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Genetic Relationships among Four Saudi Arabian Sheep Populations

Research Article

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ABSTRACT

Four Saudi Arabian indigenous sheep populations including Najdi, Hbsi, Arb, and Naemi were genotyped for 16 microsatellite markers recommended by the food and agriculture organization (FAO). This study aims to provide information on the genetic structure of the breeds analyzed and give indications and proposals for the cross breeding program. All loci were polymorphic in all populations and locus combinations were at Hardy-Weinberg equilibrium except ILSTS044, ILSTS005, MAF209, HUJ616, OARFCB226 and SRCRSP09 ($P<0.05$). There was substantial genetic variability within sheep populations, with average heterozygosity range of 0.759-0.811 based on expected hetrozygosity. It was observed that the four sheep populations showing the lowest level of inbreeding on the basis of heterozygote deficiency. The lowest genetic distance (0.013) was obtained between Hbsi and Najdi and the highest genetic distance (0.146) between Arb and Hbsi. Estimates of inbreeding coefficient (F_{IS}) were significant for three breeds, except for Arb breeds ($P<0.05$). The sequence results of the 16 microsatellite markers were sequenced and then phylogenetic tree based on unbiased distances was drawn using MEGA 4. To study the genetic relationships among sheep populations, a principal coordinate analysis (PCA) based on Nei standard distances was performed which indicated a conservation program is needed in these sheep population since most of them are in danger of inbreeding.

KEY WORDS genetic relationship, heterozygosity, microsatellite, phylogenetic tree, Saudi Arabia, sheep.

INTRODUCTION

Sheep plays an important role in modern agriculture and possess many heritable traits that have economic importance. Sheep genetic resources in Saudi Arabia were formed under the impact of different processes. Geographical isolation, genetic drift, selection and crossbreeding, and the transhumance of population, contributed to the current picture of sheep breeds and varieties in the Kingdom where the main feature is the presence of good quality breeds. The Najdi is the principal native sheep breed in the eastern province of Saudi Arabia. It is a fat-tailed black coated animal with a coarse fleece (Ali and Naomi, 1992; Muneeb *et al.* 2012) Naemi is also native, fat tailed white coated distinguish brown head color and distributed western part of the Kingdom (Peter *et al.* 2005). Hbsi and Arb breeds are distributed randomly throughout Saudi Arabia.

This study used microsatellite markers because they are a powerful tool for tracking alleles through a population and to estimate genetic variability and inbreeding (Ligda *et al.* 2009; Molaee *et al.* 2009). Over the past decade, extensive efforts have been made by the international sheep gene

mapping community to develop a useful sheep linkage map by microsatellites which provide a large number of genetic information (Peter *et al*. 2007; Dalvit *et al*. 2008; Santos-Silva *et al*. 2008). To promote the use of common markers, FAO is proposing an updated, ranked list of microsatellite loci for the major livestock species (FAO, 2007).

The aim of this study is the use of molecular data for evaluating genetic variability and genetic relationships of the Saudi Arabian sheep populations. The genetic relationship of Saudi Arabian sheep breeds have been not studied previously in the Kingdom, and this paper, we present an analysis based on individual genotypes at 16 microsatellites sequences with a view obtaining a deeper insight into relationship within and between four breeds.

MATERIALS AND METHODS

Distribution

The present study deals with the sheep genetic diversity in Saudi Arabia for the first time to such an extent, covering four local sheep breeds analyzed in 16 microsatellites. Furthermore, the sampling was performed on flocks covering the whole breeding region of each breed. In Figure 1 highlights the map of sample distribution which was taken from the Kingdom.

