

Genetic distance between the Polish Red, Czech Red and German Red cattle estimated based on selected *loci* of protein coding genes and DNA microsatellite sequences

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The investigations covered the representatives of three red cattle breeds: Polish Red (PR, n = 65), Czech Red (CR, n = 54) and German Red (GR, n = 28). The allele frequency was analysed for five protein genes: *CSN3*, *LGB*, *GH*, *PRL* and *Pit-1* as well as 13 microsatellite sequences. In the populations of PR and GR cattle the frequencies of protein *loci* were similar, with a clearly marked domination of one allele. Only in the CR cattle, allele frequencies were similar for all the protein *loci* analysed (with the exception of *Pit-1*) with no clear dominance of any one allele. Out of the 13 microsatellite sequences analysed six showed a similar distribution of the allele frequencies in all three breeds, while the remaining ones were characterized by a wide variation. On the basis of the results obtained the genetic distance was estimated between breeds. The smallest genetic distance was observed between the PR and GR population. In turn, between the PR and CR as well as CR and GR populations, the genetic distance proved to be similar, the most similar being obtained on the basis of Nei method.

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In most countries the red cattle are currently included in preserve breeding programmes as in the past they have been eliminated from mass breeding by high producing and specialized breeds. Only after some years it proved that red cattle are ideal for breeding in the mountains or in regions lacking good pastures, where the use of contemporary, highly specialized breeds did not lead to the results expected. However, as a result of the earlier activities, the populations of individual red cattle breeds became very small and the inbreeding effects were strengthened. One of the solutions for reconstructing those breeds would be the use of their common gene pool, provided the genetic distance between them is small. This would make it possible to use animals from one population to increase the gene pool of another, especially one strongly threatened by the unfavourable inbreeding effect, and would not simultaneously lead to a change of traits characteristic of these cattle. The analysis of the genetic distance and similarity between breeds can be conducted on the basis of numerous genetic markers, including proteins and DNA microsatellite sequences.

In our geographical region (among others in Poland, the Czech Republic, Germany and Denmark) several red cattle breeds can still be found. Studies conducted on these cattle and including them in the gene resources protection programmes, render it possible to use the gene frequency to determine the relations between the breeds. The present study aimed at examining the relations between three red cattle breeds and determining their genetic similarity. The studies included the animals currently maintained under protective breeding, using the polymorphism of protein coding genes as well as DNA microsatellite sequences.

Material and methods

The material consisted of samples obtained from animals of three populations of red cattle included in gene resources protection programmes: Polish Red (PR, n = 65), Czech Red (CR, n = 54) and German Red (GR, n = 28).

The DNA was isolated from blood drawn from the jugular vein according to Gemmell and Akiyama [1996]. Next, using the PCR-RFLP method, the genotypes of five protein coding genes were determined: prolactin, κ -casein, β -lactoglobulin, growth hormone and Pit-1 [Medrano and Aguilar-Cordova 1990, Schlee *et al.* 1992, Woollard *et al.* 1994, Mitra *et al.* 1995] as well as 13 DNA microsatellite sequences [Moore *et al.* 1992, 1994, Bishop *et al.* 1994, Ferretti *et al.* 1994, Kossrek *et al.* 1994, Lang and Plante 1994]. On this basis the frequencies of individual alleles were determined and those were next used to estimate the genetic distance between populations. Using the software PHYLIP ver. 3.5c [Felsenstein 1993] the genetic distance was estimated according to the methods by: (i) Nei, (ii) Cavali-Sforza and (iii) Reynolds.

Results and discussion

The frequencies of individual protein alleles are presented in Table 1. In the populations of PR and GR cattle the frequencies of protein *loci* occurred similar, with a clearly marked dominance of one allele. Only in the CR sample the allele frequencies were similar for all the protein *loci* analysed (with the exception of Pit-1) with no clear dominance of any one allele.

Table 1. Allele frequency of the proteins across cattle populations analysed

Protein	Allele	Polish Red	Czech Red	German Red
Prolactin	<i>A</i>	0.869	0.559	0.909
	<i>B</i>	0.131	0.441	0.091
κ -casein	<i>A</i>	0.685	0.559	0.814
	<i>B</i>	0.315	0.364	0.174
	<i>E</i>	0.000	0.076	0.012
β -lactoglobulin	<i>A</i>	0.192	0.441	0.321
	<i>B</i>	0.808	0.559	0.679
Growth hormone	<i>L</i>	0.769	0.490	0.849
	<i>V</i>	0.231	0.510	0.151
<i>Pit-1</i>	<i>A</i>	0.137	0.049	0.079
	<i>B</i>	0.863	0.951	0.921

In the samples of PR and GR cattle prolactin was characterized by a significant frequency of allele *A* (0.869 and 0.909, respectively), while in the CR the frequency of alleles *A* and *B* was almost identical (0.559 and 0.441, respectively). In populations of Lithuanian cattle also a considerable dominance of allele *A* (from 0.77 to 0.97) over allele *B* [Skinkyte *et al.* 2005, Miceikiene *et al.* 2006] was observed. Another study of the polymorphism of prolactin [Klauzińska 2002] in the PR cattle demonstrated the frequency of allele *A* and *B* to reach 0.13 and 0.87, respectively.

