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MALDI-TOF MS analysis of Y-SNPs in ancient samples

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Abstract. Studying ancient central Asian, Siberian and South American populations with classical markers (nuclear microsatellites and mitochondrial DNA sequence polymorphisms) allowed us to investigate parental relationships among individuals from burial sites revealing funeral practices. Ancient DNA studies can also provide information on the origins and the history of population from the past. Focussing on biallelic markers which have a lower mutation rate than repeat polymorphism, it is possible to address events corresponding to longer periods of time. In the frame of our anthropological studies on ancient DNA samples from Mongolia, Siberia, Yakutia and South America, we concentrated efforts on three Y chromosomal SNPs (TAT, M242 and RPS4Y) known to have specific allelic distributions in these populations or to be informative regarding the peopling of America (M242 and RPS4Y). Facing ancient samples where DNA is strongly degraded and scarce requires the use of technologies which can provide information from only short fragments of intact template. In this context, we developed a primer extension and matrix-assisted laser desorption ionization time-of-flight mass spectrometry-based multiplexed reaction for the investigation of these three polymorphisms. The tested ancient male specimens were recovered from a necropolis located south of Lake Baïkal in northern Mongolia (Egyin Gol valley). Distinct SNP profiles were obtained enlightening the ethnic heterogeneity of the Xiongnu tribe which was only foreshadowed by the STR marker analysis.

Keywords: Ancient DNA; Y-SNP; MALDI-TOF mass spectrometry

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1. Introduction

Beyond their extreme interest, ancient DNA studies present some technical difficulties among which the risk of contamination by modern DNA and the minute quantities as well as the degraded nature of the surviving DNA molecules. If the risk of contamination can be minimized by following scrupulously stringent guidelines, the quality and/or the quantity of the DNA extract cannot easily be improved. DNA profiling systems based on SNPs appeared to be a solution because of the reduced size fragment of interest. In this context, we decided to investigate SNPs by a primer extension and Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)-based method allowing multiplexed analysis.

Considering the past populations actually under study in our laboratory (Mongolian, Siberian and Native American), we have focused on 3 relevant Y-SNPs: Tat, RPS4Y and M242 (Fig. 1). Tat [1] (T→C) is restricted to a subset of the populations of Asia and northern Europe and may have arisen in Mongolia. $RPS4Y_{711}$ [2] (C→T) is restricted to eastern Asia and America raising a Native American founder lineage outside M45 characterized by differentiated STR alleles. M242 [3] (C→T) is a descendant lineage of M45, ancestral to M3 and found both in Central Asia and America.

2. Material and methods

The tested ancient male specimens were recovered from a necropolis located south of Lake Baïkal in northern Mongolia (Egyin Gol valley). DNA was extracted as previously described [4] and quantified by real-time PCR (Quantifiler™ Human DNA quantification kit, ABI Prism 7000, Applied Biosystems).

Fragments shorter than 150 bp, containing the RPS4Y, M242 and TAT loci, were amplified simultaneously by PCR. The allelic discrimination of the SNPs was achieved by simultaneous primer extension and its products analysed by MALDI-TOF MS (Ultraflex TOF-TOF, Bruker Daltonics) directly after magnetic bead assisted purification. The PEX primers were designed to yield products in the 5.4–8.2-kDa mass range. The minimal mass difference between two analytes was 60 Da. The triplex was previously validated on modern DNA samples of different DNA content.

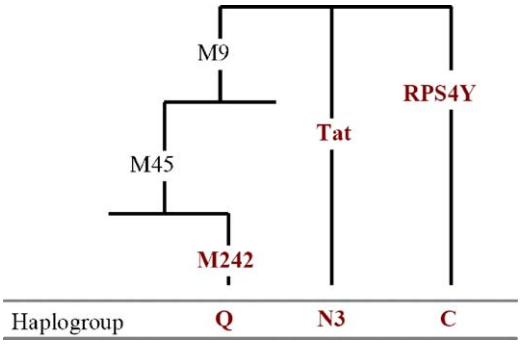

Fig. 1. Phylogeographic tree showing the investigated loci and the haplogroups they define.

Table 1
Results obtained on 3 ancient specimens

Sample	M242 allele	TAT allele	RPS4Y allele	Haplogroup
19EG	C	C	C	N3
70EG	–	–	T	C
112EG	T	T	C	Q

3. Results

Among the ancient samples under study, only 3 allowed genotyping of the targeted markers. Consistently with results of a dilution test performed on modern DNA samples, when 30 pg DNA input was all that was available, preferential amplification of the shortest fragment was induced (sample 70EG). For the other ancient samples tested, no conclusions could be drawn because either their DNA content was to scarce or the reaction yield was weak and the products not unambiguously distinguishable from background signal. This latter is due to the detection method which is very sensitive but not specific to nucleic acids. Whatever the detection method employed, we have observed that the DNA quantity remains the primary obstacle to DNA analysis.

Interestingly, distinct SNP profiles were obtained from the analyzed samples, revealing that these 3 ancient specimens belong to 3 major NRY clades (Table 1).

4. Conclusion

Whereas the very reduced number of ancient Egyin Gol specimens examined does not allow any upshot, the present work demonstrates the ethnic heterogeneity of the Xiongnu tribe which was only foreshadowed by the STR marker analysis.

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