Animal Science Papers and Reports vol. 22 (2004) no. 1, 45-56
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Genetic variation in nine European cattle breeds as determined on the basis of microsatellite markers. III. Genetic integrity of the Polish Red cattle included in the breeds preservation programme

Grzegorz Grzybowski, Beata Prusak

Polish Academy of Sciences Institute of Genetics and Animal Breeding,
Jastrzębiec, 05-552 Wólka Kosowska, Poland

(Received January 5, 2004; accepted February 27, 2004)

On the basis of DNA microsatellite polymorphism at 26 *loci* tested within the European Concerted Action AIRE 2066 for the Analysis of Genetic Diversity to Preserve Future Breeding Options, a determination was made of the genetic structure of 147 Polish Red (PR) cattle, included in the National Rare Livestock Breeds Preservation Programme (NRLBPP). The examined PR cattle population was characterized by a high genetic variation (a total of 193 alleles identified, H_o = 0.695, H_e = 0.703, mean number of alleles per *locus* = 7.4). An analysis of the genetic distance (D_{ps}), including information on the presence (or absence) in the genome of alleles specific for the breed, confirmed that 80% of PR animals included in the NRLBPP comprised a separate genetic group, differing from populations of other European cattle breeds. The results show the uniqueness of the gene pool of PR cattle included in the NRLBPP. Despite the crossing with other breeds widely applied in the past, the present PR material does, to a considerable degree, remain genetically distinct. Thus, it is anticipated that basing on the existing preserved population the reconstruction of a pure, or almost pure PR cattle can be achieved.

KEY WORDS: cattle / genetic variation / microsatellite markers / preservation programme

Poland is a signatory of the programme for protection of genetic resources in agriculture [Anonymous 1996b], which aims at the reconstruction of native animal breeds, characterized by a high potential as regards adaptation traits. Beside Polish Whitebacked cattle, the restitution of which is still at a initial stage [Anonymous 1998, 2003], the National Rare Livestock Breeds Preservation Programme (NRLBPP) includes Polish

Red (PR) cattle. The programme is conducted on a total of about 150 cows, maintained in two preserve herds and with the use of stored bulls' semen, and embryos.

Basing on the polymorphism of DNA microsatellite *loci*, selected for analyses within the European Concerted Action AIRE 2066 for the Analysis of Genetic Diversity for Preserve Future Breeding Options [Anonymous 1996a], studies were undertaken to determine the genetic structure of certain European cattle breeds. The results already published as Part I of this study [Lubieniecka *et al* 2001] presented the degree of genetic within-breed variation in relation to the Angler, German Simmental, Brown Swiss, Swiss Simmental, Holstein and Eringer cattle. Against this background, the DNA microsatellite polymorphism was characterized in Polish Red (PR) cattle preserved population, as well as in Polish Black-and-White (PBW) and Polish Red-and-White (PRW) cattle. Moreover, in Part II [Grzybowski and Prusak 2004] a determination was made of the scale of the potential gene migration (N_em) between these breeds and, on the basis of the D_A and D_{SW} genetic distances, also of the topology of phylogenetic trees for the populations tested.

The present Part III aimed at determining the proportions of alleles common for the nine breeds mentioned and on this basis at analysing the genetic uniqueness of the population of PR cattle included in the NRLBPP.

Material and methods

The 147 Polish Red cows used in this study belong to two, geographically distant NRLBPP groups. One, comprising 48 animals (north group), is maintained in north-east Poland at the Research Station for Ecological Agriculture and Preserve Animal Breeding, Popielno, belonging to the Polish Academy of Sciences. The remaining 99 cows (south group) are maintained in small farms in the south of Poland, in the Carpathian foot-hills. The number of alleles and their frequency was identified separately for the north and south group, but when comparing with other breeds both groups were treated jointly (data pooled).

The selection criteria and description of the 26 DNA microsatellite *loci* used for a comparative analysis of the variation of cattle breeds considered, as well as the molecular procedure for allele identification, are presented in Part I of this study [Lubieniecka *et al* 2001]. Information about the number of the animals tested, as well as the results referring to the number and size of alleles identified in Angler, German Simmental, Brown Swiss, Swiss Simmental, Holstein and Eringer breeds, were obtained from a website database [Anonymous 1996a]. Respective information referring to the PBW and PRW cattle came from earlier studies conducted by Lubieniecka [2001].

