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Polymorphism of *TYRP1* at position 215 indomestic wool-and-meat and meat sheep breeds

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The study was conducted in 2009-2013 in flocks of wool-and-meat sheep, i.e. Polish Merino (3 flocks from Wielkopolskie Voivodeship and 8 flocks from Kujawsko-Pomorskie Voivodeship), old-type Polish Merino (1 flock from Wielkopolskie Voivodeship, 5 flocks from Mazowieckie Voivodeship and 8 flocks from Kujawsko-Pomorskie Voivodeship), Corriedale (2 flocks from Podlaskie Voivodeship) and Żelaźnieńska Sheep(2 flocks from Podlaskie Voivodeship and 1 flock from Łódzkie Voivodeship), and meat sheep flocks, i.e. Berrichon du Cher (1 flock from Wielkopolskie Voivodeship), Suffolk (2 flocks from Wielkopolskie Voivodeship) and Charollais (2 flocks from Wielkopolskie Voivodeship). The total number of genotyped individuals (from 2 to 11 years old) was 1,732 (1,359 ♀ and 373 ♂). Genotypes identification was performed with respect to the occurrence of C and T alleles of the brown colour gene (*TYRP1*). The distribution of alleles and genotypes of the locus encoding brown colour was found to clearly differentiate sheep with different head and leg colour from the other meat and wool-and-meat breeds, with white or cream-coloured coats. Each of the breeds showed a characteristic distribution of alleles and genotypes, which may be evidence of their genetic distinctiveness. It also appears that, based on the frequency of occurrence of this determinant in European sheep breeds, we can expect different paths of origin for these breeds than in the case of sheep bred e.g. in Asia. Furthermore, it would be worth comparing the frequency of occurrence of *TYRP1* determinants with objective measurements of sheep wool colour.

KEY WORDS: sheep / *TYRP1* / distribution of alleles and genotypes

A study on the coat colour of sheep was conducted in Poland on breeds with coloured coats as compared to the European mouflon (*Ovis aries musimon*) [7]. At a time of intensive wool production, breeding work has become reduced to its most desired type, i.e. white or cream-coloured. The foreign literature includes a number of studies in this area, published by geneticists [1, 2, 6, 8]. These studies have found numerous sites in the DNA chain determining this trait in sheep. Of particular interest are studies on the

gene encoding brown coat colour, as pointed out by Deng et al. [1], who conducted observations on sheep with dark coat colour in comparison to white sheep. The study determined the frequency of the brown coat colour allele of the *TYRP1* gene at position 215. Therefore it was decided to study the frequency of alleles of the *TYRP1* locus in selected Polish breeds [4] of wool-and-meat sheep and meat sheep characterized by white wool. The information obtained in the study will be useful in studies on the origin of sheep [3].

Material and methods

The study was carried out in 2009-2013 on flocks of wool-and-meat sheep, of the breeds Polish Merino (3 flocks from the Wielkopolskie Voivodeship and 8 flocks from Kujawsko-Pomorskie Voivodeship), old-type Polish Merino (one flock from the Wielkopolskie Voivodeship, 5 flocks from the Mazowieckie Voivodeship and 8 flocks from the Kujawsko-Pomorskie Voivodeship), Corriedale (2 flocks from the Podlaskie Voivodeship), and Żelaźnieńska (2 flocks from the Podlaskie Voivodeship and one flock from the Łódzkie Voivodeship), and on flocks of meat sheep, i.e. Berrichon du Cher (one flock from the Wielkopolskie Voivodeship), Suffolk (2 flocks from the Wielkopolskie Voivodeship) and Charollais (2 flocks from the Wielkopolskie Voivodeship). The animals studied were aged 2 to 11 years (Tab. 1). The flocks from which samples were tested were selected at random. Blood was collected from the jugular vein into EDTA test tubes for the purpose of DNA isolation for molecular genetic analysis. The frequency of alleles and genotypes of the locus encoding the occurrence of brown coat colour—*TYRP1* at position 215—was determined [1].

