

Daniel Franklin,^{1,2} Ph.D.; Charles E. Oxnard,^{1,2} M.B.Ch.B., Ph.D., D.Sc.; Paul O'Higgins,³ M.B.Ch.B., Ph.D., D.Sc.; and Ian Dadour,¹ Ph.D.

Sexual Dimorphism in the Subadult Mandible: Quantification Using Geometric Morphometrics*

ABSTRACT: There have been numerous attempts, with varying degrees of success, to differentiate males from females on the basis of the immature skeleton. We investigate here whether the mandible can discriminate immature individuals by sex; the techniques we apply are from the field of geometric morphometrics. The application of these methods in forensic anthropology is still relatively new; thus, an important aspect of this research is that it demonstrates potential applications in this discipline. The sample comprises 96 known age and sex subadult individuals; the three-dimensional coordinates of 38 landmarks are analyzed using the shape analysis software *morphologika*. Multivariate regressions indicated no significant sexual dimorphism in the subadult sample; this result is supported by poor cross-validated classification accuracy (59%). Our results suggest that the subadult mandible is not dimorphic (to the extent that dimorphism is not evident within the sample we studied); thus, sex determination using previously described criteria is likely to yield poor results.

KEYWORDS: forensic science, forensic anthropology, subadult, mandible, sex assessment, population affinity, geometric morphometrics, morphological variation

The determination of sex from human skeletal material is of fundamental importance for any forensic investigator. The most favored approaches are selected in the hope of providing a high degree of accuracy, but also for suitability of assessment of material that is often damaged or fragmentary (1–2). Although these methods have proved feasible when the recovered material is adult, when applied to the subadult skeleton, the accuracy of sex determination falls markedly. This, the inability to reliably assign sex in the subadult age range is a significant problem facing even the most experienced forensic anthropologist (3).

There are examples in the literature of attempts to differentiate males from females using different elements of the immature skeleton (4–7). Of direct relevance to the present study is the research of Loth and Henneberg (8), who, based on their nonmetric examination on a South African subadult sample, claimed that shape differences in the symphyseal region and anterior body of the mandible can be used to predict sex with above 80% accuracy. In a blind test of that technique, however, Scheuer (9) showed that when applied to different population samples, sex classification accuracy declined considerably to 64%.

In recent years, geometric morphometric methods have become increasingly common for studying human skeletal biology in both physical, and of late, forensic anthropology (10). These methods have been used to a greater extent because they are versatile and allow detailed assessment of differences among specimens (11). This paper is the first in a series of studies designed to apply three-dimensional geometric morphometric methods to problems in

forensic anthropology. The aim of the present study is to use techniques not previously applied to answer the following question: given a large enough sample, can the mandible be used to discriminate subadult individuals by sex?

Materials and Methods

Material

The present study examines 96 subadult mandibles from three distinct populations. The composition and origin of the mandible series are described in Table 1. The entire sample comprises “known individuals”; thus, the sex, local population, and a statement of age are documented for each specimen.

Data Acquisition

A total of 38 bilateral three-dimensional landmarks were designed and acquired using a Microscribe G2X portable digitizer and Inscribe-32 software (Immersion Corporation, San Jose, CA). Landmarks were chosen to correspond to those commonly used in the traditional metric and geometric morphometric systems (10,12–15) and should thus be familiar to most physical and forensic anthropologists. In addition, a series of new landmarks were designed to characterize the shape of the posterior ramus, lateral body, and symphyseal region (see Table 2 and Fig. 1 for a complete description and illustration).

Shape Analysis

The shape analysis software *morphologika* (www.york.ac.uk/res/fme) is used to analyze the three-dimensional coordinates of the landmarks. As there are now many studies in the literature utilizing the techniques of geometric morphometrics implemented in this software, we provide only a succinct overview of our methods; the reader is directed to consult the following sources for a more detailed description (11,16–19).

¹Centre for Forensic Science, The University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia.

²School of Anatomy and Human Biology, The University of Western Australia, 35 Stirling Hwy, Crawley, Western Australia.

³Functional Morphology and Evolution Research Unit, The Hull York Medical School, Heslington, York YO10 5DD, U.K.

*Presented at the 58th Annual Meeting of the American Academy of Forensic Sciences in Seattle, Washington.

