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Genetic Differentiation of Draa Indigenous Breed and Relationships with Other Goat Populations Assessed by Microsatellite DNA Markers

Research Article

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ABSTRACT

Moroccan goats are characterized by the presence of different populations identified only based on their phenotypes. The objectives of this study were to assess the genetic differentiation of the Draa goat breed and to analyze its genetic structure and its relationships with other local populations using 12 microsatellite markers. The screening was done in South Eastern and Southern Morocco on 192 animals form 5 populations, including Draa, Atlas, Barcha, Ghazzalia breeds, and from a set of goats showing highly variable phenotypes grouped together into "undefined goats" population. Population structure was assessed by standard diversity indices, multivariate statistics, analysis of molecular variance and bayesian clustering techniques. The mean allelic richness was 6.526, varying from 2.777 to 9.669. More than 88.4% of the total variance was distributed between individuals and only 1.85% was due to differences between populations. The Draa breed had the lowest observed heterozygosity (0.579), the highest inbreeding coefficient (0.161) and a higher number of deviations from Hardy-Weinberg equilibrium. Moreover, it had the highest genetic distances from the other populations. Bayesian clustering showed a high level of admixture between populations, with a single well defined cluster identifiable within Draa breed. It was concluded that the studied Moroccan goat populations have a substantial but weakly structured genetic diversity, with the exception of Draa breed which shows a higher degree of differentiation and population substructure.

KEY WORDS Draa breed, genetic diversity, goat, microsatellites, morocco.

INTRODUCTION

Characterization is the first approach to sustain use of a genetic resource (Yakubu *et al.* 2010). Ideally, it should encompass both the phenotypic descriptions based on a number of indicators and morphological measurements (FAO, 2012), and the genetic characterization through molecular markers (FAO, 2011). Microsatellites Markers have been increasingly used in genetic characterization studies of farmed animals (Sunnucks, 2001) and particularly in the assessment of genetic relationships (FAO, 2008). These DNA markers are still used in such studies (Aljumaah *et al.* 2015; Seilsuth *et al.* 2016; Lenstra *et al.* 2017). The Moroccan goat population is over 6.2 million heads and is ranked in the 13th position worldwide in terms of goat's numbers (http://faostat3.fao.org/browse/Q/QA/E). Until the beginning of the 21st century, only the Draa goat has been considered as a breed, while other goat populations have traditionally been named as the names of their geographical location, e.g. "goat of the North" or "black goat of the

mountains". In the last decade, some subpopulations of the black goat were described as separate on the basis of slightly different morphologies (Atlas, Barcha and Ghazzalia) (Ibnelbachyr *et al*. 2015). However, these phenotypic different should be verified with genetic analysis evidences. The highly distributed genetic variability in Moroccan goats may reflect the levels of the ancestral genetic diversity existing in the first individuals that colonized Morocco and/or the existence of an extensive and recurrent inter-population gene flow (Benjelloun *et al*. 2011). In fact, livestock populations which are geographically, ecologically or culturally isolated may have been affected by different natural and artificial selection (FAO, 2003). Therefore, it can be expected that there are a degree of likeness between Draa breed and other goat populations of Morocco. The aim of this study was to use microsatellite markers to characterize Draa breed and four other Moroccan goat populations.

MATERIALS AND METHODS

The screening was done in the cradle of the Draa goat (South-Eastern Morocco) and its neighbouring areas in south-eastern and southern Morocco (Figure 1). The sampling location was established based on the geo-referenced cells of 2500 km². Within each cell, three herds were randomly selected and 3 to 4 unrelated adult goats were selected per herd, until 30 to 50 samples were collected per population, as recommended by FAO for genetic diversity studies (FAO, 2011). The investigated goat populations were Draa, Atlas, Barcha, Ghazzalia and other populations that were grouped together and named as "undefined goats". Phenotypic traits were recorded, and then blood samples were taken from the jugular vein using vacutainer tubes. At the end, 150 genotypes were analyzed: 35 from Atlas, 31 from Barcha, 36 from Draa, 32 from Ghazzalia and 16 from ''Undefined goats''. These animals, 55 males and 95 females, were aged approximately from 2 to 6 years.

