Genetic Diversity of sheep genotypes for identification of uniqueness genes in the Barbarine breed

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Abstract

Two africain sheep genotypes (Tunisian fat-tail Barbarine breed "BB", a native Nigerian population "NS") and one Northern American (Tunis sheep "TS") were genotyped. The objectives of this study were to compare genetic diversity among these three genotypes and to identify the genetic distance between "TS" and its origin the Tunisian "BB" from which some animals were exported to USA since 1799. Five microsatellites were used and main results showed a total of 56 alleles. The average number of alleles by locus was 6.2. Values of this parameter were 5.2, 6.6 and 6.8 for "TS", the "BB" and the "NS", respectively. Expected heterozygoty varied from 0.49 for OarFCB128 and 0.83 for OarCP34 locus. Expected and observed heterozygoty were (0.67 and 0.57), (0.71 and 0.69) and (0.76 and 0.67) for "TS", "BB" and "NS", respectively. Values of Fis and Fit varied from -0.14 to 0.42 and from -0.06 to 0.58, respectively. Multilocus values Fst values indicated that 15% of the global genetic variation was explained by a population difference. Genetic distances between The "BB" and "TS" and The "BB" and the "NS" genotype were 0.38 et 1.46, respectively showing strong ties between Tunis sheep and Barbarine sheep.

Key-words: Sheep, genotyping, DNA, Barbarine, diversity

INTRODUCTION

Small ruminant resources are playing very important roles worldwide. Beside providing meat, milk and fiber to a growing population, their socio and cultural dimensions still constitute valuable added values, especially in developing countries which own approximately 80 percent of the world's animal genetic resources (ICAR, 2000). The majority of livestock encountered is raised generally under medium to low input production environments. The livestock breeds' distribution is strongly related to production environment characteristics as is their ongoing development. Production environments offer a variety of situations world wide. In Tunisia, vast genetic resources are reflected by the availability of nine ecotypes within the fat-tail "Barbarine" sheep breed. From this breed, in 1799 ten head were shipped to the United States as a gift to the U.S from the ruler of Tunisia, and in 1896, the American "Tunis Sheep" Breeder's Association was organized. The outstanding property of the Tunis sheep is the ability of the ewes to breed at any time of year. This makes them valuable for hothouse lamb production. The present study was based on two African sheep genotypes (Tunisian fat-tail Barbarine breed "BB", a native Nigerian population "NS") and one Northern American (Tunis sheep "TS") with the objectives to compare genetic diversity among these three genotypes and to identify the genetic distance between them.

MATERIAL AND METHODS

Sample and DNA extraction

A total of 53 blood samples of genetically unrelated animals of the three genotypes were randomly collected from their respective breeding tracts. Genomic DNA was isolated from white blood cells by two methods: the first related to standard procedure using proteinase K digestion followed by phenol-chloroform extraction method of (sambrook et al., 1989) and the second using automated extraction using iPrep purification instrument and stored at -20°C. A total of 5 microsatellites loci (OarFCB20, OarFCB128, MAF209, OarCP34, MCM527) recommended by FAO sheep and goat biodiversity were included in this investigation (table 1) to estimate various genetic variation in three sheep breeds. Primers pair of microsatellite markers were synthesized by Invitrogen. Polymerase chain reaction (Biorad, C1000 thermal Cycler, USA) was carried out in 25 µl reaction volume containing 1.5 or 2.0 or 2.5 mM MgCl2 specifically to primer used (table 1), 0.5 mM dNTPs, 0.5 mM of each

primer, 40 ng of template DNA and 1 U of Taq DNA polymerase (Promega Corporation. Madison, WI, USA). Thermal amplification conditions are reported in table (2).

The amplified products were resolved on automated electrophoresis (Biorad, Experion Automated Electrophoresis Station, USA)

Table 1. Primers sequences, size range and chromosomal location for microsatellite loci used.

