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Phenotypic characterization and microsatellite markers based genetic evaluation of Kalahandi goats

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ABSTRACT

Sporadic information is available on morphological traits of Kalahandi, a medium size non-descript goat population of Kalahandi district of Odisha, but no information is found on its genetic diversity. Therefore, an attempt was made to measure the genetic diversity in Kalahandi goat population using 25 microsatellite markers. Genomic DNA isolated from blood samples drawn at random from 50 individuals were utilized for this study. PCR amplified products were used for genotyping on the automatic DNA sequencer. The data were analysed applying different softwares to estimate the various measures of genetic diversity. The average number of observed allele was 11.08 and the effective average number of allele was 4.29. The observed heterozygosity ranged from 0.11 to 0.98 and the expected heterozygosity from 0.11 to 0.87 respectively. Average PIC value ranged from 0.45 (ILSTS044) to 0.91 (OMHC1) with the average value 0.79. Average Fis value for markers tested in Kalahandi goat population ranged from 0.07 at ILSTS030 to 0.67 at OarAE129. Among the negative values it ranged from –0.36 (RM088) to –0.02 (ILSTS029). The mean F value was 0.02. There was no genetic bottleneck observed in Kalahandi goat population. The results suggested that all the microsatellite markers were highly polymorphic and suitable for molecular characterization of Kalahandi goats. There was substantial genetic variation and polymorphism across the studied loci in Kalahandi goats and the population was not in H.W equilibrium but in mutation drift equilibrium. The low inbreeding observed in Kalahandi flocks is a favourable parameter to formulate the appropriate breeding strategies to enhance heterozygosity in the population.

Key words: Genetic diversity, Kalahandi goat, Microsatellite

Genetic diversity is important to–meet current and future production needs in varied and frequently changing climatic conditions, allow a sustained genetic improvement and facilitate rapid adaptation to changing breeding objectives (Crawford and Littlejohn 1998). Microsatellites have become the markers of choice for many applications. Their abundance, high level of polymorphism manifested as the occurrence of a large number of alleles per locus, and co-dominant inheritance has facilitated their extensive use in genome mapping, phylogenetic inference and population genetics (Crawford and Littlejohn 1998, Jouquand *et al.* 2000, Moioli *et al.* 2001). The genetic diversity of European, Indian and other countries goat breeds have been well researched, however, most of these studies pertain to the well characterized and documented breeds. Inadequately studied or lesser known goat populations are yet to be a subject of intense research. Such populations form about 70% of the total goat population of the country. In the present investigation an attempt was made to study the genetic variability in Kalahandi goats of Odisha state using microsatellite markers.

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MATERIALS AND METHODS

Data recording and blood sampling: Visits were made to the breeding tract of Kalahandi goats. The body measurements were recorded for height at wither, body length, chest girth, paunch girth, face length, ear length, horn length and tail length of 132 animals belonging to different flocks, age and sex. Body weights were taken of adult (>18 months) animals of both sexes. Blood samples were collected from jugular vein using EDTA coated vaccutainer tubes from 50 animals of different parentage.

DNA extraction and PCR amplification: Genomic DNA was extracted using standard phenol/chloroform extraction protocol (Sambrook *et al.* 1989). The extracted DNA was checked for quality and quantity. A total of 25 fluorescently labelled microsatellite markers were chosen based on the

degree of polymorphism reported in the literature (FAO 2004). They were further optimized and tested for polymorphism using genomic DNA extracted from individual animals. Only forward primers of each pair were labelled with 1 of the 4 fluorophore, that is, pentachlororo-6-carboxyflouroscien (NED), 6 carboxyflouroscien (FAM), phosphoramidites (VIC) and PED, which were synthesized and supplied by Applied Biosystems (ABI). The polymerase chain reaction (PCR) mixture with the final volume of 10 µl consisted of 50ng of genomic DNA, 10 pmol of each primer, 10mM dNTPs, 0.5U Taq polymerase and 10x buffer. The amplification was carried out for 35 cycles with initial denaturation at 95°C for 10 min, second denaturation at 95°C for 30 sec; annealing with different temperatures for 1 min, extension for 45 sec at 72°C and final extension for 7 min at 72°C.

Genotyping and allele detection: After ensuring the amplification, the PCR products were run on automated DNA Sequencer. The PCR products were mixed with 0.3 µl of Liz 500 as internal lane standard and 9.20 µl of Hi-Di Formamide per sample. The resulting mixture was denatured by incubation for 5 min at 95°C. These denatured samples were run on automated DNA sequencer. The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software (version 3.0).

