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Mitochondrial DNA polymorphism in Moroccan goats☆

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ABSTRACT

The present study characterizes the mitochondrial DNA diversity of Moroccan goats. 150 goats of different phenotypic entities were sampled over four geographic regions covering most of the Moroccan territory and the HVI segment of their mitochondrial DNA (mtDNA) control region was sequenced. The 150 Moroccan goats represented 97 haplotypes for this mtDNA fragment. Most of this diversity was present within phenotypic entities and within geographic regions. This weak genetic structure may result from the fact that all haplotypes were already mixed in the populations that colonized Morocco and/or the existence of recurrent gene flows from Mediterranean routes. Comparing the Moroccan haplotype diversity to that of 21 haplotypes representative of the worldwide diversity showed that all the Moroccan goats studied belonged to the A haplogroup that is preponderant in the world. The haplotypes of the Northern region appeared to be less diverse, what would probably reflect a stronger founder effect in this region.

1. Introduction

Small ruminants have a major socio-economic and ecological role throughout the world and allow the production of 11.1 million of tons of meat per year. This is especially true in Morocco, where the breeding of small ruminants interests more than 65% of the rural population (MADRPM, 2004) and plays an essential role in the rural economic activity. However, only a few studies have described the genetic structure of these local populations until now. For goats, it is difficult to distinguish well-defined Moroccan breeds. However, several investigations have described typical populations with specific localization and phenotypic characteristics. The three major local populations are: (i) the Black population of the Atlas with two subpopulations; (ii) the Northern population; (iii) and the population Draa.

The study of microsatellites and casein genes highlighted the strong genetic polymorphism of such Moroccan populations (Tadlaoui Ouafi et al., 2002). They are characterized by a high diversity and a significant heterogeneity due to an uncontrolled mixing between various populations. A comprehensive study of mitochondrial and Y chromosome DNA diversity in Northern African goats (Pereira et al., 2009) confirmed the high level of variability and suggested that their colonization was influenced by recurrent gene flows through maritime routes (instead of a unidirectional terrestrial route) as

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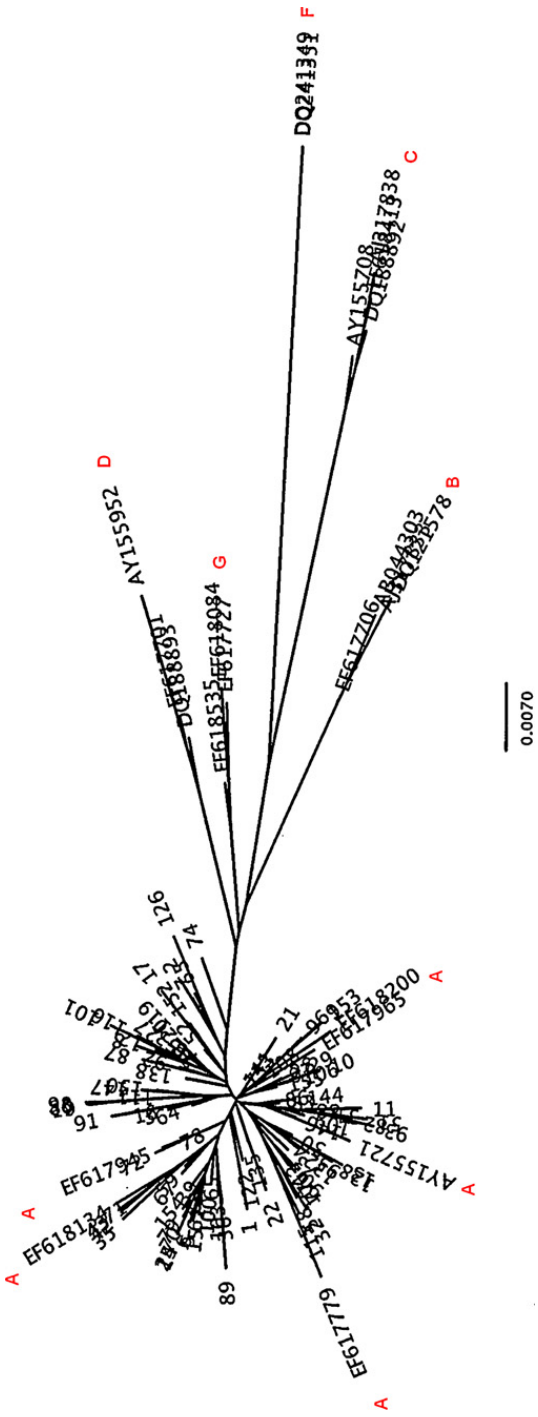

Fig. 1. Neighbor-joining tree of the 97 Moroccan haplotypes and 21 reference haplotypes representing the worldwide diversity. Codes starting with two letters represent the GenBank Accession numbers of the 21 reference sequences, the other numbers correspond to the Moroccan haplotypes. The red letters give the name of the 6 haplogroups identified in the world. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Distribution of genetic variance in populations and geographic regions revealed by AMOVA.

