Assessment of genetic diversity in balangir sheep breed by microsatellite DNA profiling

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ABSTRACT

Balangir, one of the important sheep breeds of Odisha state was investigated genetically utilizing microsatellite markers. Markers were selected on the guidelines of FAO's DADIS (Domestic Animal Diversity Information System) MoDAD programme. The number of alleles, its frequencies, heterozygosities and heterozygote deficiency were estimated. A total number of 103 alleles were detected across nineteen microsatellite markers. Observed number of alleles varied from 2.0 to 9.0 with the mean of 5.421±1.774 which is higher than mean expected number of allele (3.360±1.118). The observed heterozygosity in the population varied from 0.239 to 0.714 with the mean of 0.495±0.098. Balangir sheep possesses sufficient genetic variation and thus has scope for the improvement.

Keywords: Balangir, diversity, Indian sheep, microsatellite

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INTRODUCTION

India has 42 descript breed of sheep along with a majority of non-descript animals (Acharya 1982). Various indigenous breeds are the results of thousands of years of selection, evolution and development of wild species in the process of domestication suitable to local agro-climatic conditions. The vast animal genetic diversity of Eastern and South-Eastern India is under-developed in terms of production and genetic improvement. Economic returns from them are less. Comprehensive identification of this germ pool has not been carried out so far. Balangir sheep is found in Balangir, Sambalpur and Sundergarh districts of Odisha. It is a dual purpose meat and wool type breed. Balangir animals are medium sized. The fleece colour is white or light brown or of mixed colours. Few animals are also black. Ears are small and stumpy. Males are horned and females are polled. Tail is medium, long and thin. Fleece is extremely coarse, hairy and open. Legs and belly are devoid of wool (Fig. 1). Preliminary step for the sustainable development and exploitation of domestic animal genetic resources is the assembling of knowledge with reference to the genetic variability in the breeds. Earlier studies on genetic structure exploited polymorphism of phenotypic traits or biochemical markers. Molecular techniques of population composition and genetic associations involve direct investigation of the DNA. The effectiveness of microsatellite markers for the evaluation of genetic diversity and relationships among livestock breeds has been well established (Barker et al. 2001). An assessment of genetic variation is a crucial element in determining breeding strategies and for effective and meaningful improvement and conservation programme of any breed. In this paper, we present genetic variability of Balangir sheep using microsatellite markers.

MATERIALS AND METHODS

Sample collection: Blood sampling was done as per the guidelines of FAO' MoDAD (Measurement of Domestic Animal Diversity) programme (FAO, 1995). Thus, fifty unrelated blood samples were collected from 14 flocks (5 or <5 samples per flock) of various villages of Balangir sheep breeding tract (Fig. 2) so as to make them representative of population. Owners were questioned in order to avoid close relationships as no pedigree data was available for the breed under field condition. Thus analyzed animals could be considered as a representative sample of the breed as they were collected from different flocks from the main breeding tract with efforts to avoid closely related individuals. Whole blood (8-10ml) was taken from Jugular vein

using vacutainer containing EDTA as anti-coagulant.

