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ASHLEY L. HUMPHRIES, ANN H. ROSS

CRANIOFACIAL SEXUAL DIMORPHISM IN TWO PORTUGUESE SKELETAL SAMPLES

ABSTRACT: One of the goals of anthropological research is to investigate biological human variation of past and present populations. Of particular interest is the study of sexual dimorphism, which can shed light on the human condition and aid in the identification of unidentified remains. When dealing with human skeletal remains, one of the four pillars of the anthropological protocol is the estimation of sex. Problems arise when applying sexing methods to different populations. Consequently, a skeletally robust female may appear to be "male", particularly in light of cross-population comparisons. The purpose of this study is to evaluate sexual dimorphism of the craniofacial complex in two local Portuguese samples (Coimbra, F=40, M=39; Lisbon, F=27, M=28). An index of sexual dimorphism or ISD was used to assess the level of sexual dimorphism within each sample (Lisbon ISD=3.71; Coimbra ISD=3.07). The Student's t-test indicates that the degree of sexual dimorphism is not significantly different between Coimbra and Lisbon (P=0.31). However, Mahalanobis D^2, which was computed to examine differences among the groups, indicates that Lisbon females differ significantly from the other samples and the pattern of sexual dimorphism coincides with the ISD results. The disparity of the Lisbon females may indicate the possible influence of immigration or genetic diversity left behind by the numerous population influxes on the Iberian Peninsula and warrants further study.

KEY WORDS: Craniometric variation – Sexual dimorphism – Index of sexual dimorphism

INTRODUCTION

One of the goals of anthropological research is to better understand human biological variation of past and present populations and to investigate the biological, genetic, and environmental forces responsible for that variation. Of particular interest is the phenomenon of sexual dimorphism, which can aid in the identification of unknown remains and shed light on the human condition regarding health, differential exposure to disease and trauma, and evolutionary trends. It has been demonstrated that skeletal characteristics, including levels of sexual dimorphism, vary among populations and across time (İşcan 2005, Jantz, Meadows Jantz 2000, King *et al.* 1998, MacLaughlin, Bruce 1986, Meadows, Jantz 1995). In a drastically changing world with a growing ability to migrate to different geographic locations, it is essential to continually investigate skeletal variation and sexual dimorphism of various populations within and between geographic regions (İşcan 2005, Kimmerle *et al.* 2008, Ross *et al.* 2011). Reassessment and refined assessment of regional samples assists in developing more accurate identification methods for the medico-legal system and bioarchaeological investigations, and may provide information regarding evolutionary trends in sexual dimorphism.

Human populations differ in relation to body size and shape, as do the males and females within each population, which has been well documented by numerous studies (İşcan *et al.* 1995, İşcan 2005, Jantz, Meadows Jantz 2000, Kimmerle *et al*. 2008, Macho 1990, Meadows, Jantz 1995,

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Steyn, İşcan 1998). In fact, sexual dimorphism contributes greatly to intraspecies variation and constitutes a major source of variation within and between species (Willmore *et al.* 2009). Males and females appear very different, which was supported by Kimmerle and colleagues (2008) who found significant size differences between males and females in European and African American crania using geometric morphometric techniques, finding that males, on average, are larger. Yet, compared to other primates, human levels of sexual dimorphism are low (Frayer, Wolpoff 1985). While levels of sexual dimorphism are lower in humans, differences are still apparent and may be influenced by a combination of sexual selection, energetic intake, nutrition, body composition, genetics, cultural practices, and human migration, which may differentially impact the levels of sexual dimorphism between human populations (Frayer, Wolpoff 1985, Hall 1982).

A combination of intrinsic (genetic) and extrinsic (environmental) factors contribute to population differences in body size and proportions and influence secular change and sexual dimorphism within populations (Hamilton 1982). However, the individual influences of these factors are difficult to tease apart. This has implications for both forensic and bioarchaeological investigations as problems may arise when applying sex estimation methods to different populations because they may innately differ in stature, physique, or general robustness due to differential effects of intrinsic and extrinsic factors. For example, Macho (1990) compared femora from several African populations and a South African population of European descent and demonstrated through multivariate analyses that different patterns of sexual dimorphism exist among the groups. Furthermore, Macho (1990) detected significant differences in sexual dimorphism in adjacent African tribes.

