CONTENTS

ORIGINAL PAPERS

Genetic diversity between seven Central European cattle breeds as revealed by microsatellite analysis

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ABSTRACT: This study compares the genetic variation in seven cattle breeds from the territory of Central Europe. Eleven polymorphic microsatellite loci were used to detect differences in the polymorphism of genetic markers in Czech Pied (Simmental), Slovakian Pied (Simmental), Slovakian Pinzgau, Holstein, Polish Red, German Red, and Czech Red breeds. For these loci, allele frequencies, heterozygosity, polymorphism information content, effective population size, and genetic distances were evaluated. The phylogenetic tree was constructed using the unweighted pair group method with arithmetic mean and it showed that the Central European Red breeds tended to cluster together, whereas the Holstein was the most divergent from the remaining breeds. These data are discussed in the context of the known origin of respective breeds.

Keywords: cattle breeds; gene resources; heterozygosity; microsatellites

The restriction of gene pool by dwindling of a breed or by a substantial decrease in the population is undesirable from both economic and breeding aspects. Moreover, it also causes a cultural loss. The Czech Republic began to pay a closer attention to this problem only recently. In 1995, in compliance with the Convention on Biological Diversity, the National Programme for the Preservation and Utilisation of Genetic Resources of Farm Animals was adopted. One of the parts of this programme is aimed at the protection of the local cattle breeds considered by the FAO to be endangered, namely, the Czech Red and Czech Pied cattle. The general goal of this study was to examine the genetic relationships between Central European cattle breeds as expressed by microsatellite markers and to evaluate the data in the context of the known origin of respective breeds. The diversity between the breeds was of particular interest as its knowledge is crucial for the ongoing effort to preserve the above-mentioned local breeds.

Recent progress in gene mapping supplied the cattle industry with a large number of bovine microsatellite markers which make use of highly polymorphic multi-site loci found in the genome of many organisms and thus are highly useful for genotyping. A number of authors reported the genetic diversity of commercial breeds such as Holstein and Hereford (e.g. Janik et al., 2002; Maudet et al., 2002). On the other hand, the genetic diversity of endangered populations considered here (Czech Red, Czech Pied, Polish Red, and German Red cattle) was described only in one paper (Čítek and Řehout, 2001). Slovakian Pied and Slovakian Pinzgau have not been studied by means of genetic markers until now.

MATERIAL AND METHODS

Central European Cattle Breeds. Czech Red cattle (hereinafter CR) are an original breed of brachycerous red cattle from the territory of Bohemia and Moravia. CR have yellowish red colour of hair, yellow horns with dark tips, medium body frame, vivacious temperament, and good fer-

tility. CR cows produce high quality milk in the amount of about 3 000 kg during lactation, with a higher content of fat. Currently, only a small population of the CR remains in the Czech Republic (the effective population size (N_e) is 11, the values of N_e were taken from the materials of the EAAP Working Group on Animal Genetic Resources, see: http://www.tiho-hannover.de/einricht/zucht/eaap/). Consequently, the cryopreservation of their semen and embryos and grading-up with the utilization of red breeds of a mountain type (Polish or German) are carried out. Czech Pied (Simmental) cattle (CP) belong to the group of European Simmental dual-purpose spotted cattle breeds established during the 19th century, mainly through the grading-up of local cattle breeds (for example Czech Red) with Swiss Simmental cattle. After World War II the number of original breeding bulls and cows of CP descended steeply due to the intensification of milk production (intensification efforts resulted in selections and crossings with dairy cattle breeds). In the herd-book of the Czech Republic there are about 285 000 females registered at present (N_e = 2 772).

Slovakian Pied (Simmental) cattle (SP) are spread mainly in the regions of southern Slovakia, representing a dual-purpose cattle breed. As far as the origin of this breed is concerned, it arose from upgrading the native cattle breeds (Red-brown and Grey-brown cattle as well as Bern and other breeds imported during the 17th and 18th centuries) with Simmental cattle. The colour of Slovakian Pied ranges from pale yellow to dark yellow, from red to reddish brown, and may also include grey. The colour of the head is predominantly white. SP have the following useful properties: high fertility, outstandingly docile nature, hardiness, good mothering ability, and high quality milk. In the 50ies of the 20th century the Slovak Pied breed was recognized as an independent one. In 2002 there were about 50 000 purebred cows (N_e = 575) in the Slovak Republic.

