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Characterization of Indian cattle breeds, Ongole and Deoni (*Bos indicus*) using microsatellite markers

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Characterization of Indian cattle breeds, Ongole and Deoni (*Bos indicus*) using microsatellite markers

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Running Title:
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Abstract:

Molecular characterization of cattle breeds is important for the prevention of germplasm erosion by cross breeding. The present study was carried out to characterize two Indian cattle breeds, Ongole and Deoni using microsatellite markers. Using 5 di-and 5 tri- nucleotide repeat loci, 17 Ongole and 13 Deoni unrelated individuals were studied. Of the ten loci, eight revealed polymorphism in both the breeds. The di-nucleotide repeats loci were found to be more polymorphic (100%) than tri-nucleotide repeat loci (60%). A total of 39 polymorphic alleles were obtained at 4.5 alleles per locus in Ongole and 4.1 in Deoni. The average expected heterozygosity was 0.46 ($\pm$0.1) and 0.50 ($\pm$0.1) in Ongole and Deoni breeds, respectively. The PIC values of the polymorphic loci ranged from 0.15 to 0.79 in Ongole and 0.13 to 0.80 in Deoni breeds. Six Ongole specific and three Deoni specific alleles were identified. The two breeds showed a moderate genetic relationship between themselves with a F_{ST} value of 0.10.

Key Words: *Bos indicus,* Deoni, Heterozygosity, Microsatellite, Ongole, PIC

Background

The Indian cattle breeds, also known as '*zebu* cattle' (*Bos indicus*) are broadly categorized into dairy, draft and dual purpose breeds depending upon their utility either in dairying or in agricultural work. The dual-purpose breeds have specific qualities like disease resistance, heat tolerance, ability to survive and reproduce under stress and low feed input. Ongole and Deoni are the dual-purpose breeds from southern part of India. Ongole breed contributed to the development of some of the exotic breeds like 'American Brahman', 'Santa Getrudis' etc. (1) and is used extensively for beef purpose in Latin American countries (2). Deoni is yet another breed serving the needs of the people of semi-arid hilly areas. Short calving interval, massive body and fairly well developed udder in cows of this breed indicate its dual-purpose nature.

Zebu cattle are used in cross breeding programs as they can adapt to hot and humid climates (3,4). But a number of these breeds are now subjected to fast genetic degradation because of intensive cross breeding with high milk producing exotic breeds and reduction of emphasis on draft ability due to mechanization of agriculture and transport. As a result, some of the native draft breeds are on the verge of extinction. Hence, there is an urgent need to conserve these breeds. Breed characterization is the primary step in any conservation programme. The accuracy of phenotypic characterization of domestic cattle is often affected by the influence of the environment and the underlying genetic complexity. A number of studies have been initiated to characterize the European cattle breeds using the molecular techniques like microsatellite markers (5,6,7,8,9). Microsatellite markers, by virtue of

their codominant and multiallelic nature prove to be efficient in genetic diversity studies, pedigree evaluation and genetic mapping as compared to other molecular markers like RAPD, RFLP and ISSRs (10). Microsatellites have become markers of choice in characterization of cattle breeds (8,11,12, 13). Many studies have indicated that the deepest roots of cattle phylogeny occurs between Indian cattle and those of Europe (14). Inspite of the evolutionary significance of the Indian cattle breeds, the available literature on characterization of these breeds using the reliable molecular markers is scanty.

In the present study, we undertook the characterization of Ongole (n=17) and Deoni (n=13) cattle breeds using 5 di- and 5 tri- nucleotide repeat microsatellite markers. The two breeds showed a moderate genetic relationship (F_{ST} = 0.10). A few breed-distinguishing alleles were identified, which can be used to differentiate the two breeds.

