

## Genetic diversity in Bhutanese yak (*Bos grunniens*) populations using microsatellite markers

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### Summary

Eight cattle microsatellite markers were used for genetic analyses of three Bhutanese yaks (*B. grunniens*) populations (western, central and eastern). There was substantial genetic variability within yak populations, with average heterozygosity range of 0.644 to 0.680. Neighbour-joining tree constructed from Nei's standard genetic distances ( $D_s$ ; Nei 1972) grouped western and central Bhutan yaks in one clade ( $D_s = 0.01$ ) separate from eastern Bhutan yaks ( $D_s = 0.20$  and  $0.27$ , respectively). The genetic distances between the yak from eastern Bhutan and the other two regions suggest that the populations have been separated for at least 4000 years and that they have exchanged <2 migrants per generation. Based on these results, Bhutanese yak populations are categorised into two types: 1) western and central Bhutan yaks, and 2) eastern Bhutan yaks. Implications of these findings on yaks conservation and breeding programmes are discussed.

**Keywords:** Bhutan, conservation, genetic distance, genetic diversity, heterozygosity, yak

### Introduction

Knowledge of the genetic relationships among breeds or populations of yak will be useful in planning for conservation of yak genetic resources, in designing breed comparison experiments and in predicting heterosis in crosses between breeds (Cai and Wiener 1995). Populations, which are genetically very different, should be considered for separate conservation. The genetic relationships among populations can be estimated from gene frequencies at microsatellite loci. The aim of this paper was to assess the genetic diversity within Bhutanese yak populations.

### Materials and methods

A total of 169 yaks were sampled from three yak populations (western Bhutan 106, central Bhutan 32 and eastern Bhutan 31). No more than one-tenth of each herd or village population was sampled to ensure that the animals sampled were as far as possible unrelated. The eight cattle microsatellite loci studied were TGLA53, TGLA122, TGLA73, AGLA293, BM2113, BM1824, CSSM066 and ETH3. PCR amplification was performed on an OmniGene Thermal Cycler (Hybaid, UK). The number of alleles per loci, observed heterozygosity ( $H_o$ ) and the unbiased estimates of heterozygosity ( $H_e$ ) were estimated using computer package Biosys-1 (Swofford and Selander 1989). Dendrogram was constructed using the neighbour-joining (NJ) method (Saitou and Nei 1987). Nei's standard distances ( $D_s$ ; Nei 1972), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ), neighbour-joining trees and bootstrap values were computed using the computer package DISPAN (Ota 1993). The software MICROSAT version 1.4 (Minch 1996) was utilised for estimating fixation index ( $F_{st}$ ) of Reynolds et al. (1983). From simulation studies,  $D_A$  (revised Nei's genetic distance; Nei et al. 1983) has been shown to be superior for clarifying the evolutionary relationship of closely related populations, however,  $D_s$  was more appropriate for estimating evolutionary time (Takezaki and Nei 1996). As in the present data set there was no apparent difference in the tree topology obtained by  $D_A$  and  $D_s$ , only the standard genetic distance of Nei (1972), was used. Approximate divergence time between yak populations was estimated by substituting distance values in the equation,  $D_s = 2\alpha t$  (Nei 1976), where  $t$  is the time of divergence between populations and  $\alpha$  is the mutation rate. The value of  $\alpha$  was assumed to be  $1.1 \pm 0.5 \times 10^{-4}$  as derived by Crawford and Cuthbertson (1996) for microsatellite loci in sheep. The number of effective migrants per generation ( $Nm$ ) was calculated from the equation  $E(F_{st}) = 1/(1 + 4Nm)$  (Reynolds et al. 1983).

## Results

Genetic variability parameters are presented in Table 1. There was no significant difference in the mean number of alleles per locus among the three yak populations. Similarly, the heterozygosity values among yak populations were not significantly different.