Slovakian Pinzgau (SPg) cattle are another breed traditionally kept in the territory of Slovakia. Like the Slovak Pied breed, SPg also arose from the upgrading of native Red and Grey-brown Carpathian cattle with the Pinzgau breed imported from Austria. Breeding activities were developed after 1870. This breed is also classified as dual-purpose cattle. SPg have pigmented skin under a chestnut red coat and white markings on the back, tail and barrel. It adapts readily and easily to unfavourable conditions like pastures in mountainous and sub-mountainous areas. At present a further increase of milk production is of main interest, therefore crosses with Holstein (Red) are produced. For comparison, crosses with Yorkshire and Lowland Red Pied cattle were predominant in the past. There were about 5 000 individuals in the purebred form in 2002 (N_e = 262).

Polish Red cattle (PR) are an autochthonous original Slavic breed. In the 1880's, red cattle from Denmark, Germany and Sweden were used to improve the various local strains of red Polish cattle. This crossing resulted in the formation of PR. The herd-book was established in 1895. PR are extremely robust dairy animals with red coloured coat. However, PR mature rather late: first calves are dropped at 3 years or later. It is a meat dairy type and is used for draught purposes. Many herds have been crossed with Angeln cattle, which are related to the breeds concerned, therefore pure PR are relatively rare. There are about 100 000 (purebred and crossbred) females in Poland (N_e = 559).

German Red cattle (GR) are an autochthonous original breed which belongs phylogenetically to the Central European Red group. This breed is dual-purpose cattle with good muscling, medium framed, undemanding, robust, prolific and long-lived. GR have reddish brown colour of coat and wax-yellow horns with dark tips. Crossing with Red Danish cattle and later with Angeln cattle made the frame considerably larger and increased the milk yield and the fat content. There are few animals with the original bloodstock left (1 150 females in the herd-book, N_e = 136).

Sampling. Blood samples were collected from 511 adult animals belonging to seven breeds. The studied populations included Czech Pied (n = 60), Slovakian Pied (n = 67), Slovakian Pinzgau (n = 83), Holstein (n = 102), Polish Red (n = 63), German Red (n = 42), and Czech Red (n = 94) cattle. Genomic DNA was isolated from whole blood using the NucleoSpin Blood Kit (Clontech Laboratories, Palo Alto, CA, USA).

Microsatellite analysis. A set of the ISAG (International Society of Animal Genetic) recommended microsatellite loci was adopted, namely, the following 11 loci were studied: BM2113, BM1824, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126 and TGLA227. PCR amplifications were performed in multiplex reactions using 14 μl reaction volumes with 80–100 ng of template DNA, 3 μl of reaction buffer (10 × Stockmarks

Buffer), 4 µl of dNTP mix (1.25 mmol/l), 5.5 µl of Primer mix (20 nmol/l) and 2.5 U of Taq Gold polymerase. These components were taken from the StockMarks for Cattle Bovine Genotyping Kit (Applied Biosystems, Foster City, CA, USA). The reactions were carried out in the TGradient 96 thermal cycler (Whatman Biometra, Goettingen, Germany) using a 15-minute initial denaturation at 95°C, followed by 31 cycles of 30 seconds at 94°C, 45 seconds with 50% ramp rate to 61°C, 45 seconds with 80% ramp rate to 72°C. The final exposure at 72°C was prolonged to 60 minutes. Samples were diluted with 90 µl of water. The analysis of PCR products and allelic size computations were performed using ABI PRISM 310 (Applied Biosystems) and GeneScan analysis software.

Statistical analysis

The hypothesis that each population exhibits Hardy-Weinberg proportions (HWE) was tested using the GENEPOP package (Raymond and Rousset, 1995). Genotypic linkage disequilibrium was estimated between all locus pairs with GENEPOP. The measures of gene diversity (namely, the mean number of alleles per locus NA, the number of private alleles PA, the expected H_{exp} and observed heterozygosity H_{obs} under HWE) were used to

quantify the degrees of genetic variation within, and between, populations (Falconer, 1989). The effective population size (EA) and polymorphism information content (PIC) were calculated with the GENETIX software package (Belkhir et al., 1996) using the formula of Botstein et al. (1980) and of Hartl and Clark (1989), respectively. The following genetic distance measures were calculated (using GENETIX) to estimate the similarity between breeds: standard genetic distance D_S according to Nei (1972), distance D_R as defined by Cavalli-Sforza and Edwards (1967), Rogers (1972), chord distance D_C, and Nei et al. (1983) D_A distance. The unweighted pair group method with arithmetic mean (UPGMA, Michener and Sokal, 1957) was used to construct the phylogenetic tree. The principal component analysis (PCA) was performed using SAS software (SAS, 1999–2001).

