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Genetic Characterization of Hamra Goat Population in Two Different Locations of Morocco Using Microsatellite Markers

Research Article

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ABSTRACT

In this study, genetic diversity of two different populations of Hamra goat breed of Morocco was investigated in 60 different samples (including 30 from Beni Arouss and 30 from Rommani) using fifteen microsatellite markers. A total of 145 alleles were detected with average number per locus of 8.67 and 8.07 in Beni Arouss and Rommani goats, respectively. The Shannon's information index ranged from 1.58 in Rommani goats to 1.66 in Beni Arouss goats. The expected and the observed heterozygosity average over loci varied from 0.62 to 0.72 in Rommani and from 0.64 to 0.75 in Beni Arouss goats. Six markers in Beni Arouss goats and five in Rommani goats showed a significant deviation from Hardy-Weinberg equilibrium. The F_{IS} values were 0.110 and 0.108 for Beni Arouss and Rommani goats, respectively. A low genetic differentiation was indicated by F_{ST} values across the two goat groups. The genetic distance of Nei between the two groups was 0.046 indicating a low genetic differentiation. This was confirmed by the analysis of molecular variance (AMOVA) that showed that 99.15% of variation was distributed within genetic groups. The presence of two clusters (K=2) for microsatellite markers suggested a high level of population admixture. It was concluded that both groups (Beni Arouss and Romani) presented a high similarity and may be considered as belonging to the same population.

KEY WORDS genetic diversity, Hamra goat, microsatellite marker, Morocco.

INTRODUCTION

Goats have been domesticated 10000 years ago in the Middle East (Luikart *et al.* 2001; Fernandez *et al.* 2006). The breeds have been developed based on traditional knowledge and improved through human interventions and natural selection (FAO, 2009). However, there is worldwide recognition of the need for the conservation of livestock diversity and for the characterization of breeds and populations including their genetic differentiation and relationships. The goat diversity analysis based on microsatellite markers have been successfully used because of their high mutation rate, abundance and distribution throughout the genome, neutrality, co-dominance nature and easy automation of analytical procedures. Several studies (Bruno-de-Sousa *et al.* 2011; Dixit *et al.* 2012; Bosman *et al.* 2015; Nafti *et al.* 2016) have been conducted to investigate the genetic diversity of goats in the world. Dixit *et al.* (2012) compared the genetic diversity of 20 Indian goat breeds and Bosman *et al.* (2015) investigated the genetic diversity of the south African commercial dairy goat population. In addition, Bruno-de-Sousa *et al.* (2011) and Nafti *et al.* (2016) used the microsatellites to identify the population structure of Portuguese goat breeds and southern Tunisia goat populations, respectively.

In Morocco, 95% of goat inventory, estimated to 6.2 million goats (FAOSTAT, 2015), is represented by indigenous populations. Nowadays, four local breeds are officially characterized and recognized by the Ministry of Agriculture: Draa goat in oases and Barcha, Laghzalia and Atlas goats in Atlas Mountains. Among the remaining indigenous populations, the Hamra goat is in the process of recognition.

This population is located in two regions that are far from each other (about 400 km); Beni Arouss (BA) located in North and Rommani (RO) found in the centre of Morocco. However, animals of these two regions have a remarkable resemblance with regard to their external appearance (color and morphology), but the Ministry of Agriculture does not know if these two groups are belonging to the same Hamra population or not.

This paper reports on the first molecular investigation of Hamra goat population and the comparison of its level of diversity in two groups using fifteen microsatellite DNA markers in order to determine the level of similarity between the Beni Arouss and Rommani goat population.

MATERIALS AND METHODS

Description of Hamra population

The Hamra goat population is characterized by its red coat color. Both males and females of this population are horned. Usually, the females are not bearded, while the males have a beard. The height at withers averaged 63 cm and 65 cm for females and males, respectively (Hilal *et al.* 2014).

Sample collection and DNA extraction

Fresh blood was collected from a total of 60 unrelated Hamra goats (30 in Beni Arouss and 30 in Rommani regions) based on the information provided by farmers. Sampling was carried out from 15 flocks (9 in Beni Arouss and 6 in Rommani regions). The blood was drawn from the jugular vein in vacationer tubes coated with EDTA as blood anti-coagulants. DNA was isolated from the whole blood using a commercial extraction and purification kit (DNeasy™ Blood Kit, Qiagen®), according to the manufacturer's protocol.

