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GENETIC VARIATION AND RELATIONS IN DIFFERENT GOAT POPULATIONS OF BANGLADESH

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Abstract

The genetic relation among different populations and breeds of goats in Bangladesh was studied using 15 microsatellite markers. A total of 181 goat samples- 36 from Black Bengal of western part (BBW), 39 from Black Bengal of central part (BBC), 40 from Black Bengal of Hilly region (BBH), 28 from exotic breeds (JAM) and 39 from crossbred (CRW) were genotyped. The allele number per locus ranged from 2 to 12. The average number of alleles per population was 5.69 ± 2.32, 5.38 ± 2.02, 5.23 ± 2.28, 6.08 ± 2.33, and 5.77 ± 2.35 for BBW, BBC, BBH, CRW and JAM, respectively. Heterozygosity was found in the range from 0.5049 ± 0.0222 (BBC) to 0.5751 ± 0.0262 (JAM). The genetic distance (D_A) between BBC and JAM was the highest (0.0627) and that between JAM and CRW was the lowest (0.0037). In the phylogenetic dendrogram, BBC and BBH grouped in the same cluster, while CRW and JAM formed another cluster. BBW formed a third cluster between those two clusters. The closest genetic relation between BBW and CRW indicates the introgression of exotic genes occurred more in BBW than BBC and BBH.

Key words: Bangladeshi goats, Microsatellite markers, Genetic variation, Dendrogram

Introduction

The goat is a very important and promising animal genetic resource in the developing countries, especially in Asia and Africa region. Of the total goat population, 92.76% of goats are found in Asia and Africa. China, India, Pakistan and Bangladesh possess 35.36, 25.46, 10.79 and 7.05% of the total goat of Asia, respectively (FAOSTAT, 2009). Bangladesh has the 4th highest population of goat in Asia. Bangladesh possesses 20.75 million goats at present (DLS, 2007). This species was neglected in the past. In the recent years, farmers and government are showing interest to utilize this species to increase the supply of meat and to alleviate poverty through creation of employment. Attention is being paid for their genetic improvement.

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For genetic improvement of any species, genetic characterization is the first and foremost priority. Genetic characterization can be done using isozymes, genomic DNA, mtDNA and chromosomes. Use of microsatellite marker is currently the marker of choice for a wide range of molecular genetic studies such as establishing genetic linkage maps (Kappes *et al*., 1997), analysis of mating system and population structures (Bruford and Wayne, 1993; Queller *et al*., 1993) and reconstruction of phylogenetic relationships among populations (Bowcock *et al*., 1994; Roy *et al*., 1994; Takezaki and Nei, 1996; MacHugh *et al*., 1997). Microsatellite markers has been used successfully for study of genetic diversity and relationship of goat breeds/populations by Yang *et al.* (1999), Luikart *et al*. (1999), Saitbekova (1999), Chenyambuga *et al.* (2004)**,** Jim'enez-Gamero *et al.* (2006), Thilagam *et al.* (2006) and Dixit *et al.* (2010). Katsumata *et al*. (1984) studied isozymes for the characterization of Bangladeshi goat. But there is no report on characterization of Bangladeshi goats based on microsatellite markers. The present study was, therefore, done to find out the genetic variation and relation among different populations of goats in Bangladesh using microsatellite markers.

Materials and Methods

Sample collection, DNA extraction and quantification

All the goats of Bangladesh were divided into five populations based on geographical distribution and history of breeding and management systems. These were (i) BBW- Black Bengal goats in the western part of the country, (ii) BBC- Black Bengal goats in the central part of the country, (iii) BBH- Black Bengal goats in the hilly area of the country, (iv) JAM- imported Indian goats consist of Jamnapari, Sirohi and Beetal, and (v) CRW- crossbred between JAM and Black Bengal goat. Forty samples were collected from each population. However 30 samples were collected from JAM. So a total of 190 samples were collected for five populations (Fig. 1). Samples were collected only from adult goats of subsistent farmers taking a ratio of 1:3 for male and female. Samples were collected avoiding related animals. Blood was collected in venoject tube, treated with anticoagulant and carried to Animal Genetics Laboratory of Bangladesh Agricultural University and preserved at 4^0C until DNA extraction. DNA was extracted following a salting out protocol after IAEA (2004). The DNA samples were quantified in 0.8% agarose gel electrophoresis.

