# **RESEARCH ARTICLE**

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# The talus of the pre-Hispanic population from Punta Azul (El Hierro, Canary Islands): Variability and sexual dimorphism of nonmetric traits

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## Abstract

The aim of this study was assessing the prevalence of os trigonum, talo-calcaneal facets, and squatting facet variations among pre-Hispanic individuals (around 940 ± 30 BP) placed in the collective burial cave of Punta Azul (El Hierro, Canary Islands), and to assess whether prevalence differed among sexes. A total of 149 tali (70 right and 79 left) belonging to adult individuals were found in a comingled context. Sex determination was achieved as follows: genetic sexing of a subsample of 35 tali was assessed using paleogenomic procedures. Anthropometric measurements of the genetically sexed bones were used to calculate an accurate discriminant function that was later applied to the remaining 114 tali. We observed marked differences in the os trigonum pattern among male and female tali, whereas no relevant differences were observed regarding the other two traits. A very high prevalence of squatting facets was observed in both sexes, higher than the reported frequencies for other population groups. The summarized conclusions in this study are the following: (a) the high prevalence of the squatting facets observed in both males and females could indicate the performance of a similar activity that requires a squatting position, such as shellfishing. (b) We observed a predominance of the type C talo-calcaneal facet configuration among the population that indicates a maximal mobility of the talar joint, well adapted to displacement and/or activities such as goat herding in the steep landscape of El Hierro. (c) The statistically different os trigonum pattern among sexes may suggest a greater activity associated to jumping and climbing among males, possibly related to goat herding in the steep landscape of the island of El Hierro.

#### KEYWORDS

Canary Islands, genetic sexing, lower limb traits, nonmetric traits, physical activities, talar discriminant function sexing

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## 1 | INTRODUCTION

Some activities of daily living may cause morphological changes in bones, either due to the bone-forming consequences of the traction exerted by muscles involved in some repetitive actions or by the variable effects of loading changes on bone. Nonmetric traits are minor variants of phenotypic expression that can vary in etiology. Some of these morphological traits have been used as variables to identify family groups within cemeteries or as ethnic indicators. In contrast, other traits can be used to detect activity and posture patterns in skeletal remains (Cox & Mays, 2000). Therefore, some nonmetric traits can be treated as activity markers, while others are influenced by both genes and environment stress (Hauser & Stefano, 1989; Mann & Murphy, 1990). The latter would be the case of variations observed in elements such as the *os trigonum*.

The os trigonum trait was first reported by Rosenmüller in 1804 and then fully described by Sewell (1904) as an accessory ossicle that appears after birth and usually fuses between the age of 8-11 years old. This ossicle's lack of fusion may be due to genetic and activity factors during ontogenesis (Grogan et al., 1990), such as repetitive microtrauma by hyperplantarflexion in actions like jumping or intensive running where the foot is quickly extended (Peace et al., 2004). Also, as commented later in depth, sex-associated variations of the so-called squatting facets, or the talo-calcaneal facets configuration, may inform about differences in certain activities performed by males and females (Kuramoto et al., 2009; Pandey & Singh, 1990). Therefore, assessing the prevalence of certain traits in each population and exploring possible differences regarding sex is paramount in reconstructing the daily living and social structure of past populations. Among the pre-Hispanic population of El Hierro, the main activities were related to goat and sheep herding and shellfishing (Arnay-de-la-Rosa et al., 2009; Jiménez Gómez, 1993). It is unclear whether these activities were performed differently between males and females. This is important because previous research on other pre-Hispanic samples of other islands have indicated differing results. On the one hand, no differences were found between males and females regarding os trigonum and talo-calcaneal facets (Rodríguez Martín & Martín Oval, 2009). However, other studies have shown sexual differences in Gran Canaria regarding the upper limb (Santana Cabrera, 2010). Thus, given these different results between the pre-Hispanic populations of these islands, it is very important to assess if there are differences between males and females in the pre-Hispanic population of El Hierro regarding traits related to ankle activity.

The first task we had to face is accurate sex assessment of the included tali. This bone lacks major morphological differences among sexes, so it is necessary to perform a discriminant function analysis combining several measurements that differ among males and females, as other authors have made using several different bones (Alemán Aguilera et al., 1999; Asala et al., 2004; Dibennardo & Taylor, 1983; Garoufi et al., 2020; González-Reimers et al., 2000).

The major problem of this approach is that a discriminant function obtained from a given population may not be accurate when applied to

another. Moreover, anthropological measurements may evolve in the same population over time (Bertsatos & Chovalopoulou, 2018), and this may also alter accuracy. In other words, sexual dimorphism changes over time among populations (González Reimers et al., 2015). Therefore, we should acquire particular discriminant functions based on each population of a specific territory and chronological period for precise sex estimation.

To obtain discriminant functions, it is essential to have a gold standard. This would allow researchers to obtain trustworthy mean values and confidence intervals of the anthropometric measurements of male and female remains.

