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Full Length Research Paper

Genetic variation in the population of three Polish cattle breeds included into the programme of genetic resources protection and Holstein-Friesian breed, estimation on the basis of polymorphism of 24 microsatellite DNA sequences

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The study focused on determining and characterizing genetic variation of three Polish cattle breeds [Whiteback (WB), Polish Red (PR) and Polish Black-and-White (PBW)] included in the programme of genetic resources conservation. The obtained results were related to the genetic variation within the Holstein-Friesian (PHF) breed of Black-and-White variety. Overall, 214 alleles were identified within four examined breeds, including 189 in WB, 178 in PR, 168 in PBW and 158 in PHF. Almost 13% of the identified alleles were the specific ones and the majority of them were determined within WB and PR cattle, 10 alleles in each breed. The greatest genetic distance of 0.013 was established between PR breed and PHF. PR and WB cattle were located in the same clad of neighbour-joining tree which proves their distinction from PBW and PHF cattle.

Key words: Local cattle breeds, genetic variation, microsatellites.

INTRODUCTION

The concept of biological diversity preservation is gaining global understanding and thus is supported by many governments and non-governmental institutions. The International Convention on Biological Diversity during Earth Summit in Rio de Janeiro in 1992 reached a significant milestone in this area. In 1996, Poland joined in the programme of implementing global strategy for animal genetic resources and their conservation, undertaken by FAO. The first cattle breed included in the programme was Polish Red (PR) breed in 1999, followed by Whiteback (WB) breed in 2003, Polish Red-and-White breed in 2007, and Polish Black-and-White breed in 2008; all of them have been raised in Poland for hundreds of years. Although they represent a multipurpose type, they are mostly reared for milk production. The main purpose of protecting local animal breeds is to preserve a pool of genes characteristic for a given population as an achievement of local breeding tradition and also a durable and important element of cultural heritage.

In the case of endangered species and breeds, it is very important to recognize their genetic diversity on the genome level in order to monitor changes in the genetic structure of the population triggered off by breeding practices (Groeneveld et al., 2010). In the analysis of genetic variation of breeds included in the genetic resources conservation, microsatellite sequences are used most frequently because of their valuable characteristics: frequent occurrence, high polymorphism, balanced division within a genome, their hereditary abilities consistent with Mendel's rules, and also, the ease of their identification using polymerase chain reaction (PCR) method and electrophoresis (Weber and May, 1989;

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Wang et al., 1998; Martin-Buriel et al., 2007). A European programme of cattle genetic diversity assessment based on 30 recommended microsatellite sequences (FAO, 2004) was initiated to enable the implementation of the FAO programme. The main objective of the study was to determine genetic diversity on the basis of 24 microsatellite deoxyribonucleic acid (DNA) sequences of three polish local cattle breeds and PHF breed.

MATERIALS AND METHODS

The study examined 260 animals of three Polish local breeds which were included into genetic resources conservation programme together with Holstein breed, selected for its high milk yield. These were WB breed (100), Polish Black-and-White (PBW) breed (50), PR breed (60) and 50 animals of Holstein-Friesian (PHF) breed of Black-and-White variety; is common all over the world and also in Poland. The initial biological material consisted of peripheral blood from which DNA was isolated with the use of a commercial set QIA amp DNA BLOOD MINI KIT (Life Technologies Poland). The genetic structure analysis was conducted on the basis of 24 microsatellite markers chosen out of 30 recommended by FAO and ISAG (BM1818, ETH225, BM1824, BM2113, SPS115, HEL1, INRA005, INRA063, ILSTS005, ILSTS006, TGLA53, ETH10, HEL5, HEL9, HEL13, INRA023, INRA035, INRA037, CSRM60, CSSM66, TGLA122, TGLA227, INRA032, TGLA126) located on 17 chromosomes. The PCR primer sequence, chromosome location, allele range and Genbank accossiation numbers, if possible, of the selected microsatellites are shown in Table 1.

