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Morphological and genetic characterization of Ganjam sheep

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Summary
Ganjam is an important sheep breed in the Orissa State in the eastern region of India. They are reared mainly for meat. The present study was conducted to characterize the Ganjam breed both phenotypically and genotypically at the DNA level using microsatellite markers. A survey was conducted in the breeding tract to study the habitat, body biometry, management practices and reproductive and productive performance of Ganjam sheep. A total of 604 animals were studied for morphological characteristics. The animals are medium sized with hairy fleece. Their coat colour varies from brown to dark tan. The average flock size is 35. Measurements were recorded for body weight, body length, height at withers, chest girth, ear length and horn length in 366 adult animals. A set of 25 microsatellite markers was used to assess the genetic variability in 50 DNA samples extracted from randomly collected blood samples of unrelated Ganjam sheep across their breeding tract. A total of 137 alleles were identified across the 25 markers. The allele diversity (5.48), mean observed heterozygosity (0.623) and gene diversity (0.685) estimates elucidated substantial genetic diversity within the Ganjam breed. The Mode Shift Test implied that a reduction in the effective population size or a recent genetic bottleneck was very unlikely in this indigenous breed of sheep. The within population inbreeding estimate values for the investigated population (0.087) showed a low rate of inbreeding.

Keywords: *Ganjam sheep, genetic characterization, India, phenotypical characterization*

Résumé
La race Ganjam est une race ovine importante de l'état d'Orissa, dans la région orientale de l'Inde, élevée principalement pour la viande. La présente étude a été conduite pour caractériser la race Ganjam d'un point de vue phénotypique ainsi que génotypique au niveau de l'ADN en utilisant les marqueurs microsatellites. L'enquête a été effectuée dans la zone d'élevage pour étudier l'habitat, la biométrie corporelle, les pratiques de gestion, la performance de reproduction et de production du mouton Ganjam. Au total, on a étudié les caractéristiques morphologiques de 604 animaux. Les animaux ont une taille moyenne et une toison velue. La couleur de la robe varie du marron au fauve foncé. La taille moyenne des troupeaux est de 35 animaux. Les mensurations ont été enregistrées pour le poids corporel, la longueur du corps, la hauteur au garrot, la circonférence de poitrine, la taille des oreilles et des cornes de 366 animaux adultes. Un assortiment de 25 marqueurs microsatellites a été utilisé pour évaluer la variabilité génétique dans 50 échantillons d'ADN extraits du sang recueillis au hasard parmi des moutons Ganjam sans relation dans leur zone d'élevage. Au total, on a identifié 137 allèles dans les 25 marqueurs. Les estimations de la diversité allélique (5,48), de l'hétérozygosité moyenne observée (0,623) et de la diversité génétique (0,685) ont mis en évidence une diversité génétique substantielle au sein de la race Ganjam. Le test de déplacement de mode laisse supposer que la réduction de la taille effective de population ou un goulet d'étranglement génétique récent était très improbable dans cette race indigène de moutons. Dans le cadre de la consanguinité de la population, les estimations (F_{IS}) pour la population examinée (0,087) ont indiqué un faible taux de consanguinité.

Mots-clés: *mouton Ganjam, Ind, caractérisation phénotypique, caractérisation génétique*

Resumen
La Ganjam es una importante raza ovina del Estado de Orissa de la región oriental de la India, criada principalmente para la producción de carne. El presente estudio fue realizado para caracterizar la raza Ganjam fenotípica y genéticamente -a nivel de ADN usando marcadores moleculares de tipo microsatélite. Se llevó a cabo una encuesta como parte de la mejora genética realizada con objeto de estudiar el hábitat, la biometría corporal, las prácticas de manejo, así como los rendimientos reproductivos y productivos de la oveja de raza Ganjam. Se estudiaron las características morfológicas sobre un total de 604 animales. Los individuos de esta raza son de mediano tamaño y vellón abierto. El color del manto varía desde el castaño hasta el canela oscuro. El tamaño medio de los rebaños se sitúa en 35 ejemplares. Las medidas tomadas fueron el peso corporal, el diámetro longitudinal, la alzada a la cruz, el perímetro torácico, la longitud de la oreja y la del cuerno sobre un total de 366 animales. Un conjunto de 25 marcadores moleculares de tipo microsatélites fue utilizado para evaluar la variabilidad genética en 50 muestras de ADN extraídas aleatoriamente de muestras de sangre pertenecientes a ovejas de la raza Ganjam no emparentadas como parte de acciones encaminadas a su mejora genética. Se identificaron un total de 137 alelos diferentes a través de 25 marcadores moleculares. La diversidad de los alelos (5,48), heterocigosidad media

