

Polymorphism of brown coat-coding gene (*TYRPI*) in position 215 in national sheep breeds and the European mouflon (*Ovis aries musimon*)*

Roman Niżnikowski, Grzegorz Czub, Krzysztof Głowacz,
Marcin Świątek, Magdalena Ślęzak

Warsaw University of Life Sciences SGGW,
Faculty of Animal Sciences, Sheep and Goat Breeding Division,
ul. Ciszewskiego 8, 02-787 Warszawa

The studies were conducted with 1805 animals (1165 ♀ and 640 ♂): the European mouflon (*Ovis aries musimon*), its crossbreeds with Polish Heath sheep, the sheep of four breeds, characterized by mixed wool coat (Polish Heat sheep, Świniarka sheep, Polish Mountain Sheep of white and colour variety) and Polish Merino. All animals were subjected to identification of brown colour coat gene (*TYRPI*) in respect of evaluation of occurrence of C and T alleles. Summing up the studies, it was stated that the distribution of genes and genotypes of brown coat-coding gene clearly differentiated the European mouflon (dominance in the frequency of occurrence of T allele and TT genotype) as compared to the remaining groups of domesticated sheep. Each of the sheep breeds revealed specific system of distribution of alleles and genotypes what may be an evidence of their genetic separateness. On the grounds of the frequency of occurrence of the discussed phenomenon in the European sheep breeds, we may suppose that there are different ways of their origin as compared to the sheep, being bred e.g. in Asia. Confirmation of the mentioned thesis requires further work in the discussed field, with consideration of various conditions that determined the colour of coat of the sheep.

KEY WORDS: sheep / *TYRPI* / distribution of alleles and genotypes

At the time of intensive wool production, coat colour in sheep in Polish breeding was limited to the one considered most desirable, i.e. white or light cream. In research conducted abroad this trait has been frequently investigated by geneticists [1, 2, 4, 5]. These studies have indicated numerous loci in the DNA strand, which determine this trait in sheep. Particularly interesting findings were reported concerning the gene encoding the brown coat colour, indicated by Deng et al. [1], who conducted observations on sheep characterised by dark colouring of the skin, muscles, internal organs and the coat in comparison to those with the white colour character.

*The study was part of international non-cofinanced project no. 625/N-WĘGRY/2009/0

In this study analyses concerned the frequency of incidence of the gene encoding brown colour character (*TYRPI*) and the distribution of its incidence in the ancestral species, i.e. the European mouflon (*Ovis aries musimon*), as well as the white-coloured dual purpose wool and mutton type Polish Merino and mixed coated sheep breeds, among which many were characterised by coat colours other than white or light-cream. Moreover, this genetic background may prove useful in studies on the origin of sheep [4], which is considered to be of increasing interest also in terms of traditions of sheep rearing in various regions worldwide.

Material and methods

The experimental material comprised Polish Merino (2 flocks) from the Wielkopolskie province, mixed colour coated sheep from the Małopolskie, Łódzkie and Podkarpackie provinces (29 flocks), European mouflons (3 flocks) and European mouflon x Polish Heath sheep crosses (2 flocks) from the Wielkopolskie and the Lubuskie provinces. Animals aged 2 to 11 years were analysed (Table 1). In randomly selected flocks blood samples were collected from the jugular vein to EDTA test tubes to isolate genomic DNA for molecular

Table 1
Summary of experimental material used in the study in 2009-2012

Breed	Sex		Number of ewes and rams in experiments
	♀	♂	
European mouflon	119	71	2010 – 2 ♂; 2011 – 2 ♀, 6 ♂; 2012 – 117 ♀, 63 ♂
European mouflon x Polish Heat sheep	8	9	2010 – 4 ♀, 6 ♂; 2011 – 4 ♀, 3 ♂
Polish Heat sheep	368	400	2009 – 126 ♀, 143 ♂; 2010 – 127 ♀, 128 ♂; 2011 – 115 ♀, 129 ♂
Swiniarka sheep	134	29	2009 – 33 ♀, 14 ♂; 2010 – 106 ♀, 16 ♂
Polish Mountain Sheep – white	145	15	2010
Polish Moutain Sheep – colour	173	14	2011
Polish Merino	218	102	2010 – 104 ♀, 43 ♂; 2011 – 97 ♀, 51 ♂; 2012 – 17 ♀, 8 ♂
Total within gender	1165	640	
Total	1805		

and genetic analyses. The frequency of genes and genotypes of the *TYRP1* gene encoding the brown coat colour was determined.

