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Blood protein polymorphism in three sheep breeds from the south of Tunisia

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Abstract

Biochemical polymorphism was studied in the native ovine Barbarine (BAR) and Queue Fine de l'Ouest (QF) breeds as well as in the exotic D'man (DMN) breed in the South of Tunisia using a panel of six protein coding loci: Haemoglobin (Hb), Carbonic anhydrase (Ca), Albumin (Al), Transferrin (Tf), X-protein (X-p) and Arylesterase A (EsA). Polymorphic loci were detected by means of horizontal starch gel electrophoresis. All tested loci were polymorphic. The Tf locus exhibited the highest number of alleles while the other loci showed two alleles in all sampled breeds. Estimates of expected heterozygosity were almost twice those of observed heterosigositiy in all sheep populations and the high level of heterozygosity was observed in DMN breed. Genetic distances observed between BAR-DMN and between QF-DMN were larger than those found between BAR-QF breeds.

Key worlds: Tunisian sheep, Blood protein, Polymorphism, Genetic distance, Genetic diversity

Introduction

Major sheep breeds in Tunisia are the fat tailed Barbarine (BAR) which accounts for around 60% of the sheep population, the Queue Fine de l'Ouest (QF), the Noire de Thibar (NT) and the exotic prolific D'man (DMN) meat breeds and the Sicilo-Sarde dairy breed. The DMN breed was introduced last decade from Morocco into the Tunisian oases. Production performances of Tunisian breeds are limited compared to those of their counterparts reared in temperate countries. These limited performances may not only be explained by breeds' genetic potentials but also traditional management practices. On the other hand, local breeds are resistant to pathogens and are well adapted to the rather difficult conditions (harsh climatic conditions, poor-quality food, etc.). These unique characteristics are results of the evolutionary forces and their interactions over long periods of time. However, resistance and adaptation capabilities might have been reduced because of intermixing, sub-structuring and/or consequent genetic drift in these sheep population over time. Therefore, the investigation of genetic diversity and similarity between and within breeds is necessary to provide useful genetic information essential for developing effective management plans for the conservation and improvement of these genetic resources. Studies on Tunisian sheep population were focused on productive and reproductive performances (Atti et al., 2001; Rekik et al., 2002; Rekik et al., 2008) but there were no reports to our knowledge on genetic diversity at the biochemical and DNA levels. Blood protein system has been widely used to characterize genetic diversity in domestic animals (Jordana et al., 1999; Deza et Al., 2000; Sun et al., 2007; Boujenane et al., 2008), while many authors have associated blood protein with quantitative and adaptability traits (Gueny et al, 2003; Pieragostini et al., 2006).

Genetic diversity of indigenous sheep breeds in Tunisia has not been sufficiently studied. The objective of this study was to provide information at the biochemical level about genetic structure and diversity of some Tunisian breeds reared in the south-west of Tunisia, employing blood protein markers.

Materials and Methods

This study was conducted in 2009 in the Research Unit of Macromolecular Biochemistry and Genetics (BMG), Faculty of Science of Gafsa and the Regional Center of Research in Oases Agriculture (CRRAO). A total of 160 blood samples were taken from three sheep breeds reared in the south west of Tunisia. Blood samples were equally collected from the

BAR, DMN, QFG (Queue Fine de l'Ouest from the Gafsa region) and QFT (Queue Fine de l'Ouest from the Tozeur region) sheep populations. Blood samples were randomly collected from adult and presumably unrelated animals of both sexes. Whole blood was collected from the jugular vein into 8 ml vacuum tubes containing the K3EDTA anticoagulant. Blood samples were centrifuged at 4°C for 20 min at 3000 rpm to separate serum and red cells. Red cells were washed three times in saline solution (NaCl) and lysed with cold distilled water. Serum was stored at – 20°C while hemolysate was stored at – 80°C prior to electrophoretic analysis.

Six protein coding loci: Haemoglobin (Hb), Transferrin (Tf), Albumin (Al), Carbonic anhydrase (Ca) and X-protein (X-p) were typed using horizontal starch gel electrophoresis. The electrophoretic assay and references are shown in Table 1. Precipitation of Haemoglobin was achieved where 0.5 ml of thawed erythrocyte was mixed with 0.4 ml ethanol (40%) and 0.2 ml chloroform and the mixture was then stirred vigorously. After 30 min at 4°C, the extract was centrifuged at 3000 rpm for 15 min and the supernatant was used for electrophoresis. Alcohol-chloroform extracts were used for typing Ca. Following electrophoresis, the gel was sliced into 3 mm thick strips. Each slab was stained for 10-15 minute using concentrated nigrosine or amido black-10B solution; it was thereafter covered by a de-staining solution using a 1:5:5 mixture of glacial acetic acid: methanol: distilled water until it was clear for reading and photographing. Alleles at each locus were designated alphabetically according to their migration rate. Plasma Arylesterase (EsA) phenotypes were determined using the quick tube test developed by (Tucker et al., 1967).

