Journal of Animal Research: v.8 n.3, p. 449-453. June 2018

DOI: 10.30954/2277-940X.06.2018.18

Genetic Structure and Differentiation of Different Strains of Nellore Sheep

B. Punya Kumari, S. Vani*, K. Harini, J.K. Sheela Manjari, M. Mohan Kishore and J. Surekha

Department of Animal Genetics and Breeding, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, INDIA

**Corresponding author: S Vani; Email: vanireddy12786@gmail.com*

Received: 22 Feb., 2018 **Revised:** 20 April, 2018 **Accepted:** 28 April, 2018

ABSTRACT

The aim of present study was to assess the genetic diversity, genetic distance and establish genetic relationship between Palla, Jodipi, Nellore Brown and Macherla brown strains of Nellore sheep breed from their breeding tracts in Andhra Pradesh, India based on 24 ovine specific microsatellite markers so as to support breed conservation and improvement decisions. All used microsatellites were amplified well and exhibited polymorphism. A total of 200 unrelated DNA samples from Palla, Jodipi, Nellore brown and Macherla brown strains of Nellore sheep breed were genotyped to find out within and between strain genetic diversity indices. The mean number of alleles (11.71); effective number of alleles (8.74) reflected the existence of substantial amount of diversity among the strains. The mean value of Shannon index is 2.25, which clearly indicates high gene diversity within populations. Observed and expected heterozygosity in the populations were found to be 0.35 and 0.88 respectively. The mean estimates of F_{IS}, F_{ST} and F_{IT} were 0.890, 0.039 and 0.895 respectively. The Nei's standard and D-genetic estimates using UPGMA method revealed low genetic distance and high genetic identity among the strains of Nellore sheep. All the four strains were genetically distinct and divided into three clusters with low degree of admixture at k = 4 based on Structure analysis. Ewans-Watterson test was conducted to test the neutrality of microsatellites. The rate of gene flow (N_m) between different strains of Nellore sheep was found to be 5.72.

Keywords: Genetic structure, Genetic distance, Phylogeny, Microsatellites, Nellore sheep strains

There are forty two indigenous breeds of sheep in India reared for meat and wool which play an important role in the biodiversity and livelihood of a large proportion of small and landless labourers. Nellore sheep is a tallest and best mutton producing breed of India known for heat tolerance, disease resistance and thrives well in harsh conditions. There are different strains of Nellore sheep distributed in Nellore, Chittoor, Kadapa, Kurnool, Anantapur, Prakasam, Guntur, Krishna and other north coastal districts of Andhra Pradesh. Considering the importance of meat in the area in which it is located, efforts have been initiated to conserve different strains of Nellore breed of sheep at molecular level using microsatellites to understand the genetic structure, relationship and differentiation in between them which will be helpful for the breeders to formulate breeding strategy for the improvement of the breed.

MATERIALS AND METHODS

Sampling and PCR based profiling

A total of 50 number blood samples were collected from each strain of Nellore sheep unrelated by ancestry from their home tract and genomic DNA was isolated as per the standard procedure of Sambrook and Russell (2001). A total of 24 microsatellite markers chosen randomly from the list recommended by FAO were amplified using a thermal cycler with a PCR reaction mixture (25µ1) containing 25 mM $Mgcl_2$, 10mM dNTPs, 10x buffer, 60 pM of each primer, 50 ng of template DNA and 1 unit of Taq DNA polymerase. The PCR products were genotyped using 6% denaturing polyacrylamaide gel and then visualized after silver staining. The allele sizes were

determined with the help of 50 bp DNA marker. Samples were scored manually either as homo or heterozygote for each loci.

Statistical analysis

Allelic profile was calculated using the POPGENE 1.31 software version (Yeh *et al.,* 1999). Shannon index was calculated for determining diversity index (Shannon and Weaver, 1949). Polymorphism information content was calculated using PIC calculator. Nei's genetic distance was calculated using UPGMA method. Genetic structure and the degree of admixture of four sheep populations were studied using the Bayesian clustering procedure of STRUCTURE ver.2.3 (Pritchard *et al.,* 2000). Ten independent runs for each 'K' value ranging from 2 to 10 were carried out to identify the most probable 'K' value that best fit the data. Structure harvester (Earl and Von Holdt, 2011) which implements the Evanno method (Evanno *et al.,* 2005) was used.

