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ORIGINAL ARTICLE

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Sexual dimorphism: a comparative study between the prehispanic inhabitants from El Hierro and other populations of the world

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SUMMARY

Sex estimation based on tibial measurements can be achieved using discriminant functions combining several parameters. However, functions differ from one population to another, because sexual dimorphism may be more or less marked among different ancestry or ethnic groups. Calculation of one of these functions with the dimensions of populations other than that from which the function was obtained may misclassify a different proportion of males or females than when calculated with the dimensions of the original population. By dividing the proportions of correctly classified individuals when the function was applied to the population from which it derived and that of El Hierro (Canary Islands), we can calculate an index of male trait expression and an index of female trait expression, and, by addition of both indices, an index of sexual dimorphism. Therefore, it is possible to compare the degree of sexual dimorphism among several populations, at least regarding those measurements included in the function. Based on this fact we have calculated several functions (reported in the scientific literature), obtained from tibiae of modern black, white, and Japanese populations, and from medieval Croatians and prehispanic inhabitants of Gran Canaria (ap. 1000 BP), with the dimensions of the prehispanic population of El Hierro, genetically sexed, also with an antiquity of ap. 1000 BP. Despite the different antiquity, the population of El Hierro was more dimorphic that the modern Japanese one, but less dimorphic than most of the other groups with which it was compared, especially when functions including distal epiphyseal breadth and minimum shaft perimeter (near the distal end of the tibiae) were calculated: in these cases, dimorphism was lower for the population of El Hierro, due to the fact that, although male trait expression index was higher, many females of El Hierro were misclassified as males because of the abnormally thick distal diaphyseal and epiphyseal breadths of El Hierro inhabitants.

Key words: Sexual dimorphism – Bone sexing – Discriminant functions – Canary islands – Prehispanic population

INTRODUCTION

A correct sexual assignment of a given bone or bone fragment is of paramount importance for the archaeologist, anthropologist, and in forensic medicine. Correct sex estimation is a relatively easy task when the entire skeleton is available, but sexing of long bones usually relies on discriminant functions which include several dimensions taken following standard criteria. In this way, sex has been assessed from several long bones such as

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femur (Iscan and Ding, 1995; DiBennardo and Taylor, 1983); scapula (Dabbs and Moore-Jansen, 2010), humerus (Dittrick and Suchey, 1986) and other upper limb bones (Holman and Bennet, 1991; Albanese, 2013); tibia (Iscan and Miller-Shaivitz, 1984); mandibles (Franklin et al., 2008); cranial bones (Kalmey and Rathburn, 1996); short bones (Steele, 1976; Bidmos and Asala, 2003; Bidmos and Dayal, 2003); metacarpal bones (Barrio et al., 2006), and even teeth (Pereira et al., 2010), among other studies.

It is important to keep in mind, however, that discriminant functions differ according to major ancestral or even ethnic differences. A function which was obtained from a certain population group may accurately assign the correct sex in a certain proportion of cases. If we apply the same function to a different population group, it may occur that the proportion of correctly classified males or females, or both, is higher than that obtained for the population from which the function derived. For instance, fewer “foreign” males may be misclassified as females and fewer “foreign” females as males. By dividing the proportions of correctly classified individuals obtained for the foreign population from that obtained for the original one, we can calculate an index which represents the ratio of accuracy of the function of correctly classifying sex in the foreign population compared with the original one. If the function shows better accuracy for the correct diagnosis of both sexes in the foreign population than in the original one, then we can conclude that the foreign population shows a more intense dimorphism, of course regarding those parameters which entered the function.

In this kind of studies, it is of paramount importance that the diagnosis of sex is firmly based. Diagnosis of sex based on anthropometric features, even when the entire skeleton is available, is still associated with some uncertainty. Recently, genetic analysis based on the differences in the amelogenin gene in males and females allow accurate sexing of ancient bones (Faermann et al., 1995; Stone et al., 1996). In this sense, we have performed a study on the ancient population of El Hierro (Canary Islands), being able to accurately sex 52 tibiae (Ordóñez et al, 2013).

