Indian Journal of Animal Sciences **80** (11): 1134–37, November 2010

Genetic architecture of Black Bengal and Chegu goats

R K VIJH[1], M S TANTIA[2], R BEHL[3] and BINA MISHRA[4]

National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

Received: 23 January 2010; Accepted: 2 August 2010

Key words: Black Bengal goats, Chegu goats, Genetic architecture, goats

Microsatellites are the markers of choice to decipher the population genetic parameters and have exceptionally high variability. India has 20 recognised breeds of goats (Acharya 1982) distributed throughout the country. The heterologous loci were polymorphic in goat and were utilized for demographic studies (Behl *et al.* 2003). Most of the studies in the Indian goats are restricted to morphological attributes and very little work is available on the genetic level (Kumar *et al.* 2005a,b, Gour *et al.* 2005). Black Bengal goats are widely distributed in eastern India, meat type with a kidding rate of 1.6 with 50% twining, and are famous for quality Morocco leather. There is no threat to the breed as far as the census size is concerned. Chegu goats are found in Lahul Spiti and Kangra valleys of Himachal Pradesh. The breed has its economic importance with pastoralist based farming system of high altitude cold arid region. The population size is 8000. The breed is famous for fine Pashmina (down undercoat) production but due to low yields of Pashmina is now being reared for meat. We carried out microsatellite analysis of these 2 phenotypically distinctive populations of North-West mountainous region (Chegu) and eastern India (Black Bengal). The present study was undertaken to examine the population structure, ancestral proportion and differentiation in the two populations.

The blood samples from the tract of 2 goat populations, viz. Black Bengal and Chegu were collected at random from unrelated individuals in EDTA vacutainers and transported at 4°C. A total of 40 samples were collected from Black Bengal from 25 flocks in West Bengal and 35 Chegu goats from 16 flocks in Himachal Pradesh. The DNA was isolated using standard laboratory protocol (Sambrook *et al.* 1989). The PCR conditions were standardized for 22 heterologus primers and the amplified products were run on polyacrylamide gel electrophoresis followed by silver staining of the gels (Bassam *et al.* 1991). The gels were scored and genotype data was generated for these loci. The data obtained on these 22 microsatellite loci was subjected to analysis to obtain the expected and observed heterozygosity values in the 2 breeds using POPGENE software (Yeh *et al.* 1999). The software FSTAT 2.9.1 (Goudet 1995) was used to compute values of standard genetic diversity indices, their variances and estimate of F_{ST}. The F statistics values F_{IS}, F_{IT} and F_{ST} were estimated using Jack-knifing over loci and the confidence interval were generated using 10 000 permutations with the GDA software (Lewis and Zaykin 2002). The analysis of molecular variance (AMOVA) was carried out using the software ARLEQUIN version3.0 (Excoffier *et al.* 2005). The assignment of individuals was carried out using the "leave one out" procedure (Efron 1983). The Bayesian clustering method (as implemented in the software structure) was used to infer the population structure and simultaneously assigns individuals to the populations of their origin was implemented (Pritchard *et al.* 2000). The model assumes that there are K populations (where K is unknown), each K is characterized by a set of allele frequencies at each locus. The posterior probabilities of K (i.e., the likelihood of K as a proportion of the sum of the likelihood for different values of K) were estimated assigning uniform prior values of K between 2 and 4. The presence of structure in the 2 populations was inferred using the increase in likelihood values as obtained from the data analysis. The results presented in the study are based on 100 000 iterations, following a burn in period of 10 000 iterations.

Alleles (211) were amplified using 22 loci. The number

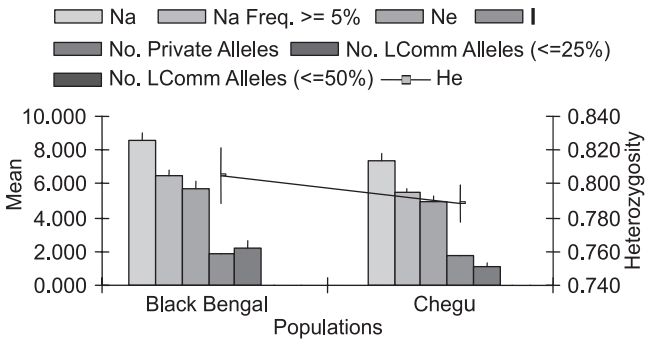

Fig 1. Allelic patterns across populations.

