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RAPD in goat and application of Shannon's index and AMOVA

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Abstract

This experiment was conducted in the Department of animal breeding and genetics , Ranchi Veterinary college, Birsa Agricultural university, Ranchi. Fifty blood samples of Black Bengal Goat were collected from different villages of Purulia district of West Bengal and another fifty blood samples of unrelated individuals of Black Bengal type goat from Jharkhand were collected from different villages of Ranchi district *(This goat population is referred as Jharkhand Black goat in this study)*. Ten Random primers were used to determine the RAPD polymorphism in the two populations, out of which only eight primers were used as Primer-1 & primer-2 did not produce any significant amplification products. A total of 18 bands were generated from 8 random primers. Data were analyzed by using a computer program POPGENE. Shannon's Information index was observed to be 0.6792 and 0.5898 for Black Bengal and Jharkhand Black respectively. It was found from AMOVA that the percentage variation component among the population and within population were 1.69983 and 98.30017 respectively. The variation within population was much more marked than between the two populations. Therefore there is ample scope for further improvement in its productivity through appropriate breeding strategies.

Key words : AMOVA, Shannon's information index, PCR, RAPD, MAS, Conservation etc.

Inroduction

Black Bengal goat is believed to be derived from wild Bezoar of *Capra aegragrus* (Herre, W. and Rohrs, M., 1973) with infiltrated blood from Markhor i.e. *Capra falconeri.*.

Black Bengal and Black Bengal type goats of Jharkhand are black in color but differ in morphological characters such as body weight and these two goat populations also show significant divergence to each other in molecular study based on Microsatellite marker (Kumar. S., 2007). In addition to available information on phenotype and/ or geographic distribution, the information on genetic variation within and between breeds is warranted to differentiate them on genetic basis. The genetic information may further help to choose best conservation improvement options for genetic resources The need for conservation of livestock diversity and for characterization of breeds and populations including their genetic differentiation and relationship is also recognized word wide (FAO, 1995 a, b).

The characterization data of Indian goats is imprecise because earlier studies based on morphological and biochemical markers did not present a true picture of their relationships. The present investigation was proposed to be undertaken to evaluate gene flow, phylogeographic history, genetic structure and differentiation of Black Bengal and Black Bengal type goat of Jharkhand using RAPD PCR technique which

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took into account Shannon's information index& Amova besides various measures like, dendrogram gene frequency, observed and effective number of alleles etc to reach at conclusion. RAPD is a PCR based technique for which involves the use of single arbitrary short primers (8-12 nucleotides), resulting in the amplification of many discrete DNA. The RAPD technology has provided a quick and efficient screen for DNA- sequence polymorphisms at a very large no of loci. A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simply species richness (i.e., the number of species present); they also take the relative abundances of different species into account. (Begon *et al.* 1996). Analysis of Molecular Variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about differentiation. A variety of molecular data-molecular marker data (for example, RFLP or AFLP), direct sequence data, or phylogenetic trees based on such molecular data-may be analysed using this method (Excoffier, *et al.* 1992). It is a statistical model for the molecular variation in a single species, typically biological. The name and model are inspired by ANOVA. The method was developed by Laurent Excoffier, Peter Smouse and Joseph Quattro at Rutgers University in 1992.

So combining the aim of breed conservation, selective breed propagation and breeds' characterization, the current research was pursued precisely with an objective to study genetic variation prevailing among Black Bengal and Black Bengal type goat in west Bengal and Jharkhand respectively, to study genetic relationship between both goat populations and to study population structure and gene flow between these two goat populations.

Materials and Methods

Blood samples of 50 unrelated individuals (10 ml each) from each goat population were collected from the respective places of their geographical distribution. Fifty blood samples of Black Bengal Goat were collected from different villages of Purulia district of West Bengal. Fifty blood samples of unrelated individuals of Black Bengal type goat from Jharkhand were collected from different villages of Ranchi district *(Further this goat population is referred as Jharkhand Black goat in this article)*.

Genomic DNA was isolated and purified from white blood cells using proteinase K digestion and standard phenol: chloroform extraction as per the standard protocol described by Sambrook *et al.* (1989). Quality and quantity check of isolated genomic DNA sample was done by agarose gel electrophoresis. Amplified products appeared as sharp orange color bands under UV Transilluminator due to the intercalation of ethidium bromide. In case of Black Bengal and Jharkhand black each 48 random samples out of 50 collected were amplified using the 10 primers on the basis of standardized protocol for RAPD.

Statistical analysis of the amplified product was carried out. Genetic diversity within each population was determined as the observed and expected number of alleles (Kimura and Crow, 1964) and Shanon's Information Index (Lewontin, 1972) using Popgene software (Yeh *et al*, 1999). Ewans-Watterson test was performed to test the neutrality for RAPD markers.