Based on the aforementioned statements, in the present study we compare sexual skeletal dimorphism of the tibiae of the prehispanic population of El Hierro with that of several other populations of the world. We also tried to discern which of the tibia dimensions are more or less dimorphic among the population of El Hierro when compared with the foreign populations.

MATERIALS AND METHODS

Study population

It belongs to the prehispanic inhabitants of El Hierro, in the Canary Islands. All the sample was found in a single burial volcanic cave (Punta Azul, located in the southern part of the Island), which contained remains of about 120 individuals. Corpses were intermingled at the time of excavation. Although no radiocarbon dates are available for this sample, the antiquity of the bones found in a similar burial cave located nearly to Punta Azul yielded an antiquity ranging from 875-1000 BP.

As previously published, it was possible to define sex in 52 tibiae corresponding to adults buried in this cave (Ordóñez et al., 2013). Briefly, the sexing method included amplification of a fragment of the amelogenin gene, which is present both in the X chromosome and in the Y chromosome, but with differences that allow sex determination by polymerase chain reaction (PCR). Indeed, the first intron of the amelogenin gene includes a 6 base pairs (bp) deletion for the X chromosome (Sullivan et al., 1995), a deletion which is absent in the Y chromosome. For a more sensitive detection of the different fragments, the amplification of the amelogenin gene was made through qPCR, using two Taqman probes that are specifically attached to the X and Y chromosome (Fregel et al., 2011), as well as an internal control to detect the presence of inhibition (Hudlow et al., 2008). The TaqMan Universal PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA) was used in the real time thermal cycler 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

This sexing methodology has several advantages. The small length of the fragments (71bp) allows for the quantification of severely degraded DNA, whereas the use of two distinct primer sets for X and Y chromosome amplification helps reduce allelic dropout in low copy number analysis. To avoid allelic-dropouts, at least three independent amplifications were performed in those samples sexed as females. In this way the possibility of erroneously sexing a male as a female, as a consequence of the elimination of the Y -allele during the first cycles of the PCR was diminished. Furthermore, when possible, two independent extractions of the same individual were performed in the same laboratory, with a time separation and made by different investigators.

As detailed elsewhere (Ordóñez et al., 2013) for genetic assessment of sex, only intact tibiae were chosen. The sample included 31 left tibiae (21 males and 10 females), and 21 right ones (13 males and 8 females) Although left and right tibiae were quite different, and probably belong to different individuals, the possibility that some pairs belong to the same individual cannot be fully excluded.

Anthropometric measurements

The following tibial measurements were obtained using standard methods (Martin, 1914; Martin and Saller, 1957).

Tibial length, from the medial malleolus to the lateral condyle taken with an osteometric board.

Circumference at the nutrient foramen level with a plastic covered cloth tape.

Minimum shaft circumference, usually located near the distal end of the tibia, also measured with a plastic covered cloth tape. Anteroposterior and transverse diameters at the nutrient foramen levels, with a sliding caliper.

Proximal epiphyseal breadth, as the maximum distance between the condyles.

Distal epiphyseal breadth, as the distance between the medial malleolus and the centre of the fibular notch.

These measurements were performed both to left (31 cases) and right bones (21 cases).

Compared populations

We have selected data derived from published discriminant analyses looking for sex differences using anthropometric parameters of the tibiae. Specifically, we looked for studies which included individuals of different ancestry (black, white, Asians), modern, and ancient individuals (about 1000-1200 BP), and selected those functions in which several variables were combined. The utilized functions are listed in Table 1 (the number of the functions is the same they have in the original papers where they were published), and the populations from which they were obtained are:

- A modern Japanese population composed of 90 skeletons. Three functions (Japan 1 to Japan 3) include a multivariate analysis with some of the parameters mentioned before (Iscan et al., 1994)

- A North American sample from the Terry Collection of the Smithsonian Institution composed of 40 white men, 39 white women, 40 black men and 40 black women. Six functions in the white and black populations (Iscan and Miller-Shaivitz, 1984) were compared. These functions were obtained from the left tibiae.

- Two functions derived from South African white population preserved at the Universities of Witwatersrand and Pretoria (South Africa 9,2; Stein and Iscan,1997).

