Study of genetic distances between cattle breeds of Central Europe

J. Čítek[1], L. Panicke[2], V. Řehout[1], H. Procházková[1]

[1]Department of Animal Breeding, University of South Bohemia, České Budějovice, Czech Republic
[2]Research Institute for the Biology of Farm Animals, Dummerstorf, Germany

ABSTRACT: Genetic distances were studied among Czech Red cattle, German Red, Czech Pied, Polish Red, Czech Black and White, and German Black and White cattle. DA genetic distances were calculated, and trees were constructed using the Neighbour-Joining method. Evaluating the genetic distances by microsatellites, the lowest value was between Czech and German Black and White breeds. A surprisingly high value was found between Czech and German Red breeds, and the highest values between German Red breed and both German and Czech Black and White populations came up to expectations. In the phylogenetic tree made using microsatellites, the German and Polish Red breeds clustered, but Czech Red breed was not joined with them. The other cluster was obtained for Czech Black and White and German Black and White. The tree made of protein markers differed slightly. Because the populations of Czech and German Red breeds are small and also because of organizational issues, the common protection of Central-European red populations and breeding them as a gene pool are recommended.

Keywords: genetic distances; cattle breeds; microsatellites; protein markers

Recently, maintaining the diversity of domesticated animals has become an important problem especially because of the industrialisation of agriculture in developed countries. Many old breeds are facing extinction and genetic variability in small populations is restricted by inbreeding and genetic drift. Thus, many valued genes and genotypes successful in different conditions are endangered. Inbreeding in the industrially exploited breeds is increasing due to a reduced effective population size.

The genetic diversity and genetic relationships between breeds of cattle have often been studied using polymorphic loci of blood groups or milk and blood proteins. Molecular markers, especially microsatellites, have become popular recently above all due to their high polymorphism. Microsatellites have been described as length variations within tandem arrays of short nucleotide motifs. Microsatellite loci are unequivocally defined by specific sequences of primers in PCR.

Thanks to their high degree of polymorphism and frequency in vertebrate genomes, microsatellites have a broad application in animal genetics, including the evaluation of inter-breed genetic similarities. They seem to be very useful for clarifying the evolutionary relationships between closely related populations (Rubinsztein et al., 1995; Arranz et al., 1996; Takezaki and Nei, 1996; Ritz et al., 2000). MacHugh et al. (1998) analysed the relationships between breeds and found a remarkable degree of breed clustering. Hanslik et al. (2000) investigated the genetic differences between American and European Holstein populations, Czerneková et al. (2006) studied the genetic diversity of Central European cattle.

Other authors used blood groups and protein polymorphisms to evaluate genetic variation, even though the latter are presently studied more often to explain their relationship to performance (Maj et al., 2004; Kučerová et al., 2006, e.g.). Studying these polymorphisms, the aim is to identify the

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QTL affecting the important traits (Freyer and Vukasinovic, 2005). Blott et al. (1998) identified two major breed groups using blood group and serum protein polymorphisms, French, Italian and Channel Island breeds with Simmental and Gelbvieh and the second group consisting of British and North European breeds. Medjugorac (1995) studied the relationships between breeds mostly from the Balkans and the Alps.

The estimation of genetic distances simplifies the comparison of populations. Out of many methods, Nei's standard genetic distance and DA distance are often used (Nei, 1972, 1976; Nei et al., 1983). Also Nei's minimal and maximal distance, Manhattan matrix, etc. are used. Laval et al. (2002) compared different methods of distance estimation.

The aim of this paper is to analyse the relationship of breeds for conservation purposes by the allelic frequencies of microsatellites and protein markers.

MATERIAL AND METHODS

Animals

The analysis was performed on Czech Pied cattle (Czech Simmental, n = 48), Czech Black and White cattle (n = 42), and German Black and White cattle (n = 42). The German Black and White animals originated from a modern commercial population of milk cattle raised in eastern federal countries. Further, Czech Red cattle (n = 54), German Red (n = 28) and Polish Red (n = 65) breeds, which are endangered gene resources, were involved in the study.

