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20 YEARS OF COLLABORATIVE GENETIC RESEARCH BETWEEN INSTITUTO CANARIO DE BIOANTROPOLOGÍA AND INSTITUTO NACIONAL DE TOXICOLOGÍA Y CIENCIAS FORENSES (1997-2017)

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Abstract. Since its foundation, the Instituto Canario de Bioantropología (ICB, Canary Institute of Bioanthropology) has been aware of the importance of studying human remains not only from a bioanthropological and paleopathological perspective, but also from a molecular point of view. To carry out these studies, since 1997, the ICB establishes a collaboration agreement with the Instituto Nacional de Toxicología y Ciencias Forenses (INALTOX, National Institute of Toxicology and Forensics Sciences, Ministry of Justice).
In this paper we review the research lines that, using aDNA, were opened in the fields of paleodemography and paleopathology, as well as the future lines of research.

Keywords. aDNA. Paleodemography. Paleopathology. Instituto Canario de Bioantropología. Instituto Nacional de Toxicología y Ciencias Forenses.

1. INTRODUCTION

Since its beginnings, the collaboration between the ICB and the INALTOX is part of a large-scale study whose goals are:

- To contrast the hypotheses on the pre-Hispanic settlement of the Canary Islands and Tenerife in general.
- To contrast the hypothesis on the interinsular isolation of the pre-Hispanic population.
- To determine demographic patterns within the different menceyatos (pre-Hispanic kingdoms) of the island of Tenerife.
- To determine patterns of settlement in Tenerife after the conquest.
- To study infectious diseases in different places.

Approaching all of them from the point of view of Physical Anthropology, Archeology and, mainly, Genetics.

2. THE GENETIC STUDIES

As we know, the main problems associated with the genetic studies of ancient human remains are the low amount of DNA present in the samples, its high state of degradation and the high probability of contamination with exogenous DNA, mainly from the researchers who manipulate the remains.

At the genetic level, our main objectives are:

- Check if it is viable to obtain DNA, mainly mitochondrial (its high number of copies facilitates the study) by our laboratories from human bone and teeth from archaeological sites, both aboriginal and post-conquest, in the island of Tenerife and, to a lesser extent, to the rest of the archipelago.
- Check if the environmental conditions of the archaeological sites and burial methods influence the amount of DNA collected.
- Optimize a process to obtain, purify and amplify DNA.
- The possibility of obtaining, from archeological bone and teeth, bacterial DNA markers from infectious agents like *Y. pestis*, *S. enterica* Ser. Typhi, …

3. MATERIAL AND METHODS

For this purpose, we have used a total number of 60 teeth from deposits located in Tenerife (22 from 16th century and 29 from the pre-Hispanic era and unk-

nown antiquity); Lanzarote (3 pieces) and the Mediterranean island of Crete (6 teeth of the 17th century)

The genetic analysis consisted in the extraction of DNA, amplification by PCR of the Hypervariable Region I (HVI) and subsequent analysis of the polymorphism of the amplification products by means of cyclic sequencing techniques with labeled terminators and detection by automatic sequencing.

3.1. DNA extraction

All the teeth were subjected to a cleaning process in order to eliminate exogenous DNA. For this purpose, sodium hypochlorite and UV radiation were used. Then we proceeded to grind the pieces with mechanical means (with the help of liquid N_2 in most cases)

In all pieces, the DNA was extracted by the phenol / chloroform method after proteolytic digestion with proteinase K in presence of DTT and SDS during a period ranging between 12 and 72 hours.

After phenolic separation, the aqueous phase has been purified by various methods as technological advances have progressed. Thus, a quarter of the pieces (15 teeth) were subjected to a purification with Centricon-30 filters (Milipore); another quarter was subjected to purification using Centricon-YM100 filters; 20 pieces were purified by silica columns QIAamp® DNA Mini Kit; and 10 were purified by the use of guanidine thiocyanate columns GENECLEAN® Kit For Ancient DNA.

For each group of pieces, a negative extraction control was performed.

Always, sterile and / or decontaminated material by UV irradiation was used and sterile reagents were used and also treated with UV radiation.

The entire extraction process was carried out in laminar flow cabinets previously irradiated, at least for 30 minutes, with UV light.