Microsatellite amplification and analysis

Genomic DNA was extracted using the salting out procedure (Miller *et al.* 1988). Sixteen microsatellite markers, which are recommended by FAO-ISAG panel were amplified and analyzed (Table 1). The PCR amplifications were performed in a final volume of 28 µL containing 2 µL of genomic DNA (on average 50 ng/µL), 1 µL of each primer (80 nmol/mL) and 22 µL of the following PCR Mastermix solution (SuperMix from Invitrogen, Life Technologies): 22 m*M* Tris-HCl, 55 m*M* KCl, 1.65 m*M* $MgCl_2$, 22 U/mL *Taq* polymerase and 220 µ*M* of each dNTPs. The following PCR protocol was used: an initial denaturation step (95 ˚C for 5 min) followed by 30 cycles of a denaturation step (94 ˚C for 15 s), an annealing step (annealing temperature of the primers, varied from 55 to 65 ˚C, for 45 s) and an extension step (72˚ for 90 s). The 30 cycles were followed by a final extension (72 ˚C/30 min).

The PCR products were resolved on a 3500 DNA fragment analyzer (Applied Biosystem) and the obtained data were analyzed with GeneMapper 5.0 software. Standard DNA samples provided by the ECONOGENE Project (www.econogene.eu) were also genotyped and used to adjust allele length.

Statistical analyses

The measure of genetic polymorphism such as number of alleles, the observed (Ho) and expected (He) heterozygosity, were calculated using GENETIX software version 4.03 (Belkhir *et al*. 2004). Allelic richness (Na), which is an unbiased estimator of the number of alleles, was calculated using FSTAT software version 2.9.3 (Goudet, 2001). The GENETIX software was used to estimate the power of these markers to discriminate between populations via the coefficients of genetic differentiation G_{ST} (Nei, 1978) and to carry out a factorial correspondence analysis at the population level. Population structure was analyzed through a Bayesian clustering procedure implemented in the version 2.3.4 of STRUCTURE package (Pritchard *et al*. 2000). For each value of K (K=2 to K=10), five independent runs were carried out under an admixture model with correlated allele frequencies and with no prior information on the population of origin.

A burn-in-period of 50000 was followed by 100000 Monte Carlo Markov Chains iterations. Two parameters were calculated to estimate the best fitting number of clusters, i.e. K value: i) the mean values of log likelihood, lnPr(X|K), calculated for each K over 10 runs, and ii) the posterior probabilities of K, Pr(X|K), from the mean lnPr(X|K) values according to Pritchard *et al*. (2000).

Graphical representations of structure analyses were obtained using DISTRUCT software (Rosenberg, 2004). Version 3.5 of ARLEQUIN package (Excoffier *et al*. 2005) was used to perform an Analysis of Molecular Variance (AMOVA; Excoffier *et al*. 1992) and to calculate the value of the coefficient of inbreeding (F_{IS}) and F_{ST} (Wright, 1968) distances between populations. This F_{ST} distance matrix was subsequently used to build a neighbour-network with version 4.13.1 of SPLITSTREE software (Huson and Bryant, 2006).

RESULTS AND DISCUSSION

Polymorphism and heterozygosity of the studied loci

In total, 160 alleles were found and allelic richness values varied between 2.770 and 9.669. Twelve loci were polymorphic, with mean effective number of alleles ranged from 5.853 to 9.669 and heterozygosity from 0.546 to 0.885 (Table 1).