Figure1. Black bengal goat genomic (48 samples) amplification with primer sigma06

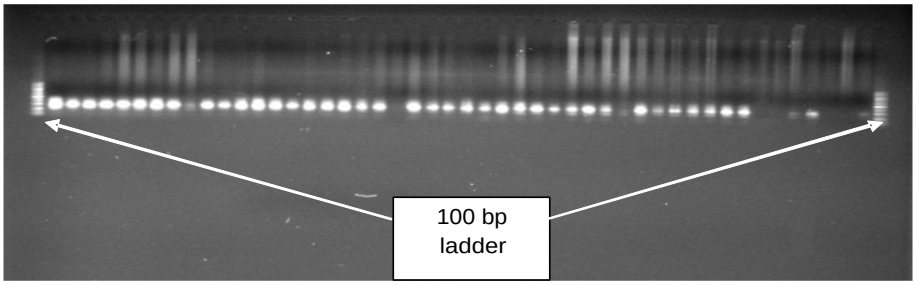
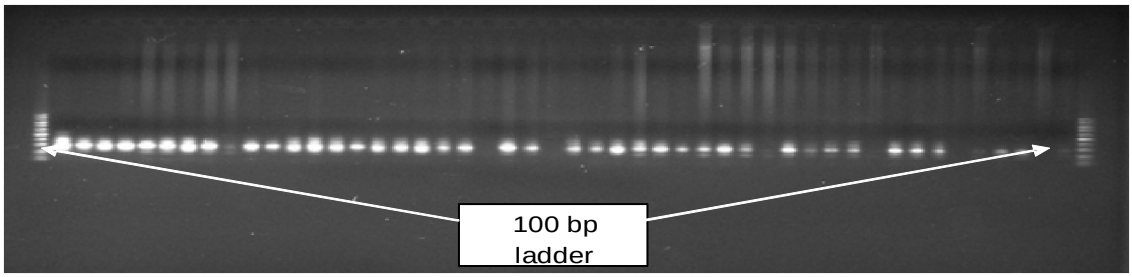


Figure 2. Jharkhand black goat genomic (48 samples) amplification with primer sigma 06



AMOVA programme included in the Arlequin software package) was used to study genetic differentiation

Result and Discussion

Shannon's index

Shannon's Information index was moderately high in both the populations. The overall mean was observed to be 0.6792 and 0.5898 for Black Bengal and Jharkhand Black respectively(Tables1).In case of Black Bengal the maximum value of shannon's information index (I) was observed to be 0.6713 for loci sigma06-2 and sigma09-2 and minimum value was found to be 0.0739 for locus sigma04 (Table 1). Similarly in case of Jharkhand black ,minimum value of shannon's information index was found to be 0.4506 for locus sgma06-3 followed by 0.4154 for locus sigma08-1 and maximum value of shannon's information index was 0.6897 for loci sigma06-1 and sigma10-2 followed by 0.6853 for locus sigma10-1. Rajan E.K. and Mariamuthu.G. (2006) did a preliminary examination of genetic diversity in False vampire bat Megaderma *lyra* .According to their findings the lowest genetic diversity estimated by Nei's genetic diversity index and Shannon's index was observed in population IV (h = 0.21 ± 0.11; S = 0.22 ± 0.14),while the highest value was observed in populationI (h = 0.26 ± 0.13; S = 0.28 ± 0.16).They concluded from their finding that no population in *M. lyra had* a higher degree of diversity. Diversity indices provide more information about community composition than simply species richness (i.e., the number of species present); they also take the relative abundances of different species into account,(Begon *et al.* 1996). Diversity indices provide important information about rarity and commonness of species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure (Magurran, A. E. 1988; Rosenzweig, M. L. 1995).

Mufti *et al.* (2009) studied the genetic diversity of of Red Chittagong Cattle using Randomly Amplified Polymorphic DNA Markers.The approach was to determine the genetic variation of Red Chittagong cattle and to find out the genetic status for their future improvement and conservation lowest diversity were found in Anwara (0.2925) and Chandonish (0.2147) respectively. Hence in the present study it can be concluded based on the data that as the shanon's index is relatively high it is indicative of greater gene diversity in both the populations

Amova

During the analysis of molecular variance between the two populations,18 RAPD loci were considered. It was found from AMOVA that the percentage variation component among the population and within population were 1.699 and 98.300 respectively (TABLE2.). The variation within population was much more marked than between the two populations. This revealed the fact that Jharkhand black might be a strain variant of the Black Bengal.

Kumari & Thakur(2014) mention that in order to quantify the percentage of molecular variance due to difference among different populations and significance is tested by a non-random permutation approach described by Excoffier *et al.* (1992) using the AMOVA programme included in the Arlequin software package (Excoffier *et al.*, 2007).

AMOVA showed that there was approxmately 1.7 % variation between both the populations and 98.3 % within the population. Building of dendrogram with use of genetic distance was done with popgene & visualized by mega software on the basis of individual goat.

