



Microsatellite markers based genetic diversity and differentiation of Balangir goat population of Odisha

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ABSTRACT

This study was executed to investigate the genetic diversity of Balangir, a local goat population from Odisha, and its differentiation from the geographically closest registered goat breeds, Ganjam and Black Bengal by utilizing 22 polymorphic microsatellite markers. The genotypic status of individuals at each locus was identified by an automated DNA sequencer and allelic data was analyzed for genetic diversity parameters. It resulted in the acquisition of original and comparable information confirming the genetic distinctness of the Balangir goat population. The observed number of alleles varied between 4 (RM4) and 16 (OMHC1) with 8.23 ± 0.64 alleles per locus in Balangir goats. A moderate level of observed heterozygosity (0.57 ± 0.05) indicated sufficiently existing genetic diversity in this goat population which varied between 0.09 (OarJMP29) and 1 (ILSTS22). Expected heterozygosity (0.64 ± 0.04) oscillated between 0.18 (ILSTS34) and 0.89 (OMHC1). Within the population, heterozygosity deficiency estimate (F_{IS}) was significantly positive (0.13 ± 0.05) and 73% of the investigated loci contributed towards the homozygote excess. Consequently, significant deviation from Hardy–Weinberg equilibrium was observed at 12 of 22 analyzed loci. Assessment of Balangir goat population for mutation drift equilibrium indicated that the population did not suffer a reduction in effective population size in the last few generations. Four different approaches utilized to study genetic relationships (F statistics, analysis of molecular variance, phylogenetic relationship genetic distance, and individual assignment) indicated that the Balangir goat population was genetically differentiated from the two registered goat breeds. The study highlighted that Balangir is discrete Indian goat germplasm with sufficient genetic variability and needs to be registered as a goat breed of India.

Keywords: Balangir goat, Bottleneck, Diversity, Inbreeding, Microsatellite, Odisha

Goats (*Capra hircus*) are the smallest domesticated ruminants, widely spread across the world. They play an important economic role in India and other developing countries (MacHugh and Bradley 2001, Paim *et al.* 2019). In India, goats are distributed in varied geographical regions from the high altitude Himalayas to Rajasthan deserts and humid coastal areas (Joshi *et al.* 2004). Indian goat population has evolved mainly through adaptation to local agro-ecological conditions and to some extent through artificial selection for different traits (Tantia *et al.* 2018). As per 20th Livestock Census (www.dahd.nic.in/), the goat population in India was 148.88 million registering an increase of 10.1% over the previous census in 2012 (19th Livestock Census 2012). India has 34 registered goat breeds (www.nbagr.res.in) which correspond to only 41% of the indigenous goat population. The majority of the goat population (59%) is still referred to as the non-descript (Shivahre 2017). Hence, there is a need to characterize non-

descript populations and to establish their distinctness from the registered goat breeds for the formulation of conservation plans to ensure maintenance of indigenous caprine germplasm diversity.

Characterization of the new goat population and establishing their uniqueness from the registered breeds have gained momentum in the last decade in India. One such population is the Balangir goat of Odisha state. Ganjam is the only goat breed (INDIA_GOAT_1500_GANJAM_06008) registered from the state, although its total goat population is 6.39 million. Crossbreeding of indigenous goat germplasm of Odisha with Black Bengal goat breed (INDIA_GOAT_2100_BLACKBENGAL_06004) was taken up for genetic up-gradation of the existing stock. Molecular characterization of genetic diversity is helpful in the planning of conservation programs and in understanding existing variability in a population, its differentiation, structure, origin, and domestication. In recent years, different studies have been reported for genetic diversity estimation of various Indian goat breeds using microsatellites markers (Mishra *et al.* 2010, Vijn *et al.* 2010, Zaman *et al.* 2013, Singh *et al.* 2015, Raghavendra *et al.*

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2017, Tantia *et al.* 2018, Verma *et al.* 2019).