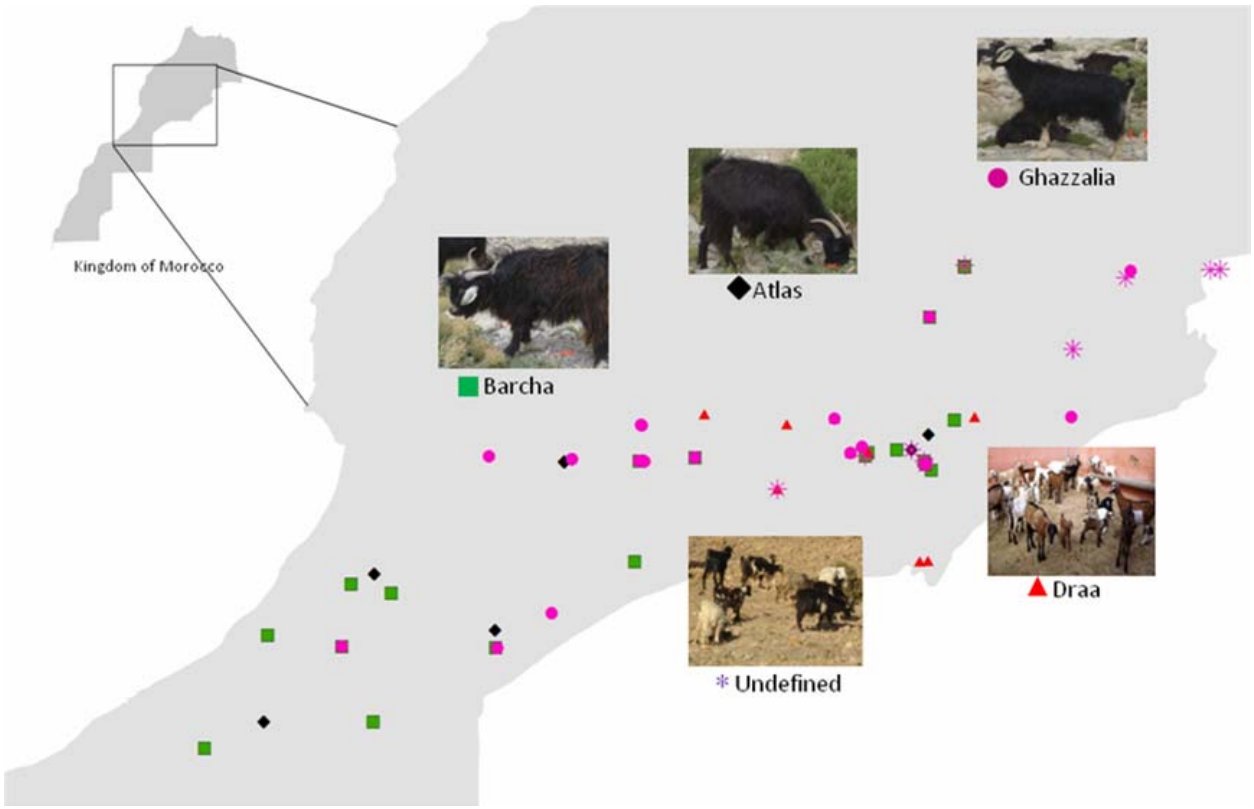

Figure 1 Goat sampling locations are shown on the map of Morocco
Different colors correspond to different breeds / populations
Some sampling points are overlapping since they correspond to individuals from the same or different populations in the same location

Table 1 Allelic richness (Ne), expected heterozygosity (He), observed heterozygosity (Ho), coefficients of differentiation (G_{ST} and F_{ST}) of microsatellite DNA markers used

Microsatellite	Size range	Anealing temperature (˚C)	Number of alleles	Dye	Ne	He	Ho	G_{ST}	F_{ST}
Duplex 1									
MAF209	100-104	55	04	NED	3.185	0.492	0.480	0.032	0.024
TGLA53	126-160	55	13	VIC	8.008	0.793	0.735	0.052	0.038
Duplex 2									
ETH10	200-210	55	05	PET	3.488	0.629	0.619	0.021	0.008
ILSTS005	172-218	55	04	VIC	2.770	0.235	0.200	0.126	0.120
Duplex 3									
SRCRSP05	156-178	55	10	NED	7.137	0.798	0.774	0.024	0.012
SRCRSP08	215-255	55	12	FAM	7.454	0.814	0.797	0.017	0.001
Duplex 4									
SRCRSP09	099-135	58	15	FAM	9.259	0.813	0.798	0.018	0.001
SRCRSP23	081-119	58	14	VIC	9.022	0.852	0.809	0.045	0.039
Duplex 5									
ILSTS087	135-155	58	12	NED	5.853	0.546	0.522	0.034	0.021
ILSTS11	250-300	58	10	FAM	6.557	0.698	0.663	0.029	0.017
Duplex 6									
CSRD247	220-247	58	11	VIC	7.644	0.839	0.836	0.024	0.011
McM527	165-187	58	07	NED	3.524	0.424	0.396	0.057	0.056
Duplex 7									
MAF65	116-158	58	14	VIC	9.669	0.885	0.855	0.030	0.018
OarFCB48	149-173	58	10	PET	7.441	0.825	0.795	0.029	0.022
Simplex									
OarFCB20	093-112	58	09	NED	6.195	0.709	0.687	0.035	0.020
MAF 70	134-168	65	10	FAM	7.217	0.785	0.766	0.016	0.001
Mean			10		6.526	0.696	0.671	0.037	0.026

NED: fluorescent dye of ABI yellow color; VIC: fluorescent dye of ABI green color; PET: fluorescent dye of ABI red color and FAM: fluorescent dye of ABI blue color.

These loci had G_{ST} and F_{ST} values ranged from 0.001 to 0.126. The least polymorphic loci were MAF209 (3.185 alleles on average), MCM527 (3.524 alleles), ETH10 (3.488 alleles) and ILSTS05 (2.770 alleles), and were excluded from the subsequent analyses, as recommended by Barker (1994).

