One of the main principles of the FAO's Global Strategy for the Management of Farm Animal Genetic Resources is to preserve the biodiversity of farm animals by monitoring and conservation of native breeds threatened with extinction (Hammond, 1997). In Poland, the Genetic Resources Conservation Programme covers 13 breeds and varieties of sheep: Corriedale, Kamieniecka, Olkuska, Pomorska, Świniarka, Uhruska, Wielkopolska, Wrzosówka, Żelaźnieńska, Coloured Merino, Polish Mountain Sheep of coloured variety, Podhale Zackel and old-type Polish Merino. Due to the small size of the flocks, it is important that the genetic differences between these flocks should be monitored and inspected. An important indicator that provides indirect information about the degree of differences between animals in a flock is genetic variation, which can be calculated using class I markers such as blood groups and proteins (Kaczor et al., 1996; Rychlik and Duniec 2000; Rychlik et al., 2004) and

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class II markers, which include mini- and microsatellite DNA sequences and single nucleotide polymorphisms (SNP) (Arranz et al., 2001; Rychlik et al., 2007 b; Słota et al., 2007; Kijas et al., 2009).

The aim of the study was to compare the genetic structure (determined based on the study of blood group and protein polymorphism) of Polish Mountain Sheep of white variety and that of Polish Mountain Sheep of coloured variety, included in the Genetic Resources Conservation Programme.

Material and methods

Investigations were made of 108 Polish Mountain Sheep (POG) of coloured variety from 3 flocks and 110 Polish Mountain Sheep of white variety also from 3 flocks. All of 218 animals originated from the Podhale region.

Erythrocyte antigens were determined using 16 standardized test reagents: anti-Aa, Ab, Bb, Bc, Bd, Be, Bf, Bg, Bi, PLB-17, Ca, Cb, Da, Ma, R and 0. Polymorphic variants of transferrin and haemoglobin were determined by horizontal starch gel electrophoresis.

Statistical analysis included calculating the frequency of alleles at different loci using direct gene counting, and calculating the degree of heterozygosity (Nei and Roychoudhury, 1974) and the effective number of alleles per locus (Kimura and Crow, 1964). Based on the observed and expected number of haemoglobin (HBB) and transferrin (TF) genotypes, genetic equilibrium was evaluated according to Hardy-Weinberg's law. Significant differences were determined by chi-square test according to a formula provided by Stratil (1970).

Results

Data obtained on the diversity of genetic markers in coloured and white variety of POG sheep are shown in Tables 1–4.

Table 1 summarizes the frequency of blood group alleles in A, B, C, D, M and R systems, the frequency of plasma protein (transferrin) and erythrocyte (haemoglobin) alleles, and significant differences in the frequency of these markers.

In the A blood group system, the A^- allele was most frequent in the coloured variety of POG sheep (0.5370) and the A^a allele in the white variety of POG sheep (0.5136). The A^{ab} allele was found only in the latter group of sheep (0.0545). In this group, highly significant differences were found in the frequency of the A^{ab} allele and significant differences for the A^- allele.

In the B blood group system, 49 alleles were found, of which $B^{fPLB\text{-}17}$ (0.1296 in the coloured variety of POG sheep) and B^- (0.1273 in the white variety of POG sheep) were the most frequent. In addition, B^f (0.1111), B^- (0.0787), B^{fi} (0.0694) and B^{ci} and $B^{dfPLB\text{-}17}$ (both 0.0509) alleles were highly frequent (above 5%) in the coloured variety of POG sheep. In the flock of POG sheep of white variety, the most frequent alleles were $B^{fPLB\text{-}17}$ (0.1091), B^{ci} (0.1045), $B^{dfPLB\text{-}17}$ (0.0818), B^{bg} (0.0636) and B^c (0.0545).

Table 1. Frequencies of blood groups (EA), haemoglobin (HB) and transferrin (TF) alleles in investigated population of Polish Mountain Sheep of coloured (POG c.v.) and white variety (POG w.v.)

