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The polymorphism of 24 microsatelite loci in 4 Polish cattle breeds

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The aim of the studies was to evaluate the polymorphism of 24 microsatellite DNA sequences in 4 Polish cattle breeds, i.e. the Whitebacks (BG) –100 animals, the Polish Red (RP) – 60 animals, the Polish Black-and-White (BW) – 50 animals and the Polish Holstein-Friesian of Black-and-White variety (PHF-HO) – 50 animals. The most polymorphic loci of all races were: TGLA53, TGLA227, TGLA122, INRA037 and HEL9, in which 17, 15, 14, 13 and 12 different alleles were identified, respectively. Allele 186-bp at ILSTS005 locus was the most frequent in BG (0.675), allele 101 bp in locus INRA035 was the most frequent in RP and BW (with the value of 0.742 and 0.730, respectively) and 163 bp allele in HEL5 locus of race PHF--HO (0.637). Using a total of 24 loci, the probability to exclude a wrong parent for each of the races was 0.999.

KEY WORDS: polymorphism / microsatelite sequencing / Polish cattle breeds

Molecular genetics in animal breeding was used as early as at the turn of 1950's and 60's, what allowed for the detection of chromosomal mutations in bulls, boars and foxes. In 1980's genetic engineering was used together with biotechnology to breed transgenic cattle, among other species [1, 8]. Currently, it is finding genetic diversity at the genome's level of endangered species and races that occupies an important position in animal breeding. This allows for studying the polymorphism of DNA genetic markers, among which microsatellite sequences are most frequently examined [13]. The term "microsatellite" was proposed in 1989 by Litt and Luty [7]. It is the most common group of 2nd class markers that manifests polymorphism in over 90% of cases. Such sequences are present in all eukaryotic genomes and are characterized by a basic pattern consisting of a few nucleotides (1-6) that repeats from 10 to 50 times. Their length is from 60 to 300 pairs of bases [2, 10]. Microsatellites are encountered primarily in non-coding genes, however, they may also be identified in flanking sequences or, less often, in coding sequences. They are characterized by even distribution every 6000 to 10 000 bp [6, 16]. The function of microsatellites has not been fully discovered yet [5]. Most probably, their distribution in genome increases or decreases the expression of genes [9].

Such features of microsatellites as frequency, significant polymorphism, even distribution in genome, inheritance according to Mendel's laws, ease of identification with PCR and electrophoresic methods allow for a wide use of these markers. They are used for characterization of population structure and its inbreeding, estimating genetic variability, controlling the origin, defining genetic distance between populations, races and animal breeds, phylogenetic research, identification of quantitative genes' traits, construction of gene maps and performing marker assisted selection (MAS) [3, 12].

There are about 4000 microsatellite markers which have been described so far, and which are of significant interest for researchers. They are used for genome mapping (BovMap), intra- and extra-population differentiation, individual identification and qualitative traits' loci (QTL), origin control, diagnosing blood cell chimerism and infertility of heifers from male-female twinnings [11, 14].

The aim of the present work was to assess the polymorphism of 24 microsatellite DNA sequences in 4 Polish cattle breeds: the Whitebacks (BG), the Polish Red (RP), the Polish Black-and-White (BW) and the Polish Holstein-Friesian of Black-and-White variety (PHF-HO).

Material and methods

The research was conducted on 210 animals representing 4 Polish cattle breeds: the Whitebacks (BG) – 100 animals, the Polish Red (RP) – 60 animals, the Polish Black-and-White (BW) – 50 animals, and the Polish Holstein-Friesian of Black-and-White variety (PHF-HO) – 50 animals. The input material in a form of peripheral blood was taken to disposable tubes with EDTA anticoagulant stored at 4°C for a period of up to 12 hours, and then frozen until the day of analyses at –20°C.