RESULTS AND DISCUSSION

Intra population genetic variation

All the 24 ovine specific microsatellite markers were amplified well and all of them were found to be polymorphic. The observed, effective number of alleles, Shannon's index, observed and expected heterozygosity were presented in Table 1. A total of 281 alleles were observed across all loci in all the four strains studied. The observed number of alleles ranged from 9 (OarCP34, OarVH72) to 16 (OarCP49) with a mean of 11.71 ± 1.92. The effective number of alleles across all the loci ranged from 5.47 (OarVH72) to12 (OarCP49) with a mean of 8.74 ± 1.36. Some of the alleles screened for microsatellite markers were found to be specific to a particular strain with low frequency. The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of strains within a breed. The results were in agreement with those of other sheep breeds in India (Kumarasamy *et al.,* 2009; Pramod *et al.,* 2009; Ramachandran *et al.,* 2015).

The observed heterozygosity for different strains in the present study ranged from 0.11 (OarHH64) to 0.52 (MAF214) with a mean of 0.35. Takezaki and Nei (1996) stated that for markers to be useful for measuring genetic variation, these should have an average heterozygosity ranging from 0.3 to 0.8 in the populations. This again confirmed that these markers were sufficient to measure the genetic variation. The average expected heterozygosity (0.88) was found to be higher than the average observed heterozygosity (0.35) which revealed that the population had retained the presence of several alleles although at lower frequencies.

Table 1: Summary of genetic variation and heterozygosity in four different strains of Nellore sheep

Locus	Sample Size	na	ne	I	H0	He
BM1314	200	11	9.04	2.28	0.39	0.89
BM6506	200	11	9.36	2.30	0.29	0.89
BM6526	200	11	8.58	2.25	0.43	0.88
BM757	200	12	8.05	2.24	0.44	0.88
BM8125	200	11	7.95	2.14	0.34	0.87
BM827	200	13	10.74	2.43	0.3	0.90
CSSM31	200	10	7.51	2.11	0.27	0.86
CSSM37	200	10	7.61	2.12	0.43	0.87
HSC	200	11	9.14	2.28	0.45	0.89
HUJ616	200	11	7.33	2.13	0.4	0.86
INRA63	200	10	7.39	2.12	0.42	0.86
MAF214	200	15	9.66	2.41	0.52	0.90
OarCP20	200	13	8.85	2.30	0.44	0.89
OarCP34	200	9	8.07	2.12	0.49	0.88
OarCP49	200	16	12.00	2.56	0.46	0.92
OarFCB128	200	12	9.52	2.36	0.23	0.90
OarFCB48	200	15	8.99	2.38	0.19	0.89
OarHH35	200	13	9.98	2.40	0.27	0.90
OarHH41	200	13	9.35	2.35	0.25	0.89
OarHH47	200	10	9.53	2.28	0.22	0.90
OarHH64	200	14	9.50	2.39	0.11	0.89
OarJMP29	200	10	6.83	2.04	0.29	0.85
OarJMP8	200	11	9.28	2.30	0.45	0.89
OarVH72	200	9	5.47	1.82	0.33	0.82
Mean		11.71	8.74	2.25	0.35	0.88
Std. dev.		1.92	1.36	0.16	0.05	0.02

The Shannon's index is a parameter for determining diversity index. The Shannon's index values ranged from 1.82 (OarVH72) to 2.56 (OarCP49) with a mean value of

2.25 ± 0.16 which clearly indicates the existence of gene diversity in the population. The high amount of variation indicates scope for conservation. In general, the average higher values of genetic diversity indices observed indicate the basis of its heterogeneous nature being represented by four morphologically different groups of animals.

