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PRELIMINARY EVALUATION OF THE GENETIC STRUCTURE OF ŚWINIARKA SHEEP BASED ON BLOOD GROUPS AND POLYMORPHIC PROTEIN VARIANTS*

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Abstract

The aim of the study was to identify the genetic structure of Świniarka sheep based on blood group and blood protein polymorphism. A total of 96 sheep originating from flocks in the Świętokrzyskie and Małopolskie provinces were analysed. The effective number of alleles and the degree of heterozygosity were calculated based on the frequency of individual blood group, haemoglobin and transferrin alleles. The mean values of these indicators, calculated from the genetic markers used, are 3.37 and 0.4608, respectively. The analysis of genetic equilibrium, performed in HBB and TF systems, showed a discrepancy between the observed and expected number of genotypes in the TF system. This result can be attributed, among others, to rigorous selection for breed characteristics during the period when the Świniarka breed was restored.

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

Genetically determined antigens located on the surface of erythrocytes, known as blood groups, as well as polymorphic variants of blood proteins have been used since the 1960s to characterize populations, especially those of farm animals. One example in this context are studies on the genetic characterization of different sheep breeds (Rychlik et al., 1997; Diez-Tascón et al., 2000; Arranz et al., 2001; Rychlik et al., 2007 a, b).

These studies are of special importance in protected breeds. Because the animals of protected breeds are small in number, breeding work is based not only on the evaluation of phenotypic variation and breeding documentation (Olech et al., 1996), but also on genetic variation. The latter is most often evaluated indirectly using, for example, the polymorphism of blood groups and proteins that are class I markers (Kaczor et al., 1996; Kmieć, 1997; Rychlik and Duniec 1999, 2000; Rychlik et al., 2002, 2004) and

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the polymorphism of minisatellite and microsatellite DNA sequences that are class II markers (Radko et al., 2006; Rychlik et al., 2007 b; Słota et al., 2007).

Previous analysis of the genetic structure of conservation breed sheep was performed for Wrzosówka (Janik et al., 1996; Rychlik et al., 2006, 2007 a), Coloured Merino (Rychlik et al., 2007 b), Olkuska (Rychlik et al., 1997), Kamieniecka (Kaczor and Rychlik, 2004) and Corriedale (Kaczor and Rychlik, 2005) breeds.

The aim of the study was to analyse genetic differences in Świniarka sheep based on class I markers (blood groups and polymorphic variants of haemoglobin and transferrin).

Material and methods

A total of 96 Świniarka sheep originating from two flocks included in the genetic resources conservation programme were analysed for blood group and protein polymorphism. Of these, 46 came from the Świętokrzyskie province and 50 from the Małopolskie province.

Erythrocyte antigens were determined using the authors' own test reagents: anti-Aa, Ab, Bb, Bc, Bd, Be, Bf, Bg, Bi, PLB-17, Ca, Cb, Da, Ma, R, and 0. All the 16 reagents used were subjected to international standardization in comparison tests organized by the International Society for Animal Genetics. Polymorphic variants of transferrin and haemoglobin were determined using starch gel horizontal electrophoresis.

Statistical analysis included calculating allele frequencies in particular loci using the direct gene number counting method, 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 calculated, the state of genetic equilibrium was evaluated in accordance with the Hardy-Weinberg law. Statistical significance of the differences was analysed using the Chi^2 test.

Results

The analysis of class I markers in the flocks studied revealed data on the polymorphism of erythrocyte antigens in 7 blood group systems (A, B, C, D, M, R, I) and the polymorphism of plasma protein (transferrin) and erythrocyte protein (haemoglobin).

The frequency of blood group, haemoglobin and transferrin alleles is presented in Table 1.

In the A blood group system, A^- allele was the most frequent and A^b allele did not occur in the flocks studied.

A total of 24 alleles were observed in the most polymorphic B system. Of these, alleles $B^{bfPLB\text{-}17}$ (0.1719) and B^{dg} (0.1302) were the most frequent. The lowest frequency

was characteristic of allele $B^{cPLB-17}$ (0.0052). Alleles $B^{beiPLB-17}$, B^{c} and B^{di} were also very infrequent (0.0104).