Present address: [1,3]Principal Scientist, [3]Senior Scientist, [4]Research Associate (e mail: rameshnjh@gmail.com).

Table 1. Hetrozygosity and number of alleles in 2 goat breeds

Locus	Black Bengal					Chegu				
	Na	Ne	Ho	He	PIC	Na	Ne	Ho	He	PIC
OARFCB11	12	5.86	0.68	0.84	0.82	7	5.40	0.74	0.83	0.82
OARFCB20	9	5.97	0.83	0.84	0.81	8	4.77	0.74	0.80	0.81
OARFCB48	13	9.97	0.83	0.91	0.87	7	6.08	0.63	0.85	0.87
OARFCB304	12	8.38	0.68	0.89	0.88	11	8.03	0.69	0.89	0.88
OARCP34	7	6.43	0.88	0.86	0.82	8	4.94	0.83	0.81	0.82
ETH10	10	5.45	0.75	0.83	0.81	10	4.84	0.80	0.81	0.81
ETH152	8	4.44	0.75	0.78	0.80	8	6.00	0.60	0.85	0.80
ETH225	5	3.26	0.68	0.70	0.72	5	3.80	0.69	0.75	0.72
ILSTS001	9	5.12	0.68	0.81	0.78	8	4.10	0.74	0.77	0.78
ILSTS005	11	9.28	0.83	0.90	0.87	9	6.27	0.63	0.85	0.87
ILSTS011	7	5.52	0.78	0.83	0.80	6	4.44	0.69	0.79	0.80
ILSTS19	9	5.69	0.65	0.83	0.82	7	5.70	0.74	0.84	0.82
ILSTS022	7	4.78	0.68	0.80	0.77	6	4.12	0.71	0.77	0.77
ILSTS029	9	5.93	0.58	0.84	0.79	7	4.04	0.83	0.76	0.79
ILSTS033	8	5.18	0.60	0.82	0.74	6	3.15	0.69	0.69	0.74
ILSTS038	7	5.51	0.65	0.83	0.81	7	5.24	0.74	0.82	0.81
ILSTS044	9	4.41	0.38	0.78	0.78	7	4.63	0.63	0.80	0.78
ILSTS049	12	9.38	0.73	0.90	0.86	11	6.03	0.80	0.85	0.86
ILSTS052	4	2.19	0.48	0.55	0.66	6	4.34	0.63	0.78	0.66
ILSTS059	8	4.61	0.73	0.79	0.82	8	6.81	0.69	0.87	0.82
ILSTS065	7	5.44	0.78	0.83	0.77	6	3.75	0.69	0.74	0.77
ILSTS072	5	3.76	0.70	0.74	0.72	5	3.32	0.66	0.71	0.72
Mean	8.55± 0.269	5.75± 0.218	0.69± 0.013	0.81± 0.009	0.80± 0.006	7.41± 0.201	4.99± 0.145	0.71± 0.008	0.80± 0.006	0.80± 0.006

Na, number of observed alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information content.

of alleles was 188 in Black Bengal and 163 in Chegu breed. The mean number of alleles in Black Bengal and Chegu were 8.54±0.52 and 7.41±0.36 and the effective number of alleles were 5.75 and 4.99 alleles respectively (Table 1). The mean heterozygosity values were 0.81±0.02 and 0.79±0.01 for the 2 breeds (Fig. 1). The maximum observed and expected heterozygosity in Black Bengal was for locus OARCB34 and ILSTS029, respectively. These values were 0.83 (ILSTO72) and 0.60 (ILSTS049) in Chegu breed. 69 alleles of 22 loci were absent in one or the other population (Private alleles). The F_{IS} values were calculated using Weir and Cockerham (1984), and Robertson and Hill (1984) in both the populations. Out of 22 loci, 9 loci in Black Bengal and 13 loci in Chegu deviated significantly from HWE pointing towards existence of sub-population structure. The F_{IS} values were 0.151 and 0.112 for Black Bengal and Chegu populations respectively. The global F_{IS} value was found to be 0.135. The hierarchical analysis of molecular variance revealed 9.33% of the variance between the populations. The estimates were calculated using Arlequin software (Excoffier *et al.* 2005) using pair-wise differences method (Weir 1996). Among individuals within population component contributed

