

ASSESSMENT OF MICROSATELLITE SEQUENCE SUITABILITY FOR GENETIC STRUCTURE ANALYSIS OF PUŁAWSKA PIGS FROM CONSERVATION BREEDING*

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Abstract

The polymorphism of three microsatellites (FST, IGF1, Swc9) was investigated in 150 Puławska gilts and sows. The pigs originated from herds included in the Puławska Pig Genetic Resources Conservation Programme. Genetic variation for the population was determined based on main coefficients, i.e. polymorphic information content (PIC) and heterozygosity (\hat{H}). Out of the analysed microsatellite loci, the highest number of alleles (7) was established for Swc9 and the highest number of genotypes (12) for IGF1. The estimates of PIC ranged from 0.446 (FST) to 0.679 (IGF1), and heterozygosity (\hat{H}) in the analysed population varied between 0.427 (FST) and 0.653 (Swc9).

Key words: pigs, Puławska breed, microsatellites, polymorphism

Genetic variation, observed within a breed or population, is one of the highly informative sources of animal breeding level. To determine the genetic structure of pigs, a number of genetic markers can be employed, such as blood groups, microsatellites, and genes. In this regard, microsatellite sequences have become the focus of ongoing research.

Microsatellites that have wide-ranging applications in the field of genetics, by virtue of their ubiquitous nature, equal distribution throughout the genome, high heterozygosity and polymorphism levels as well as relative ease and accuracy of identification procedures and analyses prove to be efficient in genetic diversity studies (Ellegren et al., 1993; Rohrer et al., 1994). Therefore, they are likely to be successfully applied in the genetic characterization of a small-sized population such as that of Puławska pigs. This breed, known as Gołębska in the interwar period and after World War II, and as Puławska since 1951, is the oldest indigenous breed of pigs in Po-

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land. The breed became threatened with extinction and in 1996 it was included in the Conservation Breeding Programme that aims to determine and maintain high genetic diversity of the existing Puławska pig population (Walkiewicz et al., 2005).

Therefore, a set of traits typical of this breed could be preserved, including high reproductive performance, maternal ability, innate tractability, and good quality attributes of meat and fat (Walkiewicz et al., 1997, 2000). It is also noteworthy that estimation of the Puławska breed genetic structure is consistent with the current global priority that focuses on the recognition and conservation of farm animal biodiversity (Martinez et al., 2000; Megens et al., 2008).

The objective of the present study and analysis was to determine the polymorphism of microsatellite sequences for Puławska pigs maintained in 9 reproductive herds.

Material and methods

The study comprised a total of 150 Puławska gilts and sows. The animals were maintained in 9 farms located in the Lublin region under the Puławska Pig Conservation Breeding Programme.

Biological material was represented by blood collected from the external jugular vein (*vena jugularis externa*) of each gilt or sow using disposable syringes (Monovette, Sarstedt) with EDTA as anticoagulant.

DNA recovery from blood leukocytes was performed after the Kawasaki method (1990) modified by Coppieters et al. (1992), using commercial kits (Dneasy® Blood & Tissue Kit, Qiagen) according to the manufacturer's instructions.

Primer pairs for IGF1 and Swc9 were designed using Primer 3 online software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi), and those for FST after Shimasaki et al. (1988).

Each sample of 20 µl capacity contained 5 µl RedTaq™ReadyMix™PCR (SIGMA), 0.2 µl of each primer (0.2 µM), 3.6 µl water (SIGMA) and 2 µl DNA (20 µl). Alleles within the microsatellite sequence loci were identified by the PCR process (PTC-200 Peltier thermal cycler) and capillary electrophoresis using fluorescein-labelled primers 6-FAM (6-carboxyfluorescein), HEX (4,7,2',4',5'7'-hexachloro-6-oxycarboxyfluorescein) or TET (4,7,2',7'-tetrachloro-6-carboxy fluorescein).

PCR conditions for Swc9 and IGF1 were as follows: 94°C for 3 min followed by 33 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s, followed by final extension at 72°C for 7 min and completed by cooling for 10 min at 10°C.

The PCR thermal profile for FST comprised the following steps: 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, final extension at 60°C for 10 min, and cooling at 4°C.

Electrophoresis was conducted on the ABI PRISM™ 310 genetic analyser equipped with a laser-induced fluorescence detection system for DNA fragments. The capillary electrophoresis data were analysed by 310 ABI PRISM Data Collection and 310 Genescan Analysis software version 2.1.

