

Genetic admixture of North-African ovine breeds as revealed by microsatellite loci

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Abstract

North-African countries are endowed with a wealth of sheep resources adapted to a wide range of environments and production systems. In this study, genetic diversity was estimated in seven sheep breeds, including two Algerian (Hamra n=35 and Ouled-Djellal n=50) and two Moroccan (Béni-Ighil n=50 and D'men n=49) local breeds as well as two French (Corse n=50 and Lacaune n=50) and one sub-Saharan (Foro-Foro n=46) breed, using six microsatellite markers.

All markers were highly informative and a total of 109 alleles were detected with a proportion of private alleles of 0.023. Observed heterozygosity average over loci was 0.66 ± 0.195 . The estimated within-population inbreeding FIS was significant in all studied breeds. 6.1% of the genetic diversity in the total population could be attributed to differences among the breeds. Significant gene flow among North-African breeds was detected. Population structure analysis revealed showed that all African breeds are closely related and were distinguished in the same cluster; however, the European breeds belonged to a different cluster.

Key words: *Algeria, French, genetic diversity, microsatellites, Morocco, sheep*

Introduction

Sheep were domesticated at the beginning of the Neolithic era (7500 years before J.C.) in the Middle-East (Peters et al 1999; Vigne et al 1999), it was then spread in the whole world, adapting to a wide range of environments, from the grassland of north of Europe or New

Zealand to the hot and dry areas and even semi-desert of Africa or Australia (Tabet-Aoul 1999). Thus, there exists a great diversity of breeds or populations adapted to different contexts. Initially, morphological characters (coat colour, horns, etc.) as well as the polymorphism of biochemical markers (blood and milk proteins) have been used for genetic characterisation of breeds and populations. However, these markers reflect only some loci duly identified in the domestic animals.

The development of molecular genetics techniques allowed access to DNA polymorphisms in both coding and not coding sequences. Indeed, molecular DNA markers are commonly used for the generation of genetic maps in livestock species as well as for characterizing the genetic diversity of domestic animal populations or breeds (Ligda et al 2009; Calvo et al 2011; Niu et al 2012; Agaviezor et al 2012). In this context, microsatellite markers have been widely used owing to their high level of polymorphism and their distribution over the genome. The usefulness of microsatellites has been extensively documented for sheep characterization to elucidate genetic relationships among closely related populations (Peter et al 2007; Álvarez et al 2012; Kijas et al 2012). However, such data are still lacking for North-African breeds. Therefore, the aim of this study was to explore the genetic structure of two Algerian (Ouled-Djellal and Hamra) and two Moroccan (Béni-Ighil and D'man) sheep breeds compared to two French (Lacaune and Corse) and one sub-Saharan (Foro-Foro) sheep breed using microsatellite markers, through the analysis of the genetic diversity within and among breeds and their relationships.

The Ouled-Djellal breed is considered as the principally Algerian meat breed representing more than half of ovine livestock. This breed is distributed over the country but it is mainly found in the middle and eastern regions. The Ouled-Djellal sheep is a heavy sheep with a little strong skeleton and a short white fleece (Chellig 1992).

The Hamra breed is derived several decades ago from the Moroccan Béni-Ighil raised on the high atlas by the tribe Béni-Ighil from where it draws its name. The breed is primarily localized in the Western steppes close to the Moroccan border and showed a significant census decrease last years because of the massive cross breeding with Ouled-Djellal by the stockbreeders leading to its replacement in many areas. Hamra is small size sheep known for its high meat organoleptic qualities as sheep of Oranie, and is distinguished from the other breeds by a head and legs dark chestnut tending towards the red, the wool being white with guard hair going to brown the russet-red (Chellig 1992).

The D'men breed, localized in Moroccan south-east, is well known for its high level of prolificity. It is a breed with coarse wool covering the top of the body only, color black or brown dark. The end of the tail is white.

The Lacaune breed; accounting for about 20% of the French sheep. It's named from the mounts of Lacaune located at the south-west of France. Moreover, this breed is specialized for milk production or meat production, the conformation of this breed remains the same, only the characteristics related to production specialization differ. Its weight varies between 65 and 100 kilogrammes. The colour of the fleece is black.