Primers	Sequence (Forward + reverse)	Tm/MgCl2	Size range	Chromos	Reference
OarFCB20	AAATGTGTITAAGATTCCATACAGTG GGAAAACCCCCATATATACCTATAC	52/ 2,5	87-139	2	BUCHANAN et *al*. (1994)
OarFCB128	CAGCTGAGCAACTAAGACATACATGCG ATTAAAGCATCTTCTCTTTATITCCTCGC	51/1,5	71-74	2	BUCHANAN and CRAWFORD (1993)
MAF209	GATCACAAAAAGTTGGATACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	60/2	131-161	17	BUCHANAN and CRAWFORD (1992)
OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	51/1,5	100-165	3	EDE et al. (1994)
MCM527	GTCCATTGCCTCAAATCAATTC AAACCACTEACTACTCCCCAA	50/2	166-205	5	HUIME ***et*** ***al.*** (1995)

Table 2. Thermal amplification program for PCR

Cycle	Denaturation	Annealing	Extension
1	95°C- 5mn		
35	95°C - 30 s	50°C ; 62°C -30 s	72°C -30 s
1			72°C -10 mn

The genetic diversity of the three populations: “BB”, “TS” and “NS” breeds has been evaluated on the basis of microsatellite DNA.

Statistical analysis

Different measurements of within breed genetic variations, observed number of alleles (na), effective number of alleles (ne), observed heterozygosity (Ho), expected heterozygosity (He) were estimated using GenALEx software package (version 6.2).

To assess the population genetic structure of the three sheep breeds. D-statistics parameters (total inbreeding estimate), θ (measurement of population differentiation), f(within-population inbreeding estimate) that are analogous to Fit, Fst and Fis (Wright’s 1978), respectively were estimated using GenALEx.

Microsatelitte allele frequency data was applied to calculate genetic distance estimates by employing genetic distance methods.

Results and discussion

Genetic variability

A total of 56 alleles were detected. The average number of alleles by locus was 6.2. Mean number of alleles observed in the three genotypes were 5.2, 6.6 and 6.8 for Tunis sheep "TS", Barbarine sheep "BB" and Niger sheep "NS" respectively. Expected heterozygoty varied from 0.49 for OarFCB128 and 0.83 for OarCP34 locus.The highest average observed (Ho) and expected heterozygosity (He, gene diversity) values were seen in "BB" (Ho=0.69, He=0.71) followed by "NS" (Ho=0.67, He=0.76) and "TS" (Ho=0.57, He=0.67) (Table 3).

Table 3. Average observed (Ho) and expected heterozygosity (He) values for the tree breeds TS, NS, BB

Locus	Breeds	Size range	Na (Ne)	Ho (He)
OarFCB128	TS	71-130	4 (1.6)	0.3 (0.38)
	BB	71-130	4 (2.03)	0.3 (0.49)
	NG	69-107	4 (2.36)	0.25 (0.57)
MAF209	TS	123-159	4 (3.15)	0.69 (0.68)
	BB	135-157	5 (2.96)	0.85 (0.66)
	NG	131-153	7 (5.19)	0.85 (0.80)
OarFCB20	TS	91-135	8 (4.39)	0.61 (0.77)
	BB	93-133	8 (4.54)	0.9 (0.78)
	NG	89-129	7 (5.40)	0.9 (0.81)
MCM527	TS	181-199	4 (3.41)	0.84 (0.70)
	BB	165-199	8 (3.84)	0.95 (0.74)
	NG	165-199	9 (6.10)	0.8 (0.83)
OarCP34	TS	107-135	6	0.38 (0.81)
	BB	107-131	8	0.45 (0.83)
	NG	107-133	7	0.55 (0.75)
MEAN	TS		5.2 (3.59)	0.57 (0.67)
	BB		6.6 (3 .92)	0.69 (0.71)
	NS		6.8 (4.63)	0.67 (0.76)

Genetic differentiation

Heterozygote deficiency analysis revealed that all the three populations exhibited deviations from HWE ($p<0.05$) at several loci. This deviation could be linked to high positive F_{IS} (within-population inbreeding estimate) values observed in the investigated sheep populations (table 4) and might be attributed to a number of factors such assortative mating (sample relatedness), linkage with loci under selection or null alleles. And from flock structure of BB

and NS breeds, it is apparent that rams and ewes are housed and grazed together thereby no controlled mating is practiced at farmers' level.

Table 4. Within population inbreeding estimates (F_{IS}) in the three sheep genotypes

Locus	TS	BB	NG
OarFCB128	0.24	0.43	0.58
MAF209	0.03	-0.36	-0.03
OarFCB20	0.24	-0.13	-0.08
MCM527	-0.16	-0.26	0.07
OarCP34	0.55	0.48	0.24

Population differentiation explained by fixation indices F_{it}, F_{is} and F_{st} for each of the five loci across three sheep breeds are given in table (5). Mean estimates of F-Statistics obtained were F_{it}=0.24, F_{is}=0.11 and F_{st}=0.15. The overall high values of F_{st} across all the loci reflected the substantial degree of breed differentiation. Values of Fis and Fit varied from -0.14 to 0.42 and from -0.06 to 0.58, respectively. Multilocus values Fst values indicated that 15% of the global genetic variation was explained by a population difference.

