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A Suvarna
Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

D Anil Pavan Kumar
Principal, Animal Husbandry Polytechnic, Karimnagar, Telangana, India

P Jaya Laxmi
Professor and Head, Department of Animal Genetics and Breeding, College of Veterinary Science, Mamnoor, Telangana, India

RMV Prasad
Professor and Head of Livestock Farm Complex, College of Veterinary Science Rajendranagar, Telangana, India

Corresponding Author:
A Suvarna
Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Studies on genetic variability in sheep prevalent at Nagarjuna Sagar region of Telangana using microsatellite markers

A Suvarna, D Anil Pavan Kumar, P Jaya Laxmi and RMV Prasad

Abstract
Using ten microsatellite markers, the genetic diversity of the local sheep that are common in Telangana's Nagarjuna Sagar region were genotyped. Ten microsatellite markers and a total of 82 alleles were examined. OarAE129 and OarCP38 had the fewest alleles (6 each), whereas MAF214 had the most alleles (11), with a mean of 8.2 alleles. With a mean value of 7.221, the effective number of alleles ranged from 4.810 (OarAE129) to 9.970 (MAF214). While the allele frequencies ranged from a minimum of 0.050 (BM8125, INRA063, MAF214) to a maximum of 0.315 (OarAE129), the allele sizes varied from a minimum of 112bp in OarCP38 to a maximum of 264bp in MAF214. The mean heterozygosity that was observed and predicted, respectively, was 0.104 and 0.855. The mean inbreeding coefficient across the board was 0.878. The F_{IS} values were between 0.832 to 0.941 (OarCP34 to BM8125). The study's inbreeding estimations were all positive, indicating a severe shortage of heterozygotes. The PIC values for the ten microsatellite loci varied from 0.762 (OarAE129) to 0.890 (MAF214) and all ten loci (100%) were determined to be highly polymorphic. It was discovered that the mean PIC value across all ten loci was 0.836. The chi-square test showed that all 10 loci were significantly deviating from the Hardy-Weinberg equilibrium adding to our understanding of the genetic makeup of this indigenous sheep. High genetic variability within the breed was found by the results.

Keywords: Genetic variability, allele frequency, microsatellite markers, native sheep

Introduction
One of the significant small ruminant livestock species in India that makes a significant contribution to the agrarian economy is sheep. India ranks third in the world for sheep population, with an estimated 74.26 million sheep and 44 recognised breeds, according to the 20th livestock census (2019). Sheep make up roughly 13.8% of all livestock in the nation. According to BAHS, 2019 Telangana has the highest number of sheep in the nation (19.1 million), and it also has highly productive sheep breeds including Deccani and Nellore. Farmers favour and raise a specific breed of indigenous sheep known as "Chikkajala gorre" that were likely developed over many years in some regions of Telangana, particularly in the Nagarjuna Sagar area of Nalgonda, Nagarkurnool, and Suryapet districts. Because of its hardiness, disease resistance, capacity to grow on limited food, and tolerance of heat, these farmers favour the local sheep. Thus, 10 FAO-recommended ovine specific microsatellite markers were used in the current study to examine the genetic variability of local sheep existent in the Nagarjuna Sagar area of Telangana.

Materials and Methods
Of the 100 blood samples of native sheep, 30 were collected from Nagarkurnool district (Amrabad, Padra, Akkaram & Maddimadugu villages), 50 were collected from Nalgonda district, while the remaining 20 were collected from Suryapet district (Palakeedu, Mattampally & Mellacheruvu villages).

Genomic DNA and microsatellite markers
The phenol-chloroform method was used to extract DNA from blood samples. Genomic DNA quality and quantity were assessed using electrophoresis on 0.8% agarose gels and a UV spectrophotometer, respectively. A total of 10 microsatellite markers sets specific for native sheep were used in the study as recommended by FAO (http://www.fao.org/dad -is). the markers analyzed were BM8125, HUJ616, INRA063, MAF214, OarAE129, OarCP34, OarCP38, OarFCB128, OarHH47 and OarJMP29.

