Sheep Genetic Resources of India

GAROLE

The prolific microsheep of India

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Introduction

The present day sheep populations are the result of mutation and genetic drift, as well as selection imposed by humans, available nutrition, endemic parasites, diseases and climate. Potentially there is much unrecognized beneficial genetic variation present in the rare, especially semi managed breeds and indigenous sheep populations, which form important reservoirs of non-exploited resources. These are also the major contributors to the rural economy in India and provide stability of subsistence living. The Garole sheep is one such example of sheep in India that plays a vital role in the economic sustenance of landless labourers and marginal farmers in the Sunderban region of West Bengal
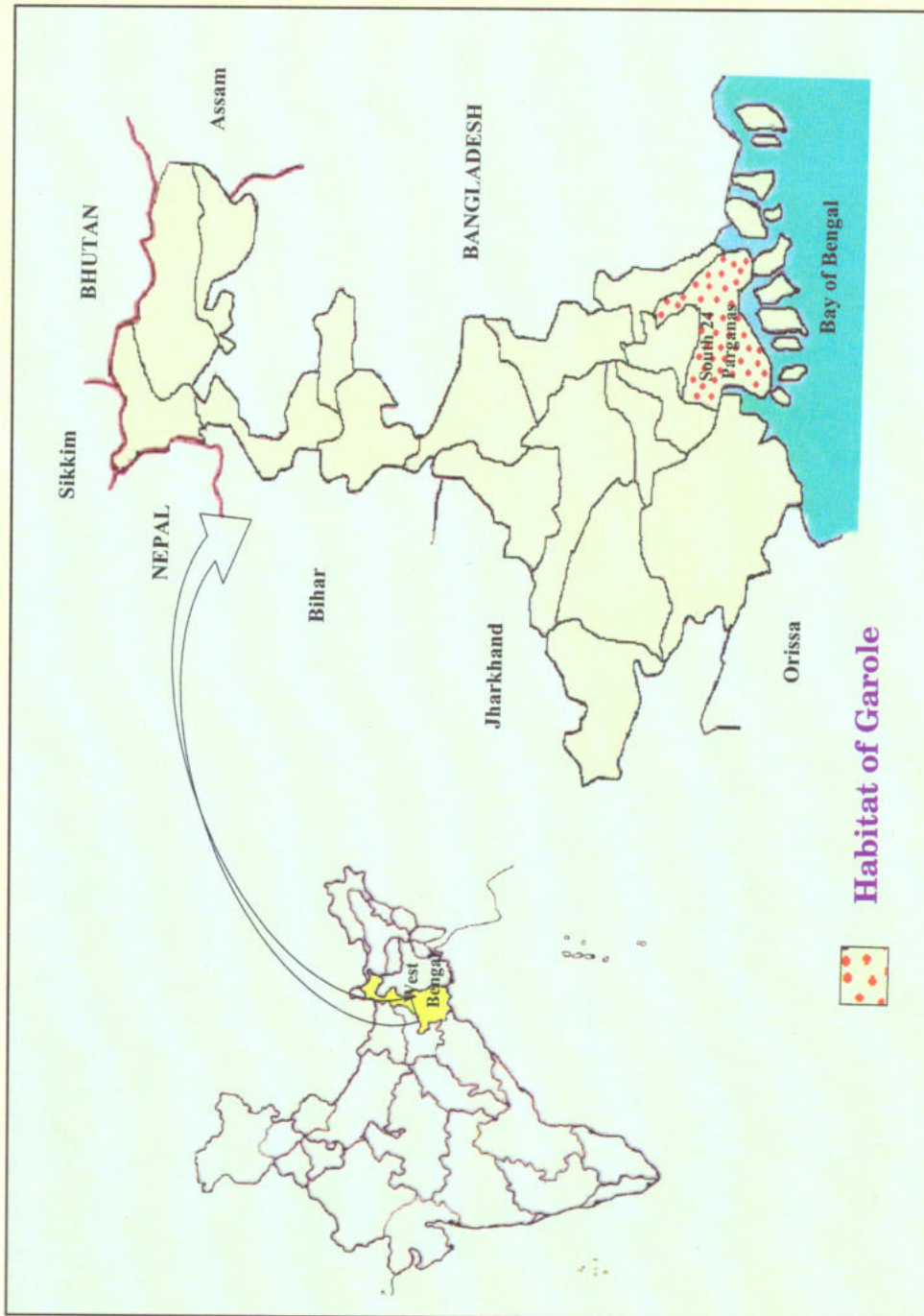
The Garole sheep, a small sized (microsheep) breed reared for meat and mostly known for high reproductive performance is found in hot, humid, swampy Ganges delta of West Bengal. The uniqueness of this breed lies in its high rate of prolificacy, ability to breed round the year, disease resistance and grazing habit in knee deep marshy saline area. The Haldar community that mainly comprises of landless labourers and marginal farmers rears the Garole sheep. This breed has gained prominence as the contributor of Booroola gene to the Australian flocks (Davis 2002) in the late 18th century. These microsheep undoubtedly have outstanding qualities and given attention could economically boost the small scale agricultural production system in the Sunderban region. Therefore the assessment of the phenotypic and molecular genetic characteristics is imperative for genetic improvement and conservation of this unique sheep genetic resource of India. Molecular genetic characterization using molecular markers viz., microsatellites provides useful information on the evolution of breeds and gene pool development that are important for decision concerning breed conservation.