The genetic variation was estimated based on the following statistical indices:

– frequency of alleles and genotypes for each satellite locus and gene computed with the least square method,

- heterozygosity coefficient (\hat{H}) evaluated after Ott (1992) and Weir (1990),
- polymorphic information content (PIC) for microsatellite loci and genes estimated using the formula given by Botstein et al. (1980).

The statistical analyses were performed using the SAS software package.

Results

The allele frequency of the microsatellite loci was calculated for 150 Puławska sows maintained in 9 breeding herds. A total of 15 alleles and 28 genotypes were observed. As regards the number of alleles, Swc9 was the most polymorphic with 7 alleles, whereas in terms of the number of genotypes, IGF1 locus was the most polymorphic with 12 genotypes (Table 1). The results obtained also showed the presence of genetic diversity in each breeding herd, as reflected in a variable number of genotypes for each locus (Tables 2, 3 and 4).

Table 1. Genetic variation indices in 3 microsatellite loci in the Puławska pig (n=150)

Locus	Identified allele size (bp)	Number of alleles	Allele	Allele frequency	Genotype	Genotype frequency	PIC ¹	\hat{H} ²
FST	142-146	3	142	0.647	142-142	0.433	0.446	0.427
			144	0.103	142-144	0.100		
			146	0.250	142-146	0.327		
					144-144	0.053		
					146-146	0.087		
IGF1	124-132	5	124	0.027	124-126	0.040	0.679	0.633
			126	0.227	124-130	0.013		
			128	0.387	126-126	0.040		
			130	0.117	126-128	0.153		
			132	0.243	126-130	0.073		
					126-132	0.107		
					128-128	0.200		
					128-130	0.067		
					128-132	0.153		
					130-130	0.027		
					130-132	0.027		
					132-132	0.100		
Swc9	230-244	7	230	0.067	230-230	0.033	0.654	0.653
			232	0.357	230-242	0.067		
			234	0.017	232-232	0.133		
			238	0.167	232-238	0.140		
			240	0.007	232-240	0.013		
			242	0.367	232-242	0.293		
			244	0.020	234-244	0.033		
					238-238	0.047		
					238-242	0.100		
					242-242	0.133		
		242-244	0.007					

¹PIC – Polymorphic Information Content; ² \hat{H} – degree of heterozygosity.

Table 2. Genetic variation indices in IGF1 locus in the individual herds of Puławska pigs (n = 150)

Herd	Number of alleles	Allele	Allele frequency	Genotype	Genotype frequency	PIC ¹	\hat{H}^2
1	3	142	0.773	142-142	0.636	0.344	0.273
		144	0.091	142-144	0.182		
		146	0.136	142-146	0.091		
				146-146	0.091		
2	3	142	0.714	142-142	0.571	0.386	0.286
		144	0.071	142-144	0.143		
		146	0.214	142-146	0.143		
				146-146	0.143		
3	2	142	0.929	142-142	0.857	0.124	0.143
		146	0.071	142-146	0.143		
4	3	142	0.682	142-142	0.455	0.434	0.455
		144	0.182	142-144	0.182		
		146	0.136	142-146	0.273		
				144-144	0.091		
5	3	142	0.618	142-142	0.412	0.427	0.412
		144	0.059	142-144	0.118		
		146	0.324	142-146	0.294		
				146-146	0.177		
6	3	142	0.733	142-142	0.533	0.370	0.400
		144	0.067	142-144	0.133		
		146	0.200	142-146	0.267		
				146-146	0.067		
7	3	142	0.522	142-142	0.261	0.532	0.522
		144	0.174	142-146	0.522		
		146	0.304	144-144	0.174		
				146-146	0.044		
8	3	142	0.500	142-142	0.148	0.448	0.704
		144	0.056	142-144	0.037		
		146	0.444	142-146	0.667		
				144-144	0.037		
		146-146	0.111				
9	3	142	0.611	142-142	0.445	0.489	0.333
		144	0.222	142-144	0.222		
		146	0.167	142-146	0.111		
				144-144	0.111		
		146-146	0.111				

¹PIC – Polymorphic Information Content; ² \hat{H}^2 – degree of heterozygosity.

Allele frequency for each microsatellite locus did not exceed the 70% threshold. The highest frequency (0.647) was detected in the FST locus for the allele of 142 bp. The allele of 240 bp in the Swc9 locus was shown to occur most randomly. The most frequent genotypes for each microsatellite were 142–142 in the FST locus (frequency equal to 0.433), 126–128 and 128–132 in the IGF1 locus (0.153) and 232–242 in the Swc9 locus (0.293). The least frequent genotypes were 144-144 for FST (0.053), 124–130 for IGF1 (0.013) and 232–240 for Swc9 (0.013).

