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AN ESTIMATION OF THE GENETIC DISTANCE BETWEEN POLISH RED AND OTHER RED CATTLE BREEDS ON THE BASIS OF SELECTED MILK PROTEIN *LOCI*

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ABSTRACT

The material for the study were six populations of Red Cattle, including three samples of the Polish Red cattle population (PC'69, n = 203, PC'82, n = 261 and PC'96, n = 313) and one each of the Danish Red (DC, n = 169), German Red (NC, n = 510) and Czech Red (CC, n = 14). DNA was isolated from blood, according to the method by Gemmell and Akiyama (1996). All individuals were genotyping for the following proteins: α_{S1}-casein, β-casein, κ-casein and β-lactoglobulin. The frequency of alleles was estimate and was used for the estimation of the genetic distance between populations. Using the PHYLIP ver. 3.5c software the genetic distance was determined according to 1) Nei, 2) Cavali-Sforza and 3) Reynolds. All the populations demonstrated a similar distribution of allele frequencies at individual *loci*. Only the Czech Red showed different tendencies as regards β- and κ-casein and β -lactoglobulin. This may have been the effect of the smaller population size as compared to the remaining breeds. Genetic distances estimated by these three using methods were similar. The smallest genetic distance was observed between populations PC'96 and NC (0.0007). In turn, the highest value was obtained between population DC and CC (0.0776). In the case of the genetic distance calculated according to the formulas by Cavalli-Sforza and Reynolds, the values obtained were higher then value of genetic distance estimated by Nei. The comparatively low values for the genetic distance obtained in the present studies may indicate that the breeds analysed originate from a common ancestor or lived on neighbouring geographical regions, what could lead to a frequent exchange of genes. The considerable similarity between those breeds could also be caused by a similarly conducted selection, based on similar assumptions (similar type of production). Those populations demonstrate similar gene pools what may be used for common preserve breeding programmes, which would decrease the danger of inbreeding. An analysis of the data collected over 30 years for the three populations of the Polish Red cattle, renders it possible to conclude that the frequency of individual alleles of the proteins examined are subjected to a small but continuous change.

Key words: genetic distance, polymorphism, red cattle.

INTRODUCTION

Red Cattle is currently included in a preserve breeding programme in Poland, the Czech Republic, Germany and other European countries. Due to the comparatively low level of milk production it was eliminated from commercial farming by more specialized, high yielding breeds. However, after some years, when the population of this cattle decreased considerably, it proved that Red Cattle is excellently adapted to mountain conditions and to regions deficient in rich pastures. As result of the marked decrease in numbers, the genetic variability within each population of Red Cattle also decreased and, what is more, the negative effects of inbreeding were intensified. For over a dozen years studies are being conducted on the gene pool and determination of the genetic similarity between individual breeds of Red Cattle in different European countries. A small genetic distance between breeds would make it possible to use animals from one population to increase the gene pool in another, especially one that is threatened by the negative effect of inbreeding. Such activities would be justified if enriching the gene pool would not be accompanied by changes in traits characteristic of individual breeds of this type.

For the analysis of the genetic distance and similarity between breeds or populations numerous genetic markers are used, among which proteins are also counted. Numerous studies indicate that the polymorphism of many proteins is linked with productivity and the quality of the product obtained [20, 21, 23, 29, 30, 33, 34, 36].

In our geographical region there occur several Red Cattle breeds – in Poland, the Czech Republic, Germany and Denmark. Work on the protection of the gene resources of those breeds have considerably widened the range of studies on genetic markers within individual populations of Red Cattle. Thus a possibility arises of using the results of genetic analyses for the determination of relations between breeds and their use for the protection of endangered Red breeds on an international scale. The studies presented here aimed at determining the relations between different Red Cattle breeds and their genetic similarity. Moreover, data collected from studies conducted over 30 years render it possible to evaluate changes occurring in the population of Polish Red cattle included in the preserve breeding programme. The studies have been divided into two parts: a) "historical" analysis of the material on the basis of class I genetic markers (proteins) and b) an analysis of the cow population currently maintained in preserve herds, based on the polymorphism of proteins and microsatellite DNA sequences.