Flock of Garole sheep

Garole triplets

Assam

BHUTAN

Sikkim

NEPAL

Bihar

Jharkhand

BANGLADESH

South 24 Parganas

Bay of Bengal

Orissa

West Bengal

Habitat of Garole

Breeding tract of Garole

Garole sheep are found in the Sunderban region of South 24- Paraganas district in West Bengal. Some animals are also found in parts of the North 24-Paraganas and Midnapore district adjoining the South 24-Paraganas district. The population of this breed is high in Joynagar-1, Joynagar-II Kuttali, Mathurapur-1, Mathurapur-II, Mandir Bazar, Patharpratima, Namkhana and Kakdwip blocks of the 24- Paraganas district.

The Sunderban region comes under the coastal Saline Zone of West Bengal that is part of the Indo-Gangetic delta and traversed by numerous tidal rivers and channels. This zone is mostly comprised of the southern part of the state embracing the Alipore Sadar and Diamond Harbour sub-division of the South 24-Parganas district. The Sunderban is spread over an area of approximately 4226km^2 within 21-23° N latitude and 87-89° E longitudes. The human population of this region is about three million where 90 percent of the inhabitants are below the poverty line (Saha, 1996). Ninety five percent of the people depend only on agriculture of which 85 percent belong to small and marginal farmer families. The mean annual rainfall of this region is 1763mm and ranges between 1450 and 1925mm. Average minimum and maximum temperature range between 15.5 and 32.3°C. Relative humidity remains high, over 80 percent from June to September and minimum 65 percent in December. The island areas are subjected to occasional inundation by the saline water. Soils are of tidal origin and silty clay in nature and the pH ranges between 6.4 and 7.6 (Gangopadhyay, 1991).

Management practices

Marginal farmers and landless labourers mostly from socially and economically less privileged classes of the Haldar community maintain Garole sheep. The flock size of Garole sheep ranges between 2 to 27. However, most of the farmers keep small flocks ranging between 3 and 5. The flocks

are stationary. No organized grazing land is available. The sheep are reared only on grazing on rice fallow land, bunds and natural grass cover on the roadsides and water channels. The animals are tied with a small rope and allowed to graze in the surrounding area. Mostly females and children are involved in the sheep rearing practices.

Animals tied in the field during grazing

Garole sheep grazing on bunds

In the rainy season, most of the fields become waterlogged. Garole sheep have the ability to swim and graze in knee-deep conditions in marshy land. During the monsoon, in addition to grazing, animals are fed treetops and chaffed paddy straw. Generally the sheep are not provided separate houses and are kept along with cattle. Some farmers believe that sheep and goats do not survive well together. Therefore, either they keep cattle and sheep or cattle and goats. However, some farmers maintain both sheep and goats. Sheep are pegged in front of the house or on the roadside during daytime.

