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National Academy of Agricultural Science (NAAS) Rating : 3. 03

Association of the Microsatellite Marker with Body Biometric Parameters in Koraput Sheep of Odisha

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ABSTRACT: *The present investigation was undertaken to study the association of the polymorphism of the microsatellite markers with the body biometric traits in the Koraput sheep which is a lesser known sheep of Odisha. Body biometric measures were recorded in the Koraput sheep by standard procedures using tailor's tape. The Genetic polymorphism was studied by the automated DNA sequencer and the association between microsatellite genotypes and the body biometric traits was analyzed using General Linear Model (GLM) procedure using SPSS software. This study identifies an association of marker BM827 with Punch Girth and Face Length which are important body biometry parameters. The identification of this marker within the same syntenic group with Lactoglobulin (LGB), Somatic Cell Count (SCC) and internal fat indicates the importance of this marker in the livestock production. Further studies on the identification of SNPs in candidate genes closely linked to this marker and development of haplotypes will provide the usefulness of this highly polymorphic marker in the breeding programme for the identification of the superior genotypes for the selection of the animals.*

Key words:- Koraput sheep, genetic characterization, microsatellite marker association

INTRODUCTION

India is rich in ovine biodiversity, possessing around 6.5% of sheep population of the world with a total of 65.06 million heads [1]. However, only around 25% of the country's estimated sheep population which comprises of 40 registered breeds has been exploited so far, through survey on native distribution tracts and/or their molecular characterization. These sheep groups need to be characterized at phenotypic as well as genetic level in order to assess the actual ovine diversity available in our country. This would also enable unique/ economically important ovine populations to be identified, documented and recognized at the National level, eventually leading to their conservation and improvement. Koraput sheep is one such lesser known sheep population, reared for the mutton purpose and widely distributed in Koraput, Nabarangpur, Malkangiri and Rayagada districts of Odisha (Kornel Das, 1999). Although few reports are available on the morphometric characteristics of Koraput sheep, their molecular genetic characterization using microsatellite markers and association study with the body biometric parameters is totally lacking [2 and 3]. Microsatellites are made up of core sequences of 2-6 base pairs and have repetitions of simple sequence repeat (SSR). Microsatellites are abundant, evenly distributed, highly polymorphic and co-dominantly inherited. They are often used in the association studies of disease resistance and production traits in livestock species [4]. Marker assisted selection (MAS) technology can be used for mapping of quantitative trait loci (QTLs) or economic trait loci (ETLs). Association of the microsatellite marker with the body biometric parameters might be helpful in the selection of the superior animals at an earlier age. This would assist in the improvement of the traits of economic importance like body weight, higher conformation and musculature and other production traits linked to them. Therefore, the present study was undertaken to study the association of the

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microsatellite markers with the body biometric parameters of the Koraput sheep.

MATERIALS AND METHODS

Body biometric measurement

Body biometry (Body Weight, Body Length, Height at Wither, Chest Girth, Punch Girth, Ear Length, Horn Length, Tail Length and Face Length) was recorded on 42 adult male and 319 female of Koraput sheep.

Microsatellite analysis

Blood samples were collected from 60 unrelated animals of Koraput sheep in its breeding tract. The genomic DNA from the blood samples was isolated using a standard phenol/chloroform/isoamyl alcohol extraction method [5]. A set of 24 microsatellite markers (table 1) based on the list of MoDAD (FAO) were utilized to generate data on 60 DNA samples of the Koraput sheep [6,7]. The details of microsatellite markers, primer sequences, size range, genebank accession number and chromosomal location is given in table1. The forward primer for each marker was fluorescently labeled with FAM, NED, VIC or PET dye. Amplification of the loci was performed in a 25µl final reaction volume containing at least 100 ng of genomic DNA, 5pM of each primer, 1.5mM $MgCl_2$, 200µM dNTPs, 0.5 U *Taq* DNA polymerase and 1x *Taq* buffer. A common touch down PCR programme, as suggested under MoDAD project without extension step was used for the amplification of all the twenty four markers [8]. PCR amplification consisted of 3 cycles of 45 sec at 95^0C, 1 min at 60^0 C; 3 cycles of 45 sec at 95^0C, 1 min at 57^0 C; 3 cycles of 45 sec at 95^0C, 1 min at 54^0 C; 3 cycles of 45 sec at 95^0C, 1 min at 51^0 C and 20 cycles of 45 sec at 95^0C, 1 min at 48^0 C. The amplified products were resolved on 2% agarose gel and the genotyped on an automated DNA sequencer using LIZ 500 as internal lane standard (ABI PRISM). The raw data files were extracted by Gene Mapper software version 3. Popgen3.2 [9] and GenAlEx6.5 softwares [10]. were used for the genetic diversity analysis.