The selection of a gold standard depends on the burial conditions. In the case of complete skeletons, inspection of pelvis and/or cranial features may provide useful information about sex. In the case of a modern cemetery, reliable documentation about the buried individuals can be used to aid sex identification. However, both approaches are unattainable when the skeletal remains are comingled and/or only isolated or disarticulated bones are available. In this case, genetic sexing is necessary, because it has dramatically improved the possibility to study these types of remains. In the case of burial spaces containing only a few skeletons, genetic analysis can resolve sex estimation effectively. However, the high cost of DNA analysis makes this approach unrealistic when dealing with sites containing several hundreds of individuals. Also, another significant limitation is that due to post-mortem degradation, it is not always possible to obtain enough well-preserved tissue to perform genetic analysis. Both DNA laboratory costs and possible DNA degradation are the reasons by which, sometimes, genetic sexing can only be used as a gold standard to perform discriminant functions. This is the case of many pre-Hispanic collective burials containing hundreds of commingled bones. such as Punta Azul.

The aims of this study were (1) to assess the prevalence of some nonmetric traits related to ankle activity among the pre-Hispanic populations of Punta Azul (Canary Islands) such as the degree of fusion of *os trigonum*, squatting facets and talo-calcaneal facet types and (2) to ask whether there are ankle activity differences between males and females among this population. In order to fulfill this second objective, the usage of genetic sexing as a gold standard is crucial in creating discriminant functions.

# 2 | MATERIALS AND METHODS

#### 2.1 | Burial description

In this study, we analyzed human remains from the pre-Hispanic site of Punta Azul (Figure 1). The burial site Punta Azul, on the island of El Hierro, is a funerary cave containing comingled skeletal remains of at least 120 adult individuals, according to the number of tibiae (Ordóñez et al., 2017). The cave is located in the region of El Pinar, on the coast of Taibique, and it is on the side of a cliff with an altitude of 375 m above sea level. Punta Azul is part of a naturally shaped cave network in the south-eastern part of the island that the ancient FIGURE 1 Location of Punta Azul cave [Colour figure can be viewed at wileyonlinelibrary.com]



inhabitants of El Hierro used as a funerary space. This cave network also includes the necropolises of La Cueva de La Ballena and La Cueva de Montaña de La Lajura. There are also a series of other archaeological sites of interest in the vicinity of Punta Azul, including areas of ash and coal deposits, remains of stone tool development and ceramics, and shell middens (Velasco Vázquez et al., 2005). In the surrounding region, there are also small volcanic tubes that are thought to have been inhabited by the indigenous people of El Hierro. There are also stone altars where it is thought the pre-Hispanic population burnt animal remains for ritual purposes (Hernández Pérez et al., 2002). Two radiocarbon dates from human bone extracted from the interior of Punta Azul funerary cave produced a result of 910  $\pm$  30  $_{BP}$  (1030–1210  $_{AD}) beta 382741 and 970 <math display="inline">\pm$  30  $_{BP}$ (1015-1155) beta 382740 (Ordóñez et al., 2017), suggesting that the individuals placed in this site probably lived during the 12th century.

#### 2.2 | Individuals analyzed

In total, we analyzed 149 tali belonging to adult individuals. Selection criteria consisted of choosing bones that were sufficiently preserved to allow accurate detection of at least one of the nonmetric traits described below. From the selected sample, 70 were right-sided and 79 were left-sided. We measured tali bones following du Souich Henrici et al. (2002).

## 2.3 | Sexing

#### 2.3.1 | Genetic sexing

Molecular sex determination was performed on 35 left tali (out of 79; 44.3% of the sample). In Table 1, we show the mean values of the

	Genetic sexing	N	Mean	SD	t value	P value
Maximum length	1	35	55.36	4.29	1.31	0.19
	2	44	53.83	3.89		
Width	1	35	42.14	3.75	0.65	0.52
	2	44	41.64	2.93		
Maximum length of trochlea	1	35	30.58	2.44	0.24	0.82
	2	44	30.44	2.62		
Maximum width of trochlea	1	35	35.45	3.79	0.20	0.85
	2	44	35.29	3.35		

**TABLE 1** Anthropometric data among cases selected for genetic sexing and cases not selected for genetic sexing

Note: 1 = selected for genetic sexing. 2 = not selected for genetic sexing.

anthropometric variables that were measured comparing the tali (only left tali were considered), for both the bones that were used to assess genetic sex and for those that were not selected for genetic sexing. We observe that there are no statistically significant differences between the anthropometric variables calculated using those selected for genetic sexing and those that were not. Therefore, the subsample selected for genetic sexing was representative of the whole sample recovered from Punta Azul.