Morphological Characteristics

Garole is a small sized animal with a relatively low body weight in comparison to medium and heavy breeds of sheep. It has a compact and square body with a small head, medium ears and a short thin tail. The average body weights of adult male and female are 15 kg and 13.7 kg respectively. Garole sheep have three distinct types of ears: rudimentary (1-3 cm), medium-sized (4-8 cm) and long (more than 8 cm). The average weight (pooled) at birth, 3 months, 6 months, 9 months and 12 months are 1.12± 0.02, 5.79± 0.18, 8.72 ± 0.15, 10.35± 0.11 and 11.89± 0.14 kg respectively. The average body measure-ments of adult male and female are given in Table1. (Sahana et al., 2001).

Grey and white are the predominant colours in this breed. More than 90% of sheep are white in colour and the remaining 10% are brownish black. Males are usually horned and females are polled. Birth weight of Garole sheep is about 1 kg. The udder is fairly well developed and twins can easily be sustained on milk available from the ewe. The fleece is open and very coarse. The fleece cover on the coat is not dense but covers almost the whole body and the greater part of the legs. Usually farmers do not shear their animals and utilize them for meat purpose only. They graze along field boundaries and on the verges of roads. No medication of any kind is given

Ram

Ewe

Lamb

to the sheep and they are never fed concentrates. While grazing, they are usually tended by a young girl. At night they are kept inside the shepherd's hut or in a shed near by the hut (Sahana et al., 2001).

Table 1. Average body measurements

Characteristics (Adult)	Male	Female
Body weight	15.08 ± 1.36 (kg)	13.72 ± 0.78 (kg)
Body length	45.78 ± 0.70 (cm)	42.97 ± 0.60 (cm)
Height at wither	44.00 ± 0.75 (cm)	43.69 ± 0.59 (cm)
Chest girth	59.67 ± 1.52 (cm)	55.91 ± 1.19 (cm)

Reproductive performance (Table 2)

Garole ewes breed round the year with two lambing peaks between December to February and August to September. They lamb twice in 15-18 months and age at first lambing is 14-18 months. Multiple births are common: single (41.63 %), twins (43.35%), triplets (14.81%) and quadruplets (0.21%). Overall lambing rate was 173.6 per 100 ewes lambing. Singh and Bohra (1996) reported 10-12 lambing in the lifetime of a ewe with longevity of 7-8 years.

Table 2. Reproductive performance of females

Age at puberty	226.7 ±7.88 days
Weight at puberty	9.06 ± 0.05 kg
Age of first conception	252.67 ± 10.35 days
Weight of first conception	9.85 ± 0.12 kg
Lambing interval	205.53 ± 2.23 days
Lambing rate	173.56 per 100ewes

Garole ewe with twins

Utility

Garole sheep are reared for meat production and animals are not generally sheared. However, some farmers shear wool for preparation of bedding materials. The wool traits are depicted in Table 3. Surplus sheep and lambs are sold for slaughter prior to the rainy season to avoid risk of mortality. Some farmers do not sell ewes for slaughter. However, in some places, ewes are sold after 6-7 lambings. The dressing percentages on a pre-slaughter live weight basis of male animals slaughtered at the age of nine months was reported to be 48.26 ± 0.31 (Bose,1996).

Table 3. Wool traits

Average wool yield	150 g
Staple length	5.09 cm
Fibre diameter	$67.82 \pm 2.25\mu$
Medulation	75.17%
Hairy	57.81%

Health management

Parasitic infection and diarrhoea are the main health problems in rainy season. The overall lamb and adult mortality is about 33.2 and 12.2% respectively, with higher rate of lamb mortality in rainy season (42%), followed by winter (39%) and summer (19%). There is 15.5, 36.1, 44 and 50% mortality of lambs born as single, twins, triplets and quadruplets respectively. These sheep are very much disease resistant, neither any vaccination nor any deworming is practiced. The mortality generally takes place due to sheep pox and amphistomiosis.

Husbandry

Family surveys have revealed that the landless and marginal farming communities own most of these sheep. Average numbers of sheep per family recorded in case of landless, marginal and small farmers are 1.04, 1.74, and 0.37, respectively. Seventy one per cent farmers are holding small sized flocks (less than 5 animals). The villagers generally maintain very few males for breeding purpose, and excess males and females are sold for meat purpose in the months of September-October before the Durga Puja festival and in the months of May-June before the arrival of monsoon. The population of these sheep around a village found its maximum in the months of December to March and the minimum in the months of June to August.