Association studies

The association between microsatellite genotypes and the body biometric traits was analyzed using general linear model (GLM) procedure using SPSS software version 20 and the following linear model was used:

$$Y_{ijk} = \mu + S_i + G_j + e_{ijk},$$

Where:

Y_{ijk} is the studied phenotype

μ is the overall mean

S_i is the i^{th} sex effect

G_j is the j^{th} microsatellite marker genotype effect

e_{ijk} is the random error associated with Y_{ijk} phenotype with $(0, \acute{o}^2)$

Multiple comparison of Least Square Means (LSM) was performed with the Bonferroni test. The studied microsatellite loci were tested separately (one at a time) and the significant differences between microsatellite genotypes for each locus were indicated.

RESULTS AND DISCUSSION

The body biometry parameters of the adult animals of Koraput sheep are given in table 2. Details of microsatellite markers used and the genetic diversity measures are depicted in table 1 and 3; respectively. A total of 224 alleles were identified across the 24 markers. The observed no. of alleles ranged from 3 (BM6506, CSSM47) to 16 (OarHH35) with a mean of 9.33. Effective number of alleles was lower than the observed number of alleles and ranged from 1.62 (OarFCB48) to 11.58 (OarHH35) with a mean value of 5.13. Out of the 24 markers studied, 17 markers with complete records were used to study the association of genotypes with the body biometric traits. A significant association ($P<0.05$) was observed for the marker BM827 with the Face length and Paunch girth. Genotype AB differed significantly from the genotype BB ($P=0.034$) for the Face length and the genotype CC differed significantly from the genotype DE ($P= 0.045$) for the Punch girth. However, non-significant association was observed for the remaining 16 markers for all the traits ($P >0.05$). The least square means and S.E. for these genotypes with significant effects are given in table 4. No association studies have so far been carried out between the body biometry parameters and microsatellite marker polymorphism in Indian sheep.

Cavanagh and coworkers 2010 identified a QTL close to marker BM827 for internal fat on chromosome 3 in Awassi X Merino sheep with significant effect (LOD score 2.1) while mapping the QTL for the carcass composition traits in sheep[11]. The significant association of the Punch girth with the marker BM827 might be due the presence of the QTL effect for internal fat close to this region. Punch girth is often used as an explanatory variable for the prediction of the body weight from body biometry

Table 1
Details of microsatellite markers used in the study of Koraput sheep.