This study was undertaken to get an in-depth description of the current status of the genetic diversity and population structure in the Balangir goats as well their genetic distinctness from two registered goat breeds of this region (Ganjam and Black Bengal). The results can guide the development of breeding strategies for efficient germplasm resources utilization, conservation, and development. This will also facilitate the registration of Balangir as a breed.

MATERIALS AND METHODS

Animals, distribution and sample collection: Balangir goats are mainly distributed in the three districts of Odisha; Balangir, Sonepur, and Bargarh. These were located between 20°72' and 21°20'N latitude and between 83°37' and 83°92'E longitude. Animals are of brown, black, and white color with brown having dominance. More than 5,000 animals of 500 farmers were explored for the morphometric characteristics in an extensive survey undertaken in the Indian Council of Agricultural Research (ICAR) funded project–Network project on Animal Genetic Resources. Blood samples of 50 unrelated animals were collected from the jugular vein of the animal using vacutainer tubes having EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. Samples were transported to the laboratory in an ice-box and stored at –80°C.

DNA extraction and microsatellite amplification: DNA was isolated from whole blood using the standard phenol-chloroform method (Sambrook *et al.* 2001). The resulting DNA strands were spooled out and washed twice with ice-cold 70% ethanol to remove excess salts. DNA was re-dissolved in 300–450 µl of Tris-acetate-EDTA (TAE) buffer (pH 8). The quality and concentration of DNA were checked on 0.6% agarose gel as well as by nanodrop spectrophotometer. Twenty-two FAO (<http://dad.fao.org/en/refer/library/guideline/marker.pdf>) and ISAG (International Society for Animal Genetics) recommended microsatellite markers for goats, spread across the genome were selected for microsatellite genotyping (FAO 2011). The forward primer of each primer pair was labeled with a fluorescent dye. These markers have previously been demonstrated to be polymorphic in different Indian goat breeds (Tantia *et al.* 2018, Verma *et al.* 2019). PCR was performed in a total reaction volume of 10 µL using 96 well plates. The reaction mixture consisted of 10–20 ng of DNA, 0.2 µM of each primer, and a PCR master mix consisting of 0.2 mM of each dNTP and 2 mM of MgCl₂. A negative control, consisting of all the reaction components, except for the template DNA, was also included to detect any possible contamination. Touchdown protocol was run. Details are—initial denaturation of 95°C for 1 min; amplification cycle with steps of denaturation at 95°C for 45 sec, 60–51°C with a decrease of 3°C every third cycle for 1 min, 72°C for 45 sec and 20 cycles of denaturation; 95°C for 45 sec, amplification at 48°C for 1 min, extension at 72°C for 45 sec followed by final extension step at 72°C for 5 min. The remaining 2 loci (ILSTS049 and OarAE129) were amplified

with a specific temperature protocol which consisted of initial denaturation of 95°C for 1 min; 32 cycles of 95°C for 30 sec, specific annealing temperature (58 and 60°C, respectively) for 45 sec, 72°C for 45 sec and final extension step at 72°C for 10 min. Amplification products were electrophoresed in 2% agarose gel treated with ethidium bromide for later visualization of DNA bands under ultraviolet light.

Microsatellite marker genotyping: Fluorescently-labelled DNA fragments were analyzed by capillary electrophoresis on Applied Biosystems 3130XL Genetic Analyzer. Genotyping reaction consisted of 1 µL PCR products, 8.9 µL of Hi-Di formamide, and 0.1 µL of GeneScan® LIZ 500 size standard. Fragment sizes were estimated by GeneScan analysis software (Applied Biosystems, USA) and extraction of allele size was done with the Gene Mapper 3.0 software. The extracted data were analyzed to estimate genetic diversity.

Diversity estimation: The genotype data were analyzed using GenAlEx 6.5 software (Peakall and Smouse 2012) to calculate allele frequencies at each locus for each population, the average number of alleles per population; observed (Na), and effective numbers of alleles (Ne) and heterozygosity values; observed (Ho) and expected (He), Shannon information index (I) as well as heterozygote deficit (F_{IS}) per locus across breeds and markers. Average values were expressed as Mean±SE from values at each locus. Chi-square tests of deviations from Hardy-Weinberg equilibrium (HWE) were derived.

