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Genetic sexing to determine the optimal discriminant functions for the analysis of archaeological remains from El Hierro (Canary Islands)

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ABSTRACT

A correct sex assignment of a given bone or bone fragment is of paramount importance for the archaeologist, anthropologist and in forensic medicine. Discriminant functions, combining several anthropometric measurements obtained from individuals with known sex are useful tools for this purpose, but it is essential to know exactly the sex from which the measures are obtained. This is an easy task in modern populations, but it is problematic in ancient ones, since even when the entire skeleton is available, diagnosis of sex is not 100% accurate. Sexing by genetic methods by amplifying the first intron of the amelogenin gene constitutes a much more accurate method for sexing bones and may be the gold standard for further elaboration of discriminant functions which may serve for sexing new bones dug up in future excavations. With this aim we have genetically sexed 52 (out of 59) tibiae belonging to the prehispanic population of El Hierro, in the Canary Islands, identifying 18 women and 34 men, and then, performed discriminant functions combining several anthropometric variables. These functions show a high accuracy in sex diagnosis (94.2%; area under ROC curve = 0.954 with the best of the functions), so that they allow correct sexing of tibiae or tibiae fragments (only proximal third, distal third or midshaft). Thus, genetic sexing obviates the problem of finding an accurate gold standard for the elaboration of discriminant functions for ancient bones. This method could be applied to other populations of different antiquity and different ethnicity.

1. Introduction

A correct sex assignment of a given bone or bone fragment is fundamental for the archaeologist, anthropologist and in forensic medicine. Although a correct sex estimation is a relatively easy task when the entire skeleton is available, a problem arises when parts of the skeletons, or bone fragments, are the sole remains. Sexing of long bones usually relies on discriminant functions which include several dimensions measured following standard criteria, combined in a mathematical formula. Therefore, discriminant functions for almost every bone of the entire skeleton have been obtained. They show a remarkable accuracy in diagnosing sex, especially those performed on long bones such as femur, humerus, ulna and tibia (Iscan and Miller-Shaivitz, 1984; Ubelaker, 1989). It is important to keep in mind, however, that functions differ according to major racial or even population groups of different geographical areas (Iscan et al., 1994; Iscan and Ding, 1995). A function which is obtained from a certain population group may accurately assign the correct sex in a proportion of cases of this population, but accuracy is lower if we apply the same function to a different population group. This is also true if we try to apply a discriminant function obtained from a modern population to an ancient one, even from the same geographical area, since, along centuries, and especially in the last 200 years, mobility of people has become a worldwide phenomenon, that has led to a progressive population mixing and a decrease in racial differences (Farkas et al., 2004). Moreover, there is a trend in human beings to a stature increase along the centuries, which may reach, in some populations, to10 to 30 mm per decade (Bubas et al., 2012).

Therefore, when dealing with ancient skeletal remains, ideally, a discriminant function should be available for each skeletal bone of

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the individuals of a given geographical area in a given period, in order to properly assign sex when a new bone is dug up. This is difficult to achieve, and it faces a major problem, which is the finding and definition of an accurate gold standard, a necessary previous step for elaborating a discriminant function. Even examination of the pelvis does not allow a 100% accuracy in the diagnosis of sex, so when a function is performed using ancient bones, there might be a bias in the selection of male and female samples.

In recent years it has been possible to determine sex in ancient bones by genetic methods (Stone et al., 1996; Schmidt et al., 2003; Luptáková et al., 2011). This has led to a considerable progress in this field, solving the problem of having an accurate gold standard for sex, which allows a precise assessment of metric and non-metric characteristics of men and women of a given population. However, genetic sexing is an expensive procedure. It is difficult to apply to every new bone dug up in every new excavation. Consequently, discriminant functions are still necessary. In this scenario, genetic sexing allows an accurate sex assignment, and hence, the definition of a precise gold standard, an invaluable tool for performing discriminant functions which can be applied in the future to new bone remains of individuals of similar antiquity.

Based on these facts, we performed the present study with two main objectives: 1) to assess, by genetic methods, sex of right tibiae and 2) to elaborate discriminant functions based on metric measurements of those right tibiae belonging to the prehispanic population of El Hierro, in the Canary Islands, which sex was assessed genetically.

2. Materials and methods

2.1. Sample

Fifty-nine well-preserved right tibiae were included in this study. They belong to the prehispanic population of the island El Hierro, in the Canary Archipelago, specifically to individuals buried in a volcanic cave in Punta Azul (Fig. 1). This cave is located in the southern slopes of El Hierro, at an altitude of about 150 m above sea level. The central mountains of the island El Hierro (27° North, 18° West), reach heights of 1500 m. North-eastern trade winds lose their moisture on the northern slopes of the island, so when they reach the southern coastal area they are dry, and relatively hot and strong. The cave is therefore located in an arid, windy environment, where temperatures do not vary much along the year (about 18–26°). These climatic conditions are sometimes interrupted by uncommon Atlantic storms arriving from the southwest, or by eastern-southeastern invasions of hot air from the Sahara desert.