The Corse breed is an insular French sheep from Corsica Island. It is a dairy breed, which is characterized by its small size (50-60 cm) and low weight (35 to 40 kg). The colour of the fleece may be black, white and grey or reddish

The Foro-Foro breed (used here as reference) is a sub-Saharan breed from Ivory Coast with no apparently any continuum with other breeds described here.

Materials and methods

Animals

A total of 330 blood samples were collected from seven sheep breeds (Figure 1), including two Algerian: Hamra (n=35) and Ouled-Djellal (n=50); samples collected and DNA extracted in Algeria; two Moroccan: Béni-Ighil (n=50), D'men (n=49) and two French breeds: Corse (n=50), Lacaune (n=50) in addition to the sub-Saharan Foro-Foro breed (n=46), for all these breeds DNA was provided by LaboGena, Jouy-en-Josas, France). Concerning Algerian samples Genomic DNA was extracted using salting out method as described by Miller et al (1989).

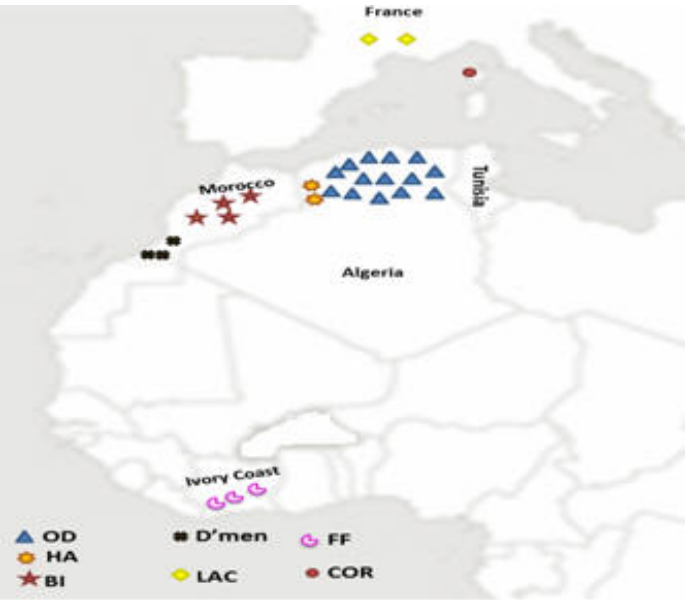


Figure 1: Geographic distribution of sampled sheep (OD: Ouled Djellel, HA: Hamra, BI: Béni-Ighil, LAC: Lacaune, COR: Corse, FF: Foro-Foro)

Microsatellites genotyping

A set of six microsatellite markers (Table 1) was chosen based on their level of polymorphism location on different chromosome, preferably unlinked following the recommendation of the Food and Agriculture Organization (FAO) and the International Society for Animal Genetics (ISAG). The microsatellites were amplified by PCR using fluorescently labelled primers. The PCR reaction included 1 X PCR Buffer, 1.5 mM MgCl₂, 250 μM dNTPs, 10 picomoles of each primer, 0.25 units of Taq polymerase and 200 ng of DNA. The PCR thermal profile started with an initial denaturation at 94°C for 5 min and 30 cycles of 30 s at 94°C for DNA denaturation, 30 s for primer annealing at 55°C and 30 s at 72°C for primer extension followed by a final extension at 72 °C for 15 min.

PCR products were analysed by denaturing polyacrylamide gel electrophoresis using an automated ABI 373 DNA sequencer (Applied Biosystems, CA, USA) and genotypes were detected using GENESCAN™ 6.7.2 version 3.0 software (Applied Biosystems, USA).

