

## Genetic variability among Polish Red, Hereford and Holstein-Friesian cattle raised in Poland based on analysis of microsatellite DNA sequences

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**Abstract.** Polymorphism of 11 microsatellite DNA loci was analysed in Polish Red (PR), Hereford and Holstein-Friesian (HF) cattle raised in Poland and genetic distance among these breeds was determined. At the 11 loci (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 and BM1824) analysed with automated DNA sizing technology, a total of 213 alleles were identified: 76 in PR, 76 in HF, and 61 in Hereford. All the microsatellite DNA markers showed high polymorphism. Polymorphism information content (PIC) calculated for each marker exceeded 0.5, except for the ETH3 locus in Hereford cattle (PIC=0.475), and heterozygosity (H) ranged from 54.1% to as much as 85.2%. The coefficient of genetic distance was 0.354 between PR and Hereford, 0.414 between HF and Hereford, and 0.416 between PR and HF cattle.

**Key words:** cattle, DNA microsatellites.

The analysis of allele distribution at highly polymorphic microsatellite loci can be used to monitor the genetic structure of populations and to detect changes in the frequency of genes due to breeding, which makes it possible to preserve the biological diversity of farm animals (Moazami-Goudarzi et al. 1997; Martín-Burriel et al. 1999; Maudet et al. 2002). This study was designed to analyse the polymorphism of 11 microsatellite loci in Polish Red (PR), Hereford and Holstein-Friesian (HF) cattle raised in Poland and to determine genetic distances among them.

The studies involved 165 head of Polish Red (51), Hereford (56) and Holstein-Friesian (58) cattle. Included in the investigation were blood samples of 20 PR heifers derived from 14 sires and different dams for each heifer, 20 PR bulls from 11 sires and different dams, and blood samples from 11 unrelated PR cows. Blood material from

Hereford animals originated from 36 cows being progeny of 19 sires and different dams, as well as 20 bulls from 8 sires and different dams. Samples representing HF cattle were taken from 32 bulls being progeny of 24 sires and different dams, as well as 26 unrelated cows.

Microsatellite polymorphism was analysed based on markers TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, and BM1824.

Genomic DNA was isolated from 40 µl of peripheral blood using proteinase K. Based on the isolated DNA, the 11 microsatellite loci were amplified by the polymerase chain reaction (PCR). Standard "Bovine PCR Typing Kit II" reagents by Applied Biosystems were used for PCR. PCR products were separated by electrophoresis in a 4% denaturing polyacrylamide gel on

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**Table 1.** Number and size range (base pairs) of alleles, Heterozygosity (H) and Polymorphism Information Content (PIC) for investigated microsatellite markers

Marker	Breed	No. of alleles	Size range (bp)	H	PIC
TGLA227	PR	8	77-103	0.824	0.803
	Hereford	6	83-97	0.752	0.711
	HF	8	81-103	0.832	0.815
BM2113	PR	8	121-141	0.830	0.808
	Hereford	7	125-139	0.818	0.801
	HF	6	125-141	0.779	0.743
TGLA53	PR	9	154-182	0.775	0.751
	Hereford	6	154-176	0.686	0.633
	HF	11	154-186	0.852	0.834
ETH10	PR	7	213-225	0.735	0.700
	Hereford	6	215-225	0.729	0.684
	HF	6	209-225	0.541	0.530
SPS115	PR	6	248-258	0.759	0.729
	Hereford	6	236-260	0.782	0.751
	HF	7	248-260	0.670	0.633
TGLA126	PR	6	115-125	0.612	0.570
	Hereford	6	115-127	0.653	0.612
	HF	6	115-123	0.611	0.550
TGLA122	PR	5	141-169	0.630	0.559
	Hereford	5	141-171	0.765	0.744
	HF	10	141-183	0.673	0.639
INRA23	PR	9	200-218	0.842	0.825
	Hereford	5	204-214	0.624	0.596
	HF	6	200-214	0.773	0.740
ETH3	PR	8	109-129	0.760	0.732
	Hereford	3	115-119	0.570	0.475
	HF	5	117-129	0.672	0.635
ETH225	PR	6	140-152	0.786	0.753
	Hereford	6	140-158	0.753	0.713
	HF	6	140-152	0.676	0.621
BM1824	PR	4	178-188	0.688	0.627
	Hereford	5	178-192	0.612	0.569
	HF	5	178-188	0.748	0.711