These sheep are reared only upon grazing on the rice fallow land and on natural grass cover. During monsoon, in addition to grazing, the farmers feed tree tops, chaffed paddy straw mixed with rice polish and rice gruel.

Molecular genetic characterization

The genetic characterization of Garole sheep was based on Food and Agricultural Organizations (FAO's, United Nations) proposed integrated programme for the global management of genetic resources using

microsatellite methodology for breed characterization. Genotype data for 25 FAO recommended microsatellites typed in 48 unrelated animals was used for the assessment of intra breed genetic variation of microsatellites and characterization of breed structure (Sodhi et al., 2003).

Sample and DNA Isolation

A total of 48 blood samples of Garole sheep were randomly collected from their native breeding tract of 24 parganas districts of West Bengal. Typically not more than 10 % of any village or flock population was sampled. Samples contained animals of both the sexes. DNA was isolated from whole blood according to standard phenol chloroform extraction technique.

Microsatellite Markers

Twenty five microsatellite markers approved and recommended by an Expert Advisory Group of International Society of Animal Genetics under the Food and Agriculture Organisation's programme (FAO, 1996) on measurement of Domestic Animal Diversity (MoDAD - http: www.fao.org/ dad-is) for the global analysis of genetic diversity in sheep were used for characterisation of Garole Sheep. Primers sequences and other details of these markers are given in Table-4.

Microsatellite Analysis

The typical assay protocol involved

i) PCR amplification of DNA by locus specific primers :PCR amplification was performed on a PTC - 100 thermocycler (MJ Research Inc, USA). Each 25μ l PCR reaction contained 60ng template DNA, 50 ng of each primer (Gemini Biotech, Texas), 200 mM of each dNTP (Promega), 0.5 units of Taq DNA polymerase (Promega) and 2.5 μ l 10x PCR standard reaction buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris.HCl, pH 9.0). A common "Touchdown" PCR programme used for amplification of all the twenty five markers involved 3 cycles of 45 sec at 95^{0}C, 1 min at 60^0 C; 3 cycles of 45 sec at 95^{0}C, 1 min at 57^{0}C;
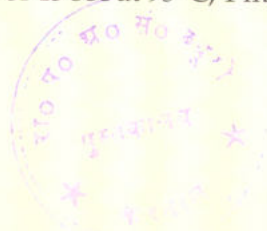
Table 4. The primer sequences and chromosomal localization of the FAO recommended microsatellite markers used in the study

S.No	Microsatellite Marker	Primer Sequences (5'-3')	Chromosomal Location
1	BM757	TGGAAACAATGTAAACCTGGG TTGAGCCACCAAGGAACC	9
2	BM827	GGGCTGGTCGTATGCTGAG GTTGGACTTGCTGAAGTGACC	3
3	BM1314	TTCCTCCTCTTCTCTCCAAAC ATCTCAAACGCCAGTGTGG	22
4	BM6506	GCACGTGGTAAAGAGATGGC AGCAACTTGAGCATGGCAC	1
5	BM6526	CATGCCAAACAATATCCA GC TGAAGGTAGAGAGCAAGCAGC	26
6	BM8125	CTCTATCTGTGGAAAAGGTGGG GGGGGTTAGACTTCAACATACG	17
7	CSSM47	TCTCTGTCTCTATCACTATATGGC CTGGGCACCTGAAACTATCATCAT	2
8	HUJ616	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAGG	13
9	OarAE129	AATCCAGTGTGTGAAAGACTAATCCAG GTAGATCAAG ATATAGAATATTTTTCAACACC	5
10	OarCP20	GATCCCCTGGAGGAGGAAACGG GGCATTTCATGGCTTTAGCAGG	21
11	OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	3
12	OarCP38	CAACTTTGGTGCATATTCAAGGTTGC GCAGTCGCAGCAGGCTGAAGAGG	10
13	OarFCB48	GAGTTAGTACAAGGATGACAAGAGGC AC GACTCTAGAGGATCGCAAAGAACCAG	17
14	OarFCB128	CAGCTGAGCAACTAAGACATACATGCG ATTAAAGCATCTTCTCTTTATTTCCTCGC	2
15	OarHH35	AATTGCATTCAGTATCTTTAACATCTGGC ATGAAAATATAAAGAGAATGAACCACACGG	4
16	OarHH41	TCCACAGGCTTAAATCTATATAGCAACC CCAGCTAAAGATAAAAGATGATGTGGGAG	10
17	OarHH47	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAAATATCATACCTCTTAAGG	18
18	OarHH64	CGTTCCCTCACTATGGAAAGTTATATATGC CACTCTATTGTAAGAATTTGAATGAGAGC	4
19	OarJMP8	CGGGATGATCTTCTGTCCAAATATGC CATTTGCTTTGGCTTCAGAACCAGAG	6
20	OarJMP29	GTATACACGTGGACACCG CTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	24
21	OarVH72	CTCTAGAGGATCTGGAATGCAAAGCTC GGCCTCTCAAGGGGCAAGAGCAGG	25
22	OMHC1	ATCTGGTGGGCTACAGTCCATG GCAATGCTTTCTAAATTCTGAGGAA	20
23	RM004	CAGCAAAATATCAGCAAACCT CCACCTGGGAAGGCCTTTA	15
24	TGLA137	GTTGACTTGTTAATCACTGAC AGCC CCTTAGACACACGTGAAGTCCAC	5
25	TGLA377	GACTGTCATTATCTTCCAGCGGAG GATCTCTGGTTGAAATGGCCAGCAG	2

