Indian J. Anim. Res.,
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijaronline.in

Genetic variability of sheep populations of Saudi Arabia using microsatellite markers

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Received: 10-05-2017 Accepted: 11-01-2018

ABSTRACT

The present study was conducted to know the genetic diversity of three Saudi sheep populations; Naeimi (NM), Herri (H) and Najdi (NJ). Genomic DNA was extracted from 156 animals of sheep comprising 47 Naeimi, 47 Herri and 62 Najdi breeds using 18 microsatellite markers. A total of 212 alleles were generated with a mean value of 11.80 alleles per locus, with a range of observed and expected heterozygosity of 0.505 to 0.875 and 0.595 to 0.854, respectively. Eleven of the microsatellites loci studied in NM, three loci in H and fifteen loci in NJ were observed to be deviated from Hardy-Weinberg equilibrium. The fixation genetic indices (Fst) among the three sheep populations were very low, ranging from 0.017 (between NJ and H) to 0.033 (between NJ and NM), indicating low population differentiation among the three sheep populations studied. The present study showed that the microsatellite markers are powerful tool in determining genetic diversity among sheep populations.

Key words: DNA sequencing, Genetic diversity, Microsatellites, Sheep population.

INTRODUCTION

In recent years, the molecular biological approaches have created an exciting new means for studying livestock genetics. Several studies had investigated the genetic diversity in sheep using microsatellites markers (Arora and Bhatia, 2004; Peter *et al.*, 2007). Sheep is one of the important domestic livestock species in Saudi Arabia, exceeding 7.2 million head (Ayadi *et al.*, 2014) and plays an important role in the livelihood of local communities since they are a good source of meat, milk and coarse wool. Najdi sheep is considered the breed of choice followed by Naeimi in central Saudi Arabia and Herri breed which is the preferable breed in the western Saudi Arabia. The extent to which Saudi sheep populations are genetically differentiated is poorly documented. In the present study, a set of eighteen microsatellite markers were used to evaluate the genetic diversity within and between three sheep breeds of Saudi Arabia (Najdi, Naeimi and Herri).

MATERIALS AND METHODS

Blood samples (10 ml) were collected from 156 sheep belonging to three breeds i.e: Naeimi (47 NM), Herri (47 H) and Najdi (62 NJ). Genomic DNA was extracted from the blood using the QIAgen DNeasy blood and tissue kit (Hilden, Germany). Eighteen microsatellites recommended by the International Society of Animal Genetics (ISAG)/FAO to study genetic diversity in sheep were used for genotyping the samples (FAO, 2011). PCR amplifications were carried out in a 25 µl reaction volume containing 100 ng of template DNA and 2 µl of each forward and reverse primer (10 µM). Master mix containing all PCR reagents including the KapaTaq polymerase enzyme (KAPA Biosystems, Boston, MA, USA) was added to the reaction. The amplification program was performed using the Gene Amp PCR system 9700 thermocycler (Applied Biosystems, Warrington, UK). The amplification protocol involved annealing temperature at 55 ˚C. The PCR products were analyzed in the ABI Prism® 3500 Genetic analyzer (Applied Biosystems, Warrington, UK).

Statistical analysis: The basic parameters like allele frequencies, observed number of alleles (Na), effective number of alleles (Ne), observed (Ho), expected (He) heterozygosities and Polymorphism Information Content (PIC) were calculated using Cervus version 3.0.3 (Kalinowski *et al.*, 2007). Wright's F-statistics were calculated by using GenePop version 4.0.10. Deviations from Hardy-Weinberg equilibrium (HWE) were calculated using GenePop. PAUP version 4.0b10. The number of genetically distinct groups were determined by two ways: principal coordinate analysis (PCoA) using software GenAlEx version 6.5 (Peakall and Smouse, 2012) and Bayesian clustering using software structure. For the structure analysis we used the admixture model but did not assume correlated allele frequencies among the breeds, nor did we use the USEPOPINFO or LOCPRIOR options. For each run we

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conducted a burn-in of 10000 replicates followed by 100000 replicates of data collection. We ran 5 iterations for each value of *K* from 1-5. The most likely value of *K* was determined by Pritchard's *ad hoc* method (Pritchard *et al.,* 2000) and Evanno's Δ*K* (Evanno *et al.,* 2005).