Genetic diversity between and within the populations

The average number of alleles was similar in the five populations (Table 2), while the "undefined" goats showed the lowest average followed by the Draa breed. The expected heterozygosity (He) was ranged from 0.635 to 0.705, while the observed heterozygosity (Ho) was 0.579 in Draa, 0.613 in Barcha, 0.614 in Ghazzalia, 0.635 in undefined goats and 0.644 in Atlas. Most of the total variance (88.4%) was distributed within individuals and only 1.85% of variance was due to differences between populations (Table 3). The inbreeding coefficient (F_{IS}) was ranged from 0.161 for Draa breed to 0.037 for the "undefined" group. All values were also statistically significant ($P<0.001$) with the sole exception of the latter population. For F_{ST} coefficient, it varied from 0.005 between Barcha and Ghazzalia to 0.040 between Ghazzalia and Draa.

The graphical representation of the factorial correspondence analysis results (Figure 2) showed the distribution of the individual genotypes along the axes corresponding to the three first major components of inertia (total inertia explained 85.3%). The scatter of the points corresponding to the individuals from Draa breed occupied a very large area of the multivariate space of the graph, stretching far from the remaining populations, which, in turn, largely overlapped on the central part of the plot surrounding the origin of the axes.

Genetic structure

The distinctiveness of Draa breed and the weak structure of the other populations were also highlighted by structure analyses (Figure 3). At K= 4 – identified as the best fitting resolution according to Pritchard *et al*. (2000); (Table 4) – a clear split appeared within the group of individuals assigned to the blue cluster at K= 2 and separating half of Draa population from the rest. Also, the appearance of the purple cluster did not create any clear split, but rather highlighted some distinctiveness of Ghazzalia breed in which the red component remained predominant (Figure 3). Conversely, both Barcha breed and the undefined group showed the lowest level of population structure what was confirmed by the average proportions of membership. Within these two populations, the genomic clusters shared by all five populations (red, purple and green) occurred almost at the same percentage, while Draa, Atlas and Ghazzalia were characterized by the prevalence of the blue, green and red components, respectively.

The average proportions of membership at each sampling location showed a geographical pattern underlying the distribution of the clusters. In particular, the blue cluster is localized mainly in a specific area in the central part of the country that corresponds to almost the whole distribution area of Draa breed.

Genetic distances and phylogenetic relationships

Nei's genetic distances between the Draa goat and the other local goats (Table 5) showed that the Draa breed had the highest distance from the other populations. Even if all these genetic distance values of Draa are almost the same, the Neighbour-network graph (Figure 4) confirmed the evidence already revealed by Structure software results: Atlas and Draa branches stem from the same basal edge, thus suggesting an ancestral genetic relationship between these breeds. In addition, the genetic distinctiveness and relative isolation of Draa breed were highlighted by the major length of its branch. Among the studied populations, Barcha breed seems to be the less differentiated, being positioned in the middle of the graph with a branch length close to zero value.

The distribution of the major variance component (about 98.2%) among and within individuals is in agreement with the findings of Benjelloun *et al*. (2011). Low levels of differentiation within sheep and goat populations have already been highlighted by several studies (Cañón *et al*. 2006; Peter *et al*. 2007). The highly distributed genetic variability in Moroccan goats may reflect the levels of the ancestral genetic diversity existing in the first individuals that colonized Morocco and/or the existence of an extensive and recurrent inter-population gene flow (Benjelloun *et al*. 2011). The observed heterozygosity, which varied from 0.579 in Draa to 0.644 in Atlas goats, are close to those found in the Canary breeds (Martínez *et al*. 2006), in some local Egyptian goats (Agha *et al*. 2008) and others African ones (Missohou *et al*. 2011), but less than Gaddi goat (Singh *et al*. 2015). In all studied breeds, the observed heterozygosity (He) was lower than the expected heterozygosity (Ho) and the Draa breed showed the most severe departure from Hardy-Weinberg equilibrium due to a deficit of heterozygoty as testified by the F_{IS} value of 0.161. This value is close to that reported in Baladi breed (Agha *et al*. 2008), in some Chinese populations (Li and Valentini, 2004) and in some breeds of India (Dixit *et al*. 2012), but less than Berari breed (Kharkar *et al*. 2015). Likewise, Bruno-de-Sousa *et al*. (2011) found a high value of inbreeding coefficient (0.124) in Preta breed in Portugal associated with reduced population size.

Table 2 Number of effective alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding coefficient (F_{IS}) with significance level

Populations	Na	He	Ho	F_{IS}	P (0.99)
Atlas	7.875	0.705	0.644	0.101	***
Barcha	7.250	0.673	0.613	0.104	***
Draa	7.125	0.694	0.579	0.161	***
Ghazzalia	7.250	0.648	0.614	0.068	***
Undefined	6.563	0.635	0.633	0.037	NS

NS: non significant.