Locus	Alleles	Frequency		Chi²
		POG c. v. n=108	POG w. v. n=110	
1.	2.	3.	4.	5.
EAA	a	0.4537	0.5136	1.57
	ab	0.0000	0.0545	12.12***
	b	0.0093	0.0227	1.25
	-	0.5370	0.4091	7.16**
EAB	b	0.0741	0.0591	0.39
	bc	0.0139	0.0182	0.13
	bcfPLB-17	0.0000	0.0045	0.98
	bci	0.0324	0.0045	4.70*
	bciPLB-17	0.0185	0.0045	1.88
	bcPLB-17	0.0000	0.0045	0.98
	bd	0.0046	0.0227	2.63
	bdfi	0.0278	0.0000	6.20*
	be	0.0139	0.0136	0.00
	befiPLB-17	0.0000	0.0091	1.97
	bei	0.0000	0.0045	0.98
	bf	0.0185	0.0045	1.88
	bfi	0.0000	0.0091	1.97
	bfiPLB-17	0.0046	0.0045	0.00
	bfPLB-17	0.0139	0.0000	3.08
	bg	0.0046	0.0636	11.42***
	bgi	0.0000	0.0091	1.97
	bi	0.0880	0.0455	3.17
	biPLB-17	0.0093	0.0000	2.05
	bPLB-17	0.0046	0.0091	0.32
	c	0.0185	0.0545	4.00*
	cdf	0.0093	0.0000	2.05
	cdfiPLB-17	0.0093	0.0136	0.18
	cf	0.0046	0.0000	1.02
	cfgiPLB-17	0.0000	0.0045	0.98
	cfgPLB-17	0.0093	0.0000	2.05
	cfi	0.0000	0.0091	1.97
	cfiPLB-17	0.0000	0.0182	3.96*
	cfPLB-17	0.0093	0.0091	0.00
	cgPLB-17	0.0046	0.0000	1.02
	ci	0.0509	0.1045	4.36*
	ciPLB-17	0.0046	0.0045	0.00
	cPLB-17	0.0046	0.0045	0.00
	d	0.0000	0.0045	0.98
	def	0.0000	0.0045	0.98
	defiPLB-17	0.0000	0.0227	4.97*
	defi	0.0139	0.0000	3.08
	dfiPLB-17	0.0093	0.0000	2.05
	dfPLB-17	0.0509	0.0818	1.68
	di	0.0046	0.0000	1.02
	e	0.0093	0.0000	2.05
	f	0.1111	0.0273	11.96***
	fgPLB-17	0.0000	0.0045	0.98
	fi	0.0694	0.0136	8.58**
	fiPLB-17	0.0185	0.0455	2.54
	fPLB-17	0.1296	0.1091	0.44
	i	0.0324	0.0227	0.38
	PLB-17	0.0185	0.0227	0.10
	-	0.0787	0.1273	2.78
EAC	a	0.1296	0.0636	5.45*
	ab	0.1852	0.0864	9.10**
	b	0.5648	0.7409	14.93***
	-	0.1204	0.1091	0.14
EAD	a	0.0741	0.1909	12.90***
	-	0.9259	0.8091	12.90***
EAM	a	0.5278	0.4909	0.59
	-	0.4722	0.5091	0.59
EAR	R	0.4537	0.5636	5.27
	0	0.5370	0.4000	8.22**
	i	0.0093	0.0364	3.57
HBB	A	0.3194	0.3409	0.23
	B	0.6806	0.6591	0.23
TF	A	0.1435	0.0818	4.16*
	B	0.1944	0.2091	0.15
	C	0.3611	0.2955	2.13
	D	0.2685	0.3545	3.76
	E	0.0324	0.0591	1.77

*P<0.05; **P<0.01; ***P<0.001.

Table 2. Frequency of haemoglobin (HBB) and transferrin (TF) genotypes in investigated sheep breeds

Locus	Genotype	Frequency	
		POG c. v.	POG w.v.
HBB	*AA*	0.0370	0.1273
	AB	0.5648	0.4273
	BB	0.3982	0.4455
TF	*AA*		0.0181
	AB	0.0555	0.0727
	AC	0.1296	0.0455
	AD	0.0926	0.0091
	AE	0.0025	
	BB	0.0025	0.0091
	BC	0.1759	0.1273
	BD	0.1389	0.1727
	BE		0.0273
	CC	0.0926	0.0909
	CD	0.1944	0.2000
	CE	0.0370	0.0364
	DD	0.0463	0.1364
	DE	0.0185	0.0545

Table 3. Number of alleles (N), effective number of alleles (E) and degree of heterozygosity (hk) in investigated groups of sheep

Locus	POG c. v.			POG w.v.		
	N	E	hk	N	E	hk
EAA	3	2.0	0.5057	4	2.3	0.5653
EAB	36	12.7	0.9211	38	13.8	0.9276
EAC	4	2.6	0.6154	4	1.7	0.4277
EAD	2	1.2	0.1327	2	1.4	0.3090
EAM	2	2.0	0.4984	2	2.0	0.4998
EAR	3	2.0	0.5057	3	2.1	0.5211
HBB	2	1.8	0.4348	2	1.8	0.4494
TF	5	3.8	0.7381	5	3.7	0.7331
Total	57			60		
		3.5			3.6	
			0.5440			0.5541

E – mean degree of effective number of alleles.
H – mean degree of heterozygosity.

