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Genetic characterization of Koraput sheep of Odisha

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India is rich in ovine biodiversity, possessing around 6.5% of sheep population of the world with a total of 65.06 million heads (Livestock census 2012). However, only around 25 to 30% of the country's estimated sheep population including 40 registered sheep breeds has been characterized so far. Besides, about 47 lesser-known sheep groups need to be characterized at phenotypic as well as genetic level in order to assess the actual ovine diversity available in our country (Bhatia and Arora 2010). Koraput sheep is one such lesser known sheep population widely distributed in Koraput and adjoining districts of Odisha. Although few reports are available on the morphometric characteristics of Koraput sheep (Kornel *et al*. 2004), their molecular genetic characterization is totally lacking. Therefore, the present study was undertaken to assess the genetic diversity measures of Koraput sheep using microsatellite markers.

Blood samples were collected from 60 unrelated animals of Koraput sheep in its breeding tract. The genomic DNA from the blood samples was isolated as per method described in Sambrook *et al*. 1989. A set of 24 microsatellite markers based on the list of MoDAD (FAO) reported by Bradley *et al*. (1997) and Di Stasio (2001) were utilized to generate the data. The forward primer for each marker was fluorescently labeled with FAM, NED, VIC or PET dye. Amplification of the loci was performed in a 25µl final reaction volume containing at least 100 ng of genomic DNA, 5pM of each primer, 1.5mM $MgCl_2$, 200µM dNTPs, 0.5 U *Taq* DNA polymerase and 1x *Taq* buffer. A common touch down PCR programme without extension step was used for the amplification of all the 24 markers. The amplified products were resolved on 2% agarose gel and the genotyped on an automated DNA sequencer using LIZ 500 as internal lane standard. Popgen3.2 (Yeh *et al*. 1999) and GenAlEx6.5 softwares (Peakall and Smouse 2005) were used for the genetic diversity analysis. The genetic bottleneck effect was inferred using sign test, standardized differences test, Wilcoxin Rank test as well as mode shift analysis implemented in the programme BOTTLENECK ver 1.2.02 (Cornuet and Luikart 1996).

The microsatellite loci analyzed were observed to be polymorphic in the investigated Koraput sheep population with ≥ 3 alleles per locus. All the markers were found to be highly informative with PIC value ≥ 0.5, except BM6506 and OarFCB48 which were reasonably informative with PIC values between 0.25 - 0.499. Thus, 91.66% markers were highly informative and 8.33% were reasonably informative. The overall PIC value of 0.72 observed in the present study is in agreement with the PIC value obtained by Yadav *et al*. 2011.This indicates that these markers are quite useful for the genetic diversity analysis. The genetic variability measures of Koraput sheep across the 24 microsatellite markers are depicted in Table 1. Eighteen of these loci had excess of homozygotes.

The allele frequencies across all the loci ranged from 0.008 to 0.653 (data not shown). A total of 224 alleles were identified across the 24 markers in Koraput sheep. The observed no. of alleles ranged from 3 (BM6506, CSSM47) to 16 (OarHH35) with a mean of 9.33. Effective number of alleles was lower than the observed number of alleles and ranged from 1.62 (OarFCB48) to 11.58 (OarHH35) with a mean value of 5.13. The overall level of allele diversity (mean observed alleles) in Koraput sheep was higher than the other recognized sheep breeds of Eastern region (Garole, Chhotanagpuri and Ganjam) which ranged from 6.048 to 7.238 (Arora *et al*. 2011). However, this allelic diversity was lower than the Indonesian sheep breed which had a mean value of 10.6 (Jakaria *et al*. 2012). The observed heterozygosity ranged from 0.27 (OarFCB48) to 1.0 (OarCP20, CSSM47) and expected heterozygosity from 0.38 (OarFCB48) to 0.92 (OarHH35). The mean observed heterozygosity (0.65±0.20) was significantly lower ($P< 0.05$) than the mean expected heterozygosity or gene diversity (0.76± 0.13). The gene diversity of Koraput sheep is quite higher than the gene diversity obtained in other sheep breeds of Southern peninsular, eastern and north-western arid and semi-arid region (He= 0.62 to 0.75) except Magra which had a slightly higher mean expected heterozygosity (He= 0.78). Earlier, workers had estimated the genetic variation in Jalauni, Muzaffarnagari, Garole and

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Table 1. Genetic variability measures in Koraput sheep across 24 microsatellite markers

Locus	Observed no. of alleles (Na)	Effective no. of alleles (Ne)	Size range (bp)	Heterozygosity observed/expected		PIC	F_{IS}
BM6506	3	1.84	191–199	0.38	0.46	0.36	0.15
OarCP20	9	5.62	72–88	1.0	0.82	0.80	–0.21
OarFCB48	4	1.62	146–152	0.27	0.38	0.35	0.28
OarVH72	7	5.68	121–133	0.89	0.83	0.79	–0.09
OarHH47	11	6.61	124–144	0.70	0.85	0.83	0.17
BM757	8	2.73	172–186	0.45	0.64	0.60	0.27
BM1314	12	5.96	141–167	0.79	0.83	0.80	0.04
BM8125	6	3.87	109–119	0.56	0.74	0.70	0.23
OarHH35	16	11.58	107–137	0.77	0.92	0.90	0.15
OarHH64	5	3.69	127–135	0.37	0.74	0.68	0.49
OarJMP8	13	7.75	115–139	0.70	0.87	0.85	0.19
OarJMP29	9	4.97	109–143	0.70	0.81	0.77	0.11
BM827	5	3.01	210–218	0.56	0.67	0.61	0.15
OarHH41	9	3.81	120–140	0.63	0.74	0.77	0.14
CSSM31	11	4.11	146–180	0.85	0.77	0.73	–0.12
CSSM47	3	2.45	148–152	1.0	0.60	0.50	–0.68
CSRD0247	13	7.54	211–239	0.81	0.87	0.85	0.05
MAF0214	13	2.82	185–229	0.68	0.65	0.61	–0.05
OarCP0049	13	7.51	83–139	0.73	0.87	0.85	0.15
BM6526	9	5.30	191–199	0.81	0.91	0.78	–0.002
OarCP34	8	3.44	112–126	0.56	0.71	0.67	0.20
INRA0063	10	5.79	173–199	0.31	0.84	0.80	0.61
OarAE129	14	6.35	133–159	0.48	0.85	0.81	0.41
OarFCB128	13	9.15	106–130	0.72	0.90	0.88	0.18
Mean	9.33	5.13		0.65	0.76	0.72	0.124
SD	3.69	2.43		0.20	0.13	0.15	