DNA was isolated from the leukocytes of blood preserved with EDTA. In order to obtain high-quality DNA that could be used multiple times after freezing and thawing, haem compounds causing DNA modifications were initially removed from the blood by removing erythrocyte lysis products. DNA was isolated from the leukocytes by chromatography on silica minicolumns from A&A Biotechnology (Gdańsk, Poland). A fraction of the DNA obtained in this manner was used as a template for amplification of the polymorphic gene fragment. Genotyping of alleles was performed with the KASPar® system. This system (www.kbioscience.co.uk) involves the use of the single nucleotide polymorphism (SNP) method with the primers given in Table 2.

The readout of the genotyped DNA samples for ewes and rams was used to present the distribution of frequencies of alleles and genotypes. This was preparation for the subsequent steps of the study.

Statistical calculations were performed in the SPSS software package, version 21.0 [10]. The chi-square test was used to assess the effect of breed and of sex within breed on the frequency of alleles and genotypes. Genetic distances between populations were determined by Nei's method [9]. The results are presented in tables and in a dendrogram.

Table 1
Characterization of the material tested according to breed, sex, and year

Breed	Sex		Number of ewes and rams in each year
	♀	♂	
Polish Merino	296	69	2010 – 40 ♀; 6 ♂ 2011 – 109 ♀; 10 ♂ 2012 – 71 ♀; 30 ♂ 2013 – 76 ♀; 23 ♂
Old-type Polish Merino	456	72	2009 – 291 ♀; 12 ♂ 2010 – 50 ♀; 18 ♂ 2011 – 34 ♀; 11 ♂ 2012 – 54 ♀; 25 ♂ 2013 – 27 ♀; 6 ♂
Corriedale	135	11	2011 – 25 ♀; 5 ♂ 2013 – 110 ♀, 6 ♂
Żelaźnieńska Sheep	284	153	2010 – 47 ♀; 62 ♂ 2011 – 38 ♀; 39 ♂ 2012 – 45 ♀; 27 ♂ 2013 – 154 ♀; 25 ♂
Berrichon du Cher	131	40	2010 – 14 ♀; 6 ♂ 2011 – 41 ♀; 16 ♂ 2012 – 24 ♀; 6 ♂ 2013 – 52 ♀; 12 ♂
Suffolk	37	19	2010 – 8 ♀ 2011 – 17 ♀; 8 ♂ 2013 – 12 ♀; 11 ♂
Charollais	20	9	2010 – 2 ♀ 2011 – 11 ♀; 4 ♂ 2013 – 7 ♀; 5 ♂
Total within sex	1359	373	
Total	1732		

Table 2
Primers and SNP genotyping of the locus of *TYRP1*

Locus	Name	Starters 3' do 5' (forward/reverse)	SNP	Localization
TYRP1	tyrosinase-related protein 1 gene	GCTCCAGGCAGAATGAAATC/ GTGACCAGAGGGTTCTCACAG	AY737511.1:215 C>T*	Exon 2

*Deng et al. [1]

Results and discussion

The results pertaining to the distribution of alleles of the gene *TYRP1* are shown in Table 3. The statistical significance of their frequency in relation to breed was high. The frequency of the C allele substantially surpassed that of the T allele in all breeds. The lowest frequency for the C allele was noted in the ewes and rams of the Suffolk breed and the ewes of the Charollais breed, and the highest in the Charollais, Berrichon du Cher and Corriedale rams