Received 6 Mar. 2006; and in revised form 25 May 2006; accepted 8 July 2006; published 8 Dec. 2006.

TABLE 1—Composition of, and institutions from which, the skeletal material used in the present study are derived.

Population	Male	Female	Age Range (years)	Institution
African American	18	19	1–17	CMNH
South African Bantu	25	17	1–17	WITS
Caucasian	10	7	2–17	NHM

CMNH, Cleveland Museum of Natural History (Hamann-Todd Osteological Collection); WITS, University of the Witwatersrand (Raymond A. Dart Collection of Human Skeletons); NHM, Natural History Museum, London (Spitalfields Coffin Plate Sample).

To eliminate the nonshape variation in the sample, the raw landmark coordinates from all mandibles are first registered using generalized procrustes analysis (GPA). This process involves translating, rescaling, and rotating the configurations relative to each other so as to minimize a total sum of squares (20). The scaling procedure adjusts the landmark coordinates such that each mandible has a unit centroid size, which is used as a biologically meaningful expression of the overall size of the landmark configuration, and thus of the mandible (20–22). In the analysis of the shape differences between mandibles, the scatter of points representing the specimens is projected from Kendall's shape space into Euclidean tangent space; this specifically allows statistical analyses to be performed using standard analytical techniques (11).

A series of principal components analyses (PCA) are used to explore the relationships between samples of male and female mandibles. The shape differences revealed by the PCA are visualized and explored using PC plots, wireframe, and rendered models. Multivariate regression analyses are used to assess the significance of sexual dimorphism and interpopulation variation in the sample; plots of fitted values against standardized residuals showed that the assumptions of regression were met. Discriminant analyses with cross-validation are then used to assess classification accuracy.

Both analyses use the PC scores from GPA/PCA of the sample. The number of variables relative to the number of individuals tends to be large in geometric morphometric analyses as there are three coordinates per landmark. This inevitably means that classification accuracy can be impaired because of the dimensionality. So, to achieve optimal group discrimination, we examine plots of discriminant classification results against the number of variables used (23). By removing the redundant higher order variables, we effectively reduce the dimensionality of the sample and optimize the efficacy of the discriminant functions. Statistical analyses are performed using Genstat 8.10 and SPSS 13.0.

Measurement Error

Measurement error in landmark acquisition is assessed by digitizing six different specimens on six different occasions. Using the method of O'Higgins and Jones (16), the six repeat sets of coordinate data from the test mandibles are submitted to GPA and PCA along with the total sample. The six repeat specimens cluster closely together on PCs 1–8, relative to the variation between individuals. The test thus showed that measurement error was exceedingly small on all significant PCs.

Results

To explore whether there is any significant sexual dimorphism in the sample, we examine the material at two levels: (i) the pooled sample and (ii) various age and population groupings.

Sexual Dimorphism in the Pooled Sample

In the PCA of the total sample, PC 1 accounted for 47.4% of the total variance and showed a significant correlation with age and centroid size (both $p < 0.001$ —Fig. 2). It should be noted that although all the populations appear to scale on PC1, there are possible divergences in ontogenetic trajectories (between the sexes as they approach adulthood) on higher order PCs (this is discussed

TABLE 2—Definitions of the landmarks used in the present study.

