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Evaluation of the genetic profile of the Pulawska breed

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Abstract. An assessment was made of the genetic variation of the Pulawska pig through the determination of polymorphism of 6 genes and 14 microsatellite sequences. The examinations covered 52 gilts included in a preservation breeding project. The identification of the alleles at microsatellite loci was performed in an ABI PRISM 310 GENETIC ANALYZER. Gene polymorphism was established by the PCR-RLFP method. On the basis of the variation of 6 genes and 14 microsatellites the mean value of the heterozygosity coefficient was estimated at 0.61, while the value of the corresponding PIC coefficient (polymorphism information content) amounted to 0.55. The probability that the genotypes of two randomly chosen individuals in a population are identical was: 6.95×10^{-3} (based on gene allele frequency) and 1.23×10^{-14} (based on microsatellite allele frequency).

Key words: genes, microsatellites, polymorphism, Pulawska breed.

Introduction

Pigs of the Pulawska breed were selected from a subpopulation of a regional eco-type, known as "laciatki" (spotted) and developed at the turn of the 19th century by crossing native pigs, maintained in the western part of the Lublin region, with Berkshire pigs. This organized breeding work, initiated by Prof. Zabielski, has been continued since the year 1933 (ZABIELSKI 1933, SURDACKI 1992) and the animals were highly valued by breeders and producers of pork. However, the changing preferences of both meat industry and the consumer market contributed to the development of pig breeds with a high content of meat in the carcass. Hence, the population of the Pulawska breed decreased, as its production possibilities in this respect remained at a clearly lower level.

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The breeding and production work confined to only a few breeds is likely to cause a significant restriction of the genetic variation in animal populations and this is one of the basic factors determining the efficiency of breeding work. Evaluating the polymorphism of the genetic structure of a given breed is important, as the variation level is a basic factor determining the selection efficiency in successive generations. An important practical effect of these investigations will be the possibility of using the results obtained for developing a modern breeding programme for this pig breed, which since 1992 has been included in the program of genetic reserve protection. The genetic structure of the Pulawska breed was characterized by JANIK et al. (1995) on the basis of blood group typing, allotypes and the polymorphism of blood proteins.

The aim of the present study was to evaluate the genetic profile of the Pulawska breed on the basis of the polymorphism of selected genes and microsatellites.

Material and methods

The studies were conducted on 52 Pulawska gilts included in a preserve breeding program. The gilts for the study were selected within 5 lines of sires. The polymorphism analysed comprised the following 6 genes: the ryanodine receptor gene (*RYR1)*, growth hormone gene (*GH)*, growth hormone releasing hormone (*GHRH)*, myogenic factor 3 (*MYF3)*, myogenin (*MYOG)*, colipase gene (*CLPS)*, and 14 microsatellites located on the six chromosomes: *S0026* (chromosome *16*), *S0061* (*16*), *S0088 (15*), *S0090* (*12*), *S0148* (*15*), *S0296* (*17*), *Sw15* (*15*), *Sw419* (*16*), *Sw964* (15), *Sw1891* (17), *Sw1983* (15), *Sw2427* (17), *TNFB* (7), and *IGF1* (5).

Blood samples were collected from the *vena cava cranialis* of all the gilts using expendable Monovette syringes (Sarsted) with K_2EDTA as an anticoagulant.

Genomic DNA was isolated from blood leucocytes according to the method by KAWASAKI (1990), as modified by COPPIETERS et al. (1992). The allele identification within the sequences of microsatellite loci was conducted by capillary electrophoresis performed in a sequencer ABI PRISM 310 GENETIC ANALYZER. Gene polymorphism was identified by the PCR-RLFP method according to the following procedures: *RYR*1 (KAMINSKI et al., 2001), *GH* (KIRKPARTICK 1992), *GHRH* (BASKIN, POMP 1997), *MYF3* (KNOLL et al. 1997), *MYOG* (SOUMILLION et al. 1997), *CLPS* (BASKIN, POMP 1998).