Table 1: Sequences of microsatellite marker primers, Chromosome location, annealing temperatures and detected allele size range

Markers	Ch. location	Origin	Primer sequence 5' 3	Allelic size range (bp)	N.of alleles	Citation
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OarFCB11	2	sheep	GCAAGCAGGTTCTTTACCACTAGCACC GGCCTGAACTCACAAGTTGATATATCTATCAC	121-143	12	(1)
OarCP49	17	sheep	CAGACACGGCTTAGCAACTAAACGC GTGGGGATGAATATTCCTTCATAAGG	85-107	12	(2)
OarHH56	20	sheep	GCAACCCACTCATCTCTCCGTGTC GAAAACCTTAAGTTCCAGCTATTAATAAGC	147-163	9	(3)
MAF36	22	sheep	CATATACCTGGGAGGAATGCATTACG TTGCAAAAGTTGGACACAATTGAGC	99-125	14	(4)
ILSTS05	7	cattle	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	188-214	14	(5)
CSSM66	9	cattle	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	160-214	28	(6)

(1): Buchanan and Crawford (1993)

(2): Ede et al (1995)

(3): Ede et al (1994)

(4): Swarbrick et al (1991)

(5): Kemp et al (1995)

(6): Barendse et al (1994)

Statistical analysis

Cervus v. 3.0.3. (Kalinowski et al 2007) software was used to analyse the number of alleles, expected heterozygosity corrected for sampling bias, observed heterozygosity and polymorphic information content (PIC). Genepop v.4 (Raymond and Rousset 1995) software was utilized to calculate the exact test for Hardy–Weinberg equilibrium, the linkage disequilibrium test between markers and the Nm value. We used Bottleneck 1.2.02 (Cornuet and Luikart 1996) to test significant deviation in allelic diversity and heterozygosity from mutation-drift equilibrium predictions. Hierarchical analysis of molecular variance (AMOVA) was assessed with Arlequin 3.5.1.2 (Excoffier et al 2005). FST values for pairwise comparisons between breeds and their significance level, mean number of alleles across populations, observed, average expected (non-biased) and average observed heterozygosities were calculated using Genetix 4.05.2 software (Belkhir et al 1998). Allelic richness and private allelic richness were rarefacted using HP-RARE (Kalinowski 2004; 2005).

Bayesian model-based hierarchical clustering, implemented in Structure 2.3.4 (Pritchard et al 2000) was used to infer the number of homogenous clusters, the range of possible Ks tested was from 1 to 10 (the real number of breeds plus 3). The most likely value of K was estimated using the ΔK statistic proposed by Evanno et al (2005) and implemented by Structure Harvester Web version 0.6.93 (Earl 2012). Genetic affinities among breeds were also examined using a neighbour-joining (NJ) tree based on Reynolds distances (Reynolds et al 1983) and 1000 bootstraps were computed in Populations version 1.2.32 (Langella 1999) and visualized in Treeview program version 1.6.6 (Page 1996). CLUMPP (Jakobsson and Rosenberg 2007) was used to estimate the number of identical repeated runs per K. The output files from CLUMPP were used as input in DISTRUCT for cluster visualization (Rosenberg 2004).

Results and discussion

Microsatellites analysis

A total of 109 alleles were detected in the 6 loci studied and the number of alleles per locus ranged from 10 (OarHH56) to 29 (OarCP 49) with a mean number of alleles per locus of 18.17 (Table 2). All markers were highly informative ($PIC > 0.5$) with PIC values ranging from 0.632 for OarHH56 to 0.901 for OarCP 49.

Table 2: Genetic variability measures at the 6 microsatellites loci analysed across the seven breeds number of detected alleles (k), number of animal (N), observed (Ho), expected (He) heterozygosity, polymorphism information content (PIC), Hardy–Weinberg (HW).

Microsatellites	k	N	Ho	He	PIC	HW
OarCP 49	29	311	0.756	0.909	0.901	***
OarFcB 11	13	317	0.798	0.838	0.816	NS
CSSM 66	24	307	0.713	0.891	0.879	***
MAF36	15	309	0.819	0.857	0.839	NS
OarHH56	10	285	0.418	0.688	0.632	***
ILSTS 05	18	274	0.478	0.795	0.778	***

NS: No significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant p -values means deviations from equilibrium

Mean proportion of private alleles was 0.023, being the number of immigrants (Nm) after correction for size 5.722, indicating a relatively high gene flow among breeds. Slatkin (1985) showed that a linear relationship exists between gene flow (Nm) and the average frequency of private alleles, low gene flow could lead to an increase of private alleles. Private allele (PA) frequencies ranged between 0.39 and 0.76; allelic richness (Ar) ranged from 3.62 to 4.07 (table 3). Ouled-Djellal breed showed the highest number of alleles per locus (11.83), and the highest allelic richness (4.07) compared with the other populations of animals in the present study. Mean number of alleles (MNA) values of Corse and Lacaune are higher than that found in the European breeds (Dalvit et al 2008; Calvo et al 2011; Salamon et al 2014).