The frequency of microsatellite alleles was estimated using the GENEPOP software [Raymond and Rousset 1995]. The heterozygosity observed (H_o) at each *locus* and for all populations was calculated as a per cent of heterozygous genotypes. In turn, the values for expected heterozygosity (H_e) were calculated according to the formula presented by Ott [1992].

The genetic difference between the PR cattle and other breeds was presented as the D_{ps} genetic distance, calculated on the basis of the occurrence of common alleles [Bowcock *et al.* 1994]. The software MICROSTAT was used for calculations [Minch 1998].

Results and discussion

Table 1 presents the number, size and frequency of DNA microsatellites identified in the north group, south group and in the whole PR cattle population examined. In total 193 alleles were identified, *i.e*. a mean of 7.4 allele per *locus*. When comparing results obtained separately for the north and south group (177 alleles with a mean of 6.8 allele per *locus* for the north group and 181 alleles with a mean of 7.0 allele per *locus* for the south group), with those recorded for the whole population (data pooled), no significant inter-group differences were observed. The allelic ladders for certain *loci* were irregular, *i.e*. the size of alleles did not increase regularly by each repeat unit. Between the north and south group considerable differences were found in the frequency of individual alleles, and a comparatively large number of unique ("private") alleles was observed (16 in the north and 12 in the south group) – Table 1. From the moment the programme for preservation of the genetic resources of PR cattle was initiated, the reproduction within the preserved population was based on semen stored in a central semen bank. Thus, the differences in the allele number and frequency observed between the north and south group, are probably a reflection of the earlier situation, when those groups remained in a geographic isolation, and cows were mated naturally to local bulls. The greater overall number of alleles identified in the south group and the higher number of "private" alleles observed in the north group, may also be related to the number of animals tested (the south group was almost twice the size of the north group). Also the observed incidence of irregular allelic ladders may be explained by the comparatively low number of animals tested. All in all, the PR cattle from the preserve breeding programme presented a very high polymorphism of DNA microsatellites. This is testified by the considerable number of alleles identified, the high mean number of alleles at one *loci* and the high degree of observed (H_o) and expected (H_e) heterozygosity. Table 2 presents a comparison of H_o and H_e between breeds considered. All the breeds included showed a much smaller level of DNA microsatellite polymorphism than the PR cattle. Only Anglers demonstrated a degree of polymorphism similar to that observed for the PR cattle: 182 alleles recorded in the population and a mean of 7.0 allele found per one *locus*. For comparison, in an analysis of the DNA microsatellite polymorphism, performed on a *loci* panel identical with that used in this study for testing PR cattle, Schmid *et al*. [1999] observed in Simmental, Holstein, Brown Swiss, Herens and Evolenard cattle 5.6, 6.5, 5.7, 6.0 and 4.8 alleles per *locus*, respectively. In turn, Martin-Burriel *et al*. [1999], analysing the microsatellite variation for six Spanish cattle breeds, observed a mean of 8.6 alleles, while MacHugh *et al*. [1997], analysing the microsatellite alleles in 20 populations of humped (zebu) and humpless cattle, recorded a mean of 8.4 alleles per *locus*. However, the results of numerous studies on the DNA microsatellite poly-

Table 1. Polymorphism of DNA microsatellites in Polish Red (PR) cattle

Locus	Allele size (bp)	Frequency: northern group	southern group	total
BM2113	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
ETH10	[illegible]	–	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
SPS115	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
TGLA53	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
INRA023	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	–	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	–	[illegible]	[illegible]
TGLA122	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
TGLA126	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
BM1824	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
ETH225	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	–	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
ETH3	[illegible]	–	[illegible]	[illegible]
	[illegible]	[illegible]	–	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
CSRM60	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
ILSTS005	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	–	[illegible]	[illegible]
	[illegible]	–	[illegible]	[illegible]