Genotyping

DNA was isolated from whole blood. Thirteen microsatellites were amplified in PCR and genotyped in ALF ExpressII (Pharmacia Biotech, Uppsala, Sweden), or on acrylamide sequencing gels and stained with silver. The microsatellite markers used are given in Table 1. Further, the polymorphic genes of growth hormone 1 (*GH1*, alleles *L*, *V*), casein kappa (*CSN3*, alleles *A*, *B*, *E*), lactoglobulin beta (*LGB*, alleles *A*, *B*), prolactin (*PRL*, alleles *A*, *B*), and pituitary growth factor (*PIT1*, alleles *A*, *B*) were used. The protein loci were genotyped using the PCR/RFLP method (Medrano and Aguilar-Cordova, 1990; Schlee et al., 1992; Woolard et al., 1994; Mitra et al., 1995). The enzymes used for restriction were as follows: casein kappa alleles *A*, *B*, restrictase *Hind*III; allele *E*, restrictase *Hae*III; lactoglobulin beta, restrictase *Hae*III; growth hormone 1, restrictase *Alu*I; prolactin, restrictase *Rsa*I; *PIT1*, restrictase *Hinf*I.

Statistical analysis

The genetic distances between breeds were calculated from allelic frequencies. Nei's DA genetic

Table 1. Microsatellites used in the analysis

Locus	Number of alleles	Length (bp)	Reference
BM6438	4	256–272	Bishop et al. (1994)
CSSM004	3	183	Moore et al. (1994)
IDVGA9	2	201–203	Ferretti et al. (1994)
BM6117	3	110–114	Bishop et al. (1994)
BM148	3	97–105	Bishop et al. (1994)
RM012	3	107–111	Kossarek et al. (1994)
BOVCASK35	4	234–238	Moore et al. (1992)
BOVIRBP	3	176–186	Moore et al. (1992)
BTOBCAM	3	180–186	Moore et al. (1992)
BOVPAI1MR	2	217–219	Moore et al. (1992)
BM4621	3	137–145	Bishop et al. (1994)
BOVSEMRN	3	202–223	Moore et al. (1992)
SRC97	3	118–124	Lang and Plante (1994)

Table 2. Average heterozygosity and its standard error

Breed	Microsatellites		Protein markers	
	H	S_E	H	S_E
Czech Red	0.403	0.074	0.434	0.085
Czech Simmental	0.506	0.053	0.415	0.072
Czech Black and White	0.417	0.059	0.388	0.056
German Black and White	0.436	0.060	0.376	0.032
Polish Red	0.415	0.067	0.317	0.039
German Red	0.431	0.072	0.272	0.054

distance (Nei et al., 1983) was used to quantify the distances. The trees were made according to the Neighbour-Joining method (Saitou and Nei, 1987). The bootstrapping method (Weir, 1996) was used to evaluate the significance of the node clusters. The gene diversity H_S and H_T and an estimator of genetic differentiation G_{ST} (Nei, 1973) were also calculated. All the computations were done by statistical package DISPAN (Ota, 1993).

RESULTS AND DISCUSSION

Cattle breeds have developed in different ways depending on regional climates, nutritional conditions and selection for different purposes. Genetic drift has also contributed to the process of breed differentiation. Here, we present the results of the study of variability of some cattle breeds in the Czech Republic. The internal genetic diversity and

Table 3. Gene diversity and gene differentiation of microsatellites

Locus	H_T	H_S	G_{ST}
BM6438	0.724	0.688	0.049
CSSM004	0.641	0.631	0.016
IDVGA9	0.231	0.215	0.070
BM6117	0.626	0.613	0.021
BM148	0.613	0.470	0.233
RM012	0.548	0.495	0.098
BOVCASK35	0.721	0.693	0.039
BOVIRBP	0.326	0.288	0.117
BTOBCAM	0.469	0.289	0.383
BOVPAI1MR	0.128	0.126	0.020
BM4621	0.529	0.468	0.115
BOVSEMRN	0.450	0.403	0.105
SRC97	0.145	0.141	0.025
All loci	0.473	0.425	0.103

Table 4. Gene diversity and gene differentiation of protein markers

Locus	H_T	H_S	G_{ST}
Prolactin	0.304	0.276	0.094
Casein kappa	0.463	0.444	0.042
Lactoglobulin beta	0.458	0.440	0.039
Growth hormone	0.420	0.384	0.086
PIT1	0.270	0.250	0.075
All loci	0.383	0.359	0.064