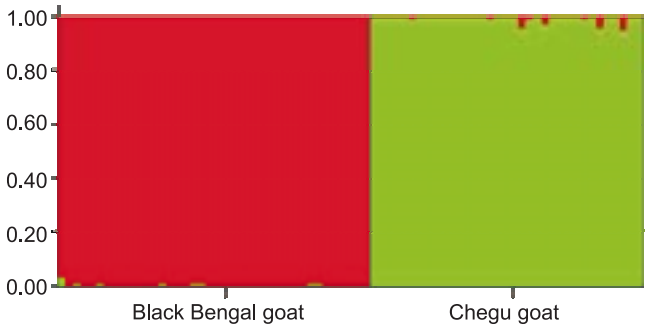

Fig 2. Estimated membership fraction in each of the K=2 inferred clusters.

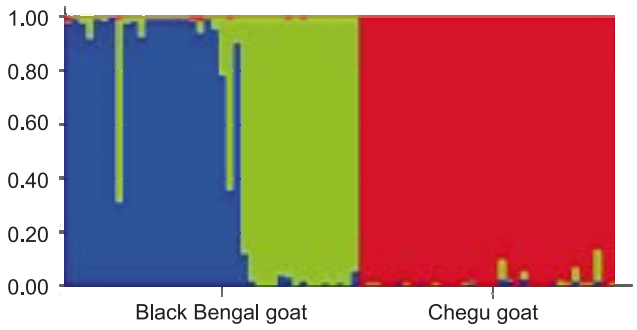

Fig 3. Estimated membership fraction in each of the K=3 inferred clusters.