Statistical analysis: The data generated on 25 microsatellites loci were statistically analysed for the assessment of genetic diversity. The PIC values, observed and expected heterozygosities were calculated using POPGENE (Yeh *et al*. 1999) and Cervus software (Botstein *et al*. 1980). The observed and effective number of alleles were calculated using POPGENE software (Kimura and Crow 1964). F-statistics were determined using F-Stat software (Goudet 2001) with a Jackknifing procedure applied on the loci by deriving their significance levels. Bottleneck hypothesis was tested using BOTTLENECK 1.2.01 as per Cornuet and Luikart (1996). This test for the departure from mutation drift equilibrium is based on the excess/deficiency of heterozygosity. The bottleneck compares expected heterozygosities at HW equilibrium to that at mutation drift equilibrium in same sample having same number of alleles. Bottleneck events were tested by three methods. The first method consisted of 3 excess heterozygosity tests i.e (i) sign test, (ii) standardized difference test, and (iii) Wilcoxon sign-rank test developed by Cornuet and Luikart (1996). The probability distribution was established using 1000 simulations under 3 models; infinite allele model (IAM), step wise mutation model (SMM) and two phase model of mutation (TPM).

RESULTS AND DISCUSSION

Phenotypic attributes and performance: Kalahandi goats are of medium size, found extensively in the Kalahandi, Nuapara district and adjoining areas in Odisha. Kalahandi goats are mostly brown/tan and white with a strip on either side of face extending from base of horns to muzzle

Fig. 1. Colour variants of Kalahandi goats.

(Fig. 1). Ears are flat, leafy and drooping; horns long flat upward and backward; legs thin and cylindrical. In breeding males a black colour ring around the neck is observed. These animals are hardy and can thrive well in harsh climatic conditions like high temperature in the native tract which goes up to 48°C in summer. Twinning is more than 60%. The flocks of size 5 to 25 are kept at small holdings under extensive and semi extensive management system without applying any proper breeding and genetic improvement strategies.

Body biometry: The average biometric estimates for height at withers, body length, chest girth, paunch girth, face length, horn length, ear length and tail length were 70.32, 69.05, 71.53, 73.84, 17.89, 15.05, 15.74 and 15.32 respectively for adult males and 65.51, 66.28, 70.36, 74.87, 15.29, 12.74, 15.01 and 13.97 respectively for females. The body weights of adult male and females were 33.42 and 30.88 kg.

Genetic diversity: Various parameters like allelic size, heterozygosity, polymorphic information contents and f – values were estimated to determine the genetic diversity in Kalahandi goats and are presented in Table 1.

Allelic variation: The allele number across the loci varied from 4.00 (ILSTS005 and ILSTS065) to 18.00 (ILSTS030 and OarFCB304). The mean number of alleles across the markers was 10.00. The effective allele size varied from 1, 12 (ILSTS044) to 7.32 (ILSTS087) with mean 4.29. The mean allelic size observed in this study was higher than that reported for Jakhrana (Kumar *et al*. 2005), Sangamneri (Verma *et al.* 2011), Zalawadi, Gohilwadi and Surti goat (Fatima *et al.* 2008), Changthangi (Mishra *et al.* 2010), and Southern goat breeds (Dixit *et al.* 2010 and 2011). The alleles more than the observed number was reported in goat breeds like Mehsana (Aggarwal *et al.* 2007), Gohilwari (Kumar *et al.* 2009) and Sirohi (Verma *et al.* 2007). The observed number of alleles across the loci was more than the effective number of alleles as per expectation. The allelic polymorphism observed here indicated the suitability of microsatellite markers for the genetic diversity in Kalahandi goats. Suitability of markers was further supported by the fact that each marker had more than 4 alleles, the number recommended by Barker (1994) to confirm the suitability of microsatellite markers.