Relationships and genetic differentiation among populations: To study the genetic differentiation of Balangir goats from the closely distributed registered breeds (Ganjam and Black Bengal), its genotype data were compared and analyzed with the data already generated for the two breeds. Different F statistics estimates, Principal coordinate analysis (PCoA), analysis of molecular variance (AMOVA), and genetic distances were applied. PCoA for the microsatellite markers, Nei's genetic distance (Nei 1972), and Cavalli-Sforza Chord distance were estimated by GenAlEx 6.5. The dendrograms of phylogenetic trees were built from different distance matrices and were visualized by MEGA4 (Tamura *et al.* 2007) using the neighbor-joining (NJ) approach. Population assignment was performed using multilocus genotypes of individuals as implemented in GenAlEx 6.5.

Bottleneck detection: Bottleneck events in the population were tested by two methods using Bottleneck v1.2.02 (<http://www.ensam.inra.fr/URLB>). The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996) like a sign test, standardized differences test, and a Wilcoxon signed-rank test. The probability distribution was established using 1,000 simulations based on allele frequency and heterozygosity under three models namely infinite allele model (IAM), stepwise mutation model (SMM), and two-phase model of mutation (TPM). The genetic bottleneck test was reconfirmed through a Mode shift indicator test based on qualitative descriptive allele frequency distribution (Luikart *et al.* 1998). Loss of rare

alleles in bottlenecked populations was detected when one or more of the common allele classes have a higher number of alleles than the rare allele class.

RESULTS AND DISCUSSION

Genetic characterization of goat breeds requires a basic understanding of the variation both within and between populations. Microsatellites are useful in conservation genetics because of the high degree of polymorphism, which makes them extremely informative and provides very high discriminating power (Glaubitz and Moran 2000). These properties of microsatellites allow a thorough assessment of the genetic variation and structure within and among populations (Bruford *et al.* 1996). Genetic diversity is essential for the long-term survival of species and populations, because it provides the raw material for adaptation and evolution, especially when environmental conditions change. The genetic diversity of many goat breeds has been investigated using microsatellite markers.

Genetic variability in Balangir goat population: All the markers were polymorphic and a total of 181 alleles were detected across the 22 loci. An exact test for genotypic linkage disequilibrium yielded no significant *P* values across the population, and therefore independent assortment of all the loci was assumed. Reasonable polymorphism in Balangir goats is evident from the allele frequency data. OMHC1 showed the highest number of observed alleles per locus (16) while RM4 showed the lowest (4) with 8.227 mean numbers of alleles (Table 1). The expected number of alleles varied from 1.21 (ILSTS34) to 8.87 (OMHC1) with a mean of 3.69 ± 0.43 . Lower values of the expected number of alleles as compared to the observed number of alleles in all the population suggested that low-frequency alleles were prevalent in this population (Sharma *et al.* 2015). The use of microsatellites with a range of polymorphisms reduced the risk of overestimating genetic variability, which might occur with the selection of only highly polymorphic microsatellite markers. Genetic variation is important as it provides the ability to adapt to ever-changing environments. Indian goats in general show the higher genetic variation that must have contributed to their adaptability (Sharma *et al.* 2015). Balangir goats also had a considerable amount of allelic diversity (Table 1), which is comparable to some of the established breeds of India such as Mahboobnagar (8.8 ± 0.55 ; Raghavendra *et al.* 2017), Bidri (8.48 ± 0.88) and Nandidurga (8.22 ± 0.66) (Tantia *et al.* 2018). Balangir allelic diversity is much higher than the Assam Hill goats (4.9 ± 2.220 ; Zaman *et al.* 2013) but is lower than the Changthangi breed of the Himalayan region (10.4 ± 3.91 ; Mishra *et al.* 2010).

