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Three-Dimensional Procrustes Analysis of Modern Human Craniofacial Form

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ABSTRACT

The objective of this study was to analyze modern human craniofacial form using 3D Procrustes superimposition in order to establish a reference model and validate it on computed tomography (CT). The sample consists of 136 specimens from five modern human regional groups. Thirty-three craniofacial landmark coordinates have been recorded using a Microscribe and calculated on CT scans for five crania from the sample. Procrustes superimposition has been performed to calculate the mean shape, and a discriminant analysis has also been carried out to estimate the variability of shape. The results show that the repeatability of measurements made on CT and on Microscribe is excellent (R = 0.99). There is no major distinctiveness in the craniofacial shape; however, discriminant function 1 separates out the European crania from the others, especially African and American. It includes the width and the length of the face, the flatness of the upper face, the prognathism of the maxilla, as well as the length and the inclination of the palate. The width of the maxilla and the palate do not show a great variability. This may be the common invariant feature responsible for the alignment of the teeth in all specimens. It may correspond to functional patterns related to masticatory constraints manifested by the important interproximal and occlusal dental wear in all specimens. This study confirms the high accuracy of measurements made on CT scan and the importance of geometric morphometrics, which provides an accurate characterization of the overall craniofacial shape and its variation within the entire population. Anat Rec, 290:268–276, 2007. © 2007 Wiley-Liss, Inc.

Key words: computed tomography; Procrustes superimposition; 3D craniofacial form; modern humans

The morphology and growth of the craniofacial skeleton in living humans have been widely described using cephalometric radiography, which requires identifying specific landmarks and calculating various angular and linear variables. However, two types of errors may occur with this approach: errors of projection and errors of identification (Baumrind and Frantz, 1971). Errors of projection are caused because the head film is a two-dimensional (2D) representation of a three-dimensional (3D) object. Errors of identification are the errors of identifying specific landmarks on the head films and are considered by many investigators as the major sources of error in cephalometrics (Hixon 1956; Bjork and Solow, 1962; Savara et al., 1966; Gravely and Benzies, 1974; Mitgaard et al., 1974). Several factors are involved and include the quality of the radiographic image, the precision of landmark definition, the reproducibility of the landmark location, the operator, and the registration procedure. The major problem is that such errors lead to

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missing subtle changes and allow only the grossest changes to be observed clearly. Broadbent's (1931) introduction of the cephalostat has stressed the importance of using and coordinating both the lateral and frontal head films to define the craniofacial form. Although the method is sound, it has often been reported as difficult to apply, and it yields less accurate measurements than true anatomic values (Grayson et al., 1988).

In recent years, computed tomography (CT) has provided 3D reconstruction of the entire craniofacial skeleton from axial slices allowing methods to evaluate all internal structures (Vannier et al., 1984; Maki et al., 1997). CT also provides digital 3D data, a source of information for morphometric analysis. An increase in the application of 3D data is expected soon with the use of new tools in order to redesign cephalometrics and incorporate advances from related fields such as geometric morphometrics (Halazonetis, 2005). Geometric morphometric methods have widely been applied to primatology (Collard and O'Higgins, 2000; Singleton, 2002), human variation (Hennessy and Stringer, 2002; Rosas and Bastir, 2002), and paleoanthropology (Ponce de Leon and Zollikofer, 2001). In traditional multivariate morphometrics, the form of a biological object is typically recorded as a set of measurements of distances and angles. However, geometric morphometrics links the set of measurements with the shape of the object. The form of the object is recorded as the coordinates of defining features (landmarks), and its geometry is thus preserved. Geometric morphometrics also distinguishes the form of an object (shape plus size) from the shape (form with scale removed) by scaling to unit size, so that it would be possible to model morphological variations without taking into consideration the size factor (TpsSmall, version 1.18; State University of New York, Stonybrook, NY) (Bookstein, 1990; Rohlf and Marcus, 1993; O'Higgins, 2000). In addition, studying the morphology of different forms by superimposition removes the need for a common reference plane. Multivariate analysis, such as principal-component analysis (PCA), can then be carried out to investigate the main shape variations.

Despite all these technical advances, no 3D reference (normal) model of modern human craniofacial form has been established yet. The objective of this study was to develop a 3D digitized craniofacial model of modern human using Procrustes superimposition and validate it on CT scan, so that it could be used as a reference for future comparative studies.