Microsatellite polymorphism detection

PCR amplification and genotyping work were done in ILRI laboratory, Nairobi. All of 190 DNA samples were amplified in PCR system (GeneAmp 9700 PCR machine) using 15 pairs of microsatellite primers selected from FAO recommendation. All the microsatellite markers were forwardly labeled with a capillary based dye: 6FAM (blue), PET (Red), VIC (Green) and NED (yellow) for the purpose of genotyping. The PCR condition has been presented in Table 1. PCR was carried out in 10 µl reaction mixture containing 4ng/µl of template DNA, 4 ng/µl each primer, 125 µM/µl dNTPs, 0.03 unit/µl of Tag polymerase and 1.5 to 2.0 mM/µl of $MgCl_2$.

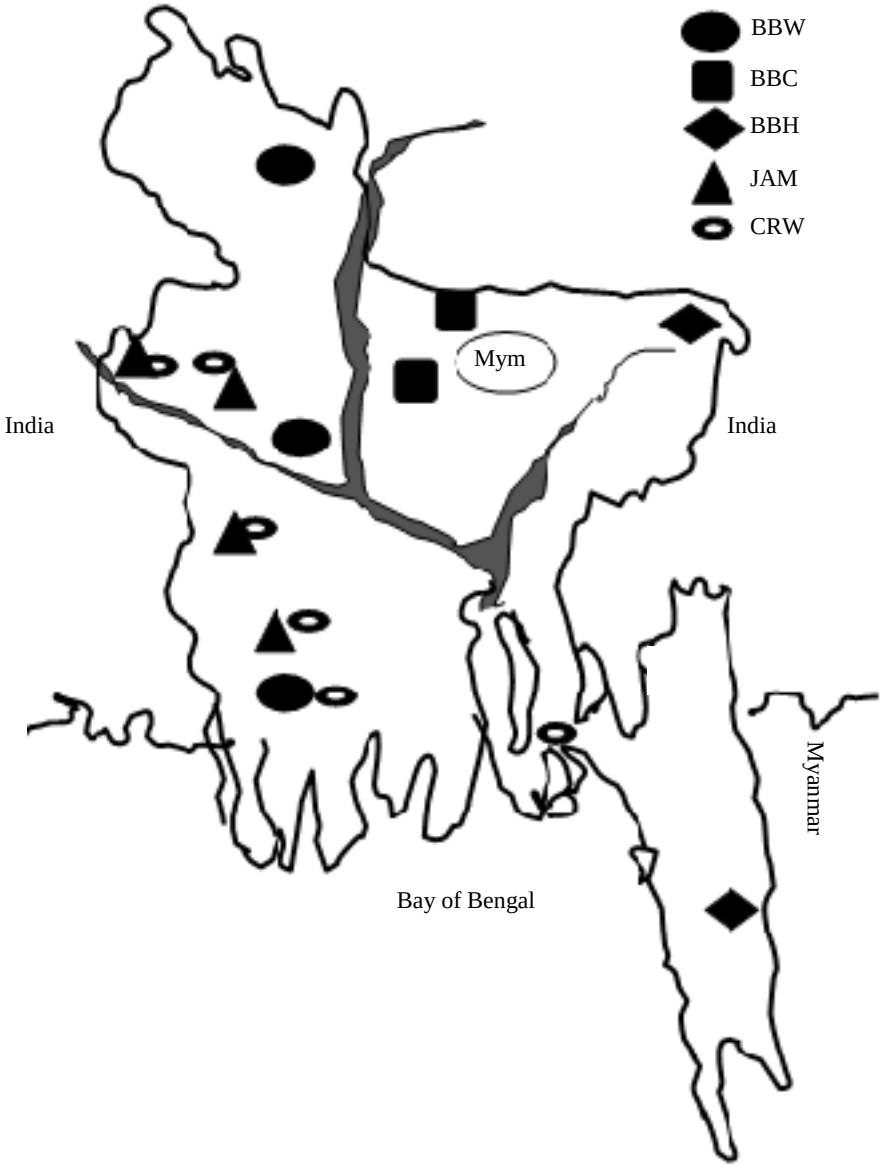

Fig. 1. Sites from where samples were collected. BBW = Black Bengal in the western part; BBC = Black Bengal in the central part; BBH = Black Bengal in the Hilly region; JAM = Imported Indian goat breed; CRW = Crossbred between Black Bengal and exotic goat breeds

