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Biometric sex assessment from the femur and tibia in a modern Greek population

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ABSTRACT

Forensic anthropologists assess sex by analysing quantitative and qualitative characters of the human skeleton. In general, the pelvis and skull are the skeletal regions used most often, but in many cases, they are missing or fragmentary. In such circumstances, where only limb bones are present, it is necessary to use techniques based on other skeletal elements. Metric traits of the long bones of the lower extremities have been reported as reliable indicators of sex. This study was designed to determine whether the two main long bones of the leg, the femur and tibia, can be used for the assessment of sex on a Greek skeletal population. The skeletal sample used in this study comes from the modern human skeletal collection that is currently housed at the National and Kapodistrian University of Athens and is known as The Athens Collection. It consists of 371 femora and 372 tibiae corresponding to 200 adult individuals (111 males and 89 females). The age range is 19–96 years for males and 20–99 years for females. The maximum lengths and epiphyseal widths were measured for the present study, and it was found that the discriminant analysis of the metrical data of each long bone provided high sex discrimination accuracies. The rate of correct sex discrimination based on different long bones ranged from 91.50 % (left femur) to 93.40 % (left tibia). Our results suggest that lower limb bones can be used effectively for sexing in forensic contexts, in addition to other sex assessment techniques.

1. Introduction

The discipline of forensic anthropology is concerned with issues such as identification of human skeletons, remains in advanced decomposition or otherwise not readily identifiable human remains. A detailed knowledge of the human skeleton is required in order to produce a biological profile. This term refers to the determination of sex and the estimation of age, stature, ancestry, and the identification of any pathological conditions and identifying features [1]. The focus of the present research is the first of these elements, the assessment of sex from the skeleton. This becomes possible due to the average body size difference between adult males and females, known as sexual dimorphism [2]. The definition also includes a series of traits that are linked to functions such as reproduction, or sexual attraction. The characteristics directly linked to reproduction are known as primary and include pelvic morphology, while those that are not, are called secondary, such as the size of the canine teeth. In the majority of primates, the most pronounced sex difference can be found in body size [3]. The smaller size of females, which is universal across human populations, is attributed to the increased energy demands linked to their reproductive ability and functions [4]. In some primates, such as gorillas, orangutans and baboons, sexual dimorphism may be extreme, with males having an average body size that is double to that of females [5]. In humans, the greater body size of males is believed to be the result of the differential growth rate of the lower limbs between the sexes [6]. It has been estimated that the body size of human males is between 8 % and 20 % larger than that of females [1,7].

The sexually dimorphic traits of the skeleton appear after puberty and include longer, more robust bones with more pronounced muscle attachments. The most marked morphological differences are present in the bones of the pelvis, while those of the skull also present a high degree of sexual dimorphism [1,7,8]. The development of methods used to assess the sex from the skeleton are particularly useful in fields such as forensic anthropology, as they enable investigators to identify victims of

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crime from a variety of contexts. Generally, there are three different types of sex determination methods: morphological, metric and molecular [1,8]. While the first two are anthropological since they require a detailed osteological knowledge, the third is "borrowed" from the field of molecular biology and provides the most accurate results, provided of course that genetic material is present within the remains [9].

The morphological methods focus on very specific structures of the pelvis, skull and some long bones [7,10], and require the completeness of the skeleton or, at the very least those elements that have the most sexually dimorphic characteristics. For an experienced anthropologist, this method is fast, non-destructive and with high accuracy levels that range between 80 % and 100 %, when both the pelvis and skull are present [8,11,12]. The metric methods on the other hand, rely on standardised measurements of various skeletal elements, most frequently long bones and are based on the observed differences in body size between the sexes [13–15]. Studies on almost all of the different long bones have been conducted, and a variety of populations have been examined [16–22]. Completeness of the skeletal elements to be measured is necessary for the use of the metric methods, even though for some elements there are measurements that can produce good results even in fragmentary material [23,24]. In some cases, it has been found that metric methods are more accurate that the morphological techniques used for the skull [25,26]. It should be noted that metric methods are population-specific, therefore standards developed on one group should not be used on another, especially if they are geographically distant [14]. Some exceptions to this have been identified, as it has been suggested that pelvic measurements have a universal application [24].