Shannon's Information Index (*I*) is a parameter indicative of the informative degree of a marker which ranged from 0.45 (ILSTS34) to 2.36 (OMHC1) with a mean of 1.44 ± 0.11 in Balangir goats (Table 1). Balangir goat had sufficient genetic variation based on its gene diversity in addition to the average number of alleles per locus. The observed and expected heterozygosity values ranged from 0.09

(OarJMP29) to 1 (ILSTS22) and from 0.18 (ILSTS34) to 0.89 (OMHC1) with an overall mean of 0.57 ± 0.05 and 0.64 ± 0.04 , respectively. Mean observed heterozygosity (0.57) in Balangir goat was less than that in several recognized goat breeds of India, viz. Changthangi (0.60; Mishra *et al.* 2010), Nandidurga (0.60; Tantia *et al.* 2018), Black Bengal (0.69; Vijn *et al.* 2010), Osmanabadi (0.71; Bhat *et al.* 2013), Sanagamneri (0.73; Nath *et al.* 2014), Berari (0.79; Kharkar *et al.* 2015), Chegu (0.80) and Gaddi (0.748) goat of Himachal Pradesh (Singh *et al.* 2015). Observed heterozygosity was lower than expected showing a departure from Hardy-Weinberg Equilibrium (HWE) and the possibility of inbreeding. Significant deviation from HWE was observed in 12 loci at $P < 0.001$. Ewens-Watterson test for neutrality revealed that all the microsatellite markers were neutral as observed *F* values lie within the upper and lower limits of 95% confidence region of the expected *F* values. Since 100% loci were neutral, selection as a cause of the decrease in observed heterozygosity was ruled out. Thus, the difference between the observed and expected heterozygosity can be due to the non-random mating among the individuals of the population. This was also reflected in the positive F_{IS} value (0.13) which ranged from -0.36 to 0.70 (Table 1). Heterozygote deficiency of 13% indicated the possibility of inbreeding in the population.

Genetic bottleneck analysis: Bottleneck influences the distribution of genetic variation within and among populations. In recently bottlenecked populations, the majority of loci will exhibit an excess of heterozygotes, exceeding the heterozygosity expected in a population at mutation drift equilibrium. Non-significant heterozygote excess based on different models, as revealed from Wilcoxon rank test (Table 2) and under IAM and TPM models of Sign test and IAM model of Standardized differences tests suggested that there was no recent bottleneck in the existing Balangir population.

The genetic bottleneck in the recent past is a significant aspect to consider for conservation because it leads to a reduction in genetic variability, inbreeding, expression of undesirable recessive alleles, and therefore diminishing the survival rates.

A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig. 1) and hence the abundance of low frequency (<0.10) alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (preceding 40–80 generations) in this population.

Differentiation of Balangir goat population: Balangir goat was most diverse, which had 181 alleles, closely followed by Black Bengal with 165 alleles and Ganjam with a total of 157 alleles (Table 2). Similarly, the mean observed number of alleles (*N_a*) and heterozygosity (*H_o*) were highest in the Balangir goat population being 8.23 ± 0.64 and 0.57 ± 0.05 , respectively. Corresponding values for the Black Bengal breed were 7.50 ± 0.48 and 0.53 ± 0.05 and the least values were observed in Ganjam as 7.14 ± 0.54 and 0.51 ± 0.05 . Ganjam had the highest

Table 1. Microsatellite diversity estimates in Ganjam and Black Bengal goat breeds and Balangir goat population