Microsatellites and PCR conditions

Fifteen microsatellite loci chosen from the recommended FAO/ISAG list (FAO, 2011) were tested. These microsatellite markers were arranged into six duplexes PCR panels (SRCRSP23/SRCRSP09, TGLA53/MAF209, SRCRSP08/SRCRSP05, OARFCB48/MAF65, ILSTS87/ILSTS11, MCM527/CRSD247) and three simplexes (MAF70, OARFCB20 and ETH10) based on their annealing temperature and their marking (Table 1).

The panel of microsatellites used in the present study has been also used earlier to study the genetic variability in other goat breeds by other researchers (Hoda *et al.* 2011; Agaoglu and Ertugrul, 2012; Bosman *et al.* 2015). PCR amplifications were performed in a final volume of 28 µL on an Applied Biosystem 2700 Thermocycler containing 2 µL of genomic DNA, 1 µL of each primer and 22 µL of SuperMix PCR kit containing 22 m*M* Tris-HCl, 55 m*M* KCl, 1.65 m*M* $MgCl_2$, 22 U/mL Taq polymerase and 220 µM for each dNTP. The PCR protocol used consisted of an initial denaturation step at 95 ˚C for 5 min, followed by 30 cycles of 15 seconds at 94 ˚C, 45 seconds at 55-65 ˚C depending on the primers used and 90 seconds at 72 ˚C and a final extension step at 72 ˚C for 30 minutes. The amplified PCR products were resolved on a 3500 DNA fragment analyzer (Applied Biosystem) and fluorescently-labelled fragments were detected and sized using GeneMapper 5.0 software (Applied Biosystems, Foster City, CA).

Statistical analyses

The genotypic data were statistically analyzed using different softwares. The POPGENE software (version 1.32) (Yeh *et al.* 1999) was used to calculate the number of alleles, the effective number of alleles, the Shannon information index, the observed and the expected heterozygosity at each locus and genetic group. Wright's F-statistics (F_{IS}, F_{IT} and F_{ST}) and the gene flow between the two groups were also calculated according to Weir and Cockerham (1984) using POPGENE software. Nei's standard genetic distance (Nei, 1978) among the two groups were estimated by using GENETIX software (version 4.03) (Belkhir *et al.* 2001). Deviations of genotype frequencies from Hardy-Weinberg expectations were assessed by exact tests based on Markov chains (20 batches, 5000 iterations and a dememorization number of 10 000) as implemented by the GENEPOP software (version 4.2) (Raymond and Rousset, 1995). Analysis of molecular variance (AMOVA) was done with the ARLEQUIN (version 3.0) (Schneider *et al.* 1997) in order to explain the partitioning of the level of genetic variation of the genetic groups. Finally, the genetic structure was performed by full Bayesian approach using the software package STRUCTURE version 2.3.4 (k=7) (Hubisz *et al.* 2009). The probability (or likelihood) of different values was tested by assessing LnPr (X|K), i.e., the likelihood of the observed distribution of genotypes given the assumed number of ancestral populations. All runs used a burn-in period of 100000 iterations and a data collection period of 100000 iterations under an admixture model with allele frequencies correlated.

Table 1 Description of microsatellite DNA markers used in the current study

Microsatellite	Chromosome	Primer sequence (forward and reverse)	Annealing temperature (˚C)	Dye	Size range
Duplex 1					
MAF209	CHI17	gatcacaaaaagttggatacaaccgtg tcatgcacttaagtatgtaggatgctg	55	NED	100-104
TGL53	BTA16	gctttcagaaatagtttgcattca atcttcacatgatattacagcaga	55	VIC	126-160
Duplex 2					
SRCRSP05	CHI21	ggactctaccaactgagctacaag tgaaatgaagctaaagcaatgc	55	NED	156-178
SRCRSP08	Not reported	tgcggtctggttctgatttcac gtttcttcctgcatgagaaagtcgatgcttag	55	6-FAM	215-255
Duplex 3					
SRCRSP9	CHI12	agaggatctggaaatggaatc gcactcttttcagccctaatg	58	6-FAM	99-135
SRCRSP23	Not reported	tgaacgggtaaagatgtg tgtttttaatggctgagtag	58	VIC	81-119
Duplex 4					
ILSTS087	BTA6	agcagacatgatgactcagc ctgcctcttttcttgagag	58	NED	135-155
ILSTS11	BTA14	gcttgctacatggaaagtgc ctaaaatgcagagccctacc	58	6-FAM	250-300
Duplex 5					
CSRD247	OAR14	ggacttgccagaactctgcaat cactgtggtttgtattagtcagg	58	VIC	220-247
MCM527	OAR5	gtccattgcctcaaatcaattc aaaccacttgactactccccaa	58	NED	165-187
Duplex 6					
MAF65	OAR15	aaaggccagagtatgcaattaggag ccactcctcctgagaatataacatg	58	VIC	116-158
OARFCB48	OAR17	gagttagtacaaggatgacaagaggcac gactctagaggatcgcaaagaaccag	58	PET	149-173
Simplex 1					
OARFCB20	OAR2	ggaaaaccccatatatacctatac aaatgtgtttaagattccatacatgtg	58	NED	93-112
Simplex 2					
ETH10	CH15	gttcaggactggccctgctaaca cctccagcccactttctcttctc	55	PET	200-210
Simplex 3					
MAF70	BTA4	cacggagtcacaaagagtcagacc gcaggactctacggggcctttgc	65	6-FAM	134-168

