Genetic Diversity of Indigenous Cattle Populations in Bhutan: Implications for Conservation

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ABSTRACT : The Genetic diversity and relationship of native Siri (Bos indicus) cattle populations of Bhutan were evaluated using 20 microsatellite markers. A total of 120 Siri cattle were sampled and were grouped into four populations according to their geographical locations which were named Siri West, Siri South, Siri Central and Siri East cattle. For each, 30 individuals were sampled. In addition, 30 samples each of Indian Jaba (B. indicus), Tibetan Goleng (B. taurus), Nepal Hill cattle (B. indicus), Holstein Friesian (B.taurus) and Mithun (B. frontalis) were typed. The mean number of alleles per loci (MNA) and observed heterozygosity (Ho) were high in the Siri populations (MNA=7.2±0.3 to 8.9±0.5 and Ho=0.67±0.04 to 0.73±0.03). The smallest coefficient of genetic differentiation and genetic distance (F_{ST}=0.015 and D_A=0.073) were obtained between Siri West and Siri Central populations. Siri East population is genetically distinct from the other Siri populations being close to the Indian Jaba (F_{ST}=0.024 and D_A=0.084). A high bootstrap value of 96% supported the close relationship of Siri South, Siri Central and Siri West, while the relationship between Siri East and Jaba was also supported by a high bootstrap value (82%). Data from principal component analysis and individual assignment test were in concordance with the inference from genetic distance and differentiation. In conclusion we identified two separate Siri cattle populations in Bhutan at the genetic level. One population included Siri cattle sampled from the West, Central and South of the country and the other Siri cattle was sampled from the East of the country. We suggest that Siri cattle conservation program in Bhutan should focus on the former population as it has received less genetic influence from other cattle breeds. *(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 7 : 946-951)*

Key Words : Siri, Cattle, Bhutan, Genetic Relationships

INTRODUCTION

The Siri cattle population is the most important livestock resource in Bhutan, providing milk, meat, draft power and manure. A recent census counted 2,23,847 Siri cattle in Bhutan (DALSS, 2001). Recognizing the value of Siri for Bhutanese conditions, the Government of Bhutan has initiated a conservation program for it. As an initial step, a nucleus herd was formed in East Bhutan with animals originating from the remote Sombeykha village in the Haa district of Western Bhutan. The decision to consider Siri West as the conservation unit was based on oral history that traces Sombeykha as the original home tract of Siri and the general belief in Bhutan that pure Siri are found there (Bourgeois-Luethi, 1999). Presently, plans are formulated to implement an open nucleus-breeding scheme.

Remarkable diversity at the phenotypic level among Siri populations in different parts of the country, however leads to the followings questions regarding the genetic background of the Siri cattle from the South, Central and East of Bhutan; the decision to restrict the conservation unit to Siri West population and the implications of an open nucleus-breeding scheme in the conservation herd located in East Bhutan.

Microsatellite markers have proven to be useful for the detection of genetic differentiation and in assessing genetic relationships among different livestock species and populations (MacHugh et al., 1998; Martinez et al., 2000; Pandey et. al., 2002). Its extensive capability to distinguish among individuals has also been utilized successfully for assigning breed identities of anonymous samples into their sources of origins (Diez-Tascon et al., 2000; Bjr∅nstad and R∅ed, 2001). Use of DNA markers to determine genetic variations and relationships among populations provides the best available tools to generate objective criteria for conservation management of animal genetic resources (Rege, 1996).

The primary aim of this study was to quantify breed diversity and differentiation in Siri cattle sampled in four different Bhutanese locations. Its second objective was to investigate the relationship of Siri with other local cattle breeds present in Bhutan (Jaba, Goleng) and in the neighbouring country (Nepal Hill cattle). The results from this study will provide inputs for policy decisions on the need of conservation; formulating conservation strategies and programs and further use of Siri populations in crossbreeding programs.
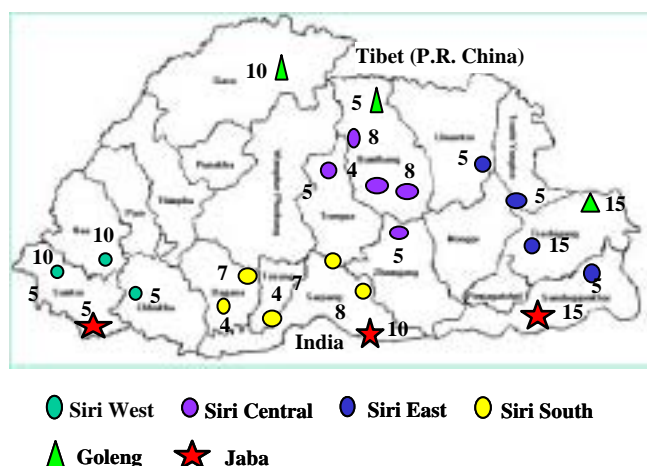
Figure 1. Map of Bhutan showing the sampling locations. The figures indicate the number of individuals sampled.