MATERIALS AND METHODS

Only crania, determined as adult from dental examination (fully erupted third molars) and in good conditions of preservation and completeness, were analyzed. They belong to the skeletal collection of the Institut de Paléontologie Humaine, Muséum National d'Histoire Naturelle, Paris. Five regional groups were studied: 34 crania from Europe (Spain, France, Italy, Croatia, Finland, and Portugal), 21 from America (Botocudo and Bogota Indians, Fuegian, Puelche-Paradero, and Navajo populations), 31 from Africa (Sudan, Tunisia, Algeria, Morocco, Congo, Dahomey, and Madagascar), 20 from Asia (Turkestane, Syria, Suse, and Dongane Kouldja populations), and 30 from Oceania (Mallicolo, New Caledonia, Java, Borneo, and Îles de Pâques). All specimens were dated to the 19th century. They have aligned teeth with no dental anomalies of form, number, volume and position, nor craniofacial deformity. Unfortunately, most of the specimens were represented by isolated crania, with obvious limitations for sex diagnosis. Consequently, sexual dimorphism was not taken into consideration in the present study.

The data have been collected in the form of three-dimensional coordinates of craniofacial landmarks using a Microscribe 3DX portable digitizer, which provides a fast and reliable method for creating accurate 3D computer models. This apparatus records each point in all three spatial dimensions. An electronic map calculates the x, y, and z of each point. Then the coordinates are downloaded to the computer.

Landmarks are defined in geometric morphometrics as homologous points that can be reliably and repeatedly located in all specimens under study (Bookstein, 1990; O'Higgins, 2000). Cranial, facial, and palatal landmarks are used in an effort to represent the craniofacial morphology as fully as possible (Figs. 1–3, Table 1). A bilateral configuration is preferred to keep the structural balance of the morphological system. In fact, morphology is best interpreted as the result of a biomechanical network between ipsilateral and contralateral components, functionally related by physical interactions (Moss and Young, 1960; Enlow, 1990). Most are sutural landmarks for two reasons: they represent boundaries of each bone, and they can be reproduced easily on CT scannograms, as new CT scanners show detailed images of the sutures. Each skull has been fixed on the table near the digitizer using plasticine to prohibit movement during and between measuring sessions. Inhibiting movement during data collection is absolutely significant to acquire valid information about the relative location of landmarks.

In order to validate the measurements made on CT scans, five crania from the same collection were digitized with Microscribe and then were CT-scanned with LightSpeed^{16} (General Electric HealthCare, Waukesha, WI) at the Centre Hospitalier National d'Ophtalmologie (CHNO) des XV–XX (Paris). We used a standardized protocol for dry and fossil skull scanning, which consisted of the following CT parameters (Badawi-Fayad et al., 2005): detector configuration = 16 × 0.625, beam collimation = 10 mm, acquisition mode = 0.562:1, slice thickness/reconstruction interval = 0.625/0.4 mm, pitch = 5.62, tube voltage = 120 kV, dose per section = 300 mAs, and no gantry tilt. The acquisition matrix was 512 × 512 with a 230 mm display field Of view (DFOV), which resulted in a nominal pixel size of 0.45 × 0.45 mm. Image data were transferred to an Advantage Window workstation (AW 4.1; GE HealthCare) to perform 3D reconstructions and 2D multiplanar reconstructions. One operator on the workstation performed image analysis. The set of 33 landmarks were collected from the 3D reconstructions and verified at the same time on the sagittal, frontal, and axial reconstructions of the same monitor.

Statistical Methods

The software used for the superimposition is APS 2.41 (Xavier Penin, Caen, France). APS is a Procrustes superimposition software designed to compute, visualize, and