Table 3
Frequency of alleles of *TYRP1* locus according to breed and sex

Breed	Sex		Allel Allele		Ogółem Total
			C	T	
Polish Merino	♀	n	427	165	592
		%	72.1	27.9	100.0
	♂	n	102	36	138
		%	73.9	26.1	100.0
Old-type Polish Merino	♀	n	711	201	912
		%	78.0	22.0	100.0
	♂	n	102	42	144
		%	70.8	29.2	100.0
Corriedale	♀	n	220	50	270
		%	81.5	18.5	100.0
	♂	n	19	3	22
		%	86.4	13.6	100.0
Żelaźnieńska Sheep	♀	n	431	137	568
		%	75.9	24.1	100.0
	♂	n	210	96	306
		%	68.6	31.4	100.0
Berrichon du Cher	♀	n	208	54	262
		%	79.4	20.6	100.0
	♂	n	67	13	80
		%	83.7	16.3	100.0
Suffolk	♀	n	45	29	74
		%	60.8	39.2	100.0
	♂	n	22	16	38
		%	57.9	42.1	100.0
Charollais	♀	n	25	15	40
		%	62.5	37.5	100.0
	♂	n	16	2	18
		%	88.9	11.1	100.0
Total within sex	♀	n	2067	651	2718
		%	76.0	24.0	100.0
	♂	n	538	208	746
		%	72.1	27.9	100.0
Total		n	2605	859	3464
		%	75.2	24.8	100.0

Influence of breed significant at P≤0.01; influence of sex in Charollais significant at P≤0.05; influence of sex in Żelaźnieńska Sheep significant at P≤0.05

and the Corriedale ewes. The frequencies of the T allele showed a reverse pattern to that of the C allele. The distribution of frequencies of alleles depending on sex was statistically significant (P≤0.05) only in the Charollais and Żelaźnieńska breeds. In Charollais rams the frequency of the C allele was significantly higher and that of the T alleles lower than in ewes, while in the Żelaźnieńska sheep a significantly lower frequency of the C allele and higher frequency of the T allele was noted in comparison with ewes. The distributions of frequencies of TYRP1 alleles can be supplemented by research on coat colour in sheep.

Table 4
Frequency of genotypes of *TYRP1* gene according to breed and sex

Breed	Sex		Genotype			Total
			C:C	C:T	T:T	
Polish Merino	♀	n	152	123	21	296
		%	51.4	41.6	7.0	100.0
	♂	n	39	24	6	69
		%	56.5	34.8	8.7	100.0
Old-type Polish Merino	♀	n	273	165	18	456
		%	59.9	36.2	3.9	100.0
	♂	n	34	34	4	72
		%	47.2	47.2	5.6	100.0
Corriedale	♀	n	87	46	2	135
		%	64.4	34.1	1.5	100.0
	♂	n	8	3	0	11
		%	72.7	27.3	0.0	100.0
Żelaźnieńska Sheep	♀	n	163	105	16	284
		%	57.4	37.0	5.6	100.0
	♂	n	71	68	14	153
		%	46.4	44.4	9.2	100.0
Berrichon du Cher	♀	n	80	48	3	131
		%	61.1	36.6	2.3	100.0
	♂	n	28	11	1	40
		%	70.0	27.5	2.5	100.0
Suffolk	♀	n	13	19	5	37
		%	35.1	51.4	13.5	100.0
	♂	n	5	12	2	19
		%	26.3	63.2	10.5	100.0
Charollais	♀	n	8	9	3	20
		%	40.0	45.0	15.0	100.0
	♂	n	7	2	0	9
		%	77.8	22.2	0.0	100.0
Total within sex	♀	n	776	515	68	1359
		%	57.1	37.9	5.0	100.0
	♂	n	192	154	27	373
		%	51.5	41.3	7.2	100.0
Total		n	968	669	95	1732
		%	55.9	38.6	5.5	100.0

Influence of breed significant at P≤0.01

A study by Kulesza et al. [5] drew attention to the differences in wool colour measurements, performed with a colourimeter, in Żelaźnieńska sheep as compared to the Corriedale breed. Żelaźnieńska sheep had cream-coloured wool, while the wool of the Corriedale sheep was snow-white. Comparison of these breeds in terms of the frequency of *TYRP1* alleles indicates substantial variation between them (Tab. 3), which necessitates consideration of the results of the present study in relation to measurements of wool colour.

With regard to the frequency of *TYRP1* alleles, the distributions presented clearly differ from tendencies noted in the European mouflon and Polish Heath sheep [7], while confirming the tendencies noted in other Polish sheep breeds, such as the coloured and white varieties of Polish Mountain Sheep and the Świniarka breed. Only in the mouflon was the T allele markedly more frequent than the C allele [7]. Only their frequency in the Polish Heath sheep was similar [7]. This indicates a different distribution of this determinant in wild sheep in comparison with domesticated sheep. These relationships differ from results obtained by other authors [1, 6, 8]. Grattin et al [2] also do not confirm the distributions of this allele in sheep in China. Thus it appears that Asian sheep [2], European mouflons and Polish Heath sheep [7] have a different origin and have undergone different domestication processes, showing a different distribution of alleles than sheep bred in Europe [3].