DNA was isolated from leukocytes derived from sheep blood preserved using EDTA. In order to ensure quality DNA suitable for analyses following multiple freezing and thawing cycles, blood was preliminarily purified from haeme compounds causing DNA modifications by removing erythrocyte lysis products. DNA was isolated from leukocytes by chromatography using silicate minicolumns by A&A Biotechnology (Gdańsk, Poland). The obtained DNA fraction was used as a template in order to amplify the polymorphic gene fragments. Alleles were genotyped using the KASPar® system (www.kbioscience.co.uk). This system is based on the method identifying point mutations or single nucleotide polymorphism (SNP); the analyses were conducted using primers listed in Table 2.

Table 2

The primers and SNP genotyping of the locus of *TYRP1*

Locus	Name	Starter 3' do 5' (forward/reverse)	SNP	Localization
<i>TYRP1</i>	gene of tyrosinase related protein 1	GCTCCAGGCAGAATGAAATC/ GTGACCAGAGGGTTCTCACAG	AY737511.1:215 C>T*	exon 2

*Deng et al. [1]

Based on the genotyped DNA samples the distributions of allele and genotype frequencies were determined within the groups of ewes and rams. This analysis was a preliminary step for the successive stages in the study.

Statistical calculations were performed using the SPSS ver. 12.0 software package [6]. The χ^2 test was applied to assess the effect of breed and sex on the frequency of alleles and genotypes of the *TYRP1* gene.

Results and discussion

Results for the distribution of the *TYRP1* alleles are given in Table 3. The assessed frequency of the allele incidence proved to be statistically non-significant both within and between the sexes. In relation to the alleles this distribution turned out to be statistically highly significant ($P \leq 0.01$), indicating definite differences between the ancestor of modern sheep, i.e. the European mouflon, and the other breed groups. Only in the mouflon the T allele was found markedly more often than the C allele, while the Polish Heath sheep was characterised by a uniform frequency of the C and T alleles. Thus the European mouflon differed from the other groups in the frequency distribution pattern of both the alleles, with the Polish Heath sheep taking an intermediate

Table 3
Summary of gene alleles *TYRPI* (n – number)

Specification		Allele		Total
		C	T	
European mouflon	ewes	47	191	238
	rams	24	118	142
	n	71	309	380
	%	18.7	81.3	100.0
European mouflon x Polish Heat sheep	ewes	12	4	16
	rams	15	3	18
	n	27	7	34
	%	79.4	20.6	100.0
Polish Heat sheep	ewes	361	375	736
	rams	415	385	800
	n	776	760	1536
	%	50.5	49.5	100.0
Swiniarka sheep	ewes	183	85	268
	rams	34	24	58
	n	217	109	326
	%	66.6	33.4	100.0
Polish Mountain Sheep – white	ewes	224	66	290
	rams	20	10	30
	n	244	76	320
	%	76.3	23.8	100.0
Polish Mountain Sheep – colour	ewes	286	60	346
	rams	24	4	28
	n	310	64	374
	%	82.9	17.1	100.0
Polish Merino	ewes	304	132	436
	rams	151	53	204
	n	455	185	640
	%	71.1	28.9	100.0
Total	ewes	1417	913	2330
	rams	683	597	1280
	n	2100	1510	3610
	%	58.2	41.8	100.0

Table 4
Summary of genotypes *TYRP1* (n – number)