3 cycles of 45 sec at 95^0 C, 1 min at 54^{0}C; 3 cycles of 45 sec at 95^0 C, 1 min at 51^0 C and 20 cycles of 45 sec at 92^0 C, 1 min at 48^{0}C

ii)   Separation of amplification products on 6% urea - PAGE sequencing gels of 30×38 cm (Biorad Sequi: Gen GT apparatus). Sizing of alleles (range) using 10 bp sequencing ladder (Gibco BRL).

iii) Visualization of amplified fragments (alleles) of DNA by silver staining (Bassam et al.,1991) followed by data recording from air-dried gels.

Statistical Analysis

Allele frequencies of 25 microsatellite loci were estimated by direct allele counting. Intra breed genetic variation of microsatellite and breed structure was quantified using observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed heterozygosity, expected heterozygosity for each microsatellite marker and their respective average diversity measures. The computer programme POPGENE 1.31 (Yeh et al.,1999) was used for these calculations. Polymorphism Information Content (PIC) was calculated in accordance with Botstein et al. (1980). Another software programme used in this study included BOTTLENECK (Piry et al., 1999) for existence of bottleneck effect in the investigated sheep population, if any.

Several genetic variability measures namely number of alleles; observed and expected heterozygosity and allele size range for each marker for the investigated breed, calculated from allele frequency data assuming the population in Hardy- Weinberg equilibrium are presented in Table 5.

Analysis of Loci

All the 25 microsatellite markers were effective for demonstration of polymorphism ($\geq$ 2 alleles). The microsatellite loci showed high level of genetic variability as exhibited by wide range of alleles , which varied from 2 (BM6506, BM8125, CSSM47) to 11 (OarHH47). Effective number of alleles varied from 1.09 (CSSM47) to 6.46 (OarHH47).
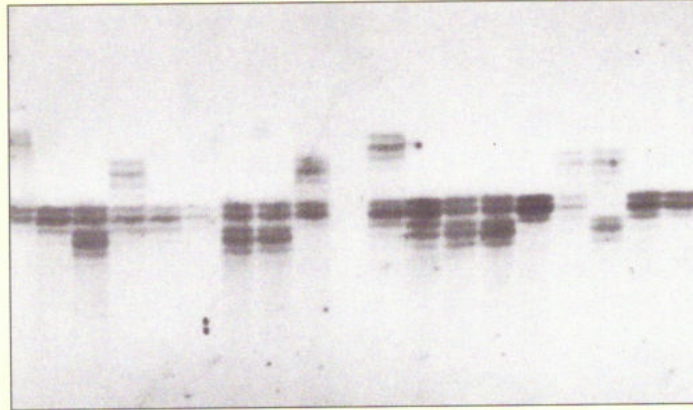
Representative silver stained urea gel showing polymorphism in Garole

Allele frequency distribution at the 25 analyzed loci varied between 0.011 (BM757, BM827, BM1314, OarHH47 OarJMP29, RM004) and 0.920(CSSM47). PIC estimates ranged from 0.082 (CSSM47) to 0.841 (OarHH47).