In forensic contexts, fragmentary remains are found frequently. This can be the result of scavenger activity, other natural processes such as flooding or soil erosion, or even human intervention aimed at preventing identification, such as dismemberment [9,27]. In Greece in particular, in recent years there has been an increase in migrant waves from surrounding areas like the Middle East that result in a significant number of individuals drowning at sea. The remains are washed upon the shores of the Greek islands or mainland and regularly end up as casework for forensic anthropologists in the country. In the majority of cases, significant disarticulation has taken place and this results in individual limbs being recovered. It is therefore very important to have methods for establishing the biological profile as part of the effort to identify these individuals [28]. In addition, having metric standards for the assessment of sex, can be particularly useful for cases of commingled remains. These are not rare and can be found in cases of mass disasters, secondary burials or mass graves [29,30].

The aim of the present work is to determine whether sexual dimorphism of the femur and tibia exists in the modern Greek population. Any significant sex differences present, will form the basis to develop equations that can be applied to the assessment of sex from the lower limbs in unidentified skeletal material from this part of the world.

2. Materials and methods

The human skeletal material used in this study comes from the Athens Collection, currently housed at the Department of Animal and Human Physiology of the University of Athens, Greece [31]. The collection, consisting of 250 skeletons, was put together by Anna Lagia (first 72 skeletons) and the rest by Constantine Eliopoulos (178 skeletons), as part of his doctoral thesis at the University of Sheffield [32]. The creation of this valuable reference collection required not only the personal labour and financial resources of both of its creators but also those of other institutions: The American School of Classical Studies at Athens, where the collection was started, the University of Sheffield, the National and Kapodistrian University of Athens and Liverpool John Moores University, which supported the most recent expansion of the collection from 225 to 250 individuals in 2010. Previously, it has been erroneously reported that the sole institution responsible for the creation of the Athens Collection was the National and Kapodistrian University of Athens [33]. The collection is fully documented with death certificate information that includes the sex, age, place of birth, occupation and cause of death for most individuals. In the sample used for the present study, both sexes are represented in relatively equal numbers (111 males and 89 females), covering a broad age range from 19 to 99 years. Table 1 illustrates the number of individuals utilised for the present study, all of whom were adult. It should be noted, however, that the skeletons of three males and two females were used even though their exact age was not known. Skeletal elements that were not complete or had signs of pathologies were not used in this research.

The measurements were taken in two different phases: during the first, which lasted three months, 202 skeletons were measured by the first author (CK). Three weeks later, a second set of measurements was taken on a random sample of 60 skeletons from the original sample. This second set of measurements was taken in order to examine the consistency of the single observer (intra-observer error). The same sub-sample of 60 skeletons was also measured by the second author (CE) for the evaluation of the consistency between two different observers (inter-observer error). In total, 187 right and 184 left femora were measured, while the count for the measured tibiae was 188 and 184, respectively. It should be noted that the fibula was not included in the present study due to its fragility and the fact that it is frequently found in a fragmented state.

The measurements were taken with a standard osteometric board with an accuracy of 1 mm and Mitutoyo Digimatic digital calipers with 0.01 mm accuracy. The following measurements were taken from the femur: Maximum length (ML), Maximum diameter of the head (MDH) and Epicondylar breadth of the femur (EBF). Measurements taken from the tibia were: Maximum length (ML), Maximum epiphyseal breadth of the proximal tibia (MEBP) and Maximum epiphyseal breadth of the distal tibia (MEBD), according to Langley et al. [23] (Table 2).

Statistical analysis was performed by use of Microsoft Excel and SPSS. Initially, the descriptive statistics and the sexual dimorphism index were calculated. Discriminant analysis was selected as the most suitable type of statistical methodology, as it is a multivariable method that enables the classification of an observation into an existing, defined group, in this case sex. The analysis that was conducted in the present study was for each bone and for each measurement, but also for the total of measurements for each skeletal element. Stepwise analysis was also performed, which indicated the most reliable measurement for each bone. In total, 12 equations were calculated for each skeletal element, and each one is a separate "sexing tool".