Table 1. Continued

Locus	Allele size (bp)	Frequency: northern group	southern group	total
INRA005	139	0.146	0.040	0.075
	141	0.542	0.596	0.578
	143	0.312	0.364	0.347
BM1818	252	–	0.025	0.017
	256	0.052	–	0.017
	258	–	0.020	0.014
	260	0.177	0.207	0.197
	262	0.219	0.167	0.184
	264	0.500	0.581	0.554
	266	0.042	–	0.014
	272	0.010	–	0.003
HEL1	101	–	0.025	0.010
	103	0.115	0.061	0.078
	105	0.438	0.505	0.483
	107	0.020	0.005	0.010
	109	0.042	0.030	0.034
	111	0.208	0.061	0.109
	113	0.177	0.313	0.276
HEL5	151	0.042	0.051	0.048
	153	0.188	0.283	0.252
	155	0.010	0.116	0.082
	159	–	0.010	0.007
	161	0.010	–	0.003
	163	0.094	0.106	0.102
	165	0.208	0.192	0.197
	167	0.438	0.242	0.306
	169	0.010	–	0.003
CSSM66	179	0.021	0.010	0.014
	181	0.073	0.040	0.051
	183	0.354	0.566	0.497
	185	0.083	0.121	0.109
	187	0.115	0.046	0.068
	189	0.146	0.126	0.133
	191	0.115	0.046	0.068
	193	0.083	0.020	0.041
	197	–	0.025	0.017
	199	0.010	–	0.003
ILSTS006	287	0.052	0.040	0.045
	289	0.245	0.227	0.233
	291	0.202	0.192	0.195
	295	0.074	0.177	0.144
	297	0.352	0.308	0.322
	299	–	0.051	0.034
	301	0.074	0.005	0.027

Locus	Allele size (bp)	Frequency: northern group	southern group	total
INRA037	[illegible]	[illegible]	0.247	0.204
	[illegible]	[illegible]	0.025	[illegible]
	[illegible]	[illegible]	–	0.007
	[illegible]	[illegible]	0.086	0.075
	[illegible]	0.146	0.146	0.146
	[illegible]	0.031	0.071	0.058
	[illegible]	0.500	0.278	0.350
	[illegible]	[illegible]	0.076	0.071
	[illegible]	0.031	0.025	[illegible]
	[illegible]	–	0.005	0.003
	[illegible]	[illegible]	0.025	0.014
	[illegible]	–	0.021	0.014
	[illegible]	[illegible]	0.025	0.017
INRA032	[illegible]	0.011	0.045	0.034
	[illegible]	[illegible]	0.106	0.109
	[illegible]	0.666	0.741	0.714
	[illegible]	0.156	0.082	0.105
	[illegible]	0.052	0.025	0.034
	[illegible]	[illegible]	–	0.003
[illegible]	[illegible]	0.104	0.056	0.071
	[illegible]	0.167	[illegible]	0.279
	[illegible]	[illegible]	0.045	0.054
	[illegible]	0.396	0.348	0.364
	[illegible]	[illegible]	0.020	0.014
	[illegible]	[illegible]	–	0.003
	[illegible]	0.052	0.020	0.024
	[illegible]	0.156	0.187	0.177
	[illegible]	[illegible]	0.020	0.014
ETH185	[illegible]	[illegible]	0.399	0.395
	[illegible]	0.177	0.051	0.092
	[illegible]	[illegible]	0.051	0.041
	[illegible]	[illegible]	0.116	0.133
	[illegible]	0.240	0.103	[illegible]
	[illegible]	[illegible]	0.020	0.017
	[illegible]	[illegible]	0.020	0.014
	[illegible]	[illegible]	0.020	[illegible]
INRA063	[illegible]	0.500	0.561	0.541
	[illegible]	[illegible]	0.338	0.330
	[illegible]	0.031	0.021	0.024
	[illegible]	[illegible]	–	0.003
	[illegible]	0.125	0.045	0.071
	[illegible]	[illegible]	[illegible]	0.022

Table 1. Continued.