Molecular sex determination was performed at the Paleogenomics laboratory at the University of La Laguna. All the processing was performed taking strict measures to avoid any contamination with modern DNA. Ancient DNA was extracted from the bone powder, obtained from the cortical, using a silica-based protocol (Dabney et al., 2013) and built into double-stranded libraries after Kircher et al. (2012). The ancient DNA libraries were sequenced on an Illumina NextSeg 500 platform at Sistemas Genómicos S.L. (Valencia, Spain). using a  $2 \times 75$  bp paired-end protocol and aiming to obtain between 2 and 5 million reads per sample. Reads were trimmed and adapters removed using AdapterRemoval v2.1.7 (Lindgreen, 2012) and then mapped to the human reference genome (hg19) using BWA v0.7.17 (Li & Durbin, 2009). Low-guality (MAPQ < 25) and duplicate reads were removed using SAMtools v1.9 (Li et al., 2009). To confirm the presence of post-mortem damage in the ancient samples, we used MapDamage v2.0.8. Molecular sex was determined using the RY estimate proposed by Skoglund et al. (2013). Given the low conservation level of DNA in the tali samples, we performed simulations to determine the minimum number of reads needed to accurately identify the molecular sex of a sample. For that, we selected six well-conserved samples from our own database (three males and three females from the pre-Hispanic population of the Canary Islands) and generated 100 independent subsamples for each of them, selecting at random a subset of variable numbers of reads mapped to the sex chromosomes (2000, 1000, 500, 400, 300, 250, 200, 150, 100, and 50) and recalculated the RY value for each replicate. Based on our results (Table S1 and Figure S1), only individuals with a minimum of 250 reads mapping to the sex chromosomes were considered in the analysis. Also based on our simulation analysis, those classified as consistent with one sex but not with the other were accepted as classified within the proposed molecular sex.

Of the 35 left tali bones selected for the genetic sex estimation, we only obtained enough endogenous DNA for providing an accurate RY estimate in 19 cases. This result was due to the poor conservation of DNA in tali from the Punta Azul site, with samples producing both low concentrations of total DNA and moderate levels of endogenous DNA (Figure S2). From those samples for which a DNA analysis was possible, 12 were males and seven females (Table S2 and Figure S3). All the samples considered in this study showed the damage patterns expected for ancient DNA (Figure S4).

## 2.3.2 | Sexing based on anthropometry

In Table 2, the mean values of the analyzed anthropometric variables can be seen among the males and females that were identified through genetic analysis. We observe highly statistically significant differences in all the parameters described in Table 1. It is worth mentioning that differences are especially marked regarding talus width (T = 5.93 P < 0.01).

We used the talus width variable to perform a discriminant function analysis. The discriminant function was defined as  $0.447 \times \text{Width} - 19.402$ . Values obtained for males were  $1.038 \pm 0.8957$ , and values obtained for females were  $-1.78 \pm 1.1681$ . This discriminant function did not misclassify any of the genetically sexed samples: We correctly classified 12 out of 12 males (100%) and six out of seven females (85.71%), with a total accuracy of 94.74%. The 95% confidence interval (CI) for males ranged from -0.7176 to 2.7936 and for females from +0.5095 to -4.0694.

We applied the discriminant formula to the remaining 114 bones (of the total sample of 149) of which the genetic sexing was not performed. We also applied the discriminant function to the 16 individuals (out of the 35) in whom genetic sexing was attempted but failed to yield a result due to poor conservation of the DNA in tali. We could correctly classify sex in 97 bones (20 males and 77 females). In 37 cases, the values obtained fell within the overlap region of the discriminant function between males and females and thus were classified as undetermined. Additionally, in 15 cases, the talus width could not be measured due to taphonomic changes (Table 3).

# **TABLE 2**t test between males andfemales regarding talus metrics

Group statistics						
Anthropometrics	Genetic sex	N	Mean	SD	t value	P value
Maximum length	Male	12	58.71	2.35	4.18	0.001
	Female	7	52.70	3.97		
Width	Male	12	45.75	1.87	5.93	>0.001
	Female	7	39.44	2.79		
Maximum length of trochlea	Male	12	32.75	0.98	5.50	>0.001
	Female	7	29.01	2.00		
Maximum width of trochlea	Male	12	38.11	2.07	4.29	>0.001
	Female	7	33.39	2.70		

TABLE 3 Frequencies of the tali used for sex estimation

	Male	Female	Undetermined	Excluded
Total	20	77	37	15
Left	13	39	22	6
Right	7	38	15	9



**FIGURE 2** Os trigonum type 1 with a sharp notch [Colour figure can be viewed at wileyonlinelibrary.com]

#### 2.4 | Nonmetric traits

# 2.4.1 | Os trigonum

According to Sewell, there are different ways the *os trigonum* can manifest on the talus bone:

• Type 1: The *os trigonum* is completely fused with a sharp notch. This variant of the trait is completely fused. The only evidence of its separation is a sharp notch in the margin of the posterior process on the posterior side of the talus bone (Figure 2). In this case, the *os trigonum* can cause a posterior extension to the posterior calcaneal facet on the calcaneus.

**FIGURE 3** Os trigonum type 2 with an inferior groove [Colour figure can be viewed at wileyonlinelibrary.com]



**FIGURE 4** Os trigonum type 3 with a well-marked notch, showing the ossicle semi-fused to the talus [Colour figure can be viewed at wileyonlinelibrary.com]

• Type 2: The *os trigonum* is completely fused with an inferior groove. The only evidence that the ossicle was actually separated is that it shows a groove on the inferior side of the talus, exactly where the ossicle has fused to the rest of the talus (Figure 3).