BTA: *Bos taurus*; OAR: *Ovis aries* and CHI: *Capra hircus*.

RESULTS AND DISCUSSION

Genetic variation among groups

The various measures of genetic variation evaluated by the number of observed (NO) and effective alleles (NE), Shannon information index (I), and the observed (HO) and expected heterozygosity (HE) for Beni Arouss and Rommani genetic groups across all loci are presented in Table 2. The microsatellites studied in Beni Arouss and Rommani genetic groups were successfully amplified and were found to be polymorphic. Total numbers of alleles observed per group were 127 and 118 alleles in Beni Arouss and Rommani groups, respectively. The observed number of alleles per locus ranged from 5 (MAF209, TGL53 and MCM527) to 13 (MAF65) for Beni Arouss group and from 5 (MAF209, MCM527 and ETH10) to 12 (SRCRSP23) for Rommani group. The mean number of alleles in Beni Arouss group seems to be higher than the Rommani group (8.67 *vs*. 8.07 alleles). Similar results had been observed in Noire-Rahalli (8.33 alleles) and Draa Moroccan goat populations (7.83 alleles) (Ouafi-Tadlaoui *et al.* 2002). The mean number of alleles of Beni Arouss and Rommani groups were higher than the three populations in South Africa: Saanen (6.80), Toggenburg (6.44) and British Alpine (6.84) (Bosman *et al.* 2015). Moreover, the allelic variation between the two groups was almost the same in different locus. The value of Shannon information index, which measures the polymorphism across loci, was highly informative with an overall mean of 1.58 in Rommani group and 1.66 in Beni Arouss group.

Table 2 Various measures of genetic variation at different loci of Beni Arouss and Rommani genetic groups of Hamra goat

Locus	Beni Arouss group					Rommani group				
	NO	NE	I	H_o	H_e	NO	NE	I	H_o	H_e
MAF209	5	1.87	0.82	0.30	0.47	5	1.99	0.83	0.47	0.51
TGL53	5	1.77	0.86	0.33	0.44	9	2.66	1.42	0.53	0.63
SRCRSP09	12	5.11	1.98	0.77	0.82	9	5.32	1.91	0.83	0.83
SRCRSP23	11	6.82	2.16	0.89	0.87	12	5.77	2.06	0.77	0.84
ILSTS087	8	2.99	1.48	0,47	0,68	7	1.80	0.99	0,43	0,45
ILSTS11	11	5.86	1.89	0.87	0,84	9	4.09	1.68	0.57	0,77
CSRD247	10	5.36	1.96	0.81	0,83	9	5.79	1.95	0.92	0,86
MCM527	5	3.09	1.23	0.29	0,69	5	1.42	0.65	0.07	0,20
ETH10	6	3.22	1.31	0.83	0.70	5	2.69	1.08	0.50	0.64
MAF65	13	6.36	2.12	0.73	0.86	11	7.83	2.23	0.80	0.89
OARFCB48	9	6.29	1.98	0.80	0.85	9	7.47	2.11	0.69	0.87
SRCRSP05	9	5.26	1.84	0.76	0.82	8	5.06	1.80	0.83	0.81
SRCRSP08	7	3.80	1.56	0.63	0.75	8	4.04	1.61	0.67	0.76
MAF70	11	5.03	1.93	0.50	0.81	9	5.01	1.81	0.43	0.81
OARFCB20	8	4.24	1.73	0.83	0.78	6	4.00	1.61	0.72	0.75
Mean±SD	**8.67±2.66**	**4.47±1.62**	**1.66±0.44**	**0.64±0.22**	**0.75±0.13**	**8.07±2.12**	**4.33±1.97**	**1.58±0.49**	**0.61±0.22**	**0.72±0.19**

NO: observed number of alleles; NE: effective number of alleles; I: Shannon's information index; H_o: observed heterozygosity and H_e: expected heterozygosity.
SD: standard deviation.