Locus	Black Bengal goat										Ganjam goat										Balangir goat									
	N	Na	Ne	I	Ho	He	F	N	Na	Ne	I	Ho	He	F	N	Na	Ne	I	Ho	He	F	N	Na	Ne	I	Ho	He	F		
ETH225	47	5	1.11	0.28	0.06	0.1	0.1	33	3	1.66	0.72	0.12	0.4	0.69	45	6	1.82	0.92	0.36	0.45	0.21									
ILSTS044	47	5	1.27	0.5	0.21	0.22	0.22	45	8	2.44	1.3	0.49	0.59	0.17	45	7	2.46	1.3	0.36	0.59	0.4									
ILSTS008	45	7	1.58	0.86	0.44	0.37	0.37	40	5	1.87	0.96	0.18	0.47	0.62	45	5	2.24	1.08	0.6	0.55	-0.08									
OarHH64	31	10	5.81	2	0.39	0.83	0.84	44	8	2.55	1.38	0.5	0.61	0.18	44	6	3.09	1.39	0.52	0.68	0.23									
ILSTS059	48	8	5.71	1.86	0.85	0.82	0.83	46	8	2.87	1.35	0.61	0.65	0.07	39	10	4.6	1.8	0.62	0.78	0.21									
ILSTS065	44	7	3.32	1.52	0.73	0.7	0.71	48	5	2.1	1.04	0.29	0.52	0.44	41	6	1.74	0.9	0.2	0.43	0.54									
OarJMP29	43	5	1.44	0.67	0.33	0.31	0.31	42	8	2.08	1.19	0.29	0.52	0.45	43	5	1.45	0.68	0.09	0.31	0.7									
ILSTS033	45	11	5.13	1.95	0.87	0.8	0.81	48	8	4.11	1.63	0.98	0.76	-0.29	46	9	2.52	1.35	0.7	0.6	-0.15									
OarFCB48	45	9	5.08	1.82	0.69	0.8	0.81	45	7	5.36	1.77	0.67	0.81	0.18	48	12	5.45	1.99	0.81	0.82	0.01									
OMHC1	43	11	7.18	2.1	0.79	0.86	0.87	48	11	7.62	2.17	0.98	0.87	-0.13	47	16	8.87	2.36	0.89	0.89	-0.01									
ILSTS005	46	4	1.84	0.8	0.35	0.46	0.46	47	4	1.57	0.74	0.13	0.36	0.65	48	7	1.97	1.01	0.52	0.49	-0.06									
ILSTS019	46	7	4.34	1.64	0.78	0.77	0.78	41	4	2.87	1.16	0.68	0.65	-0.05	48	8	5.47	1.82	0.65	0.82	0.21									
ILSTS058	36	11	7.01	2.13	0.67	0.86	0.87	43	13	3.78	1.75	0.74	0.74	-0.01	44	14	4.17	1.88	0.59	0.76	0.22									
ILSTS087	41	7	4.02	1.54	0.29	0.75	0.76	36	5	3.77	1.41	0.44	0.73	0.4	47	9	6.44	2.02	0.87	0.84	-0.03									
ILSTS029	36	9	4.21	1.71	0.81	0.76	0.77	38	9	5.18	1.84	0.32	0.81	0.61	43	6	1.62	0.87	0.33	0.38	0.15									
ILSTS049	45	7	3.4	1.44	0.69	0.71	0.71	41	7	4.2	1.6	0.49	0.76	0.36	43	9	5.44	1.9	0.7	0.82	0.15									
ILSTS30	47	7	2.67	1.4	0.51	0.63	0.63	44	8	6.14	1.86	0.68	0.84	0.19	43	9	4.68	1.88	0.65	0.79	0.17									
ILSTS34	47	8	1.8	1.02	0.36	0.44	0.45	48	7	1.57	0.83	0.29	0.36	0.2	43	6	1.21	0.45	0.16	0.18	0.07									
ILSTS022	48	4	1.38	0.55	0.17	0.27	0.28	38	3	2.02	0.8	0.37	0.51	0.27	48	7	3.8	1.5	1	0.74	-0.36									
RRM088	42	8	3.22	1.45	0.69	0.69	0.7	45	9	3.64	1.61	0.67	0.73	0.08	46	4	2.05	0.84	0.43	0.51	0.15									
RRM4	45	5	1.98	0.84	0.22	0.49	0.5	44	7	3.29	1.46	0.57	0.7	0.18	48	10	4.35	1.77	0.65	0.77	0.16									
ILSTS082	48	10	2.6	1.37	0.79	0.62	0.62	48	10	4.36	1.82	0.73	0.77	0.05	48	10	5.85	1.95	0.79	0.83	0.05									
Mean	43.86	7.5	3.46	1.34	0.53	0.6	0.13	43.27	7.14	3.41	1.38	0.51	0.64	0.24	45.09	8.23	3.69	1.44	0.57	0.64	0.13									
SE	0.94	0.48	0.4	0.12	0.05	0.05	0.05	0.91	0.54	0.34	0.09	0.05	0.03	0.06	0.55	0.64	0.43	0.11	0.05	0.04	0.05									