MATERIALS AND METHODS

The material consisted of samples obtained from six populations of Red Cattle, including three samples of the Polish Red cattle population (PC'69, n=203, PC'82, n = 261 and PC'96, n = 313) and one each of the Danish Red (DC, n = 169), German Red (NC, n = 510) and Czech Red (CC, n = 14). The data referring to population PC'69 came from the studies conducted by Michalak [25], to population PC'82 – from those by Felenczak [13], to population DC – from those by Bech and Kristiansen [3], to population NC – from those by Erhardt et al. [12], while data on the Czech Red (CC) and Polish Red (PC'96) populations were collected by the authors between year 1996 and 2003.

DNA was isolated from peripheral blood, drawn from the jugular vane, according to the method by Gemmell and Akiyama [15]. Next, genotypes of the following proteins were identified for individual animals: α_{S1}-casein, β-casein, κ-casein and β-lactoglobulin [24, 26, 32]. The frequency of individual alleles was determined on the basis of the genotype frequency, and was next used for the estimation of the genetic distance between populations. Using the PHYLIP ver. 3.5c software [14] the genetic distance was determined according to 1) Nei, 2) Cavali-Sforza and 3) Reynolds. Moreover, the same software was used to elaborate the dendrogrammes presenting a graphic form of the relations between populations. The dendrogrammes were created using the Neighbour-joining method.

RESULTS AND DISCUSSION

The frequency of individual protein alleles is presented in Table 1. All the populations demonstrated a similar distribution of allele frequencies at individual *loci*. Only the Czech Red showed different tendencies as regards β- and κ -casein and β-lactoglobulin. This may have been the effect of the smaller population size as compared to the remaining breeds.

Table 1. Frequency of alleles at the *loci* of the proteins analysed

Locus	Allele	Polish Red '69	Polish Red '82	Polish Red '96	Danish Red	German Red	Czech Red
α S1-casein	B	0.889	0.913	0.943	0.996	0.975	0.964
	C	0.111	0.087	0.057	0.004	0.025	0.036
β - casein	A	0.910	0.967	0.938	0.940	0.943	0.786
	B	0.090	0.033	0.062	0.060	0.057	0.214
κ - casein	A	0.604	0.609	0.690	0.810	0.712	0.464
	B	0.396	0.391	0.310	0.190	0.288	0.536
β -lactoglobulin	A	0.397	0.328	0.224	0.110	0.191	0.429
	B	0.603	0.672	0.776	0.890	0.809	0.571

In the case of α_{S1}-casein in all populations two alleles were observed – B and C, the frequency of allele B being clearly higher and reaching about 0.9. Ng-Kwai-Hang et al. [29], recorded for a population of Holstein cattle from upper Quebec the occurrence of three alleles for this protein: A, B and C, with a frequency of 0.003, 0.970 and 0.027, respectively. Studies conducted on dairy breeds maintained in Hungary [1, 2] – Hungarian Spotted, Hungarian Grey, Hungarian Holstein and Hungaro-Friesian, demonstrated only the presence of protein alleles B and C in those populations. In the Hungarian Spotted and Hungarian Grey cattle the frequency of those alleles in year 1993 reached 0.89 and 0.11 and 0.82 and 0.18, respectively, while in year 1996 already 0.88, 0.12 and 0.68, 0.32. The remaining two breeds were included into the studies only in year 1996 and the frequency of protein alleles reached 0.96 and 0.04 (Hungarian Holstein) and 0.89 and 0.11 (Hungaro-Friesian). Erhardt [11] reported the occurrence of alleles B and C of α_{S1}-casein in the Pinzgauer and Limpurger breeds, allele B being clearly more frequent (0.99) then allel C (0.72). Similar results were obtained by Ikonen et al. [19] for the Finish Ayrshire cattle, in which allele B also proved the most frequent (0.999). An analysis of the polymorphism of α_{S1}-casein, conducted by Dobicki et al. [10] on Black-and-White and Charolaise cattle as well as on their crosses, demonstrated that in those populations only allele B occurred, as allele A was found only in the Charolaise cattle, and that with a very low frequency (0.02). Chobotova et al. [4] observed in Slovakian Pied and Slovakian Pinzgau, the presence of allele B and C for α_{S1}-casein, with frequencies of 0.79 and 0.21 for the first population and 0.56 and 0.44 for the latter.