**FIGURE 5** Os trigonum type 4a with porous mark on the talus [Colour figure can be viewed at wileyonlinelibrary.com]



**FIGURE 7** Medial facet (right) and lateral facet (left) on the talus [Colour figure can be viewed at wileyonlinelibrary.com]



**FIGURE 8** Talus facet types. Top left = type A, top right = type

**FIGURE 6** Os trigonum type 4b with a smooth facet on the talus [Colour figure can be viewed at wileyonlinelibrary.com]

- Type 3: The os trigonum is semi-fused to the talus. This type consists of a fused ossicle but it is slightly separated by a well-marked notch (Figure 4).
- Type 4a: A separate os trigonum with a porous mark where the posterior process usually located. In this case, the ossicle was attached by fibrous tissue. This type can be frequently confused with a broken Stieda process. However, a broken Stieda process shows signs of healing and an irregular surface; therefore, the os trigonum type 4a has a smooth surface and no signs of remodeled bone (Figure 5).
- Type 4b: A separate os trigonum with a smooth facet where the posterior process is usually located. In this case, the os trigonum was attached and covered with a thin layer of hyaline cartilage and separated by a joint cavity, which may be continuous with the cavity of the posterior talo-calcaneal articulation (Figure 6).

B1, middle left = type B2, middle right = type C, bottom left = type D, and bottom right = fused type [Colour figure can be

# 2.4.2 | Squatting facets

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The facets located on the dorsal side of the talus bone are known as "squatting facets" as they are caused by friction. This friction can be caused by specific activities of daily life that require hyperdorsiflexion of the foot, causing the tibia bone to press against the talus bone, creating lateral, medial, or continuous (gutter-like) facets (Baykara et al., 2010; Garg et al., 2015; Trinkaus, 1975). Nevertheless, these facets have also been related to other movements, such as gait motion on uneven ground and frequent crouching (Oygucu et al., 1998).

The classification method used in this study was based on the present/absent classification of Baykara et al. (2010) and Verna (2016) (see Figure 7).

## 2.4.3 | Talo-calcaneal facets

The talo-calcaneal facets constitute the talus and calcaneus joint. There can be either two or three facets available on the talus bone that connect with the calcaneus dorsal side (Namburu et al., 2017). Due to observations of the different types of talo-calcaneal facets in unborn individuals, such as those of the second and third trimesters (Rehman, 2014), the talo-calcaneal facets are believed to be affected by genetic factors. Different frequencies of talo-calcaneal facets have also been observed between various ethnic populations (Bilodi, 2006). In addition to these observations, the talo-calcaneal facets are involved in the pronation and supination movements of the ankle (Norris, 2011; Placzek & Boyce, 2006). Therefore, the morphology of these facets is likely influenced by many factors including both the genetic background of the individual and the repetition of particular movements, like gait and ankle motion.

Four of the six different types of talo-calcaneal facets have been observed in the Punta Azul sample population (Figure 8):

## 2.5 | Statistics

All statistical procedures were performed with IBM SPSS Statistics for Windows, statistical software package, Version 20.0. Armonk, NY: IBM Corp. The  $\chi^2$  analyses were performed using Fisher's exact test when at least 25% of the cells showed an expected count less than 5. In all other  $\chi^2$  analyses, Pearson  $\chi^2$  was used as default. To aid in interpretation of the statistical results, the effect size was also calculated using  $\psi$  values using  $2 \times 2$  tables to indicate if the significance of a result is high, medium, or low following Cohen (1988). The Student's *t* test was used to compare continuous variables because they showed a parametric distribution according to the Kolmogorov-Smirnov test.

## 3 | RESULTS

#### 3.1 | Nonmetric trait analysis

### 3.1.1 | Os trigonum

A total of seven (5.1%) (one right talus and six left) tali present OT1. Thirty-nine (28.5%) (22 right and 17 left) tali present OT2, four (2.9%) (one right and three left) tali present OT3, 27 (19.7%) (12 right and 15 left) tali present OT4a, and a total of 22 (16.1%) (12 right and 10 left) tali present OT4b.

As observed in Table 4, only the OT4b type is observed as significantly more frequent among males (P = 0.038). Meanwhile, the rest of the *os trigonum* types appear statistically proportionate between male and female tali, while the  $\psi$  value (0.24) shows a low-strength association between sex and this trait.

Due to analyzing comingled remains, it is possible that the two tali of the same individual are included in the study. To avoid this, we performed the same statistical analyses considering left tali and right tali separately (Tables 5 and 6). This statistically significant association was not observed when left and right tali were analyzed separately. This is possibly due to the low number of cases.

Therefore, prevalence of OT4b was 16.1%, and they were more frequently observed among males.

## 3.1.2 | Squatting facets

The total frequency of tali that present the lateral facet (LF) is 145 (97.3%) (67 right and 78 left) tali, while a total number of 101 (69.2%) (47 right and 54 left) tali present the medial facet (MF).