Table 1. Comparison of genetic variability of four Siri populations of Bhutan, Goleng, Jaba, Nepal Hill cattle, Holstein Friesian and Mithun

Population	[a]MNA (SE)	[b]Ho (SE)	[c]He (SE)
Siri west (SW)	7.2 (0.3)	0.729 (0.044)	0.718 (0.026)
Siri south (SS)	8.4 (0.4)	0.711 (0.044)	0.742 (0.027)
Siri central (SC)	7.9 (0.4)	0.730 (0.033)	0.749 (0.021)
Siri east (SE)	8.9 (0.5)	0.673 (0.048)	0.753 (0.032)
Nepal hill cattle (NH)	9.1 (0.4)	0.716 (0.045)	0.796 (0.018)
Jaba (JB)	7.8 (0.4)	0.641 (0.055)	0.707 (0.038)
Goleng (GO)	8.5 (0.5)	0.643 (0.056)	0.745 (0.038)
Holstein friesian (HF)	5.8 (0.5)	0.571 (0.044)	0.699 (0.021)
Mithun (MN)	7.1 (0.5)	0.585 (0.057)	0.667 (0.028)

[a] Mean number of alleles per locus; [b] Observed heterozygosity; [c] Expected heterozygosity.

MATERIALS AND METHODS

Samples and laboratory procedures

Blood or hair samples were taken from 30 individuals of each of four Siri (B. indicus) populations (Siri West, Siri South, Siri Central and Siri East) in Bhutan (Figure 1). Sampling was done in several distantly located herds to ensure that the animals sampled were as unrelated as possible. Another 30 individuals each of Tibetan dwarf cattle Goleng (B. taurus), Indian Jaba (B. indicus), Nepal Hill cattle (B. indicus), Mithun (B. frontalis) and Holstein Friesian were typed as reference breeds. The Holstein Friesian samples were provided by the Medigenomix laboratory in Germany, where the genotyping was carried out.

The DNA was extracted from hair or blood using the DNA extraction kit from Qiagen according to the manufacturer's instructions. The 20 microsatellite markers used in this study were: BM1824, BM2113, INRA023, ETH10, ILSTS006, ETH225, MM12, INRA032, INRA037, CSSM66, HEL1, SPS115, INRA035, TGLA53, TGLA227, ETH3, TGLA126, TGLA122, HAUT24 and HAUT27. The details regarding primers sequences and chromosomal locations and the references describing the selected markers can be found at http://www.projects.roslin.ac.uk/cdiv/markers.html. The PCR conditions were accordingly to the original publication describing the markers chosen.

The PCR products were separated on polyacrylamide gels using the ABI Sequencer 377 automated DNA sequencer and ABI ROX 500 as the internal size standard. The software GeneScan[TM] 672 (ver 2.0.2, Applied Biosystems) and Genotyper[TM] (version 2.0) were used to analyse the results. The allele frequency data and the individual genotype data are available from the corresponding author.

Data analysis

Allele frequencies were calculated using the Program Excel Microsatellite Toolkit (http://oscar.gen.tcd.ie/~sdepark/ms-toolkit/) (Park, 2001). The genetic diversity of each population was assessed by calculating the mean number of alleles per loci (MNA), the observed heterozygosity (Ho), and the expected heterozygosity (He) using the software Biosys 1 (Swofford and Selander, 1989). Departure from Hardy-Weinberg (HW) and linkage equilibrium expectations was tested using GENEPOP version 3.3 (Raymond and Rousset, 1995).

Genetic distances (D_A, Nei et.al., 1983) between populations were calculated and used to construct a phylogenetic tree with the neighbour joining algorithm (Saitou and Nei, 1987). The software Dispan (Ota, 1993) was used for the purpose. In addition, Genetic distance of the Reynolds et al. (1983) (F_{ST}) index, measure of gene differentiation, was estimated using the Microsat 1.5 d (Minch et al., 1998). The tree was visualized using Treeview v1.5 (Page, 1996). To obtain a summary of breed relationships based directly on allele frequencies, a principal component analysis was performed for all populations and after excluding Mithun and Holstein Friesian from the data set using the program XLSTAT (ver 4.3, http://www.xlstat.com). The software WHICHRUN 3.2 (Banks and Eichert, 2000) was employed to obtain a maximum likelihood estimate of individual assignment to its source population.