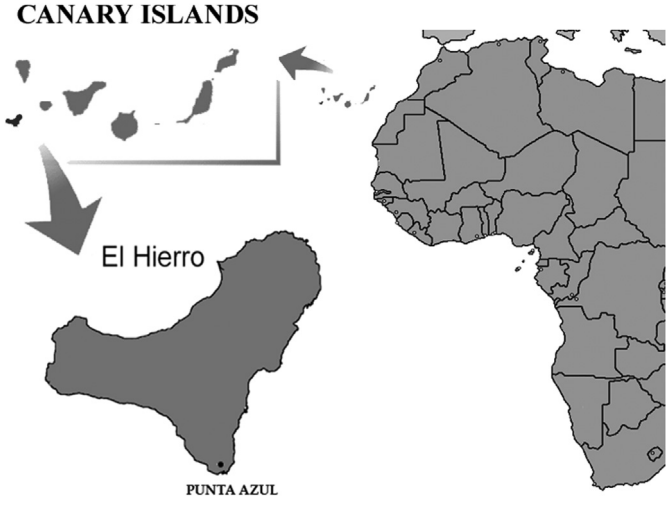

Fig. 1. The island El Hierro in the Canary Archipelago, with the burial site of Punta Azul in the most southern point of the Island.

Punta Azul is one of the most important burial caves in El Hierro, containing skeletal remains of more than 100 individuals. The cave had already been plundered when excavated by members of the Department of Prehistory of the University of La Laguna (Tenerife, Canary Islands) about 18 years ago (Velasco-Vázquez et al., 2005). It contained about 100 corpses, which were deposited on stony or vegetal layers, but not interred. This burial procedure undoubtedly favored preservation of the bones, especially in an arid environment such as that of the southern slopes of El Hierro. This material has served to perform several studies dealing with paleonutrition (Arnay-de-la-Rosa et al., 2010), assessment of bone lead and cadmium contents (González-Reimers et al., 2005), and description of several cases of Klippel-Feil disease (González-Reimers et al., 2001), an uncommon clinical entity with a seemingly high prevalence among the prehispanic population of the Canary Islands.

2.2. Genetic analysis

2.2.1. DNA extraction

The previous manipulation of the archaeological samples was always done using gloves, and under strict sterile conditions, to avoid contamination with modern DNA (Cooper and Poinar, 2000; Pääbo et al., 2004). In order to remove the possible modern DNA contaminants due to manipulation, bone surfaces were exposed to UV light for 10 min and at least 1 mm of the bone surface was mechanically polished using a dental drill. The initial decontamination step commonly used for ancient teeth samples, consisting of washing with 15% HCl and rinsing with ddH_2O, was not used due to the porosity of the bone and the negative effect of this step in endogenous DNA integrity.

Using a dentist's drill, bone powder was obtained from the cortical portion of the long bones, previously removing the superficial part of the cortical. The resulting powder was used to extract DNA using the PrepFiler Express BTA™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA, USA). To avoid contamination due to human manipulation, the samples were automatically extracted using the AutoMate Express™ Forensic DNA Extraction System (Applied Biosystems, Foster City, CA, USA). In those cases where the DNA concentration was too low to perform amelogenin analysis, several independent extractions of the same individual were made and then concentrated using the Amicon® Ultra-0.5 (Millipore) system.

2.2.2. Mitochondrial DNA quantification by qPCR

It is known that the DNA recovered from ancient samples is mostly composed by small size molecules, with abundance of multi-copy loci, like mitochondrial DNA (mtDNA) in comparison with the nuclear DNA (Higuchi et al., 1984; Pääbo, 1985).

Therefore, before performing the molecular sexing, the number of mtDNA copies was estimated by qPCR, using a Taqman mitochondrial-specific assay (Almeida et al., 2011; Fregel et al., 2011) that includes three different primer/probe sets. Two of the amplicons are human-specific and allow the detection of DNA degradation, as they are 167 and 314 bp long, respectively. The third amplicon is interspecific, and could be amplified for a wide range of organisms (from primates to insects), allowing us to detect the presence of non-human DNA. For the mtDNA real-time analysis, the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) kit was used in the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as detailed in Fregel et al. (2011).

As an authenticity criterium, only those individuals where mtDNA was amplified were subjected to the analysis of the