Table 5. DA genetic distances, microsatellites below diagonal, coding loci above diagonal

Breeds	1	2	3	4	5	6
Czech Red	–	0.0108	0.0412	0.0358	0.0394	0.0434
Czech Simmental	0.0561	–	0.0181	0.0210	0.0239	0.0259
Czech Black and White	0.0469	0.0474	–	0.0071	0.0153	0.0151
German Black and White	0.0318	0.0313	0.0210	–	0.0125	0.0112
Polish Red	0.0724	0.0704	0.0769	0.0704	–	0.0084
German Red	0.0850	0.0799	0.1101	0.0920	0.0535	–

Table 6. DA genetic distances, all loci

Breeds	1	2	3	4	5	6
Czech Red	–					
Czech Simmental	0.0435	–				
Czech Black and White	0.0453	0.0393	–			
German Black and White	0.0329	0.0284	0.0172	–		
Polish Red	0.0632	0.0575	0.0598	0.0543	–	
German Red	0.0734	0.0649	0.0837	0.0696	0.0410	–

the estimator of genetic differentiation are given in Table 2–4. In microsatellite loci, the mean proportion of genetic variation due to the interpopulation subdivision G_{ST} was 10.3% in our sample of breeds (Table 3), which is in accordance with MacHugh et al. (1998), who found a mean value of 10.4% in seven European cattle breeds. In protein markers it was only 6.4% (Table 4), and the mean value across all loci was 9.4%.

The main goal of the study was to analyse the relationship of breeds, as the internal diversity was evaluated in another study (Čítek and Řehout, 2001). The genetic distances are given in Table 5–6. DA distance is supposed to be suitable for the visualisation of relationships irrespective of the mutation model (Takezaki and Nei, 1996). The lowest value was between Czech and German Black and White breeds, but, somewhat surprisingly, a rather high value was found between Czech and German Red breeds. The highest values, between German Red breed and both German and Czech Black and White populations, were as expected.

In real phylogeny, the Czech Red breed is an original Czech cattle breed. The Czech Pied (Czech Simmental) breed arose in the 19th century from the crossing of the ancestral Czech Red popula-