Locus	*Primer sequence*	*Size range (bp)*	*Genebank accession no.*
BM6506	F- gCA CgT ggT AAA gAg ATg gC		
	R- AgC AAC TTg AgC ATg gCA C	191-199	G18455
OarCP20	F- gAT CCC CTg gAg gAg gAA ACg g		
	R- ggC ATT TCA Tgg CTT Tag CAg g	72-88	U15699
OarFCB48	F- gAg TTA gTA CAA ggA TgA CAA gAg gCA C		
	R- gAC TCT AgA ggA TCg CAA AgA ACC Ag	146-152	M82875
OarVH72	F- CTC Tag Agg ATC Tgg AAT gCA AAg CTC		
	R- ggC CTC TCA Agg ggC AAg AGC Agg	121-133	L12548
OarHH47	F- TTT ATT gAC AAA CTC TCT TCC TAA CTC CAC C		
	R- gTA gTT ATT TAA AAA AAT ATC ATA CCT CTT AAg g	124-144	L12557
BM757	F- Tgg AAA CAA TgT AAA CCT ggg		
	R- TTg AgC CAC CAA ggA ACC	172-186	G18473
BM1314	F- TTC CTC CTC TTC TCT CCA AAC		
	R- ATC TCA AAC gCC AgT gTg g	141-167	G18455
BM8125	F- CTC TAT CTg Tgg AAA Agg Tgg g		
	R- ggg ggT Tag ACT TCA ACA TAC g	109-119	G18475
OarHH35	F- AAT TgC ATT CAg TAT CTT TAA CAT CTg gC		
	R- ATg AAA ATA TAA AgA gAA TgA ACC ACA Cgg	107-137	L12554
OarHH64	F- CgT TCC CTC ACT ATg gAA AgT TAT ATA TgC		
	R- CAC TCT ATT gTA AgA ATT TgA ATg AgA gC	127-135	L12558
OarJMP8	F- Cgg gAT gAT CTT CTg TCC AAA TAT gC		
	R- CAT TTg CTT Tgg CTT CAg AAC CAg Ag	115-139	U35059
OarJMP29	F- gTA TAC ACg Tgg ACA CCg CTT TgT AC		
	R- gAA gTg gCA AgA TTC AgA ggg gAA g	109-143	U30893
BM827	F- ggg CTg gTC gTA TgC TgA g		
	R- gTT ggA CTT gCT gAA gTg ACC	210-218	U06763
OarHH41	F- TCC ACA ggC TTA AAT CTA TAT AgC AAC C		
	R- CCA gCT AAA gAT AAA AgA TgA TgT ggg Ag	120-140	L12555
CSSM31	F- CCA AgT TTA gTA CTT gTA AgT AgA		
	R- gAC TCT CTA gCA CTT TAT CTg TgT	146-180	U03838
CSSM47	F- TCT CTg TCT CTA TCA CTA TAT ggC		
	R- CTg ggC ACC TgA AAC TAT CAT CAT	148-152	U03821
CSRD0247	F- ggA CTT gCC AgA ACT CTg CAA T		
	R- CAC TgT ggT TTg TAT TAg TCA gg	211-239	EU009450
MAF0214	F- AAT gCA ggA gAT CTg Agg CAg ggA Cg		
	R- ggg TgA TCT TAg ggA ggT TTT ggA gg	185-229	M88160
OarCP0049	F- CAg ACA Cgg CTT AgC AAC TAA ACg C		
	R- gTg ggg ATg AAT ATT CCT TCA TAA gg	83-139	U15702
BM6526	F- CAT gCC AAA CAA TAT CCA gC		
	R- TgA Agg Tag AgA gCA AgC AgC	191-199	G18454
OarCP34	F - gCT gAA CAA TgT gAT ATg TTC Agg		
	R - ggg ACA ATA CTg TCT Tag ATg CTg C	112-126	U15699
INRA0063	F -gAC CAC AAA ggg ATT TgC ACA AgC		
	R - AAA CCA CAg AAA TgC TTg gAA g	173-199	X71507
OarAE129	F -AAT CCA gTg TgT gAA AgA CTA ATC CAg		
	R - gTA gAT CAA gAT ATA gAA TAT TTT TCA ACA CC	133-159	L11051
OarFCB128	F -CAg CTg AgC AAC TAA gAC ATA CAT gCg		
	R - ATT AAA GCA TCT TCT CTT TAT TTC CTC GC	106-130	L01532

[3]. The significant association of the marker BM827 with Punch girth indirectly explains the variability in the body weight which is an economically useful trait used in the selection index for the mutton type of sheep like Koraput.

BM827 was found to be segregating dependently with Lactoglobulin (LGB) on chromosome 12 in buffalo [12] and had a QTL effect on Somatic Cell Score (SCC) in cattle (http://www.ncbi.nlm.nih.gov/mapviewer). In another study, Nudda and coworkers observed the significant effect of BLG genotypes in the Sarda ewes on milk yield [13]. Further, there was a significant increase in whey protein with the increase in the SCC of the milk.

River buffalo, cattle and sheep are three domestic species of the same family (bovidae). Since marker BM827 has syntenic relationship with LGB, SCC and internal fat in these three species, this marker can be

Table 2
Body weight and Biometry of Adult Koraput sheep.

Body Character	*Male (N=42)Average ± SE*	*Female (N=319)Average ± SE*	*SignificanceZ value*
Body length (cm)	50.30± 0.63	50.93±0.22	0.94
Height at Wither (cm)	57.71± 0.52	56.44±0.18	2.30*
Chest Girth (cm)	65.78±0.55	65.92±0.22	0.23
Paunch Girth (cm)	67.21±0.88	67.29±0.29	0.08
Ear Length (cm)	5.05±0.45	6.02±0.20	1.96*
Horn Length (cm)	7.26±0.60	-	
Tail Length (cm)	9.63±0.30	9.03±0.09	1.87
Face Length (cm)	16.09±0.18	15.65±0.07	2.28*
Body Weight (Kg)	19.79±0.58	20.03±0.19	0.391

* indicates significant difference at 5% level of significance.

Table3
Genetic variability measures in Koraput sheep across 24 microsatellite markers.