- Two functions from prehispanic population from Gran Canaria, belonging to the anthropological collection of Museo Canario, in the city of Las Palmas (GC 1,2). Antiquity of these samples is about 700-1000 BP. These functions were obtained from the right tibiae (González-Reimers et al., 2000).

-Three functions derived from a Medieval Croatian population, with an antiquity of 1000-1250 BP (Slaus and Tomicic, 2005).

We calculated these functions with the dimensions obtained from the prehispanic population of El Hierro (Table 2), and we have calculated the

Table 1. Discriminant functions employed in this study

Author and function number	**Function**	**Tibia analysed**
Iscan white 5	(DEB * 0.25418) + (MinC * 0.07209) -16.84382	L
Iscan black 5	(DEB * 0.17093) + (MinC * 0.12175) -16.94451	L
Iscan white 4	(PEB * 0.23460) + (DEB * 0.09773) -21.35923	L
Iscan black 4	(PEB * 0.25400) + (DEB * 0.01385) -19.08374	L
Iscan white 6	(PEB * 0.28795) – (TL * 0.00162) – 20.14831	L
Iscan black 6	(PEB * 0.25109) + (TL * 0.00247) – 19.19673	L
Iscan white 7	(PEB * 0.23541) + (CNF * 0.04210) - 20.79471	L
Iscan black 7	(PEB * 0.24019) + (CNF * 0.01970) – 19.32619	L
Iscan white 8	(PEB * 0.20804) + (DEB * 0.08792) + (CNF * 0.05585) – (TL * 0.01019) – 20.42235	L
Iscan black 8	(PEB * 0.23173) + (DEB * 0.00904) + (CNF * 0.01716) + (TL * 0.00161) – 19.49878	L
Iscan white 9	(PEB * 0.20121) + (DEB * 0.12689) + (CNF * 0.05934) – (TL * 0.00817) + (APDNF * 0.11453) – (MinC *0.08337) -20.45133	L
Iscan black 9	(PEB * 0.23300) + (DEB * 0.03454) – 19.52740	L
Iscan Japan 3	(DEB * 0.365927) + (MinC * 0.0449891) - 18.94278	NS
Iscan Japan 2	(PEB * 0.2447224) + (CNF * 0.0213940) – 19.02956	NS
Iscan Japan 1	(PEB * 0.1529167) + (DEB * 0.2284790) – 20.58253	NS
South Africa 9	(PEB * 0.1237026) + (DEB * 0.2218177) – 19.77125	NS
South Africa 2	(PEB * 0.1078602) + (DEB * 0.24325580) + (APDNF * 0.15334320) + (TDNF * 0.06750036) – (MinC * 0.09845310) -19.48625	NS
Gran Canaria 1	(TDNF * 1.105) + (MinC * 0.498) -57.89	R
Gran Canaria 2	(TDNF * 1.315) + (PEB * 0.523) -67.76	R
Medieval croat 1	(TL * 0.007) + (PEB * 0.162) + (DEB * 0.031) - (APDNF * 0.051) + (TDNF * 0.041) + (CNF * 0.098) -24.051	L
Medieval croat 2	(PEB * 0.187) + (CNF * 0.103) – 22.803	L
Medieval Croat 3	(PEB * 0.222) + (DEB * 0.081) – 20.29	L

DEB=distal epiphyseal breadth; MinC= minimum shaft perimeter; PEB= proximal epiphyseal breadth; TL= tibial length; CNF= circumference at the nutrient foramen; APDNF= anteroposterior diameter at nutrient foramen; TDNF= transverse diameter at nutrient foramen; L=left; R=right; NS= not specified

Table 2. Single anthropometric measures (in mm) included in the discriminant functions