To assess the genetic variation on the basis of the results obtained, the following statistical indices were calculated: allele and genotype frequency for particular microsatellites and genes (computed using the least squares method), heterozygosity coefficient – $\hat{H}$ (estimated after OTT 1992) and WEIR`s (1990) formula, Polymorphism Information Content - PIC (computed according to the formula elaborated by BOTSTEIN et al. 1980), the probability of identification of

an identical genotype in two individuals randomly chosen from a population (G1 coefficient – acc. to VAN ZEVEREN et al. 1995).

Results and discussion

Frequency of alleles and genotypes at microsatellite loci

Among the 14 analysed microsatellite sequences the highest variation was recorded at locus TNFB, at which 12 alleles were identified (Table 1). Microsatellite loci S0296, Sw964, Sw1891 with 10 alleles and Sw1983 with 11 alleles also showed a high level of variability. The lowest variability, showed by 3 alleles, was observed at locus S0061. Other alleles with the coefficient q exceeding the frequency of 50%, hence widespread in the examined pigs population, turned out to be 146 bp, 162 bp and 126 bp at loci S0088, S0148 and Sw2427, respectively. An interesting arrangement was observed for a seven-allele locus S0148, at which the total frequency of two alleles, 162 bp and 170 bp, amounted to 81%, whereas the other alleles occurred rarely. A similar distribution characterized locus Sw419. Two variants, 164 bp and 166 bp, were predominant as their total frequency amounted to 87.0%.

A comparative analysis of the results obtained and the data presented in world literature showed that the high polymorphism of microsatellites observed in Pulawska pigs was consistent with the values reported by the other authors (JOHANSSON et al. 1992, Van ZEVEREN et al. 1995, FREDHOLM et al. 1993). However, clearly marked differences were observed as regards the number of alleles at each locus examined. ELLEGREN et al. (1993) found 4, 4 and 7 alleles at loci S0088, S0090 and TNFB, respectively, in an experimental material comprising crosses of the European Wild Boar and Swedish Yorkshire pigs. The number of identical alleles among Pulawska pigs was 4, 6 and 12, respectively. Another group of researchers (ROHRER et al. 1994) reported the occurrence of 5, 6 and 7 alleles at these loci, respectively. Moreover, these authors, studying crossbred pig families (25% Chester White, 25% Large White, 25% Landrace, 25% Yorkshire) × (Duroc; Fengjing; Meishan; Minzhu) reported the occurrence of 4 alleles at locus *Sw15* and 6 at locus *Sw419*. Eight alleles at locus *Sw15*, ranging in size from 148 bp to 164 bp, were identified within the population of Pulawska gilts, while locus *Sw419* was represented by 7 alleles, 156-172 bp in length. KORWIN-KOSSAKOWSKA et al. (1999), when analysing the polymorphism at locus *S0090* in pigs of the Zlotnicka Spotted and PLW breed, and their crosses, identified 6 alleles unique for the Zlotnicka Spotted breed and 8 alleles – for the PLW pigs. While comparing these data with the results obtained in the present study, it was observed that no allele 250 bp was found in the Zlotnicka Spotted and Pulawska pigs. Moreover, allele 235 bp was observed in the Zlotnicka Spotted breed

Table 1. Characterization of polymorphism at selected microsatellite loci in Pulawska pigs

