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GENETIC STUDIES CONTRIBUTION TO THE STUDY OF MATRILOCALITY IN FUNERARY PRACTICES ON THE CANARY ISLANDS

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Abstract. Through the genetic studies of the human remains of the Punta Azul Cave in the Island of El Hierro, we have been able to propose a new approach to the funerary practices of the Bimbape and Canarian aboriginal populations. We have studied 61 tibias, on which we were able to perform mitochondrial DNA analysis. All of them belonged to the same maternal lineage, H1cf. These results allow us to propose an interpretation of the funerary practices that could be related to matrilocality. The importance of the maternal lineages in the Canarian aboriginal populations has been proposed from different disciplines. This study supports some of these hypotheses and is one of the first empirical evidence of the link of the indigenous populations through their maternal lineages.

These results lead us to a more profound analysis and reflection about the role of the maternal lineages in the social organization of the Bimbapes, about the use of collective spaces as burials, and the connection with their ancestors. It also allows us to propose a line of research where more studies of this kind in other archaeological sites could help in our understanding of their funerary practices.

Keywords: ancient DNA, matrilocality, Bimbapes, funerary practices.

INTRODUCTION

One of the most common characteristics among the aboriginal populations of the Canary Islands is the collective character of many of their burial spaces (Arco Aguilar 1992-1993; Velasco Vázquez & Alberto Barroso 2005a; Alberto Barroso & Velasco Vázquez 2007). The fact that numerous individuals were placed in these sites in different moments proves their collective nature (Alberto Barroso & Velasco Vázquez 2007: 39). It is also the expression of a normalized funerary behavior from a group of people who, united to each other by ties of various kinds, intend to maintain those bonds after death, sharing the same place for their deceased (Velasco Vázquez & Alberto Barroso 2005a: 329). Beyond death, they are still part of the community, now with the status of ancestors (Velasco Vázquez et al. 2005b: 85-124).

The cult of the ancestors among the Canarian aboriginals can be seen in the archaeological record as well as in the ethnohistorical sources. Regarding the archaeological data, there are two elements regarding funerary behaviors that reflect the importance these societies gave to their ancestors. The first one is the aim to preserve the corpses, naturally or artificially, which implies a desire for the bodies to last throughout time (Méndez Rodríguez 2014). The second one is the existence of big collective "burials", where the main criterium is that the remains are placed in the same place through long periods.

The collective funerary caves, very abundant in the islands, are not only an indicator of that cult to the ancestors but also of a marked fixation to a specific territory. They can also be seen as territorial markers, with an important social and identity character for each specific group. As it has been proposed, this means collectivism may have its foundations in kinship relationships (Velasco Vázquez et al. 2005b).

The archaeological reality has made it impossible to perform bioanthropological studies to establish possible kinship relationships within the individuals in the collective burial places. These sites tend to be highly altered, and there are not many places of these characteristics that could have been studied with an appropriate archaeological methodology. Moreover, there have not been any specific genetic studies to establish possible kinship relationships between the individuals of these collective deposits, mainly because of the bad preservation of the human remains. These relationships have only been studied through non-metric traits and the calculation of the mean measure of divergence (MMD) in two

skeletal series from the Island of Gran Canaria (Lomo Juan Primo y la Guancha - El Agujero). In these works, the authors confirmed the intention of placing certain bodies in specific areas of the necropolis. This formed deposit ensembles where kinship relationships seem to play a determinant role (Cabrera López, 2010).

Ethnohistorical sources repeatedly tell us about this cult to the ancestors. For example, Alonso de Espinosa mentions it several times for the Island of Tenerife:

«*Cuando alzaban por rey a alguno, tenían esta costumbre, que cada reino tenía un hueso del más antiguo rey de su linaje envuelto en pellejuelos y guardado y, convocados los más ancianos al Tagoror, lugar de junta y consulta, después de elegido el rey, dábanle aquel hueso a besar*…»(A. Espinosa, 1980:41-42).