The present Shannon's index values were comparable to those of the Nigerian West African dwarf goats (Awobajo *et al.* 2015), but slightly higher than those reported for Zalawadi (1.25), Gohilwadi (1.44) and Surti (1.33) Indian goat breeds (Shadma *et al.* 2008). Despite the small sample size of Beni Arouss and Rommani goat groups, they exhibited high levels of polymorphism at all studied loci. The genetic diversity in the two genetic groups was very similar and high varying from 72% in Rommani to 75% in Beni Arouss groups (Table 2). The high values of heterozygosity detected for Beni Arouss and Rommani genetic groups may be attributed to the large number of alleles detected in the two groups. These values are compared to those reported for six Portuguese native goat breeds (65%-71%) (Bruno-de-Sousa *et al.* 2011), lower than those of Nafti *et al.* (2016) on four indigenous goat populations of Tunisia, but higher than those reported for Saanen (65%), Toggenburg (62%) and British Alpine (64%) (Bosman *et al.* 2015). Based on the expected heterozygosity across the two groups, there was no appreciable difference in the level of genetic diversity. Moreover, six markers (ILSTS087, MCM527, CSRD247, MAF65, SRCRSP05 and MAF70) and five markers (TGL53, MCM527, MAF65, OARFCB48, and MAF70) showed a significant deviation from Hardy-Weinberg equilibrium in Beni Arous and Rommani groups, respectively. This indicates that differences between observed and expected number of genotypes were significant ($P<0.05$) for Beni Arouss and Rommani groups.

Fixation indices

Table 3 shows Wright's F-statistics for each locus in the two genetic groups. The within-group heterozygosity deficit (F_{IS}) was almost similar in both groups (11% and 10.8%).

Thus, large differences among loci were observed in estim-ated F_{IS} of the two groups, with values ranging from -0.221 (ETH10) to 0.551 (MCM527) and from -0.022 (OARFCB20) to 0.800 (MCM527) in Beni Arouss and Rommani groups, respectively. However, most of loci of the two groups showed a heterozygote deficit as depicted by the positive F_{IS} value. In the Beni Arouss and Rommani groups, 3 and 5 markers, respectively out of the 15 markers had negative F_{IS} values. This deficiency may be related to management conditions of Hamra goats in the two regions. The positive F_{IS} value indicated an increased homozygosity or heterozygosity deficit (Mishra *et al.* 2013). This excess of homozygotes in the two genetic groups was moderate in comparison to that found by Dixit *et al.* (2009) for Kutchi (26%), Mehsana (14%) and Sirohi (36%) Indian goat breeds, and by Gour *et al.* (2006) for Jamunapari goat breeds (19%), but higher than those observed by Mahrous *et al.* (2013) for three indigenous goat breeds (Barki and Zaraibi in Egypt and Ardi in Saudi Arabia) (5.3%). The heterozygote deficiency found in the Beni Arouss and Rommani goats groups could be due to the higher rate of inbreeding, to the population subdivision (Wahlund effect), and to the presence of "null alleles" (non-amplifying alleles). The overall genetic differentiation indicated by F_{ST} values was similar and very low in the two genetic groups. The F_{ST} value over all loci averaged 0.021 in Rommani group and 0.026 in Beni Arouss group, while the global deficit of heterozygotes (F_{IT}) varied from 0.128 in the Rommani group to 0.133 in the Beni Arouss group (Table 3). The F_{ST} value for the two groups was low compared to two Mexican cattle meta-populations (Iberian-like and Mexican Criollo) (Ulloa-Arviz *et al.* 2008) and lower than those found in three south African dairy goat populations (Saanen, British Alpine and Toggenburg) (Bosman *et al.* 2015).

Table 3 Wright's F-statistics for Beni Arouss and Rommani genetic groups for each of the 15 microsatellite markers

Locus	Beni Arouss group			Rommani group		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
MAF209	0.352	0.355	0.004	0.002	0.037	0.035
TGLA53	0.219	0.236	0.022	0.103	0.110	0.008
SRCRSP09	0.040	0.047	0.007	-0.002	0.003	0.005
SRCRSP23	0.001	0.023	0.022	0.069	0.084	0.016
ILSTS87	0.293	0.299	0.009	-0.018	-0.001	0.017
ILSTS11	-0.076	-0.045	0.029	0.282	0.291	0.013
CSRD247	0.004	0.057	0.053	-0.001	0.013	0.014
MCM527	0.551	0.606	0.121	0.800	0.805	0.026
ETH10	-0.221	-0.208	0.011	0.080	0.165	0.092
MAF65	0.157	0.169	0.015	0.017	0.033	0.016
OARFCB48	0.027	0.049	0.022	0.131	0.146	0.017
SRCRSP05	0.168	0.177	0.011	-0.017	0.025	0.041
SRCRSP08	0.131	0.140	0.011	0.079	0.092	0.014
MAF70	0.358	0.376	0.028	0.462	0.469	0.014
OARFCB20	-0.109	-0.090	0.017	-0.022	-0.002	0.020
Mean±SD	**0.110±0.205**	**0.133±0.207**	**0.026± 0.029**	**0.108±0.227**	**0.128±0.222**	**0.022±0.021**

SD: standard deviation.
F_{IS}: the within-group heterozygosity deficit; F_{IT}: the global deficit of heterozygotes and F_{ST}: the overall genetic differentiation.