Results and Discussion

In the present study, genetic polymorphism in the two cattle breeds, Ongole and Deoni was analyzed by using 10 microsatellite markers, which were known to be polymorphic in taurine populations. Two microsatellite markers ARO23 and PZE46 were monomorphic in both the breeds while the remaining eight markers showed polymorphism. These polymorphic loci gave a total of 39 alleles in both the breeds, with an average of 4.5 and 4.1 alleles per locus in Ongole and Deoni breeds, respectively. The FAO suggests five different alleles per locus are required for estimation of genetic differences between breeds. The mean number of alleles in the

present study is almost in accordance with the FAO recommendations. The monomorphic locus ARO23 showed allele size deviation in both the breeds when compared to the published data from other exotic breeds. Four alleles were reported for this locus with sizes ranging from 96 bp to 105 bp in IBRP full-sib families (15). However, in the present study, the same locus revealed a monomorphic allele of 78 bp in both the breeds. If this could turn out to be a *zebu* specific allele, it would be very useful. However, this could be only confirmed by studies involving other 24 recognized Indian *zebu* breeds.

There appears to be no correlation between the number of alleles detected and the number of SSR repeats in the SSR loci used in the study. For example, the microsatellite loci containing the 'GT' repeat motifs varying from $(GT)_{17}$ to $(GT)_{22}$ did not show any correlation with the number of alleles they revealed. However, the di-nucleotide repeat loci were more polymorphic (100%) than the tri- nucleotide repeat loci (60%). The two monomorphic loci observed in the present study (ARO23 and PZE46) are tri- nucleotide repeat loci. Three alleles specific to Deoni and six alleles specific to Ongole were observed with frequencies ranging from 3 to 24 % (Table 1). Further, some alleles were more frequent in one breed than the other (Fig. 1).

The genetic diversity in the breeds was expressed in terms of average heterozygosity. The average expected heterozygosity was 0.46 ($\pm$0.1) in Ongole breed and 0.50 ($\pm$0.1) in Deoni breed. Informativeness of the marker was expressed as PIC (16). The PIC of the polymorphic loci ranged from 0.15 to 0.79 in Ongole breed and 0.13 to 0.80 in Deoni breed (Table 2). The F_{IS} value, which indicates within breed genetic variation, for Ongole and Deoni breeds was 0.36 and 0.18,

respectively. This suggests that our sample represented individuals of inbred population of Ongole compared to Deoni. This could also be due to the small sample size and some of the individuals considered as unrelated during collection may indeed share common parentage. This is exactly the advantage of using molecular markers in revealing the actual genetic relationships. The overall genetic divergence between Ongole and Deoni, F_{ST} was 0.10. This genetic differentiation falls in the range of moderate genetic differentiation on a scale defined by Wright (17).

Conclusion

To our knowledge there has been no study involving molecular markers in comparing dual-purpose breeds of India. The present study attempts to apply microsatellite markers to analyze diversity and find genetic relationship between Ongole and Deoni. Further studies involving large samples of other *zebu* breeds with more microsatellite loci are required to understand the genetic relationships among the Indian breeds.

Materials and Methods

Blood Samples and DNA isolation

Blood samples were collected from unrelated 17 Ongole and 13 Deoni breed individuals maintained at two different livestock research stations managed by the A.N.G.R Agricultural University, Hyderabad, India, in 3ml Sodium-EDTA vacutainers. The genomic DNA was isolated and assessed for purity following standard molecular biology protocols (18).

Microsatellite analysis

Five di- nucleotide markers, BMS1716, BMS2057, BMS2270, BMS2840 and BMS2847 (19) and five tri- nucleotide markers, ARO23, ARO62 and ARO85 (15); BtDIAS1 (20) and PZE46 (21) were employed in the present study. For the loci ARO62, BtDIAS1 and PZE46, primers were designed using 'Amplify 1.2" program (22) as,

ARO62	F: CAGACACAACTGAAGCAACTC
	R: GTAGATTCCATAACAGC
BtDIAS1	F: GTAGCATCTTAATAATGCCCTC
	R: ACCCCACTCCAGCACTTTTG
PZE46	F: TTATGGCGGCTCCATATTAAC
	R: GTAACTCGGGCCCTTTCTCC

For the rest, primer sequence information was obtained from GenBank.