There are also references to the continuous use of a specific burial space by the same social group:«…*y los ponían en las cuevas que tenían dedicadas para ello, cada uno para su entierro; y esto tenían los inferiores del rey, que donde quieran que morían, se enterraban en su cueva que tenían para su sepultura; pero el rey, donde quiera que moría, lo habían de llevar a su sepultura, donde tenían sus pasados; a los cuales ponían por orden para que se conociesen; y así los ponían fajados y sin cubrirles con cosa encima*» (Abreu Galindo,1977:300).

The community also made pilgrimages to these sites as a mean to reinforce the link between the living and the dead, as it is narrated by Marín de Cubas: «*Hacían [los guanhes] largas romerías á visitar los huesos de sus sepulcros (…) y en particular había los más frecuentes en el pico del Teide y también juraban por los huesos de sus antepasados á modo de venganza ó pleito homenaje*» (Marín de Cubas (1993 [1694]: 220-221).

Ethnohistorical sources also mention the importance of maternal ancestors, highlighting the social role of some women. This would be the case of Tibiabin and Tamonante in Fuerteventura (Pérez Saavedra 1982; Cabrera Pérez 1993), or the case of Gran Canaria, where a woman - Atidamana - is related to the founding myth of the Guanarteme's lineage that detented the power at the moment of the European conquest of the Island (Onrubia Pintado 2003: 436).

Although we do not know much about the aboriginal kinship systems, the sources talk about a matrilineal component that seems to have a role in the transmission of heritage rights and lineages in most of the islands (Zurara, G.E. 1998; Navarro Mederos 2001; Cabrera Pérez 1992,1993; Jiménez Gómez 1993, Pérez Saavedra 1989; Martín de Guzmán 1982; Martín Rodríguez 1992). For example, in La Gomera, the first chroniclers perceived the existence of a matrilineal regime regarding the transmission of the domestic heritage «*Donde*

heredan los sobrinos, hijos de hermanas» (Navarro Mederos, 2001: 202). The importance of the matrilineal transmission can also be said of the aboriginal populations of Lanzarote, where the existence of polyandry can be assessed «*la mayor parte de ellas tienen tres maridos y sirven por mes, y el que debe tenerla después, la sirve todo el mes que el otro la tiene*» (Cabrera Pérez, 1992: 86).

The island of El Hierro, on which this study focuses, is no exception to the rest of the Archipelago regarding the cult of the ancestors and the collectivism as a funerary practice. Despite its meager demography, we also find big burial spaces (Jiménez Gómez, 1993). The dates of some of them show that they were used throughout long periods, even reaching a thousand years of use (Velasco et al. 2005b). One of the best-studied sites of this kind is the necropolis of La Lajura. On this site, a large number of human remains were found. Many of them were in anatomical position, while others were in secondary position as a result of a readjustment of the sepulchral space so that more individuals could be deposited. This reinforces the idea that the most important criterium within the funeral rite was that the individuals were deposited in the same place as their ancestors. The archaeologists also identified an initial preparation of the space, which has been interpreted as an act of "sacralization" of the place.

Although the site of La Lajura is one of the best excavated collective burials and therefore has helped us to understand the configuration of this type of funerary site, it is not the only one in El Hierro. In different parts of the island, researchers have found burial spaces with very similar configurations. Nevertheless, as a consequence of different circumstances, they have not been excavated with the desirable meticulousness (Trujillo Mederos 2018). Even so, from many of them, it has been possible to recover an important amount of very well-preserved human remains, that have been analyzed from different bioanthropological perspectives. Such would be the case of Punta Azul, the object of study of the present work (Ordóñez, 2017).