Table 1. Frequencies of blood group (EA), haemoglobin (HB) and transferrin (TF) alleles in the investigated population of Świniarka sheep

Locus	Allele	Frequency (n = 96)	Locus	Allele	Frequency (n = 96)
EAA	a	0.3698	**EAC**	a	0.0156
	ab	0.0052		ab	0.0312
	b	-		b	0.6876
	-	0.6250		-	0.2656
EAB	b	0.0938	**EAD**	a	0.0312
	bdfgPLB-17	0.0833		-	0.9688
	bdfiPLB-17	0.0521	**EAM**	a	0.4115
	bdfPLB-17	0.0313		-	0.5885
	beiPLB-17	0.0104	**EAR**	R	0.6250
	bf	0.0260		0	0.3750
	bfgPLB-17	0.0208	**EAI**	I	0.9427
	bfiPLB-17	0.0260		i	0.0573
	bfPLB-17	0.1719	**HBB**	A	0.2135
	bi	0.0365		B	0.7865
	biPLB-17	0.0260	**TF**	A	0.2656
	bPLB-17	0.0313		B	0.0781
	c	0.0104		C	0.4479
	cPLB-17	0.0052		D	0.1771
	ci	0.0208		E	0.0313
	d	0.0104			
	dfiPLB-17	0.0208			
	dg	0.1302			
	di	0.0104			
	fPLB-17	0.0208			
	i	0.0156			
	iPLB-17	0.0313			
	PLB-17	0.0313			
	B^{-}	0.0833			

In the C system, the frequency of allele C^{b} (0.6876) was considerably greater than the frequency of the other alleles. Allele Ca was the least frequent (0.0156).

In the other systems, the most frequent alleles were D^{-} (0.9688) in the D system, M^{-} (0.5885) in the M system and I^{I} (0.9427) in the I system.

Among haemoglobin (HBB) alleles, HBB^{B} allele was the most frequent in the analysed flocks (0.7865). In the case of transferrin, allele TF^{C} was the most frequent (0.4479) and allele TF^{E} the least frequent (0.0313) (Table 1).

Frequencies of transferrin and haemoglobin genotypes are shown in Table 2. BB genotype was the most frequent genotype in the haemoglobin locus (0.6146) and CC genotype in the transferrin locus (0.3125).

Table 2. Frequencies of haemoglobin (HB) and transferrin (TF) genotypes in the investigated group of Świniarka sheep

Locus	Genotype	Frequency	Locus	Genotype	Frequency
HBB	AA	0.0416	**TF**	BB	0.0417
	AB	0.3438		BC	0.0104
	BB	0.6146		CC	0.3125
TF	AA	0.0625		CD	0.0625
	AB	0.0625		DD	0.0625
	AC	0.1979		DE	0.0313
	AD	0.1354		EE	0.0104
	AE	0.0104			

Table 3. Number of alleles (N), effective number of alleles (E) and degree of heterozygosity in the investigated group of Świniarka sheep

Locus	Number of alleles N	Effective number of alleles E	Degree of heterozygosity h_k
EAA	4	1.89	0.4726
EAB	24	15.50	0.9355
EAC	4	1.84	0.4554
AAD	2	1.06	0.0604
EAM	2	1.94	0.4844
EAR	2	1.88	0.4686
SI	2	1.12	0.1085
HBB	2	1.89	0.4716
TF	6	3.23	0.6904
Total	48		
$\overline{E}$		3.37	
$\overline{H}$			0.4608

$\overline{E}$ – mean effective number of alleles.
$\overline{H}$ – mean degree of heterozygosity.

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

Locus	Genotype	Observed	Expected	Degrees of freedom	Chi-square test
HBB	AA	185	185.5		
	AB	274	273.5	2	0.056
	BB	100	100.7		
TF	AA	6	6.8		
	BB	4	0.6		
	CC	30	19.3		
	DD	6	3.0		
	EE	1	0		
	AB	6	4		
	AC	19	22.8	11	51.1***
	AD	13	9		
	AE	1	0.7		
	BC	1	6.7		
	CD	6	15.2		
	DE	3	0.4		

*** Statistically significant differences at $P<0.001$.

In the investigated population of sheep, the effective number of alleles ($\bar{E}$) averaged 3.37 and the calculated degree of heterozygosity ($\bar{H}$) averaged 0.4608 (Table 3). The observed and expected distribution of HBB and TF genotypes is shown in Table 4. The significance of differences between the observed and expected number of genotypes, calculated with the chi-square test, showed the lack of genetic equilibrium in the TF system.

Discussion

The management of the world's farm animal genetic resources (AnGR) has gained increasing importance in recent years. Many countries have established special institutions whose statutory goal is agricultural biodiversity or conservation of native breeds of animals (Martyniuk, 2003). As a result of international cooperation, the European Association for Animal Production (EAAP) developed criteria for assessing threat status, which were based on estimating the effective population size and predicted inbreeding increment after 50 years (Simon and Buchenauer, 1993). In 2003 the European Focal Point, acting within the framework of FAO's Global Strategy, elaborated guidelines for gene bank management and published materials concerning regional breeds of sheep in Europe.