Heterozygosity: Heterozygosity is an indicator of inbreeding in the population and is defined as probability

Table 1. No. of observed and effective allele, observed and expected heterozygosity, polymorphic information content and F-statics

Locus	Na	Ne	Ho	He	PIC	Fis	Hd
ILSTS030	18.00	6.20	0.78	0.84	0.89	0.07	–0.071
ILSTS065	4.00	1.37	0.22	0.27	0.55	0.17	–0.185
ILSTS005	4.00	1.70	0.43	0.41	0.63	–0.04	0.049
ILSTS087	14.00	7.32	0.77	0.87	0.89	0.18	–0.115
ILSTS033	12.00	4.78	0.98	0.80	0.77	–0.22	0.225
OarAE129	9.00	5.35	0.27	0.82	0.89	0.67	–0.671
ETH225	2.00	1.13	0.12	0.11	0.45	–0.05	0.091
ILSTS058	14.00	5.29	0.75	0.82	0.87	0.08	–0.085
OarHH64	16.00	4.92	0.91	0.80	0.86	–0.14	0.138
ILSTS059	9.00	4.38	0.87	0.78	0.80	–0.12	0.115
ILSTS034	9.00	3.93	0.79	0.75	0.81	–0.05	0.053
ILSTS082	11.00	3.94	0.58	0.75	0.85	0.22	–0.227
RM4	12.00	7.22	0.95	0.87	0.88	–0.10	0.092
ILSTS008	10.00	4.48	0.42	0.78	0.86	0.46	–0.462
ILSTS019	8.00	4.19	0.82	0.77	0.82	–0.07	0.065
OarFCB304	18.00	5.51	0.72	0.82	0.88	0.12	–0.122
OarFCB48	13.00	5.92	0.77	0.84	0.88	0.08	–0.083
ILSTS022	5.00	2.36	0.48	0.58	0.72	0.16	–0.172
OarJMP29	7.00	4.15	0.96	0.76	0.83	–0.25	0.263
OMHC1	17.00	6.60	0.98	0.85	0.91	–0.14	0.153
ILSTS044	4.00	1.12	0.11	0.11	0.45	–0.03	0.000
ILSTS049	9.00	4.18	0.72	0.77	0.85	0.06	–0.065
ILSTS029	11.00	2.80	0.66	0.65	0.75	–0.02	0.015
RM088	7.00	3.50	0.98	0.72	0.78	–0.36	0.361
ILSTS002	7.00	4.85	0.72	0.80	0.86	0.10	–0.100
Mean	10.00	4.29	0.67	0.68	0.79	0.02	–0.015

Na,Observed number of alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information contents; Hd, heterozygotic deficiency; Fis, f estimates.

Table 2. Test for null hypothesis for mutation drift equilibrium under three mutation models (IAM, TPM and SMM) using Sign rank, standardized differences and Wilcoxon tests in Kalahandi goat

	Sign test		
	IAM	TPM	SMM
Expected No. of loci with heterozygosity excess	14.75	14.70	14.63
Observed No.of loci with H excess	16	8	1
Probabilty	0.384	0.006	0.000
Standardized Difference test			
	IAM	TPM	SMM
T2 Value	0.647	-3.878	-11.425
Probabilty	0.258	0.000	0.000
Wilcoxon test			
	IAM	TPM	SMM
Probability (one tails for H excess)	0.149	0.990	1.000

IAM, Infinite allele model; SMM, step wise mutation model; TPM, two-phase model.

that an individual is heterozygous for the locus in population. Inbreeding resulting from mating between more closely related individuals will reduce the heterozygosity in the population. If the inbreeding coefficient is F, the expected heterozygosity reduces to (1-F) H as per Liu (1998). The observed heteozygosity in Kalahandi goat population ranged from 0.11 (ILSTS044) to 0.98 (ILSTS033, OMHCI, RM088) with mean value 0.67 whereas the expected heterozygosity ranged from 0.11 (ETH225, ILSTS044) to 0.87 (ILSTS087, RM4) with mean 0.68. The observed heterozygosity was lower than expected heterozygosity at about 50% loci (ILSTS030, ILSTS065, ILSTS087, OarAE129, ILSTS058, ILSTS082, ILSTS008, OarFCB304, OarFCB48, ILSTS022, ILSTS049, ILSTS002). However, the observed mean heterozygosity (0.67) was not significantly different from the expected mean heterozygosity (0.68). The heterozygotic deficiency was observed at ILSTS005, ILSTS033, ETH225, OarHH64, ILSTS059, ILSTS034, RM4, ILSTS019, OarJMP29, OMHC1, ILSTS044, ILSTS029 and RM088. This indicated positive deviation from Hardy-Weinberg Equilibrium at about half of the studied loci. Similar reports are available for many Indian goat breeds (Dixit *et al.* 2011).