The Punta Azul cave is located on the coast of Taibique, at about 375 meters above sea level. It is a cave 15 meters deep. In the 1940s, J. Álvarez Delgado and L. Diego Cuscoy performed the first investigations of this site. The first excavation campaign took place in 1946 and was published in 1947. At this moment, they found five complete and five fragmented skulls, 18 mandibles, and 10 femurs, as well as various fragments of long bones and several short bones, besides numerous human remains pieces of leather, of basaltic rock, and goat's horns (Álvarez Delgado 1947).

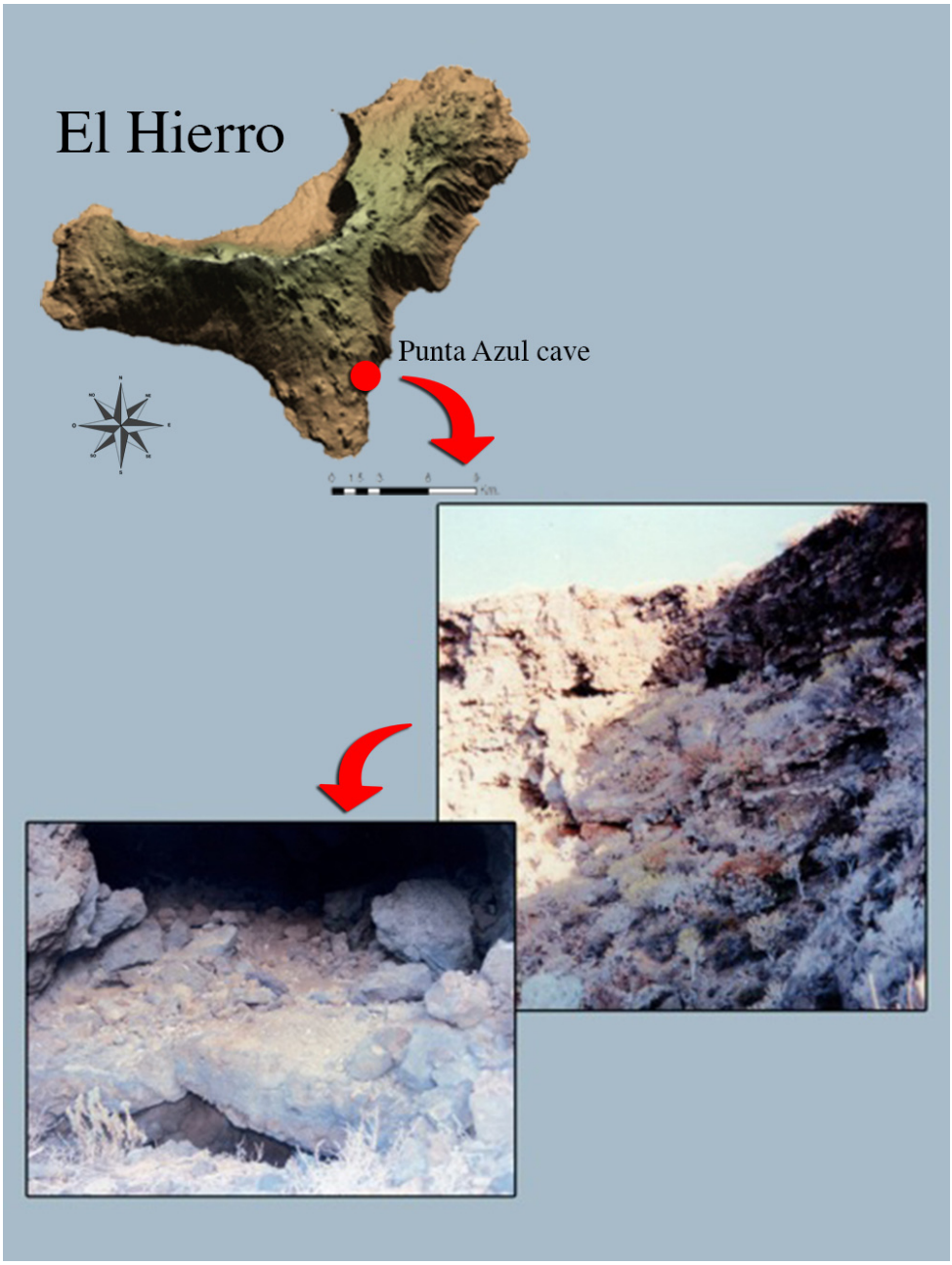

Fig. 1. Localization of Punta Azul.