PCR amplification and genotyping

The Eppendorf thermal cycler was used to carry out the PCR amplification. Each 12.5µl PCR reaction mixture contained 1 µl of 100 ng genomic DNA, 1.25 µl of 10xTaq buffer, 0.75 µl of 25 mM MgCl2, 0.25 µl of 10 mM dNTPs, 0.5 units of Taq polymerase with 0.6 µl of forward and reverse primers, and 0.755 µl of autoclaved milliQ water in the final step. Denaturation at 95 degrees Celsius for five minutes was followed by 34 cycles of denaturation at 94 degrees Celsius for one minute, annealing at various temperatures (depending on the primers), extension at 72 degrees Celsius for 30 seconds, and final extension at 72 degrees Celsius for five minutes. To resolve the bands, the amplified PCR products were electrophoresed on 2% agarose. Under the gel documentation system's UV light, the gel's bands may be seen.

Molecular genetic analysis

The number of Alleles, frequency of alleles, number of effective alleles, polymorphism information content (PIC), Hardy-Weinberg equilibrium, observed heterozygosity, expected heterozygosity and F-statistics were calculated by using POPGENE version 1.32 (Yeh *et al*., 1999) [16]. expected heterozygosity (He) of each microsatellite marker locus was measured (Nei, 1973) [12].

$$\text{Allelic frequency} = \frac{\text{No.of alleles at a locus in population}}{\text{Total no.of alleles at that locus in population}}$$

$$\text{Mean no. of alleles per locus} = \frac{Total\ no.of\ alleles\ in\ the\ population}{Total\ no.of\ loci\ studied}$$

Hardy-Weinberg equilibrium

To determine if the population was in Hardy-Weinberg equilibrium at the examined loci, the Chi-Square test of goodness of fit was performed using the observed and expected data (Falconer and Mackay, 1996) [3].

$$x^2 = \sum_{i=1}^{k} \frac{(0-E)^2}{E}$$

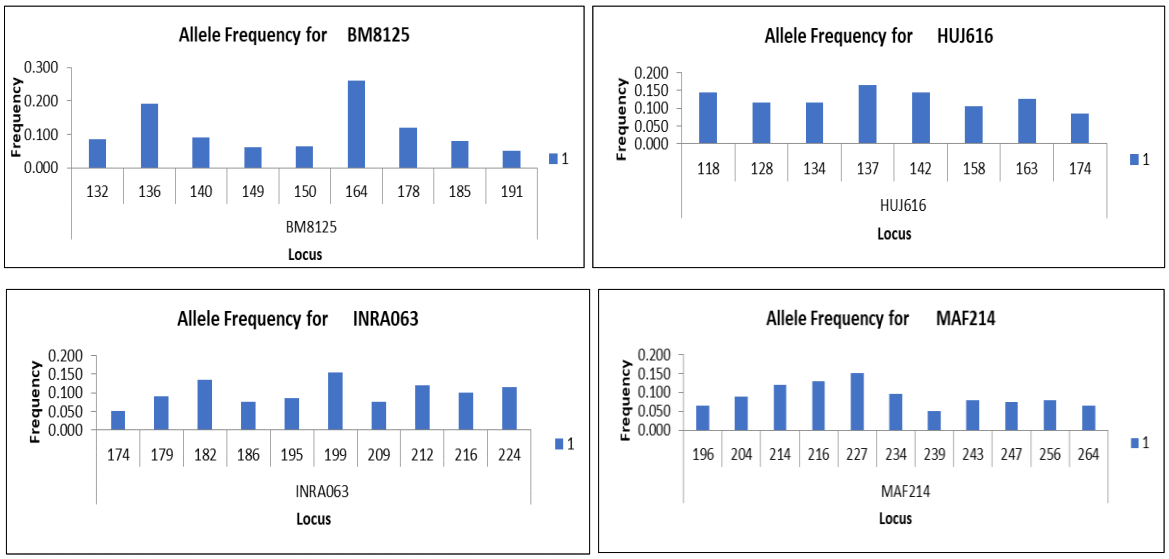
Results and Discussion

In the current investigation, a total of 82 alleles were amplified at the 10 microsatellite loci. Each locus had an average of 8.2 alleles, ranging from a minimum of 6 (OarAE129, OarCP38) to a maximum of 11 (MAF214). With a mean value of 7.221, the effective number of alleles ranged from 4.810 (OarAE129) to 9.970 (MAF214). The allele sizes ranged from 112 base pairs (bp) in OarCP38 to 264 in MAF214, and the allele frequencies from 0.050 in BM8125, INRA063, and MAF214 to 0.315 in OarAE129. The Chi-square test revealed the significant deviation of all the ten loci from Hardy-Weinberg Equilibrium which might be due to presence of unobserved null alleles or the selection process which had been progressing in the population.