Data analysis

Allele frequencies were computed by direct counting for the co-dominant loci (Hb, Tf, Al and Ca) and taking the square root of frequencies of recessive homozygotes for dominant loci (EsA and X-p) assuming genetic equilibrium. Genetic variability within breeds was evaluated by proportions of polymorphic loci and heterozygosity levels. Genetic distances among populations were calculated following (Nei, 1972). Computed distances were used to draw dendrograms by un- weighed pair-group arithmetic averages (UPGMA) according to (Sneath and Sokal, 1973). Computations were done using BIOSYS-1 (Swofford and Selander, 1989).

Results

The six studied loci were polymorphic in all breeds. Frequencies of observed alleles at the investigated loci are given in Table 2. Fifteen variants were found at 6 loci. The most frequent alleles were HbB, CaS, Xp- and EsA-. The highest number of alleles occurred at the Tf locus (five alleles) and two alleles were observed in other loci. Five alleles were detected in the BAR and DMN breeds and only four alleles were found in QFG and QFT sub- groups at the Tf locus. The TfD allele was the most frequent in BAR and DMN while the TfB was the most frequent in the QF breed. At the Al locus, the AlS allele was predominant in the BAR, QFG and QFT populations while the AlF allele was frequent in the sample of DMN sheep.

Estimates of expected heterozygosity were almost twice those of observed heterozygosity in all sheep populations (Table 3). The expected heterozygosity level was high, low and intermediate in the DMN, BAR and QF populations, respectively.

Genetic distances (Nei, 1972) among breeds showed that, as expected, sampled QFG and QFT sheep had close genetic ties. On the other hand, genetic distances observed between BAR and DMN, and QF and DMN populations were important. A dendrogram illustrating genetic distances estimated by the UPGMA method is presented in Figure 1. The dendrogram separated the breeds into two main branches, the BAR and QF native breeds and the exotic Moroccan DMN breed. Native breeds were further branched into two distinct entities, the BAR and QF (QFG and QFT) branches.

Table 1: Electrophoresis assay for the six protein loci from the literature

Locus	Gel buffer	Electrode buffer	References
Hb	Tris-Borate- EDTA pH=8.6	Tris-Borate- EDTA pH=8.7	(Tucker and Clark, 1980)
Tf	Tris citrate–borate–lithium hydroxide pH=8.67	Borate-lithium hydroxide pH=8.73	(Gahne, 1966)
Al	Tris-citrate pH=6.2	Borate-sodium hydroxide pH=8.65	(Tucker and Clark, 1980)
Ca	Tris-citrate pH=7.3	Borate-sodium hydroxide pH= 8.6	(Penedo et al., 1982)
X-p	Tris-citrate pH= 7.4	Borate-sodium hydroxide pH=8.7	(Tucker et al., 1967)

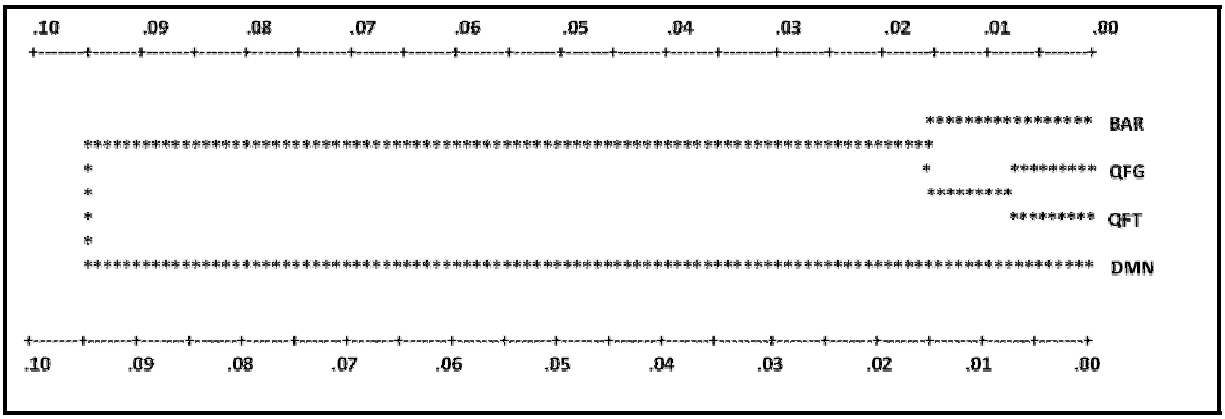

Fig 1: Cluster analysis based on the genetic distance (Nei, 1972)