	Genetically assessed sex	N	Mean	Standard deviation
Tibial length	1,00	33	352.94	22.61
	2,00	18	324,83	17.05
Proximal epiphyseal breadth	1,00	33	74.79	5.50
	2,00	18	66.94	2.98
Distal epiphyseal breadth	1,00	33	51.12	4.61
	2,00	18	46.00	5.43
Circunference at nutrient foramen	1,00	34	97.56	7.36
	2,00	18	82.94	3.61
Anteroposterior diameter at nutrient foramen	1,00	34	36.03	3.32
	2,00	18	32.56	7.11
Transverse diameter at nutrient foramen	1,00	34	24.00	1.78
	2,00	18	20.78	1.70
Maximum midshaft perimeter	1,00	34	87.03	6.96
	2,00	18	73.11	3.20
Anteroposterior diameter at midshaft	1,00	34	31.47	3.48
	2,00	18	26.56	2.38
Transverse diameter at midshaft	1,00	34	21.68	1.79
	2,00	18	19.00	1.68
Minimum shaft perimeter (distal end)	1,00	34	84.24	7.50
	2,00	18	73.72	4.59

Sex: 1=males; 2= females

Table 3. Results of the indices calculated in this study when applied to the total number of tibiae from El Hierro

	Accuracy original population (men) (a)	Accuracy original population (women) (b)	Accuracy population from el Hierro (men) (c)	Index of male trait expression (c/a)	Accuracy population from El Hierro (women) (d)	Index of female trait expression (d/b)	Index of dimorphism (c/a + d/b)
Iscan white 5	75%	84.6%	90.91	1.212	66.66	0.788	2.000
Iscan black 5	82.5	85	87.88	1.065	72.22	0.822	1.887
Iscan white 4	85	84.6	75.76	0.891	94.44	1.116	2.007
Iscan black 4	87.5	92.5	69.70	0.797	100	1.081	1.878
Iscan white 6	82.5	87.2	75	0.909	94.44	1.083	1.992
Iscan black 6	87.5	92.5	65.63	0.75	100	1.082	1.832
Iscan white 7	87.5	84.6	75.76	0.866	94.44	1.116	1.982
Iscan black 7	87.5	92.5	69.70	0.797	100	1.081	1.878
Iscan white 8	85	84.6	84.34	0.992	94.44	1.116	2.108
Iscan black 8	85	92.5	68.75	0.809	100	1.081	1.890
Iscan white 9	87.5	84.6	84.34	0.964	83.33	0.985	1.949
Iscan black 9	87.5	95	50	0.571	100	1.053	1.624
Iscan Japan 3	87	79.4	96.97	1.115	83.33	1.049	2.164
Iscan Japan 2	93.3	79.4	90.91	0.974	83.33	1.049	2.023
Iscan Japan 1	93.2	79.4	90.63	0.972	61.11	0.770	1.742
South Africa 9	89.3	92	75	0.840	94.44	1.027	1.867
South Africa 2	87.5	94	46.88	0.536	88.89	0.946	1.482
Gran Canaria 1	95.54	100	97.06	1.016	61.11	0.611	1.627
Gran Canaria 2	97.77	100	81.82	0.837	100	1.000	1.837
Medieval croat 1	92.7	91.7	78.13	0.843	100	1.091	1.934
Medieval croat 2	91.7	91.7	84.85	0.925	100	1.091	2.016
Medieval Croat 3	84.4	91.7	71.88	0.852	100	1.091	1.943

proportion of males and females from El Hierro correctly classified as males or females, respectively, with each of these functions. We further divided the proportions of correctly classified males and females from El Hierro by the proportions of correctly classified males and females when the function was applied to the population from which it was derived (these data are usually provided by the authors in the corresponding papers). So we calculated an "index of male trait expression" and an "index of female trait expression". For example, if the proportion of correctly classified females is the same in the population of El Hierro and in the population from which the function was obtained, the index is 1, and female traits are equally expressed in both populations regarding the parameters which entered the function. Conversely, if the proportion of correctly classified females is higher in the population of El Hierro, then this means that the dimensions of the female population of El Hierro adapts better to the criteria of the index of female trait expression than those of the population

Table 4. Results of the indices calculated in this study when applied only to left tibiae from El Hierro