Table 1: Genetic variability measures in native sheep prevalent at Nagarjuna Sagar region of Telangana

Microsatellite Locus	Allele in numbers	Effective Number of alleles	Allele size in Base pairs(bp)	Frequency of alleles	Hardy-Weinberg Equilibrium χ2 value d.f
BM8125	9	6.660	132-191	0.085-0.050	694.421*** 36
HUJ616	8	7.716	118-174	0.145-0.085	558.386*** 28
0.INRA063	10	9.179	174-224	0.050-0.115	751.333*** 45
MAF214	11	9.970	196-264	0.065-0.190	806.055*** 55
OarAE129	6	4.810	131-190	0.090-0.165	341.180*** 15
OarCP34	7	6.083	118-152	0.070-0.130	416.937*** 21
OarCP38	6	5.724	112-142	0.170-0.210	377.565*** 15
OarFCB128	10	9.091	120-152	0.120-0.150	674.231*** 45
OarHH47	7	6.297	140-185	0.090-0.205	451.169*** 21
OarJMP29	8	6.680	131-174	0.105-0.100	546.275*** 28
Mean	8.2	7.221			

*** Significant ($p \leq 0.001$)



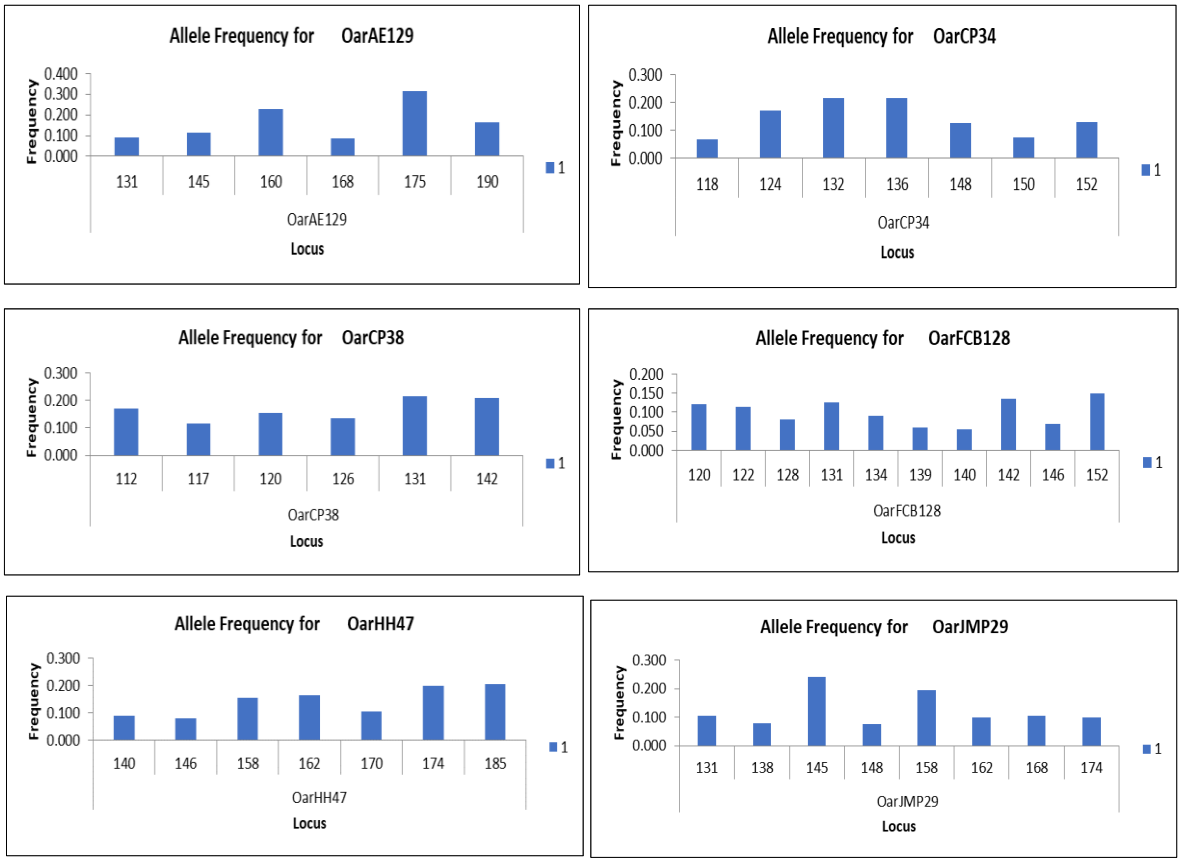


Fig 1: Allele frequency at ten microsatellite loci

Allele Diversity

BM8125

The BM8125 marker was discovered to have nine different alleles, each varying in size from 132 to 191 bp. This was greater than the variety of alleles recorded in the Nellore breed sheep (Vani *et al.*, 2017) [15], Welsh Mountain sheep (Huson *et al.*, 2015) [5], Changthangi sheep (Sharma *et al.*, 2010) [14], and Coimbatore sheep (Hepsibha *et al.*, 2014) [4].

HUJ616

The HUJ616 marker produced 8 alleles during amplification and the allele size ranged from 118 to 174bp. which was more than the range of alleles reported in Najdi sheep (Musthafa *et al.*, 2012) [10], Nellore breed sheep (Manjari *et al.*, 2018) [9].

INRA063

Ten alleles of the polymorphic marker INRA63 were discovered. Alleles reported by Sharma *et al.* (2010) [14] in Changthangi sheep and Radha *et al.* (2011) [13] in Kilakarsal sheep fell within the size range of those calculated in the current analysis (174 to 224bp), which is quite similar.

MAF214