Na, No. of different alleles; Ne, no. of effective alleles = $1 / (\text{Sum } p_i^2)$; I, Shannon's information index = $-1 * \text{Sum } (p_i * \ln(p_i))$; Ho, observed heterozygosity = No. of Hets / N; He, expected heterozygosity = $1 - \text{Sum } p_i^2$; uHe, unbiased expected heterozygosity = $(2N / (2N-1)) * \text{He}$; F, fixation index = $(\text{He} - \text{Ho}) / \text{He}$; Where pi is the frequency of the i^{th} allele for the population and Sum p_i^2 is the sum of the squared total allele frequencies.

Table 2. Test for null hypothesis for mutation drift equilibrium under three mutation models using Sign rank, Standardized differences and Wilcoxon tests

Test/ model		IAM	TPM	SMM
Sign test (Number of loci with heterozygosity excess)	Expected	13.09	13.03	12.93
	Observed	13.00	9.00	2.00
	P value	0.56472	0.06379	0.00000*
Standardized differences test	T2 value	-0.11	-4.082	-12.159
	P value	0.45614	0.00002*	0.0*
Wilcoxon rank test (one tail for heterozygosity excess)	P value	0.52537	0.96578	0.99999

*Rejection of null hypothesis / Bottleneck.

heterozygote deficiency (F_{IS} , 24%) whereas; Black Bengal and Balangir had 13% deficiency.

To describe the level of heterogeneity within and between the studied goat populations, firstly, F-statistics values were determined. The global deficit of heterozygotes across populations (F_{IT}) amounted to 32.8%. An overall 16.3% significant ($P < 0.05$) deficit of heterozygotes (F_{IS}) occurred

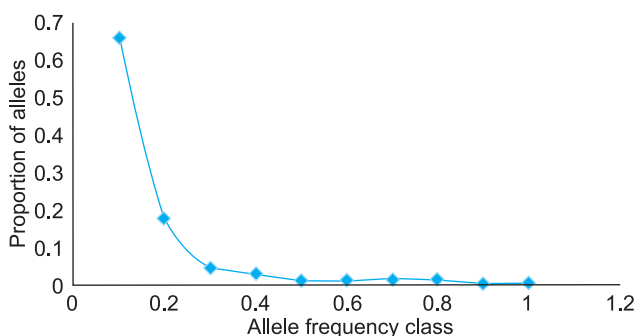


Fig. 1. Allele frequencies at different microsatellite loci in Balangir goat population.

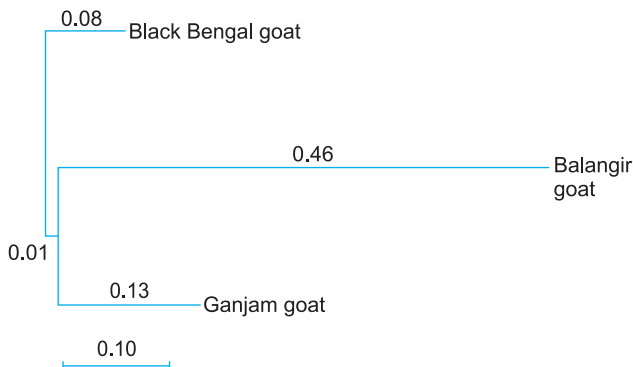


Fig. 2. Dendrogram (NJ) showing genetic relationships among goat populations based on Nei's genetic distance.