Source of variation	Within populations	Among populations	Within regions	Among regions
d.f.	3	146	3	146
% Variation	7,5	92,5	6,8	93,2
P value	$P < 0.001$		$P < 0.001$	

well as by gene flows between Morocco and the Iberian Peninsula.

The present study aimed to characterize the mitochondrial diversity of Moroccan goats (HVI segment of the control region) in relation with the haplotype diversity already described at the worldwide level (Naderi et al., 2007).

2. Materials and methods

Samples were collected from 150 goats from four main geographic regions: (i) the plains of the central region (provinces of Beni Mellal and Khouribga) (ii) the mountains of the central region (province of Azilal) (iii) the Northern Region (provinces Tangier, Larache and Chefchaouen), and (iv) the South-Eastern area (provinces of Errachidia, Ouarzazate and Zagora). These goats belonged to four entities: (i) Northern population, (ii) Black population of the Atlas, (iii) Draa breed and (iv) other phenotypes.

Tissue samples from the distal part of the ear were collected and placed in alcohol for one day, and then transferred to a tube filled with silica gel until extraction. DNA was extracted using the Qiagen DNeasy tissue kit following the manufacturer's instructions. The HVI segment of the control region was sequenced using the primers CAP-F (5′-CGTGTATGCAAGTACATTAC-3′) and CAP-R (3′-CTGATTAGTCATTAGTCCATC-5′) that amplified a fragment of 598 bp (without primers) corresponding to the positions 15,653 to 16,250 on the complete goat mitochondrial sequence of reference (Parma et al., 2003; GenBank accession number AF533441). PCR amplifications were conducted in a 25 μl volume with 2 mM $MgCl_2$, 200 mM of each dNTP, 1 mM of each primer and 1 unit of AmpliTaq Gold Polymerase (Applied Biosystems). After a 10 min period at 95 °C for polymerase activation, 35 cycles were run with the following steps: 95 °C: 30 s, 55 °C: 30 s, 72 °C: 1 min. PCR products were purified using the Qiaquick PCR purification kit (Qiagen). 35 ng of purified DNA from this PCR product was used for sequencing with the CAP-F or CAP-R primers. Sequence reactions were performed for both DNA strands with the CAP-F or CAP-R primers by using the ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems) in a 20 ml volume with 2 mM of each primer. 25 cycles were run with the following steps 96 °C: 30 s, 55 °C: 30 s, 60 °C: 4 min. Excess dye terminators were removed by spin-column purification and the products were electrophorized on an ABI 3130 PRISM DNA sequencer (Applied Biosystems) using the POP 7 polymer.

The sequences obtained were edited for correction with SeqScape v2.5 (Applied Biosystems). They were aligned together with 21 reference sequences (Naderi et al., 2007) using Mega v3.1 (Kumar et al., 2004), and then adjusted by eye. For analyses, we kept the 481 bp long region (GenBank Accession Numbers HQ455369–HQ455518) usually used for characterizing the goat mitochondrial diversity (e.g., Luikart et al., 2001; Naderi et al., 2007). AMOVA were performed on the Moroccan dataset using ARLEQUIN v3.0 (Excoffier et al., 2005) in order to test the partition of the genetic variance among and within populations and geographic areas. A median joining network representing the relationships between haplotypes was drawn using NETWORK v4.5.1.6. A Neighbor-joining tree was constructed using the Moroccan haplotypes and the 21 haplotypes representing 6 domestic goat haplogroups identified in the world (Naderi et al., 2007).

3. Results

The HV1 fragment of the control region showed a high polymorphism in Moroccan goat populations, with a haplotype diversity (Hd) of 0.9925. The 150 sequences had 98 variable sites over 481, and corresponded to 97 haplotypes. The Neighbor-joining tree (Fig. 1) made with these 97 haplotypes and 21 haplotypes representing the diversity of the 6 haplogroups found over the world (i.e., A, B, C, D, F and G, Naderi et al., 2007) showed that the 150 goats studied were from the haplogroup A. Even if the AMOVA showed a significant population effect on the mt variation ($P < 0.001$), 92% of this variation was distributed within populations (Table 1). Also, the differentiation between geographic regions was low but significant ($P < 0.001$), and about 93% of the genetic variability was distributed within regions (Table 1).