Specification		Genotype			Total
		C:C	C:T	T:T	
European mouflon	ewes	5	37	77	119
	rams	2	20	49	71
	n	7	57	126	190
	%	3.7	30.0	66.3	100.0
European mouflon x Polish Heat sheep	ewes	4	4	0	8
	rams	6	3	0	9
	n	10	7	0	17
	%	58.8	41.2	0.0	100.0
Polish Heat sheep	ewes	105	151	112	368
	rams	116	183	101	400
	n	221	334	213	768
	%	28.8	43.5	27.7	100.0
Swiniarka sheep	ewes	55	73	6	134
	rams	8	18	3	29
	n	63	91	9	163
	%	38.7	55.8	5.5	100.0
Polish Mountain Sheep – white	ewes	88	48	9	145
	rams	6	8	1	15
	n	94	56	10	160
	%	58.8	35.0	6.3	100.0
Polish Mountain Sheep – colour	ewes	115	56	2	173
	rams	11	2	1	14
	n	126	58	3	187
	%	67.4	31.0	1.6	100.0
Polish Merino	ewes	104	96	18	218
	rams	55	41	6	102
	n	159	137	24	320
	%	49.7	42.8	7.5	100.0
Total	ewes	476	465	224	1165
	rams	204	275	161	640
	n	680	740	385	1805
	%	37.7	41.0	21.3	100.0

position. This shows a different genetic pattern in wild sheep in comparison to domesticated sheep. These relationships vary from the results reported by other authors [1, 4, 5]. They do not confirm the distribution of this gene in sheep reared in China [2]. Thus it may be assumed that Asian sheep differ from sheep reared in Europe both in their origin and the course of domestication processes, as shown by the diverse distribution of the alleles [3].

The genotype distribution (Table 4), indicating highly significant ($P \leq 0.01$) differences between the Mouflon and the other sheep groups, confirms the thesis proposed based on the allele distribution (Table 3). Only in the European mouflon the dominant share of the TT homozygotes was recorded. The CC homozygotes were found in all the breed groups; however, in the Mouflon x Polish Heath sheep crosses, Polish Mountain Sheep of both types and in the Polish Merino they appeared with the definitely highest frequency. High shares of the *TYRP1* heterozygotes were found in the Polish Heath and Swiniarka sheep. A comparative analysis of sheep with coat colours other than white showed no relationship with the frequency of the *TYRP1* genotypes. Nevertheless, the genotype pattern in the European mouflon differs markedly from that in the domesticated sheep [1, 2, 4, 5].

In conclusion it may be stated that the distribution of genes and genotypes of the brown coat colour encoding gene (*TYRP1*) definitely distinguishes the European mouflon from the other groups of domesticated sheep. In turn, each sheep breed showed a specific distribution of alleles and genotypes, which may be evidence for their genetic divergence. Based on the frequency of this genetic variation in the European sheep breeds it may be assumed that their origin differs e.g. from that of sheep reared in Asia [4]. Further studies on the subject, including also other factors determining coat colour in sheep, are required to verify this thesis.

REFERENCES

1. DENG W.D., YANG S.L., HUO Y.Q., GOU X., SHI X.W., MAO H.M., 2006 – Physiological and genetic characteristics of black-boned sheep (*Ovis aries*). *Animal Genetics* 37, 586-588.
2. GRATTEN J., BERARDI D., LOWDER B.V., MC REE A.F., VISSER P.M., PEMBERTON J.M., SLATE J., 2007 – Compelling evidence that a single nucleotide substitution in *TYRP1* is responsible for a coat-colour polymorphism in a free-living population of Soay sheep. *Proceedings of the Royal Society* 274, 619-626.
3. HEINDLEDER S., JANKE A., WABMUTH R., 2001 – Molecular data on wild sheep genetic resources and domestic sheep evolution. *Archiv fur Tierzucht*, Special issue, 44, 271-279.
4. KUSHIMOTO T., VALENCIA, J.C., COSTIN G.-E., TOYOFUKU K., WATABE H., YASUMOTO K.-I., ROUZAUD F., VIEIRA W.D., HEARING, V. J., 2003 – The Melanosome: an ideal model to study cellular differentiation. *Pigment Cell Research* 16(3), 237-244.

5. PARISET L., CAPPUCIO I., AJMONE-MARSAN P., BRUFORD M., DUNNER S., CORTES O., ERHARDT G., PRINZENBERG E-M., GUTSCHER K., JOOST S., PINTO-JUMA G., NIJMAN I.J., LENSTRA J.A., PEREZ T., VALENTINI A. and ECONOGENE CONSORTIUM, 2006 – Characterization of 37 breed-specific Single-Nucleotide Polymorphisms in sheep. *Journal of Heredity* 97 (5), 531-534.
6. Statistical Product and Service Solution base version 12.0 for Windows. SPSS inc. USA 2004.