

# Relationships between the endangered Pustertaler–Sprinzen and three related European cattle breeds as analysed with 20 microsatellite loci

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## Summary

We estimated the genetic relationships between the endangered German Pustertaler–Sprinzen cattle breed and the Pinzgauer, Vosges and Simmental breeds – decided upon after consultation of the available historical literature. Within-breed diversity of the four breeds was also assessed. Twenty microsatellite markers were amplified in 27–50 unrelated individuals from populations of each breed. Within-breed variation was estimated from average heterozygosity values and mean number of alleles. Breed relationships were evaluated by genetic distance and a neighbour-joining tree was calculated from these estimates. Bootstrap resampling of loci tested the robustness of the tree topology obtained. A tree was also constructed from distance matrices using individual animals as operational taxonomic units. From both the average heterozygosity values and mean number of alleles calculated, the Pustertaler breed appears to be no more genetically impoverished than the other breeds analysed. The breed tree showed an 85% support for the Pustertaler–Pinzgauer grouping, and this result is echoed in the genetic distance values and allele-sharing individual tree.

**Keywords:** breed relationships, European cattle, genetic conservation, genetic diversity, microsatellite

To determine which breeds should be selected for conservation, it is necessary to devise objective criteria with which to assess variability, especially as breed conservation is a complex problem and involves assessing several contributing factors; for example, cost of implementation, and cultural and economic importance of the breeds involved. Although criticism has

recently been levelled at the reliability and relevance of microsatellite markers for conservation programmes (Ruane 1999), the results obtained from such surveys can provide useful insights into population structures and may help address specific conservation questions. When using microsatellite data, it is important not only to look at ‘taxonomic distinctiveness’ (Hall & Bradley 1995) and between population variation, but also at within-population diversity, as measured by the average heterozygosities and mean numbers of alleles calculated from microsatellite analyses. World-wide, there are more than 780 cattle breeds, with 305 of these being native to Europe (FAO 1995). It has been shown by microsatellite methods that, despite gene flow between populations, European cattle represent separate gene pools and possess unique combinations of alleles (MacHugh *et al.* 1994, 1998). This is most likely due to the introduction of herd books and selective breeding in the early 19th century and more recent breeding technology.

The Pustertaler cattle are an authentic Short-headed breed of cattle originating in the Puster Valley of the South Tyrol. The breed declined in the late 19th century and were subsequently outcrossed with both Simmental and Pinzgauer cattle (Feliuss 1995) with a view to increasing breed size. The total population size was estimated at 60 individuals in 1994, and the last Pustertaler animals are now seriously endangered and enjoy protected status under the ‘critically-maintained’ World Watch List of the Food and Agriculture Organisation (FAO 1995). Animals from the Vosges breed were included in this study, as it has been suggested that this breed originated from cattle introduced during the Napoleonic era (Sambraus 1992), and others believe Vosges to represent the ancestral Pustertaler population (J. Knox, personal communication).

Genotype data, from 20 microsatellite DNA markers typed in 138 animals, have been

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**Table 1.** Breeds included in the analysis and their country of origin, including the number of individuals sampled (in parentheses). The average heterozygosities and mean number of alleles (MNA) are also included

Breed	Origin of breed (No. of animals)	Average heterozygosity		MNA
		Expected	Observed	
Pustertaler	Italy (24) Germany (7)	0.69 ± 0.03	0.63 ± 0.03	5.3
Pinzgauer	Germany (20) Austria (10)	0.71 ± 0.03	0.67 ± 0.04	6.0
Vosges	France (27)	0.68 ± 0.03	0.68 ± 0.04	5.4
Simmental	France (50)	0.58 ± 0.04	0.56 ± 0.04	5.2

analysed here to assess the population structure of these European cattle breeds. Hair or blood samples were collected from between 27 and 50 individuals from each of the four breeds: Pustertaler, Pinzgauer, Vosges and Simmental. As far as could be ascertained, unrelated animals were chosen, and genomic DNA was extracted as described previously (Loftus *et al.* 1999). The breeds, their geographic origins and sample sizes involved are shown in Table 1. The 20 microsatellites chosen for this analysis were: *BM1818*, *BM2113*, *BM1824*, *CSSM66*, *CSRM60*, *ETH3*, *ETH10*, *ETH152*, *ETH225*, *HEL1*, *HEL5*, *HEL9*, *HEL13*, *ILSTS005*, *ILSTS006*, *INRA005*, *INRA023*, *INRA032*, *INRA035*, and *INRA063*. This represents a panel of 20 loci from 30 markers agreed by the International Society for Animal Genetics (ISAG, Tours, France, July 1996) from a collaborative study of variability in European cattle ([http://www.ri.bbsrc.ac.uk/cdiv\\_www/homepage.htm](http://www.ri.bbsrc.ac.uk/cdiv_www/homepage.htm)). The data from the Simmental breed have been published previously (Schmid *et al.* 1999).