RESULTS AND DISCUSSION

All the eighteen microsatellite loci were amplified. The values of Na, Ne, Ho and He for the three populations are given in (Table 1). The number of alleles per locus ranged from 4 (in OarAE129) to 18 (DYMS1 in 18), with an average value of 11.8 alleles per locus. A total of 212 alleles were observed among the 156 sheep using 18 microsatellite loci. The total number of alleles observed in NM, H and NJ populations were 159, 144 and 152, respectively. Out of these 212 alleles, 60 were designated as private alleles. Out of these 60 private alleles, 31 were observed in NM, 15 in H and 12 in NJ breeds populations. For all 18 microsatellite markers in three sheep populations, the mean value were recorded as: the mean of number of alleles (NA) was 11.8, mean number of alleles (MNA) was 8.426, mean of effective allele number (MNE) was 4.506, mean of observed heterozygosity (MHO) was 0.754, and mean of expected heterozygosity (MHE) was 0.752, respectively. The mean of Polymorphic Information Content (PIC) for the 18 marker was 0.750. Mean values for F_{IS}, F_{IT} and F_{ST} were -0.003, 0.033 and 0.036, respectively were observed in F-statistics for each of the 18 analyzed loci in the three sheep populations. The NM sheep breed was genetically closer to H and NJ sheep breeds and the pair-wise genetic distances were 0.031, 0.033 and 0.017 respectively (Table 2).

HWE results showed that 7 loci in NM sheep population followed HWE, 15 loci in Herri and 5 loci in in Najdi at $p<0.05$. PCoA identified 3 distinct clusters with each cluster comprised of all sheep of one breed (Figure 1). Axis 1 explained 38.1% of the variance and axis 2 explained an additional 21.7%. Axis 1 discriminated all 3 breeds whereas axis 2 discriminated H and NM breeds from NJ. For the Structure analysis, the most likely value of *K* was 3, corresponding to the 3 breeds of sheep (Figure 2). All individuals had an assignment coefficient (*q*) >0.9 to their own breed, indicating very little admixture among the breeds.

The present study is a significant contribution towards the genetic characterization and diversity of three phenotypically distinguished populations of Saudi Sheep; namely Naeimi, Herri and Najdi using 18 microsatellite markers. This range of allele frequency was comparable with those observed in four sheep populations of Romania (Jakaria *et al.,* 2012). It was higher than those observed in Kilakarsal sheep population and in Vembur sheep population of South India (Radha *et al.,* 2011; Pramod *et al.,* 2009). Ricardo *et al.* (2016) has shown that the observed number of alleles per locus ranged from 10 to 23 in the Colombian sheep, and Yilmaz *et al.* (2015) found a range of 16 to 31 in Turkish sheep populations. It is known that mean number of alleles in a population is highly dependent on sample size and the presence of unique alleles of low frequencies. As the sample size increases, the number of alleles increases. Large numbers of allele frequencies as high as 270 and 352 were found by some investigators (Sassi-Zaidy *et al.,* 2014; Yilmaz *et al.,* 2015). Private alleles defined in this study as alleles unique to a single population were observed to be 31, 15 and 7

Table 1: Number of alleles (Na), Mean effective number of alleles (Ne), observed (H_O) and Expected (H_e) heterozygosities for each locus of the three different sheep populations, Naeimi (NM), Herri (H) and Najdi (NJ).

Locus	NM				H				NJ			
	Na	Ne	Ho	He	Na	Ne	Ho	He	Na	Ne	Ho	He
ILSTS005	10.000	4.476	1.000	0.777	8.000	4.564	0.851	0.781	6.000	4.131	0.661	0.758
MCM527	10.000	6.153	1.000	0.837	12.000	6.818	0.851	0.853	11.000	5.543	0.774	0.820
SRCRSP5	6.000	3.227	1.000	0.690	5.000	3.251	0.532	0.692	5.000	3.270	0.419	0.694
OARFCB128	8.000	5.978	0.915	0.833	5.000	4.046	0.830	0.753	9.000	5.233	0.806	0.809
HUJ616	11.000	5.137	0.979	0.805	4.000	3.092	0.638	0.677	6.000	2.770	0.661	0.639
OAEHH47	12.000	5.970	0.809	0.833	12.000	7.764	0.702	0.871	11.000	7.073	0.790	0.859
ILSTS11	8.000	3.327	0.787	0.699	7.000	5.368	0.872	0.814	6.000	3.614	0.823	0.723
DYMSI	14.000	7.126	0.851	0.860	15.000	7.889	0.957	0.873	15.000	7.099	0.710	0.859
BM8125	9.000	5.185	0.872	0.807	5.000	3.273	0.957	0.694	8.000	3.458	0.629	0.711
OarFCB226	11.000	5.349	0.894	0.813	8.000	3.574	0.723	0.720	10.000	4.381	0.823	0.772
OarAE129	3.000	2.177	0.489	0.541	4.000	2.625	0.702	0.619	3.000	2.658	0.532	0.624
OarJMP29	11.000	6.044	0.745	0.835	9.000	4.392	0.787	0.772	12.000	4.491	0.758	0.777
SRCRSP9	7.000	3.064	0.660	0.674	6.000	3.595	0.915	0.772	6.000	3.138	0.661	0.681
MAF214	5.000	3.030	0.617	0.670	9.000	3.722	0.745	0.731	6.000	2.397	0.581	0.583
OARCP34	6.000	4.463	0.851	0.776	6.000	4.387	0.660	0.772	6.000	4.541	0.774	0.780
OARFCB304	10.000	2.464	0.681	0.594	13.000	5.642	0.851	0.823	13.000	6.932	0.774	0.856
MAF209	7.000	3.176	0.936	0.685	9.000	4.042	0.617	0.753	8.000	3.460	0.726	0.711
MAF65	11.000	7.219	0.319	0.861	7.000	3.557	0.404	0.719	11.000	3.943	0.790	0.746
Mean	8.833	4.643	0.800	0.755	8.000	4.533	0.755	0.658	8.444	4.341	0.745	0.751
SE	0.658	0.377	0.044	0.022	0.763	0.369	0.035	0.016	0.658	0.353	0.026	0.019