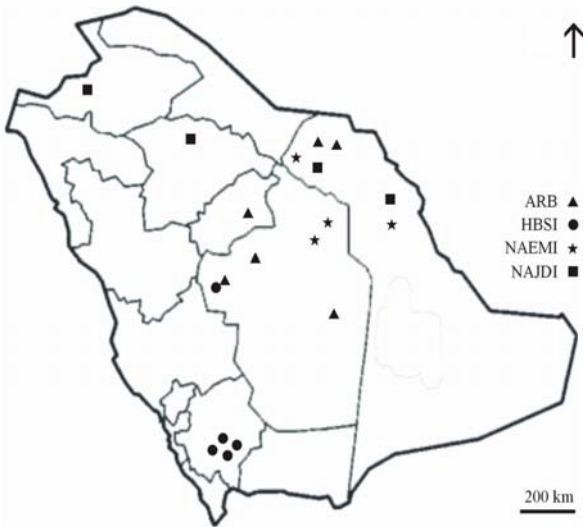

Figure 1 Geographical distribution of the sampled breeds

Samples

Blood samples were collected from a total of 172 genetically unrelated animals from four breeds in Saudi Arabia: Najdi (48 animals), Hbsi (40), Arb (40) and Naemi (44). Animals were sampled from their native breeding locations over the country and breed was determined by phenotypic appearances (Figure 1). In an attempt to minimize the bias sampling between the animals sampled, a maximum of two herds per village and a maximum of two animals per flock were sampled. Samples were collected to represent the population and the origin of the males and females were evenly sampled.

Peripheral blood samples were collected in EDTA tubes. Genomic DNA was isolated from 1 mL blood aliquots by commercial kit from GFX (GE Health Care, Uppsala, Sweden) according to the manufacture's instruction. Isolated DNA concentrations were measured quantity and quality by spectrophotometer (Spectronic GENESIS 10, USA) based on absorbance at 260 and 280 nm, and DNA samples were concentrated at 100 ng/µL.

Estimates of genetic relationships between varieties, between and within population (flocks) diversity and population structure were based on allelic variation at 16 microsatellite loci. Microsatellite markers were chosen for their polymorphism and the absence of null alleles segregating at the loci. Primer sequences, size ranges, multiplexing information and PCR protocols of the markers are available from the FAO's website (FAO, 2004) which comprises a list of markers ranked by typing efficiency, as used within the International Society for animal genetics (ISAG) project (www.projects.roslin.ac.uk). The following microsatellites were genotyped: MCM42, OARFCB20, OARVH72, TGLA53, DYMS1, ILSTS044, ILSTS005, MAF209, BM8125, OARFCB11, OARJMP29, HUJ616, OARFCB226, SRCRSP09, HSC and OARHH47.

PCR amplification

Polymerase chain reaction (PCR) was performed using a Gene Amp® PCR System 9700 (AB applied Biosystem, CA, USA) according to Touch down method, as described by Crawford *et al*. (1995). Only the forward primer of the each primer pair was labeled with the four of the following fluorescent dyes: FAM-blue, PET-red, NED-yellow and VIC-green provided by applied Biosystems™ (CA, USA). Genotyping was performed on ABI 3100 (AB applied Biosystem, CA, USA) automated DNA analysers using standard methodologies (Pariset). Microsatellite fragment sizing was performed by the Gene Mapper® version 4.0 (Applied Biosystems™, CA, USA) and the size standard peaks were defined by the user. Allele calling was performed with the software and checked manually to avoid any false calling.

Statistical analysis

The loci per locus frequencies and tests of genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE) were carried out using the exact tests of the FSTAT software (Goudet, 2002). The GENEPOP 3.4 program (Yeh *et al*. 1999) was employed for the calculation of mean observed heterozygosity (H_o) and mean expected heterozy-

gosity (H_e) for populations. Nei standard genetic distances were estimated using the FSTAT and GENEPOP software packages (Nei, 1972). The genetic relationships among the four analysed populations were evaluated by the UPGMA method.

In order to define the degree of differentiation among the populations studied, Mega 4 software (www.megasoftware.net) was used to build the phylogenetic tree, based on the Reynold's genetic distance, using the neighbor-joining method (Saitou and Nei, 1987; Paetkau *et al.* 1995).

RESULTS AND DISCUSSION

Genetic variability parameters are presented in Table 1 and Figure 2. Although varying among populations, mean observed heterozygosity was lower than the mean expected heterozygosity for all the populations (Table 1). The average gene diversity was 0.786 for the four studied sheep sites. Najdi had the highest gene diversity for all the loci (H_e=0.811) with average number of loci per population (10.25), while Arb had the lowest gene diversity (H_e=0.759) with average number of loci per population (8.00) (Table 1). The mean number of loci per locus is 9.6 in the selected 4 breeds. All populations had substantial levels of genetic variation as shown by the Ho and mean unbiased estimates of gene diversity (H_e). The lowest genetic distance (0.013) was obtained between Hbsi and Najdi and the highest genetic distance (0.146) between Arb and Hbsi (Table 2).