Locus	Allele size (bp)	Frequency		
		northern group	southern group	total
HEL9	141	0.010	0.030	0.024
	147	0.021	0.011	0.014
	149	0.010	0.030	0.024
	151	–	0.081	0.054
	153	0.646	0.429	0.500
	155	0.021	0.010	0.014
	159	0.021	0.081	0.061
	161	0.052	0.106	0.088
	163	0.021	0.045	0.037
	167	0.104	0.056	0.071
	169	0.094	0.116	0.109
	175	–	0.005	0.003
HEL13	188	0.427	0.369	0.388
	190	0.073	0.242	0.187
	192	0.396	0.283	0.320
	194	0.104	0.106	0.105
ETH152	195	0.156	0.167	0.163
	197	0.594	0.611	0.605
	199	0.156	0.106	0.122
	201	0.031	0.071	0.058
	203	0.021	0.015	0.017
	207	0.042	0.030	0.034

Table 2. Polymorphism of DNA microsatellites in nine European cattle breeds

Breed	Alleles identified	Mean number of alleles	Number of unique[1] alleles	H_o	H_E
Angler	182	7.0	14	0.677	0.702
German Simmental	177	6.8	13	0.634	0.666
Brown Swiss	148	5.7	6	0.664	0.666
Swiss Simmental	144	5.5	1	0.595	0.598
Holstein	168	6.5	4	0.679	0.687
Hringar	154	5.9	1	0.636	0.628
Polish Red	193	7.4	5	0.695	0.703
Polish Black-and-White	160	6.2	1	0.679	0.673
Polish Red-and-White	171	6.6	4	0.701	0.697
All breeds	276	10.6	49	0.662	0.669

[1] "Private" alleles.

morphism in different cattle breeds, e.g. Arranz *et al*. [1996] and Canon *et al*. [2000], cannot be directly compared with the results of analyses conducted according to the criteria accepted in the European programme [Anonymous 1996a], principally due to the different *loci* panels tested and the different procedure used for allele identification.

As a rule, the mean number of alleles at one *locus* is considered to be a good indicator of the degree of genetic variation when the population is at equilibrium between mutation and genetic migration, and the sizes of populations compared are similar. According to MacHugh *et al*. [1997], this last condition does not necessarily need to be met, as the degree of genetic variation estimated on the basis of the mean number of alleles occurring at one *locus* is a better reflection of the history of the population than would appear from the number of animals examined.

Table 3 presents the DNA microsatellite alleles common for PR cattle and eight German and Swiss breeds, as well as unique alleles occurring only in a given breed (known as a breed's "private" alleles). From the overall pool of 276 alleles identified, 81 (29.3%) proved to be common for all breeds (a mean of 3.1 common alleles per *locus*). The greatest numbers of common alleles were observed at *locus* BM2113 (6 out of 10 identified), TGLA53 (6 out of 17 identified), and HEL5 (5 out of 11 identi-

Table 3. Number of alleles identified in 26 DNA microsatellite *loci* in nine European cattle breeds

Locus	Angler	GS	BS	SS	Holstein	Friesian	PR	PBW	PRW	Total
BM1818	5	9	6	6	6	6	8	7	4	13
BM1824	9	6	5	4	5	5	5	4	3	9
BM2113	8	8	8	7	7	7	8	6	6	10
CSRM60	7	6	6	6	6	5	7	6	6	10
CSSM66	10	10	9	9	10	7	10	8	9	14
ETH10	5	4	4	3	7	4	8	7	7	8
ETH152	8	7	4	4	6	5	6	6	6	9
ETH185	9	7	7	4	8	6	8	8	9	11
ETH225	6	7	6	6	7	6	8	6	6	9
ETH3	6	6	4	6	6	6	9	7	7	11
HEL1	7	5	6	6	7	6	7	4	5	9
HEL13	4	3	4	3	3	4	4	4	5	6
HEL5	8	8	7	7	6	6	9	6	5	11
HEL9	12	6	5	4	9	8	12	9	12	18
ILSTS005	2	2	2	2	2	2	4	2	2	4
ILSTS006	7	7	5	6	5	6	7	6	7	10
INRA005	4	3	3	3	3	3	3	3	2	4
INRA023	7	9	7	7	7	10	9	7	8	13
INRA032	6	5	4	5	5	5	6	6	6	7
INRA037	9	9	6	7	6	8	13	7	9	15
NRA063	4	9	5	6	4	3	6	4	5	10
MM12	5	11	6	6	5	6	9	6	5	11
SPS115	5	5	6	4	7	8	6	4	6	9
TGLA122	8	9	9	8	12	8	8	10	14	20
TGLA126	8	7	4	5	5	6	4	5	5	8
TGLA53	13	9	10	10	13	8	9	12	12	17
Total	182	177	148	144	168	154	193	160	171	276

GS – German Simmental; BS – Brown Swiss; SS – Swiss Simmental; PR – Polish Red; PBW – Polish Black-and-White; PRW – Polish Red-and-White.

fied). In turn, 49 alleles (17.8%) may be considered "private". The greatest numbers of such alleles were found at *loci* BM1818 (6 out of 13 identified) and HEL9 (5 out of 15 identified), and in German Anglers and German Simmentals (14 and 13, respectively). In the PR cattle five private alleles were identified.