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observada (0.623) y la diversidad de genes (0,685) estimada, aclara que existe una gran diversidad genética dentro de la raza Ganjam. La prueba "Mode Shift" indica que tanto una reducción del tamaño efectivo de la población como un reciente cuello de botella de la población han sido muy poco probables en esta raza autóctona ovina. Asimismo, se observó un bajo nivel del endogamia dentro de la población objeto de estudio (0,087).

Palabras clave: *Oveja Ganjam, India, caracterización fenotípica, caracterización genética*

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Introduction

Ganjam is one of the recognized sheep breeds of the eastern region of India. The animals are mainly distributed in Orissa State and derive their name from the Ganjam District of the state. According to the Basic Animal Husbandry Statistics from the Government of India (2006), the indigenous sheep comprise about 99.25 percent of the total sheep population of Orissa. The Ganjam breed animals are well adapted to the agroclimatic conditions and vegetation of the region. Families that are less privileged economically, especially of the *Gola* community, normally rear these sheep for mutton under a fairly simple and extensive management system with the help of their family members. Despite their economic importance to the rural poor for living and sustenance, Ganjam sheep are inadequately characterized (Acharya, 1982; Mishra *et al.*, 2004). The present study was therefore undertaken to evaluate the characteristics of the Ganjam breed both phenotypically and at the DNA level to have a holistic picture (information) of this breed. The results of this study may prove useful in suggesting some guidelines for suitable improvement and conservation of this breed.

Methodology

The breeding tract was visited, and the distribution of the breed was identified through discussions with various officers of the Animal Husbandry Department. The survey was conducted in the Ganjam, Nayagarh, Cuttack, Gajpati, Rayagada, Koraput, Phulbani, Khorda and Puri Districts of Orissa State for phenotypic characterization of this breed under field conditions. Information on the morphological characterization and performance parameters of Ganjam sheep was collected through interaction with the farmers, personal observations and on the spot recording from 49 farmers' flocks totaling more than 1500 sheep.

Genetic characterization of Ganjam sheep was achieved using ovine microsatellite markers (Table 1; FAO, 1996) that are highly polymorphic, codominantly inherited and ubiquitous in nature (Litt and Luty, 1989). Fresh blood samples were randomly collected from 50 unrelated Ganjam sheep across their breeding tract in line with Global Project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD) recommendations (FAO, 1996). Blood sampling was coordinated with owners and veterinary officers. DNA was extracted from the white blood cells using the standard phenol/chloroform/isoamyl alcohol extraction protocol (Sambrook, Fritsch and Maniatis, 1989).

The loci were amplified by polymerase chain reaction in 25-µl volumes using 100-ng template DNA, 200 µM of each deoxyribonucleotide triphosphate, 50 ng of each primer, 1.5 mM of $MgCl_2$, 0.5 U of Taq DNA polymerase and 1× Taq buffer. A common touchdown programme as suggested under the MoDAD Project with no extension step was used. Polymerase chain reaction products were checked on 2 percent agarose gel, and the alleles were resolved on a 6 percent denaturing polyacrylamide gel by silver staining according to Bassam, Gustavo and Gresshoff (1991). The genotype of each individual animal at 25 different loci was recorded by direct counting. Allelic size range was estimated using a 10-bp sequencing ladder as a standard molecular weight marker.

Statistical analysis

The allele number for each locus was scored manually from the silver stained gels. The effective number of alleles was calculated as the reciprocal of the homozygosity.