In Table 7, we can observe that the LFs were distributed proportionately as expected between both sexes, which has

			Sex Est.						
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	$\psi$ value		
OT1	Absent	Count	16	70	-	0.25	-		
	Present	Count	2	3					
OT2	Absent	Count	14	45	0.31	-	-		
	Present	Count	4	28					
OT3	Absent	Count	18	71	-	1.0	-		
	Present	Count	0	2					
OT4a	Absent	Count	15	64	-	0.69	-		
	Present	Count	3	9					
OT4b	Absent	Count	13	67	-	0.03	0.23		
	Present	Count	5	6					

**TABLE 4**  $\chi^2$  test results between male and female tali with *os trigonum* trait presence and absence

Abbreviation: OT, os trigonum.

			Sex Est				
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	<i>ψ</i> value
OT1	Absent	Count	11	33	-	1.0	-
	Present	Count	1	3			
OT2	Absent	Count	9	24	-	0.72	-
	Present	Count	3	12			
OT3	Absent	Count	12	35	-	1.0	-
	Present	Count	0	1			
OT4a	Absent	Count	10	32	-	0.63	-
	Present	Count	2	4			
OT4b	Absent	Count	9	34	-	0.09	-
	Present	Count	3	2			

**TABLE 5** Left tali  $\chi^2$  test results between male and female with *os trigonum* trait presence and absence

Abbreviation: OT, os trigonum.

			Sex Est.	Sex Est.				
			Male	Female	Fisher's exact test Sig. (2-sided)	$\psi$ value		
OT1	Absent	Count	5	37	0.14	-		
	Present	Count	1	0				
OT2	Absent	Count	5	21	0.37	-		
	Present	Count	1	16				
OT3	Absent	Count	6	36	1.0	-		
	Present	Count	0	1				
OT4a	Absent	Count	5	32	1.0	-		
	Present	Count	1	5				
OT4b	Absent	Count	4	33	0.19	-		
	Present	Count	2	4				

**TABLE 6** Right tali  $\chi^2$  test results between male and female with *os trigonum* trait presence and absence

Abbreviation: OT, os trigonum.

			Sex Est	Sex Est.						
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	ψ value			
LF	Absent	Count	0	2	-	1.0	-			
	Present	Count	20	75						
MF	Absent	Count	2	25	0.08	-	-			
	Present	Count	18	51						

**TABLE 7**Frequencies and  $\chi^2$  test ofthe lateral facet (LF) and the medial facet(MF) between males and females

resulted in a non-significant result. The same was seen when right and left tali were analyzed separately (Tables S3 and S4). On the other hand, we observe that in the case of the MF, the distributions are slightly uneven, as there are more cases of this trait in males. However, the differences within this trait are not statistically significant.

# 3.1.3 | Talo-calcaneal facets

Type A is present in a total of two (1.4%) (one right and one left) tali. A total of 30 (20.7%) (10 right and 20 left) tali present type B1 talo-calcaneal facet, while a total of 39 (26.9%) (19 right and 20 left) tali present type B2. A total of 72 (49.7%) (38 right and

### **TABLE 8** Frequencies and $\chi^2$ test between talo-calcaneal facet types between males and females

			Sex Est.	Sex Est.					
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	ψ value		
Type A	Absent	Count	18	74	-	0.04	0.28		
	Present	Count	2	0					
Type B1	Absent	Count	16	63	-	0.73	-		
	Present	Count	4	11					
Type B2	Absent	Count	18	50	0.08	-	-		
	Present	Count	2	24					
Type C	Absent	Count	9	36	0.97	-	-		
	Present	Count	11	38					
Type D	Absent	Count	19	74	-	0.21	-		
	Present	Count	1	0					
Type facet fused	Absent	Count	20	74	-	1.0	-		
	Present	Count	0	1					

**TABLE 9** Left tali frequencies and  $\chi^2$  test between talo-calcaneal facet types between males and females

			Sex Est.				
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	<i>ψ</i> value
Type A	Absent	Count	12	37	-	0.26	-
	Present	Count	1	0			
Type B1	Absent	Count	10	29	-	1.0	-
	Present	Count	3	8			
Type B2	Absent	Count	12	24	-	0.07	-
	Present	Count	1	13			
Type C	Absent	Count	6	22	0.61	-	-
	Present	Count	7	15			
Type D	Absent	Count	12	37	-	0.26	-
	Present	Count	1	0			
Type facet fused	Absent	Count	13	37	-	1.0	-
	Present	Count	0	1			

34 left) tali present type C and only one (0.7%) (one left) talus presents type D and fused type was also only present in one (0.7%) (one left) talus.

Regarding the talo-calcaneal facets (Table 8), the distributions of these facet types were relatively balanced, with the exception of facet type A (P = 0.043) which resulted as statistically significant between males and females (the only two cases observed were found in males). Although, there were no significant differences between males and females when the left and right tali with talo-calcaneal facets were considered separately (see Tables 9 and 10) and this may be due to sample size. The  $\psi$  value (0.28) indicates a low-strength for this talo-calcaneal facet type. There are some cases where there were slightly

different distributions between the sexes, such as in the case of type B2. However, differences were not statistically significant regarding the distributions of the traits apart from facet type A.

