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A Proposed Method for the Identification of Race in Sub-Adult Skeletons: A Geometric Morphometric Analysis of Mandibular Morphology*

ABSTRACT: The identification of biological race (ancestry) in skeletal material is an important aspect of forensic investigations. While techniques for race determination are well established for adult skeletons, identification of race in sub-adult specimens has not been widely addressed. The present study investigates racial differences in the mandibular morphology of sub-adult specimens using geometric morphometric analyses. One hundred and seventy-four mandibles from five morphologically distinct samples were digitized and subjected to general Procrustes analysis. Results showed significant morphological differences between the samples and obtained cross-validation results of over 70% accuracy in identification of unknown individuals using the complete mandible. It is suggested that these techniques could provide a method for the identification of race in sub-adult individuals.

KEYWORDS: forensic science, race determination, sub-adults, geometric morphometrics, mandibular morphology, morphological variation

Establishing the biological race (ancestry) of skeletal remains is a vital part of forensic identifications. Race, along with sex, stature, and age at death, is one of the four principal parameters used when determining the biological identity of an individual in a forensic context (1). Although there are a number of established techniques for identifying race in both cranial and post cranial adult skeletons (2–4), the identification of race in sub-adults is presently considered virtually impossible (5,1).

In adult skeletons, race can be difficult to determine and categorize, as there are no "pure" races, rather continuous variations onto which artificial delimiters have been imposed. Many anthropologists (e.g., Brace (6)) believe that a more valid approach to biological variation is the consideration of clines of traits rather than the definition of distinguishable populations. In forensic anthropology, where the aim is to identify a specific unknown individual from skeletal material, this approach is not suitable. Such identification involves the comparison of individualizing data from a missing person to similar data recovered from the skeletal remains and the attempt of a positive match (7). The determination of ancestry is vital in this context because it provides the possible assignment of an individual to a biological group within which they would have been recognized in a specific society.

Traditionally, studies of skeletal variation between populations have depended on the application of multivariate statistical methods to sets of defined linear distances. Most of this work has concentrated on the cranio-facial region, where morphological variation is greatest (2,8,9), although some work has been undertaken on post-cranial material using similar techniques (10). The discriminant functions obtained from such studies cannot, however, be directly applied to sub-adult skeletal material, due to the confounding influence of large scale ontogenetic allometric changes occurring between birth and adulthood (11). Comparative datasets of adult measurements cannot thus be directly applied to individuals who have not completed their skeletal growth. In recent years, geometric morphometric techniques have been successfully applied to analyses of population variance in adult skeletons (3,12). As these techniques provide a method of partitioning size from shape in a biological entity, geometric morphometrics also can be applied to the comparison of form across a range of ages (13).

The present study sets out to answer two questions. First, can variation in mandibular morphology be used to separate distinct geographic samples? The analyses pertaining to this question will be referred to as Step 1. Although the mandible has generally not been considered a good indicator of adult population diversity (14), limited research has been undertaken in this area. Angel and Kelly (15), for example, discussed the differences between whites and blacks with regard to the amount of inversion to the posterior edge of the mandibular ramus, while Kean and Houghton (16) discussed the 'rocker jaw' feature characteristic of Polynesian skulls.

The second question is whether mandibular morphological variation is practical for use in forensic investigations where skeletal remains may not be complete. As the bones of the calvaria and face often do not survive inhumation intact (1), it is important to examine whether any proposed identification technique can be applied to partial skeletal material. An analysis of two separate sections of the mandible, the mandibular corpus and ramus, will therefore be undertaken in Step 2 to test whether the applicability of any method identified in Step 1 holds true for incomplete mandibular material.