Moreover, the African breeds of the present work (Hamra, Ouled-Djellal, Béni-Ighil, D'men and Foro-Foro) showed MNA values higher than that observed in Kenyan sheep breeds and some South African sheep breeds (Peters et al 2010; Muigai et al 2009) and lower than that showed in the Balami and Yankasa Nigerian sheep breeds (Agaviezor et al 2012).

Four microsatellites showed significant ($p < 0.05$) departures from the Hardy–Weinberg proportions. Expected (He) and observed (Ho) heterozygosities for each breed are given in table 3. Ho average over loci was 0.66 ± 0.195 , whereas He was 0.783 ± 0.098 , being $He > Ho$ in all breeds. The lowest value of observed heterozygosity is noticed in Foro-Foro (0.591) and the highest was detected in Ouled-Djellal followed by Béni-Ighil.

Wright's FIS coefficient ranged from 0.0981 in Hamra to 0.243 in Foro-Foro breed (Table 3). The estimated FIS was significant in all studied breeds.

Table 3: Genetic diversity measures in each breed. Standard deviations are in brackets.

	H_e^a	H_o^b	MNA ^c	A_R^d	PA ^e	FIS (IC 95%) ^f
HA	0.740 (0.130)	0.668 (0.212)	8.33	3.62	0.41	0.098 (0.008-0.158)*
OD	0.820 (0.101)	0.710 (0.196)	11.83	4.07	0.68	0.135 (0.059-0.189)*
BI	0.781 (0.106)	0.677 (0.146)	11.33	3.88	0.61	0.135 (0.05940-0.18790)*
D'men	0.774 (0.117)	0.661 (0.151)	11.33	3.79	0.49	0.147 (0.063-0.214)*
COR	0.804 (0.089)	0.654 (0.193)	10.66	3.92	0.52	0.18766 (0.104 - 0.248)*
Lac	0.783 (0.093)	0.651 (0.201)	9.83	3.76	0.76	0.16938 (0.091 - 0.228)*
FF	0.778 (0.047)	0.591 (0.266)	7.83	3.63	0.39	0.24333 (0.159 - 0.299)*

^a Unbiased expected heterozygosity.

^b Mean observed heterozygosity.

^c Mean number of alleles per loci.

^d Allelic richness (rarefacted)

^e Private alleles frequency

^f 10000 Bootstrap over FIS by population, IC 95% = confidence interval at 95%.

* Significant *p*-values ($p < 0.01$).

OD: Ouled Djellal, HA: Hamra, BI: Béni-Ighil, LAC: Lacaune, COR: Corse, FF: Foro-Foro)

Genetic differentiation

The global *FIT* was 0.207 (0.100 - 0.323) at 95% confidence interval and after 1000 bootstraps. In the overall population the homozygote excess (*FIT*) was caused mainly by a significant homozygote excess within breeds *FIS* 0.155 (0.050 - 0.271) and partially by the genetic differentiation among breeds *FST* 0.061 (0.042 - 0.080). The homozygote excess may be explained by the father effect for the Corse and Foro-Foro breeds by the inbreeding for Algerians and Moroccan breeds. For the Lacaune breeds, this result may be explained by the intensity of selection. The *FST* values observed in this study were higher than that showed for the Egyptian sheep breed (El Nahas et al 2008; Ghazy et al 2013) and lower than that observed in Nigerian sheep breeds (Agaviezor et al 2012). Moreover, these values were near to that detected in Ethiopian sheep breeds (Gizaw et al 2007).

The AMOVA analysis revealed that 79.26% ($p < 0.001$) of the total genetic variation occurred among individuals, whereas 14.57% ($p < 0.001$) is due to individuals within populations and only 6.15% ($p < 0.001$) among populations.