Eleven different alleles were created by the MAF214 marker, with the sizes of the alleles varying from 196 to 264 bp. In Nellore brown sheep, there were 12 different alleles identified by prior researchers (Manjari *et al.*, 2018) [9].

OarAE129

During amplification, the OarAE129 marker produced six alleles, with an allele size range of 131 to 190bp. According to prior researchers, there were 7 alleles in Karakul sheep (Nanekarani *et al.*, 2011) [11], 10 alleles in Deccani sheep and 8 alleles in Nellore sheep (Amareswari *et al.*, 2018) [1].

OarCP34: Seven different alleles were produced by the OarCP34 marker, with the allele sizes varying from 118 to 152 bp. These were less alleles than those reported by Jyotsana *et al.* in 2012 for Marwari and Dumba sheep, Kevorkian *et al.* in 2010 for the Meat line Palas sheep breed of Rome and Nellore brown sheep (Manjari *et al.*, 2018) [9].

OarCP38

The allele size for the marker OarCP38 ranged from 112 to 142bp, and six were generated during amplification. These were completely consistent with the allele range found in Coimbatore sheep as reported by Kumarasamy *et al* (2009) [8].

OarFCB128

The OarFCB128 marker was discovered to have ten different alleles, with the sizes of the alleles varying from 120 to 152 bp. Alleles reported by Sharma *et al.* (2010) [14] in Changthangi and Radha *et al.* (2011) [13] were almost all within the range of those recorded in the current work.

OarHH47

Seven alleles were created by the OarHH47 marker, with the sizes of the alleles ranging from 140 to 185bp. which were less alleles than those reported in sheep from the Najdi, Coimbatore, and Nellore brown populations (Musthafa *et al.*, 2012; Hepsibha *et al.*, 2014; Manjari *et al.*, 2018) [10, 4, 9].

OarJMP29

The eight alleles produced by the OarJMP29 marker during amplification had a size range of 131 to 174 kb. The allele sizes observed in the current study were consistent with the allele sizes predicted by Amareswari *et al.* (2018) [1] in Deccani and Nellore sheep and Manjari *et al.* (2018) [9] in Nellore brown sheep.