4. Discussion

4.1.1. Moroccan goats' diversity in the worldwide context

The very high haplotype diversity obtained for the Moroccan goats is similar to that measured by Naderi et al. (2007) on 1440 haplotypes from the A haplogroup across the world. It also confirms the high diversity already described in Moroccan and other Northern African goats (Pereira et al., 2009). This high diversity would result from the heterogeneity of domestic goat populations that first colonized Morocco. Because the goat domestication process is recent at the evolutionary timescale (about 10,000 years ago), only a few mutations should have occurred since the initial steps of domestication despite the high mutation rate of the control region. Thus, the high diversity observed now would result from the capture of a large part of the wild diversity during domestication. This has been confirmed by the comparison of the genetic diversity of domestic goats to that of their wild ancestor, showing among others large initial effective population size for domestic goats (see Naderi et al., 2007).

4.2. Structure of Moroccan diversity

The high level of variability observed within populations and geographic regions is consistent with the high diversity found within geographical regions at the worldwide scale (Sultana et al., 2003; Joshi et al., 2004; Chen et al., 2005; Pereira et al., 2005; Naderi et al., 2007). The median joining network of the Moroccan haplotypes (Fig. 2) confirms the high diversity of haplotypes within geographic regions. However, it points out a tendency for a higher similarity between haplotypes from the Northern region. The results are in accordance with two non-exclusive hypotheses that are (i) the high heterogeneity of founder populations in Morocco due to a mix of haplotypes in the first domesticated populations (Naderi et al., 2007), and (ii) the recurrent influx of genetic diversity via the Mediterranean Sea (Pereira et al., 2009). The higher similarity between Northern haplogroups would probably result

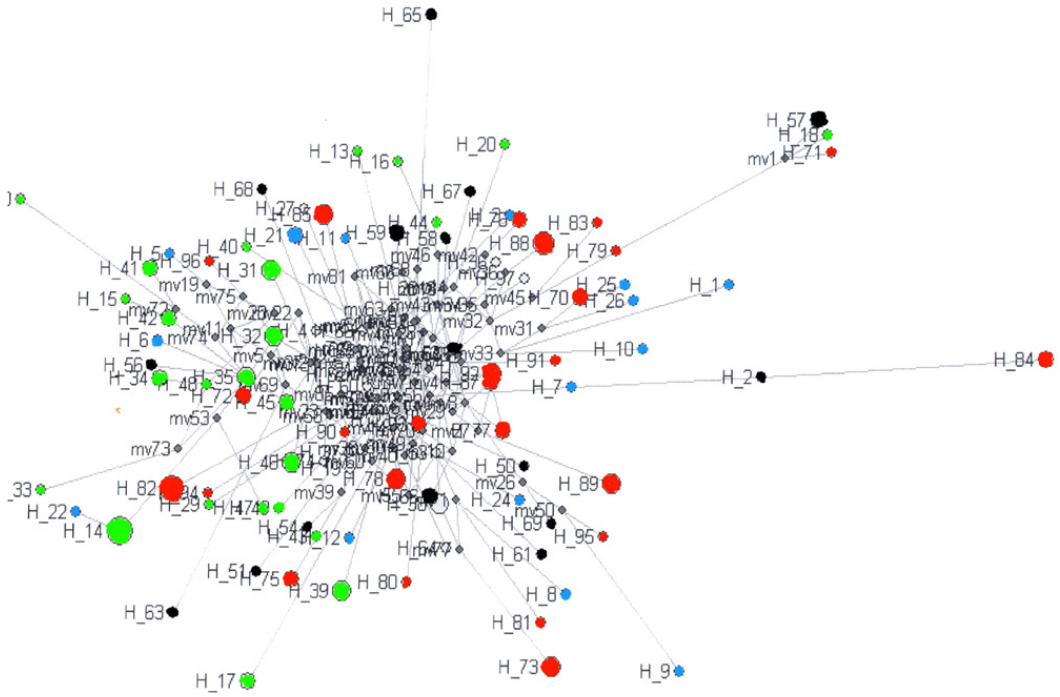

Fig. 2. Phylogenetic network based on the HVI control region haplotypes. The different colours are related to the geographical origin. Green: Northern region; Red: South-Eastern region; Blue: mountains of the center; Black: plains of the center. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

from a strong founder effect, probably from populations originating in the Iberian Peninsula, given the past occurrence of admixture across the strait of Gibraltar (Anderung et al., 2005; Pereira et al., 2009).

5. Conclusion

The 150 Moroccan goats studied are all from the A haplogroup that is predominant in the world and especially in Western Europe. It appears that, as elsewhere in the world, these populations are characterized by a high mitochondrial diversity that is very weakly structured according to regions and breeds/populations. This variability would reflect the diversity already present in the first domesticated individuals that arrived in Morocco and/or the existence of recurrent gene flows from Mediterranean routes. Despite the high diversity within geographic regions, the Northern population seems more homogeneous, probably because of a stronger founder at the origin of these populations.

Complementary studies on selected genes would allow understanding the adaptive history of these populations in relation with local conditions (i.e., climate, pathogenic context, breeding system, etc.).

Conflict of interest

None.

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