There is also no evidence of secular change ceasing, so it is of anthropological interest to continue to investigate trends in human variation so that appropriate samples are used to develop identification criteria for contemporary populations. For example, it has been demonstrated by Jantz and Meadows Jantz (2000) and Meadows and Jantz (1995) that skeletal elements have exhibited striking changes over the past 1.5 centuries in African and European Americans. A study presented by Ross *et al.* (2011) examined morphometric cranial variation among Spanish and Portuguese skeletal samples and identified varying patterns of regional variation, sexual dimorphism, and secular change indicating a size related change overtime in the Spanish population. Additionally, İşcan *et al.* (1995) examined sexual dimorphism in modern Japanese cranial dimension and found that the population has gone through significant microevolutionary changes. Their study revealed that sexual dimorphism in Japanese crania may have decreased as a result of an increase in size of females.

Accurate and timely estimation of biological sex is one of the most important features in the progression of an investigation involving unidentified human remains and for bioarchaeological research because many of the methods used for the estimation of age and stature are both population and sex specific (Brooks, Suchey 1990, İşcan, Loth 1986a, b, İşcan 2005, Spradley *et al.* 2008). The estimation of sex helps to approximate the sexual composition of large samples for demographic investigations and aids in narrowing the pool of missing persons in forensic investigations.

Traditional methods used to determine biological sex from skeletal remains consists of visually assessing a set of features on the *os coxae*, cranium, and mandible and scoring them on a scale that ranges from "Male" to "Indeterminate" to "Female" (Bruzek 2002, Buikstra, Ubelaker 1994, Phenice 1969, Walrath *et al.* 2004, Williams, Rogers 2006). These traits are then averaged by the investigator to estimate biological sex. However, such an assessment can be influenced by the observer's training and level of experience and the features chosen for examination (Walrath *et al.* 2004, Williams, Rogers 2006).

In addition, estimates can be difficult if the observer is not familiar with the overall pattern of variability within the population from which the sample is drawn (Walrath *et al.* 2004). As a result, the visual assessments of these traits have been regarded as subjective and the assessment of any particular trait may vary among observers based on training and experience. For this reason, metric analyses are often preferred or are used to support the visual assessment because they are considered more objective. It should be noted however, that considerable debate still exists concerning which of the two, morphological or metrical methods, are best at estimating sex. Likely, it is a combination of the two. Nevertheless, metric analyses have much to offer especially in quantifying and illustrating the degree of difference within a population overtime and among populations (Kimmerle *et al.* 2008, Rosas, Bastir 2002, Ross *et al.* 2011). While nonmetric sex estimation methods exist, metric assessments of skeletal change can provide a better idea of the type of changes in body size and proportions that have occurred through time, as well as better describe the differences that exist between populations.

A number of metric sex estimation methods have been developed for various skeletal elements and many have proven reliable if applied correctly (Dabbs, Moore-Jansen 2010, King *et al.* 1998, Steyn, İşcan 1998). It is essential to have sex estimation standards for various skeletal elements because not all elements may be available for examination. For example, the pubis portion of the *os coxae* is often damaged due to taphonomic processes since it is primarily composed of cancellous bone (Ross, Cunningham 2010). While the *os coxae* is the best indicator of sex because of its obstetrical requirements, the cranium has been found to be a reliable indicator of sex especially when using metric techniques (Franklin *et al.* 2005a, Giles, Elliot 1963, Steyn, İşcan 1998). Craniometric analyses can provide a wealth of information regarding various aspects of human variation,

which makes it a promising element for analysis as it not only sheds light on sexual dimorphism but also provides information regarding biological relationships and ancestry (Relethford 1994). In addition, the measurements taken rely on standard anatomical landmarks, which ultimately reduce levels of intra- and inter-observer error.

The purpose of this study is to present a comparative craniometric study of among-group variation for two samples from Portugal to examine if there are differences in sexual dimorphism between regionally distinct samples from Coimbra and Lisbon. This paper will shed light on the anthropological issue of human variation and sexual dimorphism.