Methods

This study included a comparative sample of five morphologically distinct groups: African Americans, Native Americans (the Arikara from the Plains area of the United States), Caucasians, Inuit, and Pacific Islanders. A total of 174 individuals was included, and the composition of each sample is given in Table 1. Adults were

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TABLE 1—*Study sample.*

Population	Total Number of Individuals	Number of Adults	Age of Youngest Individual (years)	Collection*
African American	39	9	0.75	CMNH
Native American	41	8	1.5	UTK
Caucasian	42	9	0.75	NHM
Inuit	33	11	2	DC
Pacific Islander	19	4	6	NHM;DC
Total	174	41		

*Collection abbreviations: CMNH = Cleveland Museum of Natural History, Ohio; UTK = University of Tennessee, Knoxville; NHM = Natural History Museum, London; DC = Duckworth Laboratory, University of Cambridge.

TABLE 2—*List of mandibular landmarks collected.*

Landmark Definitions

1. Gnathion
2. Pogonion
3. Infradentale
4. Most posterior point situated on the labial alveolar surface behind the second incisor
5. Most posterior point situated on the labial alveolar surface behind the canine
6. Most posterior point situated on the labial alveolar surface behind the most posterior erupted tooth (or crypt for tooth)
7. Mentale
8. Gonion
9. Coronion
10. Most inferior point on the mandibular notch
11. Condylion mediale
12. Condylion laterale
13. Point at which a horizontal line drawn from landmark 17 intersects with the posterior border of the ascending ramus
14. Point at which a vertical line drawn from landmark 4 intersects with the inferior border of the mandibular body
15. Point at which a vertical line drawn from landmark 5 intersects with the inferior border of the mandibular body
16. Point at which a vertical line drawn from landmark 6 intersects with the inferior border of the mandibular body
17. Point of intersection of the labial alveolar surface with the ascending ramus

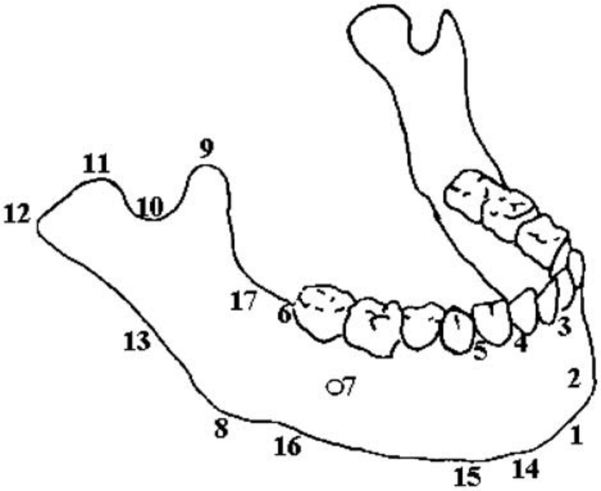

FIG. 1—*Landmarks on the mandible.*

determined by the full eruption of the permanent dentition. Sub-adult individuals were assigned a biological age based upon the standards given by Ubelaker for non-white populations (17). These estimated ages are approximate at best and thus used only for graphical purposes, not statistical analysis. Sex was not assigned to unknown individuals and has not been included as a variable in this study. Where sex is known, individuals have been sampled to avoid bias.

Seventeen homologous unilateral landmarks were digitized from the skeletal surface of each mandible. Eight of the landmarks (see Table 2) represent standard osteological points, following the definitions given in White (18). Definitions of the remaining landmarks are given in Table 2. The landmarks were selected to allow a good representation of mandibular morphology and care taken to include only landmarks that are identifiable on both adult and sub-adult specimens. Figure 1 shows the position of these landmarks on the mandible.

Three-dimensional coordinates of the landmarks (Table 2) were collected using a Microscribe 3DX desktop digitizing system (Immersion Corporation, San Jose, CA). The digitized coordinates were superimposed using generalized Procrustes analysis (GPA) in *morphologica* (© Paul O'Higgins and Nicholas Jones, University College, London). GPA registers series of forms by removing translational, rotational, and reflected differences and scaling them according to centroid size (19). Procrustes-based registration methods do not introduce bias into the distribution on specimens where landmarks vary independently and have been shown to have highest statistical power in practical applications (20,21). Centroid size, the measure of size used throughout this study, is defined as the square root of the sum of squared Euclidean distances from each landmark to the centroid (the mean of the landmark coordinates) (22). The removal of reflected differences allows the use of either side of the mandible, thus increasing the sample size available. Although not perfectly symmetrical, the near symmetry of the mandible allows for the collection of landmarks from one side only. The use of unilateral landmarks maximizes the size of the available sample while retaining maximum morphological information from the mandible (23).