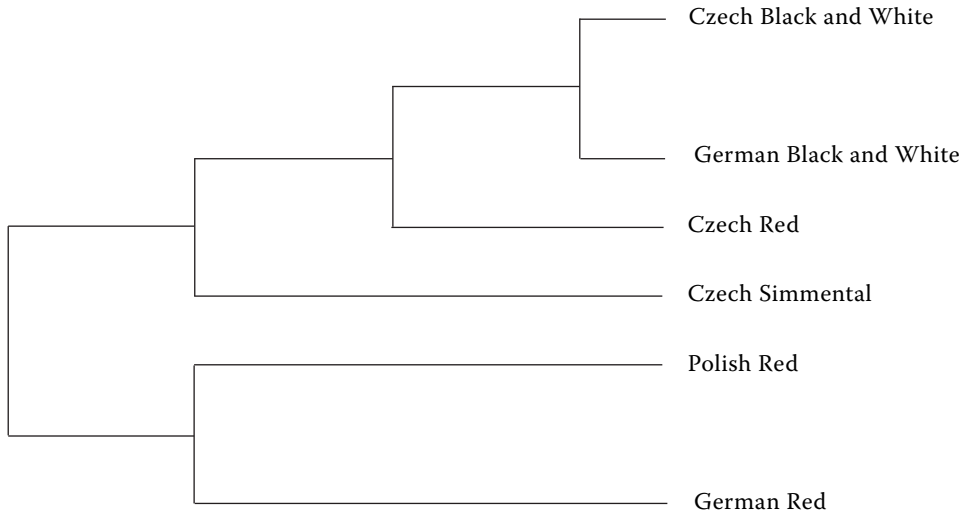

Figure 1. Dendrogram Neighbor-Joining method, DA distances, microsatellites

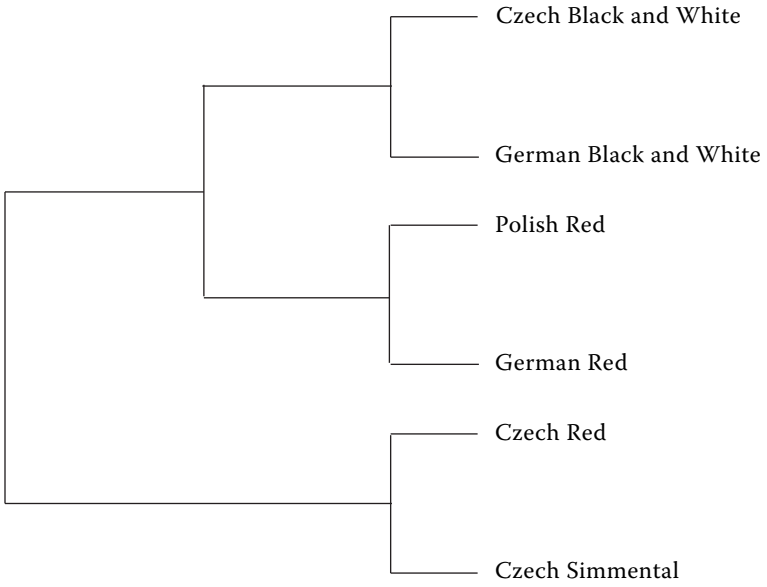

Figure 2. Dendrogram Neighbor-Joining method, DA distances, protein markers

tion with many breeds, predominantly Simmental (Čítek et al., 1997). The black and white population in the Czech Republic came into being from cross-breeding between Czech Pied cattle and Black and White cattle of European and American (Holstein-Friesian) origin in the last decades, therefore it has a similar origin to the German Black and White breed. The German, Polish and Czech Red breeds belong to the Central-European group of ancestral red cattle.

The phylogenetic trees (Figure 1–3) were made of DA distances by the Neighbour-Joining method. The trees are unrooted, i.e. it is not possible to deduce the phylogeny. Therefore, the trees are used as a descriptive tool in this paper, not for evaluating the time of separation, which is not important as the populations divided recently.

The tree made of microsatellites (Figure 1) joins the German and Polish Red breeds, but Czech Red cattle have not clustered with them. Simianer (undated) also found the Czech Red population standing alone among two groups of German Red breeds and the Gelbvieh group, Czerneková et al. (2006) found the Czech, Polish and German Red breeds clustering together. A lower bootstrap value was obtained for Czech Black and White and German Black and White. The tree based on frequencies of protein markers (Figure 2) showed also relatively high bootstrap support for Polish and German Red breeds, and a lower value for Czech and German Black and White breeds. In addition to the tree of microsatellites, Czech Red and Czech Simmental make a divided cluster. Some differences between microsatellites and proteins could be explained by genetic drift in low numbered red breeds, as the analysis showed obvious differences in allelic frequencies especially in the loci for prolactin, casein kappa, and growth hormone between German Red and other red breeds. The tree constructed of both markers (Figure 3) was quite similar to the tree of microsatellites.

Takezaki and Nei (1996), MacHugh et al. (1998), Laval et al. (2002) concluded that the diversity observed in microsatellite loci among closely related populations was not caused by mutations; therefore they regarded the influence of genetic drift as crucial. Arranz et al. (1996) reported similar results comparing genetic distances and dendrograms from 5 microsatellites and 15 protein markers. Del Bol et al. (2001) found tight clusters of autochthonous alpine Italian cattle breeds; Holstein and original German Brown were some distance away. We have found similar close relationships between black and white populations, and between Polish and German Red breeds. Mommens et al. (1999) evaluated the relationship of five Belgian breeds and African N'Dama by means of microsatellites. American bison served as an outgroup. The dendrogram displayed a geographical topology with three major lineages as expected, but the clustering of the local breeds was unclear. Hansen et al. (2002), evaluating the genetic distances between Canadian breeds based on microsatellites, emphasized the difficulty in scientifically estab-