	Accuracy original population (men) (a)	Accuracy original population (women) (b)	Accuracy population from el Hierro (men) (c)	Index of male trait expression (c/a)	Accuracy population from El Hierro (women) (d)	Index of female trait expression (d/b)	Index of dimorphism (c/a + d/b)
Iscan white 5	75%	84.6%	95	1.266	60	0.709	1.975
Iscan black 5	82.5	85	90	1.091	60	0.706	1.797
Iscan white 4	85	84.6	89.47	1.053	80	0.946	1.999
Iscan black 4	87.5	92.5	68.42	0.782	100	1.081	1.863
Iscan white 6	82.5	87.2	78.95	0.957	90	1.032	1.989
Iscan black 6	87.5	92.5	63.16	0.722	100	1.082	1.804
Iscan white 7	87.5	84.6	80	0.914	100	1.182	2.096
Iscan black 7	87.5	92.5	70	0.800	100	1.081	1.881
Iscan white 8	85	84.6	84.21	0.991	90	1.064	2.055
Iscan black 8	85	92.5	68.42	0.805	100	1.081	1.886
Iscan white 9	87.5	84.6	89.47	1.023	80	0.946	1.969
Iscan black 9	87.5	95	52.63	0.601	100	1.053	1.654
Iscan Japan 3	87	79.4	95	1.092	100	1.259	2.351
Iscan Japan 2	93.3	79.4	95	1.018	80	1.008	2.026
Iscan Japan 1	93.2	79.4	94.70	1.016	70	0.882	1.898
South Africa 9	89.3	92	78.9	0.872	90	0.978	1.850
South Africa 2	87.5	94	42.10	0.481	80	0.851	1.332
Medieval croat 1	92.7	91.7	78.95	0.852	100	1.091	1.943
Medieval croat 2	91.7	91.7	85.00	0.926	100	1.091	2.017
Medieval Croat 3	84.4	91.7	68.42	0.811	100	1.091	1.902

from which the function was obtained.

By addition of both indexes we obtained an "index of dimorphism" of the population of El Hierro in relation with the compared population: an index of less than 2 means that the population of El Hierro is less dimorphic, whereas an index greater than 2 means that the population of El Hierro is more dimorphic, always in relation with the reference population and the analysed parameters.

RESULTS

Results regarding the crude values of the anthropometric parameters are shown in Table 2. In table 3 we show the proportions of correctly classified males and females from El Hierro, compared with the data obtained by the authors when they applied the functions to the populations from which the functions derived. We also show the male and female traits expression indices, as well as the dimorphism index. We have also shown the data when only left tibiae are included (Table 4). As shown in Table 1, whether the functions were obtained on right tibiae, left tibiae, or both tibiae, is specifically stated in some of the works mentioned in this manuscript, but not in others.

DISCUSSION

The population of El Hierro was, in general, less dimorphic than the original populations from which the functions derived, except for the functions 7 and 8 for the white population analysed by Iscan, the modern Japan population and the medieval Croatian one. However, true increased dimorphism –cases in which both males and females were more separated than the result obtained on the original population- were found only with the two functions derived from Japanese population. In the remaining cases, an index of dimorphism greater than 2 was obtained at the expense of exaggerated values of the indices of female or male traits expression but with low values of those of the other sex. The differences with the Japanese population are logical, given the lower degree of sexual dimorphism observed among Asians (Ubelaker, 1980).

Besides the equations derived from the Japan population, the lowest female trait expression indices – those derived from the functions with which the proportions of females misclassified as males were highest- were observed in those equations in which minimal shaft perimeter was included. This can be easily explained when we look at the crude data of the population of El Hierro: minimum shaft perimeter among females is among the highest values of the populations analysed. On the contrary, the highest female trait expression scores were observed when parameters such as perimeter at the nutrition foramen were included, or, to a lesser degree, proximal epiphyseal breadth, and tibial length. In other words, tibiae of females among El Hierro were "dysmorphic": relatively short bones with relatively wider epiphyseal breadths – especially the distal one-, and thick diaphysis at the distal end of the bones.