RESULTS

Genetic variation

The mean number of alleles per loci (MNA) and heterozygosity estimates computed across 20 loci for each population are shown in Table 1. Nepal Hill cattle showed the highest MNA of 9.1±0.4. The MNA among Siri populations were not significantly different but were higher compared to the MNA in Holstein Friesian (5.8±0.5).

Table 2. Genetic distance between four Siri populations of Bhutan (SW: Siri west, SS: Siri south, SC: Siri central, SE: Siri east), Jaba (JB), Goleng (GO), Nepal hill cattle (NH), Mithun (MN) and Holstein friesian (HF) calculated using D_A (Nei et al., 1983) below the diagonal and Reynold et al. (1983) F_{ST} above the diagonal

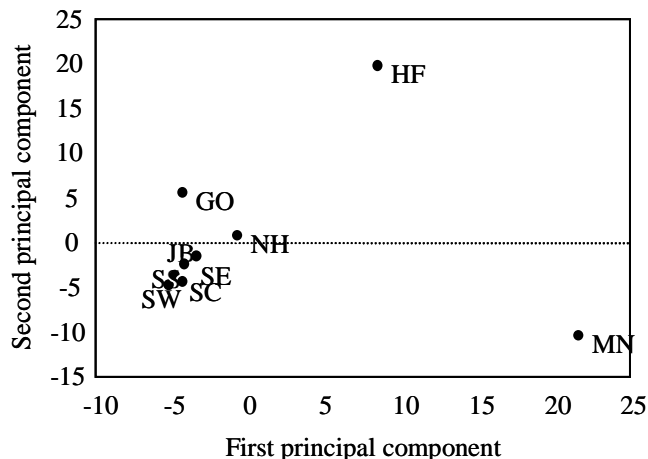|	SW	SS	SC	SE	NH	JB	GO	HF	MN
SW	-	0.022	0.015	0.053	0.070	0.054	0.106	0.233	0.265
SS	0.083	-	0.020	0.041	0.050	0.051	0.098	0.218	0.250
SC	0.073	0.084	-	0.044	0.059	0.061	0.096	0.212	0.241
SE	0.136	0.130	0.126	-	0.046	0.024	0.059	0.203	0.228
NH	0.191	0.151	0.163	0.139	-	0.068	0.069	0.161	0.210
JB	0.128	0.126	0.137	0.084	0.163	-	0.075	0.249	0.269
GO	0.213	0.199	0.205	0.147	0.178	0.153	-	0.183	0.265
HF	0.495	0.465	0.478	0.427	0.378	0.482	0.352	-	0.322
MN	0.508	0.484	0.424	0.406	0.426	0.485	0.503	0.646	-

Figure 2. Neighbour joining dendogram showing the genetic relationships among four Siri populations, Jaba, Goleng, Nepal Hill cattle, Holstein Friesian and Mithun. The tree is based on Nei et al. (1983) genetic distances. The numbers indicate bootstrap values in percentage (1,000 replicates).

Figure 3. PC of allele distribution for all 30 individuals from each of the populations investigated. The first and the second PC summarized 37% and 27% of the total variation, respectively.
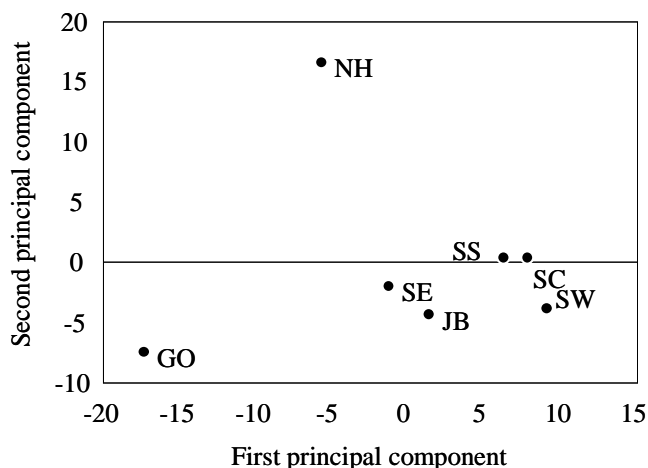
Figure 4. PC of allele distribution for all 30 individuals from each of the cattle populations investigated excluding Mithun and Holstein Friesian. The first and the second PC summarized 29% and 21% of the total variation, respectively.

Lower observed heterozygosity (Ho=(0.673±0.04) was obtained in East Siri cattle. However, its value was still higher than that of Holstein Friesian, (Ho=0.571±0.04). Mithun and Goleng show low MNA and observed heterozygosity (Table 1).