# 4 | DISCUSSION

El Hierro is the smallest island of the Canary Archipelago, with an area of 273 km<sup>2</sup>. Despite this, the island reaches 1500 m of altitude over sea level, in such a way that its landscape is characterized by steep slopes and cliffs. It is known that the economy of the inhabitants of El Hierro was mainly based on sheep/goat-herding with a minority input

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**TABLE 10** Right tali frequencies and  $\chi^2$  test between talo-calcaneal facet types between males and females

			Sex Est.				
			Male	Female	Pearson $\chi^2$ Sig.	Fisher's exact test Sig. (2-sided)	<i>ψ</i> value
Туре А	Absent	Count	6	37	-	0.15	-
	Present	Count	1	0			
Type B1	Absent	Count	6	34	-	0.51	-
	Present	Count	1	3			
Type B2	Absent	Count	6	26	-	0.65	-
	Present	Count	1	11			
Type C	Absent	Count	3	14	-	1.0	-
	Present	Count	4	23			
Type D	Absent	Count	7	37	-	-	-
	Present	Count	0	0			
Type facet fused	Absent	Count	7	37	-	-	-
	Present	Count	0	0			

from agriculture and shellfishing (Arnay-de-la-Rosa et al., 2010). As we will comment on later, these herding-related activities might be responsible for the high prevalence of some traits in the tali of the Punta Azul sample population.

Although uncertainty exists regarding pathogenesis of several nonmetric traits, some of them develop in relation to repetitive movements associated with certain activities. In order to understand the way of life of the pre-Hispanic people of the Canary Islands, it is important to discern if specific activities were equally performed by males and females. Therefore, accurate sex assessment is a crucial step that we were able to achieve using discriminant function analysis based on genetic sexing as the gold standard. Discriminant function analysis allowed accurate sexing of 72.3% of the measurable bones, a result slightly lower than those reported by other authors, such as Steele (1976), Dibennardo and Taylor (1983), and Iscan and Miller-Shaivitz (1984) on American black and white populations or in Japanese ones (Iscan et al., 1994). Several other researchers have also performed discriminant function analysis on other populations (e.g., Bidmos & Dayal, 2003; Dittrick & Suchey, 1986; Mokoena et al., 2019; Slaus & Tomicic, 2005; Steyn & Iscan, 1997; and Alemán Aguilera et al., 1999, who obtained accuracy values between 80% and 90% [depending on the bone] on a modern Mediterranean population).

The relatively low proportion of accurately sexed individuals not only depends on the degree of sexual dimorphism of the analyzed bone but also on the fact that not all the samples selected for genetic sexing yield enough DNA.

Genetic sexing of an adequate number of a given bone has evolved as a major tool in assessing sex of comingled remains. Although sequencing costs have dropped in recent years, sex estimation based on ancient DNA is still expensive for large samples. Moreover, even if ancient DNA analysis were possible for all samples not all the samples subjected to genetic sexing yield enough wellpreserved DNA to obtain accurate results. This depends on taphonomic alterations (some of them related to geographical latitude, Campos et al., 2012) and on the type of bone, so that petrous bone (Pinhasi et al., 2015), the auditory ossicles (Sirak et al., 2020) or metatarsals and metacarpals (Ordóñez et al., 2015; Zupanc et al., 2021) constitute the best sources for endogenous DNA extraction.

Although previous DNA analysis on the Punta Azul site indicated exceptional preservation of the human remains with endogenous rates reaching values higher than 30% (Fregel et al., 2019), in this research, DNA extraction was necessarily restricted to tali, a type of bone that is not within the usual sources of ancient DNA. This explains why we were only able to determine genetic sex in only 19 out of the 35 cases selected for analysis (54%). A low number of accurately sexed cases limit the number of variables that can be included in a discriminant function analysis, a fact that probably explains the relatively low accuracy obtained.

In any cases, the discriminant function allows the accurate sex estimation of the majority of the total sample (approximately 65%, a proportion that rises to 73% if we consider only the bones without taphonomic alterations) using only width measurements. This figure is intermediate among those yielded by discriminant functions performed on long bones, and those performed on other bones, such as subadult iliac bones, with an accuracy ranging between 57% and 65% (Garvin et al., 2021). This discriminant function, the first performed on tali of the pre-Hispanic population of the Canaries, can be applied to other populations from the island of El Hierro and possibly to others from the archipelago.

Using this sex estimation tool, we found significant sex differences in the case of *os trigonum* and in the very uncommon type A talo-calcaneal facet morphology but a similar prevalence in both sexes for the remaining forms of talo-calcaneal facets and the squatting facets.