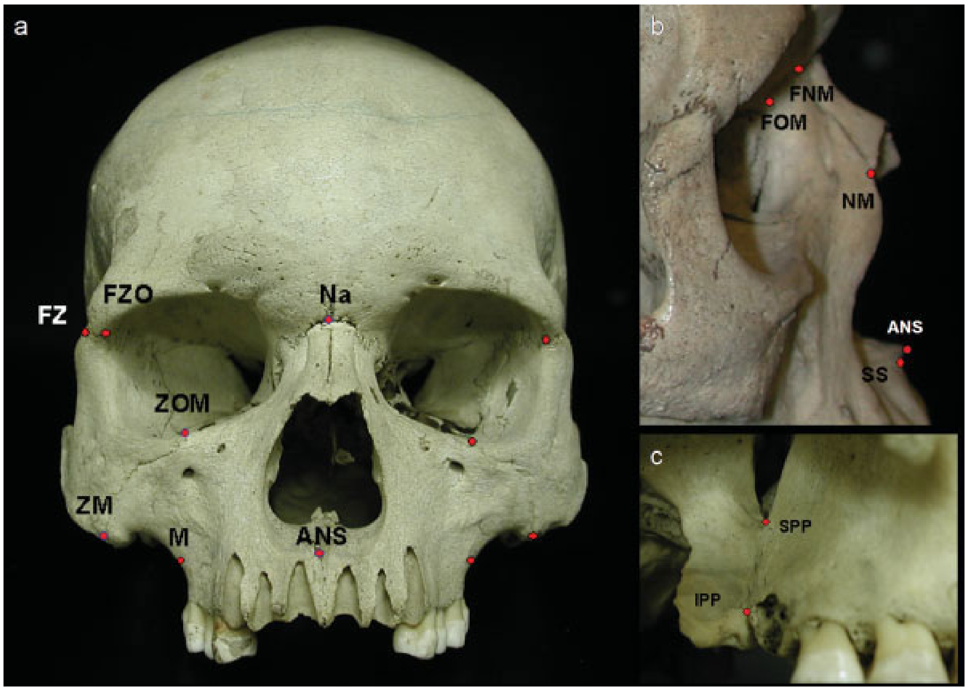

Fig. 1. European specimen with facial landmarks. **a:** Anterior view. **b:** Lateral view. **c:** Inferolateral view.

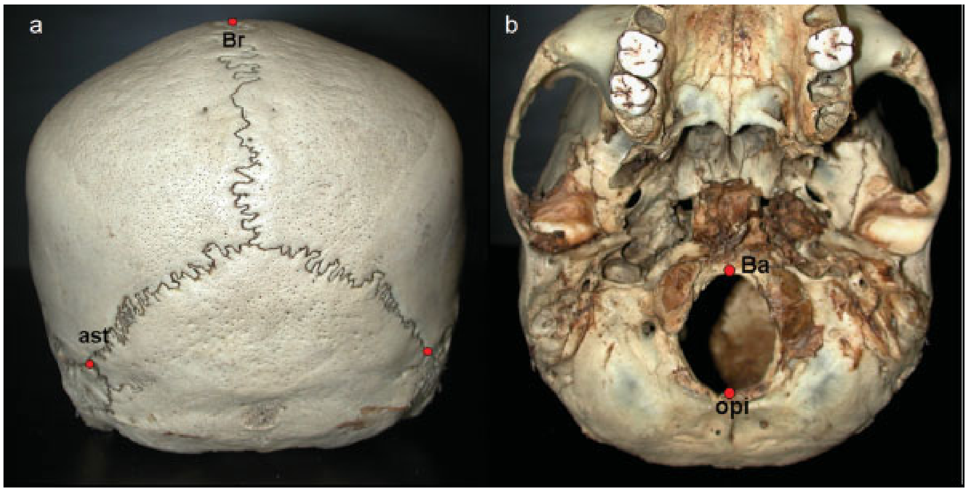

Fig. 2. European specimen with cranial landmarks. **a:** Posterior view. **b:** Inferior view.

test the significance of the morphological variation associated with a continuous (e.g., size) or qualitative (e.g., taxonomy) biological factor. Each specimen is represented by a finite number of anatomical points called landmarks. Procrustes superimposition is an iterative least-square adjustment of all the figures after size normalization. It includes three phases: scaling, translation, and rotation. Once a common scale has been applied, the skulls are all the same size and can be superimposed. The translation stage is then performed to make the geometrical centers fit exactly with one another. All the skulls can be rotated in order to minimize the gaps between the homologous anatomical points. Since all the 33 predefined anatomical points are used during these stages, no reference plane is required. Centroid size is used as size index. It is defined as the square root of the sum of squared distances of a set of landmarks from their centroid (Slice, 1996). A multivariate analysis of variance (MANOVA) has been performed on Systat to test differences in size between groups, and multivariate regression has been used to test allometry on APS.