At the κ -casein *locus* three alleles were found – *A*, *B* and *E*. However, allele *E* was characterized by a very low frequency: in the PR breed it did not occur at all and was most frequent in the CR cattle. κ -casein is a milk protein on the polymorphism of which numerous investigations have been conducted. In a study covering red cattle breeds and their crosses [Čitek *et al.* 1997a, Čitek *et al.* 2000, Juszczak *et al.* 2001, Kubarsepp *et al.* 2005, Miceikiene *et al.* 2006], the frequency of alleles *A*, *B* and *E* ranged from 0.461 to 0.93, from 0.07 to 0.481 and from 0.021 to 0.058, respectively.

The data available on β -lactoglobulin indicate a considerable dominance of allele *B* (from 0.559 to 0.808), though it was clearly lower in the sample of CR cattle. The studies conducted by Juszczak *et al.* [2001] on PR cattle also showed a dominance of allele *B* on a similar level (0.742). Similar results were reported for the Lithuanian Red breed in which allele *B* dominated (0.923) over the remaining alleles: *A* (0.068) and *C* (0.009) – Miceikiene *et al.* [2006]. In the Estonian Red cattle the frequency of allele *B* reached 0.63 [Kubarsepp *et al.* 2005].

At the growth hormone *locus* the occurrence of two alleles was observed – *L* and *V*, with a similar frequency in the PR and GR breeds, while the CR was characterized by an equal frequency of both alleles. In the Polish Black-and-White breed Dybus [2002a] and Zwierzchowski *et al.* [2002] reported allele frequencies (*L* from 0.81 to 0.87 and *V* from 0.13 to 0.19) similar to those observed in the present study for PR and GR cattle. Also Skinkyte *et al.* [2005] observed similar allele frequencies in the Lithuanian populations of Black-and-White (*L* – 0.70 and *V* – 0.30) and Red cattle (0.77 and 0.23, respectively). Studies by Sørensen *et al.* [2002] demonstrated a dominance of allele *L* over allele *V* in the Danish Holstein and Danish Red breeds (0.93 and 0.85, respectively), but in the Danish Jerseys the dominance was very small (allele *L* – 0.51, allele *V* – 0.49).

In all three populations analysed at *locus Pit-1* most often appeared allele *B* – its frequency amounting to 0.86 in the PR and 0.95 in the CR cattle. Allele *B* appeared most often also in the population of Polish Black-and-White cattle (about 0.75) – Zwierzchowski *et al.* [2002], Dybus *et al.* [2004] – and Holstein-Friesian in Italy (0.81 vs. 0.19 for allele *B* and *A*, respectively) – Renaville *et al.* [1997]. Dybus *et al.* [2003] demonstrated allele *B* (0.73) to appear most frequently also in the Limousine breed.

The frequency of microsatellite sequences is presented in Table 2. Six out of 13 sequences demonstrated a similar arrangement of allele frequencies within all three breeds examined. The *BM148* sequence was characterized by a different frequency of allele in every breed: in the PR dominant was allele 4, in the CR – allele 3 and in the German Red – allele 2. Microsatellite *RM012* showed a similar allele frequency in the population of CR and GR cattle in which allele 1 proved to be dominant, while in the PR animals allele 1 was definitely the most rare and allele 2 dominated. Microsatellite sequence *BOVIRBP* was characterized by a similar distribution of allele frequencies, allele 1 occurring exclusively in the population of PR cattle. An analysis of the *BTOBCAM* sequence demonstrated that in the CR breed allele 3 was clearly dominant, while allele 1 did not occur at all. In the PR cattle it was also allele 3 that was dominant, but in GR – allele 2. The frequency of the *BOVSEMRN* sequence alleles showed a similar distribution in all three breeds (allele 2 dominated), but in the CR cattle the frequencies showed less variation between alleles. In the case of the microsatellite sequence *SRC97*, allele 1 occurred most often, and in the CR breed it was the only allele identified.

Differences in allele frequencies in three analysed populations were highly significant for two protein *loci* – prolactin and growth hormone. For another two *loci* studied (κ -casein and β -lactoglobulin) the differences were significant. Only for the *locus Pit-1* no differences were identified between populations. For the four analysed microsatellite *loci* – *BM148*, *RM012*, *BTOBCAM* and *BOVSEMRN* – the differences between populations were found highly significant, for the *locus* *BM6438* were highly significant, while only significant for the *locus* *BM4621*. For the remaining microsatellites considered no significant differences in allele frequencies were identified.