Table 3 Analysis of molecular variance (AMOVA)

Source of variation	d.f.	Sum of squares	Components of variance	Percentage	P-value
Among populations	4	46.584	0.10252	1.85	< 0.001
Among individuals within populations	135	806.734	0.53969	9.74	< 0.001
Within individuals	140	685.500	4.89643	88.40	< 0.001
Total	279	1538.818	5.53864	-	-

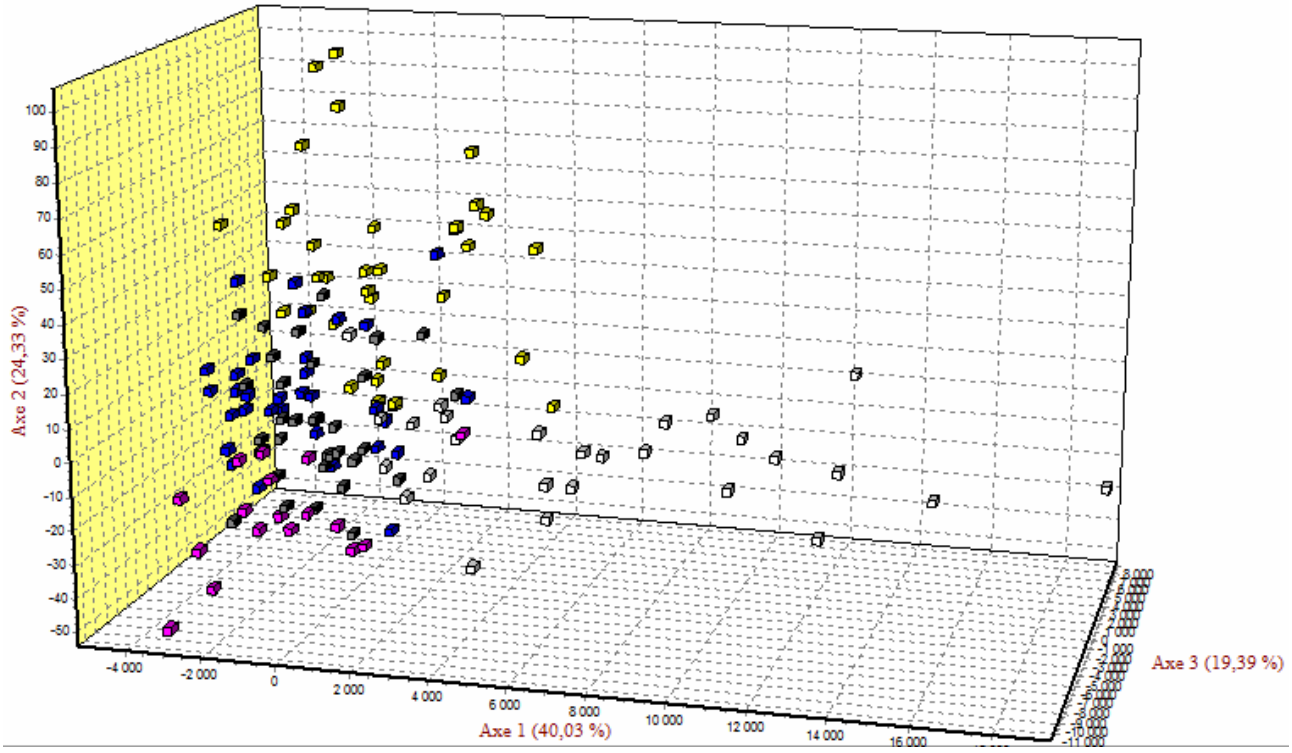

Figure 2 Factorial correspondence analysis showing multivariate relationships among individual genotypes of Moroccan goats (Atlas in yellow, Barcha in red, Draa in white, Ghazzalia in bleu and undefined goats in rose color)

A deviation from equilibrium indicates a departure from random mating and suggests that the higher than expected number of homozygotes probably due to inbreeding. The reduced numbers of Draa goats and the selection for prolificacy and milk under which the breed has been submitted for a longtime might be the cause of its deviation from equilibrium. Thus, the possibility that Draa subpopulations may have suffered from genetic bottlenecks, at least in part of the breed's distribution area can't be ignored. Indeed, this event has been reported in several studies in sheep (Fatima *et al*. 2008) and goats (Korkmaz Ağaoğlu and Ertuğrul, 2012). In the case of Draa breed, its closed geographical area, its reduced population size, the small size of herds and the selection performed by breeders are factors that may have caused its high level of inbreeding.