Measurement of shape entails calculating the average shape of the population and estimating the variability of shape. Calculation of the average is trivial and can be accomplished by calculating for each point the average x-, y-, and z-coordinates. The estimate of average shape

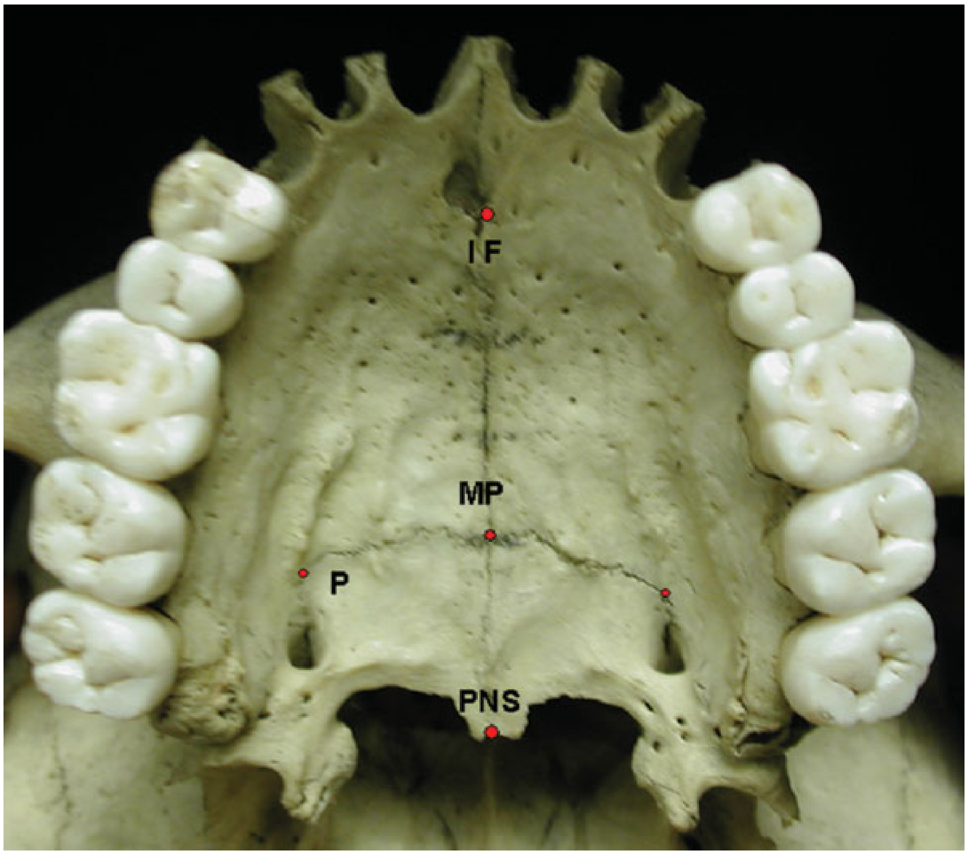

Fig. 3. European specimen with palatal landmarks.

is very important, but the most difficult to estimate is the variability of shape. The five regional data sets have been pooled, and a discriminant analysis has been carried out to find the shape variability, within the pooled data set, that differentiated the five groups. The Procrustes residuals and Procrustes mean are calculated using APS and stored. Then the Procrustes residuals are subjected to PCA, using Numerical Taxonomy and Multivariate Analysis Program NTSYS (version 1.80; Exeter Software, New York, NY). The eigenvectors and percentages explained by the PCs are stored to disk. The Procrustes residuals are projected onto the eigenvectors using NTSYS, and the first 14 PC scores, which explained 70% of the total variance, are analyzed via discriminant analysis. All 14 PC scores are entered into the model. The discriminant function coefficients, eigenvalues, and scores are recorded. Classification statistics are also recorded.