Analysis of Breed

High mean number of alleles per locus (6.20) displayed high genetic variation in Garole breed. Average of higher number of alleles in the present study also suggested utility of used microsatellites in construction of higher resolution linkage maps of ovine genome.


PIC value in increasing order across 25 markers in Garole sheep

Table 5: Genetic variability measures in Garole sheep across 25 microsatellite markers

Marker	No. of alleles		Heterozygosity		Alleles Size
	na	ne	Ho	He	Range (bp)
BM757	8	4.070	1.000	0.754	177-187
BM827	4	1.408	0.441	0.289	208-214
BM1314	9	5.796	0.772	0.827	150-174
BM6506	2	1.985	0.642	0.496	194-212
BM6526	5	3.574	0.543	0.720	158-174
BM8125	2	1.209	0.148	0.173	114-118
CSSM47	2	1.092	0.004	0.084	132-134
HUJ616	5	4.634	0.953	0.784	115-129
OarAE129	4	2.367	0.645	0.577	152-168
OarCP20	5	2.462	0.512	0.593	71-87
OarCP34	6	4.704	0.793	0.787	110-126
OarCP38	7	4.129	0.658	0.757	177-201
OarFCB48	6	3.207	0.547	0.688	147-167
OarFCB128	7	4.517	0.571	0.778	96-134
OarHH35	10	4.309	0.686	0.768	122-150
OarHH41	10	5.364	0.724	0.813	121-141
OarHH47	11	6.464	0.816	0.845	129-161
OarHH64	7	3.085	0.514	0.675	118-136
OarJMP8	7	4.476	0.613	0.776	117-131
OarJMP29	8	5.267	0.541	0.819	130-146
OarVH72	5	3.935	0.673	0.745	120-136
OMHC1	5	3.828	0.611	0.738	187-197
RM004	6	3.741	0.504	0.732	140-158
TGLA137	9	5.135	0.577	0.805	141-167
TGLA377	5	2.554	0.590	0.608	75-102
Mean	**6.20**	**3.732**	**0.603**	**0.665**	

Observed heterozygosity average on all loci was 0.603 and insignificantly lower than mean expected heterozygosity estimates of 0.665. The high heterozygosity values reflected the presence of large number of polymorphic loci in Garole breed. Failure of significant differences between observed and expected heterozygosities suggested random mating in Garole. In the context of setting priorities for conservation Barker (1999) reported that breed with highest average heterozygosity should be preferred in choosing breeds that otherwise have equal priority.

The high estimates of PIC greater than 0.50 further substantiated the suitability of used set of markers to applications such as genetic distance measurements, linkage-mapping programmes and parentage control in sheep as well. Similar tendencies of three variables viz., mean number of alleles, mean heterozygosity and mean PIC estimates observed in the present finding reflected Garole population under mutation drift equilibrium (Hanslik et al., 2000). These measurements, however, behave differently when a population bottleneck is followed by a rapid population expansion (Kimmel et al.,1998). The data subjected to Mode shift test analysis (Luikart et al., 1998) revealed no mode-shift distortion and hence no genetic bottleneck effect

Normal L-Shaped curve depicting no Mode -shift in Garole sheep
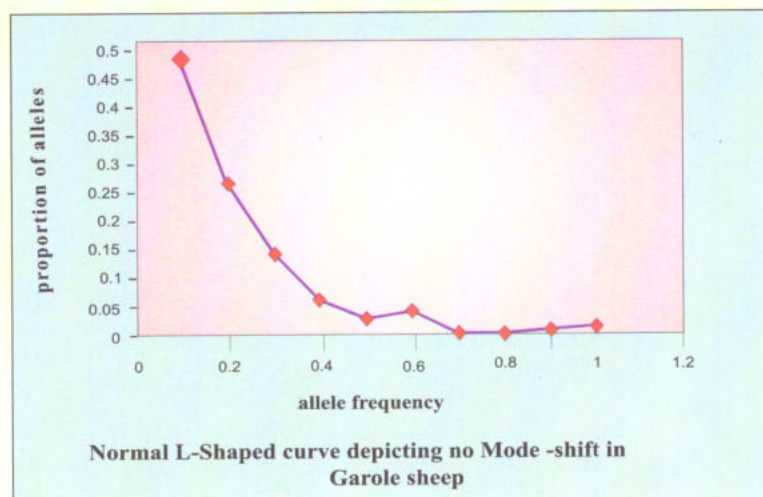
in the investigated breed. These finding further suggested absence of any recent reduction in the effective population size and non- bottlenecked Garole population under mutation drift equilibrium.