Number	Landmark	Definition
Bilateral points: 1–17 (right); 22–38 (left)		
1 and 22	Coronion (co)	The most superior point on the coronoid process
2 and 23	Mandibular notch (mn)	The most inferior point on the mandibular notch
3 and 24	Condylion mediale (cdm)	The most medial point on the mandibular condyle
4 and 25	Condylion superior (cs)	The most superior point on the mandibular condyle
5 and 26	Condylion laterale (cdl)	The most lateral point on the mandibular condyle
6–8 and 27–29	Posterior ramus (pr)	A set of three instrumentally determined points (equally spaced between condylion superior and gonion) taken on the posterior border of the ramus
9 and 30	Gonion (go)	The most lateral external point of junction of the horizontal and ascending <i>rami</i> of the lower jaw
10–12 and 31–33	Mandibular body (mb)	A set of three instrumentally determined points (equally spaced between gonion and lateral gnathion) taken on the inferior border of the mandibular body
13 and 34	Lateral gnathion (lg)	Point at which a vertical line from landmark 14 and 35 intersects with the inferior border of the mandibular body
14 and 35	Lateral Infradentale (lid)	The mid-point of a line tangent to the outer margins of the cavities of the lateral incisor and canine teeth
15 and 36	Mentale (ml)	The most inferior point on the margin of the mandibular mental foramen
16 and 37	Posterior alveolar (pa)	The most posterior point situated on the labial alveolar surface behind the most posterior erupted tooth (or crypt for tooth)
17 and 38	Anterior ramus (ar)	Point at which the minimum breadth transects the anterior border of the ramus
Midline points		
18	Gnathion (gn)	The middle point on the lower border of the mandible in the sagittal plane
19	Pogonion (pg)	The most projecting point of the chin in the standard sagittal line
20	Mandibular symphysis (mns)	The deepest point at the mandibular symphysis curvature (between the infradentale and pogonion landmarks)
21	Infradentale (id)	The mid-point of a line tangent to the outer margins of the cavities of the two mandibular central incisor teeth

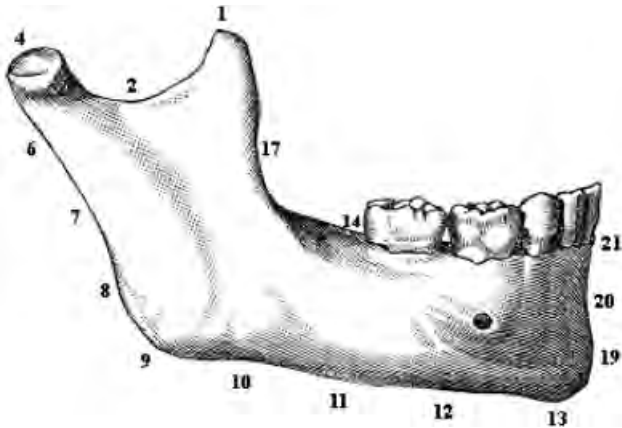


FIG. 1—Lateral view of the mandible; only selected landmarks are shown (see Table 2 for key).

further in a forthcoming publication). This PC therefore represents aspects of scaling shared by both sexes. The shape changes visualized by morphing from the negative to the positive extremes of PC1 are shown in Fig. 2 and demonstrate common features associated with “ontogenetic allometry” (10) of the mandible. From Fig. 2, it is evident that in the younger specimens the ramus is relatively short with a straight anterior border and a very obtuse gonial angle, such that the condyle is almost in line with the body; in the older specimens, the ramus is relatively longer with a curved anterior border and the gonial angle is reduced. During growth, the symphysis also increases in relative size and becomes more anteriorly projected.

Sex—Multivariate regressions of shape (performed using PCs 1–18 [optimal PCs for classification as assessed by discriminant function plots]—accounting for 90.8% of the total variance) against sex, age, and size indicate no significant sexual dimorphism in the pooled sample (Wilks’ $\Lambda = 0.749$, corresponding to an F statistic of 1.397 with 18 and 75 df [$p = 0.158$]). This result is supported by the poor classification accuracy of the cross-validated discriminant analysis performed using PCs 1–18: male 29/53 (55%); female 28/43 (65%); and overall 57/96 (59%).

Population—In contrast, multivariate regressions of shape (performed using PCs 1–36 [optimal PCs for classification as assessed by discriminant function plots]—accounting for 97.4% of the total

variance) against population, age, and size indicate significant interpopulation variation in the pooled sample (Wilks’ $\Lambda = 0.067$, corresponding to an F statistic of 4.267 with 74 and 110 df [$p < 0.0001$]). This result is supported by good classification accuracy of the cross-validated discriminant analysis performed using PCs 1–36: Bantu (83%); African American (81%); and Caucasian (70%).

Sexual Dimorphism: Various Age and Population Groupings

To further examine whether there is evidence of sexual dimorphism in the immature mandible (particularly in the more developed subadults), we examine different age groupings within a single sample, African Americans, selected because of the relative completeness of sampling across age classes. The results are shown in Table 3, and demonstrate that in only one of the subadult age groupings examined (15–17 years of age) did the analysis come close to, but not achieve, a significant level. Although there are large gaps in the data because of age-biased samples, examination of the Bantu and Caucasian populations also yielded no evidence of significant sexual dimorphism (not shown).