PCR conditions were empirically determined (Table 2). Twenty nanograms of genomic was used as template in a 10 μl PCR reaction consisting of 5 picomoles of each primers, 1 to 1.5 mM $MgCl_2$ (MBI Fermentas), 1 μl of 10 X PCR buffer (750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1% Tween-20),100 μM dNTPs, 0.5 U Taq polymerase (MBI Fermentas), in Genamp 9600^{TM} thermalcycler (Perkin Elmer). The PCR cycling conditions used were: 96°C for 1 min. (Initial Denaturation), 94 ^{o}C for 30 sec. (Denaturation), 49°C-58 ^{o}C for 30 sec. (Annealing), 72 ^{o}C for 1 min 30 sec. (Extension) and 72 ^{o}C for 10 min. (Final extension). The PCR samples were electrophoresed on 3.5% Metaphor^{TM} agarose (BMN) gel with 1 kb plus (Invitrogen) and pUC/MspI digest (MBI Fermentas) DNA size markers. To confirm the number of

alleles and to determine the allele size, Fluorescence based SSR analysis (23) was carried out. Denatured amplified samples were separated on a 5% denaturing polyacrylamide gel containing 7 M Urea after adding 2 μl of formamide gel loading buffer and 0.3 μl of ROX-500™ Genescan ruler (Perkin Elmer).

Data analysis

The allele size variation on the Metaphor Agarose gels was studied using Quantity One software (Bio Rad). The Genescan gels were analyzed using GeneScan 3.1 and Genotyper 2.1 software. The individuals are genotyped based on allele size data. Allele frequency and heterozygosity were calculated using MS Tools v3 (24). *F*-statistics were used as a measure of diversity within and between breeds respectively and were estimated using *F-STAT* program (25).

The PIC was calculated by using the formula (16):

$$PIC = 1 - \sum_{i=1}^{k} P_i^2 - \sum_{i=1}^{k-1} \sum_{j=1}^{k} 2P_i^2 P_j^2$$

Figure legends

Fig 1. Allele frequency distribution of the polymorphic microsatellite loci in Ongole and Deoni breeds

Table 1. Breed specific alleles in Ongole (n=17) and Deoni (n=13) breeds

Locus	Allele Size (bp)	Frequency	Breed
BMS1716	185	0.04	Deoni
BMS2057	72	0.15	Deoni
	110	0.06	Ongole
BMS2270	70	0.03	Ongole
	110	0.04	Deoni
BMS2840	211	0.03	Ongole
	241	0.09	Ongole
BMS2847	217	0.24	Ongole
BtDIAS1	342	0.21	Ongole

Table 2. PCR conditions and informativeness of microsatellite loci in two Indian cattle breeds, Ongole and Deoni

Locus name	Repeat type	Tm (°C)	$MgCl_2$ (mM)	PIC	
				Ongole	Deoni
ARO23	$(AGC)_8$	49	1.5	0.00	0.00
ARO62	$(AGC)_7$	52	1.5	0.15	0.37
ARO85	$(AGC)_8$	52	1.0	0.62	0.68
BMS1716	$(GT)_{20}$	58	1.5	0.34	0.65
BMS2057	$(GT)_{17}$	58	1.5	0.79	0.80
BMS2270	$(CA)_{23}$	58	1.5	0.45	0.62
BMS2840	$(GT)_{19}$	58	1.5	0.76	0.70
BMS2847	$(GT)_{22}$	58	1.5	0.56	0.35
BtDIAS1	$(TGC)_{11}$	56	1.0	0.46	0.13
PZE46	$(CCT)_8$	52	1.5	0.00	0.00

Abbreviations

PIC: Polymorphism Information Content; F_{IS} and F_{ST}: F-Statistics indices; IBRP: International Bovine Reference Family Panel;

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References

1. Taneja V K: **Dairy breeds and Selection.** In *Smallholder dairying in the tropics*. Edited by Falvey L, Chantalakhana C. International Livestock Research Institute, Nairobi, Kenya; 1999: 74.
2. Beja-Pereira A, Alexandrino P, Bessa I, Carretero Y, Dunner S, Ferrand N, Jordana J, Laloe D, Moazami-Goudarzi K, Sanchez A, Canon J: **Genetic characterization of southwestern European bovine breeds: a historical and biogeographical reassessment with a set of 16 microsatellites.** *Journal of Heridity* 2003, **94:** 243-250.
3. Koger M: **Effective crossbreeding systems utilizing zebu cattle.** *Journal of Animal Science* 1980, **50:** 1215-1220.
4. Turner JW: **Genetic and biological aspects of zebu adaptability.** *Journal of Animal Science* 1980, **50:** 1201-1205.