After a long period of abandonment, the studies of this site were reprised in the 1990s (Trujillo Mederos 2018). This time, the archaeologists found a highly altered space, and they decided to recover the human material by anatomical regions. They retrieved more than 4000 pieces belonging to at least 100 adult individuals and 17 subadults. As far as it was possible, due to the highly altered archaeological context, the researchers saw that the funerary ritual could be similar to the one later described for La Lajura.

Within the framework of the study of the genetic characterization of the aboriginal population of El Hierro, and taking into account the social element in the organization of collective sepulchral spaces, this study addresses for the first time the mitochondrial DNA (mtDNA) analysis of a large, well-preserved bone sample, recovered from the cave of Punta Azul.

MATERIAL AND METHODS

Among the material recovered in the burial, there were 169 tibia fragments. For this study, we chose 61 adult tibias. They were 29 right and 32 left. The criterium for their selection was that they were well preserved. Thanks to the study of the amelogenin gene, it was possible to establish the sex of 54 tibiae, with 36 men and 18 women. Two of the tibiae were dated by Carbon 14. They would have a chronology between 1050 and 1210 AD (Ordóñez et al. 2017). Various studies performed on this material have allowed confirming its good macroscopic and microscopic preservation. Among them, we must highlight those related to paleopathology (Mas Pascual et al. 2000; Arnay de la Rosa et al. 2010) and those that have to do with paleodiet and paleonutrition (Arnay de la Rosa et al. 1994; Velasco Vázquez et al. 1997).

Sample selection and sampling

We chose those tibias that were intact and with the minimum of fissures or alterations of their cortical bone tissue. The bones were manipulated using gloves at all times and in sterile conditions to avoid contamination with modern DNA (Cooper y Poinar, 2000; Pääbo et al. 2004).

As a measure to remove the possible contaminant DNA, we covered the tibiae with aluminum foil, leaving only a small surface exposed for the sample extraction. Then, we mechanically sanded this surface to remove approximately 1 mm of the bone surface. Afterward, using a dentist's drill, we obtained bone powder from the cortical bone through the previously sanded surface.

DNA extraction

We extracted the DNA from the bone powder using the PrepFiler™ BTA method in an AutoMate Express™ (Applied Biosystems) equipment. The PrepFiler™ BTA method uses silica covered magnetic beads, and it has proven to be highly effective in the recuperation of degraded DNA (Betancor et al. 2011). Furthermore, the automatized extraction of the samples implies a decrease in handling time, and hence a lower exposition to modern contaminant DNA. The DNA was isolated from bone powder using a protocol based on guanidine thiocyanate and the use of silica columns (Maca Meyer et al. 2001; Maca Meyer et al. 2005; Casas et al. 2006). All the analyses were carried out following the authenticity criteria required for ancient DNA (aDNA) studies (Cooper y Poinar, 2000, Pääbo et al. 2004).

Real-Time Quantification

We quantified the samples through quantitative polymerase chain reaction (qPCR) to check their preservation state. The aim was to estimate the number of mtDNA and nuclear DNA copies present in the samples. The quantification was made using the 7500 Real-time PCR system from Applied Biosystems. For nuclear DNA detection, we performed an independent amplification of small fragments of the X and Y chromosome in the amelogenin locus (71bp). This procedure also allows us to know the genetic sex of the individuals. The qPCR also included an internal PCR control (IPC), that can identify possible inhibition issues (Fregel y Delgado, 2011). We performed the mtDNA quantification following previously published protocols (Almeida et al. 2011; Fregel et al. 2011), optimized for the use of the Applied Biosystems equipment.