Locus	Alleles length (bp)	Alleles (bp)	Frequency of alleles (%)	$\hat{H}$	PIC	G1
1	2	3	4	5	6	7
S0026	90-98	90	15.3	0.77	0.72	0.10
		92	4.5			
		94	12.3			
		96	12.3			
		98	35.6			
S0061	165-179	165	16.7	0.61	0.53	0.23
		177	52.9			
		179	30.4			
S0088	146-162	146	62.5	0.54	0.46	0.29
		148	27.2			
		156	2.3			
		162	8.0			
S0090	240-252	240	10.8	0.77	0.72	0.09
		242	18.6			
		244	38.2			
		246	16.7			
		248	14.7			
		252	1.0			
S0148	146-176	146	3.6	0.63	0.57	0.19
		150	3.6			
		156	3.6			
		162	56.0			
		170	25.0			
		172	7.1			
		176	1.1			
S0296	160-186	160	4.4	0.84	0.79	0.06
		162	20.7			
		164	29.4			
		166	12.0			
		170	7.6			
		172	4.4			
		174	17.4			
		178	2.1			
		182	1.0			
		186	1.0			
Sw15	148-164	148	19.8	0.77	0.71	0.10
		150	39.6			
		152	7.3			
		154	20.8			
		156	7.3			
		158	1.0			
		162	2.1			
		164	2.1			
Sw419	156-172	156	1.0	0.62	0.54	0.22
		160	3.0			
		162	2.0			
		164	47.0			
		166	40.0			
		168	5.0			
		172	2.0			

Table 1– cont.

1	2	3	4	5	6	7
Sw964	216-244	216	3.8	0.85	0.81	0.05
		218	10.0			
		220	1.3			
		224	5.0			
		228	1.3			
		230	13.8			
		232	6.2			
		240	23.7			
		242	26.2			
		244	8.7			
Sw1891	98-124	98	2.4	0.85	0.81	0.05
		108	6.1			
		110	7.3			
		112	8.5			
		114	17.1			
		116	11.0			
		118	15.9			
		120	1.2			
		122	29.3			
		124	1.2			
Sw1983	160-190	160	1.0	0.79	0.75	0.08
		164	14.7			
		172	1.0			
		176	3.9			
		178	40.2			
		180	15.7			
		182	8.8			
		184	2.9			
		186	1.0			
		188	8.8			
		190	2.0			
Sw2427	114-132	114	8.5	0.65	0.60	0.17
		116	5.3			
		120	3.2			
		126	56.4			
		128	17.0			
		132	9.6			
TNFB	152-180	152	4.1	0.90	0.87	0.02
		154	4.1			
		156	3.1			
		158	5.1			
		160	11.2			
		162	17.3			
		164	5.1			
		170	3.1			
		174	7.1			
		176	10.2			
		178	18.4			
		180	11.2			
IGF1	124-132	124	5.3	0.75	0.69	0.12
		126	28.7			
		128	38.2			
		130	14.9			
		132	12.9			
Mean				0.73	0.64	1.23×10^{-14}

as the shortest variant at locus *S0090*, whereas the minimum allele size identified in Pulawska gilts was 240 bp. However, the frequency of the alleles examined proved to be more similar in the Pulawska gilts than that reported for the Zlotnicka Spotted breed.

The polymorphism of the selected genes

The polymorphism of 6 genes (*GH*, *GHRH*, *MYOG*, *MYF3*, *CLPS*, *RYR1*), which may be considered as candidate genes for growth rate and carcass quality, was examined in the Pulawska breed. In the examinations undertaken within the population of Pulawska gilts, the polymorphism of the growth hormone gene was identified in exon 2 and intron 2, using endonucleases *Hae*II and *Msp*I, respectively. The allele frequency at the loci analysed demonstrated a high differentiation. It was manifested by the high (82.4%) frequency of allele *Msp*I$^+$ at the *GH*/*Msp*I locus. The frequency of alleles at locus *GH*/*Hae*II remained at a similar level, which was also reflected in the number of individual *GH*/*Hae*II genotypes within the Pulawska gilts, i.e. homo- and heterozygotes. Forty nine per cent of Pulawska gilts appeared to be of genotype "+/–" at the *GH*/*Hae*II locus, whereas homozygous genotype "+/+" was the most frequent genotype (70.6%) regarding locus *GH*/*Msp*I. A similar distribution of *GH* genotypes was presented by SCHELEANDER et al. (1994) for the Austrian breed Edelschwein. KIRKPATRICK (1992) also reported the frequency of growth hormone gene variants identified in the Yorkshire pigs similar to those obtained for Pulawska gilts – 50% and 85% for *Hae*II$^+$ and *Msp*I$^+$ alleles, respectively. KRENKOVA et al. (1999) stated that in (Large White × Landrace) × Large White or Large White × Pietrain crosses a higher frequency at both considered loci was shown by allele "+". KORWIN-KOSSAKOWSKA et al. (1999), while testing Zlotnicka Spotted × PLW crosses, also found the prevalence of frequency of allele "+" at both *GH*/*Hae*II and *GH*/*Msp*I loci.