The polymerase chain reaction (PCR) was performed separately for each primer in a reaction of 15 µl containing 3.85 µl of H20, 1.5 µl of buffer (10x), 5 µl of $MgCl_2$ (25 mM), 0.075 µl of starters (10 pmoles), 1.25 µl of dNTP mix (2 mM of each), 0.025 µl of *Taq*Gold polymerase (5 U/µl) and 0.5 ng/µl of genomic DNA. Termocycler (MJ Research PTC 225) program was as follows: initial denaturalization at 95°C for 15 min: 31 cycles of denaturalization at 94°C for 45 s; hybridization at annealing temperature (Table 1), elongation at 72°C for 30 s and final elongation at 72°C for 60 min. The PCR products were subjected to electrophoretic separation using capillary electrophoresis technique in a 3100 Avant Genetic Analyze apparatus. The length of microsatellite alleles was estimated in relation to the internal length standard ROX 350.

In order to obtain accurate lengths of amplified fragments, they were related to the reference sample from Roslin Institute (University of Edinburgh). The results were collected with the use of 3100-Avant ABI PRISM Data Collection, and then analyzed with the use of Gene Mapper Software 3.5. Observed heterozygosity (Ho), expected heterozygosity (He), Hardy – Weinberg equilibrium test (HWE) and polymorphism information content (PIC) were estimated with the use of Cervus v. 3.0.3. The genetic distance value DA according to Nei (1978) was estimated with the use of a computer program Populations v. 1.2.30. The obtained values were used to create a phylogenetic tree using N-J method (neighbour – joining), with the use of Tree view (win.32) v. 1.5.2.

RESULTS

In 24 microsatellite loci, 214 alleles were identified: 189 in WB, 178 in PR, 168 in PBW and 158 in PHF. The most polymorphic of all breeds were TGLA53, TGLA227, TGLA122, INRA037 and HEL9, in which 17, 15, 14, 13 and 12 different alleles, were identified respectively. The fewest alleles appeared in locus ILSTS005, INRA005 and TGLA126 (5 in each). Among 214 identified alleles, 61% were alleles shared by all 4 assessed breeds. The highest number of common alleles was noted in TGLA53 (11 out of 15 identified), the lowest in ILSTS005 (2 out of 4), and in INRA035 (2 out of 5). Almost 13% were specific alleles, appearing only in a particular breed. Most of them were found in WB and PR breed; 10 in each.

In the case of the whiteback breed it was: 159 bp in locus ETH225; 182 bp and 184 bp in HEL13; 182 bp and 194 bp in ILSTS005; 283 bp in ILSTS006; 145 and 147 bp in INRA005; 95 and 105 bp in TGLA227 and polish red: 171 bp in BM1824; 122 bp in BM2113; 207 bp in ETH10; 153 bp in ETH225; 188 bp in ILSTS005; 205 bp in INRA023; 103 bp in INRA035; 142 bp in INRA037; 79 and 107 bp in TGLA227 (Table 2). The lowest and the highest H_o in a single locus were recorded in PR breed: in locus INRA035 it was 0.217, in locus ILSTS006 and INRA037 it was 0.900 (Table 3). Among other breeds the value fluctuated from 0.290 in INRA035 to 0.890 in TGLA227 within WB; from 0.300 in INRA035 to 0.860 in BM2113 within PBW, and from 0.451 in ILSTS005 to 0.882 in HEL9 within PHF. The average value for this marker fluctuated from 0.626 within PBW breed to 0.699 within PR breed.

In the case of expected heterozygosity, the lowest value (0.418) was noted in locus INRA035 in PR breed and the highest (0.876) in locus TGLA53 in WB breed. Mean He, calculated for all 24 markers, was the highest in WB breed (0.711), and the lowest in PBW breed (0.677). Although the Hardy-Weinberg equilibrium test proved that in WB, PC and PBW breeds deviation from the equilibrium was only noted in one locus INRA035 ($P<0.05$), the contemporary research results fail to explain this phenomenon unequivocally. The highest polymorphism information content was determined in WB breed and PR breed in locus TGLA53 (0.859 and 0.842). In PBW cattle population the highest value was recorded in BM2113 (0.851), and within PHF, in TGLA227 (0.851).