We used the pairwise D values to build the phylogenetic tree in Figure 3, which provides one method of summarizing the genetic relationships between study sites. As can be seen, only two population clusters received strong support from the data. A description of the used markers, including number of alleles per marker, the heterozygote deficiency within population values (F_{IS}), and deviation from Hardy-Weinberg equilibrium across breeds for Hbsi, Najdi, Naemi, Arb sheep, is given in Table 3.

Both the analyzed sheep populations showed significant ($P<0.05$) heterozygote deficit, being 1.8, 0.5, 1.2 and 2.7% respectively in Hbsi, Najdi, Naemi, Arb. The average F_{IS} values for most of the loci in four breeds were significantly different ($P<0.05$) from zero.

However, the main cause of the lack of heterozygotes in the investigated populations can be attributed to inbreeding. In the livestock system considered, rams breed with all the ewes in the flock and, therefore, the heterozygote deficiency is likely to arise from the relationship of individuals used for reproduction. The high level of genetic differentiation detected in the Hbsi sheep breeds (Table 1 and 2) suggested large extent of genetic exchange is less in others. A most probable cause of great genetic similarity among neighboring breeds was observed by other researchers (Trexler, 1988; Beja-Pereira *et al.* 2003) as well as in this study.

Figure 2 Microsatellite mapping of PCR-amplified DNA from the sheep breeds in Kingdom of Saudi Arabia

Genotypic data from 16 microsatellites was used to assess the genetic diversity Najdi, Hbsi, Arb and Naemi sheep. No PCR-based genetic studies for comparison of these breeds were found in the Kingdom. From this point of view, this was the first study to investigate the genetic diversity of the sheep breeds. In the global test of deviation from Hardy-Weinberg equilibrium, the deviations from the expected value may be due to a variety of causes: population subdivision owing to genetic drift (Lawson *et al.* 1989).

Table 1 Genetic variability parameters in four Saudi Arabian sheep populations

Populations	Sample size	H_o±SD	H_e±SD	MNA±SD	F_{IS}
Hbsi	40	0.746±0.017	0.811±0.019	10.25±2.46	0.082
Najdi	48	0.739±0.016	0.796±0.023	9.94±2.91	0.072
Naemi	44	0.736±0.017	0.776±0.022	10.19±2.61	0.052
Arb	40	0.746±0.017	0.759±0.022	8.00±2.42	0.018

H_o: observed heterozygosity; H_e: expected heterozygosity and F_{IS}: inbreeding coefficient.
MNA: mean number of loci per locus.
SD: standard deviation.

Table 2 Matrix of Nei's original measures of genetic identity and genetic distance among four Saudi Arabian sheep populations: the Nei (1972) standard genetic distance (below diagonal) and genetic identity (above diagonal)

Population	Hbsi	Najdi	Naemi	Arb
Hbsi	-	0.013	0.025	0.037
Najdi	0.029	-	0.022	0.020
Naemi	0.088	0.067	-	0.037
Arb	0.146	0.084	0.127	-

Table 3 The used markers are located, number of alleles per marker, and heterozygote deficiency within population (F_{IS}) values per marker and breed

Locus	Number	Hbsi	Najdi	Naemi	Arb
MCM42	11	0.915	0.359	0.684	0.306
OARFCB20	14	0.450	0.826	0.184	0.666
OARVH72	09	0.896	0.011	0.765	0.452
TGLA53	12	0.748	0.590	0.168	0.593
DYMS1	18	0.005**	0.000***	0.084	0.009**
ILSTS044	15	0.260	0.020*	0.006**	0.302
ILSTS005	13	0.005**	0.004**	0.129	0.031*
MAF209	11	0.018*	0.005**	0.001**	0.203
BM8125	08	0.503	0.074	0.536	0.884
OARFCB11	12	0.561	0.211	0.146	0.438
OARJMP29	15	0.654	0.421	0.854	0.453
HUJ616	13	0.769	0.775	0.563	0.040*
OARFCB226	15	0.046*	0.400	0.158	0.448
SRCRSP09	09	0.542	0.014*	0.029*	0.078
HSC	16	0.353	0.406	0.420	0.946
OARHH47	13	0.100	0.006**	0.625	0.501
Mean	12.8	0.426	0.258	0.334	0.397
Deficiency	-	0.018	0.005	0.012	0.027