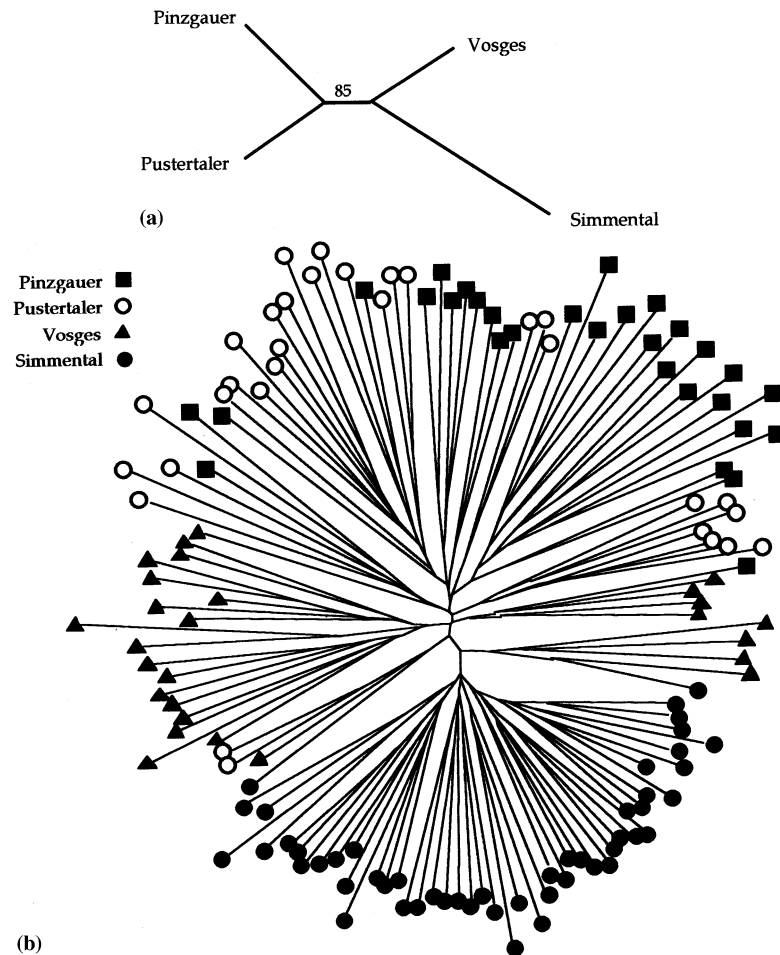
To assess the levels of variability at the DNA level in the four breeds, both the mean number of alleles (MNA) and gene diversity (average heterozygosity) values were measured. Unbiased estimates of expected gene diversity, with associated standard errors, were calculated according to Nei (1987), and observed heterozygosity values were calculated from the data. Allele frequencies were determined by direct counting. Interbreed genetic distances (*Da*) were estimated as described in Nei *et al.* (1983) (Table 2) using the DISPAN computer programme (T. Ota, Center for Human Genetics, Boston University). *Da* distances are considered the most appropriate with which to obtain correct tree topology from microsatellite data (Takezaki & Nei 1996), especially when drift is an issue. The neighbour-joining method (Saitou & Nei 1987) was used to construct a dendrogram

of breed relationships from the genetic distances (Fig. 1a) using the PHYLIP package (Felsenstein 1989), and a bootstrap value was generated by resampling loci over 1000 replicates. Another neighbour-joining tree was constructed using the allele-sharing statistic suggested by Bowcock *et al.* (1994) (Fig. 1b), with individual animals acting as operational taxonomic units (OTUs).

The average heterozygosities, MNA and genetic distances computed here (Tables 1 and 2) are similar to those obtained with other European cattle breeds (MacHugh *et al.* 1994). From both the former values it appears that the Pustertaler breed exhibits a similar level of genetic variability to other breeds. The average heterozygosities and MNA values for the Simmental and Vosges cattle are comparable to those obtained from similar microsatellite methods by MacHugh *et al.* (1997) and Moazami-Goudarzi *et al.* (1997), respectively. The topology of the breed tree (Fig. 1a) indicates strong support for the Pustertaler breed being more closely-related to the Pinzgauer breed (a bootstrap value of 85% with respect to the loci sampled); a grouping which is consistent with historical breed data (Feliuss 1995). The results from both neighbour-joining trees (Fig. 1a,b) are concordant with the conventional view that the Pinzgauer contributed to the gene pool of the Pustertaler breed in the past. However, a remarkable level of tree structure can be seen from