The polymorphism of the *GHRH* gene within the population of 52 Pulawska gilts was expressed by the alleles A and B frequency ratio – A : B = 1 : 3. A high frequency of allele B in the analysed population resulted from the more frequent occurrence of the BB genotype – 61%. A similar distribution of GHRH alleles was reported by BASKIN and POMP (1997), who while analysing the polymorphism in the GHRH gene in the European Wild Boar × Large White and Meishan × Large White crosses, defined allele B frequency as amounting to 64%.

The attempts to estimate the RFLP polymorphism within the *MyoD* family genes (*MYOG* and *MYF3*), coding for transcription factors contributing to the muscle growth and differentiation, also seem to be of interest. In Pulawska pigs the polymorphism of the MYOG gene in region 3' was manifested by an almost equal frequency of alleles *A* and *B*. Thus, in the group of 52 pigs examined heterozygous individuals dominated (42.0% of the population studied). These data correspond to the results obtained by SOUMILLION et al. (1997) for the Dutch Landrace breed – genotype *AB* was observed in 40% of the individuals tested,

whereas in the Hampshire pigs the q coefficient value amounted to 0.56. These authors did not find genotype *BB* in the analysed populations of Wild Boar and Duroc pigs. KANIAK and JASEK (1999), while estimating the frequency of the MYOG genotypes for gilts belonging to the PL, PLW and Belgian breeds, recorded a high frequency of genotype *BB* (32%) only in the PLW pigs. In the other pig breeds and lines the frequency of allele *B* did not exceed 20%. However, CIEŚLAK et al. (2000) noted that the frequency of allele *B* in the Zlotnicka Spotted and PLW breeds, amounted to 0.3 and 0.4, respectively. The results presented may imply a genetic similarity between the Pulawska and PLW breeds as regards the frequency of *MYOG* gene variants. This fact may be explained by an addition of the PLW pig blood to the Pulawska breeding stock throughout the years 1961-1975.

In the case of gene MYF3, the allele *A* prevailed in the group of gilts analysed (83.7%). As regards the frequency of *MYF*3 genotypes, it was observed that most of the animals showed genotype *AA* (83%).

CIEŚLAK et al. (2000) reported that the frequency of allele *A* in Pietrain pigs ranged from 0.5 to 0.8, depending on the herd; in the Zlotnicka Spotted breed it amounted to 0.95, whereas in the PLW × Pulawska crosses – 0.64. KNOLL et al. (1997) stated that allele A was observed in 73% of animals of the Duroc breed.

Among the genes examined, results characterizing the polymorphism of the colipase gene (CLPS) are of special interest. The examined pig population of the Pulawska breed shows a markedly dominant frequency of allele *A* – 98.9%. Simultaneously, the variability observed at the *CLPS* locus was restricted to two genotypes: *AA* – 98.0% and *AB* – 2.0%. The results obtained make it possible to observe that the frequency of allele *B* in the Pulawska breed is low. However, further studies, conducted on a larger group of animals, are necessary to confirm this observation. As it follows from other reports concerning this subject, the frequency of allele *A* is much higher than the frequency of allele *B*. This is confirmed, for instance, by the frequency of variant B in 14 crosses of the European Wild Boar × LW and Meishan × LW that did not exceed 26% (BASKIN, POMP 1997).

Of special significance for the breeding efficiency and production of pigs is the gene of stress susceptibility, which results both from its direct positive effect on the muscle tissue development – and thus carcass meat content, and from its negative impact manifested by the occurrence of meat of a lowered technological value and a decrease of reproduction results (KURYŁ, WRÓBLEWSKI 1991, KOĆWIN-PODSIADŁA 1998, KRZĘCIO 1999, GRONEK 1999). As Pulawska pigs comprise the maternal component, the breeding programme for this breed assumes a complete elimination of allele $RYR1^T$ from the population.