Estimates of the expected heterozygosity were calculated according to Nei (1978). A measure of heterozygote deficiency or excess (average within population inbreeding estimate [F_{IS}]) was estimated according to Wright's method (1978). The software package POPGENE 3.2 (Yeh *et al.*, 1999) was used for the analyses. The polymorphism information content was calculated according to Botstein *et al.* (1980). The population was analyzed for the presence of genetic bottlenecks via the Sign Test, Standardized Differences Test and Wilcoxon Test (under the infinite allele mode, two phase model and stepwise mutation model of microsatellite evolution) using BOTTLENECK software (Piry, Luikart and Cornuet, 1999).

Results and Discussion

Habitat

Based on the present survey observations, Ganjam sheep breed are distributed in the Ganjam, Gajpati, Rayagada, Koraput, parts of Phulbani, Nayagarh, Khorda and Puri Districts of Orissa (Figure 1). Some animals of this breed, although in less pure form, are also found in Cuttack District. However, according to earlier reports,

Table 1. The primer sequences and chromosomal localization of the used microsatellites.

S. No	Microsatellite marker	Primer sequences (5–3′)	Chromosomal location
1	BM757	F-TGGAAACAATGTAAACCTGGG R-TTGAGCCACCAAGGAACC	9
2	BM827	F-GGGCTGGTCGTATGCTGAG R-GTTGGACTTGCTGAAGTGACC	3
3	BM1314	F-TTCCTCCTCTTCTCTCCAAAC R-ATCTCAAACGCCAGTGTGG	22
4	BM6506	F-GCACGTGGTAAAGAGATGGC R-AGCAACTTGAGCATGGCAC	1
5	BM6526	F-CATGCCAAACAATATCCAGC R-TGAAGGTAGAGAGCAAGCAGC	26
6	BM8125	F-CTCTATCTGTGGAAAAGGTGGG R-GGGGGTTAGACTTCAACATACG	17
7	CSSM31	F-CCAAGTTTAGTACTTGTAAGTAGA R-GACTCTCTAGCACTTTATCTGTGT	23
8	CSSM47	F-TCTCTGTCTCTATCACTATATGGC R-CTGGGCACCTGAAACTATCATCAT	2
9	HUJ616	F-TTCAAACTACACATTGACAGGG R-GGACCTTTGGCAATGGAAGG	13
10	OarAE129	F-AATCCAGTGTGTGAAAGACTAATCCAG R-GTAGATCAAGATATAGAATATTTTTCAACACC	5
11	OarCP20	F-GATCCCCTGGAGGAGGAAACGG R-GGCATTTCATGGCTTTAGCAGG	21
12	OarCP34	F-GCTGAACAATGTGATATGTTCAGG R-GGGACAATACTGTCTTAGATGCTGC	3
13	OarFCB48	F-GAGTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG	17
14	OarFCB128	F-CAGCTGAGCAACTAAGACATACATGCG R-ATTAAAGCATCTTCTCTTTATTTCCTCGC	2
15	OarHH35	F-AATTGCATTCAGTATCTTTAACATCTGGC R-ATGAAAATATAAAGAGAATGAACCACACGG	4
16	OarHH41	F-TCCACAGGCTTAAATCTATATAGCAACC R-CCAGCTAAAGATAAAAGATGATGTGGGAG	10
17	OarHH47	F-TTTATTGACAAACTCTCTTCCTAACTCCACC R-GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	18
18	OarHH64	F-CGTTCCCTCACTATGGAAAGTTATATATGC R-CACTCTATTGTAAGAATTTGAATGAGAGC	4
19	OarJMP8	F-CGGGATGATCTTCTGTCCAAATATGC R-CATTTGCTTTGGCTTCAGAACCAG'AG	6
20	OarJMP29	F-GTATACACGTGGACACCGCTTTGTAC R-GAAGTGGCAAGATTCAGAGGGGAAG	24
21	OarVH72	F-CTCTAGAGGATCTGGAATGCAAAGCTC R-GGCCTCTCAAGGGGCAAGAGCAGG	25
22	OMHC1	F-ATCTGGTGGGCTACAGTCCATG R-GCAATGCTTTCTAAATTCTGAGGAA	20
23	RM004	F-CAGCAAAATATCAGCAAACCT R-CCACCTGGGAAGGCCTTTA	15
24	TGLA137	F-GTTGACTTGTTAATCACTGACAGCC R-CCTTAGACACACGTGAAGTCCAC	5
25	TGLA377	F-GACTGTCATTATCTTCCAGCGGAG R-GATCTCTGGTTGAAATGGCCAGCAG	2