The highest genetic diversity was revealed within PR and PHF breeds with the estimated genetic distance D_A (according to Nei, 1978) of 0.103 for both breeds. In the case of WB and PBW breeds, their differentiation from PHF breed was lower and was recorded at the level between 0.057 to 0.0663. A graphic view of the received genetic distance values is a phylogenetic tree calculated with the use of N-J method. WB and PR were included in the same group. Separate branches belonged to PBW and PHF breeds (Figure 1).

DISCUSSION

A high genetic diversity within WB and PR breeds was observed. The number of alleles identified within WB breed (189) and PR breed (178) was very similar to the number obtained for those breeds by Żurkowski et al.

Table 1. Characteristics of microsatellite loci selected for the research.

Number	Locus	Chromosome	Primer sequence	Annealing temp. (°C)	Genbank (Accession numer)
1	BM1818	23	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	56	G18391
2	BM1824	1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	58	G18394
3	BM2113	2	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	58	M97162
4	CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	60	-
5	CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	58	-
6	ETH10	5	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	60	Z22739
7	ETH225	9	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	60	Z14043
8	HEL1	15	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	54	X65202
9	HEL5	21	GCAGGATCACTTGTTAGGGA AGACGTTAGTGTACATTAAC	52	X65204
10	HEL9	8	CCCATTCAGTCTTCAGAGGT CACATCCATGTTCTCACCAC	52	X65214
11	HEL13	11	TAAGGACTTGAGATAAGGAG CCATCTACCTCCATCTTAAC	52	X65207
12	ILSTS005	10	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	54	L23481
13	ILSTS006	7	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	54	L23482
14	INRA005	12	CAATCTGCATGAAGTATAAATAT CTTCAGGCATACCCTACACC	54	X63793
15	INTA023	3	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	52	X67830
16	INRA032	11	AAACTGTATTCTCTAATAGCTAC GCAAGACATATCTCCATTCCTTT	58	X67823
17	INRA035	16	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	58	X68049
18	INRA037	10	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	58	X71551
19	INRA063	18	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	58	X71507
20	SPS115	15	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCCTAGTTTGGCTGTG	58	X16451
21	TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	60	-
22	TGLA122	21	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	58	-
23	TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	58	-
24	TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	56	-

Table 2. Number of identified, common and specific alleles of assessed breeds

Locus	Number of identified allele					Common allele	Specific allele			
	WB	PR	PBW	PHF	Total		WB	PR	PBW	PHF
BM1818	7	6	7	6	7	258,260,262,264,266	-	-	-	-
BM1824	5	6	4	5	6	175,177,179,185	-	171	-	-
BM2113	10	11	9	8	11	126,128,134,136,138,140,142,144	-	122	-	-
CSSM66	10	10	10	8	11	179,181,183,185,187,189,193,197	-	-	199	-
CSRM60	7	7	5	5	7	93,97,99,101,103	-	-	-	-
ETH10	7	8	7	7	8	211,213,215,217,219,221,223	-	207	-	-
ETH225	9	9	7	7	10	137,141,143,147,149,151	159	153	-	-
HEL1	5	4	7	5	7	103,105,111,113	-	-	-	-
HEL5	8	7	7	5	8	155,157,163,165,167	-	-	-	-
HEL9	11	8	11	11	12	153,159,161,163,165,169,171	-	-	-	149
HEL13	7	5	3	3	7	188,190,192	182,184	-	-	-
ILSTS005	4	3	2	2	5	184,186	182,194	188	-	-
ILSTS006	9	7	7	7	9	287,289,293,295,297,301	283	-	-	-
INRA005	5	3	3	3	5	139,141,143	145,147	-	-	-
INRA023	9	8	7	7	10	201,207,209,211,215	-	205	-	-
INRA032	6	6	5	6	6	176,178,180,182,184	-	-	-	-
INRA035	5	5	4	4	7	99,101	-	103	-	-
INRA037	11	11	10	8	13	120,126,128,130,132,134,146	-	142	138	-
INRA063	5	6	6	3	6	177,179,181	-	-	-	-
SPS115	7	8	8	7	8	244,246,248,250,252,254,256	-	-	-	-
TGLA53	15	15	14	15	17	145,149,151,153,155,157, 159,161,167,169,171	-	-	-	-
TGLA122	10	9	11	11	14	150,154,156,158,166,168	-	-	172	170,180
TGLA126	5	5	5	5	5	119,121,123,125,127	-	-	-	-
TGLA227	12	11	9	10	15	81,83,87,89,91,93,97	95, 105	79,107	-	99
Total	189	178	168	158	214	131	10	10	3	4