The Procrustes registered landmark coordinates lie in Kendall's shape space, a non-Euclidean space (24). Slice (25) has, however, suggested that Procrustes registered coordinates actually lie in a hemispherical variant of Kendall's shape space, where they remain almost identical to their projections in tangent space. For aid in statistical analysis, the coordinates in this study are projected into linear tangent space, using the method of Dryden and Mardia (26).

Principal components analysis (PCA) is performed using *morphologica* (© Paul O'Higgins and Nicholas Jones, University College, London) and calculates the principal axes of variation within the sample. The morphological variability explained by each principal component (PC) can be visualized readily by reconstructing a hypothetical mean shape and warping it to represent shapes at different points along the PCs (27). A correlation analysis with centroid size was undertaken for each PC in order to identify the effects of allometry on that component. Mahalanobis' distances were calculated to quantify inter-population shape differences, taking into consideration the variance and covariance amongst the groups. The Mahalanobis' distances were calculated using SAS (© SAS Institute Inc., Cary, NC). The significance of these shape differences was assessed using Hotelling's T^2.

Discriminant analysis with crossvalidation was used to classify individuals into pre-defined groups. Each individual was assigned a probability of belonging to a given group based on the distance of its discriminant function from that of each group mean. Cross-validation was employed, as it provides a better assessment of

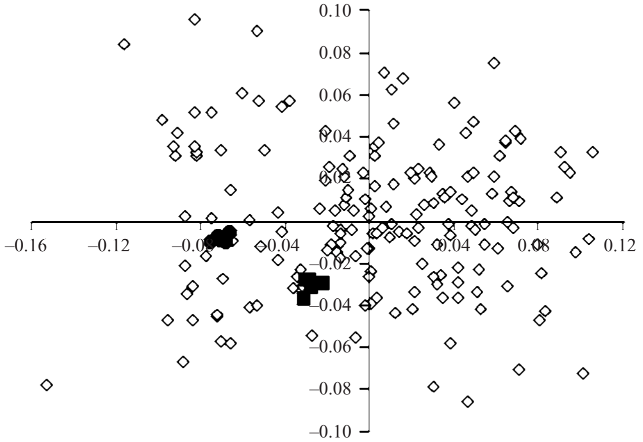

FIG. 2—*Precision of measurement. The five repeat specimens, together with the remainder of the total sample, are submitted to PCA. PC 1 is plotted on the horizontal and PC 2 on the vertical axis. Black circles and black rectangles, repeated individuals; open diamonds, the remaining individuals.*

classification accuracy than standard discriminant analysis. During crossvalidation, classification is carried out for each individual in turn, and the discriminant function used in each case is constructed with that individual removed. Every individual is therefore reclassified as if it were an unknown specimen, providing an unbiased assessment. The crossvalidation analyses are carried out using SAS (© SAS Institute Inc., Cary, NC).

To test the degree of intra-observer measurement error in digitizing the landmarks, five repeats of each landmark were taken for two separate specimens and analysed using the method of O'Higgins and Jones (27). Two of the five repeats were carried out on a different day to avoid a bias in measurement through "remembered" landmark positions. All other specimens in the study were measured only once. To assess differences due to variability within the sample, the five repeat sets of landmark data are submitted to GPA and PCA along with the remainder of the total data sample. PCs 1 and 2 are plotted in Fig. 2. The five repeats of each of the test specimens are plotted as black circles and rectangles, respectively. The remaining specimens are plotted as open diamonds. The two repeat sets are clustered tightly together on both PCs, indicating that errors of precision are small with respect to sample variability and hence unlikely to have unduly influenced the results of the study.