the breed exists only in the Koraput, Phulbani and part of Puri Districts of Orissa (Acharya, 1982). The area mostly consists of saline and sandy coastal soil. The temperature ranges from a minimum of 11.5 °C in winter (i.e. December–January) to a maximum of 39 °C in summer (i.e. May–June). The region receives annual rainfall of about 134 cm from the southwesterly monsoons, and 74 percent of this rainfall is received during June to September. Cyclones and hail are common in this area. Irrigation is mainly by tubewell and open wells. Rice (*Oriza sativa*) is the major crop; pulses (Family Leguminosae), groundnut (*Arachis hypogaea*), potato (*Solanum tuberosum*) and sugarcane (*Saccharum officinarum*) are also cultivated.

Morphological characterization

Physical characteristics were established for 604 animals (37 rams, 329 ewes and 238 lambs). The animals are medium sized, slender and leggy. The backline and noseline are straight. The coat colour ranges from brown to dark

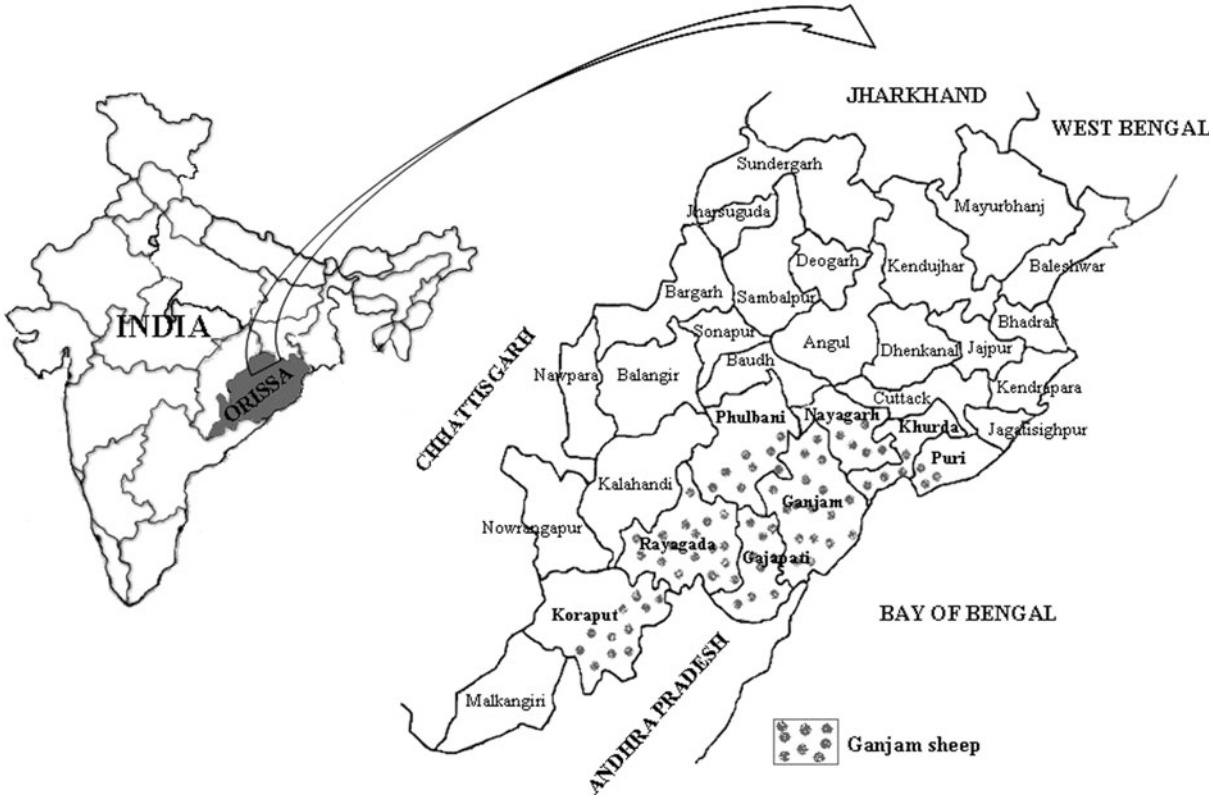

Figure 1. Distribution of Ganjam sheep.