MATERIALS AND METHODS

Materials

The materials used in this study consist of 134 adult crania of known sex from two Portuguese identified skeletal collections, the Lisbon Luís Lopes Collection and the Coimbra Medical School Skull Collection. The sample composition is presented in *Table 1*. The Lisbon sample consists of 27 females and 28 males and is curated at the Bocage Museum in Lisbon, Portugal. The Lisbon sample represents identified individuals from the 20th century, born between 1805 and 1972 and who died between 1880 and 1975 (Cardoso 2006). The Coimbra sample consists of 40 females and 39 males and is curated at the *Museo Antropologico de Coimbra* (University of Coimbra, Coimbra, Portugal). The Coimbra sample represents identified individuals from the end of the 19th century and early 20th century, born between 1802 and 1890 and who died between 1895 and 1903 (Cunha, Wasterlain 2007). Only adult crania were included in this study.

The sixteen traditional craniometric measurements, or interlandmark distances (ILDs), that were used in this study are listed in *Table 2*. The ILDs for the Coimbra sample were collected using spreading and sliding callipers. This procedure entails placing the ends of the callipers on the appropriate anatomical landmarks and reading the distance (in mm) between the two points. The coordinate data for the Lisbon sample were collected using a MicroScribe G2X digitizer and the program ThreeSkull, written by Steve Ousley (2004) was used to calculate the ILD's. This procedure entails placing the tip of the MicroScribe stylus on an anatomical landmark and depressing the attached foot pedal, which records the *x, y,* and *z* coordinates for that particular landmark. The landmarks collected by the digitizer correspond with the endpoints of the ILDs from which spreading or sliding callipers are placed to collect the traditional craniometric measurements. The program ThreeSkull calculates the distance (in mm) between the landmarks making them equally comparable with traditional ILD data collected using callipers. Furthermore, Franklin and colleagues (2005b) demonstrated that three-dimensional landmark coordinates collected by a digitizer can be successfully transformed for use in traditional ILD studies.

Statistics

First, the degree of sexual dimorphism in the Coimbra and Lisbon samples was assessed with an index of sexual dimorphism (ISD), a commonly used method to

TABLE 1. Sample composition.

Sample	Females	Males	Period
Lisbon (Luís Lopes Collection)	27	28	20th Century
Coimbra (Medical School Skull Collection)	40	39	End of 19th and Early 20th Century

TABLE 2. Traditional craniometric measurements.

#	Abbreviation	Interlandmark distances	Description of measurements
1	GOL	Maximum Cranial Length	Distance between glabella (g) and opisthocranion (op)
2	BNL	Cranial Base Length	Distance between basion (ba) and nasion (n)
3	BBH	Cranial Height	Distance between basion (ba) bregma (b)
4	XCB	Maximum Cranial Breadth	Max. width of skull from euryon (eu) to eury (eu)
5	WFB	Minimum Frontal Breadth	Distance between the two frontotemporale (ft)
6	ZYB	Bizygomatic Breadth	Distance between zygion (zy) and zygion (zy)
7	AUB	Biauricular Breadth	Distance between both auriculare (au)
8	OBH	Orbital Height	Distance between the superior and inferior orbital margins
9	OBB	Orbital Breadth	Distance between dacryon (d) and ectoconchion (ec)
10	DKB	Interorbital Breadth	Distance between both dacryon (d)
11	EKB	Biorbital Breadth	Distance between both ectoconchion (ec)
12	FRC	Frontal Cord	Distance between nasion (n) to bregma (b)
13	PAC	Parietal Cord	Distance between bregma (b) and lambda (l)
14	OCC	Occipital Cord	Distance between lambda (l) and opisthion (o)
15	NLH	Nasal Height	Distance between nasion (n) and nasospinale (ns)
16	NLB	Nasal Breadth	Distance between both alare (al)

assess levels of sexual dimorphism in extant hominines (Lockwood 1999). While numerous other more robust methods are available to assess levels of within-species variability such as multivariate methods, mixed models and more recently the MI measure as proposed by Ipiña and Durand (2004, 2010), we chose the ISD mean method for its simplicity of calculation and because our samples are derived from the same modern population with low levels of sexual dimorphism compared to extant hominines and hominoids, the samples are sexed (e.g. from collections with known demographics) and the samples were not derived from unknown individuals such as those commonly encountered in fossil samples. However, other methods to assess the levels of sexual dimorphism would be more appropriate if the sample under study cannot meet the above criteria. Individual ISDs were calculated for each of the sixteen variables. The mean ISD was then calculated by averaging the sixteen individual ISDs in order to compare average sexual dimorphism between Coimbra and Lisbon. A Student's *t*-test was computed on the mean ISD values to examine intrasexual variability between the Lisbon and Coimbra groups. The index of sexual dimorphism is calculated as:

$$ISD = \left[\text{male mean} / \text{female mean} - 1\right] \cdot 100 . \qquad (1)$$