Genetic distance between the three red cattle breeds

Table 2. Allele frequency of the microsatellites across cattle populations analysed

<i>Locus</i>	<i>Allele</i>	Polish Red	Czech Red	German Red
<i>BM6438</i>	1	0.073	0.119	0.104
	2	0.355	0.355	0.271
	3	0.161	0.421	0.344
	4	0.411	0.105	0.281
<i>CSSM004</i>	1	0.421	0.558	0.432
	2	0.254	0.154	0.307
	3	0.325	0.288	0.261
<i>IDVGA9</i>	1	0.057	0.079	0.039
	2	0.943	0.921	0.961
<i>BM6117</i>	1	0.203	0.218	0.193
	2	0.458	0.487	0.466
	3	0.339	0.295	0.341
<i>BM148</i>	1	0.028	0.040	0.012
	2	0.370	0.081	0.500
	3	0.185	0.757	0.179
	4	0.417	0.122	0.309
<i>RM012</i>	1	0.014	0.515	0.560
	2	0.843	0.394	0.440
	3	0.143	0.091	0.000
<i>BOVCASK35</i>	1	0.214	0.053	0.200
	2	0.357	0.405	0.425
	3	0.286	0.237	0.238
	4	0.143	0.303	0.138
<i>BOVIRBP</i>	1	0.063	0.000	0.000
	2	0.054	0.088	0.034
	3	0.884	0.912	0.966
<i>BTOBCAM</i>	1	0.051	0.000	0.068
	2	0.286	0.027	0.875
	3	0.663	0.973	0.057
<i>BOVPAI</i>	1	0.016	0.065	0.093
	2	0.984	0.935	0.907
<i>BM4621</i>	1	0.063	0.184	0.023
	2	0.828	0.684	0.733
	3	0.109	0.132	0.244
<i>BOVSEMNR</i>	1	0.214	0.224	0.300
	2	0.777	0.434	0.620
	3	0.009	0.342	0.080
<i>SRC97</i>	1	0.873	1.000	0.936
	2	0.063	0.000	0.043
	3	0.063	0.000	0.022

Tables 3 and 4 present the values obtained for the genetic distance. The smallest genetic distance, as estimated by Nei's method (Tab. 3), was recorded between the PR and GR populations (0.1121). Between PR and CR and between CR and GR those

Table 3. Genetic distance between populations as estimated according to Nei (above the diagonal) and Cavali-Sforza (below the diagonal)

Breed	Polish Red	Czech Red	German Red
Polish Red	–	0.1573	0.1121
Czech Red	0.1977	–	0.1577
German Red	0.1730	0.1815	–

Table 4. Genetic distance between populations as estimated according to Reynolds

Breed	Polish Red	Czech Red	German Red
Polish Red	–		
Czech Red	0.1810	–	
German Red	0.1589	0.1721	–

values were similar and amounted to 0.1573 and 0.1577, respectively. The values obtained for the genetic distance on the basis of the method by Cavalli-Sforza (Tab. 3) and Reynolds (Tab. 4) were higher, but here also the distance between the PR and GR breed appeared the smallest. The differences in the values obtained for the genetic distance between the remaining pairs of breeds were higher than when using Nei's method.

The genetic distance renders it possible to estimate the similarity between populations and determine their origin. Numerous studies on this field were conducted on different breeds of cattle [Kidd and Cavalli-Sforza 1974, Graml *et al.* 1986, Grosclaude *et al.* 1990, Moazami-Goudarzi *et al.* 1997].

The development of preserve breeding led to an increased interest in the genetic distance and search for similarities between endangered breeds or species. As European breeds of red cattle appear already only in small populations they become the objects of numerous studies. Rehout *et al.* [1998] analysed the genetic distance between five cattle populations: Czech Spotted, Czech Black-and-White (CBW), Czech Red (CR), German Black-and-White (GBW) and F1 crosses, the latter obtained from crossing the CR with Czech Spotted. The genetic distance was estimated on the basis of the polymorphism of five microsatellite *loci* and the growth hormone *locus*. Calculating the genetic distance according to three methods showed the least similarity between the GR and CR breeds and the F1 crosses, as also between the Czech Spotted and F1 crosses. The smallest distance was reported between the CBW, CR and Czech Spotted.