Inter population genetic variation

The mean estimates of F-statistics (Wright's fixation indices) *viz.,* within population inbreeding estimate (F_{IS}), measurement of population differentiation (F_{ST}), total inbreeding estimate (F_{IT}), and Gene flow (N_m) among the four strains of Nellore sheep breed are shown in table 2. The within population inbreeding estimate (F_{IS}) which indicate heterozygosity deficit ranged from 0.751 (BM6526) to 0.955 (BM827) with a mean of 0.890 whereas the total population had a mean heterozygosity deficit of 0.895 that ranged from 0.757 (BM6526) to 0.956 (BM827). The F_{ST} estimates for gene differentiation values ranged from 0.021 (OarHH47) to 0.077 (OarHH64) across all loci. The highest degree of genetic uniformity between different strains of Nellore sheep was found to be supported by relatively high level of gene flow (6.033) between these strains. The high homogeneity or lack of heterozygotes among these strains might be due to inbreeding or non random mating as evidenced by overall positive F-value. Moreover due to uncontrolled mating in India sheep populations at the farmer level, a breeding group most likely comprises a dominant male generally excludes subordinates males, and presumably sire most of the offspring. In addition, the breeding groups will be expected to be inbred with the unequal sex ratio of breeding animals causing inbreeding to accumulate. Farmers should put efforts to avoid as much as possible, by exchanging rams frequently from other flocks. The results are similar to those reported for other breeds (Dashaba *et al.,* 2011).

Table 2: Global F-Statistics and gene flow at different microsatellite loci in different strains of Nellore sheep breed

Locus	FIS	FIT	FST	Nm*
BM1314	0.878	0.882	0.035	6.890
BM6506	0.941	0.944	0.059	3.935
BM6526	0.751	0.757	0.024	10.331
BM757	0.778	0.789	0.047	5.054
BM8125	0.939	0.943	0.059	3.993
BM827	0.955	0.956	0.027	9.070
CSSM31	0.926	0.931	0.062	3.762
CSSM37	0.839	0.845	0.036	6.687
HSC	0.912	0.916	0.038	6.305
HUJ616	0.951	0.954	0.047	5.074
INRA63	0.916	0.919	0.036	6.618
MAF214	0.879	0.883	0.028	8.538
OarCP20	0.931	0.932	0.022	10.978
OarCP34	0.784	0.795	0.049	4.896
OarCP49	0.927	0.929	0.029	8.493
OarFCB128	0.936	0.938	0.033	7.345
OarFCB48	0.934	0.938	0.057	4.125
OarHH35	0.880	0.883	0.025	9.832
OarHH41	0.895	0.899	0.038	6.385
OarHH47	0.903	0.905	0.021	11.764
OarHH64	0.849	0.860	0.077	3.000
OarJMP29	0.897	0.900	0.037	6.579
OarJMP8	0.919	0.922	0.029	8.371
OarVH72	0.840	0.847	0.042	5.716
Mean	0.890	0.895	0.039	6.033

Nei's standard (D_S) genetic distance and genetic identity (D_A) values showed lowest degree of divergence between the four strains of Nellore breed (Table 3). Nei's original measure of genetic distance was calculated using UPGMA method. The D_A based genetic distance values revealed high levels of uniformity between the four strains of Nellore sheep. Neighbour joining tree based on pairwise Nei's genetic distance was presented in Fig. 1.

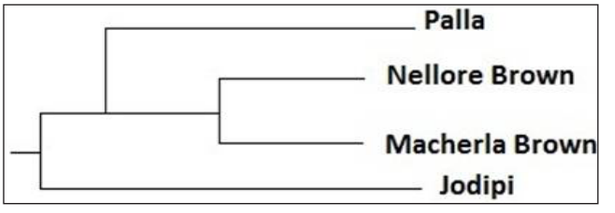

Fig. 1: Neighbour joining tree based on pair wise Nei's genetic distance among four sheep strains of Nellore sheep breed

Genetic distance estimates were used to calculate the divergence time ('t' in generation) between the four strains as calculated as D=2αt, whereas 'D' is the standard genetic distance of Nei and 'α' is the assumed mutation rates (1.2×10^{-3}) of microsatellite loci (Weber and Wong, 1993).

Assuming a constant rate of divergence and a generation interval of 3 to 4 years, the estimated time of divergence between Palla with Jodipi, Nellore brown and Macherla brown, Jodipi with Nellore brown and Macherla brown and Nellore brown with Macherla brown based on Nei's genetic distance were 170.42, 124.08, 259.1, 177.50, 146.67 and 139.17 generations (Table 4).