The frequency of β-casein alleles (A and B) was arranged similarly in all the populations examined, with the exception of the Czech Red cattle. The frequency of allele A reached over 0.9 and only in the Czech Red cattle it amounted to 0.786. Comparatively numerous studies were conducted on the polymorphism of this protein in various breeds of dairy cattle [1, 2, 10, 11, 19, 29]. The authors of those studies observed that a maximum of five β-casein alleles occurred, arranged in various combinations in the populations analysed. Their frequency ranged from 0.17 to 0.56 for allele A1, from 0.42 to 0.80 for allele A2, from 0.003 to 0.11 for allele A3, from 0.001 to 0.16 for allele B and from 0.005 to 0.044 for allele C. Studies on beef and dual purpose cattle [4, 10] demonstrated the following frequencies for the alleles of this protein: from 0.25 to 0.74 for allele A, from 0.05 to 0.50 for allele B, from 0.09 to 0.37 for allele C and from 0.12 to 0.25 for allele E.

In the first part of the studies presented the presence of two alleles was observed at the κ-casein locus: A and B, of which allele A was clearly more frequent (0.6□0.8), with the exception of the Czech Red cattle population, in which allele B was somewhat more frequent. Kappa-casein is a milk protein, on the polymorphism of which numerous studies have been conducted. Similar studies, covering the Red Cattle breeds and their crosses [6, 9, 20] showed the frequency of alleles of this protein to range between 0.46 and 0.93 for allele A, 0.07 and 0.48 for allele B and 0.06 for allele E. In populations of different dairy cattle breeds [1, 2, 4, 10, 11, 19, 20, 29, 36] the following frequency of individual polymorphic forms of κ -casein were recorded: allele A – from 0.42 to 0.79, allele B – from 0.08 to 0.58, allele C – from 0.003 to 0.03, allele E – from 0.003 to 0.31, allele F – (Finish Ayrshire breed) <0.001 and for allele G – (Pinzgauer cattle) 0.003. In beef and dual purpose breeds the distribution of alleles and their frequency, presented in many works [4, 7, 8, 10, 28] was as follows: allele A – 0.41–0.90, allele B – 0.10-0.59, allele E – 0.01-0.08.

The data obtained for β-lactoglobulin demonstrate a higher frequency for allele B (from 0.571 to 0.89) in all the populations analysed, with the exception of the Czech Red breed, for which this superiority was small (0.56-0.57). In the populations of German Red and Czech Red cattle the frequency of both alleles tended to be the same. In studies conducted by Juszczak et al. [20] on Polish Red cattle allele B was also dominant at a similar level (0.74). Similar studies were conducted also on other dairy cattle breeds [1, 2, 4, 10, 11, 19, 20, 29, 35, 36]. It was observed that for β-lactoglobulin two alleles occurred most frequently: A (0.17-0.52) and B (0.48-0.83). In a majority of dairy breeds allele B proved to be clearly the most frequent. In beef and dual purpose cattle the superiority of this allele was not so clear and in some cases (e.g. Charolaise) the allele frequencies showed a reverse tendency, with allele A being the more frequent. The following frequencies were observed in different populations for individual forms of β-lactoglobulin: allele A – from 0.38 to 0.71 and allele B – from 0.29 to 0.62 [4, 10, 35].

Tables 2 and 3 present the values for the genetic distance, estimated between the populations analysed. When calculated according to Nei, the smallest genetic distance was observed between populations PC'96 and NC (0.0007). In turn, the highest value was obtained between population DC and CC (0.0776). In the case of the genetic distance calculated according to the formulas by Cavalli-Sforza and Reynolds, the values obtained were higher, but the relations were similar, *i.e.*, the smallest distance was observed between population PC'96 and NC and the largest between population DC and CC. Dendrogrammes (Fig. 1-3) constitute a graphic presentation of those results.