Method Error

Error has been assessed using the analysis of variance applied to Procrustes methods (Penin, 1997). Goodall's statistics (1991) allows us to test the significance of differences between samples of the same size. To test the method error with Microscribe, landmark data were col-

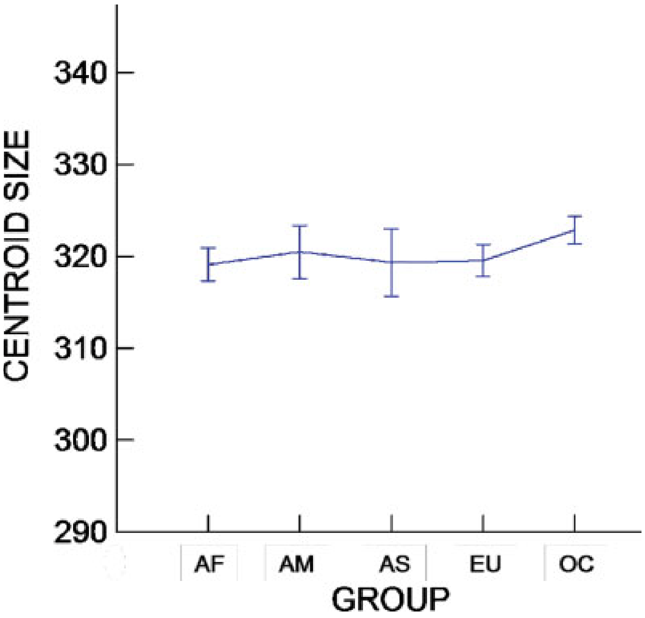

Fig. 4. Centroid size variability within each group (mean and standard error). AF, Africa; AM, America; AS, Asia; EU, Europe; OC, Oceania.

TABLE 1. List of landmarks

Facial landmarks

Na (nasion)
ANS (anterior nasal spine)
SS (subspinale)
FZO (frontozygorbitale) – Right (R) and Left (L)
FZ (frontozygomatique) – Right (R) and Left (L)
ZOM (zygorbitomaxillare) – Right (R) and Left (L)
ZM (zygomaxillare) – Right (R) and Left (L)

M (maxillare) – Right (R) and Left (L)
FNM (frontonasomaxillare) – Right (R) and Left (L)
FOM (frontorbitomaxillare) – Right (R) and Left (L)
NM (nasomaxillare) – Right (R) and Left (L)
SPP (superior Pterygo-palatine) – Right (R) and Left (L)
IPP (inferior Pterygo-palatine) – Right (R) and Left (L)

Cranial landmarks

Br (bregma)
Opi (opisthion)
Ba (Basion)
Ast (asterion) – Right (R) and Left (L)

Palatal landmarks

P (palatine) – Right (R) and Left (L)
IF (incisal foramen)
MP (maxillo-palatine)
PNS (posterior nasal spine)