The analysis of genetic structure was performed on the basis of 24 microsatellite markers located in 17 cattle chromosomes, selected from 30 chromosomes recommended by Roslin Institute, Edinburgh [18]. The isolation of genomic DNA from blood was performed with QIAamp DNA BLOOD MINI KIT (Life Technologies Polska). The chain reaction of polymerase was done in MJ Research PTC 225 thermocycler. PCR reaction was done in the reagents' volume of 15 µl including 3.85 µl H_20, 1.5 µl buffer (10x), 5 µl $MgCl_2$ (25 mM), 0.075 µl starters (10 pmol), 1.25 µl dNTP mix (2 mM each), 0.025 µl of polymerase Taq (5 U/µl) and 3 µl of DNA sample (0.0130-.0587 µg/ µl). The thermal profile of PCR was properly seleted: initial denaturation at 95°C for 10 min., 31 cycles consisting of proper denaturation at 94°C, 45 sec., binding starters – 61°C, 45 sec. and synthesis – 72°C, 60 sec.; the proper synthesis – 60 min. at 72°C and 120 min. at 25 °C. Electrophoretic differentiation with PCR product analysis was performed with the use of capillary electrophoresis in 3100 Avant Genetic Analyze equipment. The length of microsatellite alleles was defined with reference to ROX 350 internal length standard. In order to obtain precise lengths of the amplified fragments, they were referred to reference sample from Roslin Institute. The results were collected with 3100-Avant ABI PRISM Data Collection, and analysed with Gene Mapper Software 3.5. The frequency of alleles, probability to exclude a wrong parent (PE) and combined probability to exclude a wrong parent (PE_C) were defined for each microsatellite. The calculations were performed with software Cervus v. 3.0.3.

Results and discussion

214 different allele were identified in 24 analysed microsatellite loci, including 189 alleles in the Whitebacks (BG), 168 in the Polish Black-and-White (BW), 178 in the Polish Red (RP) and 158 the Polish Holstein-Friesian variety (PHF-HO). In all races the following loci were the most polymorphic: TGLA53, TGLA227, TGLA122, INRA037 and HEL9, in which the following number of alleles were indentified respectively: 17, 15, 14, 13 and 12. In loci ILSTS005, INRA005 and TGLA126 the smallest number of alleles were identified: 5 alleles in each.

With the definition of alleles' frequency, one may analyse the correlations between species and populations [15]. The results obtained for cattle breeds are presented in tables 1-5. The distribution of different alleles in a locus varies. It has been stated that the allele of 186 bp in length in locus ILSTS005 was the most frequent (0.675) (Table 3). In contrast, the research by Żurkowski et al. [17] on 50 animals BG demonstrated higher frequency for the allele of 192 bp in HEL13 (0.7294), which reached the frequency of 0.565 in the present research (Table 3). The above-mentioned 186 bp allele in locus ILSTS005 had similar frequency in the research by Żurkowski et al. [17] (0.6458). Yet, the range of the identified alleles was 182-194 bp in the present research, while in the quoted research only two alleles were defined: 184 and 186. Out of 178 alleles in the Polish Red and 168 alleles in the Polish Black-and-White, allele 101 bp located in locus INRA035 demonstrated the highest frequency with the values: 0.742 for RP and 0.730 for BW (Table 4). On the other hand, allele 163 bp in locus HEL5 had the highest frequency in the Polish Holstein-Friesian variety (0.637). In BG and BW it reached the values of 0.425 and 0.440 respectively, while in RP as little as 0.1 (Table 2).

There were also numerous alleles with the frequency below 2% – so called rare alleles. They constituted 25% in the Whitebacks, 19% in the Polish Black-and-White and 23% in the Polish Holstein-Friesian variety.

The probability of exclusion (PE) is an important parameter in the research based on microsatellite markers and allele frequency. The indicator is used to define the utility of microsatellite DNA to control the origin on the basis of wrong parent exclusion. The utility of a single locus and combined probability of wrong parent exclusion are both estimated on the basis of all analysed loci. International Society of Animal Genetics (ISAG) advises to use minimum 9 microsatelite markers to control the origin of cattle (BM1824, BM2113, ETH3, ETH10, ETH225, SPS115, TGLA122, TGLA126, TGLA227).

The lowest value of PE on the basis of one locus was in ILSTS005 in all the breeds and amounted to the following values: BG – 0.182; BW – 0.181; RP – 0.187 and PHF-HO – 0.180 (Table 6). Such values are related to the small number of identified alleles and their high frequency in the locus. This concerns allele 186 bp in BG, RP and BW and allele 184 bp in PHF-HO and proves a limited use of the selected loci in the identification research. Żurkowski et al. [17] also quote the lowest values of PE in this locus: BG – 0.1764, RP – 0.1825 and BW – 0.1422.