RESULTS AND DISCUSSION

Genetic diversity

Genetic diversity can be evaluated on the basis of the number of alleles per locus, heterozygosity and polymorphic information content. The number of alleles and size of PCR products are similar to data from the IRRF bovine male linkage map

Table 1. Characterization of 11 bovine microsatellite loci which are recommended by the International Society for Animal Genetics

Locus	Fluorescent label	Data from the IRRF bovine male linkage map*			Results from the present study	
		chromosome	No. of alleles	product size (bp)	No. of alleles	product size (bp)
TGLA227	FAM	18	10	78–96	14	77–103
BM2113	FAM	2	9	121–141	10	121–141
TGLA53	FAM	16	22	145–188	17	154–188
ETH10	FAM	5	not given	198–234	9	209–225
SPS115	FAM	15	not given	235–265	8	244–260
TGLA126	JOE	20	7	113–125	8	111–125
TGLA122	JOE	21	13	135–181	18	141–183
INRA023	JOE	3	not given	193–235	11	198–218
ETH3	NED	19	not given	90–135	10	109–131
ETH225	NED	9	7	139–151	9	140–158
BM1824	NED	1	7	180–192	7	178–190

*http://cagst.animal.uiuc.edu/genemap/loci.list

Table 2. The mean effective population size (*EA*), mean observed (*H*$_{obs}$) and expected heterozygosity (*H*$_{exp}$), mean polymorphism information content (PIC), mean number of alleles per locus (*NA*), and total number of private alleles (*PA*) in seven cattle breeds

Breed	*EA*	*H*$_{obs}$	*H*$_{exp}$	PIC	*NA*	*PA*
Czech Pied	4.597	0.779	0.764	0.757	7.90	2
Slovakian Pied	3.308	0.654	0.650	0.642	7.45	2
Slovakian Pinzgau	4.337	0.759	0.751	0.745	9.00	7
Holstein	3.736	0.806	0.697	0.685	5.82	0
Polish Red	4.285	0.748	0.747	0.741	8.45	4
German Red	3.907	0.675	0.716	0.711	7.00	2
Czech Red	4.251	0.789	0.751	0.749	7.18	1

(Table 1). Allele frequencies are available from the corresponding author upon request. All loci were polymorphic and the number of alleles varied from seven in BM1824 to 18 in TGLA122. This indicates that the microsatellites used in the present study were suitable for genetic diversity analysis. Private alleles were present in all populations except the Holstein one (Table 2). However, these alleles were all present at low frequencies. The highest and the lowest variability in microsatellite loci were found for Slovakian Pinzgau and Holstein cattle accordingly. Thus, the mean number of alleles per locus ranged from six (HO) to nine (SPg). Clearly, the low number of alleles exhibited by Holstein cattle results from an intensive human selection (see Hanslik et al., 2000 for discussion).

All populations were characterized by the values of the observed (*H*$_{obs}$) and expected (*H*$_{exp}$) heterozygosity, polymorphic information content, and by the effective number of alleles. The results are summarized in Table 2. There are common trends in all these parameters discernible for the studied breeds. Thus, the Czech Pied breed exhibits the highest values of *H*$_{exp}$, PIC, and *EA*. It is followed by the Czech Red, Slovakian Pinzgau, Polish Red, German Red, Holstein, and Slovakian Pied breed (Table 2). The value of the expected heterozygosity, which was found to be 0.764, 0.751, 0.751, 0.747, 0.716, 0.697, and 0.650 for CP, SPg, CR, PR, GR, HO, and SP, respectively, can be compared with the available literature data. Namely, the average heterozygosity of Holstein cattle is similar to the results found using microsatellite techniques by Schmid et al. (1999) (*H*$_{exp}$ = 0.69), Hansen et al. (2002) (*H*$_{exp}$ = 0.69), and Maudet et al. (2002)