3.2. Amplification of the HVI region by PCR

The amplification of the HVI region was carried out using different pairs of primers. In a preliminary way, the primers described by Wilson *et al*. (1995) to amplify the HVI region in two overlapping fragments of approximately 260-270 bp. However, in view of the low yields obtained, it was decided to perform an analysis with a set of seven pairs, described by Maca-Meyer *et al*. (2004), that allow obtaining overlapping fragments between 80 and 130 bp. These primers are showing in Table 1.

Table 1. Pairs of primers used in the amplification of the hypervariable region I of the mitochondrial genome, according Maca-Meyer (2004).

Primers		Ta	Fragment size (pb)
Forward	Reverse		
L1F 5' - CTCCACCATTAGCACCCAAAGC - 3'	H1R 5' - AGCGGTTGTTGATGGGTGAGTC - 3'	50º C	112
L2F 5' - GGAAGCAGATTTGGGTACCAC - 3'	H2R 5' - TGGTGGCTGGCAGTAATGTACG - 3'	50º C	82
L3F 5' - CACCCATCAACAACCGCTAT - 3'	H3R 5' - TGATGTGGATTGGGTTTTTATGTA - 3'	46º C	112
L4F 5' - ACGGTACCATAAATACTTGACCA - 3'	H4R 5' - TTGGAGTTGCAGTTGATGTGTGATA - 3'	50º C	127
L5F 5' - CAAGCAAGTACAGCAATCAACC - 3'	H5R 5' - CTGTTAAGGGTGGGTAGGTTTG - 3'	46º C	103
L6F 5' - CACCCCTCACCCACTAGGAT - 3'	H6R 5' - TGGGGACGAGAAGGGATTTG - 3'	50º C	111
L7F 5' - AGCCATTTACCGTACATAGCACA - 3'	H7R 5' - TGATTTCACGGAGGATGGTG - 3'	46º C	107

3.3. Amplification of markers rpoß-PLAC (*Y. pestis*) and narG-narG / stnarGR

The search of specific markers of the pathogens *Y. pestis* and *S. enterica* Ser. Typhi was performed using the primers set described by Drancourt *et al*. (1998) and Papagrigorakis *et al*. (2005). The primers used are shown in Table 2.

Both for the amplification of the HVI region of mtDNA and the rpoß, pla and narG markers, a hot start protocol was used.

Table 2. Pairs of primers used in the amplification of specific markers of the pathogens, according to Drancourt *et al.* (1998) and Papagrigorakis *et al.* (2005).

Primers			Ta	Fragment size (pb)
Y. pestis	*rpoß*	5'-AACACCTTATCGTCGTGTACGT-3'	58º C	160 pb
		5'-AATCTTCTAAAAAGCGGCCTTCA-3'		
	pla	5'-CTTGGATGTTGAGCTTCCTA-3'	53º C	148 pb
		5'-GAGATGCTGCCGGTATTTCC-3'		
S. enterica Ser. Typhi	*narG*	5'-TTCACTTCCTGCCATGAGGAGCG-3'	58º C	360 pb
		5'-TCAACCCATGGGGTGAACTG-3'		

The efficiency of the amplification was evaluated by agarose gel electrophoresis and staining with ethidium bromide (recently replaced by GelRed™ Nucleic Acid Gel Stain).

3.4. Analysis of the genetic polymorphism of the amplified regions by automatic sequencing

The PCR products were purified by ultracentrifugation and subsequently sequenced by cyclic sequencing with labeled terminators using the "dRhodamine Terminator Cycle Sequencing Kit" (Applied Biosystems). The extension products were analyzed by an ABI-Prism 310TM automatic sequencer (Applied Biosystems). Finally, Sequencing Analysis and SeqEd programs were used to edit sequences.

4. RESULTS

To achieve our objectives, we have analyzed a total of 60 teeth, of which mtDNA was in 24 (approximately 40%). Their origin is summarized in the attached table (Table 3).

Table 3. Summary of the results obtained in the study of the different archaeological sites mentioned in this work.