The results presented here indicate that the DNA microsatellite polymorphism is so wide that the probability of finding two animals with identical genotypes at all the *loci* analysed is close to zero. Accepting this as a basis for determining genetic relations between breeds, the genetic distance D_{ps} as allele shearing distance [Bowcock *et al*. 1994] calculated on the basis of the proportion of common alleles was estimated between 20 randomly selected animals from each breed. Contrary to other values describing the genetic distance between populations, *i.e.* D_A and D_{SW} that were discussed in Part II of this study [Grzybowski and Prusak 2004], the D_{ps} distance indicates the genetic similarity between individuals within a population.

The comparison based on the values for distance D_{ps} is presented in Figure 1. The topology of the phylogenetic tree thus created covers 180 lines – 20 lines correspond to 20 individuals from each breed and are marked with the same colour. Their interrelations are characterized by the presence (or lack) of genetic links between breeds. Among the animals analysed, representing nine cattle breeds (marked with different colours) from Germany, Switzerland and Poland, only 29, *i.e*. 16% of all the animals tested, did not group with animals of the same breed. As result, the topology of the tree presents a characteristic image, manifested in the form of well-separated coloured sets. The image proves that the procedure used for estimating the D_{ps} genetic distance, based on the occurrence of microsatellite DNA alleles common for individual breeds, is a valuable tool for an objective estimation of genetic relations taking place between breeds, and in particular, renders it possible to estimate the effect of a given breed, introduced in the process of genetic upgrading of animals from other populations (what is of special significance for estimating the status of PR cattle in preserve breeding). In this context, the lack of a well-defined set for the PRW cattle, must be accepted as an important element of the topology of the D_{ps} tree. The PRW breed is located in a heterogenic set composed of several sub-groups, including also PBW and Holstein cattle. In this, genetically differentiated group (many-coloured in the Figure) are located 85% of PRW animals, almost all (95%) Holsteins and a small per cent of the PBW cattle. This indicates that there is a more pronounced genetic similarity between the Holstein and PRW than between the Holstein and PBW breed. Moreover, the topology of the tree presented indicates that Swiss breeds (Brown Swiss, Eringer and Swiss Simmental) are characterized by a high degree of genetic consolidation – all the animals from each of those breeds grouped together (shown as well-defined sets marked with green, violet and grey-yellow, respectively). Also the Angler cattle may be considered highly consolidated genetically, as all the animals of the breed are grouped in the same set. A similar situation is observed for the population of German Simmentals – almost all animals (19 out of the 20 tested) group together. In the case of PR cattle, with their set placed beside that for the Angler breed, out of the 20 animals 16 (80% of the total)