The highest values ranged from 0.721 in TGLA53 in the Polish Red cattle, 0.728 in BM2113 in the Polish Black-and-White, 0.731 in TGLA227 in Polish Holstein-Friesian variety, up to 0.745 in TGLA53 for the Whitebacks.

Table 1
Allele frequency in locus: BM1818, BM1824, BM2113, CSSM66, CSRM60

Locus	Allele	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
BM1818	256	0.010	0.008	0.010	–
	258	0.065	0.042	0.080	0.010
	260	0.460	0.142	0.330	0.333
	262	0.110	0.150	0.140	0.196
	264	0.295	0.641	0.380	0.353
	266	0.035	0.017	0.030	0.088
	268	0.025	–	0.030	0.020
BM1824	171	–	0.017	–	–
	175	0.240	0.325	0.170	0.265
	177	0.145	0.125	0.250	0.186
	179	0.325	0.308	0.310	0.274
	185	0.285	0.208	0.270	0.255
	187	0.005	0.017	–	0.020
BM2113	122	–	0.008	–	–
	126	0.165	0.167	0.170	0.196
	128	0.220	0.241	0.190	0.294
	130	0.005	0.042	–	–
	132	0.035	0.017	0.050	–
	134	0.125	0.058	0.130	0.108
	136	0.150	0.175	0.100	0.176
	138	0.055	0.142	0.120	0.078
	140	0.210	0.075	0.130	0.010
	142	0.025	0.067	0.090	0.118
	144	0.010	0.008	0.020	0.020
CSSM66	179	0.065	0.042	0.070	0.049
	181	0.015	0.033	0.040	0.069
	183	0.145	0.242	0.210	0.108
	185	0.250	0.308	0.460	0.343
	187	0.105	0.092	0.040	0.039
	189	0.145	0.075	0.020	0.226
	191	0.030	0.100	–	–
	193	0.160	0.042	0.080	0.156
	195	0.015	0.008	0.010	–
	197	0.070	0.058	0.050	0.010
	199	–	–	0.020	–
CSRM60	91	0.020	0.008	–	–
	93	0.100	0.317	0.070	0.196
	97	0.100	0.175	0.080	0.157
	99	0.160	0.100	0.170	0.108
	101	0,195	0.133	0.290	0.108
	103	0,380	0.100	0.390	0.431
	105	0,045	0.167	–	–

Table 2

Allele frequency in locus: ETH10, ETH225, HEL1, HEL5, HEL9

Locus	Allele	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
ETH10	207	–	0.008	–	–
	211	0.040	0.042	0.020	0.029
	213	0.030	0.017	0.010	0.049
	215	0.250	0.458	0.310	0.186
	217	0.445	0.300	0.430	0.490
	219	0.115	0.025	0.120	0.049
	221	0.045	0.092	0.090	0.118
	223	0.075	0.058	0.020	0.079
ETH225	137	0.125	0.233	0.090	0.137
	139	0.015	0.017	0.010	–
	141	0.030	0.092	0.050	0.107
	143	0.085	0.092	0.080	0.020
	145	0.015	0.008	–	0.020
	147	0.285	0.133	0.210	0.363
	149	0.390	0.225	0.510	0.255
	151	0.040	0.142	0.050	0.098
	153	–	0.058	–	–
	159	0.015	–	–	–
HEL1	103	0.050	0.217	0.050	0.010
	105	0.450	0.358	0.430	0.431
	107	0.015	–	0.010	–
	109	–	–	0.010	–
	111	0.125	0.250	0.090	0.088
	113	0.360	0.175	0.400	0.461
	117	–	–	0.010	0.010
HEL5	153	0.035	0.067	0.020	–
	155	0.280	0.167	0.330	0.255
	157	0.080	0.100	0.100	0.020
	159	0.005	0.025	–	–
	163	0.425	0.100	0.440	0.637
	165	0.050	0.300	0.020	0.020
	167	0.110	0.241	0.080	0.068
	169	0.015	–	0.010	–
HEL9	149	–	–	–	0.010
	151	0.020	–	0.120	0.010
	153	0.380	0.475	0.300	0.235
	155	0.105	–	0.050	0.049
	157	0.015	–	0.010	0.029
	159	0.075	0.050	0.030	0.039
	161	0.090	0.100	0.090	0.275
	163	0.115	0.150	0.130	0.039
	165	0.035	0.050	0.050	0.128
	167	0.015	0.017	0.020	–
	169	0.100	0.067	0.180	0.176
	171	0.050	0.091	0.020	0.010