Step 1

In the principal components analysis of the combined sample of five groups, PC 1 explained 33.6% of the total variance in the sample and showed a strong correlation with centroid size ($p < 0.0001$, $r = 0.80$; Fig. 3). Shape changes visualized by morphing from the negative to the positive extremes of PC 1 show differences that are associated with ontogenetic allometry of the mandible. These changes include the move from a more relatively posteriorly positioned sloping angle in the younger specimens (negative extreme; Fig. 3–1) to a relatively more vertical position in the adult individuals (positive extreme; Fig. 3–2). A further change is seen in the ramus, which lengthens and becomes more relatively vertical with the move to the positive axis (Fig. 3–2).

TABLE 3—*Mahalanobis' distance matrices: Five populations (using 48 principal components).*

	African American	Native American	Caucasian	Inuit	Pacific Islander
African American	0.00				
Native American	3.79	0.00			
Caucasian	5.29	4.63	0.00		
Inuit	4.16	3.05	4.48	0.00	
Pacific Islander	4.63	4.07	4.47	4.72	0.00

TABLE 4—*Mahalanobis' distance matrices: Three populations (using 48 principal components).*

	African American	Native American	Caucasian
African American	0.00		
Native American	4.24	0.00	
Caucasian	6.14	5.72	0.00

TABLE 5—*Results of cross validation analysis: Five populations.*

	African American %	Native American %	Caucasian %	Inuit %	Pacific Islander %	Total %
African American	69.23	12.82	5.13	7.69	5.13	100.00
Native American	2.44	73.17	0.00	17.07	7.32	100.00
Caucasian	2.38	2.38	76.20	9.52	9.52	100.00
Inuit	3.03	24.24	9.09	63.64	0.00	100.00
Pacific Islander	5.26	21.05	0.00	5.26	68.43	100.00

Table 3 gives the Mahalanobis' distances between the five samples described in Table 1. The distance between the African American and the Caucasian samples was greatest; the smallest distance occurred between the Native American and the Inuit samples (Table 3). All distances were statistically significant ($p < 0.0001$). This analysis was repeated using only the African American, Native American, and Caucasian samples, to aid comparison of results with traditional race determination studies from the U.S. The Mahalanobis' distances between the reduced set of three samples are given in Table 4. Again, the greatest distance was between the African American and the Caucasian samples. The shortest distance was that between the African American and the Native American samples.

Results of the crossvalidation using linear discriminant function analysis are given in Tables 5 and 6 for the five and three group samples, respectively. All analyses were carried out using the total shape variance within the samples. For the analysis of all five groups, an average of 70.1% of the individuals were assigned to the correct group (Table 5). The highest percentage of accurate identification was for the Caucasian sample. The lowest percentage of accurate identification was found for the Inuit. There was no correlation between the percentage of correct identification and either total or sub-adult sample size. The individuals that were incorrectly assigned to their group came from the entire sample age range, with no clustering in any particular age range (Fig. 4).

For the analysis of the reduced sample of three groups, an average of 87.6% of the individuals were assigned to the correct group. The African American sample had the least number of correctly assigned individuals, while the Caucasian sample had the largest percentage of correctly assigned individuals (Table 6).