5. Kantanen J, Olsaker I, Holm LE, Lien S, Vilkki J, Brusgaard K, Eythorsdottir E, Danell B, Adalsteinsson S: **Genetic diversity and population structure of 20 North European cattle breeds**. *Journal of Heredity* 2000, **91**: 446-457.
6. Ciampolini R, Moazami-Goudarzi K, Vaiman D, Dillmann C, Mazzanti E, Foulley JL, Leveziel H, Cianci D: **Individual multilocus genotypes using microsatellite polymorphisms to permit the analysis of the genetic variability within and between Italian beef cattle breeds.** *Journal of Animal Science* 1995, **73**: 3259-3268.
7. Peelman LJ, Mortiaux F, Van Zeveren A, Dansercoer A, Mommens G, Coopman F, Bouquet Y, Burny A, Renaville R, Portetelle D: **Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds.** *Animal Genetics* 1998, **29:** 161-167.
8. Canon J, Alexandrino P, Bessa I, Carleos C, Carretero Y, Dunner S, Ferran N, Garcia D, Jordana J, Laloe D, Pereira A, Sanchez A, Moazami-Goudarzi K: **Genetic diversity measures of local European beef cattle breeds for conservation purposes.** *Genetics Selection Evolution* 2001, **33**: 311-332.
9. MacHugh DE, Loftus RT, Cunningham P, Bradley DG: **Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers.** *Animal Genetics* 1998, **29**: 333-340.
10. Nagaraju J, Reddy KD, Nagaraja GM, Sethuraman BN: **Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm *Bombyx mori.***

Heridity 2001, 86**:** 588-597.

11. Edwards C, Bradley DG, MacHugh DE: **A panel of Y-specific microsatellite markers suitable for studies of genetic differentiation in cattle and related species.**
Animal Genetics 2000, **31**: 127-130.
12. Loftus RT, Ertugrul O, Harba AH, El-Barody MA, MacHugh DE, Park SD, Bradley DG: **A microsatellite survey of cattle from a centre of origin: the Near East.**
Molecular Ecology 1999, **8**: 2015-2022.
13. MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG: **Microsatellite DNA variation and the evolution domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*).**
Genetics 1997, **146**: 1071-1086.
14. Bradley DG, Loftus RT, Cunningham P, MacHugh DE: **Genetics and domestic cattle origins.**
Evolutionary Anthropology 1998, **6**: 79-86.
15. Band M, Ron M: **Creation of a SINE enriched library for the isolation of polymorphic $(AGC)_n$ microsatellite markers in the bovine genome.**
Animal Genetics 1996, **27:** 243-248.
16. Botstein D, White RL, Skolnick M, Davis RW**: Construction of a genetic linkage map in man using restriction fragment length polymorphism.**
American Journal of Human Genetics 1980, **32**:314–331.
17. Wright S: *Evaluation and the genetics of Populations. Volume 4. Variability within and among Natural Populations.* University of Chicago Press, Chicago; 1978.

18. Sambrook J, Russell DW: *Molecular Cloning A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; 2001.

19. Stone RT, Kappes SM, Keele JW, Beattie CW: **Characterization of 109 bovine microsatellites**. *Animal Genetics* 1997, **28:** 63-65.

20. Shukri NM, Holm LE, Brusgaard K: **A polymorphic trinucleotide microsatellite in cattle: BtDIAS1.** *Animal Genetics* 1996, **27:** 373.