The same alteration seems to be present in the male tibiae: the index of male trait expression is higher when the parameter minimum shaft perimeter was included in the function, despite the fact that tibial length was shorter. In order words, the observed diaphyseal thickness, especially at the distal end of the bone, affected both males and females

An important problem which must be considered in the discussion of our results is the fact that the population included in the Iscan series and in the Steyn and Iscan studies on South African people deal with modern individuals, and that of El Hierro, with individuals with an antiquity of approximately 1000 years. Undoubtedly, differences in antiquity should correlate with differences in stature (Styne and McHenry, 1993). Therefore, it is to be expected that the male trait expression index of the population of El Hierro should be lower than that of modern populations. This was generally observed, but not when the distal breadth parameters (minimum shaft circumference and distal epiphyseal breadth were included. Conversely the index of female trait expression which should be higher than 1 among the population of El Hierro (theoretically smaller bones) was in fact lower than 1 when these parameters were included. These results reinforce the conclusion that bones from El Hierro population showed abnormally thick distal ends of tibiae.

The only comparable population in antiquity is the Medieval Croatian one, which showed similar indices of dimorphism despite higher values of tibial length and taller stature. Besides genetics (Lettre, 2009), socioeconomic conditions and nutrition (Lifshitz, 2009) may also play a role in adult stature. Coastal fishing, shellfish recollection and sheep- and goat herding were the main economic activity of the pre-urban prehispanic population of El Hierro (Abreu-Galindo, 1977). Only the prehispanic population of Gran Canaria showed a similar economic pattern (Arnay-de-laRosa et al., 2010) and also a similar antiquity, but again, when minimum shaft perimeter entered the functions, misclassification of females is considerable (female trait expression index of only 0.611). In addition, tibial length among El Hierro population was shorter. These data, indirectly, support the probably diverse origin of the prehispanic population of the Canaries, although, undoubtedly, they all share a North African origin (Maca-Meyer et al., 2004).

Therefore, the prehispanic population of El Hierro was more robust –both males and females, with some dysmorphic features: wider distal shafts and relatively smaller circumference at the nutrition foramen, although, in absolute terms the perimeter at the nutrition foramen was similar to that of other populations- or even greater.

What factors determine these changes? The population of El Hierro is a robust one. In many cases, tibiae show vascular marks, suggestive of increased vascularisation destined to an increased muscular mass (Trujillo-Mederos et al, 2013). Bending and torsional strength yielded unusually high values, especially among females (González-Reimers et al., 2014), who showed striking values of cortical thickness. Moreover, in study performed several years ago, no osteoporosis was observed (Velazco-Vázquez et al, 1999). Increased cortical thickness is influenced by several factors: indeed, genetics play a role, as well as sex steroids, but also IGF-1 and mechanical loading (Callewaert et al., 2010). The highly abrupt slopes of the island El Hierro (central mountains reach 1500 m, and total area of the island is only 273 square kilometres), and the economy, based on sheep- and goat herding, may have contributed to the robustness of their inhabitants.

The study here presented allows a comparison of sexual dimorphism among different populations, and may also suggest which of the anthropometric features carries more information regarding sexual dimorphism, always in comparison with other populations. It may also serve to disentangle some "dysmorphic" features of the population of El Hierro, raising new questions about lifestyle. It has also some limitations: when modern functions are applied to ancient populations, it must be taken into account the diachronic differences in stature and skeletal size. Unfortunately, discriminant functions on past populations are less frequent in the scientific literature, in part due to the difficulty in establishing an accurate sexing to be used as a gold standard. Accurate sexing is, undoubtedly an important issue. In the present study we clearly show that the application of a discriminant function obtained from a foreign population may lead to an unacceptably low accuracy in the diagnosis of sex.

In conclusion, we have compared sexual dimorphism of the population of El Hierro with that of modern populations and also medieval Croatians and prehispanic inhabitants of Gran Canaria. The population of El Hierro was more dimorphic than the modern Japanese one, but less than most of the other populations with which it was compared. Population of El Hierro showed abnormally thick distal ends of the tibial shaft and distal epiphyseal breadths, especially among females, which explains why many of them were misclassified as males when the functions obtained from foreign populations were calculated with the dimensions from El Hierro population. The abnormally thick distal ends of the tibiae may be interpreted as a trait of robustness, and is in agreement with other observations on this population.

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