Molecular analysis: Genomic DNA was isolated as per the method described by Sambrook et al. (1989) with minor modifications. The quality and quantity of isolated DNA was determined using agarose gel electrophoresis (0.8%) and Nanodrop spectrophotometer (GE Healthcare). A single band on agarose gel and 1.8-2 OD 260/280 ratios for 47 samples indicated the recommended quality of purified DNA. A set of 19 microsatellite markers (BM8125, OarAE129, RM4, BM827, OarHH64, ILST005, OarJMP8, BM6526, HUJ616, BM757, BM6506, OMHC1, OARJMP29, OarHH47, CSSM31, OarHH35, OarCP38, OarFCB128, OarCP34) was selected based on the guidelines of FAO's DADIS (Domestic Animal Diversity Information System) MoDAD programme for generating data in a panel of 47 animals. Polymerase Chain Reaction (PCR) was carried out on about 50-100 ng genomic DNA in a 25 ml reaction volume using BIO RAD iCycler. The reaction mixture consisted of 200 mM each of dATP, dCTP, dGTP and dTTP, 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM MgCl2, 0.75 unit Taq DNA polymerase and 4 ng/ml of each primer (Sigma Genosys). The "touchdown" PCR protocol was used with initial denaturation of 95ºC for 1 min, 3 cycles of 95ºC for 45 sec , 60ºC for 1 min; 3 cycles of 95ºC for 45 sec, 57ºC for 1 min; 3 cycles of 95ºC for 45 sec, 54ºC for 1 min; 3 cycles of 95ºC for 45 sec, 51ºC for 1 min; 20 cycles of 95ºC for 45 sec, 48ºC for 1 min. PCR products were electrophoresed on 2 % agarose gel and visualized over UV light after Ethidium bromide staining to detect the amplification. Resolution of alleles on 6% denaturing polyacrylamide gels (Fig. 3), silver staining and scoring of genotypes was done as per Sharma et al. (2006a). Observed and expected number of alleles, heterozygosity estimates and heterozygote deficiency (FIS) were calculated as implemented in POPGENE software (Yeh et al. 1999).

Figure 1. Balanger Sheep

Figure 2. Sampling location of Balangir sheep population

RESULTS AND DISCUSSION

All the 19 microsatellite loci amplified effectively and generated specific allelic patterns from which individual genotypes could be assessed. A total of 103 alleles were detected in Balangir sheep across the 19 loci investigated. Allele frequencies are presented in Table 1. Reasonable amount of polymorphism in Balangir Sheep is evident from the allele frequency data. The estimated parameters of the genetic variation in Balangir sheep such as observed and effective number of alleles, observed, expected and Nei's expected heterozygosity and heterozygote deficiency (FIS) at each of the microsatellite locus are presented in Table 2.

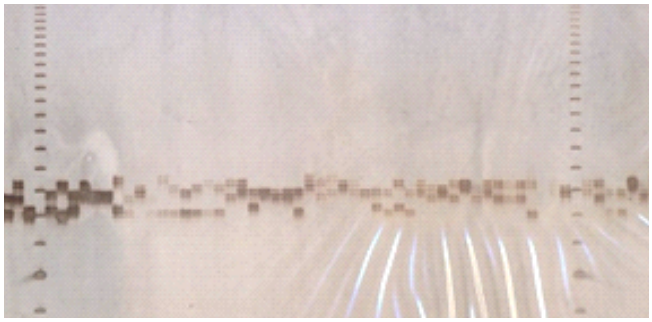

Table 1. Allele frequencies of microsatellite loci in Balangir sheep

Locus	Allele								
	A	B	C	D	E	F	G	H	I
BM8125	0.0978	0.0978	0.7609	0.0435					
OarAE129	0.0341	0.2386	0.3409	0.3864					
RM4	0.0476	0.6310	0.2976	0.0238					
BM827	0.0851	0.0426	0.4894	0.3830					
OarHH64	0.0638	0.4787	0.0319	0.3085	0.1170				
ILST005	0.0652	0.1630	0.6739	0.0978					
OarJMP08	0.0213	0.1596	0.0638	0.3085	0.3723	0.0745			
BM6526	0.2717	0.3913	0.0109	0.0543	0.2717				
HUJ616	0.0426	0.3404	0.2128	0.3298	0.0745				
BM757	0.0256	0.3205	0.5256	0.1282					
BM6506	0.4659	0.5341							
OMHC1	0.0319	0.1064	0.1064	0.0319	0.4787	0.1915	0.0532		
OarJMP29	0.0114	0.0227	0.2386	0.3068	0.0227	0.1591	0.2386		
OarHH47	0.0111	0.1000	0.4000	0.0778	0.1444	0.1222	0.0778	0.0667	
CSSM31	0.2143	0.0857	0.1571	0.0571	0.0857	0.2143	0.1571	0.0286	
OarHH35	0.0106	0.0106	0.1064	0.0532	0.0213	0.0745	0.0957	0.3085	0.3191
OarCP38	0.0116	0.0233	0.2326	0.2093	0.3488	0.1744			
OarFCB128	0.0795	0.0795	0.0909	0.4205	0.2045	0.1250			
OarCP34	0.0111	0.1667	0.2111	0.2333	0.3778				