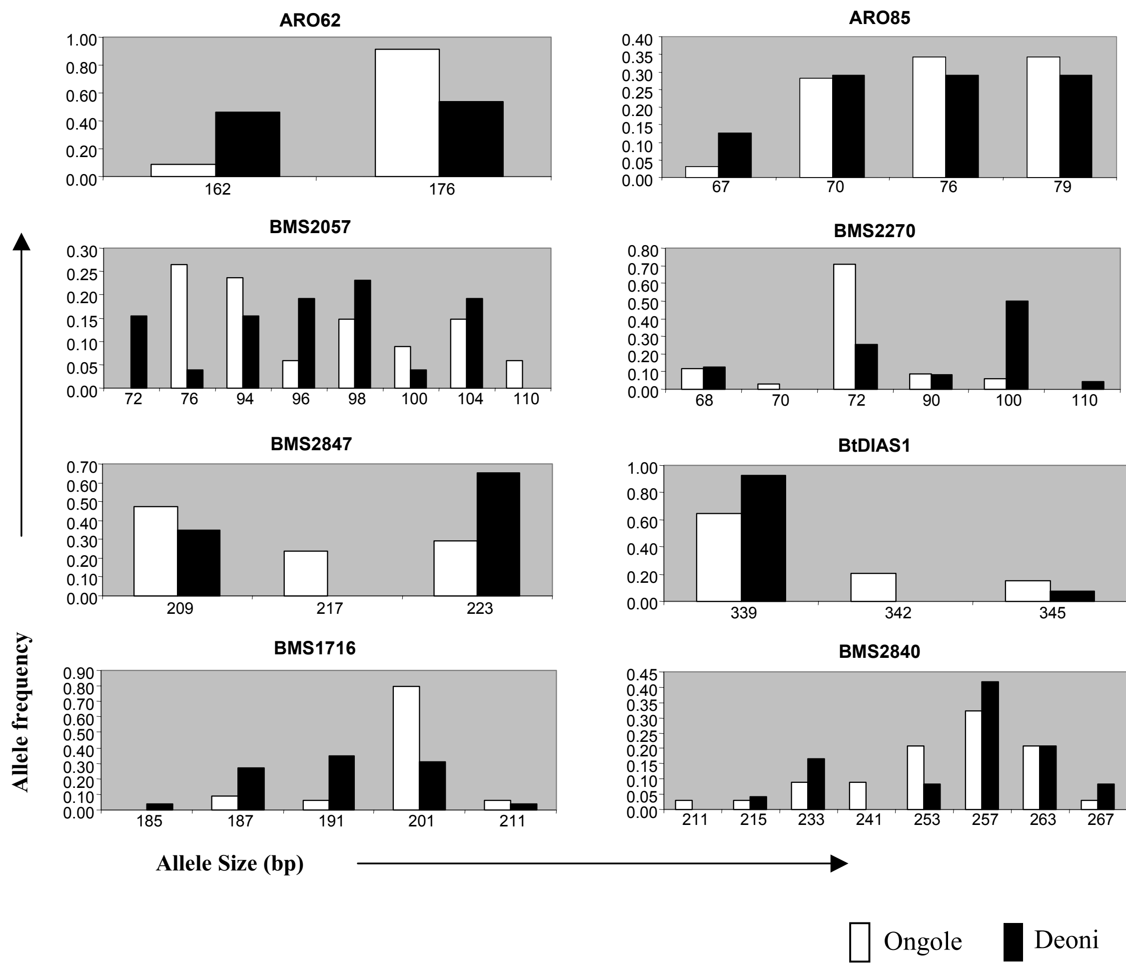
21. Motavalian M, Rando A, Urciuoli M, Senese C, Di Gregorio P, Masina P: ***PZE46 and PZE114*: two bovine polymorphic microsatellite loci isolated from a placenta cDNA library**. *Animal Genetics* 2002, **33:** 159-160.

22. Engels WR: **Contributing software to the Internet: the *Amplify* program.** *Trends in Biochemical Sciences* 1993, **18**: 448-450.

23. Nagaraju J, Kathirvel M, Ramesh Kumar R, Siddiq EA, Hasnain SE: **Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence -based ISSR-PCR and SSR markers.** *Proceedings of National Academy of Sciences, USA* 2002, **99**: 5836-5841.

24. **The Excel Microsatellite Tool Kit** (http://acergentcdie/~sdepark/ms-toolkit/)

25. Goudet J: **FSTAT (Version 12): A computer program to calculate F-statistics**. *Journal of Heredity* 1995, **86:** 485-486.


ARO62
1.00
0.80
0.60
0.40
0.20
0.00
162
176
ARO85
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
67
70
76
79
BMS2057
0.30
0.25
0.20
0.15
0.10
0.05
0.00
72
76
94
96
98
100
104
110
BMS2270
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
68
70
72
90
100
110
BMS2847
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
209
217
223
BtDIAS1
1.00
0.80
0.60
0.40
0.20
0.00
339
342
345
BMS1716
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
185
187
191
201
211
BMS2840
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
211
215
233
241
253
257
263
267
Allele frequency
Allele Size (bp)
Ongole
Deoni