Table 2. Genetic distance according to Nei'a (above the diagonal) and according to Cavali-Sforza (bellow the diagonal)

Specification	Polish Red '69	Polish Red '82	Polish Red '96	Danish Red	German Red	Czech Red
Polish Red '69	x	0.0045	0.0243	0.0674	0.0339	0.0152
Polish Red '82	0.0126	x	0.0104	0.0428	0.0167	0.0282
Polish Red '96	0.0409	0.0196	x	0.0111	0.0008	0.0593
Danish Red	0.1448	0.1006	0.0337	x	0.0061	0.1206
German Red	0.0609	0.0324	0.0020	0.0197	x	0.0732
Czech Red	0.0264	0.0657	0.1014	0.2347	0.1291	x

Table 3. Genetic distance according to Reynolds

Specification	Polish Red '69	Polish Red '82	Polish Red '96	Danish Red	German Red	Czech Red
Polish Red '69	x					
Polish Red '82	0.0084	x				
Polish Red '96	0.0457	0.0214	x			
Danish Red	0.1471	0.1087	0.0379	x		
German Red	0.0666	0.0367	0.0023	0.0228	x	
Czech Red	0.0212	0.0438	0.0939	0.2090	0.1191	x

The genetic distance renders it possible to determine the similarity between populations and identify their origin. Numerous studies aiming at the estimation of the genetic distance between different populations of farm animals covered various cattle breeds [17, 18, 22, 27]. Analyses were also made of the relation existing between the similarity between breeds (genetic distance) and the heterosis expected when crossing those breeds [16]. It was observed that an earlier evaluation of the genetic distance between breeds makes it possible to used for crossing those breeds which ensure the highest heterosis effect.