Table 2: Allelic frequencies in the studied loci

		Sheep population			
Locus	Alleles	BAR	QFG	QFT	DMN
Hb	*A*	0.125	0.10	0.213	0.287
	B	0.875	0.90	0.788	0.712
Ca	*F*	0.237	0.325	0.325	0.30
	S	0.762	0.675	0.675	0.70
AL	*F*	0.436	0.363	0.387	0.712
	S	0.564	0.637	0.613	0.287
Tf	*A*	0.293	0.038	0.050	0.150
	B	0.134	0.488	0.463	0.025
	C	0.220	0.188	0.200	0.025
	D	0.305	0.287	0.287	0.550
	E	0.049	0.000	0.000	0.250
EsA	*+ve*	0.125	0.175	0.225	0.300
	-ve	0.875	0.825	0.775	0.700
X-p	*+ve*	0.075	0.225	0.125	0.375
	-ve	0.925	0.775	0.875	0.625

BAR: Barbarine, QFG: Queue Fine de l'Ouest in Gafsa, QFT: Queue Fine de l'Ouest in Tozeur; and DMN: D'ma

Discussion

The six loci used in this study were polymorphic. The predominance of HbB, CaS, Xp-, and EsA- in Tunisian breeds agrees with results reported in other sheep populations (Sun et al., 2007; Boujenane et al., 2008). However, (Tsunoda and Sato, 2001) found that the Xp+ allele was predominant in the Indian sheep population. Only two variants for Al were found in this study in agreement with results advanced by (Mwacharo et al., 2002) in Merino and by (Gauly and Erhardt, 2002) in Rhon sheep populations. Similar results were reported for the Tf loci in Spanish merino (Nguyen et al., 1992) and Tong (Sun et al., 2007) sheep populations. The predominance of TfD in BAR and DMN is in agreement with reports on the Makueni, Siaya, Kakamega, Kajiado Kenyan (Mwacharo et al., 2002) and the Rambouillet-Spanich merino (Nguyen et al., 1992) sheep populations. The TfB allele was more frequent than the TfD allele in the QF breed in agreement with results found in Uda and Rhon sheep populations (Ibeagha-Awemu and Erhardt, 2004). However, no TfE allele was found in the QF breed.

Levels of observed heterozygosities mirrored BAR, QF and DMN population structure and sizes. The DMN population is distributed in small size nuclei in different locations and defect of heterozygotes may therefore be probably due to the Wahlund effect. Heterozygosity estimates were in the range of those reported for other sheep breeds using blood protein markers (Mwacharo et al., 2002; Boujenane et al., 2008). (Takezaki and Nei, 1996) recommended an average heterozygosity between 0.3 and 0.8 in the population, the case in this study (Table 3), for markers to be useful for measuring genetic variation. Levels of genetic variation in this study were similar to those reported in other sheep breeds using blood protein polymorphisms (Mwacharo et al., 2002; Boujenane et al., 2008). Nevertheless, heterozygosity levels in this study are higher than those advanced by (Mwacharo et al., 2002) for African sheep breeds but they are lower than those reported for Świniarka sheep populations (Rychlik et al., 2009).

The Nei genetic distance (Figure 1) revealed a clear separation between the Tunisian and exotic breeds. Gene exchange between Tunisian breeds may have occurred from uncontrolled or in some occasions deliberate mating between BAR and QF animals. On the other hand, the DMN population has been managed in closed nuclei of small sizes since its introduction from Morocco in the oases. Close genetic relationships were also reported among Spanish dairy (Ordas and San Pimitivo, 1986) and among African (Ibeagha-Awemu and Erhardt, 2004) sheep breeds.

Table 3: Parameters of genetic variability in sheep populations in the South of Tunisia

Population	Average number of alleles per locus	Percentage of polymorphic loci	Observed heterozygosity	Expected heterozygosity
BAR	2.5±0.5	100	0.220±0.078	0.368±0.094
QFG	2.3±0.3	100	0.225±0.081	0.399±0.066
QFT	2.3±0.3	100	0.262±0.088	0.418±0.062
DMN	2.5±0.3	100	0.142±0.077	0.462±0.033
Across Populations	2.4±0.35		0.212±0.081	0.412±0.063

BAR: Barbarine, QFG: Queue Fine de l'Ouest in Gafsa, QFT: Queue Fine de l'Ouest in Tozeur; and DMN: D'man

Conclusion

The results presented in this paper are the first to report on the genetic structure of sheep populations at the biochemical level in the south of Tunisia. The degree of polymorphism revealed by the six blood protein loci provides important information on the genetic structure. Native breeds have close genetic relationships and are genetically distant from the exotic DMN breed. Observed heterozygosities in all breeds were low which may warn on the efficiency of actual breeding practices. Results from this study are preliminary because of small sample size and limited protein markers used in the analysis. Further investigation shall be planned to include additional loci in order to help implement conservation and genetic improvement programmes for sheep in Tunisia.

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