The lack of differentiation observed between the two groups of Hamra goat population may be explained by the similarities in environment and breeding practices, as well as the maintained gene flow among the Hamra population although located in two different locations. The value of gene flow between the two genetic groups was 13.4 indicating a high rate of genetic flow between them. This may result from the higher mobility of goats from one region to the other and to the considerable exchange of genetic material among these goats. The low level of differentiation among the Hamra goat population of the two genetic groups was also confirmed by the standard genetic distance of Nei (0.046) showing a close relationship between them. Moreover, this reduction in genetic differentiation may result from the migration that had a greater effect than mutation or drift (Laval *et al.* 2000). Toro and Maki-Tanila (2007) suggested that the high genetic diversity observed within population groups could arise from overlapping generations and population mixtures from different geographical locations.

The analysis of molecular variation (AMOVA) within and among the two genetic goat groups of Hamra is shown in Table 4. The AMOVA showed that 99.15% and 0.85% of variation was distributed within and among the two genetic groups, respectively, suggesting that the two groups were submitted to the same geographical and environmental selections in recent history.

These results are lower than 4.8% among west African dwarf (WAD) goat populations (Awobajo *et al.* 2015) and than 7.5% among Spanish Guadarrama goat breed (Serrano *et al.* 2009). However, this analysis showed a high level of gene flow among the two groups, which is the most probable cause of this variation.

Table 4 Distribution of genetic variance in studied groups revealed by AMOVA

Source of variation	Degree of freedom	Sum of squares	Components of variation	Percentage
Among genetic groups	1	7.225	0.04089	0.85
Within genetic groups	118	563.017	4.77133	99.15
Total	119	570.242	4.81222	

To choose the appropriate number of inferred ancestral (K) populations, value of K varied from 2 to 7. However, the best value of the number of inferred ancestral populations [Ln Pr(X|K)] (-3312.7) was obtained for K= 2. A graphic representation of assumed ancestral populations is shown in Figure 1.

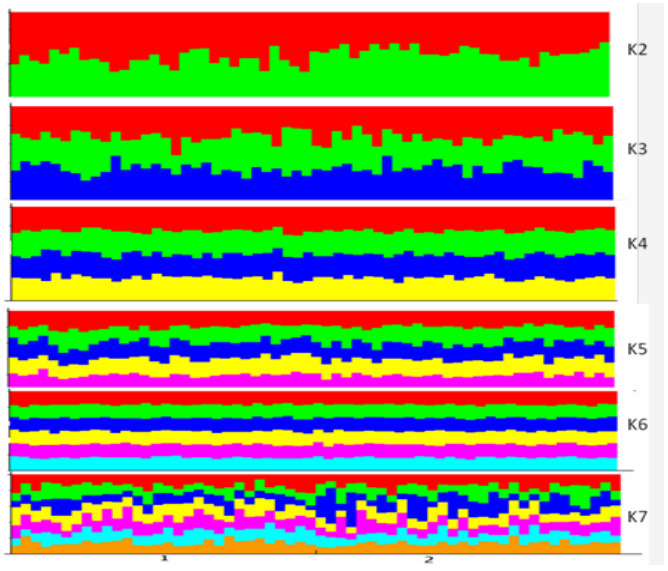

Figure 1 Clustering assignment of the two Hamra goat genetic groups provided by STRUCTURE analyses

The presence of two clusters suggested a high level of population admixture. In fact, genetic components seem to be shared by the two genetic groups which suppose a gene flow between them. The high level of admixture in the two genetic groups confirms their high similarity as indicated by genetic parameters.

CONCLUSION

All microsatellite markers used in the present study were shown to be highly polymorphic for the molecular characterization of Beni Arouss and Rommani goats. The results confirm a high similarity among Hamra goats in the two locations. The two goat groups were weakly structured, but they present an important pool of genetic diversity and high level of admixture. Therefore, the genetic information obtained in this study would help the Ministry of Agriculture in the process of breed recognition and would make a valuable contribution to the utilization of this goat population.

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