3. Results

The descriptive statistics in Table 3 indicate that all the mean values of the measurements for males are greater than those for females and that the differences are statistically significant. This, coupled with the sexual dimorphism index (SDI), suggests that sexual dimorphism is present in the leg bones of the sample under study. When it comes to laterality, or the difference between the bones of the two sides, it is apparent that there are differences, but these are not consistent with a particular side, as in some cases the ones of the right side are larger, while in others those of the left. A laterality test, not presented here, showed that the differences between the two sides are not statistically significant.

Intra- and inter-observer error was calculated, and it is presented in

Table 1
Distribution of the leg bones studied according to sex and side.

		Femur		Tibia	
	No of Individuals	Left	Right	Left	Right
Males	111	103	105	102	105
Females	89	81	82	82	83
Total	200	184	187	184	188

Table 2
Selected variables, abbreviation, source and measurement number.

Variables	Abbrev	Source
Femur		
Maximum Length (Left)	MLL	Langley et al. 2016, No 75
Maximum Length (Right)	MLR	Langley et al. 2016, No 75
Epicondylar Breadth (Left)	EBL	Langley et al. 2016, No 77
Epicondylar Breadth (Right)	EBR	Langley et al. 2016, No 77
Maximum Diameter of the Head (Left)	MDHL	Langley et al. 2016, No 78
Maximum Diameter of the Head (Right)	MDHR	Langley et al. 2016, No 78
Tibia		
Maximum Length (Left)	MLL	Langley et al. 2016, No 86
Maximum Length (Right)	MLR	Langley et al. 2016, No 86
Maximum Epiphyseal Breadth Proximal (Left)	MEBPL	Langley et al. 2016, No 87
Maximum Epiphyseal Breadth Proximal (Right)	MEBPR	Langley et al. 2016, No 87
Maximum Epiphyseal Breadth Distal (Left)	MEBDL	Langley et al. 2016, No 88
Maximum Epiphyseal Breadth Distal (Right)	MEBDR	Langley et al. 2016, No 88

Table 4. The results indicate that the reproducibility of the measurements is very good, as the error rates within and between observers is very low [14].

For the present study, an analysis was performed for each individual bone and for each measurement taken from each bone. The final result was the creation of 12 discriminant functions for each bone. Each of these functions is an individual sexing tool, in which the measurements are entered and the sex is predicted along with the corresponding correct classification rate. The result is a number; if it is greater than zero, the remains are those of a male, and if the value is below zero, the remains are those of a female. A step-wise discriminant function was also performed in order to determine the measurement with the greatest accuracy.

For the femur, the statistical analysis indicates that the most accurate sex assessment is provided when all three variables are combined. In particular, for functions F3 for the left femur and F5 for the right femur, accuracy is 91.5 % and 88.9 %, respectively. When it comes to the cross-validated results, they reach accuracies of 89.9 % for the left and 87.4 % for the right femur. The step-wise analysis showed that the most important measurement was the epicondylar breadth of the right femur, with 87.9 % accuracy (both F2 and F6 functions). Table 5 shows all the results for the femur.

The same analyses were performed for the tibia, and the results demonstrate that the measurement with the highest score in correct sex classification is that of the maximum epiphyseal breadth of the left proximal tibia with an accuracy rate of 93.4 % and for the right tibia 92.3 % (functions F8 and F11, respectively).

Step-wise analysis for the tibia indicated that the most reliable measurement was the maximum epiphyseal breadth of the right proximal end (function F2). Table 6 displays all the results for the tibia, and it can be seen that in general, the results are more accurate than those of the femur.

4. Discussion

The results of the present study indicate that there is sexual dimorphism present in the population under study. Previous studies on the same sample have resulted in the same conclusion, even though they focused on different skeletal elements [13–14,34–35]. Our results confirm the presence of significant differences between the two sexes

Table 4
Inter- and intra-observer error for the femur and tibia.