Polymorphic information content (PIC): The PIC is a parameter indicative of the informative degree of a marker. PIC for all the 25 markers is shown in Table 1. The PIC value ranges from 0 to 1, however, average PIC in this study ranged from 0.45 (ILSTS044) to 0.91 (OMHC1) with the average value 0.79. Most of the markers had PIC values higher than 0.5, (except for locus ETH225 and ILSTS044) which is a useful indicator of genetic variability and forms the basis for developing breeding or genetic improvement strategy for a population.

F estimates: Inbreeding coefficients for all markers are given in Table 1. Average Fis value for markers tested in Kalahandi goat population ranged from 0.07 (ILSTS030) to 0.67 (OarAE129). Among the negative values it ranged from –0.36 (RM088) to –0.02 (ILSTS029). The mean F value was 0.02, which indicated the nominal amount of inbreeding in the population. The higher negative Fis indicated presence of heterozygosity suggesting that Kalahandi goat populations might have been managed under controlled mating system by avoiding mating between the close relatives. This is in contrast to what has been observed in other Indian goat breeds like Marwari (Kumar *et al.* 2005), Attapaddy (Aggarwal *et al.* 2006), Sirohi (Verma *et al.* 2007), Kutchi (Dixit *et al.* 2008), Gohilwari (Kumar *et al*. 2009), Southern Indian goat breeds (Dixit *et al*. 2010), Changthangi (Mishra *et al*. 2010), Konkan Kanyal (Mishra *et al*. 2012).

Genetic bottleneck: In Kalahandi goat population, under Sign test, the expected numbers of loci with heterozygosity excess were 14.75 (IAM), 14.70 (TPM) and 14.63 (SMM). The values were substantially higher than the observed numbers of loci 8 and 1 with heterozygosity excess under TPM and SMM respectively. So the null hypothesis that the population is under mutation-drift equilibrium was

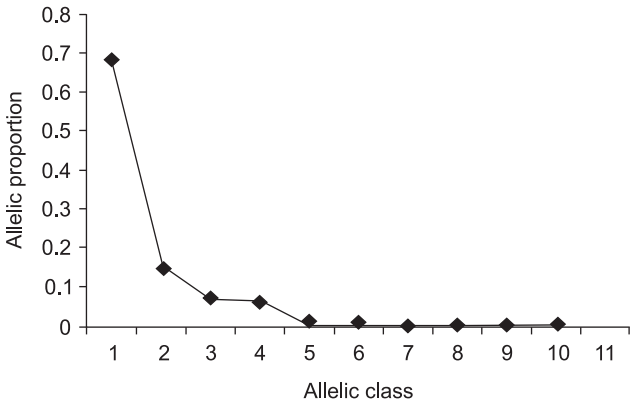

Fig. 2. Mode shift curve

accepted. The expected number of loci (14.75) with heterozygosity excess under IAM was not significantly (P>0.05) lower than the observed numbers of loci (16) with heterozygosity excess. So, the null hypothesis was not accepted under IAM for the sign test. Standard difference test (T2 statistics) in this population provided the significant (P<0.05) gene diversity deficit under TPM (–3.878) and SMM (–11.425). In IAM there was heterozygosity excess (0.645) but not significant (P>0.05). Positive values of the bottleneck statistic T2 are indicative of gene diversity excess caused by a recent reduction in effective population size, while negative value are consistent with a recent population expansion without immigration or immigration of some private (unique) alleles in population. Under Wilcoxon rank test, probability values were 0.149, 0.990 and 1.00 for IAM, TPM and SMM; these were nonsignificant (P<0.05). So, null hypothesis of mutation drift equilibrium was accepted under all the 3 models. The mode shift indicator i.e. qualitative method of estimation of bottleneck, showed the normal L-shaped curve (Fig. 2) in graphical representation of proportion of alleles verses class of frequency distribution. The L shaped curve indicated the abundance of low frequency (<0.10) alleles. This showed that population had not undergone bottleneck at least in the recent past where the probability of low frequency alleles loss is very high.

The results suggested that all the microsatellite markers were highly polymorphic and suitable for molecular characterization of Kalahandi goats. There was substantial genetic variation and polymorphism across the studied loci in Kalahandi goats and the population was not in H.W equilibrium but in mutation drift equilibrium. The low inbreeding observed in the Kalahandi flocks is a favourable parameter to formulate the appropriate breeding strategies to enhance the heterozygosity in the population.

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