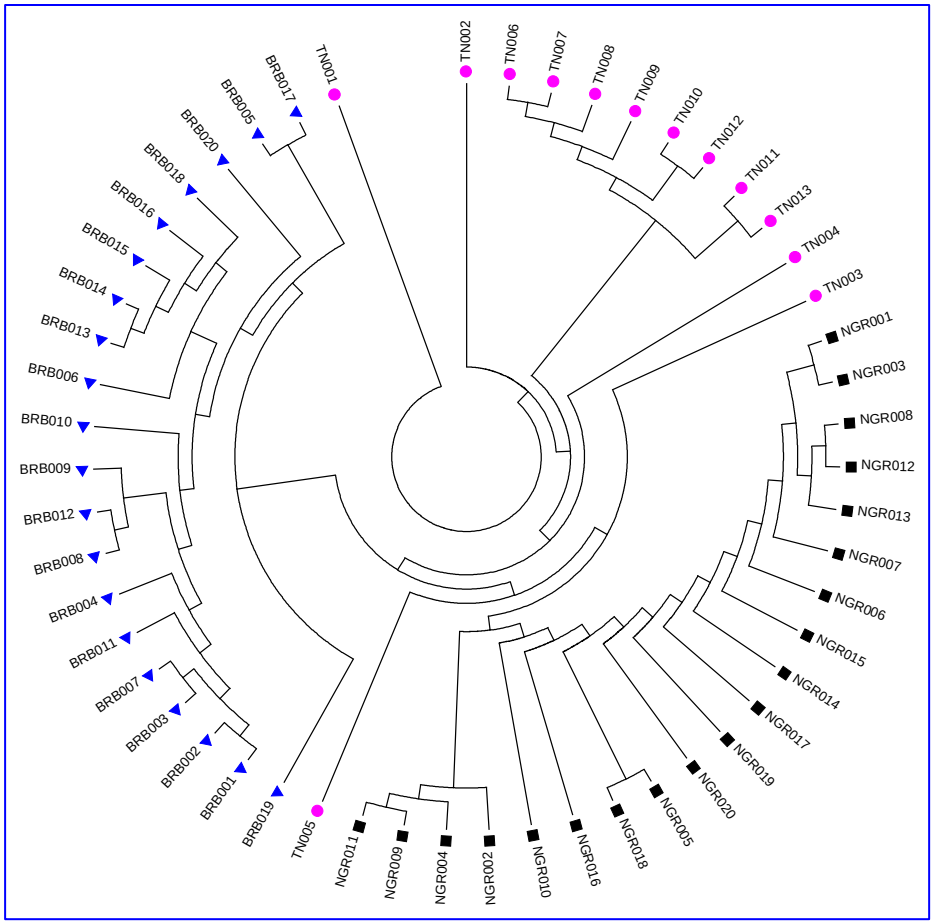
Table 5. Estimators of F-statistics at each locus across the three sheep breeds

Locus	F_{IT}	F_{IS}	F_{ST}
OarFCB128	0.578	0.418	0.275
MAF209	0.083	- 0.111	0.175
OarFCB20	0.087	-0.02	0.106
MCM527	-0.059	-0.137	0.069
OarCP34	0.488	0.425	0.109
Mean	0.236	0.115	0.147

Phylogenetic relationship

To evaluate relationships among the three breeds, genetic distances were calculated by different methods using the allelic frequency data. The Nei's genetic distance estimates for the three breeds pair combinations are 0.38, 1.46 and 1.52 for "TS"-"BB", "BB"-"NS" and "TS"-"NS" respectively.

Presence of strong differentiation between BB-NS and TS-NS may be attributed to the geographical isolation of “NS”. The close relationship between “TS” and “BB” may be explained on the basis of sharing common breeding tracts, in fact, “TS” breed has its origin from “BB” breed.



Conclusion

The results of this study contributed to the knowledge of the genetic structure of the three sheep genotypes and showed a strong tie between Tunis sheep and Barbarine sheep. This investigation suggested that Barbarine breed and Tunis sheep breed may be given a priority in identifying specific genes.

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