Many efforts are undertaken to preserve genetic diversity by protecting vanishing breeds and small populations that can serve as a source of valuable genes in the future (Martyniuk, 1996; Hammond, 1997; Notter, 1999). In Poland, 13 sheep breeds and varieties (among them Świniarka) were included in the genetic resources conservation programme.

The intensive selection of different species of breeding animals, which was carried out in Poland and abroad in the last decade, gave preference to most productive breeds. This reduced the size of native populations accustomed to extensive husbandry conditions and local environment. Many of these (including Świniarka) were considered extinct in the second half of the 20th century. As a result of restoration efforts, a small number of Świniarka sheep was found in Poland. After rigorous selection for breed characteristics, the current population shows satisfactory levels of breed uniformity and type. Despite poor productivity, Świniarka sheep are characterized by extraordinary resistance to adverse environmental conditions, are undemanding in terms of feed and highly resistant to disease.

To achieve breeding progress, it is necessary to measure genetic variation, which should be recorded on a regular basis. Estimation of the degree of genetic variation within breeds is carried out using the effective number of alleles ($\bar{E}$), the degree of heterozygosity (hk) and the total number of alleles. The high values of these parameters are evidence that a given breed is genetically diverse.

In the analysed Świniarka flocks, the mean effective number of alleles ($\bar{H}$) is higher than that for Coloured Merino (2.80) (Rychlik et al., 2007 b) and Wrzosówka flock investigated in 2001–2005 (2.90) (Rychlik et al., 2007 a), but lower compared to the other sheep breeds studied, i.e. Corriedale (3.44) (Kaczor and Rychlik, 2005), Kamieniecka (3.81) (Kaczor and Rychlik, 2004), Olkuska (4.44) (Rychlik et al., 1997) and

Wrzosówka, which was investigated in 1990–1995 (4.48) (Rychlik et al., 2006) and 1996–2000 (3.60) (Rychlik et al., 2006). The mean effective degree of heterozygosity ($\bar{H}$) in the Świniarka population analysed was below 0.5, a value regarded as the most favourable considering the degree of genetic variation in a population. This parameter was also lower than 0.5 in three out of seven other native breeds analysed: 0.414 in Corriedale (Kaczor and Rychlik, 2005), 0.431 in Coloured Merino (Rychlik et al., 2007 b) and 0.495 in Wrzosówka sheep investigated in 2001–2005 (Rychlik et al., 2007 a). This is evidence that these small flocks show little genetic variation. It should be noted, however, that in the other breeds, ($\bar{H}$) values were high: 0.594 in Olkuska sheep (Rychlik et al., 1997) and 0.537 in Kamieniecka sheep (Kaczor and Rychlik, 2004).

The analysis of genetic equilibrium in the sheep population studied, based on HBB and TF systems, revealed a discrepancy between the observed and expected number of genotypes in the TF genotype. The upset genetic equilibrium could have resulted from the relatively small number of sheep and rigorous selection for breed characteristics during the period when the Świniarka breed was restored.

In summary, the present study provided information on the genetic structure and variation in a small population of the native Świniarka sheep.

A certain genetic variation is necessary to achieve breeding progress and therefore it should be measured. The present study and the results obtained could serve as a starting point for further monitoring of variation in Świniarka sheep while being a valuable source of information for efforts aimed at preserving the genetic resources of this breed.

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Wstępna ocena struktury genetycznej owiec rasy świniarka na podstawie grup krwi i polimorficznych wariantów białek

STRESZCZENIE

Celem badań było poznanie struktury genetycznej owiec rasy świniarka w oparciu o polimorfizm grup i białek krwi. Analizie poddano 96 owiec pochodzących ze stad województwa świętokrzyskiego i małopolskiego. Na podstawie częstości występowania poszczególnych alleli grup krwi, hemoglobiny i transferyny obliczono efektywną liczbę alleli oraz stopień heterozygotyczności. Średnie wartości

tych wskaźników obliczone w oparciu o zastosowane markery genetyczne wynoszą odpowiednio: 3,37 i 0,4608. Przeprowadzona w układach HBB i TF analiza równowagi genetycznej wykazała brak zgodności między obserwowaną i oczekiwaną liczebnością genotypów w układzie TF. Wynik ten można tłumaczyć między innymi ostrą selekcją prowadzoną na cechy rasowe w okresie restytucji świniarki.