Type of deposit	Situation	Antiquity	Number of pieces analyzed	Burial characteristics	Number of pieces from which DNA was extracted	Aproximate success rate (%)
Historics	Los Silos (Tenerife)	s. XVI	22	Interior of a church. Stable environmental conditions	12	54
	Rethymno (Creta)	s. XVIII	6		3	50
Pre-Hispanics	El Almendro (Tenerife)	—	9	Cave. South Zone of the Island	2	22
	Anaga (Tenerife)	—	4	Cave. Laurisilva (Subtropical Green Forest). Humidity abundant	0	0
	Tegueste (Tenerife)	—	9	Middle mountain region	6	66
	Others (Tenerife)	—	7	Middle mountain region	3	42
	Lanzarote	—	3	Extreme variations of temperature between day and night	0	0
Total			60			33

Our initial strategy for the amplification of the HVI region of mitochondrial DNA was based on the use of two overlapping fragments between 260 and 270 bp (Wilson, 1995; González-Mamely, 2002). However, due to the high degradation state in which the mtDNA obtained from these residues, sufficient amount of amplification product was not generated to perform the sequencing reactions. Therefore, we have changed to an analysis with a set of 7 pairs of primers that allow obtaining overlapping fragments between 80 and 130 bp (Maca-Meyer, 2004).

Six dental pieces from six individuals buried in a Church of Rethymmo, on the island of Crete, dating from the 17th century and contemporaries of the plague epidemic that ravaged the island during the Turkish siege of Crete, were also analyzed in our laboratory in order to determine if the individuals had been victims of the plague, following the protocols of Drancourt *et al*. (1998) and Papagrigorakis *et al*. (2005), an attempt was made to determine whether genomic remains of *Y. pestis* and *S. enterica* Ser. Typhi still existed in the teeth. The analysis allowed to find in these pieces very fragmented DNA, both of human origin (it could be sequenced mitochondrial from it) and bacterial, but, unfortunately, no specific markers of *Y. pestis* or *S. enterica* Ser. Typhi were found.

We have observed that although there are significant differences in the performance of amplification between teeth of children and adults, there are no differences in performance between intact and fragmented teeth, which agrees with the greater presence of DNA in the dentin than in the pulp.

Nor does there seem to be a great difference in performance in terms of the antiquity of the piece, but it does refer to the environment in which it was collected. High temperature variations and high environmental humidity do not allow obtaining DNA with the protocols used in our laboratory.

5. CONCLUSIONS

Considering the results obtained during these 20 years of collaboration between the ICB and the INALTOX, we conclude that it is technically feasible to carry out an analysis of the genetic variability, to begin, of the HVI region of $_{mt}$DNA and, subsequently, of markers of Y chromosome, from a larger number of ancient human remains; understanding that the study of this genetic variety would complement what we already know about the genetic structure of current and ancient Canarian populations.

Likewise, the methods and techniques used may also be extrapolated to the genetic study of diseases, both infectious and metabolic, that affected Canarian populations in the past.

6. BIBLIOGRAPHY

DRANCOURT, M., ABOUDHARAM, G., SIGNOLI, M., DUTOUR, O. & RAOULT, D. (1998). Detection of 400-year-old Yersinia pestis DNA in human dental pulp: An approach to the diagnosis of ancient septicemia. *Proceedings of National Academy of Sciences*, 95: 12637-12640

GONZÁLEZ-MAMELY, I., HERNÁNDEZ-LUIS, A., SÁNCHEZ, J.J., RODRÍGUEZ-MARTÍN, C. & CUELLAS, J.A. (2002). Resultados preliminares en el análisis de ADN mitocondrial a partir de restos humanos del S. XVII en el convento de San Sebastián (Los Silos). *Eres Arqueología Bioantropología*, 10:139-146.

MACA-MEYER, N., ARNAY, M., RANDO, J.C., FLORES, C., GONZÁLEZ, A.M., CABRERA, V.M. & LARRUGA, J.M. (2004). Ancient mtDNA analysis and the origin of the Guanches. *European Journal of Human Genetics*, 12:156-62

PAPAGRIGORAKIS, M.J., YAPIJAKIS, C., SYNODINOS, P.N. & BAZIOTOPOULOU-VALAVANI, E. (2006). DNA examination of ancient dental pulp incriminates typhoid fever as a probable cause of the Plague of Athens. *International Journal of Infectious Diseases*, 10 (3): 206-214

WILSON, M.R., DIZINNO, J.A., POLANSKEY, D., REPLOGLE, J. & BUDOWLE, B. (1995). Validation of mitochondrial DNA sequencing for forensic casework analysis. *International Journal of Legal Medicine*, 108:68-74