The coefficient of heterozygosity (\hat{H}), calculated for the analysed population, ranged from 0.427 for FST to 0.653 for Swc9. The corresponding polymorphic information content was shown to be the highest for the IGF1 locus (0.679).

Table 3. Genetic variation indices in IGF1 locus in the individual herds of Puławska pigs (n = 150)

Herd	Number of alleles	Allele	Allele frequency	Genotype	Genotype frequency	PIC ¹	\hat{H}^2
1	2	3	4	5	6	7	8
1	4	126	0.091	126-128	0.182	0.522	0.364
		128	0.546	128-128	0.364		
		130	0.045	128-130	0.091		
		132	0.318	128-132	0.091		
2	5	124	0.036	124-126	0.071	0.677	0.643
				126-126	0.143		
				126-128	0.143		
				126-130	0.071		
				126-132	0.071		
				128-128	0.143		
3	5	124	0.036	124-126	0.072	0.654	0.643
				126-128	0.143		
				126-130	0.071		
				126-132	0.072		
				128-128	0.143		
				128-130	0.071		
4	4	126	0.136	126-128	0.091	0.607	0.818
				126-130	0.091		
				126-132	0.091		
				128-130	0.091		
				128-132	0.455		
				132-132	0.182		
5	5	124	0.088	124-126	0.176	0.578	0.467
				126-126	0.059		
				126-128	0.294		
				126-130	0.059		
				126-132	0.059		
				128-128	0.294		
6	4	126	0.200	126-126	0.067	0.645	0.533
				126-130	0.067		
				126-132	0.200		
				128-128	0.267		
				128-130	0.133		
				128-132	0.133		

Table 3 – contd.

1	2	3	4	5	6	7	8
7	5	124	0.044	124-126	0.044	0.669	0.696
		126	0.217	124-130	0.044		
		128	0.413	126-128	0.217		
		130	0.087	126-130	0.044		
		132	0.239	126-132	0.130		
				128-128	0.217		
				128-130	0.044		
				128-132	0.130		
				130-132	0.044		
				132-132	0.087		
8	5	124	0.019	124-130	0.037	0.708	0.667
		126	0.296	126-126	0.074		
		128	0.259	126-128	0.185		
		130	0.241	126-130	0.148		
		132	0.185	126-132	0.111		
				128-128	0.111		
				128-130	0.074		
				128-132	0.037		
				130-130	0.074		
				130-132	0.074		
		132-132	0.074				
9	4	126	0.138	126-128	0.056	0.643	0.611
		128	0.444	126-130	0.056		
		130	0.168	126-132	0.165		
		132	0.250	128-128	0.278		
				128-130	0.111		
				128-132	0.167		
				130-130	0.056		
				130-132	0.056		
				132-132	0.056		

¹PIC – Polymorphic Information Content; ² \hat{H} – degree of heterozygosity.

Table 4. Genetic variation indices in Swc9 locus in the individual herds of Puławska pigs (n = 150)

Herd	Number of alleles	Allele	Allele frequency	Genotype	Genotype frequency	PIC ¹	\hat{H} ²
1	2	3	4	5	6	7	8
1	4	230	0.046	230-242	0.090	0.627	0.689
		232	0.318	232-238	0.273		
		238	0.272	232-242	0.364		
		242	0.364	238-242	0.273		
2	4	230	0.071	230-242	0.143	0.621	0.786
		232	0.393	232-232	0.143		
		238	0.177	232-238	0.213		
		242	0.359	232-242	0.286		
				238-242	0.143		
				242-242	0.072		

Table 4 – contd.