The number of observed alleles varied from 2 (BM6506) to 9 (OarHH35) with the mean of 5.421±1.774. As per FAO ⩾ 4 different alleles per locus are required for evaluation of genetic differences between breeds. Thus all the loci except BM6506 are indicative of adequate polymorphism and their appropriateness for assessing genetic variation. The use of microsatellites with a range of polymorphism reduced the risk of overestimating genetic variability, which might occur with microsatellite exhibiting only high polymorphism. The observed number of alleles for all the 19 loci exceeded the effective number of alleles which varied from 1.667 to 6.250 with a mean of 3.360±1.118. Mean number of alleles (MNA) in Balangir breed is of similar magnitude as observed for some other Indian sheep breeds viz. 5.04 in Muzaffarnagari (Arora and Bhatia 2004), 4.94±0.14 in Madras Red sheep (Prema et al. 2008), 5.7 in Magra (Arora and Bhatia 2006) and 5.6 in Shahabadi (Pandey et al. 2009). MNA was higher in Garole (6.2, Sodhi et al. 2003), Hassan (7.4, Sharma et al. 2006b) and Changthangi sheep (8.76, Sharma et al. 2010) as compared to Balangir sheep.

The average observed heterozygosity (Nei's) was 0.495±0.098 which was less than the average expected heterozygosity, 0.676±0.119 (Table 2). The average expected heterozygosity (Nei, 1973) within the Balangir population varied from 0.400 (BM8125) to 0.840 (CSSM31) with an overall mean of 0.668±0.117. Balangir sheep encompassed substantial measure of genetic variation derived from its gene diversity as compared against the genetic variation described in various breeds scrutinized in India and worldwide. The average observed heterozygosity in Balangir sheep (0.495) is similar to some other Indian sheep breeds viz. Nali and Chokla 0.47 (Sodhi et al. 2006), Garole 0.44 (Mukesh et al. 2006), Rampur-Bushair sheep 0.515 (Pandey et al. 2008), Bellary 0.512 (Kumar et al. 2007), and Swiss sheep breed Mouflon 0.450 (Saitbekova-Stahlberger et al. 2001). However, higher average heterozygosity than Balangir sheep was reported in other Indian sheep breeds: Magra sheep breed 0.597 (Arora and Bhatia 2006), Hassan 0.533 (Sharma et al. 2006b) and Spanish sheep breeds: Latxa 0.661, Black-