(*H*$_{exp}$ = 0.686). The expected heterozygosities obtained for Czech Red, Polish Red, German Red, and Czech Pied cattle are higher than the data reported by Čítek and Řehout (2001). They found the average heterozygosities of 13 microsatellites to be 0.396, 0.409, 0.415, and 0.495 for CR, PR, GR, and CP accordingly. However, completely different microsatellites and quite a narrow range of variability (up to four alleles only) were considered by Čítek and Řehout (2001). These factors, instead of the changes in populations, could be the main sources of apparently lower genetic diversity reported by them. The microsatellite DNA data for Slovakian breeds, i.e. SP and SPg, are reported here for the first time. Slovakian Pied cattle have a low value of heterozygosity (see above), which might indicate that this breed is relatively well-conserved. A remarkable feature of Slovakian Pinzgau is the high number of alleles per locus (nine). This clearly suggests that this breed is highly variable, which is convenient from the aspect of its preservation in the genetic resources.

The HWE test showed that all loci deviated from HWE when analyzed across populations (data not shown). These deviations are likely caused by a small number of analyzed individuals and relatively high polymorphism at the loci investigated.

Breed relationships

Several genetic distance measures were applied to gene frequency data in order to estimate the level of similarity between respective breeds (see Material and Methods). Tables 3 and 4 show the
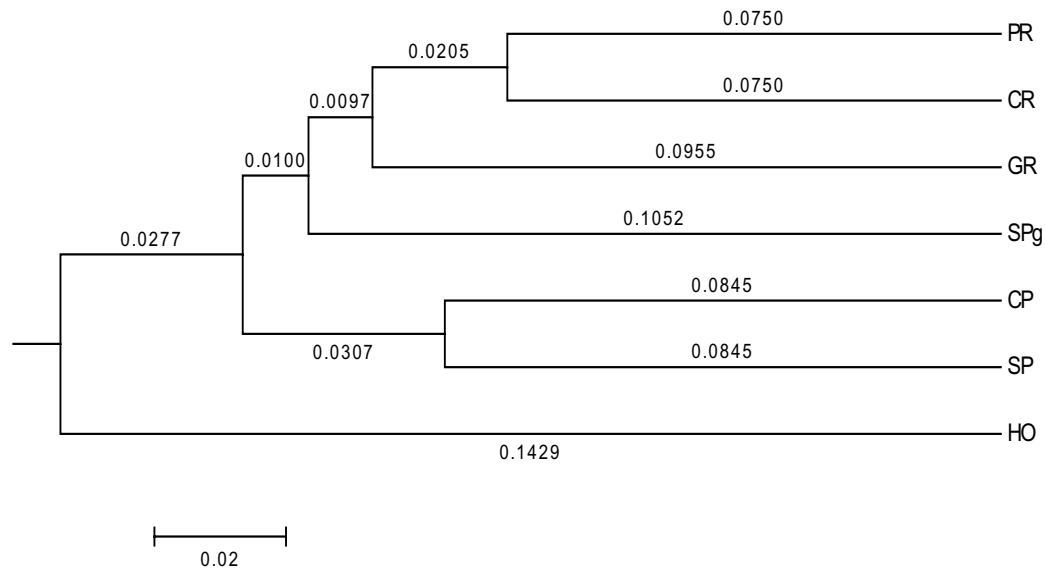
Figure 1. The phylogenetic tree constructed from the genetic distance measures of Nei (1972) by the UPGMA method (see the text). Branch lengths of the nodes are shown. The populations are Czech Pied (CP), Slovakian Pied (SP), Slovakian Pinzgau (SPg), Holstein (HO), Polish Red (PR), German Red (GR), and Czech Red (CR)