All loci (except BM1824, TGLA126 and TGLA1227) deviated from HW (p<0.005). However, there was no systematic deviation of one locus in all populations or one population across all loci (data not shown).

Genetic distances and breed relationships

The values of genetic distance D_A and gene differentiation index are presented in Table 2. The smallest genetic distance and coefficient of differentiation were obtained between Siri West, Siri Central and Siri South populations. Siri East was the most distinct of the Siri population being closer to the Indian Jaba than to any other Siri populations. As expected, Holstein Friesian and the Mithun are clearly separated from the other investigated populations (Table 2).

The dendrogram constructed from the D_A distance is shown in Figure 2. Three clusters are identified, the B. taurus cluster (Holstein Friesian and Tibetan Goleng), the B. indicus (Bhutan Siri and Jaba) cluster with the exception of the Nepal Hill cattle and the Mithun (B.frontalis). The Nepal Hill cattle is linked to the taurine cattle B. taurus although the relationship is supported with a low bootstrap value (31%). Within the Bhutanese Siri sub cluster, the separation of Siri East from the other Siri populations was supported with a high bootstrap value of 82%. A high bootstrap value of 96% was also obtained for supporting Siri South, Siri Central and Siri West on the same cluster.

The principal component scores for the first two components are plotted in Figure 3. The first component, which accounted for 37% of the total variation and the second component, 27% of the variation, separated clearly

Table 3. Population assignments of individuals from four Siri populations from Bhutan, Goleng, Jaba, Nepal Hill cattle, Holstein Friesian and Mithun using genotype information of 20 microsatellite markers with log of odds exceeding two

Popul-ations	n	SW	SS	SC	SE	NH	JB	GO	HF	MN	% Errors
SW	30	28	1	0	0	0	1	0	0	0	7
SS	30	4	24	2	0	0	0	0	0	0	20
SC	30	3	1	26	0	0	0	0	0	0	13
SE	30	0	0	0	27	0	3	0	0	0	10
NH	30	0	1	0	0	29	0	0	0	0	3
JB	30	0	0	0	0	0	30	0	0	0	0
GO	30	0	0	0	1	0	0	29	0	0	3
HF	30	0	0	0	0	0	0	0	30	0	0
MN	28	0	0	0	0	0	0	0	0	28	0

the Mithun and Holstein Friesian from the other populations. To analyse more particularly the relationship among the Siri populations, a second PC analysis was performed (Figure 4) excluding the Mithun and the Holstein Friesian populations. The first component accounted for 29% of the total variation and the second component 21%. The Goleng and Nepal Hill cattle are now clearly separated from the other populations and the Siri cattle populations separated in two groups, with Siri East being closer to Jaba, while Siri West, Siri Central and Siri South are clustering together (Figure 4).

The data from the population assignment test obtained using WHICHRUN is shown in Table 3. The misclassification error rate among the Siri populations ranged from 7% in Siri West to 20% in Siri South. The misallocated individuals from Siri Central, Siri West and Siri South were mostly placed within these three populations while the ambiguous samples from Siri East were misclassified in the Jaba population.

DISCUSSION

Genetic variability within populations

The MNA and heterozygosity (Ho and He) were higher for Siri populations (Table 1) compared to selected Holstein Friesian. This indicates that the Siri populations have remained fairly outbred. High MNA and heterozygosity in the Nepal Hill cattle could be due to gene flow from crossing with introduced Jersey and Brown Swiss (Shrestha, 1998). Genetic evidences for such crossing are supported by the result of the dendrogram, which placed the Nepalese cattle with the two taurine.

The low observed heterozygosity in Goleng and Mithun is not surprising as their population sizes in Bhutan are less than 500 (DALSS, 2001). Pure Mithun breeding in Bhutan takes place in only two government farms and the bulls are distributed to the farmers. The source of Mithuns in Bhutan is the north-eastern states of India where they are reared as semi wild animals (Payne and Hodges, 1997). Increase in

human activities that encroaches its habitat has lead to reduced Mithun population size and exposure to inbreeding (Gupta and Gupta, 2000). The Tibetan Goleng is mainly found in the high altitude areas of Bhutan bordering Tibet and it is used to cross with yak (Winter and Tshewang, 1989). However, Goleng bulls have become rare in Bhutan, following the official closure of the boundary with Tibet. Despite its low *Ho*, the high MNA and *He* observed in Goleng cattle may be suggestive of some gene flow from yak.