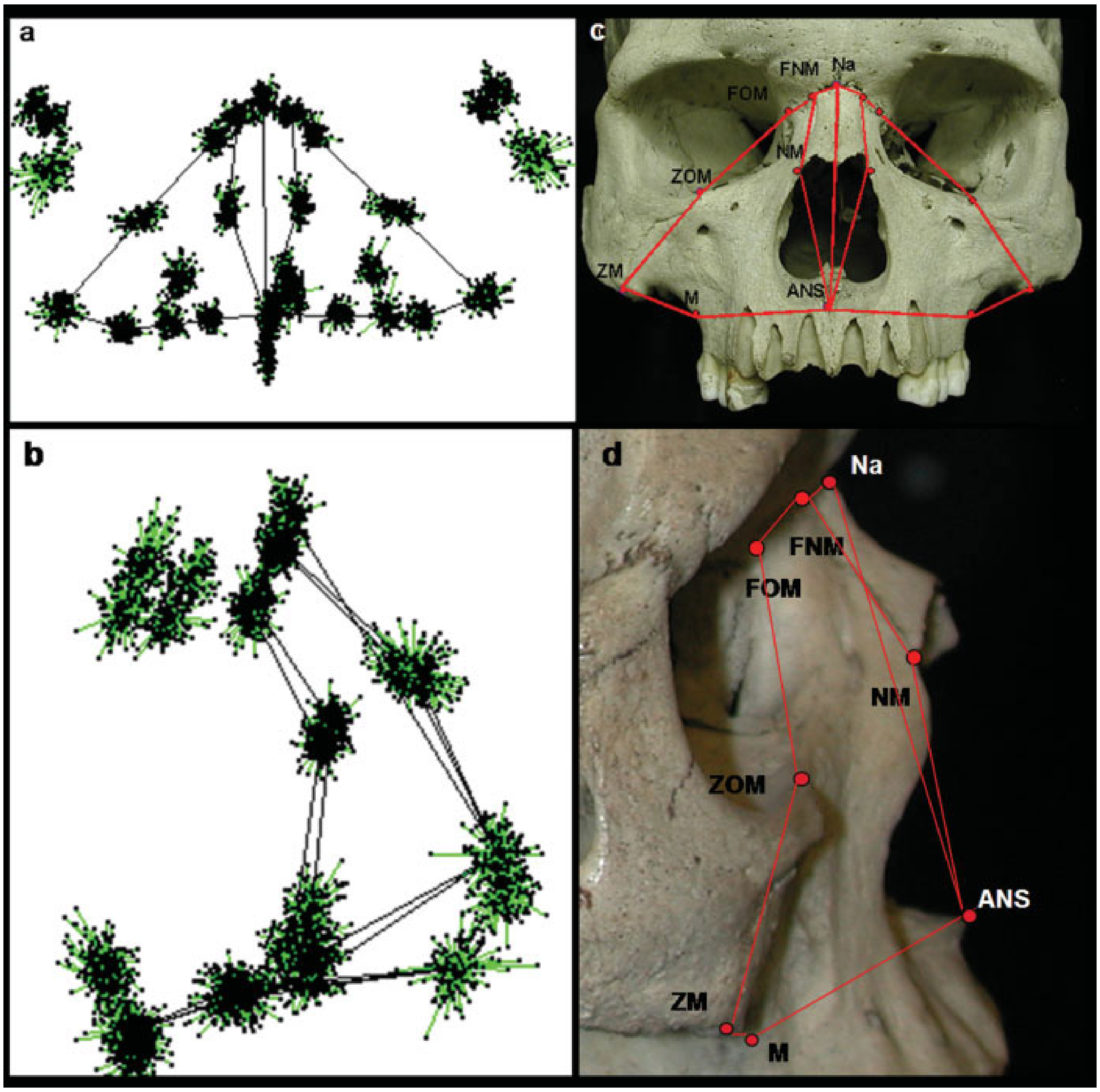

Fig. 5. Frontal (**a**) and lateral (**b**) view of the mean craniofacial shape of the 136 subjects. For visualization purposes, some facial landmarks are linked by lines called links (**c** and **d**).