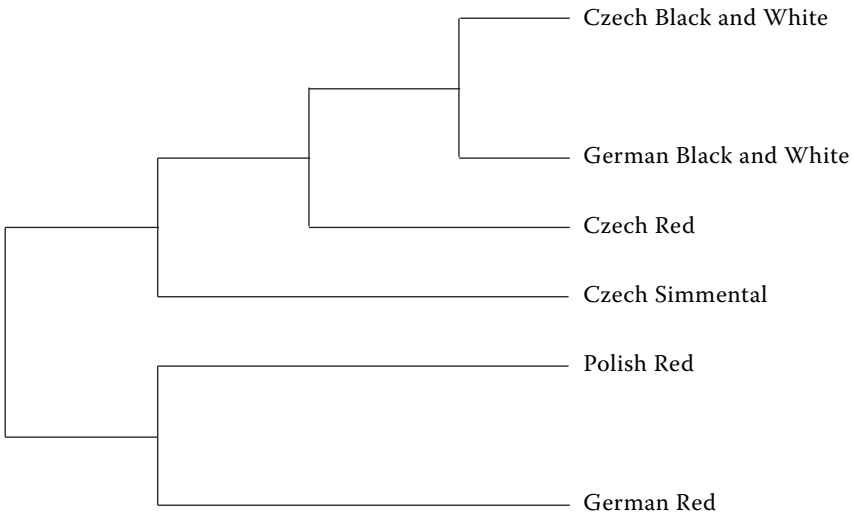

Figure 3. Dendrogram Neighbor-Joining method, DA distances, all loci

lishing unique breeds. In the preservation of gene reserves, Ciampolini et al. (1995) considered the use of microsatellites as effective for the study of genetic similarity both within and between breeds and for the detection of genetically homogeneous subgroups. Kim et al. (2002), evaluating genetic diversity based on 13 microsatellites using DA distance and N-J tree, found the group of Chinese and Korean cattle, the Japanese Black cattle was clearly distinct. Microsatellites are commonly held to be sufficient for the explanation of evolutionary relationships (Takezaki and Nei, 1996; Basedow, 1998; Peelman et al., 1998).

The interpretation of results should be done very carefully also with regard to the character of the population analysed. In this analysis, the low numbered Czech Red breed and German Red breed are susceptible to genetic drift, as mentioned above. The methodical aspects are also very important because of input assumption. Many authors have found differences between the methods used for the description of a breed's phylogeny. Martin-Burriel et al. (1999) found different clustering of Neighbour-Joining and UPGMA methods with Nei's and Cavalli-Sforza's distances, the latter being more consistent with the real breed's phylogeny. Kustermann (1994) also reported differences between clustering UPGMA and Ward, and Cavalli-Sforza's and Reynolds, Weyr and Cockerham's distances, respectively. However, in our populations tight correlations were obtained comparing Nei's, Cavalli-Sforza's and Reynolds, Weyr, Cockerham's distances (data not shown). Similarly, Nagamine and Higuchi (2001) found very high correlations between distances and small differences in accuracy.

It is important to realise that the phylogenetic trees are theoretically based on biological models which do not apply in farm animals (Simianer, 1999). Most of the methods have been developed to describe differences between the species, but differences between the breeds are differences within species. The time span in evolution is millions of years, in the breed history it is c. 150–200 years (e.g. Czech Red and Czech Pied cattle) or even only 20 to 40 years (Czech Pied and Czech Black and White cattle). The evolution and breed genesis differ also in the processes that are the fundamentals of differentiation. In evolution, the segregation of species is presumed, so that selection, mutation and drift cause further differentiation, while in breed making, crossing occurs very often. Thus, clustering and potential differences from expectations are to be evaluated in connection with the development of breeds and all the circumstances influencing their relationships.

CONCLUSIONS

Our results confirmed the close relationship between Czech Black and White and German Black and White, and, similarly, for Polish Red and German Red breeds. Czech Red breed is distinct from the two red populations. However, because of small population sizes of Czech and German Red breeds and also because of organizational issues, the common protection of Central-European red populations and breeding them as a gene pool are recommended. In prudent breeding practice, this approach could prevent the increase of inbreed-

ing, and the loss of genetic variability in the gene reserves.

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Corresponding Author

Doc. Ing. Jindřich Čítek, CSc., Department of Animal Breeding, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic
Tel. +420 387 772 591, fax +420 387 772 593, e-mail: citek@zf.jcu.cz