Conclusion

The native sheep of Telangana are genetically diverse, as shown by estimates of genetic variability such as high gene diversity with high heterozygosity and the inbreeding (F_{IS}) values estimated across all the loci studied, which indicated high inbreeding in the native sheep of Telangana. Despite the fact that this native sheep is not in danger of extinction, the genetic diversity of this unique sheep should be utilized.

References

1. Amareswari P, Prakash MG, Ekambaram B, Mahendar M, Krishna CH. Molecular genetic studies on Nellore and Deccani sheep using microsatellite markers. Indian journal of Animal Research. 2018;52(6):805-810.
2. BAHS. Basic Animal Husbandry Statistics. Department of Animal Husbandry Dairying & Fisheries. Ministry of Agriculture, Govt. of India. 20th livestock census (2012-2019). Ministry of Agriculture Department of Animal husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi; c2019.
3. Falconer DS, Mackay TF. Introduction to quantitative genetics (4th edition); c1996.
4. Hepsibha P, Karthickeyan SMK, Guru V. Microsatellite marker based assessment of genetic structure of Coimbatore breed of Sheep (*Ovis aries*) in Tamil Nadu. Indian Journal of Biotechnology. 2014;13:203-206.
5. Huson KM, Haresign W, Hegarty MJ, Blackmore TM, Morgan C, McEwan NR. Assessment of genetic relationship between six populations of Welsh Mountain sheep using microsatellite markers. Czech Journal of Animal Science. 2015;60:216-224.
6. Jyotsana B, Jakhesara S, Prakash V, Rank DK, Vtaliya PH. Genetic features of Patanwadi, Marwari and Dumba Sheep breeds (India) inferred by microsatellite markers. Small Ruminant Research. 2012;93:57-60.
7. Kevorkian SEM, Georgescu SE, Manea MA, Zaulet M, Hermenean AO, Costache M. Genetic diversity using microsatellite markers in four Romanian autochthonous sheep breeds. Romanian Biotechnological Letters. 2010;15(1):5059-65.
8. Kumarasamy P, Prema S, Ganapathi P, Karthickeyan SMK, Kanakaraj P. Molecular Characterization of Coimbatore Breed of Sheep (*Ovis aries*) in South India. The Icfai University Journal of Genetics and Evolution. 2009;11:1-10.
9. Manjari JS, Kumari BP, Ekambaram B, Reddy YR, Amol T. Studies on genetic Variability in Nellore brown sheep using microsatellite Markers. International. Journal. Of Livestock. Research. 2018;11:149-157.
10. Musthafa MM, Aljummah RS, Alshaik MA. Genetic diversity of Najdi sheep based on microsatellite analysis. African Journal of Biotechnology. 2012;11:14868-14876.
11. Nanekarani S, Amirinia C, Amirmozafari N. Genetic analysis of Karakul sheep breedusing microsatellite markers. African Journal of Microbiology Research. 2011;5(6):703-707.
12. Nei M. The theory and estimation of genetic distance. In: Morton N E (Ed) Genetic Structure of Populations. Univ of Hawaii, Honolulu; c1973. p. 45-54.
13. Radha P, Sivaselvam SN, Kumarasamy P, Kumanan K. Genetic diversity and bottleneck analysis of Kilakarsal sheep by microsatellite markers. Indian Journal of Biotechnology. 2011;10:52-55.
14. Sharma R, Pandey AK, Singh LV, Maitra A, Arora R, Bhatia S, Mishra BP. Microsatellite based diversity estimation of Changthangi-a high altitude sheep breed of India. Indian Journal of Animal Sciences. 2010;80(5):436-440.
15. Vani S, Punyakumari B, Gnanaprakash M, Rao VH. Microsatellite marker analysis in Nellore breed of sheep. International Journal of Science, Environment and Technology. 2017;6(1):702-707.
16. Yeh FC, Yang RC, Boyle T. POPGENE version 1.32: Microsoft Windows-based freeware for population genetic analysis, quick user guide. Center for International forestry Research, University of Alberta, Edmonton, Alberta, Canada; c1999. p. 1-29.