Out of the 52 gilts examined, 21 proved to have allele $RYR1^C$ in their genotype, while 14% appeared to be susceptible to stress (the $RYR1^TRYR1^T$ genotype).

Among the many pig breeds tested for genotype *RYR1*, the highest frequency of genotype $RYR1^TRYR1^T$ was shown by the Pietrain breed. The fragmentary studies conducted in Poland within particular lines of those pigs indicated that 45-96%

of them were homozygous in regard to susceptibility to stress (OSTROWSKI, BLICHARSKI 1998, KOĆWIN-PODSIADŁA 1998). This is a specific trait, observed also in crosses with the Pietrain pigs. KRENKOVA et al. (1999) determined the frequency of allele $RYR1^T$ in crosses with a share of the LW, Landrace and Pietrain breeds at 0.69, while the frequency of genotype $RYR1^TRYR1^T$ – at 38%. A relatively low share of animals susceptible to stress was recorded in the Yorkshire and Hampshire breeds (0.3%-1.9%) and in some populations of white pig breeds, e.g. Edelschwein bred in Austria and Landrace breeds (2% and 5%, respectively) (SCHELEANDER et al. 1994).

Heterozygosity and polymorphic degree of the population of Pulawska pigs

The heterozygosity coefficient ($\hat{H}$) and the polymorphism information content coefficient (PIC) were computed for all the loci analysed in the present study (Tables 1 and 2). Out of the 6 genes examined, a higher variation, expressed by the value of $\hat{H}$ and PIC coefficients, was demonstrated by the following genes: *MYOG* (0.51 and 0.37), *GH* (genotyped with *Hae*II; 0.51 and 0.38) and *RYR1* (0.49 and 0.36). The $\hat{H}$ and PIC coefficients obtained for the remaining genes, i.e. *MYF3*, *GHRH* and *GH* (genotyped with *Msp*I) ranged from 0.28 to 0.45 and from 0.24 to 0.34, respectively. The lowest variation among the genes examined was recorded at locus *CLPS* (H = 0,02).

Table 2. Characterization of polymorphism in several genes in Pulawska pigs

Locus	Alleles	Frequency of (%)		$\hat{H}$	PIC	G1
		alleles	genotypes			
RYR1	*C*	60.0	*CC* – 34.0	0.49	0.36	0.39
	T	40.0	*CT* – 52.0			
			TT – 14.0			
GH/	*Hae*II$^-$	50.0	HaeII^{--} – 25.5	0.51	0.38	0.38
*Hae*II	*Hae*II$^+$	50.0	HaeII^{-+} – 49.0			
			HaeII^{++} – 25.5			
GH/	*Msp*I$^-$	17.6	MspI^{--} – 5.9	0.30	0.25	0.55
*Msp*I	*Msp*I$^+$	82.4	MspI^{-+} – 23.5			
			MspI^{++} – 70.6			
GHRH	*A*	32.9	*AA* – 26.8	0.45	0.34	0.41
	B	67.1	*AB* – 12.2			
			BB – 61.0			
MYOG	*A*	53.0	*AA* – 32.0	0.51	0.37	0.38
	B	47.0	*AB* – 42.0			
			BB – 26.0			
MYF3	*A*	83.7	*AA* – 67.3	0.28	0.24	0.57
	C	16.3	*AC* – 32.7			
CLPS	*A*	98.9	*AA* – 98.0	0.02	0.02	0.96
	B	1.1	*AB* – 2.0			
Mean				0.37	0.28	6.95×10^{-3}

Many scientists present the opinion that the principal trait recommending the use of microsatellites as genetic markers is their high polymorphism. The results obtained for the examined population of the Pulawska pigs confirm this opinion. The degree of heterozygosity, recorded for individual microsatellite loci, was on the average higher by 40%, when compared to the genes analysed and amounted to about 0.74. The highest variation among the microsatellites analysed in the present study was observed at the following loci: *TNFB*, *Sw1891*, *Sw964* and *S0296*, and was reflected in the value of coefficient $\hat{H}$ (0.90; 0.85; 0.85 and 0.84; respectively). Relatively low values of this coefficient were recorded for loci *S0088*, *S0061* and *Sw419* (0.54, 0.61 and 0.62, respectively).