Magra sheep by silver staining method (Sodhi *et al*. 2003, Arora and Bhatia 2006, Arora *et al*. 2008). Although the two methodologies cannot be compared as such still it indicates that higher genetic diversity in Koraput sheep.

Within population heterozygote deficit (F_{IS}) values ranged from –0.09 (OarVH72) to 0.61 (INRA0063) with a mean value of 0.124, thereby exhibiting a significant deficit of heterozygotes. The observed positive F_{IS} in the investigated sheep population might be due to the non-random mating and use of fewer rams for the breeding purpose. However, the high gene diversity estimates observed in the present study does not support inbreeding. As this value is derived from 24 microsatellite markers it is unlikely to be affected by segregation of non amplifying (null) alleles. The existence of population substructure (Wahlund effect) due to sampling from different flocks in different villages of the distribution area appears to be the most probable explanation. The exact reason of this 12.4% deficit of heterozygotes is difficult to predict due to non availability of pedigree information in the field conditions. The positive F_{IS} value for Koraput sheep is comparable to those reported previously for most Indian sheep breeds (Arora *et al*. 2011a), except for Ganjam and Chotanagpuri sheep from eastern agro-ecological region and Madgyal from southern peninsular region, other breeds exhibited a positive F_{IS} value. Further the positive Fis values reported in other Indian sheep breeds varied from a minimum of 0.70% in Patanwadi to a maximum of 23.4% in Garole.

Ganjam is a well recognized breed of this region with breeding tract overlapping with that of Koraput sheep. Koraput sheep had higher values for allele diversity (9.33), mean observed heterozygosity (0.65) and gene diversity (0.76) as compared to that of Ganjam sheep which had corresponding values as 5.48, 0.623 and 0.685; respectively (Arora *et al*. 2010), indicating that this sheep has sufficient genetic diversity for its genetic improvement.

In the present study, sign test revealed substantial differences between the observed and expected number of loci with heterozygosity excess. Four microsatellite loci had heterozygosity excess, while remaining showed significant ($P \leq 0.01$) heterozygosity deficiency under the assumption of stepwise mutation model (SMM). The results of the tests further revealed mutation drift equilibrium under two phase (TPM) and infinite allele model (IAM). Standardized difference test also showed significant heterozygosity deficiency (T2= -5.894, $P \leq 0.01$) in Koraput sheep under SMM, however, Wilcoxon test revealed that the population had undergone recent bottleneck assuming the IAM. The allele frequency distribution or mode-shift indicator discriminates many bottlenecked populations from stable

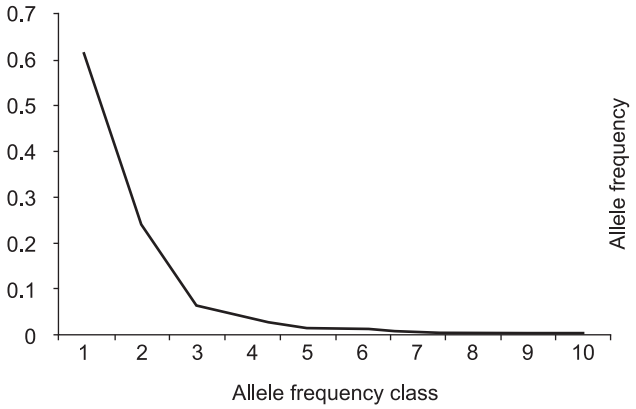

Fig. 1. L-shaped curve depicting no mode-shift in Koraput sheep.

populations (Luikart *et al*. 1998). A typical L-shaped curve (Figure 1) for the distribution of the allelic frequencies was obtained in the Koraput sheep population under study as has been observed in most of the studies in indigenous sheep (Yadav *et al*. 2011; NBAGR monographs on sheep genetic resources of India). This further supported the hypothesis that a genetic bottleneck had not occurred in this population.

SUMMARY

The genetic characterization of Koraput sheep population found in Odisha was accomplished using 24 microsatellite markers. Genetic diversity measures including within breed variability and presence of genetic bottleneck were determined. High measures of allele (9.33) and gene diversity (0.76) were observed across the population investigated. A significant positive F_{IS} (0.12) value suggested a deficiency in the number of heterozygotes in Koraput sheep which may be attributed to population sub-structuring. The genetic variation available in a population is essential for its survival in the changing environmental conditions. Loss of genetic variation may result in reduced adaptability. For indigenous sheep populations like Koraput, changing climatic conditions, shrinking pastures, lesser availability of breeding rams may pose a threat to their survival.The availability of sufficient genetic diversity in Koraput sheep would thus help in devising suitable strategies for its genetic improvement, management and recognition at National level.

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