Moreover, as revealed by Structure results plot, a strong population substructuring is present within this breed, with almost half of its individuals forming a clearly defined cluster, which may represent a separate gene pool existing within this breed and may have caused a Wahlund effect that means a reduction of heterozygosity in our population due to subpopulation structure.

Table 4 Mean values of ln Pr(X|K) and posterior probabilities of K (Pr(X|K)) calculated according to Pritchard *et al*. (2000)

K	ln Pr(X|K)	Pr(X|K)
1	-7178.44	$3.39 \cdot 10^{-156}$
2	-6988.82	$7.62 \cdot 10^{-74}$
3	-6940.66	$6.27 \cdot 10^{-53}$
4	-6820.46	1
5	-6868.80	$1.01 \cdot 10^{-21}$
6	-7018.52	$9.63 \cdot 10^{-87}$
7	-7044.14	$7.19 \cdot 10^{-98}$
8	-7203.54	$4.27 \cdot 10^{-167}$
9	-7244.08	$1.05 \cdot 10^{-184}$
10	-7284.18	$4.06 \cdot 10^{-202}$

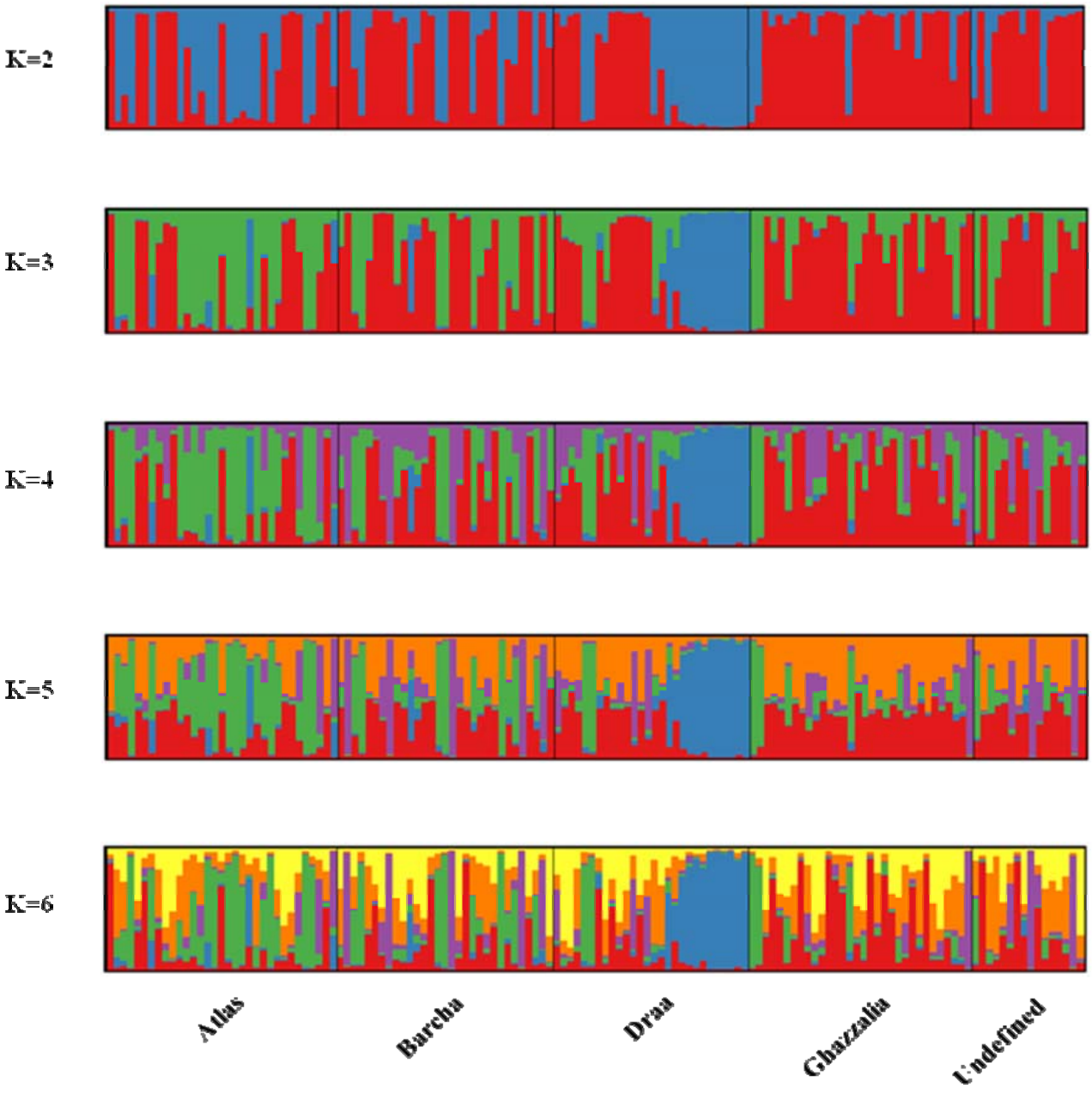

Figure 3 Bayesian clustering performed with STRUCTURE software on Moroccan goat microsatellite data