an ABI PRISM 377 laser sequencer (Applied Biosystems). Results of the electrophoretic separation were analysed with GeneScan 2.1 software and the alleles were sized with Genotyper 2.0 software. Based on the frequency of the identified alleles, the degree of heterozygosity (H) and the polymorphism information content (PIC) were calculated for each microsatellite marker studied. Genetic distances between the breeds were calculated according to Nei and Roychoudhury (1974). The unweighted pair group method (UPGM) was used to make the dendrogram of genetic distance.

The values of PIC and H were high (Table 1). PIC values calculated for each marker exceeded 0.5 except for the ETH3 locus in Hereford cattle (PIC = 0.475), while H ranged from 54.1% to as much as 85.2%. The highest polymorphism, for which PIC and H were calculated to be above 0.8, was observed at the TGLA227, BM2113 and INRA23 loci in PR, at BM2113 in Hereford, and at TGLA227 and TGLA53 in HF cattle. Lower polymorphism was displayed by the ETH10 locus (PIC = 0.530 and H = 0.541) in Holstein-Friesians, and by TGLA122 in Polish Red cattle

(PIC = 0.559 and H = 0.630). The lowest polymorphism in the present study was noted at the ETH3 locus (PIC = 0.475 and H = 0.570) in Hereford. Of the 3 alleles identified at this locus, 2 occurred with markedly higher frequencies in excess of 45%, as also reported by Stockburger et al. (1999) and Janik et al. (2002), where of the 4 alleles iden-

the genetic resources conservation programme. Another small population of cattle in Poland is formed by the single-purpose Hereford breed.

We conclude that different origins of the breeds under discussion, the population size and the purpose of breeding may account for the considerable differences shown between them.

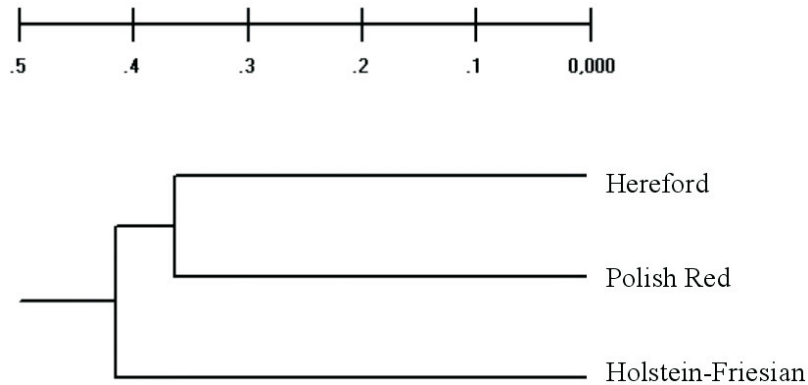


Figure 1. Dendrogram of genetic distance according to Nei and Roychoudhury (1972)

tified, 2 amounted to over 90%. At this stage of the investigations it is impossible to identify the reasons for the limited variability of the ETH3 locus in Hereford cattle.

The highest coefficient of genetic distance (0.416) was calculated between PR and HF cattle, with a similar distance separating HF from Hereford. The smallest differences were observed between PR and Hereford (0.364) (Figure 1). Microsatellite sequence-based analysis of genetic distance between different breeds of European cattle, including Hereford, was carried out by Edwards et al. (2003). Their studies showed that the coefficients of genetic distance between Hereford and 11 other analysed European breeds ranged from 0.123 for Jersey to 0.400 for Iceland cattle in Scandinavia. Relatively high coefficients of genetic distance calculated in the present study point to large differences between the investigated cattle breeds. These differences may be due to many factors. Polish Red cattle are the only native breed of dual-purpose cattle in Poland. After the Second World War, this breed was improved by crossing first with Danish Red Jersey and later with Angler. Today Polish Red cattle are threatened with extinction and have been included in

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