**Table 2.** Genetic distance (*Da*) estimates between Pustertaler and each of the other three breeds

Breed Grouping	Genetic Distance ( <i>Da</i> )
Pustertaler–Pinzgauer	0.1202
Pustertaler–Vosges	0.1240
Pustertaler–Simmental	0.1974



**Fig. 1.** Neighbor-joining dendrograms constructed from: **a**, genetic distances among the four cattle breeds; **b**, allele-sharing distances among the 138 individual animals. Both the breed tree and the individual tree suggest a closer relationship between the Pustertaler and the Pinzgauer breeds.

the allele-sharing measure tree (Fig. 1b); with 48 of the 50 Simmental animals forming a tight cluster. Of the other three breeds, definite clusters can be distinguished; 21 of the 31 Pustertaler animals form two exclusive groups, but by concentrating on the internal nodes it can be seen that, on the whole, this breed segregates with Pinzgauer individuals, as would be hypothesised from both the breed tree (Fig. 1a) and historical information. MacHugh *et al.* (1998) found a similar level of clustering among seven European cattle breeds using this microsatellite allele-sharing distance. The data were also analysed with principal component analysis (PCA). The results are not shown here but they were consistent with those obtained from the neighbour-joining trees.

The overall level of genetic variability in the Pustertaler breed is not significantly reduced compared to the three breeds, or indeed when compared to other European breeds (MacHugh *et al.* 1994). This may be expected due to the known outcrossing with Pinzgauer and Simmen-

tal in the past. Although there is a difference between the expected and observed heterozygosity values for Pustertaler, this does not appear to be significant when compared to the corresponding values from the other three breeds. From the analysis of the Pustertaler breed and the three breeds considered to be most closely associated, it clusters most closely with the Pinzgauer breed.

The genotypes obtained in this study have been submitted to the Roslin Cattle Diversity Database ([http://www.ri.bbsrc.ac.uk/cdiv\\_www/homepage.htm](http://www.ri.bbsrc.ac.uk/cdiv_www/homepage.htm)).

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## References

- Bowcock A.M., Ruiz-Linares A., Tomfohrde J., Minch E., Kidd J.R. & Cavalli-Sforza L.L. (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* **368**, 455–7.
- Felius M (1995) *Cattle Breeds – An Encyclopaedia*. Misset, Doetinchem, The Netherlands.
- Felsenstein J. (1989) PHYLIP-Phylogeny Inference Package (Version 3.2). *Cladistics* **5**, 164–6.
- Food and Agriculture Organisation of the United Nations (FAO) (1995) In: *World Watch List for Domestic Animal Diversity* (ed. by B Scherf), 2nd edn. Food and Agriculture Organisation (FAO), Rome.
- Hall S.J.G. & Bradley D.G. (1995) Conserving livestock breed biodiversity. *Trends in Ecology and Evolution* **10**, 267–70.
- Loftus R.T., Ertugrul O., Harba A.H., El-Barody M.A.A., MacHugh D.E. & Bradley D.G. (1999) A microsatellite survey of cattle from a centre of origin: the Near East. *Molecular Ecology* **8**, 2015–22.
- MacHugh D.E., Loftus R.T., Bradley D.G., Sharp P.M. & Cunningham E.P. (1994) Microsatellite DNA variation within and among European cattle breeds. *Proceedings of the Royal Society, London* **256**, 25–31.
- MacHugh D.E., Shriver M.D., Loftus R.T., Cunningham P. & Bradley D.G. (1997) Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics* **146**, 1071–86.
- MacHugh D.E., Loftus R.T., Cunningham P. & Bradley D.G. (1998) Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Animal Genetics* **29**, 333–40.
- Moazami-Goudarzi K., Laloë D., Furet J.P. & Grosclaude F. (1997) Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. *Animal Genetics* **28**, 338–45.
- Nei M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Nei M., Tajima F. & Tateno Y. (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* **19**, 153–70.
- Ruane J. (1999) A critical review of the value of genetic distance studies in conservation of animal genetic resources. *Journal of Animal Breeding and Genetics* **116**, 317–23.
- Saitou N. & Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–25.
- Samraus H.H. (1992) *A Colour Atlas of Livestock Breeds*. Wolfe Publishing Ltd., London.
- Schmid M., Saitbekova N., Gaillard C. & Dolf G. (1999) Genetic diversity in Swiss cattle breeds. *Journal of Animal Breeding and Genetics* **116**, 1–8.
- Takezaki N. & Nei M. (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**, 389–99.