Table 3. Observed heterozygosity (H_o) and expected heterozygosity (H_e), Hardy – Weinberg equilibrium test (HWE) and the polymorphism information content (PIC) of assessed cattle breeds.

Locus	Whitebacks				Polish Red				Polish Black-and -White				Holstein-Friesian Black-and-White variety			
	H_o	H_e	HWE	PIC	H_o	H_e	HWE	PIC	H_o	H_e	HWE	PIC	H_o	H_e	HWE	PIC
BM1818	0.660	0.687	ns	0.635	0.600	0.548	ns	0.506	0.580	0.726	ns	0.506	0.627	0.725	ns	0.667
BM1824	0.700	0.738	ns	0.686	0.683	0.746	ns	0.694	0.804	0.747	ns	0.691	0.667	0.762	ns	0.710
BM2113	0.770	0.841	ns	0.816	0.783	0.855	ns	0.830	0.860	0.875	ns	0.851	0.765	0.820	ns	0.787
CSSM66	0.770	0.853	ns	0.831	0.833	0.821	ns	0.793	0.700	0.734	ns	0.698	0.843	0.794	ns	0.758
CSRM60	0.810	0.773	ns	0.740	0.717	0.810	ns	0.777	0.740	0.731	ns	0.678	0.784	0.735	ns	0.690
ETH10	0.700	0.720	ns	0.679	0.667	0.691	ns	0.639	0.740	0.703	ns	0.647	0.706	0.706	ns	0.668
ETH225	0.700	0.744	ns	0.703	0.850	0.844	ns	0.816	0.660	0.683	ns	0.641	0.765	0.770	ns	0.728
HEL1	0.640	0.653	ns	0.585	0.767	0.738	ns	0.683	0.600	0.651	ns	0.577	0.608	0.599	ns	0.508
HEL5	0.660	0.722	ns	0.678	0.750	0.805	ns	0.771	0.720	0.687	ns	0.627	0.741	0.529	ns	0.466
HEL9	0.760	0.807	ns	0.786	0.750	0.730	ns	0.669	0.780	0.840	ns	0.813	0.822	0.823	ns	0.791
HEL13	0.580	0.597	ns	0.537	0.667	0.673	ns	0.603	0.560	0.608	ns	0.535	0.608	0.532	ns	0.416
ILSTS005	0.430	0.447	ns	0.355	0.492	0.444	ns	0.357	0.500	0.481	ns	0.363	0.451	0.477	ns	0.361
ILSTS006	0.800	0.790	ns	0.753	0.900	0.783	ns	0.740	0.780	0.800	ns	0.759	0.647	0.696	ns	0.630
INRA005	0.520	0.598	ns	0.532	0.567	0.496	ns	0.399	0.500	0.488	ns	0.432	0.549	0.525	ns	0.444
INRA023	0.660	0.786	ns	0.755	0.817	0.772	ns	0.734	0.620	0.787	ns	0.748	0.706	0.791	ns	0.750

Table 3. Contd.