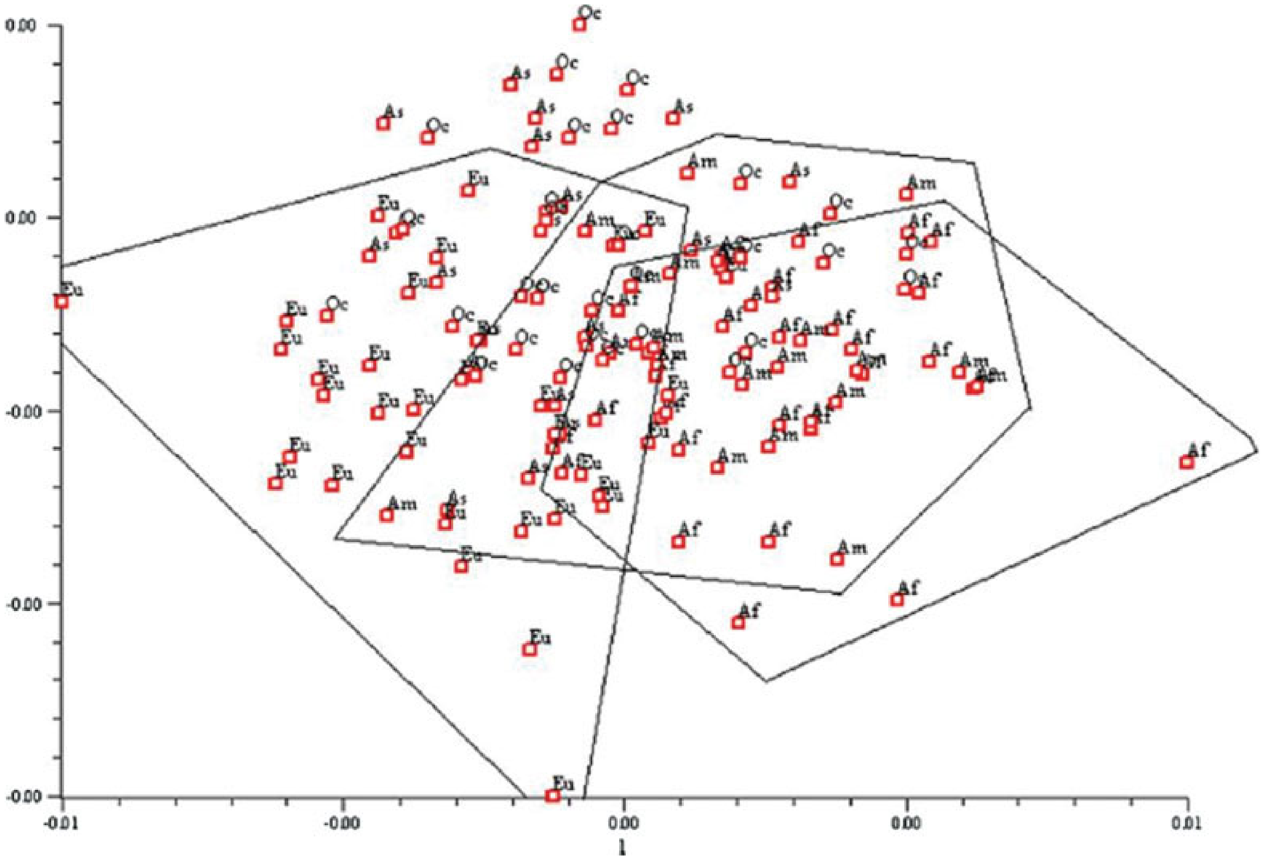

Fig. 6. Discriminant function 1 scores plotted against discriminant function 2 scores; the three most discriminant groups (European, American, and African) were delimited.

lected twice daily for six crania: once in the morning and once in the evening. At least 7 hr elapsed between measuring sessions to prevent memory-biased placement. Both recordings of the same specimen were superimposed by the generalized least squares (GLS) method and the Procrustes distances were calculated. The mean intragroup [$S_i^2 = G/L \times (L - 1) \times M$, where G = sum of squared deviations, L = number of skulls, and M = number of recordings] and intergroup [$S_g^2 = G/(M - 1)$] squared deviations were calculated. With these values, we used the method of Lessels and Boag (1987) for estimating the repeatability: $R = S_g^2/S_i^2 + S_g^2$.

To test the method error relative to CT scan, landmark data collection was repeated twice on five crania and the same test described above was used.

Validation Microscribe/CT Scan

This method was also applied to test the validation Microscribe/CT scanner. Five crania from the sample were digitized both with Microscribe and CT. Both recordings of the same specimen were superimposed by the GLS method and the Procrustes distances were calculated.

RESULTS

When testing the method error relative to Microscribe, the sum of intragroup squared deviations is 0.010 and the mean intragroup squared deviation $S_i^2 = 1.6 \times 10^{-4}$. The mean intergroup squared deviation is calculated after the superimposition of the six means of the specimens. The sum of intergroup squared deviations is 2.038, and the mean intergroup squared deviation $S_g^2 =$ 2.038. The ratio of variances gives an excellent estimated value of repeatability on Microscribe ($R = 0.99$). When testing the method error relative to CT scan, the repeatability is also excellent ($R = 0.99$).

The results of the validation CT scanner/Microscribe are as follows. The sum of intragroup squared deviations is 0.13 and the mean intragroup squared deviation $S_i^2 =$ 0.003. The mean intergroup squared deviation is calculated after the superimposition of the five means of the specimens. The sum of intergroup squared deviations is 1.78, and the mean intergroup squared deviation $S_g^2 =$ 1.78. Once again, the ratio of variances gives an excellent estimated value of repeatability ($R = 0.99$).