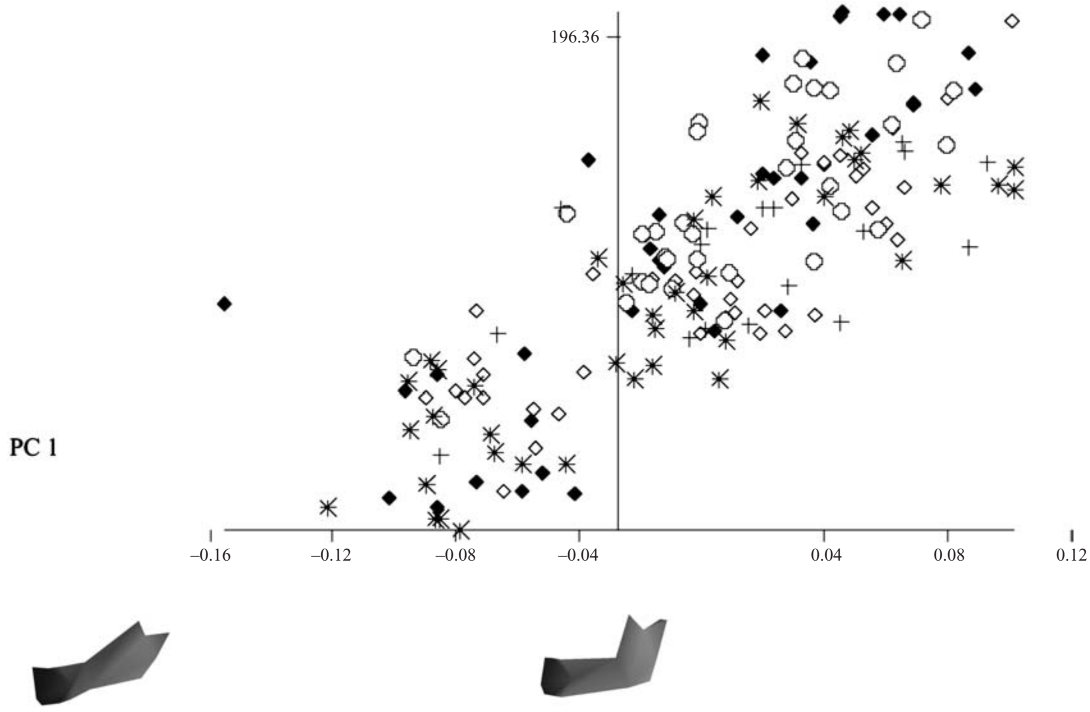

FIG. 3-1 **FIG. 3-2**

FIG. 3—*Principal component 1 versus Centroid Size. Black diamonds, African Americans; Open diamonds, Native Americans; Stars, Caucasians; Open Circles, Inuit; Cross, Pacific Islanders. Representations of the mandible at each extreme of PC 1 are given for reference (Figs. 3–1 and 3–2).*

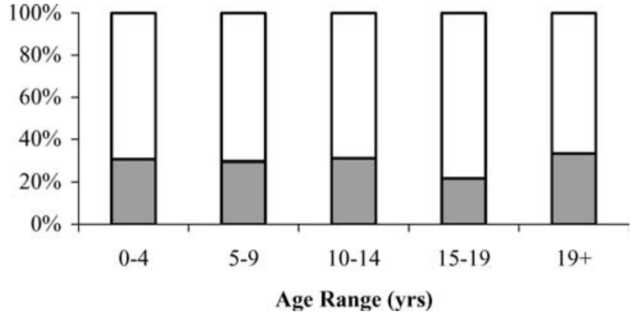

FIG. 4—*Percentage of misclassified individuals from each age range (years). The shaded area indicates the misclassified individuals.*

TABLE 6—*Results of cross validation analysis: Three populations.*

	African American %	Native American %	Caucasian %	Total %
African American	84.62	12.82	2.56	100.00
Native American	9.76	87.80	2.44	100.00
Caucasian	4.76	4.76	90.48	100.00

Step 2

The aim of the second step of the analyses was to explore the applicability of the proposed identification method to partial skeletal material. The mandible was split into two component parts, the ascending ramus and the mandibular corpus. Each section was digitized using a reduced landmark set from Step 1: the corpus included 10 landmarks (numbers 1–7, 14, 15, 16); and the ramus 8 landmarks (numbers 8–13, 16; see Table 2 for descriptions of landmarks). To aid comparison with traditional techniques, only the African American, Native American, and Caucasian samples were utilized in this analysis.