Table 3: Pair wise Nei's original measure of Genetic distance (below diagonal) and Genetic identity (above diagonal) among four strains of Nellore sheep

Population	Palla	Jodipi	Nellore Brown	Macherla Brown
Palla	****	0.664	0.736	0.733
Jodipi	0.409	****	0.653	0.704
Nellore Brown	0.305	0.426	****	0.716
Macherla Brown	0.311	0.352	0.334	****

Table 4: Estimated time divergence between four strains of Nellore sheep

Strains	Nei's Standard genetic distance	Mutation rate (α)	Divergence time	
			Generations	Years
Palla – Jodipi	0.409	1.2×10^{-3}	170.42	511.26
Palla – Nellore Brown	0.305		124.08	372.24
Palla – Macherla Brown	0.311		129.58	388.74
Jodipi – Nellore Brown	0.426		177.50	532.5
Jodipi – Macherla Brown	0.352		146.67	440.01
Nellore brown – Macherla Brown	0.334		139.17	417.51

Based on highest 'K' value obtained in the structure analysis according to evanno *et al.* (2005) revealed that four strains should be divided into three clusters (Fig. 2). From the structure analysis, the true 'K' value obtained is four. i.e., at K = 4, all the four strains were genetically distinct with relatively lower degree of admixture. Nellore brown and Macherla brown were placed in one cluster whereas Palla and Jodipi were grouped in different clusters. Proportion of membership coefficients in each of the inferred clusters with different runs of structure program are presented in Table 5. The strains were clustered based on their geographical location and genetic identity.

Table 5: Proportion of membership coefficients in each of inferred clusters with different runs of STRUCTURE program

'K' Value	Strain	Inferred clusters			
		1	2	3	4
K = 2	Palla	0.512	0.488		
	Jodipi	0.530	0.470		
	Nellore brown	0.589	0.411		
	Macherla brown	0.676	0.324		
K = 3	Palla	0.306	0.379	0.315	
	Jodipi	0.436	0.425	0.139	
	Nellore brown	0.315	0.102	0.583	
	Macherla brown	0.266	0.200	0.534	
K = 4	Palla	0.196	0.609	0.187	0.008
	Jodipi	0.631	0.027	0.129	0.213
	Nellore brown	0.001	0.130	0.725	0.144
	Macherla brown	0.125	0.09	0.533	0.252

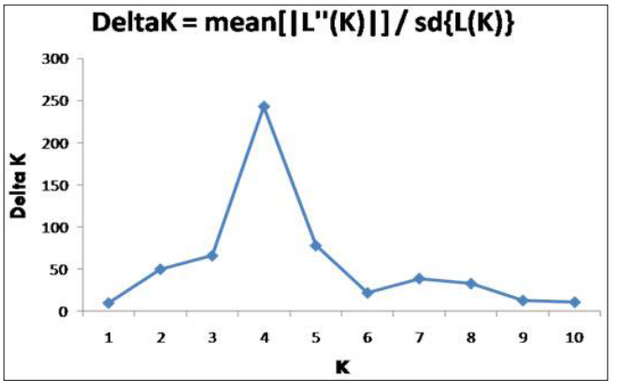

Fig. 2: Structure analysis showing highest 'K' value (Evanno *et al.,* 2005)

Ewens-Watterson test was performed to test the neutrality for microsatellite markers. The F-statistics (sum of squares of allele frequency) and limits (upper and lower) at 95% confidence region for the test were calculated using the algorithm by Manly method utilizing 1000 simulations and implemented in Popgene software. The overall test for neutrality across the four populations revealed that Mean F-values all the loci were within the lower and upper limits of the 95% confidence interval (Table 6).

Table 6: The overall Ewens-Watterson test for neutrality (statistics of natural selection)