Deviations from HW could be due to non-random mating, wrong genotyping or population sub division (MacHugh, 1996). It may also be the effect of non-amplifying alleles segregating at some of the loci (Primmer et al., 1996). In our study, the most likely explanation of HW disequilibrium observed in our Siri populations is population sub-division following sampling from a range of distinct locations within the same broad geographic area (Figure 1).

Genetic relationships among populations

The main focus of this study was to determine the genetic relationships among Siri populations from four different regions of Bhutan. All the analysis used showed close genetic relationships among Siri West, South and Central cattle, while Siri East was genetically closer to Jaba cattle than to other Siri populations. Siri cattle supposedly originated from Haa Sombeykha or Paro districts in West Bhutan where still today it is thought that the purest Siri cattle are found (Som, 1958; Bourgeois-Luethi, 1999). The close genetic similarity between Siri West, South and Central could be a consequence of the migratory system of cattle rearing in Bhutan that encourages genetic admixture (Tshering, 1995). In addition, the diffusion of Siri cattle in the other parts of the country supposedly followed the trading of bulls for horses and mules particularly with farmers from Central Bhutan (Arbenz and Tshering, 2000).

Several factors likely explain the separation of East Siri cattle from the other Bhutanese populations. First, it is believed that hybridization of Siri cattle with Mithun was common in the Eastern part of the country. Also, Jaba cattle, are readily available from neighboring Assam province in India. They represent an ideal replacement stock for the East Bhutan cattle owners being cheaper to buy, with progenies of Siri East crossed with Jaba further bred to Mithun. These would explain the close relationship of Siri East cattle to Jaba. Interestingly Siri South did not seem to have a substantial influence from Jaba (Figure 4) although Jaba cattle are also present in this part of the country (Figure 1). It appears that farmers in South Bhutan do not cross Jaba and Siri. Indeed, the F1 males are of small body conformations with small humps and perform poorly for bullock purposes (Arbenz and Tshering, 2000). Crossing

with Mithun is also not widespread in South Bhutan due to limited access to Mithun bulls.

Analyses based on likelihood estimates from microsatellite data allow identifying the most likely populations of origins of single individual (Buchanan et al., 1994; Bjrnstad, G. and K. H. Roed, 2001). The same approach applied in this study allocated the majority of Siri cattle to their populations of origins (Table 3). Interestingly, the three miss-assigned Siri West individuals were classified as Jaba animals whereas all but one other miss-assigned Siri animals were included in other Siri populations. These results are in agreement with the outcomes of the phylogenetic and PC analysis.

Felius (1996) considered Siri as the only cervico-thoracic humped zebu in the Eastern Himalayas, which is a stabilized crossbreed of B. indicus with B. taurus. Indian indigenous cattle are all B. indicus type and described as thoracic humped cattle (Payne and Hodges, 1997). Our phylogenetic (Figure 2), PC (Figure 3) and individual assignment (Table 3) analysis clearly separated Bhutanese Siri populations from Holstein Friesian and Goleng taurine cattle suggesting that if a taurine influence is present in Siri cattle it is likely to be small.

CONSERVATION IMPLICATIONS

This study revealed that Bhutanese Siri cattle could be grouped in two distinct genetic entities. Siri West, South and Central are closely related to each other. Siri East is genetically apart from other Siri populations following likely crossbreeding with Jaba cattle. It should be noted that the study did not, however, directly address or answer the question of whether Siri populations in Bhutan represent an unique breed which deserves attention for conservation or not, compared to Siri populations in other countries. Siri populations found in India (Sikkim, Darjeeling) or Nepal may have been similar to those in Bhutan. The cattle populations in these regions are, however, undergoing fast changes due to crossbreeding with exotic breeds especially the Jersey (Tantia et al., 1996). Furthermore, the Siri populations in India and Nepal are generally limited to the lower hills. In Bhutan, Siri is used in farming systems as high as 3,000 m.

The decision whether a breed is unique or not and what investments would be justified for its conservation can only partly be based on genetic evidences. Although further investigations, involving more animals and a wider geographic coverage could provide further arguments either for or against conservation, the decision to conserve the Siri breed, made by the government of Bhutan is laudable.

The conservation program may encompass Siri West, Siri Central and Siri South as a single breeding unit. However, the envisaged open nucleus-breeding scheme in the established conservation herd in East Bhutan with Siri West cattle should be viewed with caution given the genetic background of the surrounding populations of Siri East cattle. Involvement of Siri East in the replacement herd may invariably lead to the genetic loss of the supposedly indigenous Bhutanese Siri. To ensure long-term use and development of indigenous Bhutanese Siri in Bhutan, an appropriate policy decisions may be required to re-locate the existing herd or initiate an alternative conservation program in a more suitable area other than in East Bhutan.

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