We calculated the *FST* value between pairs of all breeds using Arlequin and the run was done with 5000 permutations. All estimated values were significant ($p < 0.05$) with the lowest level of differentiation observed between the Béni-Ighil and Ouled Djellal and the highest between Lacaune and Foro-Foro breeds.

Analysis with Bottleneck software showed non-significant heterozygote excess by two-phase model (TPM) along with a normal 'L'-shaped distribution of mode-shift analysis test, indicating an absence of bottleneck events for all breeds.

A neighbor-joining tree analysis using the Reynold's genetic distance (DR) revealed concordance between defined clusters of populations and their geographic origin (Figure 2 and Figure 3). Within North Africa, several population clusters had relatively low bootstrap support ($< 50\%$). The bootstrap support for a cluster including the European breeds was 57% (Figure. 2). The genetic relation observed between Hamra and Béni-Ighil breeds is in agreement with the common origin of these two breeds (Chellig 1992). Pairwise Reynolds distance values (Table 4) varied from 0.022 (between Béni-Ighil and D'men) to 0.122 (between Foro-Foro and Hamra breeds).

Table 4: Reynolds distance (DR) estimates (below the diagonal) and *Nm* (above the diagonal) among pairs of breeds.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Hamra (1)		3.54	5.21	5.35	3.24	3.24	1.94
Ouled-Djellal (2)	0.069		6.85	6.69	9.25	3.57	3.69
Béni-Ighil (3)	0.044	0.038		11.35	5.76	2.60	2.75
D'men (4)	0.043	0.039	0.022		5.00	2.56	3.40
Corse (5)	0.077	0.029	0.046	0.052		4.42	2.70
Lacaune (6)	0.078	0.068	0.095	0.097	0.056		1.99
Foro-Foro (7)	0.122	0.071	0.089	0.075	0.092	0.121	

The highest value of pairwise DR and the lowest value of *Nm* were registered in Foro-Foro indicating an absence of gene flow with the other breeds. Significant gene flow ($Nm = 11.35$) was detected between Béni-Ighil and D'men probably caused by an uncontrolled reproduction and the absence of selection programs for these breeds.