**Table 1.** Genetic variability parameters in yak populations.

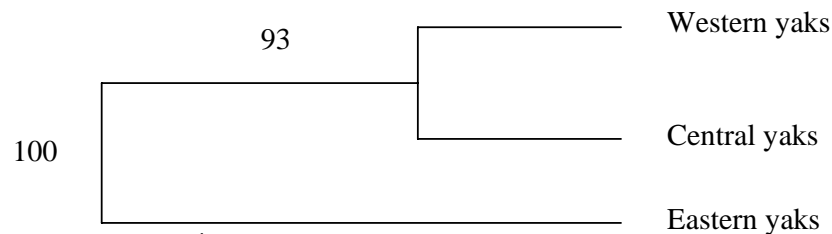
Populations	MNA	SE	Mean heterozygosity			
			<i>H<sub>o</sub></i>	SE	<i>H<sub>e</sub></i>	SE
Western region yak	7.6	1.1	0.621	0.052	0.674	0.053
Central region yak	6.1	0.8	0.598	0.042	0.680	0.051
Eastern region yak	4.8	0.6	0.601	0.069	0.644	0.044

Notes: MNA = Mean number of alleles per loci; SE = standard error; *H<sub>o</sub>* = observed heterozygosity; *H<sub>e</sub>* = expected heterozygosity (Nei 1987).

The genetic distance (*D<sub>s</sub>*) values were 0.017 (*SE*: 0.009), 0.20 (*SE*: 0.04) and 0.27 (*SE*: 0.08) between western and central, western and eastern, and central and eastern populations, respectively. The *F<sub>st</sub>* values were 0.013 (*SE*: 0.005), 0.09 (*SE*: 0.018) and 0.11 (*SE*: 0.03) between western and central, western and eastern, and central and eastern populations, respectively. The dendrogram in Figure 1 supports the divergence of eastern Bhutan yaks from other two populations with a bootstrap value of 93%. The divergence of western and central region yaks occurred comparatively more recently (<1000 years ago) while eastern Bhutan yak population have diverged from western and central populations of yak between 4000 and 16,000 years ago, respectively. *N<sub>m</sub>* estimates as 1.8, 2.5 and 19 between eastern and central, eastern and western, and western and central yak populations, respectively.

## Discussion

The mean number of alleles per loci and heterozygosity values were not significantly different among the three yak populations. The high heterozygosity values indicate that inbreeding may not be a problem at the population level. However, observed heterozygosity was always lower than the expected ones (Table 1). It could be due to population subdivision in each region, local inbreeding or the presence of null alleles. Population subdivision can occur because of the geographical isolation of yak herds. The rugged topography and mountain barriers limit movement of yaks and impose reproductive isolation. The local inbreeding might be enhanced by the dominance behaviour of the yak bull that prevents other bulls from mating (Steane 1997; Wiener 1997). Furthermore, we conceived that non-amplifying alleles might be segregating at some of the loci as all the microsatellites used were isolated in cattle *B. taurus*.



**Figure 1.** Neighbour-joining tree of yak populations based on *D<sub>s</sub>*. Figures at the nodes are bootstrap values of 1000 resampling with replacement.

The most striking result is the higher genetic distance values, which separate the eastern region yaks from the western and central yak populations. Oral history mentions that the origin of eastern region herders could be traced to Tibet. Evidences from this study suggest that the eastern Bhutan yak population have diverged from the western and central populations of yaks between 4000 and 16,000 years ago. If correct, the eastern yak population would have diverged from the other yak populations before or around the time of domestication. Alternatively, eastern region yaks might have incorporated genes from geographically distinct strains of wild yak populations or through

hybridisation with local cattle.

A common origin in Tibet could explain the close genetic similarity observed between the central and western yak populations. Also, it could be explained by genetic admixture following migration of individuals from one population to the other.

### Closing remarks

We can categorise Bhutan's yak population into two groups. Western and central region yaks could be considered as single population, distinct from the eastern Bhutan yaks. This result is important for developing a strategy for conservation purposes or designing a breeding programme. The eastern region yaks represent a unique gene pool and therefore a separate conservation policy is needed. The differences in genetic distances indicate that significant heterosis may not be achieved from the current practice of crossing between central and western region yaks.

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