tan, but some of the animals are white. Some animals have white spots on the forehead, face and body (Figures 2, 3 and 4). The ears are medium to long, and the tail is short and thin. Males are horned and females polled, and the sheep are usually not shorn. Hairy and short fleece covers the body of Ganjam animals. However, rams have long hair below the neck and hindquarters. Body measurements were recorded on 366 animals of both sexes. The averages of the various body measurements are presented in Table 2. The farmers dispose of the surplus males to local traders according to their need or breeding requirements, so the males that were measured were mostly breeding rams. The rams exhibited higher estimates for all parameters except ear length, which was slightly higher

Figure 2. Ganjam ram.

Figure 3. Ganjam ewe with lamb.

in ewes. Mishra *et al.* (2004) documented the effect of season and sex on body weight and body measurements (length, height and girth) in a flock of Ganjam sheep raised by a farmer of the *Gola* community.

Management practices

A typical flock consists of about 35 animals with 1 ram, about 26 ewes and 8 lambs or hoggets. Acharya (1982) reported the average flock size to be 60 animals (1 ram, 36 ewes and 23 lambs) with a range of 10 to 500 animals, depending on the economic condition and landholding of the farmer. Other livestock kept by some of the farmers are cattle, goats and poultry. The animals are usually housed in separate open sheds adjacent to or in part of the owner's house (Figure 4). In some cases they are also kept in the open. However, the lambs up to 1 month of age are provided with basket-type enclosures (Figure 2). Providing tree loppings to the sheep is a common practice, but no supplementations are given. The animals are allowed to graze on natural grasses (*Cynodon doctylon*, *Cenchrus cilliaris*) and shrubs (*Acacia arabica*, *Ziziphus mauritiana*) from 9 to 10 a.m. in the morning to 6 p.m. in the evening for a distance of 5–10 km. During migration this distance may increase to

Figure 4. Housing of Ganjam sheep.

Table 2. Body measurements in Ganjam sheep.

Parameter	Ram ($n = 37$)		Ewe ($n = 329$)	
	Average ±SE	Range	Average ±SE	Range
Body wt. (kg)	27.0±0.96	19–41	23.9±0.63	14–36
Body length (cm)	60.7±0.50	54–69	58.7±0.36	50–69
Height at withers (cm)	67.7±0.48	61–78	64.9±0.45	52–74
Chest girth (cm)	72.7±0.68	63–88	69.5±0.47	54–79
Ear length (cm)	11.6±0.52	7–14	12.0±0.55	3–18
Horn length (cm)	20.9±1.5			

Note: SE, standard error.

15–20 km. Most of the flocks migrate from January to June to nearby areas like Nimsala, Palur, Ambri, Sunkara, Khalikot, Rambha, Narayan, Phasla and Bhalsa.

Reproduction and breeding

Pure and selective breeding is followed in the farmer's flock. Rams are selected on the basis of body size, conformation and long horns. Mating is by natural service, and breeding takes place from July to October. The age of first breeding for males is 8–12 months with a breeding life of 2–4 years. Mishra *et al.* (2004) reported that the average age at first conception for females was 11.36 ± 0.11 months in a farmer's flock. The majority of the lambing takes place during November–February. The age at first lambing observed in the present study was 15–24 months. The lambing rate is 60–90 percent, which varies from farmer to farmer and year to year. Litter size is single, and twinning is rare. Similar observations were made by Acharya (1982) and Mishra *et al.* (2004) in Ganjam flocks. A photograph of Ganjam lambs taken during the survey is presented in Figure 5.

Health management

Only some of the farmers vaccinate their sheep against foot and mouth disease, enterotoxaemia and peste des petits ruminants and use anthelmintics like Oxyclozanil, Albendazole, Fenbendazole, Piperazine and so forth. Others neither vaccinate their sheep nor deworm them.