To further examine craniofacial sexual dimorphism among the four groups (Coimbra Males, Coimbra Females, Lisbon Males, and Lisbon Females) while directly accounting for size effects, size and shape variables were computed according to Mosimann and colleagues using the raw ILD measurements (Darroch, Mosimann 1985, Mosimann, James 1979). Here size is defined as the geometric mean (GM). Essentially, size is the product of all variables to the $1/k^{th}$ power, where k is the number of variables used. The GM or size for the 16 cranial measurements is calculated as:

$$SIZE = \left(\prod_{i=1}^{k} X_i\right)^{1/k} . \qquad (2)$$

Once the new size variable was computed, each raw cranial variable was then divided by the GM or size variable to create shape variables ($Y=X/SIZE$). The shape variables are simple ratios of the GM that measure the size of a particular region relative to the overall size of the cranium (Roseman, Weaver 2004).

A one-way analysis of variance or ANOVA using the contrast statement in the proc GLM procedure in the statistical software SAS 9.1.3 was performed to identify the differences in the newly calculated size variable among groups. Additionally, to test whether group centroids are significantly different, the degree of differentiation among the groups was measured using Mahalanobis D^2. Mahalanobis D^2 or generalized squared distance is a function of the group means and the pooled variances and covariances.

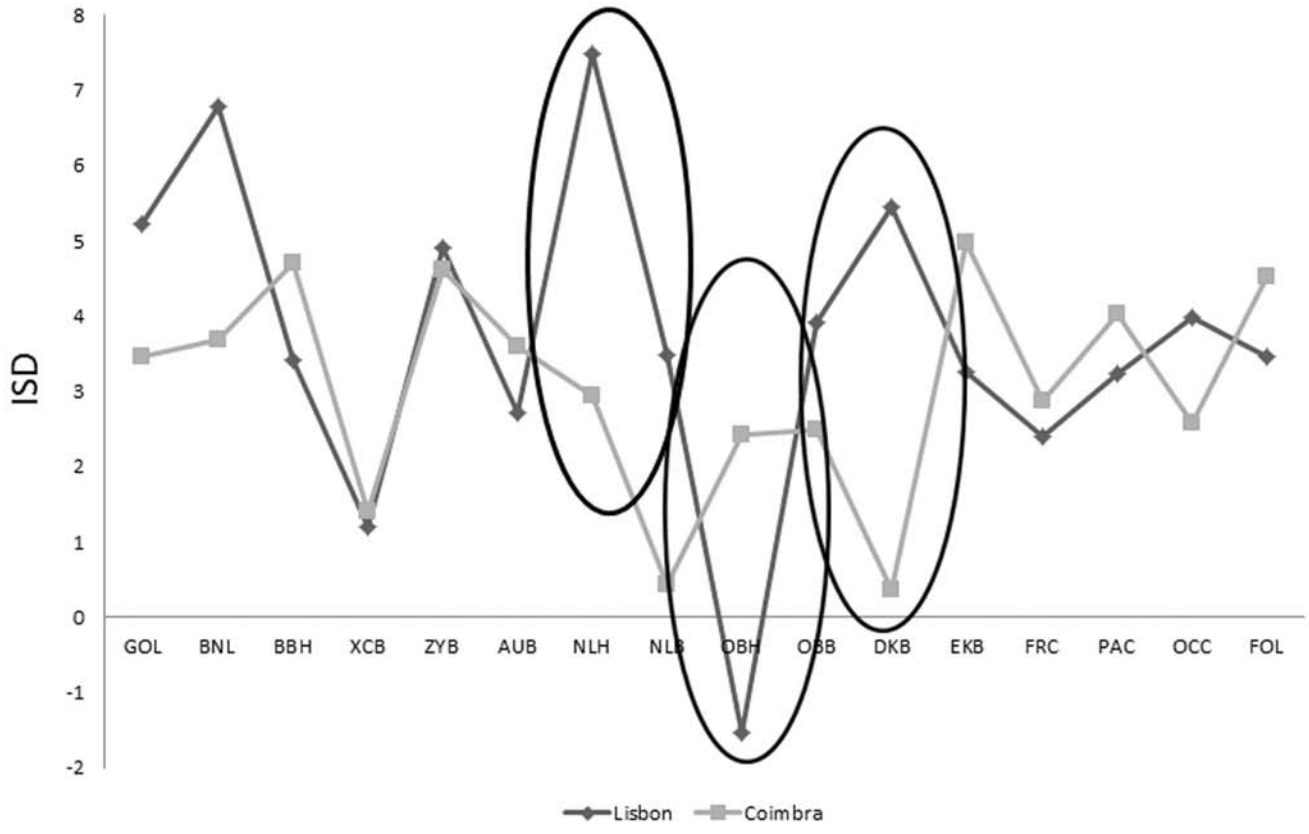

FIGURE 1. Pattern of sexual dimorphism of ISDs of all craniometric variables.

TABLE 3. Mahalanobis D^2.

	Coimbra Females	Coimbra Males	Lisbon Females	Lisbon Males
Coimbra Females	0	–	–	–
Coimbra Males	0.99	0	–	–
Lisbon Females	4.76**	4.87**	0	
Lisbon Males	2.34*	1.70	3.85*	0

*, significant at 0.05 level; **, significant at 0.0001 level.

Next, a discriminant function analysis using the cross-validation or leave-one-out method was performed on the newly transformed shape variables to obtain correct classification results. Additionally, a canonical discriminant analysis was conducted and canonical variates were derived from the newly transformed shape variables to examine between-group differences relative to within-group variation. This function extracts canonical variates, which are linear combinations of predictor variables that summarize between-population variation (Ross *et al.* 2002). This procedure reports significant canonical axes and the total canonical structure, which allow for the graphical representation of population and sex differences of significant shape variables. Lastly, a Pearson product moment correlation coefficient was conducted to measure the strength of the relationship between the size variable and canonical axes. The statistical analyses were performed using the SAS system for Windows Version 9.1.3 (SAS 9.1.3).

RESULTS