Discussion

In this study, we applied a relatively new methodology to examine an old problem in forensic anthropology: assigning sex in the subadult age range. Our investigation of the total sample suggests that it is not feasible to use the subadult mandible for sex determination. Nevertheless, some of our p values are close to significance, and as a result, although our samples are larger than most others so far reported, a further increase in sample size might well yield morphometric evidence of subadult sexual dimorphism.

In any case, the forensic scientist is faced with determining the sex of individual specimens and the results indicate that sex determination from the subadult mandible would likely only be viable from puberty. The precise age at which this could be done, however, is difficult to determine from this study. We can infer from our data that some degree of sex determination would be possible around 15 years of age (Table 3), but further investigation is necessary using adequate skeletal samples of individuals from 10 to 17 years of age. Ideally, this would use data from a single

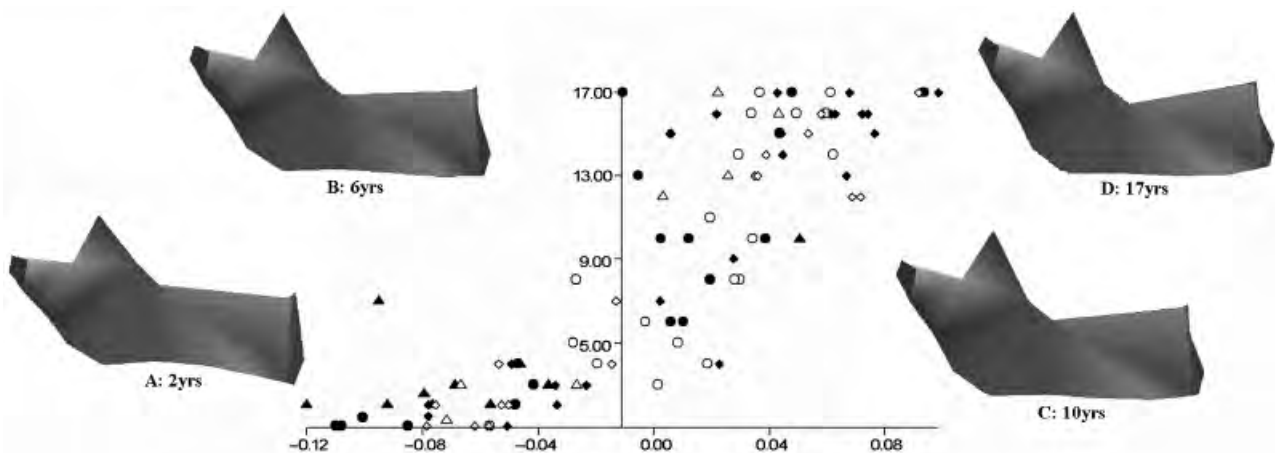


FIG. 2—Analysis of the pooled sample. PC 1 versus age (correlation $r = 0.83$, $p < 0.001$). Shaded, male; open, female; ●, African American; ▲, Caucasian; ◆, Bantu. The lateral rendered images show the variation in the shape of the mandible at different ages on PC 1. A, -0.08 (age 2); B, $+0.01$ (age 6); C, $+0.05$ (age 10); D, $+0.10$ (age 17).

TABLE 3—Probability for effect in multivariate regression analyses of shape variables on the sex, size, and age of the African American subadult sample.*

Age Range	Sex	Interaction	
		Age	Size
1–5	$p = 0.210$ (NS)	$p = 0.260$ (NS)	$p = 0.498$ (NS)
6–10	$p = 0.508$ (NS)	$p = 0.685$ (NS)	$p = 0.644$ (NS)
11–14	†	†	†
15–17	$p = 0.061$ (NS)	$p = 0.905$ (NS)	$p = 0.959$ (NS)

*Regression analyses performed using PCs accounting for 100% of sample variance.

†Insufficient specimens in age range to perform analysis.

population because sexual differentiation is under both hormonal and environmental control (24), and is thus widely variable both within and between populations.