1	2	3	4	5	6	7				
3	5	230	0.071	230-230	0.071	0.615	0.571			
		232	0.393	232-232	0.143					
		238	0.107	232-238	0.143					
		240	0.036	232-240	0.071					
		242	0.393	232-242	0.286					
				238-242	0.071					
		242-242	0.215							
4	5	230	0.071	230-230	0.071	0.615	0.571			
		232	0.393	232-232	0.143					
		238	0.107	232-238	0.143					
		240	0.036	232-240	0.071					
		242	0.393	232-242	0.286					
				238-242	0.071					
		242-242	0.215							
5	4	230	0.029	230-242	0.059	0.561	0.824			
		232	0.471	232-232	0.119					
		238	0.147	232-238	0.235					
		242	0.353	232-242	0.471					
				238-242	0.059					
				242-242	0.057					
6	4	230	0.133	230-230	0.133	0.618	0.603			
		232	0.400	232-232	0.133					
		238	0.100	232-238	0.133					
		242	0.367	232-242	0.400					
				238-242	0.067					
				242-242	0.134					
7	6	230	0.022	230-242	0.044	0.711	0.696			
		232	0.261	232-232	0.087					
		234	0.087	232-238	0.044					
		238	0.174	232-242	0.304					
		242	0.370	234-244	0.174					
		244	0.087	238-238	0.087					
				238-242	0.130					
				242-242	0.130					
8	6	230	0.019	230-242	0.037	0.639	0.409			
		232	0.407	232-232	0.296					
		234	0.019	232-242	0.222					
		238	0.222	234-244	0.037					
		242	0.296	238-238	0.185					
		244	0.037	238-242	0.074					
				242-242	0.012					
				242-244	0.037					
9	5	230	0.111	230-230	0.056	0.654	0.611			
		232	0.333	230-242	0.111					
		238	0.139	232-232	0.111					
		240	0.028	232-238	0.222					
		242	0.389	232-240	0.056					
				232-242	0.167					
				238-242	0.056					
				242-242	0.221					

¹PIC – Polymorphic Information Content; ²H – degree of heterozygosity.

Discussion

Short repeat sequences provide essential information to estimate homo- and heterozygosity levels in a population. As potential molecular markers, they indicate if the analysed population is in a state of genetic equilibrium or if the equilibrium has already been upset. Therefore, microsatellite polymorphism is widely used to characterize the genetic makeup of pig breeds all over the world (Kim et al., 2005). The intensive breeding efforts within a single breed, being largely oriented towards improvement of selected reproductive parameters usually lead to a decline in genetic diversity. Thuy et al. (2006), who compared the genetic structure of European and Vietnamese pig breeds, demonstrated distinctly lower heterozygosity compared with that of high meat breeds (Pietrain, Landrace). Polymorphic information content for the European pig breeds ranged from 0.43 to 0.49 and was lower by 0.2 compared to the Asian breeds. The PIC value for the Puławska pig corresponded with the present data and averaged from 0.446 (FST) to 0.679 (IGF1). These findings are also comparable with those reported by San Cristobal et al. (2006), who established the genetic variation in the European breeds to range from 0.43 to 0.68. Kim et al. (2005) report that genetic diversity within the native breed pigs is relatively low. The heterosis level determined for Korean pigs (0.497) was lower than for European breeds such as Duroc (0.557) and Landrace (0.624). In the present study involving 150 gilts and sows of the Puławska breed, actual heterosis levels averaged from 0.426 (FST) to 0.653 (Swc9). This implies that animal population size may significantly correlate with population genetic variation and its depression is likely to lead to inbreeding. Inbreeding, though, manifesting itself by an increase in homozygosity (inbreeding increase) among others, may cause a serious decrease in genetic diversity. Therefore, our study focused on searching and analysing the polymorphism of short repeat sequences as strongly polymorphic markers are exactly the type needed to uncover this complex interaction. Microsatellite markers may provide useful tools to detect and investigate quantitative trait loci (QTL) in a small population (Kim et al., 2006).

Out of the three microsatellite loci (FST, IGF1 and Swc9) analysed, our study with Puławska pigs revealed that the Swc9 and IGF1 loci, characterized by high genetic diversity (0.653 and 0.633, respectively), are the most useful markers to evaluate the genetic structure of this animal group.

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Ocena przydatności sekwencji mikrosatelitarnych do analizy struktury genetycznej świń rasy pulawskiej z hodowli zachowawczej

STRESZCZENIE

Wykonano badania polimorfizmu 3 mikrosatelit (FST, IGF1, Swc9) dla 150 loszek i loch rasy pulawskiej. Zwierzęta pochodziły ze stad uczestniczących w Programie Ochrony Zasobów Genetycznych Świń Rasy Pulawskiej. Zmienność genetyczną dla populacji określono na podstawie głównych

wskaźników, tj. polimorfizmu (PIC) i heterozygotyczności (\hat{H}). Spośród analizowanych loci mikrosatelitarnych najwyższą liczbę alleli (7) zanotowano dla Swc9, natomiast genotypów (12) dla IGF1. Oszacowana wartość wskaźnika polimorfizmu (PIC) wahała się w granicach od 0.446 (FST) do 0.679 (IGF1), natomiast heterozygotyczność (\hat{H}) badanej populacji pozostawała na poziomie od 0.427 (FST) do 0.653 (Swc9).