in the analyzed loci because of inbreeding within the populations. Wright (1978) suggested that F_{ST} value of greater than 0.15 indicates moderate genetic differentiation among the populations. Accordingly, the multi-locus F_{ST} values identified high breed differentiation as 20.7% of the total genetic variation was due to unique allelic differences between the populations. The pair-wise F_{ST} values were of similar magnitude between Balangir and Ganjam (0.196) and Balangir and Black Bengal (0.193) indicating almost equal differentiation of Balangir goat population from both Ganjam as well as Black Bengal goat breeds. Minimum population differentiation was recorded between the two registered goat breeds, Ganjam and Black Bengal (0.081). Stable genetic flow among populations was evident from a high number of migrants from one population to another ($N_m = 2.207 > 1.00$). Prevalent field conditions are facilitating higher gene flow among the three goat groups explored in the present study. These include the absence of breed/population-specific breeding policy perpetuating crossbreeding or uncontrolled mating, altogether resulting in gene flow and introgression in the field. Secondly, visualization of breed relationship by constructing phylogenetic tree based on Nei's genetic distance distinguished Balangir goat from both Black Bengal and Ganjam goat breeds following Neighbor-joining (NJ) approach. Balangir was on a separate node close to the Ganjam goat (Fig. 2). The pair-wise Nei's genetic distance identified the least genetic differentiation between Black Bengal and Ganjam (0.333), highest divergence between Ganjam and Balangir (1.625), and intermediate value (1.329) between Black Bengal and Balangir goat.

Thirdly, AMOVA revealed that 14% of total genetic variance resulted from genetic differentiation between three populations (Table 3).

Table 3. Partitioning of molecular variance within and between goat populations and the level of population sub-structure (F_{ST})

Source of variation	d. f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	F_{IS}	F_{IT}	N_m	P
Among populations	2	312.076	1.498	14	0.145*	0.383	0.475	1.479	0.001
Within populations									
Among individuals	141	1727.844	3.393	33					
Within individuals	144	787.5	5.469	53					
Total	287	2827.42	10.359	100					

*Significant deviation of pair-wise fixation index (F_{ST}) value through 99 permutations from zero ($P < 0.001$); d.f., degree of freedom

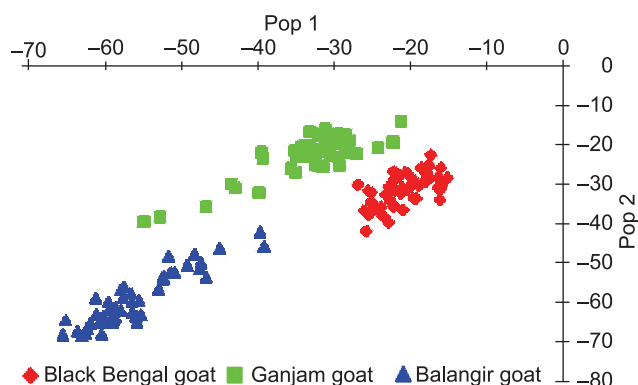


Fig. 3. Population assignment of individual animals of three Indian goat populations.

The other 86% was due to the within-population components of the genetic variance. The pairwise fixation index (F_{ST}) value (0.145) provided by AMOVA through 99 permutations differed significantly from zero at $P < 0.001$.

Fourthly, the population assignment test based on the likelihood method with the leave one out procedure assigned 100% of the individuals correctly to their respective populations, exhibiting distinctiveness of these groups (Fig. 3).

In conclusion, a genetic relationship among the Balangir goat population and two registered goat breeds of India was established. Altogether results confirmed the distinct identity of Balangir goats that was considered to be a non-descript population, till now. The important information generated by microsatellite markers on genetic variation and population structure will pave the way towards the management and conservation of Balangir goats.