Table 5 Genetic distances matrix between identified populations (Nei, 1978)

Populations	Atlas	Barcha	Draa	Ghazzalia	Undefined
Atlas	0.000	-	-	-	-
Barcha	0.017	0.000	-	-	-
Draa	0.068	0.071	0.000	-	-
Ghazzalia	0.037	0.008	0.086	0.000	-
Undefined	0.056	0.016	0.083	0.018	0.000

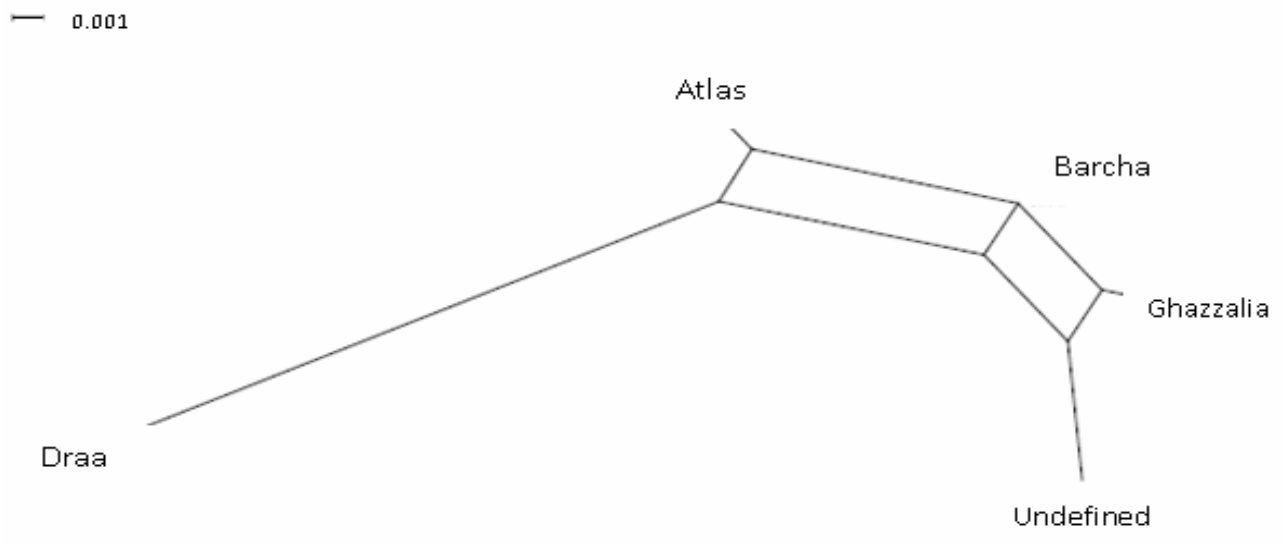

Figure 4 Neighbour-network based on F_{ST} distance matrix between the studied populations (Atlas, Barcha, Draa, Ghazzalia and Undefined goats)