distances as quantified by the D_S, D_R, D_C, and D_A values. The UPGMA tree computed from Nei's standard genetic distances is shown in Figure 1. The phylogenetic reconstruction based on this approach assumes the validity of the infinite-allele model for microsatellites and is accurate when the evolutionary rate is comparable for all populations. We suppose that both these prerequisites hold for the breeds studied here. As expected, the black and white Holstein breed is found to be divergent from the remaining breeds (Figure 1). As for the lowest distance between breeds, the D_S and D_R results are slightly different from their counterparts based on the D_C and D_A measures. Thus, according to the D_S and D_R criteria the lowest distance is found between Czech Red and Polish Red, and the second lowest between Czech Pied and Slovakian Pied, while these results are swapped when estimated using the D_C and D_A data. However, the differences in the distances between CR and PR and between CP and SP are fairly small for all the measures (cf. Tables 3 and 4). Both the common origin of Central European Red breeds (i.e. CR, PR, and GR) and the fact that in the regeneration of CR Polish and German Red bulls are utilized, are clearly manifested in the genetic distances. There are only marginal differences in the respective D_S, D_R, D_C, and D_A data for these breeds and these

Table 3. Genetic distances between cattle populations, Nei's standard distance (1972, D_S) is above the diagonal and Roger's distance (1972, D_R) is below the diagonal

Breed	CP	SP	SPg	HO	PR	GR	CR
CP	–	0.102	0.181	0.374	0.198	0.208	0.171
SP	0.169	–	0.326	0.577	0.319	0.294	0.298
SPg	0.185	0.273	–	0.267	0.190	0.280	0.182
HO	0.267	0.347	0.245	–	0.332	0.420	0.332
PR	0.198	0.265	0.194	0.278	–	0.163	0.091
GR	0.219	0.270	0.242	0.306	0.198	–	0.151
CR	0.183	0.250	0.195	0.272	0.150	0.184	–

CP = Czech Pied, SP = Slovakian Pied, SPg = Slovakian Pinzgau, HO = Holstein, PR = Polish Red, GR = German Red, CR = Czech Red

Table 4. Genetic distances between cattle populations, Cavalli-Sforza and Edwards' distance (1967, D_C) is above the diagonal and the distance according to Nei at al. (1983, D_A) is below the diagonal

Breed	CP	SP	SPg	HO	PR	GR	CR
CP	–	0.633	0.669	0.683	0.671	0.665	0.669
SP	0.497	–	0.663	0.688	0.664	0.651	0.661
SPg	0.554	0.546	–	0.665	0.667	0.672	0.666
HO	0.578	0.589	0.550	–	0.672	0.677	0.673
PR	0.559	0.550	0.551	0.558	–	0.653	0.651
GR	0.550	0.531	0.561	0.569	0.528	–	0.652
CR	0.554	0.544	0.550	0.561	0.526	0.528	–

CP = Czech Pied, SP = Slovakian Pied, SPg = Slovakian Pinzgau, HO = Holstein, PR = Polish Red, GR = German Red, CR = Czech Red

values are among the lowest found for the seven breeds considered here (Tables 3 and 4). There is a very close similarity between Czech Pied and Slovak Pied breeds (see above). This is not surprising because the cattle were subjected to analogous breeding activities in the former Czechoslovakia. The similarity of Czech Pied and Czech Red breeds is less pronounced. This indicates that Czech Pied cattle diverged from Czech Red cattle, which was utilized in its grading-up in the past (cf. Central European Cattle Breeds). The Slovakian breeds are also quite divergent (Figure 1). This is an expected result, as Slovakian Pied are closely related to Simmental cattle, while Slovakian Pinzgau belong to the Pinzgauer group.

The genetic relationships were also studied by performing the principal component analysis of the metapopulation consisting of the complete set of allelic frequency data for all seven breeds (Figure 2).

The first three principal components explained 90% of the total variation, namely, the first component 71%, the second 12%, and the third 7%. The PCA results are consistent with the variations revealed by the analysis of the genetic distance matrices described above. Thus, the first principal component clearly separates Holstein and Slovakian Pied from the remaining breeds, and shows the very close similarity of Czech Red and Polish Red cattle. An analogous distribution is provided by the second principal component. In addition, the third component clusters the Czech Red, Polish Red and German Red breed together (cf. Figure 2).