Table 3
Allele frequency in locus: HEL13, ILSTS005, ILSTS006, INRA005, INRA023, INRA032

Locus	Allele	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
HEL13	182	0.010	–	–	–
	184	0.015	–	–	–
	186	0.005	0.008	–	–
	188	0.265	0.333	0.240	0.461
	190	0.125	0.200	0.220	0.029
	192	0.565	0.426	0.540	0.510
	194	0.015	0.033	–	–
ILSTS005	182	0.005	–	–	–
	184	0.315	0.297	0.390	0.618
	186	0.675	0.686	0.610	0.382
	188	–	0.017	–	–
	194	0.005	–	–	–
ILSTS006	283	0.005	–	–	–
	287	0.040	0.050	0.050	0.019
	289	0.155	0.217	0.200	0.363
	291	0.040	–	0.040	0.010
	293	0.275	0.200	0.210	0.216
	295	0.255	0.258	0.240	0.363
	297	0.215	0.259	0.250	0.019
	299	0.005	0.008	–	–
	301	0.010	0.008	0.010	0.010
INRA005	139	0.135	0.033	0.120	0.078
	141	0.555	0.625	0.680	0.618
	143	0.280	0.342	0.200	0.304
	145	0.025	–	–	–
	147	0.005	–	–	–
INRA023	199	0.040	–	0.040	0.010
	201	0.090	0.208	0.180	0.019
	203	0.035	–	0.050	0.147
	205	–	0.008	–	–
	207	0.170	0.108	0.030	0.255
	209	0.110	0.142	0.180	0.098
	211	0.160	0.133	0.170	0.304
	213	0.005	0.017	–	–
	215	0.375	0.376	0.350	0.167
	217	0.015	0.008	–	–
INRA032	176	0.120	0.058	0.100	0.068
	178	0.075	0.067	0.130	0.049
	180	0.505	0.675	0.460	0.461
	182	0.175	0.134	0.120	0.157
	184	0.100	0.058	0.190	0.245
	186	0.025	0.008	–	0.020

Table 4
Allele frequency in locus: INRA035, INRA037, INRA063, SPS115, TGLA126

Locus	Allele	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
INRA035	99	0.245	0.183	0.200	0.402
	101	0.620	0.742	0.730	0.569
	103	–	0.033	–	–
	105	0.035	–	0.030	–
	109	0.030	0.025	–	–
	115	0.070	–	0.040	0.019
	119	–	0.017	–	0.010
INRA037	120	0.035	0.217	0.030	0.137
	122	0.005	0.017	–	–
	124	0.005	0.017	–	0.010
	126	0.095	0.067	0.150	0.088
	128	0.205	0.100	0.070	0.166
	130	0.015	0.058	0.010	0.010
	132	0.500	0.316	0.600	0.479
	134	0.015	0.075	0.020	0.010
	136	0.025	–	0.030	–
	138	–	–	0.020	–
	142	–	0.033	–	–
	144	0.010	0.017	0.020	–
	146	0.090	0.083	0.050	0.100
INRA063	177	0.530	0.534	0.690	0.569
	179	0.335	0.258	0.180	0.353
	181	0.040	0.050	0.060	0.078
	183	–	0.017	0.010	–
	185	0.060	0.133	0.020	–
	187	0.035	0.008	0.040	–
SPS115	244	0.600	0.459	0.610	0.500
	246	0.010	0.025	0.120	0.118
	248	0.165	0.158	0.060	0.177
	250	0.120	0.100	0.050	0.078
	252	0.050	0.075	0.090	0.069
	254	0.035	0.042	0.020	0.029
	256	0.020	0.133	0.040	0.029
	258	–	0.008	0.010	–
TGLA126	119	0.485	0.592	0.620	0.620
	121	0.330	0.300	0.200	0.200
	123	0.055	0.025	0.030	0.030
	125	0.045	0.008	0.020	0.020
	127	0.085	0.075	0.130	0.130