Comparable results were reported for other species of farm animals. On the basis of 23 microsatellite markers identified in 4 cattle breeds maintained in Belgium, PEELMAN et al. (1998) reported that the heterozygosity coefficient ranged from 0.5 to 0.8. GRALAK et al. (1998) observed an equally high degree of microsatellite polymorphism in Arabian horses (the $\hat{H}$ value ranged from 0.4 to 0.8). Similar values of this coefficient were obtained in studies conducted on pigs by Van ZEVEREN et al. (1995).

The mean value of the heterozygosity coefficient for the Pulawska gilts, calculated on the basis of 20 genes and microsatellites, amounted to 0.61. This is comparable to the results reported by Van ZEVEREN et al. (1995), who calculated this coefficient on the basis of the polymorphism at 7 microsatellite loci in the pig breeds maintained in Belgium obtaining $\hat{H} = 0.54$ for Pietrain pigs, and $\hat{H} = 0.63$ for Large White. FREDHOLM et al. (1993), when analysing the polymorphism at 24 microsatellite loci in the Duroc, Landrace, Hampshire, Yorkshire pigs maintained in Denmark, obtained the $\hat{H}$ coefficient amounting to 0.48, 0.51, 0.46 and 0.58, respectively. The polymorphism of erythrocyte antigens, blood proteins, lipoproteins allotype and *RYR1* gene was examined by ŻURKOWSKI et al. (1995) for the Zlotnicka Spotted and Polish Large White pigs and the heterozygosity coefficient obtained amounted to 0.34 and 0.38, respectively.

The results obtained for the genetic variation in the Pulawska pig population studied were subjected to a statistical analysis to determine the probability of observing identical genotypes in two individuals randomly chosen from a herd (Tables 1, 2). The highest genetic compatibility for the gilts examined was observed when the colipase gene was made the criterion as 96 individuals out of a 100 would possess an identical genotype. Such high values were reported also for *GH* genes (*Msp*I enzyme used for genotyping) and *MYF3* – in these cases the probability of the occurrence of two identical genotypes exceeds 50%.

The values obtained for microsatellite loci were clearly lower. Taking into consideration this group of markers, two individuals randomly chosen out of a population of the Pulawska pigs would be most likely to show a genetic compatibility in relation to locus *S0088* (G1 = 0.29). However, only in 2 out of 100 cases the animals would exhibit an identical arrangement of alleles at locus *TNFB*. For the other loci coefficient G1 ranged from 0.05 to 0.23.

Van ZEVEREN et al. (1995) reported considerably lower values for an analogous probability coefficient for microsatellites *S0017*, *S0019*, *S0020*, *S0021*, *S0029* – 0.03-0.54, depending on the locus and breed. These authors stated that in the case of for instance locus *S0020,* choosing two individuals of the Large White breed showing a genetic compatibility may occur in 37 cases out of a 100, whereas for the Belgian Landrace or Pietrain breeds – in 54 cases out of a 100.

The mean probability of a random choice of two genetically identical individuals of the Pulawska breed, computed on the basis of 6 genes, amounted to $G1 = 6.95 \times 10^{-3}$, while on the basis of 14 microsatellites – $G1 = 1.23 \times 10^{-14}$. The result obtained, although quite high, may be regarded as satisfactory, due to the, among others factors, low variation observed for genes *CLPS*, *MYF3* and *GH* (the *Msp*I enzyme used for genotyping).

Taking into consideration the small size of the population of Pulawska pig included in the preserve breeding program, the parameters of the genetic structure obtained indicate that it is possible to undertake breeding work using the basic factor, that is the genetic variation in a herd.

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