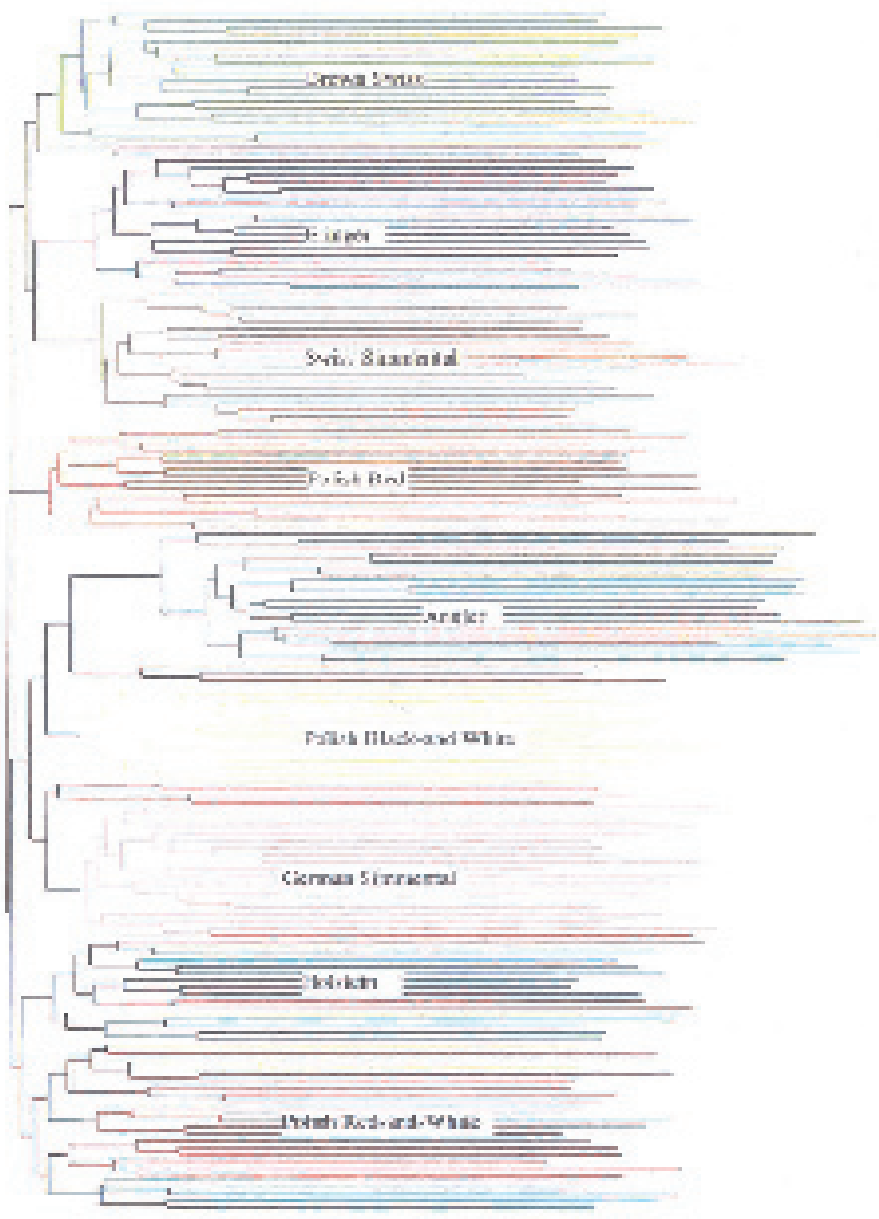

Fig. 1. Genetic integrity of the Polish Red cattle included in the breed preservation programme, shown against the background of selected European breeds (N-J tree was constructed by means of D_{ps} distance).

grouped together. The remaining PR animals grouped together with the Anglers or German Simmentals. The results obtained indicate that although the PR cattle, covered by the preserve breeding programme, are not as strongly genetically consolidated as are Swiss breeds, they are still clearly genetically distinct. It is worth emphasising, that although the PR cattle set was located in close neighbourhood of the single coat-colour red Angler cattle, it „grows out" of a single branch joined with the wide basis common for all breeds. This basis may be accepted as the level reflecting the specificity of the *Bos taurus* species. Thus, the tree topology shows that in the genealogy of the PR cattle there are no transitional branches, what means that this material reaches with its roots directly to the *Bos taurus* level.

The PR breed originates from the *brachyceros* cattle, appearing since pre-historic times in Central Europe and Scandinavia. Adametz [1901] described it even as *Bos brachceros polonicus*. The beginnings of its organized breeding on Polish territory fall to the last years of the XIX century. Due to the military operations and territorial changes that took place after World War II the population of PR cattle decreased dramatically, but still in the years 1965-1966 constituted about 18% of the cattle kept in Poland [Szarek *et al*. 1993]. Since 1959 some PR cows were mated to Danish Red and, though to a small degree, also Jersey bulls [Szarek *et al*. 1993]. Simultaneously, into regions where PR cattle was traditionally maintained the Polish BW („Polish Friesian") breed was gradually introduced. Moreover, basing on earlier results obtained throughout the seventies, the PR cattle were upgraded by crossing with Angler breed. Although these data constitute only small, selected episodes from the history of the PR cattle, they confirm that this material was affected by numerous other breeds [Czaja *et al*. 1996].