Table 5
Allele frequency in locus: TGLA53, TGLA122, TGLA227

Locus	Allele	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
TGLA53	145	0.215	0.042	0.110	0.010
	149	0.120	0.050	0.100	0.098
	151	0.160	0.250	0.420	0.245
	153	0.160	0.092	0.090	0.226
	155	0.035	0.058	0.020	0.029
	157	0.080	0.083	0.010	0.020
	159	0.045	0.042	0.090	0.118
	161	0.065	0.225	0.050	0.039
	163	0.015	0.058	–	–
	165	0.025	0.008	–	–
	167	0.035	0.017	0.020	0.029
	169	0.015	0.008	0.040	0.058
	171	0.010	0.042	0.010	0.010
	173	–	–	0.010	0.020
	175	–	0.008	0.020	0.020
	177	0.010	–	0.010	0.58
	179	0.010	0.017	–	0.020
TGLA122	148	0.095	0.042	0.120	–
	150	0.285	0.433	0.500	0.314
	152	0.005	0.017	0.010	–
	154	0.115	0.033	0.040	0.186
	156	0.195	0.183	0.060	0.137
	158	0.070	0.175	0.100	0.029
	164	0.010	0.017	–	0.010
	166	0.045	0.050	0.010	0.049
	168	0.170	0.050	0.130	0.197
	170	–	–	–	0.029
	172	–	–	0.010	–
	174	0.010	–	0.010	0.010
	176	–	–	0.010	0.029
	180	–	–	–	0.010
TGLA227	77	0.005	0.092	0.010	–
	79	–	0.033	–	–
	81	0.225	0.184	0.350	0.137
	83	0.220	0.117	0.180	0.098
	87	0.065	0.083	0.010	0.098
	89	0.110	0.050	0.120	0.118
	91	0.085	0.317	0.120	0.137
	93	0.055	0.033	0.030	0.049
	95	0.020	–	–	–
	97	0.195	0.075	0.150	0.235
	99	–	–	–	0.020
	101	0.010	–	0.030	0.059
	103	0.005	0.008	–	0.049
	105	0.005	–	–	–
	107	–	0,008	–	–

Table 6
Probability of exclusion (PE) and combine probability of exclusion (PE_C) in investigated cattle breeds

Locus	Whitebacks (BG)	Polish Red (RP)	Polish Black-and-White (BW)	Polish Holstein-Fresian Black-and-White variety (PHF-HO)
BM1818	0.445	0.326	0.484	0.470
BM1824	0.486	0.501	0.491	0.516
BM2113	0.674	0.697	0.728	0.631
CSSM66	0.698	0.646	0.530	0.594
CSRM60	0.570	0.617	0.487	0.506
ETH10	0.498	0.451	0.454	0.491
ETH225	0.525	0.675	0.460	0.553
HEL1	0.383	0.483	0.379	0.306
HEL5	0.495	0.608	0.434	0.282
HEL9	0.643	0.532	0.674	0.639
HEL13	0.342	0.394	0.327	0.223
ILSTS005	0.182	0.187	0.181	0.180
ILSTS006	0.579	0.559	0.587	0.424
INRA005	0.333	0.216	0.253	0.255
INRA023	0.594	0.560	0.578	0.577
INRA032	0.468	0.318	0.489	0.463
INRA035	0.311	0.218	0.218	0.221
INRA037	0.478	0.659	0.411	0.493
INRA063	0.337	0.381	0.279	0.264
SPS115	0.379	0.529	0.402	0.484
TGLA53	0.745	0.721	0.615	0.715
TGLA122	0.652	0.537	0.508	0.622
TGLA126	0.388	0.294	0.325	0.439
TGLA227	0.674	0.669	0.604	0.731
PE_C	0.999	0.999	0.999	0.999

The results obtained show that the use of single microsatellite locus gives the probability of wrong parent exclusion at the level of 18% (for ILSTS005 in PHF-HO) to 74% (TGLA53 in BG), while the probability of the wrong parent exclusion on the basis of the analysis of 24 microsatellite markers (PE_C) was estimated at 99.9% (Table 6).

On the basis of the definition that a polymorphic locus is the one in which the frequency of an allele is lower than 0.95 [4] one may state that all analysed loci are polymorphic. The differences in alleles' frequency in the analysed cattle breeds may result in factors which are independent on the breeder's will, such as genetic drift, which may cause fluctuations

and differences in alleles' frequency between different populations of the same breed. The most visible effect of the drift is noticeable in small populations and in case of rare alleles. Their effect accumulates in time. Migration (genes' flow) can also be another cause of changes in gene frequency.

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