Locus	Min.F	Max.F	Mean	SE	L95	U95
BM1314	0.0909	0.9513	0.3163	0.0139	0.1689	0.6255
BM6506	0.0909	0.9513	0.3237	0.0143	0.1712	0.6276
BM6526	0.0909	0.9513	0.3200	0.0152	0.1641	0.6513
BM757	0.0833	0.9465	0.3040	0.0144	0.1549	0.6240
BM8125	0.0909	0.9513	0.3191	0.0141	0.1681	0.6157
BM827	0.0769	0.9418	0.2774	0.0104	0.1467	0.5365
CSSM31	0.1000	0.9560	0.3457	0.0165	0.1768	0.6746
CSSM37	0.1000	0.9560	0.3504	0.0163	0.1757	0.6565
HSC	0.0909	0.9513	0.3242	0.0153	0.1646	0.6416
HUJ616	0.0909	0.9513	0.3256	0.0157	0.1647	0.6361
INRA63	0.1000	0.9560	0.3542	0.0176	0.1765	0.6881
MAF214	0.0667	0.9325	0.2441	0.0082	0.1341	0.4846
OarCP20	0.0769	0.9418	0.2770	0.0106	0.1447	0.5701
OarCP34	0.1111	0.9608	0.3865	0.0209	0.1940	0.7431
OarCP49	0.0625	0.9278	0.2304	0.0075	0.1268	0.4596
OarFCB128	0.0833	0.9465	0.2990	0.0123	0.1571	0.5968
OarFCB48	0.0667	0.9325	0.2464	0.0085	0.1335	0.4736
OarHH35	0.0769	0.9418	0.2747	0.0107	0.1465	0.5572
OarHH41	0.0769	0.9418	0.2800	0.0106	0.1474	0.5358
OarHH47	0.1000	0.9560	0.3549	0.0166	0.1845	0.6953
OarHH64	0.0714	0.9371	0.2666	0.0107	0.1343	0.5114
OarJMP29	0.1000	0.9560	0.3464	0.0172	0.1847	0.6977
OarJMP8	0.0909	0.9513	0.3274	0.0162	0.1671	0.6542
OarVH72	0.1111	0.9608	0.3860	0.0207	0.1854	0.7524

CONCLUSION

In conclusion the present study is an attempt to understand the genetic structure, diversity and to establish the relationship among different strains of Nellore sheep using microsatellite DNA markers and there is a need of genetic management to reduce the inbreeding and intermixing. Further investigations would be required to clarify the relationship between the strains of Nellore sheep breed of Andhra Pradesh.

ACKNOWLEDGEMENTS

We sincerely thank RKVY for providing the financial support for running the project entitled "Cytogenetic and molecular characterization of Livestock breeds of A.P". We extend our deep sense of gratitude to SVVU for providing necessary laboratory facilities needed throughout the work.

Conflict of interest

The authors exhibit no conflict of interest in the research work done and in the submission of manuscript.

REFERENCES

Dashaba, G.R., Aslaminejada, A., Nassiria, M., Esmailizadehb, A.K. and Saghia, D.A. 2011. Analysis of genetic diversity and structure of Baluchi sheep by microsatellite markers. *Trop. Subtrop. Agro. ecosys.,* **14**: 1047–54.

Earl, D. and Von Holdt, B. 2011. Structure Harvester: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.,* **4**: 359–61.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol. Ecol.,* **14**: 2611–20.

Kumarasamy, P., Prema, S., Ganapathi, P., Karthickeyan, S.M.K. and Kanakaraj, P. 2009. Molecular characterization of Coimbatore breed of sheep (*Ovis aries*) in South India. *J. Genet. Evol.,* **3**: 56–65.

Pramod, S., Kumarasamy, P., Rosalyn Mary Chandra, A., Sridevi, P. and Rahumathulla, P.S. 2009. Molecular characterization of Vembur sheep (*Ovis aries*) of South India based on microsatellites. *Indian J. Sci .Technol.,* **2**: 55–58.

Pritchard, J.K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* **155**: 945–59.

Ramachandran, A., Thiruvenkadan, A.K., Kathiravan, P., Saravanan, R., Pannerselvam, S. and Elango, A. 2015. Microsatellite based phylogeny of Indian sheep. *Indian J. Anim. Sci.,* **85(11)**: 1209-1214.

Sambrook, J. and Russel, D. 2001. Molecular cloning. A laboratory manual. Cold spring Harbour Laboratory press, Cold spring Harbour, New York.

Shannon, C.E. and Weaver, W. 1949. The mathematical theory of communication. (University of Illinois Press IL)

Takezaki, N. and Nei, M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite data. *Genetics,* **144**: 389–99.

Weber, J.L. and Wong, C. 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet.,* **2**: 1123-1128.

Yeh, F.C., Boyle, T., Rongcai, Y., Ye, Z. and Xian, J.M. Popgene. 1999. Version 1.31, A Microsoft Window Based Free Ware for Population Genetic Analysis, University of Alberta and Centre for International Forestry Research, Edmonton.