Čitek and Řehout [2001] conducted studies aiming at determining the genetic variation in different populations of cattle on the basis of an analysis of the polymorphism of 13 microsatellite *loci* and five protein *loci*. Six breeds were considered: Czech Spotted, CBW, GBW, Czech Red (CR), German Red (GR) and Polish Red (PR). A decreasing tendency was observed for the variation in the GR and PR cattle, principally in relation to the protein *loci*. An analysis was conducted of the probability of finding in two breeds the animals with identical genotypes. The lowest values were obtained for the following pairs: CR-GR, CR-Czech Spotted, CR-PR, while the highest for GBW-CBW, CR-CBW and PR-GR, what is compliant with the origin and forming of those breeds over the ages. That study demonstrated also a high risk occurring of genetic drift and inbreeding, which in small populations can limit the genetic diversity to a considerable degree and in a comparatively short time.

Lubieniecka [2001] analysed the polymorphism at 26 microsatellite *loci* in three Polish (PR, Black-and-White and Red-and-White) and six European (Angler, German Simmental, Eringer, Holstein, Swiss Simmental and Brown Swiss) cattle breeds. The highest genetic differentiation was demonstrated between the German and the PR and Polish Red-and-White cattle breeds. The values for the genetic distance obtained rendered it possible to separate three groups among the nine breeds analysed, consisting of breeds originating from one country. This may indicate a considerable effect of the geographical origin on the genetic structure of the population. Lubieniecka [2001] also analysed the genetic distance and demonstrated that 80% of PR animals group together, and only 20% are closer to those of other breeds (Black-and-White, Red-and-White, German Simmental). Indirectly, this indicates that 80% of animals in a preserve breeding is composed of “purebred animals”, while the remaining 20% contain genes of another breeds. All those data, as also the sample polymorphism at microsatellite *loci* indicate that the PR breed, despite a limited population and the earlier conducted work on its “improvement” through crossing with other breeds, still retains its genetic specificity.

Grzybowski and Prusak [2004ab] analysed the gene transfer between populations of nine European cattle breeds, including the PR, and estimated the genetic distance between them on the basis of the polymorphism at 26 microsatellite *loci*. It was observed that the greatest transfer of genes took place between breeds originating from the same geographical regions. In turn, the genetic distance, estimated in their study, indicated that the PR cattle are most similar to the Angler breed, what confirmed the participation of Anglers in the improvement of the former. On the basis of the *loci* examined Grzybowski and Prusak [2004ab], analysed the specificity of the PR cattle, currently maintained within a preserve breeding programme. It was found that PR are characterized by a high genetic variation and that as many as 80% of those animals comprise a separate group within the cattle phylogenetic tree, while only 20% groups with the Angler and German Simmental breeds.

The comparatively low values obtained for the genetic distance in the present study may indicate, that the breeds analysed originate from a common ancestor or inhabited

neighbouring geographical regions and thus a frequent exchange of genes could take place. Similarities between the breeds examined could also be caused by the fact that their selection was conducted in a similar way, based on similar assumptions and leading to similar goals (similar utilization programme). Those are populations with a similar gene pool, that may be used in preserve breeding to decrease inbreeding and to reconstruct the exceptionally small population of Czech Red cattle. However, only the most similar populations should be used. Of interest is the increasing genetic distance, estimated between three populations in the present analysis as well as reported earlier. This may indicate that the preserve breeding of the breeds discussed, together with the attempt to retain and reintroduce them into common use, is probably conducted differently in each country and thus leads to a decrease in the similarity between those breeds.

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Dystans genetyczny między rasami polską czerwoną, czeską czerwoną a niemiecką czerwoną szacowany na podstawie wybranych *loci* genów kodujących białka i mikrosatelitarnych sekwencji DNA

Streszczenie

Badania obejmowały trzy rasy bydła czerwonego – polską czerwoną (PR, n = 65), czeską czerwoną (CR, n = 54) i niemiecką czerwoną (GR, n = 28). Przeanalizowano frekwencje alleli dla pięciu genów białek: *CSN3*, *LGB*, *GH*, *PRL* i *Pit-1* oraz 13 sekwencji mikrosatelitarnych. W populacjach PR i GR frekwencje w *loci* białek układały się podobnie, z wyraźnie zaznaczoną przewagą jednego z alleli. Jedynie w rasie CR we wszystkich analizowanych *loci* białek, oprócz *Pit-1*, frekwencje alleli były zbliżone i nie zaznaczała się wyraźna dominacja częstości występowania jednego z nich. Sześć z 13 sekwencji mikrosatelitarnych miało podobny rozkład frekwencji alleli we wszystkich trzech rasach, natomiast pozostałe charakteryzowały się znacznym zróżnicowaniem. Na podstawie uzyskanych wyników oszacowano dystans genetyczny między rasami. Najmniejszy stwierdzono między populacją PR a GR. Natomiast między populacjami PR a CR oraz CR a GR wielkość dystansu kształtowały się podobnie, a najbardziej wyrównane wyniki uzyskano metodą Nei'a.