In the study by kumar,(2006) the analysis of molecular variance (AMOVA) showed a significant amount of differentiation (8.52 %) among the groups. Individuals within the groups or breeds contributed 17.63 % of total variance but with respect to overall population showed 73.85 % of total variability .

Conclusion

All measures of genetic variations i.e. observed number of alleles, Shannon's Information indicating high polymorphism across the loci, and suggesting suitability of these markers for genetic diversity studies in goat. Combining the objectives of conserving the most of the genetic diversity and uniqueness, total of breeds that were examined, showed the higher mean number of alleles and as well as high heterozygosity. Moreover further molecular studies might be done to compare the two breeds as that would help in MAS and in finally concluding before Jharkhand black might be given separate identity as a breed or labeled as a derivative of Black Bengal.

Table 1. Shannon's Index for Black Bengal and Jharkhand Black at different locus

BLACK BENGAL		JHARKHAND BLACK	
Locus	I	Locus	I
SIGMA03	0.6500	SIGMA03	0.6036
SIGMA04	0.0739	SIGMA04	0.5841
SIGMA05	0.5930	SIGMA05	0.5623
SIGMA06-1	0.6211	SIGMA06-1	0.6897
SIGMA06-2	0.6713	SIGMA06-2	0.5623
SIGMA06-3	0.5383	SIGMA06-3	0.4506
SIGMA06-4	0.5117	SIGMA06-4	0.6713
SIGMA07-1	0.6500	SIGMA07-1	0.5383
SIGMA07-2	0.5623	SIGMA07-2	0.6211
SIGMA07-3	0.5623	SIGMA07-3	0.6036
SIGMA08-1	0.5623	SIGMA08-1	0.4154
SIGMA08-2	0.6211	SIGMA08-2	0.5383
SIGMA08-3	0.6616	SIGMA08-3	0.6036
SIGMA09-1	0.5841	SIGMA09-1	0.5623
SIGMA09-2	0.6713	SIGMA09-2	0.5841
SIGMA09-3	0.3768	SIGMA09-3	0.6500
SIGMA10-1	0.5841	SIGMA10-1	0.6853
SIGMA10-2	0.5623	SIGMA10-2	0.6897
Mean	0.6792	Mean	0.5898
St. Dev	0.6036	St. Dev	

I = Shannon's Information index [Lewontin (1972)]

The number of polymorphic loci is : 18

References

1. **Begon, M.; Harper, J. L. and C.R. Townsend.** (1996) Ecology; Individuals, Populations and Communities, 3rd edition. Blackwell Science Ltd., Cambridge, MA. Cited from Kumar, S. (2007).Thesis submitted to B.A.U.

2. **Excoffier, L., Smouse, P.E., and Quattro, J.M.** (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479-491.

3. **Excoffier, L., Laval, G. and Schneider, S.** (2007) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolut. Bioinformat. Online, **23**: 47-50. PMID: 19325852

4. **Kimura, M. and Crow, J.F.** (1964) The number of alleles that can be maintained in a finite population. *Genetics,* **49**: 725-738.

5. **Kumari,N. and Thakur, S.K.** (2014) Randomly Amplified Polymorphic DNA-A brief review, *American J. Animal and Vety Sci.* 9: 6-13.

6. **Lewontin, R.C.** (1972) The apportionment of human diversity. *Evol. Biol*., **6**:381-398.

7. **Mufti, M.R.; Mostari, P. and Deb, G.K.** (2009) Genetic diversity of red Chittagong

Table 2. AMOVA designs and result (average over 18 loci)

Source of variation	D. F.	Sum of Squares	Variance components	Percentage variation
Among populations	1	6.927	0.065	1.699
Within populations	94	355.81	3.785	98.300
Total	95	362.740	3.850	

cattle using RAPD markers. *Biotecnology,* **6(1):**57-60.

8. **Magurran, A.E.** (1988) Ecological Diversity and its Measurement. Princeton University Press, Princeton, N.J.146-156.

9. **Rajan, E.K. and Marimuthu, G.** (2006) A preliminary examination of genetic diversity in the Indian false Vampire bat Megaderma lyra. *Animal biodiversity and conservation.*. **29(2):**119-115.

10. **Kumar, S.** (2007) Molecular characterization of some goat genetic resources, Thesis submitted to BAU, Ranchi.

11. **Sambrook, J.; Fritsch, E. F. and Maniatis, T.** (1998) Molecular cloning: A Laboratory Manual 2nd ed. Cold spring Harbour; Cold Laboratory press; NY.

12. **Yeh, F.; Boyle, C.T.; Ronagcai, Y.; Ye, Z. and Xian, J.M.** (1999) POPGENE Version 3.1 (http:/ www.ualberta.Ca/-Fyeh/Fyah.). Cited from American j. Animal and vety. Sci.1 **(2)**: 17-22, 2006.

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