PCR program used for amplification of all markers was: following an activation step of 5 min at 95^0C, the PCR mixture underwent 35 cycles of 30 seconds at 95^0C (denaturation), annealing time of 1 min at 55^0C and extension time of 1 min at 72^0C. The final extension time was 10 min at 72^0C. All PCR products were quantified by 2% agarose gel electrophoresis for checking the success of PCR amplification. The amplified DNA was genotyped by automated capillary DNA sequencer Genetic Analyzer (3730 of Applied

Biosystem, USA). The internal standard was prepared by adding 10 µl of LIZ standard to 45 µl of HiDi formamide. A total of 1 µl of diluted PCR product of each co-loading was transferred to individual wells with 9 µl of standard/formamide mix and denatured for genotyping. The genotyping results were processed by the GeneMapper v 3.7 program which determined the allele sizes in each animal. A total of 181 samples including 36 from BBW, 39 from BBC, 40 from BBH, 38 from CRW and 28 from JAM were finally amplified and genotyped.

Table 1. PCR condition used for different markers

Microsatellite markers	Size bp range	Annealing temp	Mg++ concentration
ILSTS029	135-185	55ºC	2.0 mM
BMS1494	235-300	55ºC	2.0 mM
MAF035	90-130	55ºC	2.0 mM
SRCRSP3	95-135	55ºC	2.0 mM
BMS1818	240-310	55ºC	2.0 mM
OARFC20	80-130	55ºC	2.0 mM
ETH10	190-220	53ºC	1.5 mM
OARAE54	105-145	55ºC	2.0 mM
ILSTS005	160-230	55ºC	2.0 mM
SPS113	125-170	58ºC	2.0 mM
CSRD247	219-260	58ºC	2.0 mM
INRA0132	125-175	58ºC	1.5 mM
MCM527	155-195	58ºC	2.0 mM
MAF70	120-190	65ºC	2.0 mM
ILSTS011	250-300	58ºC	2.0 mM

Statistical analysis

The genotyped data were analyzed using Microsatellite Toolkit program (Minch, 1995) to calculate allele frequencies at each locus for each population, average number of alleles per population and heterozygosity values (expected and observed). Dispan program (Ota, 1993) was used to calculate D_A genetic distances between the populations and a Neibour - Joining (NJ) phylogenetic tree was constructed using the PHYLIP 3.57c package (Felsentein, 1995).

Results and Discussion

Distribution of alleles and heterozygosity within the populations

All the goat populations showed polymorphism for microsatellite markers in the present study. The allele number per loci ranged from 2 to 12 in the five populations. A number of diagnostic alleles have been identified for different populations. The mean numbers of alleles along with heterozygosity values per population are presented in Table 2. The mean numbers of alleles per population ranged from 5.23 (BBH) to 6.08 (CRW). The average observed

heterozygosity values were medium in all populations ranging from 0.5049 to 0.5751 with the lowest in population BBC (0.5049 ± 0.0187) and the highest in population JAM (0.5751 ± 0.0237). The genetic variation in the goat population of Bangladesh was first studied by Katsumata *et al*., 1984. They used isozymes as markers and studied on BBW, BBC and JAM populations of present study. They reported the existence of polymorphic loci in all these populations. However, the observed heterozygosity reported by them was lower (0.0337 to 0.0429) than the findings of present study (0.5049 to 0.5751)**.** The value of heterozygosity measured from isozymes is always lower than that measured from DNA. This is also evidence from the study of Nyamsamba, *et al.* (2003). The existence of polymorphism has also been reported in Indian and Chinese goats by a number of investigators (Ganai and Yadav, 2001; Kim, *et al.*, 2002; Sultana *et al.*, 2003; Ran *et al*., 2006; Anita and Yadav, 2007). They also reported medium level of heterozygosity in those populations.