Utility

Ganjam sheep are primarily maintained for mutton. Male lambs are sold at 5–6 months of age for US $13–15 or at 7–10 months of age for US $20–30 to the traders. Old ewes are sold for US $15–20. Rams are sold for slaughter at a price of US $38–50. The selling price of the animals is based on body size, body weight and arbitrary body score determined by butchers or middlemen. Sheep are not slaughtered for personal consumption, but some of the sheep farmers reported consumption of dead sheep. Sheep droppings are either sold or used to fertilize the farmer's own fields. Some of the farmers exchange it for straw or fodder. The income from sheep droppings is about US $0.006–0.008 per sheep per day. Milk production is 100–250 ml per day, and the lactation length is about 4 months. The milk is used for the lamb and rarely for home consumption.

Genetic characterization

Microsatellite profiles for 25 loci located on 19 chromosomes were recorded for Ganjam sheep. Allele frequencies

Figure 5. Ganjam lambs.

Table 3. Genetic diversity indices across 25 microsatellite markers in Ganjam sheep.

Locus	Na	Ne	Ho	He
BM757	3	2.23	0.609	0.551
BM827	6	4.62	0.696	0.783
BM1314	5	4.05	0.778	0.759
BM6506	3	1.76	0.444	0.433
BM6526	6	2.95	0.348	0.662
BM8125	5	2.48	0.524	0.598
CSSM31	9	7.87	0.857	0.873
CSSM47	3	1.27	0.238	0.214
HUJ616	5	3.10	0.826	0.678
OarAE129	3	2.42	0.105	0.587
OarCP20	4	2.87	0.667	0.652
OarCP34	6	4.54	0.762	0.780
OarFCB48	7	4.50	0.667	0.778
OarFCB128	3	2.57	0.667	0.611
OarHH35	9	7.10	0.565	0.859
OarHH41	4	3.21	0.609	0.689
OarHH47	8	5.67	0.800	0.824
OarHH64	5	4.45	0.600	0.776
OarJMP8	5	3.98	0.818	0.749
OarJMP29	7	5.62	0.773	0.822
OarVH72	5	2.47	0.739	0.595
OMHC1	8	5.31	0.909	0.812
RM4	6	2.93	0.217	0.660
TGLA137	7	3.88	0.818	0.743
TGLA377	5	2.87	0.545	0.652
Mean	5.48	3.79	0.623	0.685
SD	1.85	1.62	0.212	0.144

Note: Na, observed number of alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; SD, standard deviation.

ranged from 0.022 to 0.863. Genetic diversity indices are provided in Table 3. A total of 138 alleles were detected across the 25 microsatellite loci that were typed, and the actual number of observable alleles at each locus ranged from 3 (BM757, BM6506, CSSM47, OarAE129, OarFCB128) to a maximum of 9 (CSSM31 and OarHH35) in this breed. The mean number of alleles (allele diversity) was 5.48 across these loci. The effective number of alleles, which is lower than the observed number of alleles, was between 1.27 (CSSM47) and 7.87 (CSSM31). The average observed heterozygosity was less than expected. The intrapopulation observed heterozygosity ranged from 0.105 (OarAE129) to 0.909 (OMHC1). The expected heterozygosity per locus varied from 0.214 (CSSM47) to 0.873 (CSSM31) in Ganjam sheep. The values of the mean observed heterozygosity and gene diversity (mean expected heterozygosity) were 0.623 and 0.685, respectively, which were relatively similar to those of other domestic sheep breeds investigated earlier (Arora and Bhatia, 2004, 2006; Bhatia and Arora, 2007; Sodhi,

Table 4. Test for null hypothesis under three microsatellite evolution models.

	Models of microsatellite evolution		
	IAM	TPM	SMM
Sign test			
Exp. no. of loci with heterozygosity excess	14.51	14.80	14.90
Obs. no. of loci with heterozygosity excess	24*	19	16
Probability	0.00002	0.06301	0.40918
Standardized differences test			
T_2 values	4.598*	3.129*	0.854
Probability	0.00000	0.00088	0.19654
Wilcoxon Rank Test			
Probability of heterozygosity excess	0.00000*	0.00103*	0.39571

Bottleneck (rejection of null hypothesis of mutation drift equilibrium).