The PCR mix included 10 µl of Taqman Universal Master Mix no UNG (Applied Biosystems), 1 µl of the primers set, 0.5 µl of AmpliTaq Gold, 0.32 µl of BSA, and 2 µl of each sample. The PCR conditions were of 10 minutes at 95°C and 45 cycles of 15 seconds at 95°C and 60 seconds at 60°C.

mtDNA Study

For the sequencing of the hypervariable region I (HVRI) fragment (positions 16,000-16,400) of the mtDNA, we used primers pairs and PCR conditions previously published (Maca-Meyer et al. 2004). We used 7 overlapping fragments with a longitude between 100 and 150 base pairs (bp). The PCR fragments were directly sequenced, using the forward and reverse primers of the amplifications.

For the sequencing reactions, we used the BigDye v3.1 Terminator Cycle Sequencing kit from Applied Biosystems, and we ran them in an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) following the manufacturer's instructions. We analyzed the DNA sequences using the BioEdit Software v.7.0.9.0 (Hall 1999), we obtained the haplotypes through the HaploSearch software (Fregel and Delgado, 2011), and we then confirmed them through manual inspection of the electropherograms. We followed Fregel et al. (2009) for the RFLP assignation of haplogroups (H, H1, H3, U, JT, and L1'2'5'6). For the haplogroup nomenclature, we followed the phylogenetic tree Build 16 (Van Oven and Kayser, 2009).

In order to validate our results, the University of the Basque Country (UPV/EHU) replied ten samples (PA-128, PA-141, PA-173, PA-174, PA-202, PA-203, PA-213, PA-229, PA-241, PA-244, and PA-268), sequencing the HVRI (positions 15,998 - 16,400) and HVRII regions (16,504 - 429).

Additionally, we obtained genome-wide sequences from four samples (PA-102, PA-125, PA-177 y PA.217) through next-generation sequencing (NGS) to confirm sex, mtDNA and contamination levels.

RESULTS

The values obtained by the qPCR allow us to determine that remains are well preserved, with low levels of inhibition. The Punta Azul remains have an average mtDNA content of 3,000 copies (3,015.0 ± 459.8 copies/µl). The mean value of the IPC is 24.7 +/- 0.4. From the values obtained, six samples (9.8%) were discarded for the amelogenin analysis, a very low percentage when considering aDNA samples.

Sex determination was possible for 55 individuals accounting for a success ratio of 88.5%. In our sample, we had 36 men (66.7%), and 18 women (33.3%).

For the mtDNA, we had an amplification success ratio of 90.2% (55 samples). Nevertheless, the amplification and sequencing of the HVRI were only possible in 54 samples. The mtDNA composition of the sample from Punta Azul is characterized by the complete fixation of the H1cf lineage. The replication performed in the Basque Country confirmed the fixation of the H1cf haplotype. The samples classification as H1 was confirmed for all the samples except for PA-241 and PA-268 that could not be amplified.

NGS also confirmed the obtained results. There was a match in the sex and mitochondrial haplotype for all the analyzed samples. Besides, all the samples

belonged to different individuals and had very low contamination levels (PA-102: 0.09%, PA-125: 0.12%, PA-177: 0.02% y PA-217: 0.22%). All these results are in agreement with the authenticity of the results.

DISCUSSION

One of the most relevant genetic results of this study is the complete fixation of the H1cf haplogroup in the Punta Azul people. This can be a consequence of different factors. First, we must take into account that a bottleneck event could explain the decrease of the observed diversity. These types of episodes are characterized by an important decrease in the population size at a certain moment, in such a way that the following generations will have lower genetic variability, and therefore the allelic proportions in the population as a whole will be altered (Fontdevila and Moya 2000). Most probably, the first inhabitants of El Hierro found difficulties in adapting to a new environment with very different characteristics from those found in their places of origin. Although this new territory had a variety of resources, the exploitation possibilities would have been limited in the first moments due to unfamiliarity with the resources distribution and the most profitable way to exploit them. That means that a bottleneck episode was plausible at the beginning of the island's colonization. This population reduction would have been later overcome once the aborigines from El Hierro started to know and control the exploitation of the resources the island had to offer (Ruíz González 2008).