The B^{bdfi}, $B^{bfPLB-17}$, $B^{biPLB-17}$, B^{cdf}, B^{cf}, $B^{cfgPLB-17}$, $B^{cgPLB-17}$, B^{defi}, $B^{dfiPLB-17}$, B^{di} and B^{e} alleles were only noted in the flock of POG sheep of coloured variety, whereas $B^{bcfPLB-17}$, $B^{bcPLB-17}$, B^{bei}, B^{bfi}, B^{bgi}, $B^{cfgiPLB-17}$, B^{cfi}, $B^{cfiPLB-17}$, B^{d}, B^{def}, $B^{defiPLB-17}$ and $B^{fgPLB-17}$ alleles occurred only in the white variety of POG sheep. Most of these alleles occur with low frequency (below 0.01%) in the populations studied.

Table 4. Observed and expected distributions of HBB and TF genotypes

Locus	Geno-type	POG c. v.		Degree of freedom	Chi²	POG w.v.		Degree of freedom	Chi²
		Observed	Expected			Observed	Expected		
HBB	*AA*	4	11.0	2	9.66**	14	11.2	2	0.77
	AB	61	47.0			47	47.8		
	BB	43	50.0			49	51.0		
TF	*AA*	0	2.2	13	10.84	2	0.7	13	16.66
	BB	1	4.1			1	4.8		
	CC	10	14.1			10	9.6		
	DD	5	7.8			15	13.8		
	AB	6	6.0			8	3.8		
	AC	14	11.2			5	5.3		
	AD	10	8.3			1	6.4		
	AE	1	1.0			0	1.1		
	BC	19	15.2			14	13.6		
	BD	15	11.3			19	16.3		
	BE	0	0			3	2.7		
	CD	21	20.9			22	23.0		
	CE	4	2.5			4	3.8		
	DE	2	1.9			6	4.6		

*P<0.05; **P<0.01; ***P<0.001.

Comparison of the frequency of individual alleles from the B system in the two groups of animals showed significant differences for 9 alleles.

When analysing the other four blood group systems, significant differences in allele frequency were found for all the C system alleles except C^- and for both alleles from the D system. No statistically significant differences were observed for alleles from the M system and for alleles from the R system except the R^0 allele.

At the haemoglobin locus, HBB^A and HBB^B alleles were found in the analysed flocks, but their frequencies did not differ significantly. A total of 5 alleles (TF^A, TF^B, TF^C, TF^D, TF^E) were identified in the transferrin system. Considerable differences in the frequency of the TF^A allele were found in the animal groups studied.

Table 2 presents the frequency of haemoglobin and transferrin genotypes.

In the flock of POG sheep of coloured variety, the most frequent genotype at the HBB locus was *AB* (0.5648). *BB* genotype was the most frequent (0.4455) in the white variety of POG sheep. In both groups of animals, the most frequent genotype at the TF locus was CD (0.1944 for coloured variety and 0.2000 for white variety of POG sheep).

Table 3 gives the degree of heterozygosity ($\overline{H}$) and the effective number of alleles ($\overline{E}$), calculated from the frequency of individual genetic markers. In both animal groups studied, these parameters had similar values. The effective number of alleles in the coloured variety of POG sheep averaged 3.5 and was just 0.1 lower than in the

white variety of POG sheep. Small differences between the analysed groups were also noted for the mean degree of heterozygosity (0.5440 for coloured variety and 0.5541 for white variety of POG sheep).

Table 4 presents the observed and expected distribution of genotypes at the HBB and TF loci. Using Hardy-Weinberg's law, the populations were analysed for genetic equilibrium based on the differences between these distributions. Significant differences between these distributions were evaluated by chi-square test. The flocks of POG sheep of white variety were in genetic equilibrium for both the haemoglobin and transferrin loci. The coloured variety of POG sheep was in genetic disequilibrium for the haemoglobin locus.

Discussion

The conservation of threatened breeds of farm animals has been a key component of EU policy since the 20th century. In the early 1980s, first projects were developed and implemented in some countries. Over the years, the cooperation of European countries was aimed at creating a list of threatened breeds of farm animals and at elaborating criteria for the threat status which were based on predicted increase in inbreeding (Simon and Buchenauer, 1993; Martyniuk, 2003).

By analysing the distribution of allele frequencies at different loci, it is possible to monitor the genetic structure of populations and to detect changes in gene frequency due to, among others, breeding work (Lipecka, 1984; Nguyen et al., 1992; Rychlik et al., 1997; Rychlik and Duniec, 2000). This especially concerns threatened breeds of small population size, which can serve as a genetic reserve for valuable breeding traits such as health, fertility, prolificacy and good adaptation to local environment (Simianer, 2005; Rychlik et al., 2006).

