



## mtDNA lineages in two Tunisian Berber communities: Comparing diversities between villages and towns

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**Abstract.** We show that the very patchy genetic landscape that is emerging for North African mtDNA diversity is mainly due to the sampling strategy being conducted in small villages, where individual ethnic affiliation to one of the main groups (Berber or Arab) is considered to be safer. The standard diversity measures and haplogroup distribution were significantly higher in a sample from a town than one from a village. Thus, the sampling strategy is a main factor to be taken into account before the construction of a forensic database in North African highly structured countries. © 2005 Published by Elsevier B.V.

*Keywords:* mtDNA; Tunisia; Berber; Town; Village

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### 1. Introduction

Haploid markers are known to be more sensitive to genetic drift, bottlenecks and founder events due to its effective size being 1/4 relatively to autosomal. These effects can be dramatic when samplings are carried out in small villages, where inbreeding is very strong, as it has been the case of most studies conducted in North Africa aiming to compare Berber and Arab communities. We can ask, therefore, if this sampling strategy is suitable for the construction of forensic databases.

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Fig. 1. Map of Tunisia showing the location of the two Berber communities.

We tried to evaluate the biases introduced by such a sampling strategy by comparing the mtDNA haplotype diversities (HVRI and HVRII) between two north Tunisian Berber communities (Fig. 1): the town of Sejenane (over 41,000 inhabitants) and the small village of Takrouna (500 inhabitants).

## 2. Material and methods

The sampling effort was considerably higher in the small village, where close kinship was more and more difficult to rule out as the sampling proceeded, so that at a certain point for all individuals not yet sampled, a relative had been collected already. The total numbers of samples were 47 from Sejenane and 33 from Takrouna.

mtDNA was amplified using the primers L15997 (5'–CACCATTAGCACCC AAAGCT–3') and H16401 (5'–TGATTTACGGAGGATGGTG–3') for HVRI and L48 (5'–CTCACGGGAGCTCTCCATGC–3') and H408 (5'–CTGTTAAAAGTGCATACC GCCA–3') for HVRII. The temperature profile was 95 °C for 10 sec, 60 °C for 30 sec and 72 °C for 30 sec, for 35 cycles of amplification. The amplified samples were purified with Microspin™ S-300 HR columns (Amersham Biosciences), according to the manufacturer's specifications. The sequence reactions were carried out using the kit Big-Dye™ Terminator Cycle Sequencing Ready Reaction (AB Applied Biosystems), with one of the above primers, in both forward and reverse directions. A protocol based on MgCl<sub>2</sub>/ethanol precipitation was used for post-sequence reaction purification of samples, which were then run in an automatic sequencer ABI 3100.

The nucleotide positions considered for the analysis of the sample were 16024–16383 for HVRI and 73–340 for HVRII. Length variation (often scored as transversions in HVRI) was not considered [1]. Sequence classification into haplogroup was done according to [2,3]. Molecular diversity indexes were calculated using Arlequin 2.0 [4].

## 3. Results

As expected, the diversity was higher in the town sample (haplotype diversity=0.988 ± 0.008; mean pairwise differences=9.521 ± 4.446) than in the village (haplotype diversity=0.907 ± 0.024; mean pairwise differences=4.625 ± 2.328). The probability to find a haplotype match was much smaller in the town (1.203%) than in the village (9.280%). And with respect to the haplogroup

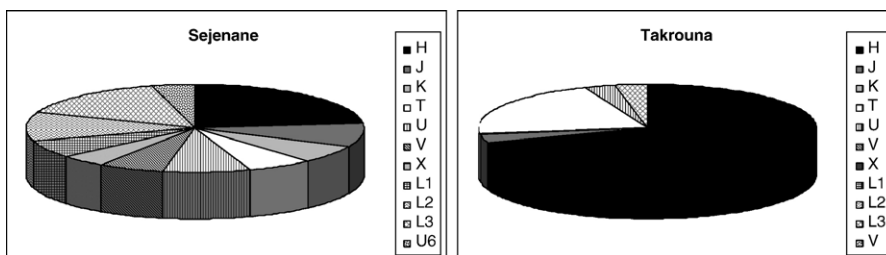


Fig. 2. Haplogroup distribution (%) in Sejenane and Takrouna.

distribution, the same higher diversity was observed for the town sample (64% Eurasian, 32% Sub-Saharan, and 4% North African), comparatively to the village one (97% Eurasian, 3% Sub-Saharan, and 0% North African) (Fig. 2).

We assayed also if, by pooling small Berber village samples, we would get a similar diversity to the town sample. This assay was limited to HVRI diversity because this report will be the first one to describe HVRI diversity in North Africa. When we pooled 47 individuals from the small village of Kesra [5] with 33 from Takrouna, we obtained still a lower diversity (haplotype diversity =  $0.897 \pm 0.028$ ; mean pairwise differences =  $4.909 \pm 2.417$ ) than the town sample (haplotype diversity =  $0.979 \pm 0.012$ ; mean pairwise differences =  $6.141 \pm 2.973$ ).

#### 4. Conclusions

These results claim some thought on the sampling strategy to be applied to the construction of forensic databases not only in Tunisia but also in the rest of North Africa and in other population coverages, where similar sampling strategies are conducted that way.

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