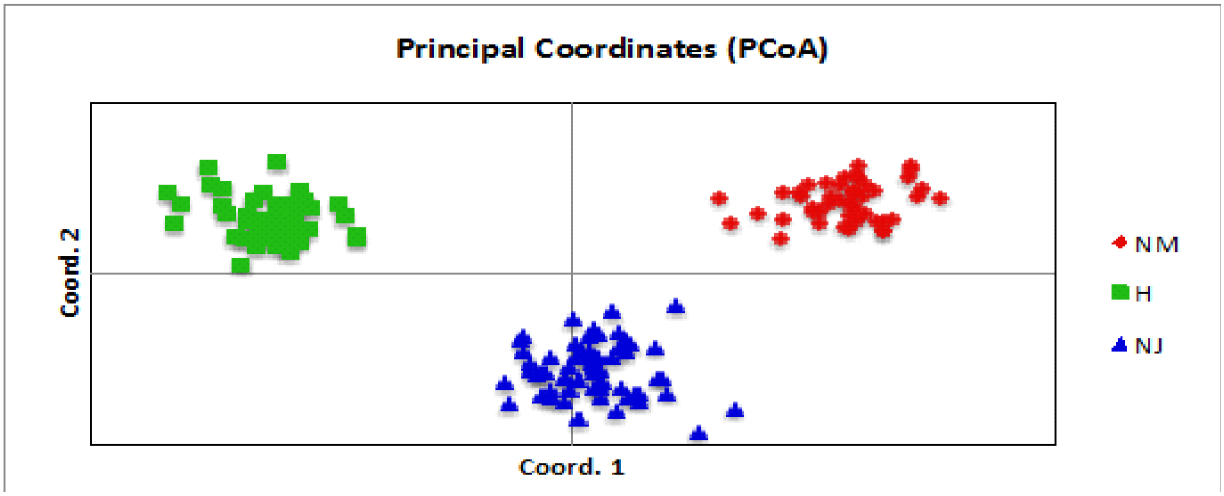

Fig 1: Principal coordinates analysis of three sheep breeds in Saudi Arabia, Naeimi (NM), Herri (H), and Najdi (NJ).

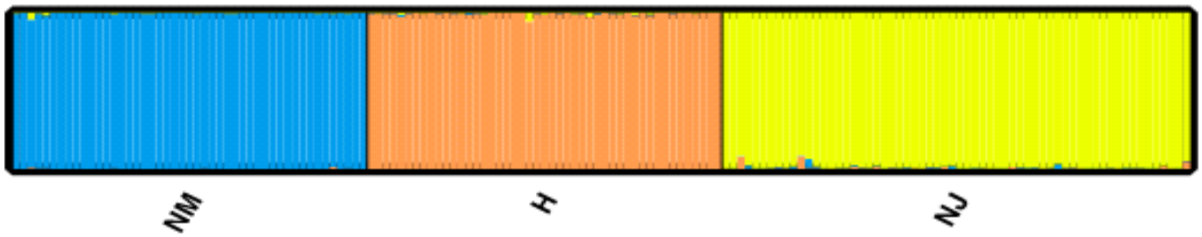

Fig 2: Structure bar plot indicating the three genetic clusters of Saudi Arabian sheep, Naeimi (NM), Herri (H), and Najdi (NJ).

alleles for Naeimi, Herri and Najdi sheep populations, respectively. Despite the low frequencies of these alleles, however, they can be distinguished among the three sheep populations and can be good indicators as breed markers. Yilmaz *et al.* (2015) designated 7 alleles in Turkish sheep breeds as private alleles having been observed in a single population. Blackburn *et al.* (2011) observed private alleles with low frequencies in Kazakh sheep breed.