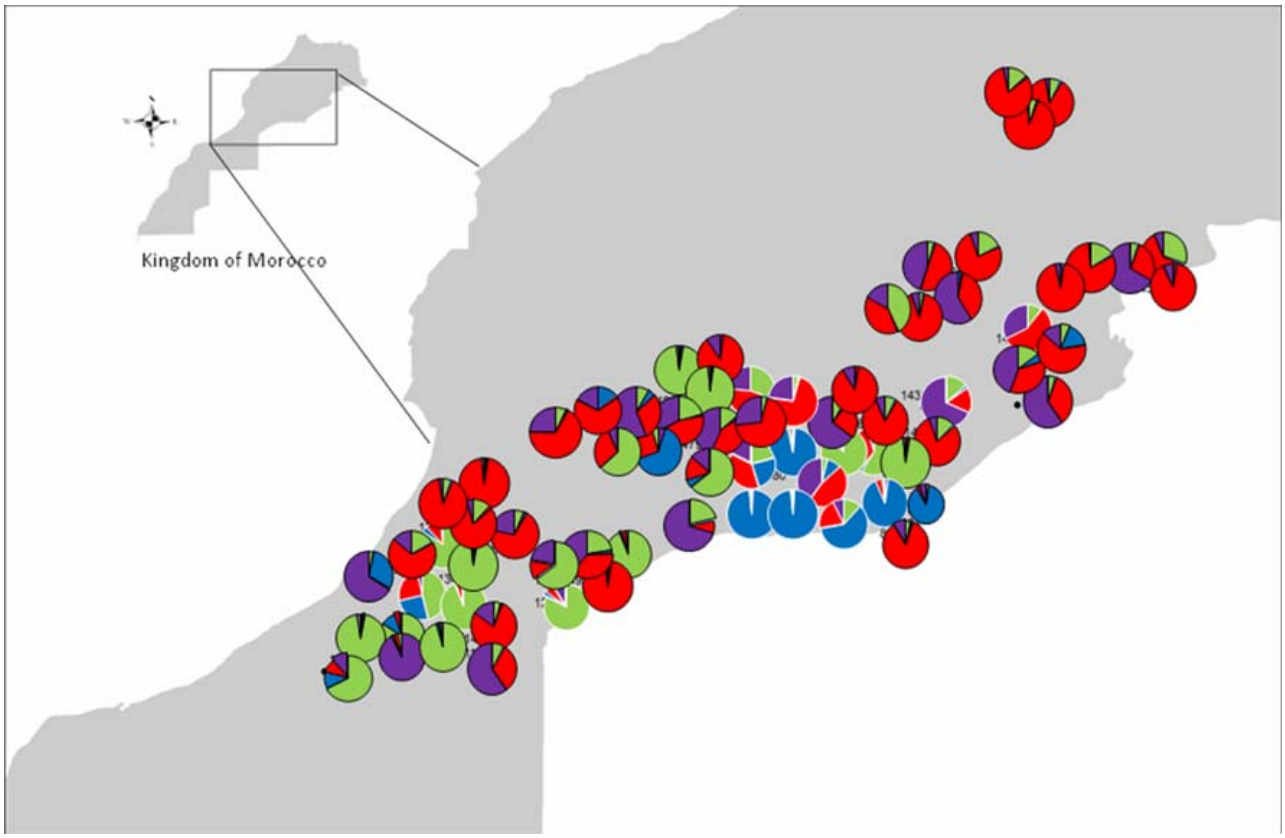

Figure 5 Proportion of membership of each sampling location on the results of Structure software run at K= 4
Each individual is represented by a pie chart that represents their genetic clusters
Pie charts with black / white outline correspond to locations where single / multiple individuals have been sampled, respectively

The genetic distances confirm the differentiation level between Draa breed and the other populations. The F_{ST} values obtained between our breeds are closed to those obtained by Al-Atiyat *et al*. (2015) between Jordan goats. Our results indicate that similarities between the Draa and other populations are low even if Draa and Atlas branches stem from the same basal edge.

An evidence which was confirmed by the findings of Tadlaoui Ouafi *et al*. (2002) who reported low values for the distance between the Draa breed and the black goat called ''R'halia'', a term identifying the black goat of the Middle Atlas in Morocco and which corresponds in our case to Atlas and Barcha populations.

Even though the genetic makeup of the Moroccan goat populations may have been influenced by gene flow and introgression coming from different areas of the Mediterranean basin during the past (Benjelloun *et al.* 2011; Pereira *et al*. 2009), nevertheless the vast majority of Moroccan goat populations likely derive from the first founders that reached Morocco in the late Neolithic. In addition, the results showed a lack of population sub-structuring for most breeds and the presence of two genomic components (i.e. red and purple in Figures 3 and 5) spread over most of the sampling area, fit well with the hypothesis of an ancestral wide genetic basis still shared today due to extensive gene flow, with the sole exception of a sub-population of Draa breed.

On the other hand, the presence of a geographical pattern in the distribution of the major genetic components could be explained either by an uneven and gradual introgression of genomic contributions from gene pools of non-native origin, or by the effect of increasing geographical distance which progressively prevents panmixia.

Indeed, a geographical gradient in genetic diversity might also reflect the adaptation of animals to the environment and their response to natural selection (Ciani *et al*. 2013).

But to investigate both such effects and the real extent of gene flow, isolation by distance and introgression, a more dense set of molecular markers (e.g. medium or high SNP panels or whole genome sequences) is needed.

CONCLUSION

The studied Moroccan goat populations were characterized by a substantial genetic diversity weakly structured between populations, with the exception of Draa breed which showed a certain degree of differentiation due either to reproductive isolation or inbreeding, possibly caused by geographical isolation, reduced population size and / or man mediated selection processes.

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