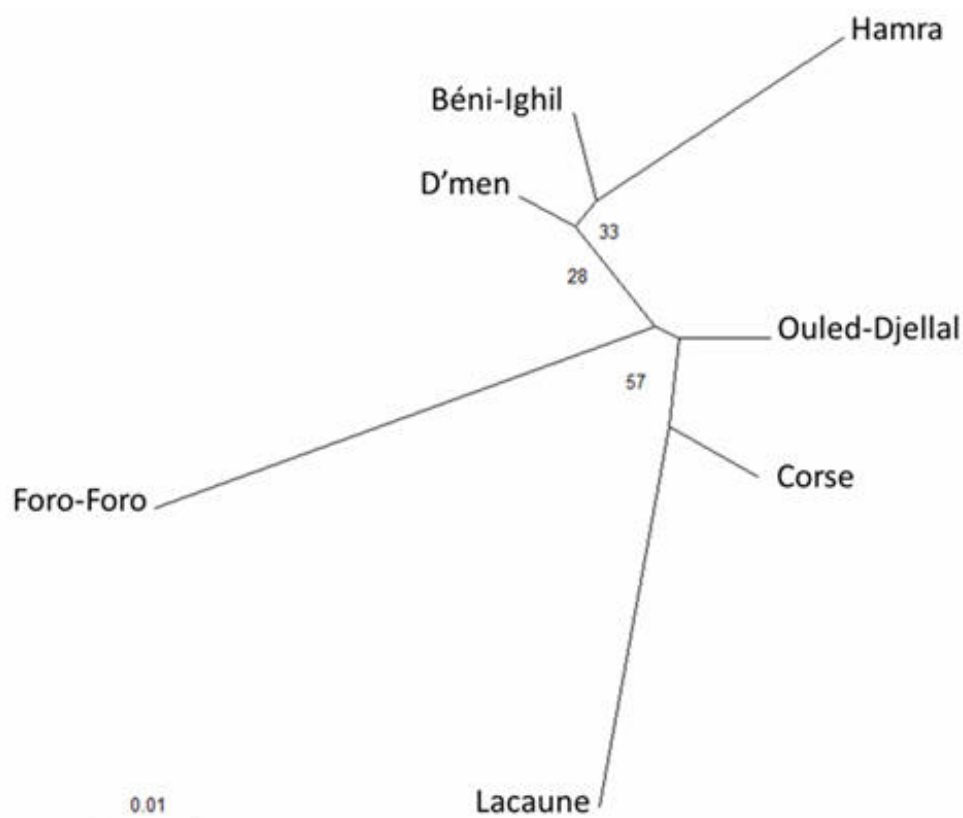


Figure 2: Genetic relationship among the seven sheep breeds using DR genetic distance according to the neighbour-joining algorithm. The values at the forks indicate the number of replicates occurrence in a bootstrap sampling of 1000 trees.