In the present study it was possible to make a comparative analysis of the DNA microsatellite polymorphism between PR cattle and only several other breeds. Although the database with information on cattle DNA microsatellites contains data referring to almost a 100 breeds and varieties of cattle from all over the world [Anonymous 1996a], only in relation to six German and Swiss breeds the data proved comprehensive enough to be fully comparable with the 26 *loci* selected for an analysis of the PR breed. For this reason, introducing other breeds into the comparative analysis (for instance Danish Red, imported in the past for upgrading the PR cattle), would certainly enrich the information on the pool of DNA microsatellites occurring in the local population. When interpreting the results obtained hitherto, the fact that as much as 80% of animals from the PR preserve-breeding population grouped together can be considered the most significant. It seems to constitute a strong and direct genetic proof, based on objective comparisons using molecular techniques, that discussions about the extent to which other breeds participated in creating the contemporary population of PR cattle included in the preserve breeding programme may refer only to a small group with an exterior typical of the breed but having probably also genes of other cattle breeds. Thus, the results obtained indicate that the PR cattle included in the NRLBPP have to a considerable extent retained its specificity, and their gene pool remains unique. It seems all the more probable that with the use of existing two groups it will be possible

to reconstruct a pure, or almost pure Polish Red cattle.

REFERENCES

1. ADAMETZ L., 1901 – Studien über das Polnische Rotvieh. Wien.
2. ANONYMOUS, 1996a – CaDBase – Cattle Diversity DataBase – The Analysis of Genetic Diversity in Cattle to Preserve Future Breeding Options – European Concerted Action Project under Food, Agriculture and Agro-Industry Research (FAIR) programme. Roslin Institute, Edinburgh. http://www.ri.bbsrc.ac.uk/cdiv_www/
3. ANONYMOUS, 1996b – Domestic Animal Diversity Information System (DAD-IS) Database. Food and Agriculture Organization of the United Nations, http://dad.fao.org/en/Home.htm
4. ANONYMOUS, 1998 – Krajowa baza danych o zasobach genetycznych zwierząt gospodarskich (Domestic database on genetic resources of farm animals). In Polish. Krajowe Centrum Hodowli Zwierząt (National Animal Breeding Centre). Warsaw, Poland. http://eto.ihar.edu.pl/gene_bank/CSHZ/
5. ANONYMOUS, 2003 – Whitebacks, old Polish cattle breed (a leaflet). Department of Cattle Breeding, Agricultural University of Lublin. e-mail: zhbyd@ursus.ar.Lublin.pl
6. ARRANZ J.J., BAYON Y., SAN PRIMITIVO F.S., 1996 – Comparison of protein markers and microsatellites in differentiation of cattle populations. ***Animal Genetics*** 27, 415-419.
7. BOWCOCK A.M., RUIZ-LINARES A., TOMFOHRDE J., MINCH E., KIDD J.R., CAVALLI-SFORZA L.L., 1994 – High resolution of human evolutionary trees with polymorphic microsatellites. ***Nature*** 368, 455-457.
8. CANON J., ALEXANDRINO P., BEJA-PEREIRA A., BESSA I., CARLEOS C., CARRETERO Y., DUNNER S., FERRAND N., GARCIA D., JORDANA J., LOLOE D., SANCHEZ A., MOAZAMI-GOUDARZI K., 2000 – The use of microsatellites for measuring genetic diversity of European local beef cattle breeds for conservation purposes. Proceedings of the 27th International Conference on Animal Genetics, Book of Abstract D030. Minnesota, July 22-26, USA.
9. CZAJA H., ADAMIK P., TRELA J., STASZCZAK S., 1996 – Hodowla bydła polskiego czerwonego – jaka była, jest i będzie (The past, presence and future of the Polish Red cattle breeding). In Polish. Sympozjum Naukowe: Hodowla Bydła w Polsce, Historia i Przyszłość (Symposium on cattle breeding in Poland – history and future). September 12-13, Olsztyn, Poland.
10. GRZYBOWSKI G., PRUSAK B., 2004 – Genetic variation in nine European cattle breeds as determined on the basis of microsatellite markers. II. Gene migration and genetic distance. ***Animal Science Papers and Reports*** 22 (1), 37-44.
11. LUBIENIECKA J., 2001 – Struktura genetyczna wybranych ras bydła hodowanych w Polsce określona na podstawie polimorfizmu loci mikrosatelitów DNA (Genetic structure of selected cattle breeds bred in Poland as determined on the basis of the DNA microsatellite polymorphism). Thesis. In Polish. Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, Poland.
12. LUBIENIECKA J., GRZYBOWSKI G., LUBIENIECKI K., 2001 – Genetic variation in nine European cattle breeds as determined on the basis of microsatellite markers. I. Within-breed variation. ***Animal Science Papers and Reports*** 19(4), 249-264.
13. MACHUGH D.E., SHRIVER D.M., LOFTUS R.T., CUNNINGHAM P., BRADLEY D.G., 1997 – Microsatellite DNA variation and the evolution, domestication, and phylogeography of Taurine and Zebu Cattle (*Bos taurus* and *Bos indicus*). ***Genetics*** 146, 1071-1086.
14. MARTIN-BURRIEL I., GARCIA-MURO E., ZARAGOZA P., 1999 – Genetic diversity analysis of six Spanish native cattle breeds using microsatellites. ***Animal Genetics*** 30, 177-182.
15. MINCH E., 1998 – MICROSAT (version 1.5e). Standford University Medical Center, Stanford, CA.
16. OTT J., 1992 – Strategies for characterizing highly polymorphic markers in human gene mapping. ***American Journal of Human Genetics*** 51, 283-290.