The Polish Mountain Sheep of coloured variety are an indigenous variety of the Zackel breed group, originating from Southern Carpathians and the Balkans. Polish Mountain Sheep and their coloured varieties were brought to the Polish Carpathian Mountains by migrating pastoral tribes. From the total population of sheep raised in Poland in the mountain areas, animals selected based on phenotypic traits were classified as the coloured variety of POG sheep, which has been included in the Genetic Resources Conservation Programme since 2003. These sheep are easy to manage and resistant to adverse environment conditions of mountain areas, while being an important part of the Podhale folk customs and landscape (Sikora, 2007).

Previous research on the genetic diversity of Polish sheep breeds included in the Genetic Resources Conservation Programme was conducted for Wrzosówka (Janik et al., 1996; Rychlik et al., 2006; Rychlik et al., 2007 a), Coloured Merino (Rychlik et al., 2007 b), Olkuska (Rychlik et al., 1997), Kamieniecka (Kaczor and Rychlik, 2004), Corriedale (Kaczor and Rychlik, 2005) and Świniarka (Rychlik et al., 2009) breeds.

The achievement of breeding progress is conditional on genetic variation, which can be expressed by the effective number of alleles (E), degree of heterozygosity (H) and total number of alleles. The high values of these parameters are evidence of considerable genetic variation within a breed.

In the population of Polish Mountain Sheep studied, $\overline{H}$ was high (0.544 for coloured variety and 0.554 for white variety), which is indicative of considerable genetic variation within the animal groups studied. Among the previously analysed 6 breeds, this parameter was higher only for Olkuska (0.594) (Rychlik et al., 1997) and highly similar for Kamieniecka sheep (0.537) (Kaczor and Rychlik, 2004). The other breeds showed lower genetic variation, with $\overline{H}$ values of 0.414 for Corriedale (Kaczor and Rychlik, 2005), 0.431 for Coloured Merino (Rychlik et al., 2007 b), and 0.461 for Świniarka (Rychlik et al., 2009). Both populations of POG sheep studied were also characterized by high values of the effective number of alleles ($\overline{E}$), which was 3.5 for the coloured variety and 3.6 for the white variety. Earlier studies reported higher values of this parameter: 3.81 for Kamieniecka (Kaczor and Rychlik, 2004), 4.44 for Olkuska (Rychlik et al., 1997) and 4.48 for Wrzosówka sheep investigated in 1990–1995 (Rychlik et al., 2006). In the other breeds, *E* was lower than the value calculated for POG sheep, reaching 2.80 in Coloured Merino (Rychlik et al., 2007 b) and 2.90 in Wrzosówka sheep studied in 2001–2005 (Rychlik et al., 2007 a), and the same as in the white variety of POG sheep (3.60) in Wrzosówka sheep investigated in 1996-2000 (Rychlik et al., 2006).

Both varieties of Polish Mountain Sheep were analysed for genetic equilibrium in the HB and TF systems. In each of these (except the HB locus in the coloured variety), there was a consistence between the observed and expected number of genotypes. The disturbed genetic balance may be attributed to the small number of analysed sheep and the use of strict selection of rams for reproduction, which increased the frequency of their genes in the flock and decreased the frequency of genes from the ewe population. In addition, the disturbed genetic equilibrium is affected by factors such as mating system, selection and migration.

The data obtained on differences in the marker loci could be useful when deciding about further directions of breeding the coloured and white varieties of POG sheep while providing a starting point for further monitoring of genetic variation in this small population of sheep.

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TADEUSZ RYCHLIK, ANNA KRAWCZYK

Polimorfizm markerów genetycznych klasy I w dwóch odmianach polskiej owcy górskiej – białej i barwnej

STRESZCZENIE

Celem badań było określenie polimorfizmu antygenów erytrocytarnych w 6 układach grupowych krwi (A, B, C, D, M, R) oraz polimorfizmu białka osocza krwi (transferyny) i erytrocytów (hemoglobiny)

u 110 owiec polskiej owcy górskiej (POG) odmiany białej i 108 owiec POG odmiany barwnej. W obu grupach zwierząt, na podstawie częstości występowania badanych markerów genetycznych obliczono średnią efektywną liczbę alleli ($\overline{E}$) oraz średnią wartość stopnia heterozygotyczności ($\overline{H}$). U owiec odmiany barwnej wskaźniki te przyjęły wartości 3,5 dla $\overline{E}$ i 0,5440 dla $\overline{H}$. U osobników POG odmiany białej wartości te były bardzo zbliżone i wynosiły odpowiednio 3,6 oraz 0,5541. Wysoki poziom polimorfizmu markerów genetycznych w wybranych loci świadczy o znacznym zróżnicowaniu genetycznym badanej populacji POG i może stanowić ważne źródło informacji do opracowania programów hodowlanych wspierających zachowanie różnorodności genetycznej w małych populacjach zwierząt.