The mean index of sexual dimorphism or ISD for all sixteen craniometric variables reveals that the mean value for males is 3.71 per cent larger than that for females in the Lisbon sample. For the Coimbra sample the mean value for males is 3.07 per cent larger than that for females. The average ISD for all measurements is somewhat larger for the Lisbon sample suggesting that they are somewhat more sexually dimorphic. However, the Student's *t*-test did not find significant intrasexual variability between group ISDs for Coimbra and Lisbon (P=0.31). *Figure 1* presents the individual ISDs for all 16 craniometric variables and illustrates that the Coimbra and Lisbon samples share a relatively similar pattern of sexual dimorphism in many areas with the exception of a different pattern of sexual dimorphism in the nasal and orbital region.

The ANOVA using the contrast statement in the proc GLM procedure in the statistical software SAS used to identify the differences in the newly calculated size variable between groups, indicates that no significant difference in size was observed between Coimbra and Lisbon females (P=0.99) or between Coimbra and Lisbon males (P=0.85) (*Table 4*).

TABLE 4. Contrast statement GLM procedure for SIZE variable. No significant difference in size between Coimbra and Lisbon females and between Coimbra and Lisbon males.

Group	P-value
Coimbra Females – Coimbra Males	<0.0001
Coimbra Females – Lisbon Females	0.99
Coimbra Males – Lisbon Females	0.0002
Coimbra Females – Lisbon Males	<0.0001
Coimbra Males – Lisbon Males	0.85
Lisbon Females – Lisbon Males	0.0003

The Mahalanobis D^2 values are presented in *Table 3*. The Mahalanobis squared distances provide information about group similarity and relatedness. Interestingly, it was revealed that the Coimbra males are not significantly different from Coimbra females and Lisbon males (see *Table 3*).

The correct classification results of the discriminant function analysis are presented in *Table 5* and show a correct classification of 35 per cent for Coimbra females, 70 per cent for Lisbon females, 31 per cent for the Coimbra males, and 43 per cent for Lisbon males. The higher classification rate for the Lisbon females suggests that they are more dissimilar from the rest of the series. This was also indicated by the Mahalanobis distances. Additionally, since the ANOVA revealed no significant difference in size between the Coimbra and Lisbon females, it indicates that the greater correct classification rate for the females is not related to size, but to shape.

Figure 2 is a graphical representation of the two significant canonical axes accounting for roughly 89% of the total among-group shape variation with 64 per cent on CAN1 and 25 per cent on CAN2 (Wilks' lambda=0.41;

TABLE 5. Percent of crossvalidated correct classification based on the transformed shape variables discriminant analysis (*n*).