17. RAYMOND M., ROUSSET F., 1995 – A population genetics software for exact tests and ecumenicism. ***Journal of Heredity*** 86, 248-249.
18. SCHMID M., SAITBEKOVA N., GAILLARD C., DOLF G., 1999 – Genetic diversity in Swiss cattle breeds. ***Journal of Animal Breeding and Genetics*** 116, 1-8.
19. SZAREK J., FELEŃCZAK A., CZAJA H., 1993 – Stan hodowli polskiego bydła czerwonego (pc) i jej perspektywy (Presence and perspectives of the Polish Red cattle breeding). In Polish. ***Problemy Zagospodarowania Ziem Górskich*** 36, 35-45.

Grzegorz Grzybowski, Beata Prusak

Zmienność genetyczna dziewięciu europejskich ras bydła określona na podstawie markerów mikrosatelitarnych. III. Odrębność genetyczna bydła polskiego czerwonego z hodowli zachowawczej

S t r e s z c z e n i e

Na podstawie polimorfizmu mikrosatelitów DNA w 26 *loci* wybranych do zunifikowanych analiz w europejskim programie ochrony zasobów genetycznych (*European Concerted Action AIRE2066 for the Analysis of Genetic Diversity to Preserve Future Breeding Options*), określono strukturę genetyczną bydła pc (PR) objętego programem hodowli zachowawczej oraz umiejscowiono ten materiał w drzewie filogenetycznym bydła. Wykazano, że na tle ras europejskich, badaną populację bydła pc charakteryzuje wysoka zmienność genetyczna (ogółem zidentyfikowano 193 allele, $H_o = 0{,}695$, $H_e = 0{,}703$, średnia liczba alleli w *locus* = 7,4). Analizy dystansu genetycznego (D_{ps}), w których wykorzystano informacje dotyczące obecności (lub nieobecności) w genomie tzw. alleli specyficznych dla rasy dowodzą, że 80% osobników rasy pc tworzy odrębną grupę genetyczną, odbiegającą od innych populacji bydła europejskiego. Jedynie mała grupa bydła pc grupuje się w drzewie filogenetycznym wspólnie z osobnikami rasy angler lub niemiecki simental. Wyniki wskazują na unikalność puli genów bydła pc objętego programem hodowli zachowawczej. Mimo licznych krzyżowań z innymi rasami, materiał ten w znacznym stopniu zachował swą rasową odrębność. Wydaje się więc możliwe, że korzystając z utworzonej stawki zwierząt (grupa północna i grupa południowa), uda się odtworzyć czystorasową bądź zbliżoną do czystorasowej, populację tego bydła.