TABLE 7—*Mahalanobis' distance matrices: Partial mandible (Ramus: 21 principal components; Corpus 27 principal components).*

Ramus	African American	Native American	Caucasian
African American	0.00		
Native American	3.00	0.00	
Caucasian	3.14	2.25	0.00
Corpus	**African American**	**Native American**	**Caucasian**
African American	0.00		
Native American	2.17	0.00	
Caucasian	3.28	3.80	0.00

TABLE 8—*Results of cross validation analysis: Partial mandible.*

	African American %	Native American %	Caucasian %	Mean %
Ramus	79.49	68.29	71.43	72.95
Corpus	56.41	65.00	79.07	67.20

The samples can be separated by statistically significant Mahalanobis' distances in the separate analyses of both the mandibular ramus and the mandibular corpus (Table 7). The distances given by the analysis of the mandibular ramus repeated the patterns identified in Step 1, with the largest distance between the Caucasian and the African American populations (Table 7). The distances obtained from the mandibular corpus did not follow this pattern but were statistically significant ($p < 0.0001$) (Table 7). The results of the crossvalidation gave an average of 73% of correctly identified individuals for the mandibular ramus and 67.2% for the mandibular corpus (Table 8).

Discussion

Traditional methods for determining race from the skeleton cannot easily be applied to sub-adult material (2,8). A strong correlation between centroid size and PC 1 shown in Step 1 demonstrates that the greatest amount of mandibular morphological variation within the sample groups is determined by the relative size and by inference age of the individual mandible. Despite this, the results of the present study suggest that geometric morphometric analytical techniques can be used to overcome this issue and differentiate between groups based on mandibular morphology alone, regardless of the age of the individual.

This study aims to explore whether geometric morphometric methodology allows for a comparable approach to race identification with that presented by more traditional analyses. Initially, five samples were analyzed, and morphological distances between the five groups proved to be statistically significant. Crossvalidation analysis successfully placed individuals into their correct sample groups with a mean accuracy level of 70% for the five groups. When the number of groups was decreased, in order to provide comparison with existing studies of population diversity (3,13), the distances remained statistically significant, and the crossvalidation accuracy rose to 88% on average. Along with showing that the proposed technique produces results comparable with findings from other studies (3,13), these results also suggest that limiting the number of samples increases the chance of correct identification. In order for the proposed technique to be applicable outside the United States, a number of different sample groups needs to be included in the identification process. The five sample groups used in the analysis were chosen to demonstrate the applicability of the technique on geographically distinct samples outside of the more commonly used groups. The use of this technique as a universal identification tool would thus benefit from a much wider range of samples that can be applied selectively to a variety of forensic contexts in a given geographic area.

Having shown that distinct sample groups can be separated using geometric morphometrics, the second question to be addressed by this study was the applicability of the proposed technique to incomplete skeletal remains. As sub-adult skeletal material frequently does not survive inhumation intact (1), Step 2 explored the potential of using geometric morphometric analysis for the identification of geographic ancestry in partial mandibular material. Group separation was achieved for both the mandibular ramus and the corpus, based upon the biological distances between the groups. This shows that the morphological variation found in the mandible can be identified with only partial sections of the bone. Crossvalidation produced mean levels of identification accuracy for the mandibular corpus and ramus of 67% and 73%, respectively, using a sample of three groups. These results indicate a reduction in accuracy compared to those obtained from the complete mandible, but the levels of accurate placement remain high. This suggests that an attempt at racial determination can be made using partial mandibular remains. The choice of sections of the mandible for this analysis was a crude split between the ramus and the corpus in order to demonstrate the technique. It is suggested that further work be undertaken to assess the most appropriate landmarks for use, as the levels of accuracy will vary depending on the landmarks available in the specific remains under investigation.

Although an exploratory study, this paper proposes that geometric morphometric techniques present a method for determining geographic ancestry in sub-adult mandibles. It is suggested that additional work be carried out to explore further the applications of this method. As previous studies have shown, greater variation between samples is found in the facial skeleton (9,13), and thus the use of full cranial material may provide greater levels of accuracy in the correct placing of unknown sub-adult individuals. It is therefore suggested that this study be extended to include analysis of the full cranio-facial skeleton. Additionally, comparisons with other cranial bones would also be beneficial toward more accurate identification. Finally, a wider range of representative samples is required for comparative analysis in order to extend the application of this process to wider contexts and to different countries, with a varying composition of racial groups.

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