Intra-observer error			
Femur	**ML**	**MDH**	**EB**
Left	0,270	0,976	0,970
Right	0,110	0,541	0,473
Tibia	**ML**	**MEBP**	**MEBD**
Left	0,387	0,688	0,919
Right	0,264	0,603	1,157
Inter-observer error			
Femur	**ML**	**MDH**	**EB**
Left	0,125	0,520	0,650
Right	0,096	0,745	0,620
Tibia	**ML**	**MEBP**	**MEBD**
Left	0,475	0,993	1,356
Right	0,224	0,865	1,238

Table 3
Summary statistics of the femur and tibia.

		Males				Females				SDI
Variable	Side	Mean	SD	CI*	No	Mean	SD	CI	No	
Femur										
ML	L	452.97	24.63	4.81	103	411.67	20.31	4.49	81	9.11
	R	448.78	23.51	4.66	100	411.85	20.43	4.67	76	8.23
MDH	L	47.75	2.79	0.55	101	41.69	2.42	0.54	80	12.69
	R	47.62	2.65	0.53	99	41.88	2.24	0.49	82	12.05
EB	L	83.31	3.81	0.75	103	73.55	3.49	0.79	81	12.91
	R	83.47	3.73	0.72	105	73.83	3.56	0.80	79	11.55
Tibia										
ML	L	372.08	21.22	4.19	101	335.55	19.32	4.25	82	9.81
	R	371.16	20.42	4.01	102	336.07	19.01	4.15	83	9.45
MEBP	L	77.74	3.49	0.68	102	68.95	3.19	0.71	80	11.30
	R	77.84	3.31	0.64	105	69.00	3.41	0.77	77	11.35
MEBD	L	49.70	3.11	0.62	100	44.17	2.61	0.57	82	11.13
	R	49.97	2.92	0.58	100	44.12	2.82	0.62	82	11.70

All measurements in millimetres.
* CI = Confidence Interval (95 %).

Table 5
Discriminant equations for the femur.

Functions		MLL	MLR	MDHL	MDHR	EBL	EBR	(Constant)	Original (%)	Cross-Validated (%)
F1	Femur L-R all	0,1465	−0,1406	0,3278	−0,2804	0,1203	0,5626	−58,0486	88,40	82,90
F2	Femur L-R all stepwise						0,7276	−56,8976	87,90	87,90
F3	Left Femur all	0,0162		0,1288		0,5823		−58,1437	**91,50**	**89,90**
F4	Left Femur all stepwise					0,7100		−55,4008	86,90	85,90
F5	Right Femur all		0,0075		0,1354		0,6258	−58,1728	**88,90**	**87,40**
F6	Right Femur all stepwise						0,7253	−56,7040	87,90	87,90
F7	Left Femur ML	0,0792						−34,0049	80,40	80,40
F8	Left Femur MDH			0,8722				−38,7730	84,90	84,90
F9	Left Femur EB					0,7215		−56,3075	86,90	86,90
F10	Right Femur ML		0,0746					−31,8475	74,90	74,90
F11	Right Femur MDH				0,9377			−41,7750	86,90	86,90
F12	Right Femur EB						0,7181	−56,1950	87,90	87,90

Table 6
Discriminant equations for the tibia.

Functions		MLL	MLR	MEBPL	MEBPR	MEBDL	MEBDR	(Constant)	Original (%)	Cross-Validated (%)
F1	Tibia Left-Right all	−0,0653	0,0884	0,3606	0,5689	−0,3937	0,1275	−63,6559	92,20	90,80
F2	Tibia Left-Right all stepwise				0,7894			−57,7289	**92,30**	**92,30**
F3	Left Tibia all	0,0323		0,7980		−0,1983		−60,4471	91,60	90,00
F4	Left Tibia all stepwise	0,0317		0,6628				−59,6155	**93,10**	**92,50**
F5	Right Tibia all		0,0175		0,7850		−0,0909	−59,3062	91,20	91,20
F6	Right Tibia all stepwise				0,7790			−56,9392	92,30	92,30
F7	Left Tibia Max Length	0,0878						−30,8655	83,10	83,10
F8	Left Tibia MEBP			0,7771				−56,7679	**93,40**	**93,40**
F9	Left Tibia MEBD					0,6580		−30,6841	84,10	84,10
F10	Right Tibia Max Length		0,0895					−31,4452	83,20	83,20
F11	Right Tibia MEBP				0,7844			−57,2761	92,30	92,30
F12	Right Tibia MEBD						0,7066	−33,0453	84,60	84,60