The genotype distribution (Tab. 4) confirms the hypothesis that was based on allele distribution (Tab. 3). The effect of breed proved highly statistically significant. The highest frequency of the CC genotype was noted for both sexes of the Corriedale and Berrichon du Cher breeds and Charollais rams, and the lowest for both sexes of the Suffolk breed. CT heterozygotes were most frequent in both sexes of the Suffolk breed, ewes of the Charollais breed and rams of the Żelaźnieńska and old-type Merino breeds. The lowest frequency was observed in rams of the Corriedale and Charollais breeds. The highest frequency of TT genotypes was noted in both sexes of the Suffolk breed and in Charollais rams, and the lowest in both sexes of the Corriedale breed and in Charollais rams.

The data presented in Table 5 and illustrated graphically in the dendrogram (Fig.) indicate that the Suffolk sheep differed most from the other breeds, as demonstrated by the highest values for indices of genetic distances. Analysis of the literature shows that homozygotic genotypes, particularly in the case of TT, have been found in the European mouflon and in Asian breeds with black wool [2, 7], while heterozygotes have been noted in Heath sheep and breeds with coloured heads and legs [1, 7]. CC homozygotes have been found in all breeds considered in the present study and those analysed in another Polish work [7]. Comparative analysis of sheep with a coat colour other than white suggests a link with the frequency of TT and CT *TYRP1* genotypes. Taking into account the results of research on wool colour in Żelaźnieńska and Corriedale sheep [5] and the different distribution of genotypes in the European mouflon than in domesticated sheep [1, 2, 6, 8], it seems reasonable to examine the results of molecular analysis of the *TYRP1* gene in relation to objective measurements of wool colour, at least in the breeds named in the work by Kulesza et al. [5].

To sum up, the distribution of alleles and genotypes of the locus encoding brown coat colour clearly differentiates sheep with different head and leg colour from other meat breeds and wool-and-meat breeds with white or cream coat colour. Each of the breeds was characterized by a specific distribution of alleles and genotypes, which may provide evi-

Table 5
Genetic distances between breeds

Breed	Polish Merino	Old-type Polish Merino	Corriedale	Żelaźnieńska Sheep	Berrichon du Cher	Suffolk	Charollais
Polish Merino		0.06	0.13	0.01	0.11	0.18	0.03
Old-type Polish Merino			0.07	0.05	0.05	0.24	0.09
Corriedale				0.12	0.02	0.31	0.16
Żelaźnieńska Sheep					0.10	0.19	0.04
Berrichon du Cher						0.29	0.14
Suffolk							0.15
Charollais							

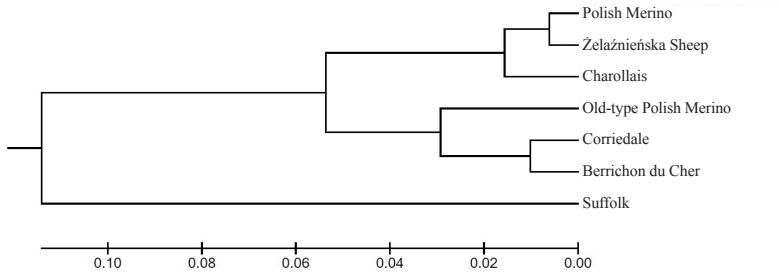

Fig. Dendrogram of genetic distances between breeds

dence that they are genetically distinct. It also appears that, based on the frequency of occurrence of this determinant in European sheep breeds, we can expect different paths of origin for these breeds than in the case of sheep bred in other parts of the world, e.g. in Asia [2, 3]. It would also be worth analysing the frequency of occurrence of *TYRP1* determinants in relation to objective measurements of sheep wool colour.

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