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REFERENCES

- 19th Livestock Census. All India Report 2012. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India, Krishi Bhavan, New Delhi.
- Bhat Z I, Kuralkar S V, Kharkar K and Kuralkar P. 2013. Genetic analysis of Osmanabadi goat breed. *Indian Journal of Animal Sciences* **83**(7): 768–71.
- Cornuet J M and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001–14.
- FAO (Food and Agriculture Organization of the United Nations) 2011. Draft Guidelines on Phenotypic Characterization. Intergovernmental Technical Working Group on Animal Genetic Resources for Food and Agriculture and Commission on Genetic Resources for Food and Agriculture, Rome, p 87.
- Joshi M B, Rout P K, Mandal A K, Smith C T, Singh L and Thangaraj K. 2004. Phylogeography and origin of Indian domestic goats. *Molecular Biology and Evolution* **21**(3): 454–62.
- Kharkar K, Kuralkar S V and Kuralkar P. 2015. Molecular genetic characterization of Berari breed of goat using microsatellite markers. *Indian Journal of Animal Research* **49**(4): 423–28.
- Luikart G, Allendorf F W, Cornuet J M and Sherwin W B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* **89**: 238–47.
- MacHugh D E and Bradley D G. 2001. Livestock genetic origins: goats buck the trend. *Proceedings of the National Academy of Sciences USA* **9**: 5382–84.
- Mishra P, Verma N K, Aggarwal R A K and Dixit S P. 2010. Breed characteristics and genetic variability in Changthangi goats. *Indian Journal of Animal Sciences* **80**(12): 1203–09.
- Nath S, Kuralkar S V, Khan W, Kharkar K and Kuralkar P. 2014. Genetic diversity analysis of Sangamneri goat. *Indian Journal of Animal Sciences* **84**(2): 212–15.
- Nei M. 1972. Genetic distance between populations. *American Naturalist* **106**: 283–92.
- Paim T D P, Faria D A, Hay E H, McManus C, Lanari M R, Esquivel L C, Cascante M I, Alfaro E J, Mendez A, Faco O, Silva K M, Mezzadra C A, Mariante A, Paiva S R and Blackburn H D. 2019. New world goat populations are a genetically diverse reservoir for future use. *Science Reports* **9**(1): 1476.
- Peakall R and Smouse P E. 2012. GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**: 2537–39.
- Raghavendra C, Saireddy S, Amareswari P and Raghunandan T. 2017. Molecular characterization of Mahabubnagar goats based on microsatellite markers. *Pharma Innovation Journal* **6**(5): 130–33.
- Sambrook J and Russell D. 2001. *Molecular Cloning: A Laboratory Manual (M)*. 3rd Cold Spring Harbor Laboratory Press, New York
- Sharma R, Kishore A, Mukesh M, Ahlawat S, Maitra A, Pandey A K and Tantia M S. 2015. Genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers. *BMC Genetics* **16**: 73.
- Shivahre P R, Verma N K, Aggarwal R A K, Sharma R, Dangi P S, Bhutia N T and Ahlawat S. 2017. Microsatellite based genetic diversity estimation in Sikkim Singharey goat population. *Indian Journal of Animal Sciences* **87**(1): 125–27.
- Singh G, Thakur Y, Kour A, Sankhyani V and Katoh S. 2015. Genetic characterization of Gaddi goat breed of Western Himalayas using microsatellite markers. *Veterinary World* EISSN: 2231–0916.
- Tamura K, Dudley J, Nei M and Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4. *Molecular Biology and Evolution* **24**: 1596–99.
- Tantia M S, Vij P K, Yathish H M, Kulkarni V S, Shettar V B, Gopala G T, Sharma H and Sharma R. 2018. Characterization of Nandidurga and Bidri goat populations of Karnataka. *Indian Journal of Animal Sciences* **88**(9): 1058–63.
- Verma N K, Aggarwal R A K, Shivahre P R, Sharma R and Savino N. 2019. Evaluation of genetic diversity in long hair Nagaland goat Sumi-Ne. *Indian Journal of Animal Sciences* **89**(1): 105–09.
- Vijh R K, Tantia M S, Behl R and Mishra B. 2010. Genetic architecture of Black Bengal and Chegu goats. *Indian Journal of Animal Sciences* **80**(11): 1134–37.
- Zaman G U, Nahardeka N, Laskar S, Ferdoci A M and Chetri A J. 2013. Molecular characterization of Assam hill goat. *American Journal of Animal and Veterinary Sciences* **8**(2): 98–103.