Fig. 1. Genetic distance by Nei – neighbor-joining method

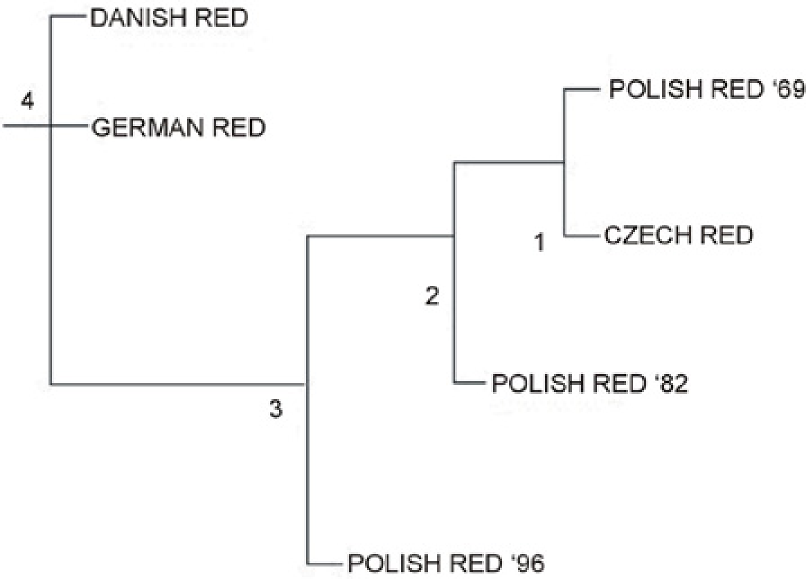


Fig. 2. Genetic distance by Cavalli-Sforza – neighbor-joining method

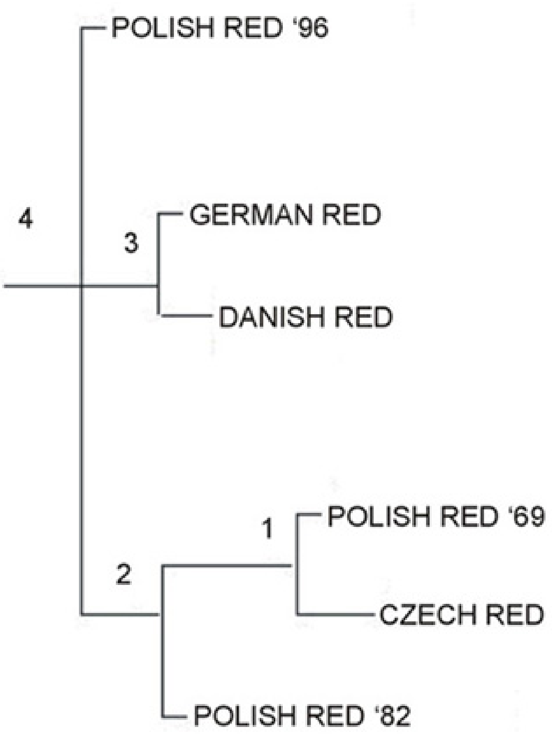
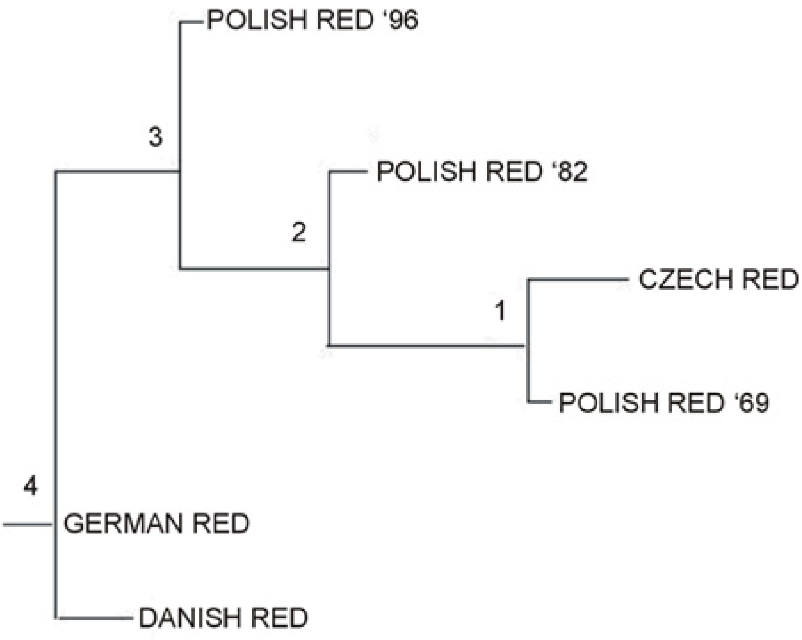


Fig. 3. Genetic distance by Reynolds – neighbor-joining method



The European breeds of Red Cattle occur nowadays in small populations, for which reason they are subjected to numerous studies. Rehout et al. [31] analysed the genetic distance between five populations of cattle: Czech Spotted, Czech Black-and-White, Czech Red, German Black-and-White and F1 crosses, obtained by crossing the Czech Red with Czech Spotted. The genetic distance was estimated on the basis of the polymorphism at five microsatellite *loci* and at the growth hormone *locus*. It was calculated according to three methods and showed that the least similar were the German Black-and-White with the Czech Red and F1 crosses as well as the Czech Spotted and F1 crosses. The smallest distance was observed between the Czech Black-and-White and Czech Red and Czech Spotted.

CONCLUSIONS

The comparatively low values for the genetic distance obtained in the present studies may indicate that the breeds analysed originate from a common ancestor or lived on neighbouring geographical regions, what could lead to a frequent exchange of genes. The considerable similarity between those breeds could also be caused by a similarly conducted selection, based on similar assumptions (similar type of production). Those populations demonstrate similar gene pools what may be used for common preserve breeding programmes, which would decrease the danger of inbreeding. However, only the most similar populations should be used this way. Of interest is the fact, that in those analyses the genetic distance tended to increase in the three populations currently maintained, in relation to the results obtained from earlier data. This may indicate that the protection of those breeds was conducted somewhat differently in each country and this may result in a decreased similarity between those breeds.

An analysis of the data collected over 30 years for the three populations of the Polish Red cattle, renders it possible to conclude that the frequency of individual alleles of the proteins examined are subjected to a small but continuous change. The greatest changes took place between year 1982 and 1996, the more frequent allele showing a tendency to strengthen. This may lead to the elimination of the remaining alleles, rarely occurring as result of the genetic drift. This means that it is necessary to monitor continuously the diversity of the gene pool of the population protected and ensure such an adaptation of the strategy as to prevent a marked decrease of their occurrence. The slow, but significant changes occurring within the population of the Polish Red cattle are also indicated by the values of the genetic distance between the three samples obtained in the sixties, eighties and the end of the nineties. The values for the genetic distance indicate that, despite the comparatively small changes in the arrangement of the allele frequency, the genetic differences have deepened.

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