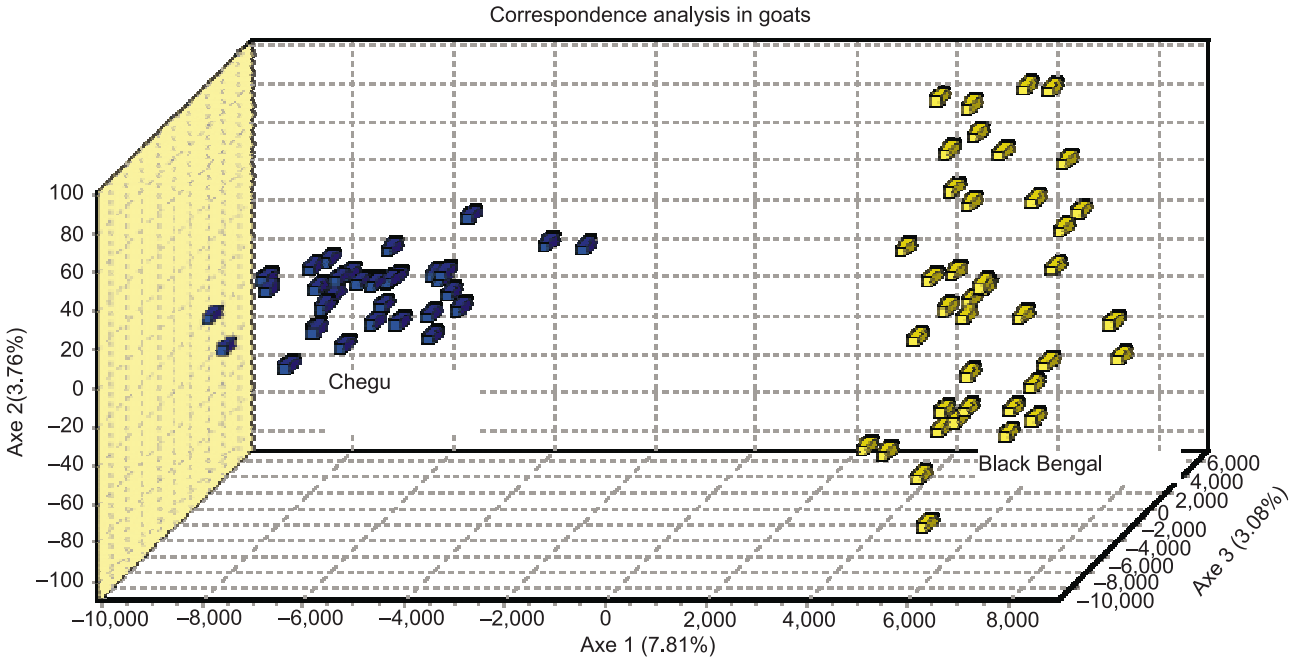

Fig 4. Correspondence analysis of two breeds of goats.

12.25% to the total variance. The among population variation (F_{ST}) and among individuals within population (F_{IS}) were significant using the permutation test. The pair wise F_{ST} value between Black Bengal and Chegu was 0.095 and was statistically significant. The linearised F_{ST} value (Slatkin 1995) was 0.105 and was statistically significant. The analysis of variance (locus wise) between these 2 populations gave higher values for among population differentiation for some of the loci compared to others. The analysis of variance revealed 15% of the variance attributable to between populations when frequency method was used. All the individuals could be assigned to their respective population with 100% accuracy using the frequency method and leave one out procedure (Efron 1983). The Bayesian analysis for assignment of individuals using STRUCTURE software (Pritchard *et al.* 2000) correctly assigned the individuals to their respective cluster. The log likelihood values obtained were –6201.4 (K=2), –0.6089.8 (K=3) and –6115.0 (K=4). Among the values K=3 gave the highest value. The clustering for K=3 and K=4 revealed only change in clustering of Black Bengal breed (Figs 2 and 3). The K=2 partitioned the 2 populations in 2 distinctive clusters while K=3 split the individuals of Black Bengal in 2 clusters. The Chegu individuals remained in earlier cluster meaning that the Chegu has only single ancestry. The correspondence analysis was carried out and it revealed that the individuals of the two populations formed distinct clusters (Fig. 4). The inter-individual distances were estimated using Allele sharing and Nei's standard genetic distances and trees constructed (Fig. 5) using Neighbour Joining Method (Saitou and Nei 1987). The mean number of alleles in these 2 populations were higher than reported in Jhakrana and Marwari (Kumar *et al.* 2005a, b) and Jamunapari goats (Gour *et al.* 2005). The values of mean observed heterozygosity were 0.69 and 0.71 in Black Bengal and Chegu respectively and these values were also higher than other 3 Indian goats studied. The higher heterozygosity pointed to allelic richness and higher gene diversity in these breeds. The F_{IS} values obtained in our study were lower than other Indian breeds and this was expected owing to more number of alleles and higher heterozygosity values. This can also be attributed to the sampling of animals

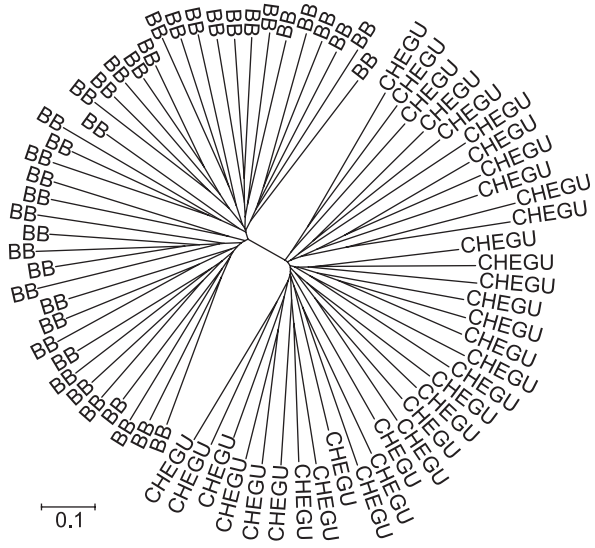

Fig 5. Neighbour joining tree based on inter individual distances based on allele sharing.

over a large area in these 2 breeds. Chegu being confined to mountainous terrain had only 1 inferred cluster suggesting no inter-mixing with other goat breeds. Black Bengal being distributed over a large area revealed ancestral contribution from at least 2 populations. The assignment was 100% for the 2 populations owing to higher F_{ST} values obtained. Vijh and Tantia (2004) also reported assignment accuracy of 100% in 4 breeds of poultry when F_{ST} values were similar to present study. Inter-individual distances and correspondence analysis separated the individuals in 2 distinct clusters as there was no admixture in the 2 populations. The results revealed that both the populations are quite distinctive separate conservation strategies need to be developed for sustenance and conservation of these 2 breeds of India in their respective breeding tracts.