When we examined the South African Bantu sample separately, we could not identify the dimorphic shape features outlined by Loth and Henneberg (8). This, in addition to the fact that the pattern and expression of sexual dimorphism varies between populations (25,26), may explain why Scheuer's (9) subsequent evaluation of their technique did not perform to expectation. Caution is thus recommended when attempting to apply sex-determination methods to individuals who are not members of the population upon which the statistics are based, as classification accuracy is likely to be reduced (1,2,27–29).

Apart from identifying sex from skeletal remains, determining age at death and population affinity (race) are two other major biological characteristics important in forensic identification (30–32). It is clear that our data show a strong correlation between the shape of the immature mandible and the age of the specimen (Fig. 2). With further testing and refinement, these techniques may provide a practical means of forensic age estimation based on mandibular morphology. Also, we have confirmed the findings of Buck and Viðarsdóttir (10) by demonstrating that different populations can be separated using geometric morphometric methods. This is especially significant to the forensic anthropologist, as it suggests that mandibular morphology is useful for determining population affinity irrespective of the age of the specimen (see also (10)).

The results of this study appear to show that population differences are more pronounced than sex differences in the subadult human mandible. We can speculate that population-specific mandibular morphology is perhaps established earlier in ontogeny, the result of inherited genetic traits. Sexual dimorphism, however, is not manifest at an appreciable level until after pubertal modifications have taken place (9). Thus, in the prepubertal age range it would be reasonable to expect that population differences will be more obvious than sex differences in the subadult mandible.

The results of this study strengthen previous findings that geometric morphometric techniques are able to describe features related to sexual dimorphism and population variation with increased sensitivity and objectivity compared with standard analytical methods (33–35). We are not implying that standard methods be disregarded; we simply aim to show how geometric morphometric methods have the potential to solve problems in forensic anthropology successfully.

Acknowledgments

We thank the following for kindly providing access to, and/or assistance with, skeletal material in their care: Dr. M. Bidmos,

Prof. P. V. Tobias and E. Mofokeng (R. A. Dart Collection), L. Jellema, and Dr. Y. Halié-Selassie (Hamann-Todd Collection); R. Kruzynski (Spitalfields Coffin Plate Series). We would also like to thank Dr. A. Cardini, Dr. J. Meyer, and Prof. L. Schmitt for helpful discussions on various statistical issues. Thanks are also due to Dr. L. Freedman for commenting on drafts of the manuscript. We disclose no financial relationship with commercial entities. This research is supported by ARC Discovery Grant DP0557157.