CONCLUSION

An extensive set of animals belonging to seven Central European cattle breeds was studied by
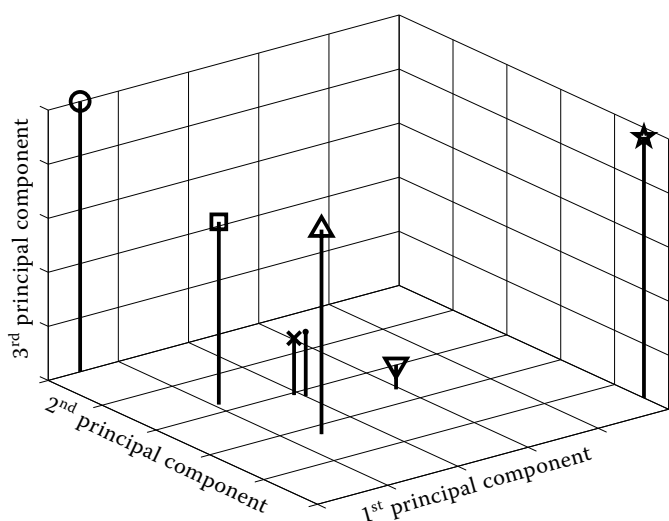

Figure 2. Principal component analysis based on allelic frequencies of 11 loci of Holstein (circle), Slovakian Pinzgau (square), Czech Pied (upward-pointing triangle), Polish Red (cross), Czech Red (point), German Red (downward-pointing triangle), and Slovakian Pied (five-pointed star) breeds

analyzing the microsatellite loci. The results were thoroughly analyzed and indicated that:

There is a close similarity between the Central European Red breeds, i.e. Czech Red, Polish Red, and German Red cattle.

There is a close similarity between Czech Pied and Slovakian Pied, i.e. within the Simmental group of cattle.

The most divergent breed is Holstein. Importantly, this breed is found to be the least variable one, which is caused by its very intensive human selection.

Czech Pied cattle exhibit the highest values of heterozygosity, polymorphic information content, and effective population size, which agrees with previous findings (Čítek and Řehout, 2001).

Of special interest are the results obtained for the Slovakian breeds because they were characterized by genetic markers for the first time. Slovakian Pinzgau are the most variable, while the Slovakian Pied have the smallest value of heterozygosity among the studied breeds.

REFERENCES

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F. (1996–2004): GENET:X 4.05, Logiciel Sous Windows (TM) Pour la Génétique des Populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France.

Botstein D., White R.L., Skolnick M., Davis R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Gen., *32*, 314–331.

Cavalli-Sforza L.L., Edwards A.W.F. (1967): Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Gen., *19*, 233–257.

Čítek J., Řehout V. (2001): Evaluation of the genetic diversity in cattle using microsatellites and protein markers. Czech J. Anim. Sci., *46*, 393–400.

Falconer D.S. (1989): Introduction to Quantitative Genetics. 3rd ed. Longman Group UK, Harlow, England.

Hansen C., Shrestha J.N.B., Parker R.J., Crow G.H., McAlpine P.J., Derr J.N. (2002): Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers. Genome, *45*, 897–904.

Hanslik S., Harr B., Brem, G., Schlötterer C. (2000): Microsatellite analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. Anim. Genet., *31*, 31–38.

Hartl D.L., Clark A.G. (1989): Principles of Population Genetics. 2nd ed. Sinauer Associates, Sunderland, MA, USA.

Janik A., Zabek T., Radko A. (2002): Identification of polymorphism at 11 microsatellite loci in Hereford cattle. Med. Weter., *58*, 867–870.

Maudet C., Luikart G., Taberlet P. (2002): Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis. J. Anim. Sci., *80*, 942–950.

Michener C.D., Sokal R.R. (1957): A quantitative approach to a problem in classification. Evolution, *11*, 130–162.

Nei M. (1972): Genetic distances between populations. Am. Nat., *106*, 283–292.

Nei M., Tajima F., Tateno Y. (1983): Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol., *19*, 153–170.

Raymond M., Rousset F. (1995): GENEPOP (vers. 1.2): Population genetics software for exact tests and ecumenicism. J. Hered., *86*, 284.

Rogers J.S. (1972): Measures of genetic similarity and genetic distance: Studies in Genetics VII. University of Texas, TX, USA.

SAS (1999–2001): SAS/STAT User's Guide. SAS Institute, Cary, NC, USA.

Schmid M., Saitbekova N., Gaillard C., Dolf G. (1999): Genetic diversity in Swiss cattle breeds. J. Anim. Breed. Genet., *116*, 1–8.

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