Turkish sheep breed displayed higher mean effective number of alleles of 7.040 (Yilmaz *et al.*, 2015) as compare to the present study. Balochi and Rakhshani sheep breed in Pakistan displayed the lowest average effective number of alleles of 2.969 (Wajid *et al.*, 2014). The mean observed heterozygosity in the present study was 0.800, 0.755 and 0.790 for Naeimi, Herri and Najdi sheep breed, respectively, with a grand mean of 0.754. This is higher than reported by other studies of Indonesian sheep (0.574), Indian sheep (0.618) and Colombian sheep (0.680) (Jakaria *et al.*, 2012; Yilmaz *et al.*, 2015 and Ricardo *et al.*, 2016). The expected heterozygosity (He), however, was recorded as 0.755, 0.658 and 0.751 for Naeimi, Herri and Najdi sheep breed, respectively, with a mean of 0.752. This was higher than reported by other studies in Indonesian sheep (0.687) (Jakaria *et al.*, 2012), but was lowest in Turkish sheep (0.870) and Colombian sheep (0.770) (Yilmaz *et al.*, 2015 and Ricardo, *et al.*, 2016). Interestingly, the lowest Ho was observed in Naeimi sheep (0.319) in the MAF65 marker and the lowest value of He was in the OarAE129 marker of Naeimi sheep (0.541). In general, all breeds showed high genetic diversity for all loci analyzed. In the present study it was to verify whether the genotypes studied were in Hardy–Weinberg Equilibrium. Results indicated that there were some genotypes with several loci that followed HWE ($P< 0.05$); 7 loci in Naeimi, 15 loci in Herri and 5 loci in Najdi sheep populations. Although these markers indicated a deficiency and excess of heterozygotes, this does not explain the deviation from HWE. Gene flow was high in some populations but lower in others. F_{IS} value for all loci was -0.003, which indicated that some moderate inbreeding has likely occurred within each population, although it does not explain the genetic variation among the three sheep populations under investigation. Outbreeding is limited due to isolation of breeding groups to specific geographical regions or even farms. In addition, F_{ST} value of 0.036 indicates little genetic differentiation has occurred. A previous study by Radha *et al.* (2011) using 25 microsatellite markers in Indian sheep indicated that seven out of 25 of the loci in the sheep populations were in Hardy-Weinberg Equilibrium.

Table 2: Genetic distance (below diagonal) and genetic identity (above diagonal), between the three populations, NM, H and NJ.

population	NM	H	NJ
NM	-	0.969	0.977
H	0.031	-	0.983
NJ	0.033	0.017	-

NM = Naeimi, H = Herri, NJ = Najdi

The mean Fst values among the three sheep populations ranged between 0.031 (between Naeimi and Herri populations), 0.033 (between Najdi and Naeimi populations) and 0.017 (between Najdi and Herri populations) indicating little genetic differentiation among Saudi sheep populations. Ferrando *et al.* (2014) also obtained close Fst value in six breeds located in the eastern Pyrenees ranging from 0.011 to 0.053. When Saudi Arabian sheep populations were compared with other populations from different countries, the lower Fst values were observed in this study (Sassi-Zaidy *et al.,* 2014).

There was no evidence that PIC less than 0.542 indicating that all microsatellite markers used in this study were highly polymorphic. The high average number of alleles per marker and the high values of PIC indicated that the 18 microsatellite markers in this study are appropriate for studying genetic diversity in Saudi sheep. Structure and PCoA results indicated a high degree of distinction among sheep breeds in Saudi Arabia with virtually no admixture. This likely is due to husbandry practices that minimize gene flow among farms raising different breeds.

CONCLUSION

In conclusion, 18 microsatellites were genotyped for 156 animals comprising 3 breeds to investigate the genetic structure of these breeds in Saudi Arabia. The results of the present study represent baseline information of genetic pattern and diversity in these sheep breeds which are commonly raised in Saudi Arabia. Hence, studying additional microsatellite markers may reveal more information on the population structure. Furthermore, larger numbers of animals from different breeds are required to establish a robust genetic analysis for genotyping and characterizing the sheep population in Saudi Arabia.

ACKNOWLEDGEMENT

We extend our appreciation to King Abdulaziz City for Science and Technology (KACST) for funding this work through project number MS-35-229.

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