Regarding talo-calcaneal facets, most correspond to C or B types that were equally distributed among males and females. The other

morphological types of talo-calcaneal facets were very uncommon, affecting less than 2% of individuals. The morphology and number of talo-calcaneal facets vary between population groups, pointing to a considerable genetic influence of this trait. As commented earlier, different facet configurations had been observed in the second and third trimesters of unborn fetuses (Bunning & Barnett, 1965; Rehman, 2014) pointing to an important genetic influence. Some studies have suggested that the talo-calcaneal facet frequencies, which have been shown to differ between various populations, are sufficient evidence to state that the talo-calcaneal joint morphology is strongly affected by genes (Garg et al., 2013; Nagar et al., 2012; Parimala & Reddy, 2016; Vučinić et al., 2020). For example, data from some European and North African samples show a predominance of three facets (Bunning & Barnett, 1965; Saadeh et al., 2000), but other European samples have shown a predominance of two facets (Forriol Campos & Gomez Pellico, 1989; Gupta et al., 1977; Uygur et al., 2009). The incongruence of the talo-calcaneal facets between ancestry groups has been discussed in a previous study (Jung et al., 2015), where Europeans have shown sometimes a predominance of two facets, while in other samples, a three-facet morphology predominates.

Other authors have suggested alternative explanations other than genes for these differences in frequency of the talo-calcaneal facets, such as ankle activity and environmental factors (Anjaneyulu et al., 2014; Bilodi, 2006; Sharada et al., 2012). The number and area of talo-calcaneal facets are strongly related to joint mobility (Kelikian & Sarrafian, 2011; Norris, 2011). Likewise, it is generally assumed that mobility is maximal among individuals with one-facet configuration (type C; Bruckner, 1987), that was the most frequently observed morphology in the series analyzed in the present study.

The same condition that allows a maximal joint mobility may be also associated with a negative counterpart. Talo-calcaneal facets with a smooth flat surface, such as the type C configuration considered in this study might be altered by pronation and supination movements of the ankle due to the talo-calcaneal facets' subjection to movements during gait and other activities, as shown and proposed by other authors (Mahan et al., 2017; Norris, 2011; Placzek & Boyce, 2006). Previous research has found marked differences between Indian and European populations regarding the pattern of talar facets that may be associated with a higher prevalence of osteoarthritis (Garg et al., 2013). This is because the morphology of these facets may influence the stability of the ankle (Bruckner, 1987; Drayer-Verhagen, 1993). Therefore, activity may also cause variable degrees of degenerative changes as a major factor in defining the morphology of these facets.

There were no differences observed among males and females regarding the frequency of type B or type C talo-calcaneal facets, being consistent with the results obtained by other authors (Jung et al., 2015; Vučinić et al., 2020), but in disagreement with the results reported by others (Lee et al., 2012). Unfortunately, in many cases, sex association is not known, because sex was not reported in most studies (Anjaneyulu et al., 2014; Bilodi, 2006; Murerwa et al., 2020; Sharada et al., 2012; Uygur et al., 2009).

In contrast with type B or type C talo-calcaneal facets, a significant association was observed regarding type A morphology and male sex. Only two cases of talo-calcaneal facet type A were observed in our series, both in males. This association was statistically significant, but this result should be cautiously interpreted given the very short number of cases in which a type A morphology was observed, so no firm conclusion can be drawn regarding its anthropological meaning.

The squatting facets appear due to a repetitive, daily resting position known as squatting (Barnett, 1954; Satinoff, 1972; Trinkaus, 1975), which consists of the contact between the talus neck and the anterior border of the inferior part of the tibia. These facets may be detected in fetuses (Oygucu et al., 1998), due to the extreme hyperdorsiflexion of the foot during intrauterine life. This observation initially suggested that squatting facets were genetic traits (Barnett, 1954), and several studies tried to search for differences in their prevalence among different ethnic groups (Singh, 1959). However, it became clear that the squatting position during adult life constitutes a major factor in developing these facets (Baykara et al., 2010; Ekanem et al., 2021; Garg et al., 2015; Jeyasingh et al., 1979). Therefore, their presence has been investigated in many population groups, including Neanderthals (Trinkaus, 1975).

We have observed a very high prevalence of lateral squatting facet. The occurrence of this trait observed by us in the Punta Azul site is higher than those reported by other groups (Ekanem et al., 2021; Oygucu et al., 1998; Pandey & Singh, 1990; Singh, 1959). A high prevalence of these facets has been attributed not only to the adoption of a squatting position at rest but also to activities related to farming (Oygucu et al., 1998). Although agriculture was part of the economy of the pre-Hispanic population of El Hierro, isotopic analysis (Arnav-de-la-Rosa et al., 2010) and the large pre-Hispanic shell middens (mainly limpets) observed on this island (Jiménez Gómez, 1993) strongly support that shellfishing was one of the most important food sources. As the collecting of limpets usually requires an individual to adopt the squatting position, our results could support the hypothesis that squatting facets might be related to this activity. In the archaeological literature, many researchers report squatting facets on skeletal remains associated with sites where considerable amounts of shell middens were present (May, 1943; Simpson et al., 2004; Turner, 1970). Furthermore, previous research reported uncertainty as to whether squatting facets were associated with any economic activity, even though the skeletal remains were found in a context of accumulated shells (Rightmire et al., 2006).