that are reflected in the dimensions of the skeletal elements examined in this research. Several other projects have focused on the bones of the lower limbs of modern populations from Croatia [36,37], Spain [18], the USA [38,39], Thailand [19] and China [40]. It is noteworthy that while all of these studies have found that males are on average larger than females, the values differ from one population to the next, confirming that this method is highly population-specific. For example, when the values obtained by the present study are compared to those by Mall et al. [41] on a German skeletal sample, it can be seen that they are on average smaller. When they are compared to those from a Chinese population [33], the values of our study on the Greeks are larger. These findings, although not quantified and one may argue are subjective in nature, offer nevertheless an indication of the variation in skeletal dimensions in different populations. This variation has been documented in past research on other skeletal elements such as the humerus [14,16,20].

In regard to sexual dimorphism, our results indicate that it is present to a great degree in the Greek population. As Table 3 indicates, the sexual dimorphism index (SDI) ranges from 8.23 % for the maximum length of the right femur to 12.91 % for the maximum epicondylar breadth of the left femur. In addition, the correct classification rates that are obtained from the application of the functions is particularly high, with 89.90 % for the left femur and 91.20 % for the right tibia. When we compare the results for the maximum length of the femur with those from the study of Mall et al. [41], we see that the accuracy rate in the Greek sample was 80.40 %, while that of the German population was 67.7 %. The cross-validated results for the femur range from 74.90 % to 89.90 %, depending on the variable examined. Of all the measurements, the results clearly show that the epicondylar breadth of the femur (EBF) is the one that has the highest discriminatory value between males and females. This is followed by the maximum diameter of the femoral head (MDH) and the maximum length (ML) of the femur (Table 5).

Between the two skeletal elements examined in the present study, it was found that the highest accuracy rates were provided by the measurements of the tibia. The cross-validated results range from 83.10 % to 93.40 %. Here, the most discriminatory variable is that of the maximum epiphyseal breadth of the proximal tibia (MEBP), followed by the maximum epiphyseal breadth of the distal tibia (MEBD) and the maximum length (ML). Another study that has found that the MEBP is the most dimorphic trait is that by Slaus & Tomicic [37] on a Croatian sample.

In similar studies of the lower limbs, equally high accuracy rates were observed. For example, for the femur, the EBF resulted in rates of 97,5% [18], 95,4% [42], 94,9% [40] and 81,4% [41]. For the tibia, results have included 88.7 % for the MEBD and 86,8% for the MEBP [39] as well as 82,2% και 85,6% for the same measurements by Slaus & Tomicic [37]. The distal breadth (MEBD) of the tibia provided the best discrimination [43].

The above comparisons reveal two very obvious trends: the first is that the epiphyseal dimensions are more sexually dimorphic and the second that the degree of sexual dimorphism varies across populations. The reliability of the epiphyseal measurements was observed not only in the present study, where the distal femur and proximal tibia provided the best results, but other authors have reached the same conclusions, for bones from both the upper and lower limbs [17–19,44–46]. Some studies have suggested that the epiphyseal areas of long bones are those that are subjected to mechanical stress during loading [47,48], and as a result they have increased dimensions. This theory could support the findings of the present study, as the vast majority of the individuals in the Athens Collection come from lower and middle socioeconomic classes and most males were engaged in manual labour, while most females were listed as homemakers. It should be noted however, that according to some authors, mechanical loading affects the dimensions that form the shape of the bone, such as the cross-section of the diaphysis [38,48–50]. The focus of the present study was different, and these traits were not recorded, therefore, we cannot reach any conclusions in regard to the Greek population. What is significant about the reliability of epiphyseal measurements for forensic purposes, is the fact that even incomplete leg bones can be used for sex assessment.