References

1. Krogman WM, Iscan MY. The human skeleton in forensic medicine. Springfield: Charles C Thomas, 1986.
2. Workshop of European Anthropologists. Recommendations for age and sex diagnoses of skeletons. *J Hum Evol* 1980;9:517–49.
3. Saunders SR. Subadult skeletons and growth related studies. In: Saunders SA, Katzenberg MA, editors. The skeletal biology of past peoples: research methods. New York: John Wiley & Sons, 1992:1–20.
4. Weaver DS. Sex differences in the ilia of a known age and sex sample of fetal and infant skeletons. *Am J Phys Anthropol* 1980;52:191–5.
5. Molleson T, Cruse K. Some sexually dimorphic features of the human juvenile skull and their value in sex determination in immature skeletal remains. *J Arch Sci* 1998;25:719–28.
6. Schutkowski H. Sex determination of infant and juvenile skeletons: I. Morphological features. *Am J Phys Anthropol* 1993;90:199–206.
7. Holcomb SMC, Konigsberg LW. Statistical study of sexual dimorphism in the human fetal sciatic notch. *Am J Phys Anthropol* 1995;97:113–25.
8. Loth SR, Henneberg M. Sexually dimorphic mandibular morphology in the first few years of life. *Am J Phys Anthropol* 2001;115:179–86.
9. Scheuer L. Brief communication: a blind test of mandibular morphology for sexing mandibles in the first few years of life. *Am J Phys Anthropol* 2002;119:189–91.
10. Buck TJ, Viðarsdóttir US. A proposed method for the identification of race in sub-adult skeletons: a geometric morphometric analysis of mandibular morphology. *J Forensic Sci* 2004;49:1159–64.
11. O'Higgins P. The study of morphological variation in the hominid fossil record: biology, landmarks and geometry. *J Anat* 2000;197:103–20.
12. Comas J. Manual of physical anthropology. Illinois: Charles C Thomas Publisher, 1960.
13. de Villiers H. The skull of the South African Negro: a biometrical and morphological study. Johannesburg: Witwatersrand University Press, 1968.
14. White TD, Folkens PA. Human osteology. 2nd ed. San Diego: Academic Press, 2000.
15. Rosas A, Bastir M. Thin-plate spline analysis of allometry and sexual dimorphism in the human craniofacial complex. *Am J Phys Anthropol* 2002;117:236–45.
16. O'Higgins P, Jones N. Facial growth in *Cercocebus torquatus*: an application of three-dimensional geometric morphometric techniques to the study of morphological variation. *J Anat* 1998;193:251–72.
17. Bookstein FL, Gunz P, Mitteroecker P, Prossinger H, Schaefer K, Seidler H. Cranial integration in *Homo*: singular warp analysis of the midsagittal plane in ontogeny and evolution. *J Hum Evol* 2003;44:167–87.
18. Mitteroecker P, Gunz P, Bookstein FL. Heterochrony and geometric morphometrics: a comparison of cranial growth in *Pan paniscus* versus *Pan troglodytes*. *Evol Dev* 2005;7:244–58.
19. Slice DE, editor. Modern morphometrics in physical anthropology. New York: Plenum Publishers, 2005.
20. Dryden IL, Mardia KV. Statistical shape analysis. New York: John Wiley & Sons, 1998.
21. Bookstein FL. Morphometric tools for landmark data: geometry and biology. Cambridge: Cambridge University Press, 1991.
22. Viðarsdóttir US, O'Higgins P, Stringer C. A geometric morphometric study of regional differences in the ontogeny of the modern human facial skeleton. *J Anat* 2001;201:211–29.
23. Baylac M, Villemant C, Simbolotti G. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. *Biol J Linn Soc* 2003;80:89–98.
24. Haralabakis NB, Spyropoulou MN. Factors affecting human growth. *Odontostomatol Proodos* 1990;44:167–77.
25. van Vark GN, van der Sman PGN, Dijkema J. Some multivariate tests for differences in sexual dimorphism between human populations. *Ann Hum Biol* 1989;16:301–10.
26. Walker PL. Greater sciatic notch morphology: sex, age and population differences. *Am J Phys Anthropol* 2005;127:385–91.

27. Franklin D, Freedman L, Milne N. Sexual dimorphism and discriminant function sexing in indigenous South African crania. *HOMO* 2005;55: 213–28.
28. Giles E, Elliot O. Sex determination by discriminant function analysis of crania. *Am J Phys Anthropol* 1963;21:53–68.
29. Johnson DR, O'Higgins P, Moore WJ, McAndrew TJ. Determination of race and sex of the human skull by discriminant function analysis of linear and angular dimensions. *Forensic Sci Int* 1989;41:41–53.
30. Rathbun TA, Buikstra JE. The role of the forensic anthropologist: the case for cooperative research. In: Rathbun RA, Buikstra JE, editors. *Human identification: case studies in forensic anthropology*. Springfield: Charles C Thomas, 1984:5–14.
31. Reichs KJ, editor. *Forensic osteology: advances in the identification of human remains*. Springfield: Charles C Thomas, 1998.
32. Burns KR. *Forensic anthropology training manual*. New Jersey: Prentice-Hall, 1999.
33. Franklin D, Milne N, Freedman L. A geometric morphometric study of cranial sexual dimorphism in selected indigenous populations of South Africa. *Am J Phys Anthropol* 2004;123(S38):96.
34. Franklin D. Cranial variation in southern Africa: a series of inter-landmark and three-dimensional multivariate morphometric studies. PhD thesis, The University of Western Australia, Australia, 2005.
35. Pretorius E, Steyn M, Scholtz Y. Investigation into the usability of geometric morphometric analysis in assessment of sexual dimorphism. *Am J Phys Anthropol* 2006;129:64–70.

Additional information and reprint requests:
Daniel Franklin, Ph.D.
Centre for Forensic Science
School of Anatomy and Human Biology
The University of Western Australia
Mail Bag Delivery Point M420
35 Stirling Highway, Crawley
Western Australia, 6009
E-mail: dfranklin@anhb.uwa.edu.au