SUMMARY

The present study was undertaken to examine the population structure, ancestral proportion and differentiation in the two populations of North-West Mountainous Region (Chegu) and eastern India (Black Bengal). A total of 40 samples were collected from Black Bengal from 25 flocks in West Bengal and 35 Chegu goats from 16 flocks in Himachal Pradesh. Both the populations were found quite distinctive and separate conservation strategies need to be developed for sustenance and conservation of these 2 breeds of India in their respective breeding tracts.

REFERENCES

Acharya R M. 1982. *Sheep and goat breeds of India*. FAO Animal Production and Health Paper 30. FAO, United Nations, Rome, Italy pp 1–190.

Bassam B J, Coetano-Anolles G and Gresshoff P M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal Biochemistry* **196**: 80–83.

Behl R, Sheoron N, Behl J, Vijh R K and Tantia M S. 2003. Analysis of 22 heterologus microsatellite markers for genetic variation in Indian goats. *Animal Biotechnology* **14**: 167–75.

Efron B. 1983. Estimating the error rate of a prediction rule: improvement on cross-validation. *Journal of American Statistical Association* **78**: 316–30.

Excoffier L, Laval G and Schneider S. 2005. An integrated software for population genetic data analysis *ARLEQUIN* ver. 3.0, http:// wwwlgbunigech/arlequin/ software

Goudet J. 1995. FSTAT A computer program to calculate F statistics. *Journal of Heredity* **86**: 485–86.

Gour D S, Malik G, Ahlawat S P S, Pandey A K, Gupta N, Gupta S C, Bisen P S and Kumar D. 2005. Analysis of genetic structure of Jamunapari goats by microsatellite marker. *Small Ruminant Research* dol: 101016/200507053.

Kumar D, Dixit S P, Sharma R, Pandey A K, Sirohi G, Patel A K, Aggarwal R A K, Verma N K, Gour D S and Ahlawat S P S. 2005a. Population structure, genetic variation and management of Marwari goats. *Small Ruminant Research* **59**: 41–48.

Kumar D, Pandey A K, Ahlawat S P S, Malik G, Sharma R, Gour D S, Dixit S P, Verma N K, Aggarwal R A K, Gupta N, Shahi B N and Gupta S C. 2005b. Genetic variability and bottleneck analysis of Jakhrana goats by microsatellite DNA fingerprinting. *Korean Journal of Genetics* **27**: 235–45.

Lewis P O and Zaykin D. 2002. *GDA software* available from http: //lewiseebuconnedu/ lewishome.

Pritchard J K, Stephens M and Donnelly P J. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–59.

Robertson A and Hill W G. 1984. Deviation from Hardy Weinberg proportions; sampling variances and use in estimation of inbreeding coefficients. *Genetics* **107**: 713–18.

Saitou N and Nei M. 1987. The neighbor joining method: A new method for constructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–25.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd edn., Cold spring Harbour, Cold spring Laboratory Press, NY.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–62.

Vijh R K and Tantia M S. 2004. Assignment of individuals to four poultry breeds of India using multilocus genotypes. *Indian Journal of Animal Sciences* **74**: 73–76.

Weir B S. 1996. Genetic data analysis II; *Methods for discrete population genetic data* Sinauer Assoc, Inc, Sunderland, MA, USA.

Weir B S and Cockerham C C. 1984. Estimating F statistics for the analysis of population structure. *Evolution* **38**: 1358–70.

Yeh F C, Yang R C, Boyle T B J, Ye Z-H and Mao J X. 1999. *POPGENE version 3.1* (http: //wwwualbertaca/-fyeh/fyeh).