Table 2. Estimates of genetic variability parameters in Balangir sheep

Locus	No. of alleles		Heterozygosity		Nei's	Heterozygote deficiency (FIS)
	Observed (No)	Expected (Ne)	Observed (Ho)	Expected (He)		
BM8125	4.0	1.667	0.457	0.404	0.400	-0.141
OarAE1 29	4.0	3.090	0.523	0.684	0.676	0.227
RM4	4.0	2.043	0.429	0.517	0.511	0.161
BM827	4.0	2.530	0.511	0.611	0.605	0.156
OarHH64	5.0	2.914	0.468	0.664	0.657	0.287
ILST S 005	4.0	2.022	0.239	0.511	0.505	0.527
OarJMP08	6.0	3.713	0.638	0.739	0.731	0.126
BM6526	5.0	3.291	0.565	0.704	0.696	0.188
HUJ616	5.0	3.607	0.489	0.731	0.723	0.323
BM757	4.0	2.525	0.436	0.612	0.604	0.278
BM6506	2.0	1.991	0.432	0.503	0.498	0.132
OMHC1	7.0	3.409	0.447	0.714	0.707	0.368
OarJMP29	7.0	4.264	0.500	0.774	0.765	0.347
OarHH47	8.0	4.495	0.533	0.786	0.778	0.314
CSSM31	8.0	6.250	0.714	0.852	0.840	0.149
OarHH35	9.0	4.414	0.425	0.782	0.773	0.449
OarCP38	6.0	3.989	0.465	0.758	0.749	0.379
OarFCB128	6.0	3.919	0.546	0.753	0.745	0.268
OarCP34	5.0	3.709	0.578	0.739	0.730	0.209
Mean	5.4 21	3.360	0.495	0.676	0.668	0.249
SD	1.774	1.118	0.098	0.119	0.117	0.146

faced Latxa 0.594, Rubin del Molar 0.600, Churra 0.661, Xalda 0.572 (Alvarez et al. 2004). This obviously signifies that this breed has sufficient but slightly lower genetic variability as compared to few other Indian breeds. In assessing diversity estimates from different studies, it should be mentioned that the values are not directly comparable, as different microsatellite set have been used by different workers. These values have only suggestive indication of diversity in the population.

Balangir sheep population exhibits high level of heterozygote deficiency (FIS = 0.249±0.146). Except locus BM8125 the entire 18 loci contribute to the overall heterozygote deficiency. It has been observed in earlier discussion that observed heterozygosity was slightly lower than expected, indicating a departure from Hardy-Weinberg Equilibrium (HWE) and possibility of inbreeding. The high heterozygote deficiency could be due to any of the following: Segregation of non amplifying (null) alleles, Wahlund effect (population substructure), genetic hitchhiking and inbreeding. Ewens- Watterson neutrality test (Manly, 1985) showed that heterozygote deficiency in the Balangir breed is not due to selection as most of loci except two viz. BM827 and OarHH64 were found to be neutral. Possibility of Wahlund effect is also ruled out as sub population only arises when population of a breed is separated by any geographical or physical barriers which are not the case in this breed. Due to uncontrolled mating in Indian sheep populations at farmer's field, a breeding group of 80-100 sheep most

likely comprises 2-4 breedable males only and presumably sires most of the offspring. Thus, this increases inbreeding in the population. Utmost care was taken to collect random samples but relatedness of few samples cannot be ruled out in absence of pedigree records that might also contribute to observed heterozygote deficiency. A similar trend of higher heterozygote deficiency has been observed in several indigenous breeds; Nali (28.4%) and Chokla (28.6%) sheep breeds of Rajasthan (Sodhi et al. 2006), Shahabadi sheep of Bihar (21.5%, Pandey et al. 2009), Sarda sheep (19%, Pariset et al. 2003), Magra sheep (16%, Arora and Bhatia 2006) and Rampur Bushair sheep (22.7%, Pandey et al. 2008). Low heterozygote deficiency was observed in Muzzafarnagari sheep (5.8%) by Arora and Bhatia 2004, Changthangi sheep (4.7%, Sharma et al. 2010), whereas, Madras Red sheep exhibited heterozygote excess (FIS -0.021, Prema et al. 2008).

Keeping in view current concerns for sustainability, maintaining environment and biodiversity there should be rethinking on the development and use of indigenous breeds among policy makers, farmers/ stakeholders in conservation and development. Balangir breed is a source of livelihood of poorest of poor in the country. Rams of the breed must be provided to the flock owners and these rams should be rotated between the flocks in an effort to minimize inbreeding. This is possible only when adequate infrastructure, financial support and human resource development in conservation is initiated on priority. Rams of the breed must also be provided under the guidance of Animal Husbandry Department as genetic measures undertaken to improve livestock will not be successful unless the livestock production system as a whole is considered.

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