Locus	*na*	*ne*	*Heterozygosity Observed/ Expected*		*PIC*	F_{IS}
BM6506	3	1.84	0.38	0.46	0.36	0.15
OarCP20	9	5.62	1.0	0.82	0.80	-0.21
OarFCB48	4	1.62	0.27	0.38	0.35	0.28
OarVH72	7	5.68	0.89	0.83	0.79	-0.89
OarHH47	11	6.61	0.70	0.85	0.83	0.17
BM757	8	2.73	0.45	0.64	0.60	0.27
BM1314	12	5.96	0.79	0.83	0.80	0.04
BM8125	6	3.87	0.56	0.74	0.70	0.23
OarHH35	16	11.58	0.77	0.92	0.90	0.15
OarHH64	5	3.69	0.37	0.74	0.68	0.49
OarJMP8	13	7.75	0.70	0.87	0.85	0.19
OarJMP29	9	4.97	0.70	0.81	0.77	0.11
BM827	5	3.01	0.56	0.67	0.61	0.15
OarHH41	9	3.81	0.63	0.74	0.77	0.14
CSSM31	11	4.11	0.85	0.77	0.73	-0.12
CSSM47	3	2.45	1.0	0.60	0.50	-0.68
CSRD0247	13	7.54	0.81	0.87	0.85	0.05
MAF214	13	2.82	0.68	0.65	0.61	-0.05
OarCP49	13	7.51	0.73	0.87	0.85	0.15
BM6526	9	5.30	0.81	0.91	0.78	-0.002
OarCP34	8	3.44	0.56	0.71	0.67	0.20
INRA63	10	5.79	0.31	0.84	0.80	0.61
OarAE129	14	6.35	0.48	0.85	0.81	0.41
OarFCB128	13	9.15	0.72	0.90	0.88	0.18
Mean	9.33	5.13	0.65	0.76	0.72	0.124
SD	3.69	2.43	0.20	0.13	0.15	

Table 4
The least square means and S.E. for these genotypes with significant effect (P<0.05)

Locus	*Trait*	*Genotype*	*Least square means ± SE (cm)*
BM827	Face length	AB	17.5± 0.65
		BB	14.34±0.57
	Paunch girth	CC	63.94±1.35
		DE	76.71±3.61

exploited for the improvement of these economically useful traits in the livestock species.

Moreover, this marker (BM827) is a probe for the target gene AFF3 in *Bos taurus* and *Ovis aries* (http://www.ncbi.nlm.nih.gov/probe) which is involved in the proper development of lymphoid tissue, essential for the growth and development of an animal (*www.genecard.org*).

In a similar study carried out in red fox on 19 microsatellite markers, 10 markers were significantly associated with four economically important traits (Body Weight, Body Length, Body Circumference and Tail length) [14]. Four out of them were reported to be associated with two characters, simultaneously. The association of the microsatellite markers with Body Weight, Height and Relative Body Mass in American Bison herds was studied by Musani and coworkers 2006 [15]. Although none of the main effects of the loci were significant, estimated ancestry and its interaction with marker loci were significantly associated with the phenotypes, illustrating the importance of including the ancestry in the models.

Microsatellite markers are often used for studying the association of disease resistance and production traits in livestock species [4, 15]. Analysis of association between three microsatellite loci BMS2508, BM1329 and OarAE101 located in the 10 cM region covering the FecB gene (*Booroola* gene) and litter size in Small Tail Han sheep indicated that BMS2508 had significant effect on litter size in the second parity ($p<0.05$) signifying the role microsatellite association mapping in the reproductive traits in sheep [16]. FAO recommended microsatellites are routinely been used for the genetic characterization of livestock species. The association of BM827 with the Face length and Paunch girth in sheep might through some light into the linkage of this marker with the evolution of this breed in the local adaptive condition on the basis of the body biometry, growth trait and production traits.

CONCLUSION: This study identifies an association of marker BM827 with Punch girth and Face length which are important body biometry parameters. The identification of this marker within the same syntenic group with LGB, SCC and internal fat indicate the importance of this marker in the livestock production. Further studies on the identification of SNPs in candidate gene closely linked to this marker and development of haplotypes will provide the usefulness of this highly polymorphic marker in the breeding programme for the identification of the superior genotypes for the selection of the animals.

ACKNOWLEDGEMENT

The Authors are thankful to the Director, NBAGR, State Animal Husbandry Department, and Orissa Livestock Resources Development Society, Odisha for the full support and cooperation during the entire research programme.

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