** (P<0.001); ** (P<0.01) and * (P<0.05).

All four sheep populations had a substantial amount of genetic variation, and none of them can be considered genetically impoverished. The different F_{IS} values of the populations reflect different levels of inbreeding (Pariset *et al*. 2003). Overall inbreeding in the four breeds are lowest ranked is Arb. The three sheep populations (Najdi, Hbsi and Naemi showing the highest level of inbreeding should be considered to be at risk as this condition can lead to reduced fitness (Table 1). This finding could be due to population subdivision in each region, local inbreeding or the presence of null alleles (Peter *et al*. 2007; Handley *et al*. 2007; Gustavo *et al*. 2000).

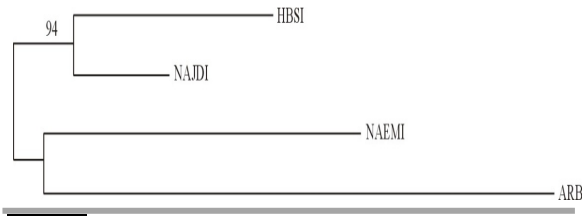

Figure 3 Genetic relationships among the 4 sheep breeds using R_D genetic distance

Since the set of microsatellites markers we used showed a little higher variability than that of the microsatellites markers used in the genetic diversity analysis of Sarda sheep breeds of central Italy (Pariset *et al*. 2003), European sheep breeds (Peter *et al*. 2007; Arranz *et al*. 1998), Iranian Sheep breeds (Molaee *et al*. 2009), desert bighorn sheep of Arizona, California, New Mexico, Alberta and Canada (Gustavo *et al*. 2000), we interpreted our higher gene diversity as reflections of both the choice of the microsatellite markers and the choice of breeds. For microsatellite distributions values genetic differentiation among the four breeds in the populations was about 3%. This value is slightly lower than that found by others at microsatellite loci in Spanish breeds (about 7% of total diversity; Kantanen *et al*. 2000), in cattle (10.7%; Laval *et al*. 2000), and in pigs (27%; (Raymond and Rousset, 1995), indicating a closer relationship. The results for population differentiation using the exact test of (Nei, 1978) were in agreement with those of our results. It is interesting to note that the spatial distribution of the flocks explained by phylogenetic tree based on (Wright, 1978) genetic distance partially fits with the relationship with the geographical locations in the study area.

CONCLUSION

Based on this criterion, the 16 microsatellite loci used in the present study can be considered useful for the evaluation of genetic diversity within and among populations and for the selection of breeding animals from divergent groups maximizing genetic variation and consequently fitness for improve the breeding for prosperous country.

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REFERENCES

Ali K.E. and Al-Naomi A.A. (1992). Copper status of Najdi sheep in Eastern Saudi Arabia under penned and grazing conditions. *Trop. Anim. Health Prod*. **24,** 115-120.

Arranz J.J., Bayon Y. and Primitivo F.S. (1998). Genetic relationships among Spanish sheep using microsatellites. *Anim. Genet.* **29,** 435-440.

Beja Pereira A., Alexandrino P., Bessa I., Carretero Y., Dunner S., Ferrand N., Jordana J., Laloe D., Moazami-Goudarzi K., Sanchez A. and Canon J. (2003) Genetic characterization of southwestern European bovine breeds: a historical and biogeographical reassessment with a set of 16 microsatellites. *J. Hered.* **94(3),** 243-250.

Crawford A.M., Dodds K.G., Pierson C.A., Montgomery G.W. and Beattie C.W. (1995). An autosomal genetic linkage map of the sheep genome. *Genetics*. **140,** 703-724.