Genetic drift would be another factor that could have produced the observed low diversity. Genetic drift is a stochastic process that happens because of the finite size of populations, in such a way that some alleles are lost by chance and not as a consequence of natural selection (Fontdevila and Moya 2000). Usually, this phenomenon is compensated by the arrival of new individuals to the community, which increases diversity. However, this is not the case of an isolated population like that of El Hierro. The small size of the island's population could have also increased the impact of genetic drift in diversity.

By last, we should mention the founding effect as a possible cause for the loss of diversity. It implies a decrease in diversity when a subset of a certain population gives origin to a new colony (Fontdevila and Moya 2000). This effect would have taken place during the colonization of the Archipelago.

In the case of the population of Punta Azul, we are probably in the presence of a combination of these three factors, although the proportion of each one of

them is quite difficult to determine. Nevertheless, if these were the only factors responsible for the low diversity, they should have affected other uniparental markers, like the Y-chromosome, in the same way. Our results also show a decrease in the genetic diversity of this marker, although it has a much less acute impact than that suffered by matrilineal lineages. This differentiated behavior of the two markers must be explained by mechanisms that go beyond biological factors. That is the reason why cultural factors should be taken into account to find possible answers to these results (Ordóñez 2017). This possibility has been discussed in previous works. We have proposed that the matrilineal behaviors observed in the aboriginal populations of the Canary Islands could have played an important role in the explanation of this phenomenon. The ethnohistorical sources seem to witness, although in a rather imprecise way, behaviors with a matrilineal base in all the Islands (Rodríguez Rodríguez, A. 2006; Santana Cabrera 2018). They are especially well documented for the Island of La Gomera, where a matrilineal system of heritage transmission is specifically described (Zurara 1998:355-356). Also, matrilineal heritage is not uncommon in North-African Berber populations (Camps 1980), where the Canarian aboriginal populations came from (Fregel et al. 2018). Regarding the island of El Hierro, the only ethnohistorical references talk about the important religious and symbolic role of some women. This would be the case of the "king's" daughter, who supposedly had special skills. She took part and acted as an intermediary in certain special celebrations (Tejera y Montesdeoca 2004:49; Frutuoso 1964:132-134).

Fig. 2. Aboriginal woman from El Hierro according to Torriani.

The results obtained for the site of Punta Azul are the first ones that allow us to achieve a greater definition on the topic of the possible existence of matrilineal-

based behaviors among the aborigines from El Hierro, at least regarding certain funeral behaviors. The fact that all the analyzed individuals have the same mitochondrial haplogroup shows us that they were all related and shared a female ancestor. This would imply choosing a specific burial space determined by its connection with the maternal lineage, that is, following a funeral matrilocality criteria (Ordóñez 2017).

CONCLUSIONS

Although El Hierro is the island with less ethnohistorical information on this subject, the results of this study seem to be one of the first empirical results to point out the link of the members of a community through a maternal lineage. This would be related to the cult to the ancestors, but not just any ancestor, as it would be with those related through the maternal lineage. Although for now, it is just a working hypothesis that should be tested in other burials of El Hierro and other islands, it still signifies an important milestone in the understanding of kinship relationships in the Islands, specifically in El Hierro.

The genetic research started in the burial site of Punta Azul has provided new data to understand the real dimension of women's roles in the Canarian aboriginal societies, specifically in the Island of El Hierro. This would be framed in current gender studies that have been started in the bioanthropological field.

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