	Coimbra Females	Coimbra Males	Lisbon Females	Lisbon Males	Total
Coimbra Females	35.0 (14)	35.0 (14)	12.5 (5)	17.5 (7)	100.0 (40)
Coimbra Males	35.9 (14)	30.8 (12)	7.7 (3)	25.6 (10)	100.0 (39)
Lisbon Females	14.8 (4)	7.4 (2)	70.4 (19)	7.4 (2)	100.0 (27)
Lisbon Males	10.7 (3)	21.4 (6)	25.0 (7)	42.9 (12)	100.0 (28)

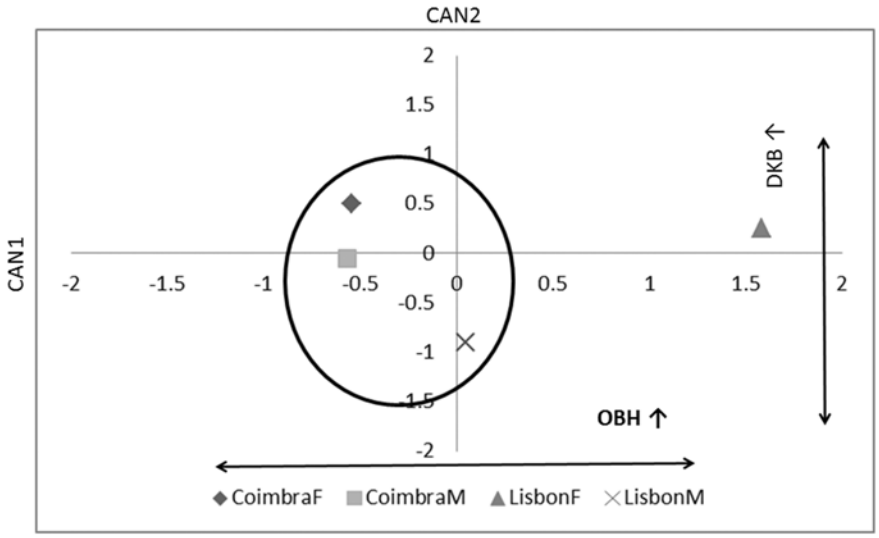

FIGURE 2. Class means on canonical variables (using transformed shape variables).

F=2.50; *df*=48, 343; *P*=0.0001). The total canonical structure, the correlation between the original variables and the canonical variates, for CAN1 and CAN2 indicates that the variation on the first canonical axis separates the groups with respect to orbit height (OBH) and basion-nasion length (BNL), while the second axis isolates the groups on interorbital distance (DKB). *Figure 2* illustrates that Coimbra males and females have narrower orbits and relatively wider interorbital distances and Lisbon males are more similar in shape with to the Coimbra series. In addition, Lisbon females have a wider and higher orbital distance and height. These results coincide with the pattern of observed in the ISD's.

The Pearson product moment correlation coefficient used to measure the strength of the relationship between the size variable and canonical axes revealed a weak relationship between the second canonical axis and the size variable (R^2=0.299, *P*=0.0004). This suggests that some variation observed on this second axis is influenced by size.

DISCUSSION

The majority of sexually dimorphic traits are polygenic, which makes explaining observed differences cumbersome. In addition, the social nature of the human species makes cultural practices an additional factor (Frayer, Wolpoff 1985). Therefore, evolutionary explanations must consider both intrinsic and extrinsic influences as well as cultural practices to interpret the differences observed (Hamilton 1982).

This study found that the overall levels of sexual dimorphism, as measured by the Index of Sexual Dimorphism, are not significantly different between the Coimbra and Lisbon samples. However, significant and interesting differences were detected encouraging further discussion. Frayer and Wolpoff (1985) note that while the magnitude of sexual dimorphism may differ greatly from population to population, it does not seem to differ on the average from region to region. While the overall mean levels of sexual dimorphism are not significantly different between the Portuguese samples used in this study, the patterns of dimorphism do slightly differ between the two samples (*Figure 1*).