The other important finding of our study is that the degree of sexual dimorphism, as well as which variable is sexually dimorphic, are both population-specific. It is well-known that body and consequently skeletal dimensions are affected by several factors. These include the environment, diet [20,21,51,52], mechanical stress and activity patterns [49,50,53], and the genetic makeup of populations [21,53,54]. Stini [51] was one of the first researchers who discussed the differential response of the two sexes to dietary stress by suggesting that the long-term lack of protein delays the skeletal development to a greater extent in males. The result is that males do not reach their maximum potential of development, and thus sexual dimorphism is reduced. Other studies have shown that those who are at the extremes of protein availability (minimum and maximum) display low sexual dimorphism, while those who are in the middle are more sexually dimorphic [55]. This theory can explain the results of the present study, as the Mediterranean diet, which is dominant in modern Greece, contains moderate amounts of protein, which favours the development of sexual dimorphism [56,57].

As mentioned earlier, mechanical loading on the skeleton is responsible for the size that individual bones will have. Consequently, the degree of sexual dimorphism will be linked to the degree of physical activity and division of labour between the two sexes [21,48]. It is therefore expected that in an urban and technologically advanced society, sexual dimorphism will be reduced, as both males and females engage in a more sedentary lifestyle. This is supported by studies that focus on societies where the opposite is observed; for example, the study by Carlson et al. [49], which examined hunter-gatherers from Australia. The long bones were evaluated for sexual dimorphism, and it was found that they were highly dimorphic. The bones of the upper limbs because of the different tasks that they were engaged in, such as the use of hunting weapons by males and food gathering by females, and the bones of the lower limbs due to the greater stress (longer distances for hunting travelled by males). Such extreme division of labour does not exist in Greece, but prior to the urbanisation of the country, the differences in the activity patterns between the two sexes were more pronounced. Males were usually engaged in manual labour, heavy in some cases, such as farming and construction, while most females stayed at home and engaged in housekeeping, which usually includes manual, yet lighter tasks. The sample examined in this study is representative of this period, during which, division of labour in Greece was pronounced.

All the factors mentioned above, which all play a role in the expression of sexual dimorphism, are interacting with the genetic background present in each population [50,53–54,58]. The fact that in each population different bones, or even different dimensions of the same bones, display the highest degree of sexual dimorphism may be related to the genes that determine their size [58]. This is believed to be the reason for the existence of differences even among populations that are close both geographically and in terms of lifestyle. It is clear that each population has a unique pattern of sexual dimorphism, therefore, the metric standards derived from a particular sample should not be used for a different population, unless there are documented similarities between the two [20,21,50]. In addition, the skeletal changes that a population may be subjected to over time have the potential to change its metric characters and make the application of functions developed on a modern sample unsuitable for an archaeological one [59].

When it comes to forensic anthropological casework, the findings generated by the present study will provide a tool for forensic practitioners in Greece and other countries in the same region, by which they can make more accurate sex assessments. Forensic anthropologists have been increasingly engaged in the development and use of quantitative methods for biological profiling methods in recent years, as these provide more robust support for the assessments that are used within a medicolegal system [60]. Such methods can be especially useful when incomplete remains are found, or when the recovered skeletal elements that contain sexually dimorphic features have been compromised due to taphonomic or other alterations.

5. Conclusion

The results of the present research indicate that there is a high degree of sexual dimorphism in the Greek skeletal population under study and that the formulae that have been produced are very successful in differentiating sex from the bones of the lower limbs. This is in line with the recent trend within forensic anthropology to standardise the methods used by practitioners by the use of quantitative approaches to sex assessment. It should be noted that the metric methods proposed by our study should only be used on skeletal remains from Greece and the surrounding region of Southeast Europe. The applicability of the methods on populations from other geographic areas should be tested on skeletal samples in which the sex is known, either through documentation, other anthropological methods, or genetic analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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