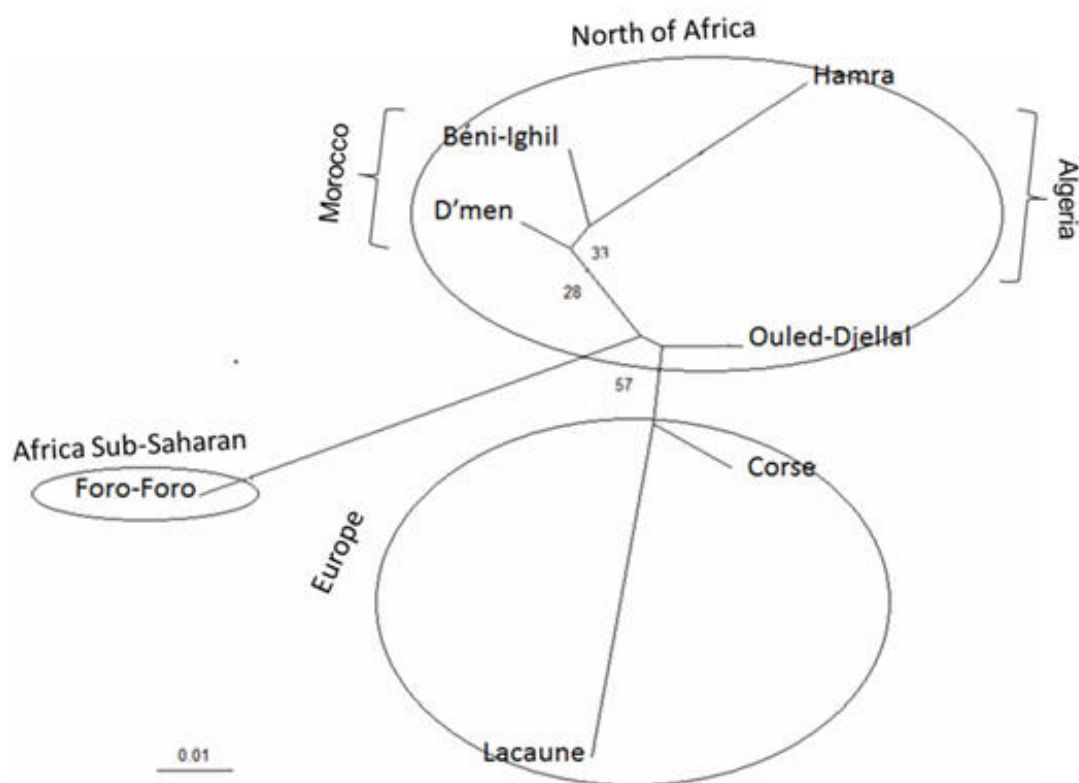


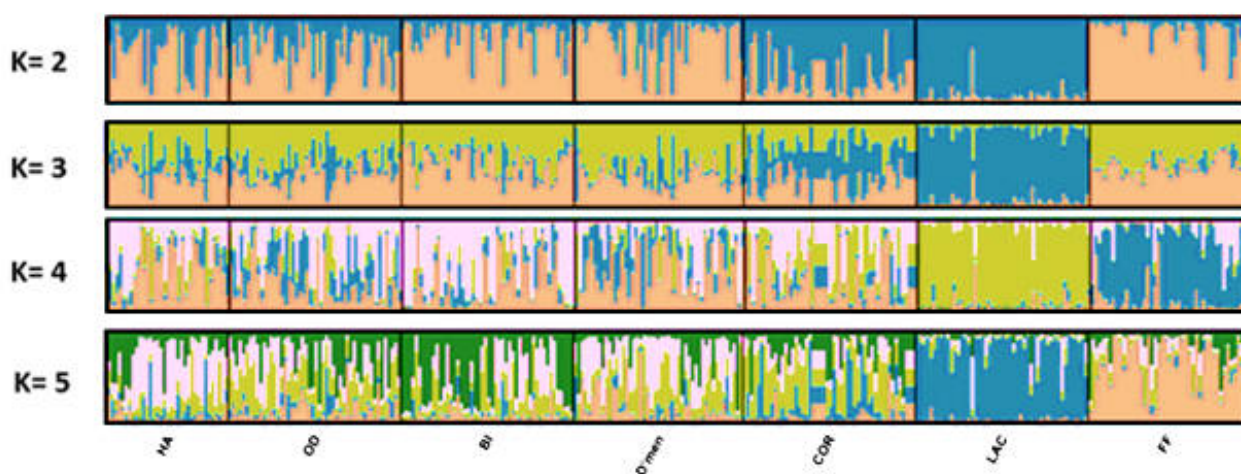
Figure 3: Unrooted neighbor-joining tree analysis, using DR genetic distance

Application of Evanno's ΔK method to STRUCTURE analysis supported $K = 2$ as the most probable number of genetically distinct populations (Table 5). The first cluster represents the Europe origin, the second the African origin.

Table 5: Proportion of membership of each of the seven sheep breeds in the two clusters ($K = 2$) inferred using STRUCTURE software.

Breed	Cluster		Sample size
	1	2	
Hamra	0.391	0.609	35
Ouled-Djellal	0.409	0.591	50
Béni-Ighil	0.206	0.794	50
D'men	0.250	0.750	49
Corse	0.695	0.305	50
Lacaune	0.930	0.070	50
Foro-Foro	0.131	0.869	46

Bar plots showing the proportion of membership of each individual to one or more of the 5 real clusters identified is presented in Figure 4. The distruct result showed that the breeds Hamra, Ouled-Djellal, Béni-Ighil, D'men, Corse and Foro-Foro) were not very well differentiated and an admixture process between these breeds had occurred.

**Figure 4:** Bar plot showing individual sheep by breed: (OD:Ouled Djellel, HA: Hamra, BI: Béni-Ighil, LAC: Lacaune,

COR: Corse, FF: Foro-Foro). Coloured zones on each vertical bar show the proportion of membership of an individual to each of one or more of 5 real clusters identified

Conclusions

- This study investigated the genetic structure of seven sheep breeds from four geographic locations (Algeria, Morocco, France and sub-Saharan Africa). Results showed a significant gene flow among North-African breeds. Besides, relatively high values of FIS were observed in all breeds studied. It would be of interest to extend the number of microsatellites loci to better investigate the genetic structure and diversity of these breeds.

The result will be more useful in defining adequate conservation and management strategies.

Acknowledgement

We would like to thank Dr. K. Moazami-Goudarzi, Dr.E. Cribiu and Dr. F. Grosclaude and A. Gaouar (Centre for Scientific and Technical Research on Arid Regions).

We are grateful to Sir. AOUISSAT and Sir L.DHIMI and their groups for their help for collecting samples of Hamra and Ouled-Djellal, also l'ITelv (Aïn-el-Hadjar and Aïn M'lila) and LaboGENA (Jouy-en-Josas) for providing samples of the breeds.

Institution at which research was done: ES-SENIA university d'Oran (laboratory of molecular biology and genetics), laboratory of génétique biochimique et cytogénétique (INRA de Jouy-en-Josas).

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Received 18 March 2014; Accepted 11 June 2014; Published 1 July 2014

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