INRA032	0.590	0.687	ns	0.650	0.450	0.520	ns	0.488	0.500	0.718	ns	0.674	0.647	0.702	ns	0.652
INRA035	0.290	0.551	***	0.496	0.217	0.418	***	0.375	0.300	0.429	***	0.379	0.471	0.520	ns	0.410
INRA037	0.630	0.692	ns	0.656	0.900	0.827	ns	0.801	0.540	0.613	ns	0.583	0.784	0.712	ns	0.672
INRA063	0.490	0.603	ns	0.532	0.683	0.633	ns	0.576	0.440	0.491	ns	0.449	0.549	0.551	ns	0.460
SPS115	0.550	0.597	ns	0.560	0.683	0.735	ns	0.701	0.560	0.603	ns	0.574	0.549	0.699	ns	0.662
TGLA53	0.770	0.876	ns	0.859	0.850	0.863	ns	0.842	0.680	0.787	ns	0.763	0.510	0.862	ns	0.839
TGLA122	0.800	0.827	ns	0.800	0.700	0.746	ns	0.709	0.620	0.710	ns	0.678	0.608	0.812	ns	0.779
TGLA126	0.620	0.647	ns	0.584	0.583	0.558	ns	0.485	0.500	0.563	ns	0.511	0.686	0.704	ns	0.641
TGLA227	0.890	0.840	ns	0.815	0.867	0.834	ns	0.808	0.720	0.800	ns	0.765	0.745	0.874	ns	0.851
-	0.658	0.711	-	0.667	0.699	0.704		0.629	0.627	0.677	-	0.659	0.662	0.696	-	0.639

ns, non significant; ***significant when is P<0.05.

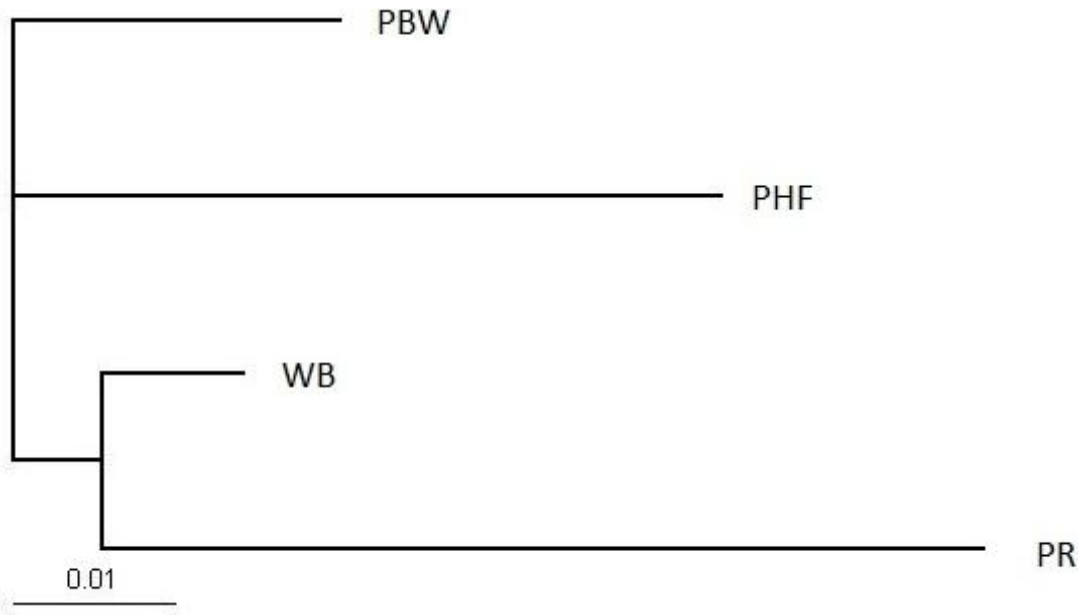

Figure 1. Phylogenetic tree of genetic distance D_A for assessed cattle breeds.

(2004); 171 alleles in WB and 181 in PR. Similarly, the number of 168 alleles was close to the number obtained by Lubieniecka et al. (2001); 160 alleles. Among all cattle breeds, locus TGLA53 turned out to be the most polymorphic because the number of alleles identified in WB, PR, and in the control group PHF was 15 and within PBW it was 14. High polymorphism of this locus in 7 European cattle breeds (including Polish Red) was pointed out by Czerneková et al. (2006) (17 alleles) and also by Grzybowski and Prusak (2004) (13 alleles in 9 European cattle breeds).