Dalvit C., Sacca E., Cassandro M., Gervaso M., Pastore E. and Piasentier E. (2008). Genetic diversity and variability in Alpine sheep breeds. *Small Rumin. Res*. **80,** 45-51.

FAO. (2004). Feed and Agriculture Organization. Secondary guidelines for development of national farm animal genetic resources management plan, measurement of domestic animal diversity (MoDAD), recommended microsatellite markers. Rome. Database available at: www.fao.org.

FAO. (2007). Feed and Agriculture Organization. The state of the world report, Rome. Database available at: www.fao.org.

Goudet J. (2002). FSTAT, a program to estimate and test gene diversities and fixation indices.

Gustavo A., Steven T., Walter M. and Philip W. (2000) Genetic variation and population structure in desert bighorn sheep. *Conserv. Genet*. **1,** 3-15.

Kantanen J., Olsaker I., Holm L.E., Lien S., Vilkki J., Brusgaard K., Eythorsdottir E., Danell B. and Adalsteinsson S. (2000). Genetic diversity and population structure of 20 North European cattle breeds. *J. Hered.* **91,** 446-457.

Laval G., Iannuccelli N., Legault C., Milan D., Groenen M.A.M., Giuffra E., Anderson L., Nissen P.E., Jorgensen C.B., Beeckman P., Geldermann H., Foulley J.L., Chevalet C. and Ollivier L. (2000). Genetic diversity of eleven European pig breeds. *Genet. Sel. Evol.* **32,** 187-203.

Handley L.J.L., Byrne K., Santucci F., Townsend S., Taylor M. and Hewitt G.M. (2007). Genetic structure of European sheep breeds. *Heredity*. **99,** 620-631.

Lawson R., Kofron C.P and Dessauer H.C. (1989). Alloenzyme variation in natural populations of Nile crocodile. *Am. Zool.* **29,** 863-871.

Ligda C., Altarayrah J. and Georgoudis A. (2009). Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. *Small Rumin. Res.* **83,** 42-48.

Molaee V., Osfoori R., Eskandari Nasab M.P. and Qanbari S. (2009). Genetic relationships among six Iranian indigenous sheep populations based on microsatellite analysis. *Small Rumin. Res.* **84(1),** 121-124.

Muneeb M., Riyadh S., Aljumaah and Al-Shaikh M.A. (2012). Genetic diversity of Najdi sheep based on microsatellite analysis. *African J. Biotechnol.* **11(83),** 14868-14876.

Nei M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*. **89**, 583-590.

Nei M. (1972). Genetic Distance between population. *Am. Natur*. **106,** 283-292.

Paetkau D., Calvert W., Stirling I. and Strobeck C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* **4,** 347-354.

Pariset L.M., Savarese C., Cappuccio I. and Valentini A. (2003). Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of central Italy. *J. Anim. Breed. Genet.* **120,** 425-432.

Peter C., Bruford M., Perez T., Dalamitra S., Hewitt G. and Erhardt G. (2007). Population structure of 57 European and Middle Eastern marginal sheep breeds. *Anim. Genet.* **38(1),** 37-44.

Peter C., Erhardt G., Hewitt G., Dalamitra S., Bruford M. and Perez T. (2005). Microsatellite markers reveal differentiation of South Eastern and Western European sheep breeds. The role of biotechnology. Villa Gualino, Turin, Italy.

Raymond M. and Rousset F. (1995). An exact test for population differentiation. *Evolution.* **49,** 1280-1283.

Saitou N. and Nei M. (1987). The Neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4,** 406-425.

Santos Silva F., Ivo R.S., Sousa M.C.O., Carolino M.I., Ginja C. and Gama L.T. (2008). Assessing genetic diversity and differentiation in Portuguese coarse-wool sheep breeds with microsatellite markers. *Small Rumin. Res.* **78,** 32-40.

Trexler J.C. (1998). Hierarchical organization of genetic variation in the Saifin Molly, Poecilia Latipinna (pisces: Poeciliidae). *Evolution.* **42,** 995-1005.

Wright S. (1978). Variability Within and Among Natural Populations. Evolution and the Genetics of Populations. Chicago, IL., University of Chicago Press.

Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H. and Mao J.X. (1999). POPGENE, the user friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.