There were no statistical differences observed between males and females regarding the squatting facets. Previous research has found statistical differences between males and females in India, possibly indicating difference in activity/stress between both sexes (Pandey & Singh, 1990). If this interpretation was accurate, our results could be suggesting that ankle stress/activity in our sample was of similar intensity among males and females. If limpet shellfishing is associated with developing these facets, then the presented data would support that males and females equally participated in this activity.

The os trigonum trait is a developmental anomaly due to the posterior process not fusing around the age of 8–11 years old (Grogan et al., 1990). This lack of fusion has been shown by previous research to be likely due to repetitive microtrauma by hyperplantarflexion in activities such as jumping or intensive running where the foot is quickly extended (Peace et al., 2004). However, a genetic component has also appeared to likely influence the appearance of the lack of fusion (Mann & Owsley, 1990). Our results indicate that the *os trigonum* types described by Sewell (1904) show statistically significant differences between males and females in the Punta Azul sample.

As demonstrated by previous research (Fregel et al., 2019; Ordóñez et al., 2017), the population of Punta Azul had a low genetic diversity. This may explain the high prevalence of *os trigonum* among this population, higher than that previously reported in a sample of ballet dancers and other samples of high ankle activity (Peace et al., 2004; Sewell, 1904; Tsuruta et al., 1981; Zwiers et al., 2018). Also, the low genetic diversity found in this population strongly supports that the differences observed between males and females are due to activity differences.

This pattern becomes clearer when focusing on the male prevalence of the *os trigonum* type 4b, an entirely separate ossicle, statistically more prominent among males. Considering previous evidence on the *os trigonum* being predominantly caused by constant mechanical trauma by hyperplantarflexion during childhood (McDougall, 1955; Peace et al., 2004), our result could indicate that males suffered more hyperplantarflexion trauma during childhood in order for the ossicle to lack complete fusion among males. It is possible that the females' *os trigonum* did not reach total separation from the talus bone because of the lack of microtrauma during childhood, like that experienced by males. Therefore, the association of *os trigonum* type 4b with male sex perhaps indicates that males were more intensely devoted than females to activities related to goat-herding that requires climbing and jumping in such a steep landscape as that of El Hierro.

## 4.1 | Limitations of this study

There are two main limitations of this study. The first limitation is the low number of genetically sexed individuals that hampers the possibility to perform discriminant function analyses using several variables and therefore decreases the accuracy of these functions. As previously commented, this is related both to the high cost of applying genetic sexing analyses to the commingled remains of burial caves containing hundreds of individuals and the effect of taphonomic changes that hinder the process of acquiring valid DNA from the included samples. The second major flaw is related to the low prevalence of some traits (such as type A talo-calcaneal facet) that complicates the conclusions regarding this trait, especially if we consider that the sample has to be divided into four subgroups (right/left; male/female). Fortunately, this was not the case of squatting facets or type 4b os trigonum trait that showed a much higher prevalence. In any case, it would be desirable (if the possibility ever existed) to confirm our results in another similar sample with the same conditions. However, the finding of a high prevalence of the squatting facets among both sexes compatible with shellfishing activities and the

higher proportion of type 4b *os trigonum* trait among males, which is perhaps related to goat/sheep herding, may be viewed as a robust confirmation of what could be inferred from chroniclers' reports and the historical context.

## 5 | CONCLUSIONS

- The results have shown that the variations in the os trigonum strongly support differences in ankle activity between males and females. The pattern observed among males suggests activities such as climbing, jumping, and running during childhood, probably related to goat-herding in the steep landscape of El Hierro. In addition, results show that males were involved in more ankle activity than females, providing evidence of different socioeconomic roles for males and females.
- 2. We observed a predominance of the type C talo-calcaneal facet (one facet) configuration among the population included in this study, a morphology that allows a maximal mobility of the talar joint, well adapted to displacement and/or activities such as goat herding in the steep landscape of El Hierro.
- The prevalence of squatting facets is very high in both sexes. We hypothesize that this high prevalence may be related to activities such as limpet shellfishing, an activity that was probably equally performed by males and females.
- 4. Generally, the aboriginal people of the Canary Islands are well known for their highly intensive activity level due to their goatherding lifestyle and the adaptive strategies to the mountainous terrain of the islands (Cabrera et al., 2011; Concepción Estévez González, 2003). Based on the presented data, it is likely that males were more heavily involved in goat-herding activities than females. On the contrary, possibly males and females were equally devoted to activities that required adopting the squatting position, such as agriculture and, probably, the collection of limpets.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHOR CONTRIBUTIONS

Samuel James Cockerill: Conception, design, data acquisition, data analysis, interpretation of data, and drafting of the manuscript. Matilde Arnay-de-la-Rosa and Emilio González-Reimers: Conception, design, and drafting of the manuscript. Alejandra C. Ordóñez: Data acquisition, data analysis, interpretation of data, and critical revision of the manuscript. Javier G. Serrano, Rosa Fregel, and Clara Díaz-Pérez: Genetic data acquisition, analysis of paleogenomic data, interpretation of genetic results, and review of the final draft. Richard G. Guamán-Chulunchana: Data acquisition.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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