Similar to the results from a study conducted by Kimmerle *et al.* (2008) who found significant size differences between males and females in European and African Americans, this study revealed a significant size difference between males and females in the two Portuguese samples. This is also in agreement with Calcagno (1981) and Uytterschaut (1986) who found that sexual dimorphism is primarily the result of size differences rather than shape differences between males and females. The results of this research also revealed that the Coimbra and Lisbon males are not significantly different in both shape and size from each another, and that size differences do not exist between the Coimbra and Lisbon females. Interestingly, while females are not significantly different in size, they do exhibit some shape differences, which is substantiated by the better classification rates for Lisbon females and is illustrated by the graphical representation of the canonical axes. This facet also helps to explain that while the mean ISDs for the Coimbra and Lisbon samples were not significantly different, slightly different patterns of dimorphism were still revealed by comparing the individual ISDs of the sixteen ILD measurements (*Figure 1*).

Rosas and Bastir (2002) investigated allometry and sexual dimorphism using geometric morphometric methods within a Portuguese population. They demonstrated that size and sex had a significant influence on the shape of the craniofacial region. Furthermore, they found no difference in the influence of size on shape (allometry) between the sexes revealing a shift in the proportions of the neurocranium and the viscerocranium, with a marked allometric variation of the lower face. In contrast, Kimmerle *et al.* (2008) found that size did not have a significant influence on shape, but that sex did.

It is generally accepted that males are more susceptible to changes when nutritional quality is altered and that females are more canalized, or less affected by nutritional shortages because of reproductive demands, fat storage, and overall smaller body size (Frayer, Wolpoff 1985). Therefore, greater secular change is typically seen in males because of differential sensitivity to environmental changes between the sexes. For example, while comparing 19th and 20th century American skeletons, Meadows and Jantz (1995) found that the male secular change was stronger than the female secular change. Interestingly, the present study detected no significant differences between the males from the two samples. Therefore, it seems unlikely that the differences detected between the Coimbra and Lisbon females would be due to an environmental factor. Another explanation for the morphological differences observed in this study may be differential mate selection in the two populations because of sexual selection. Additionally, Lisbon may be subject to the influences of immigration more so than in rural areas because it is a major port. This may introduce more genetic diversity into the gene pool. Lastly, the genetic traces left behind from various ethnic groups that occupied the Iberian Peninsula throughout history may have left a significant signature within the population.

Ross *et al.* (2011) report that morphological variability observed in skeletal samples from the Iberian Peninsula could most likely be attributed to an amalgamation of the various ethnic groups that originally populated the area. In addition, Pereira *et al.* (2000) analysed mitochondrial DNA in Portugal and found that the population presents a higher level of diversity than some surrounding populations. The authors analysed three population samples from North, Central, and Southern regions that were arbitrarily defined by the Douro and Tagus Rivers. Overall, they found all important European haplogroups from the Palaeolithic and Neolithic time periods, as well as a distinct African influence. The African influence was detected by distributions of haplogroups U6 and L, with U6 being restricted to the Northern region and L being widespread. The authors attributes these findings to two different population movements including the African slave trade as the mediator for the L sequence and Muslim rule of Iberia during the 16th century as the mediator for the U6 haplogroup. While the Coimbra and Lisbon samples fall within the central and southern regions, respectively, it is still plausible that morphological variability observed between the Coimbra and Lisbon females may be attributed to the genetic diversity left behind by various populations that came to occupy the Iberian Peninsula throughout history.

Lastly, because of the different, but relatively close time periods of the two samples, it is uncertain whether the results represent two regionally distinct Portuguese populations that differ due to separate secular trends or one Portuguese population that is experiencing secular change. Further analyses are warranted to attempt to tease out these nuances.

CONCLUSIONS

These results obviate the importance of investigating regional or geographic morphological variations and further underscore the importance of calibrating methods to reflect the biology of the local population. The varying levels of dimorphism among populations could affect the classification accuracy when attempting to assign an "unknown" to a particular reference group and further skew results for sex estimation when applying different standards across populations. The results from this study are intriguing and warrant further investigation using more robust sample sizes and more temporally distinct samples to tease out regional and/or temporal factors influencing the shape difference observed between the Coimbra and Lisbon females.

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Ashley L. Humphries
Department of Anthropology
University of South Florida
Tampa, Florida 33620-8100
USA
E-mail: ahumphri@mail.usf.edu

Ann H. Ross
NC State University
Department of Sociology and Anthropology
Campus Box 8107
Raleigh, NC 27695-810
USA
E-mail: ann_ross@ncsu.edu