Conclusion

The Garole sheep are self-sustainable in their breeding track due to their adaptability to the agro-climatic conditions, their survivability under low input system and their utility as meat animals. No organized crossbreeding is ongoing in the area because crossbred population may be less adaptable in that area. The Garole could be a most valuable breed because of its high prolificacy, ability to graze in standing water and suitability to hot and humid climates. This breed is being used in crossbreeding projects with the other breeds viz., Malpura, Deccani and Bannur (Nimbkar et al., 2003) in India, to see if any of its prolificacy traits can be incorporated into these populations.

There is a need for genetic improvement in Garole sheep in farmers flock in order to make sheep rearing more profitable and to conserve this unique genetic resoure of India. Systematic and effective planning is required in order to ensure that Garole sheep genetic resources are conserved, used and developed to contribute to the region's food security and sustainable rural development.

References

Barker J S F.1999. Conservation of livestock breed diversity. AGRI 25: 33-43.

Bassam B J,Caetano-Anolles G and Gresshoff M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry 196: 80-83.

Bose S. 1996. Studies on the productive and reproductive performance of sheep in the saline and semi saline belt of West Bengal. Ph.D. thesis, West Bengal University of Animal and Fishery Sciences of Animal and Fishery Sciences, Calcutta, India.

Botstein D, White R L, Skolnick M.and Davis R W.1980. Construction of genetic linkage maps in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32: 314 -331.

Davis G H, Galloway S M, Ross I K, et al. 2002. DNA Tests in Prolific Sheep from Eight Countries Provide New Evidence on Origin of the Booroola (FecB) Mutation1. Biology of Reproduction 66:1869-1874

FAO. 1996. Global project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD). http://www.fao.org/dad-is/

Gangopadhyay S.1991.Agricultural characteristics in the Agro-climatic zones of Indian States-WestBengal. In:Agro-Climatic Zone Specific Research, Indian Perspective under NARP,Ghose S.P.(Ed.) ICAR Publication. pp 438-462.

Hanslik S, Harr B, Brem G and Schlotterer C. 2000. Microsatellite analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. Animal Genetics 31: 31-38.

Kimmel M, Chakraborty R, King J P, Bamshad M, Watkins W S and Jorde L B. 1998. Signatures of population expansion in microsatellite repeat data. Genetics 148: 1921-1930.

Kimura M and Crow J F. 1964. The number of alleles that can be maintained in a finite population. Genetics. 49:725-738.

Luikart G, Allendorf F W, Cornuet J M and Sherwin WB.1998. Distortion of allele frequency distributions provides a test for recent population bottleneck. Journal of Heredity 89:238-247

Nimbkar C, Ghalsasi P M, Swan A A, Walkden-Brown and Kahn L P. 2003. Evaluation of growth rates and resistance to nematodes of Deccani and Bannur lambs and their crosses with Garole. Animal Science 76: 503-515.

Piry S, Luikart G and Cornuet J M. 1999. BOTTLENECK a computer programme for detecting reductions in the effective size using allele frequencies. Journal of Heredity 90:502-503.

Saha D. 1996. Share and Integration. Nimpith Rankrishna Ashram Krishi Vigyan Kendra, Nimpith, South 24-Paraganas, West Bengal, India. pp 3.

Sahana G, Gupta S C and Nivsarkar A E. 2001. Garole:The prolific sheep of India. AGRI 31:55-63.

Singh R N and Bohra S D J.1991. Garole sheep a profile. Indian Journal of Small Ruminants. 2:38-42.

Sodhi M, Mukesh M, Arora R, Tantia M S and Bhatia S. 2003. Genetic characterization of Garole sheep using microsatellite markers. Indian Journal of Dairy Science. 56 : 167-173.

Yeh F C, Boyle T, Rongcai Y, Ye Z and Xian J M.1999. POPGENE, Version 1.31. A microsoft window based freeware for population genetic analysis. University of Alberta, Edmonton.