Table 2. Mean number of alleles and heterozygosity for different populations

Population	Loci type	No. alleles	Expected heterozygosity	Observed heterozygosity
BBW	15	5.69 ± 2.32	0.5411 ± 0.0713	0.5258 ± 0.0231
BBC	15	5.38 ± 2.02	0.5311 ± 0.0734	0.5049 ± 0.0222
BBH	15	5.23 ± 2.28	0.5473 ± 0.0657	0.5171 ± 0.0219
CRW	15	6.08 ± 2.33	0.5827 ± 0.0600	0.5708 ± 0.0224
JAM	15	5.77 ± 2.35	0.5925 ± 0.0584	0.5751 ± 0.0262

Genetic distances between the populations

The D_A genetic distances between different populations are presented in Table 3. The standard genetic distance between the population pairs ranged from 0.0043 to 0.0498. The lowest genetic distance (0.0043) was observed between CRW and JAM and the highest distance (0.0498) was observed between JAM and BBC. BBW had closer relationship to CRW than BBC and BBH. The genetic relationships among different populations are shown in the phylogenetic dendrogram (Fig. 2) constructed on the basis of D_A distances and using Neibour- joining (NJ) method (Nei, 1978). In the phylogenetic dendrogram, goat populations of Bangladesh were divided into three groups, BBC and BBH clustered in the first group, BBW in the second group, and CRW and JAM in the third group separately. Such a. clustering of the goat breeds according to their geographical origin was also reported for Indian goat breeds by Thilagam *et al.* (2006) and Dixit *et al.* (2010), for Chinese goat breeds by Yang *et al.* (1999), for African goat breeds by Chenyambuga *et al.* (2004)**,** and Swiss goat breeds by Saitbekova (1999).

From the history it is evident that CRW was crossed with imported goat breeds of India. Crossbreeding is more evidence in the western part of Bangladesh than central and eastern part or hilly region for the last l0 years as revealed from the history of goat mating system in the country. Because of the introgression of genes from exotic breeds, BBW possessed more exotic genes than BBC and BBH (Faruque and Khandoker, 2007). So CRW and BBW showed closed genetic relation and had a higher mean number of alleles (6.08 ± 2.33 and

5.69 ± 2.32, respectively) than BBC and BBH. There was no introgression of genes from exotic breeds in BBH. Therefore, this population had the lowest mean number of alleles, lower heterozygosity value and highest genetic distance from JAM. Anita and Yadav (2007) also reported that Black Bengal and Jamnapari breed in India had larger genetic distance and they clustered separately in dendrogram. Therefore, BBH can be regarded as the most pure Black Bengal goat in Bangladesh.

Table 3. Genetic distance among different populations/breeds

	BBW	**BBC**	**BBH**	**CRW**	**JAM**
BBW					
BBC	0.0265				
BBH	0.0179	0.0282			
CRW	0.0043	0.0415	0.0198		
JAM	0.0189	0.0627	0.0498	0.0037	

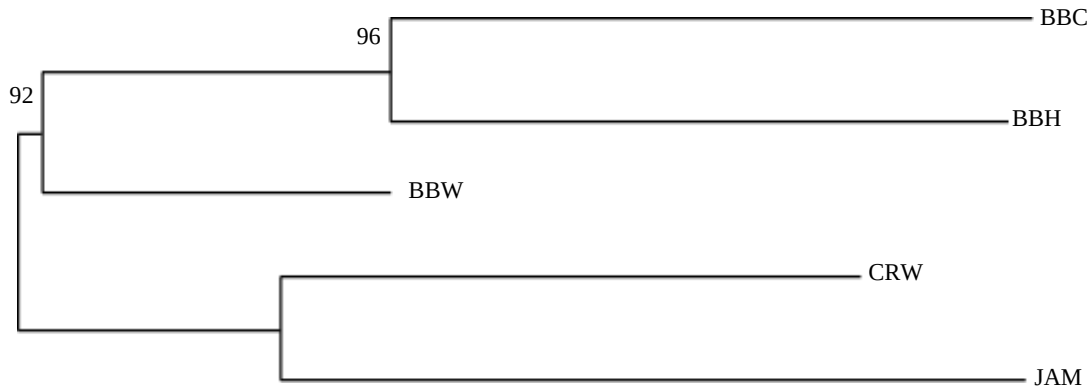

Fig. 2. Dendrogram for genetic relationship of five goat populations/breeds

BBW : Bangladesh north**, BBC :** Bangladesh central**, BBH :** Bangladesh Hilly**, JAM :** Exotic breeds, **CRW :** Crossbred between Black Bengal and exotic breeds

Conclusion

It can be concluded that all the populations of goats in Bangladesh have high genetic diversity with medium heterozygosity. There is significant introgression of genes from exotic breeds in the populations of BBW than BBC and BBH. The results of the present study would be useful in planning breeding strategy for future genetic improvement program of goat in Bangladesh.

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