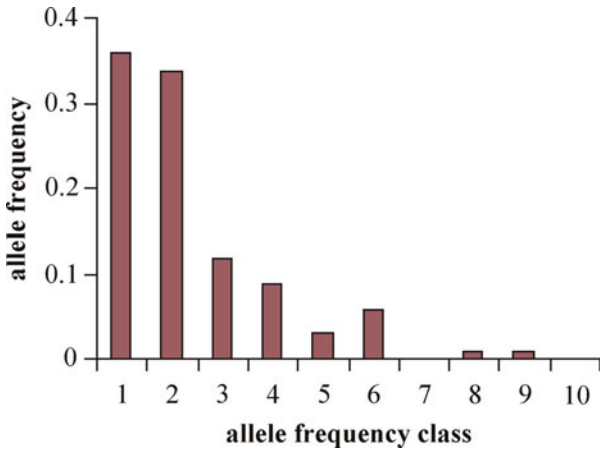

Figure 6. Mode shift analysis depicting absence of genetic bottleneck in Ganjam sheep.

Mukesh and Bhatia, 2006). The results showed that Ganjam sheep breed possesses a substantial level of genetic diversity that might be used in planning breeding strategies.

The Ganjam breed showed a significant ($p < 0.05$) deviation from Hardy–Weinberg equilibrium at four loci (BM757, BM6506, BM6526, OarVH72). This deviation might represent nonrandom mating, selection or the presence of null alleles. It was not possible to estimate the extent of null alleles because no pedigree records were available for analysis and care was taken to collect blood samples from unrelated animals only. However, there did not appear to be significant deviations from equilibrium across all loci within this population. Subsequent analyses were therefore carried out on the basis that Hardy–Weinberg equilibrium prevailed in the investigated breed.

The polymorphic information content, a parameter indicative of the degree of informativeness of a marker, exhibited a range of 0.199 (CSSM47) to 0.859 (CSSM31). In the present study the majority of the markers (84 percent) were highly informative (PIC > 0.5), followed by moderately informative (12 percent, 0.25 < PIC < 0.5) and least informative (4 percent, PIC < 0.25) markers (Botstein *et al.*, 1980). These observations suggested the additional utility of these markers for population assignment (MacHugh *et al.*, 1998) in indigenous sheep.

The BOTTLENECK program was used to test for genetic bottlenecks in the recent breeding history of this breed (Cornuet and Luikart, 1996). Under the assumption of the stepwise mutation model, the most suitable model for microsatellite evolution, neither the sign and standardized differences tests nor the Wilcoxon Signed Rank Test revealed any significant results ($p > 0.05$, Table 4). These findings indicated the absence of a genetic bottleneck in the investigated population, and the population can be considered in mutation drift equilibrium. In addition, the typical L-like distribution of the allele frequencies (Figure 6) further supported that a recent genetic bottleneck or reduction in effective population size (up to 40–80 generations) was not present in Ganjam sheep.

The F_{IS} value was 0.087 with a range of −0.241 (OarVH72) to 0.821 (OarAE129). The positive F_{IS} value that was observed was not significant ($p > 0.05$), thereby indicating a very low rate of inbreeding in the population.

Conclusion

Ganjam is an important sheep genetic resource in its habitat and contributes substantially to the income of the poor farmers rearing them. Because of the substantial genetic variation comparable with other indigenous sheep breeds of India that were investigated earlier and the absence of any systematic cross-breeding programme in the area, there is no immediate threat to Ganjam sheep from a conservation point of view. Nevertheless, Ganjam has sufficient potential for improvements in body weight and meat output, so the need for improvement programmes through selective breeding is imperative. Efforts have been initiated in this direction at the Orissa University for Agriculture and Technology, Bubhaneshwar, Orissa. The proposed programme of the Orissa State Livestock Policy Sector in 2002 (http:/www.orissa.gov.in/fisheries&ard/livestockpolicy.pdf) also involved the supply of breeding rams through selected breeders. Proper implementation and strengthening of these measures along with (i) creation of awareness among the sheep rearers for better health and management practices by training of small holders or rural technicians and (ii) provision of proper support and subsidy to the farmers by the appropriate agencies for acquiring breeding stocks will not only improve production but will also ensure the sustainable conservation of Ganjam sheep.

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