The average number of alleles in a single locus for all analyzed breeds was 8.91 (Table 1). Native breeds were characterized by a considerably higher number of identified alleles in a single locus in relation to the control group [WB (7.87), PR (7.41), PBW (7.00) and PHF Black-and-White variety (6.58)]. The analysis of the Polish Red breed genetic diversity, conducted by Grzybowski and Prusak (2004) and based on 26 microsatellite markers, displayed the mean of 7.42 alleles in a locus. The research by Czerenková et al. (2006), conducted on the basis of 11 basic microsatellite loci related to this breed, displayed the presence of 8.45 alleles in a locus. In the case of WB breed, the number of alleles in a single locus varied from 4 in ILSTS005 to 15 in TGLA53; on average 7.87 allele in a locus. The higher average number of alleles was noted in one of the first research concerning genetic identity of this breed (8.3), where only 11 STR were taken into consideration; BM1824, BM2113, ETH3, ETH10, ETH225, INRA 023, SPS115, TGLA53, TGLA122, TGLA126 ORAZ TGLA227 (Litwińczuk et al., 2006).

Within 214 identified alleles, 131 (61.2%) were common for all breeds (a mean of 5.46 common alleles per locus). The greatest number of common alleles was noted in locus BM2113 (8 out 11 identified). In comparison, Grzybowski and Prusak (2004) observed 81 (29.3%) common alleles located in 26 microsatellite markers in 9 cattle breeds (including Polish Red).The highest number of specific alleles was identified in WB and PR breeds. The presence of specific alleles in a particular breed constitutes a specific gene pool and proves its genetic distinct feature. Identification of shared and specific alleles in assessed breeds may be vital in determining genes responsible for particular biological characteristics.

Heterozygosity, which mean value according to Takezaki and Nei (1996) should be contained within 0.3 to 0.8 which proves the markers' usefulness in the genetic diversity assessment. The results obtained in each assessed breed were within the given rage. High values of this parameter in the three assessed populations included in genetic resources conservation programme indicate their high genetic diversity, particularly WB breed and PR breed, with expected heterozygosity of 0.711 and 0.704, respectively. The phenomenon was further confirmed by the highest number of identified alleles (189 and 178), and the highest number of specific alleles (10 in each). In 24 selected microsatellite loci, BM2113, TGLA53, TGLA122 and TGLA227 were characterized by the highest level of polymorphism and heterozygosity. Similarly, high values for these loci were obtained for WB breed, PR breed or PBW breed by Litwińczuk et al. (2006), Żurkowski et al. (2004) and Radko et al. (2005).

The obtained heterozygosity results were relatively high in relation to other cattle breeds raised worldwide, for example, Indian breeds: Deoni (0.59) (Mukesh et al., 2004), Kherigarh (0.574) (Pandey et al., 2006), and also 5 Swiss cattle breeds (0.60 to 0.69) (Schmid et al., 1999), or 7 Italian breeds (0.60 to 0.68) (Del Bo et al., 2001). The highest values of genetic distance between Polish Red and Holstein-Friesian breeds result from their origin and their place in taxonomy. Similarly to the first research on WB breed genetic diversity carried out by Żurkowski et al. (2004) on the basis of 24 microsatellite loci, the obtained values of genetic distance for current populations of WB and PR breeds indicate lower genetic distance between PR and WB breeds in comparison with the two remaining ones. Recurrence of this result proves that the two oldest breeds (PR and WB) have a unique pool of genes, which makes them indeed special in terms of biodiversity of farm animals.

Conclusion

WB and PR cattle constitute specific banks of genes which were eliminated during breeding practices in other high productive breeds. Among these breeds, in 24 assessed loci, 20 to 30 more genes were displayed as having relation to PHF breed, highly popular worldwide. Those genes may be connected with such traits as longevity, fertility or production of milk with more favourable nutrition properties and higher technological usefulness. The obtained results concerning genetic diversity of 3 Polish cattle breeds included in genetic resources conservation programme can be used in the future to monitor changes within these populations and to determine their independence from breeds of similar phenotype.

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