Differences in facial size are not statistically significant as calculated with the Kruskal-Wallis nonparametric rank F-test ($P = 0.6$; Fig. 4). The first five components are used in a multivariate regression on centroid size with APS to test for allometry. They have a moderate ($r = 0.36$) but significant ($P = 0.002$) relationship. The morphological transformation along the regression vector shows that an increase in size is associated with a relative vertical development of the midsagittal upper facial areas, lateral development of cheek bones, widen-

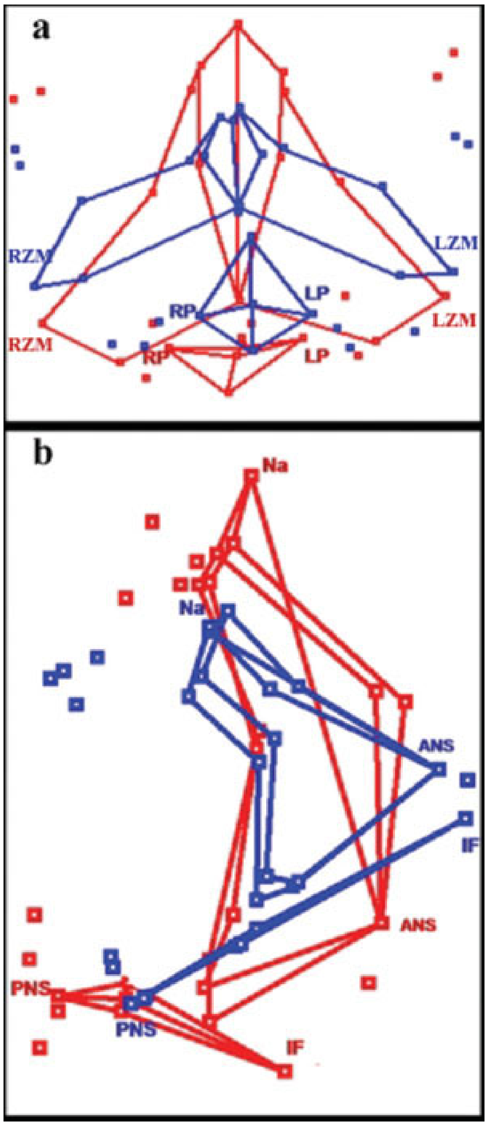

Fig. 7. **a:** Visualization of the two superimposed extremes of the face associated with the first component in frontal view. **b:** Visualization of the two superimposed extremes of the face associated with the first component in lateral view.

ing of the nasal aperture, downward maxillary development, and forward development of the palate.

Figure 5 shows the average shape of the 136 subjects superimposed by the Procrustes method. For visualization purposes, some facial landmarks are linked by lines called links (Fig. 5c and d). The percentages of the variance explained by the first three PCs are 16.3%, 8.6%, and 7.3%, respectively. Approximately 70% of the variability is incorporated into the first 14 components. The percentages of misclassifications for the five groups are as follows: European (26.4%), American (28.5%), African (38.7%), Asian (40%), and Oceanian (53.3%). This shows that the variations within these groups exceed the variations distinguishing group from group. However, the first discriminant function allows a differentiation between the three groups Europe, Africa, and America. Figure 6 plots discriminant function 1 (x-axis) scores against discriminant function 2 (y-axis) scores for the entire sample. For visualization purposes, the most discriminant groups are delimited: Europe, Africa, and America.

Multivariate regressions are performed between the first discriminant function and the first 14 PCs. The results show a high correlation with the first principal component ($R^2 = 0.51$; $F = 143.2$) and a sudden decrease with the other components, which indicates that PC1 is the most implicated in discrimination. PC1 is mostly influenced by the width and the length of the face, the relative flatness of the upper face, and prognathism of the maxilla, as well as the length and the relative inclination of the palate. Figure 7a shows the two superimposed extremes of the face associated with the first component in a frontal view: the width of the face (RZOM-LZOM), the total length of the face (Na-ANS), as well as the height of the zygoma (ZOM-ZM) are concerned. The lateral view (Fig. 7b) shows the variation in the prognathism of the maxilla (M and especially ANS), the length of the nose (FNM-NM), and the flatness of the upper face (Na, FNM, FOM, and NM), which is exaggerated by the posterior position of Na relatively to FNM and FOM. The length and the inclination of the palate (IF-PNS) also contribute to the first component. In other words, while moving toward one extreme of PC1 (the blue face in Fig. 7), faces become relatively wider and shorter, the nasal bones flatten, and the upper face lowers. Also, the maxilla becomes vertically flattened, while the prognathism increases. The palate becomes longer and more inclined anterosuperiorly.

DISCUSSION

Conventional cephalometric methods have existed for more than 60 years. One of the main applications of cephalometrics in paleoanthropology, orthodontics, and maxillofacial surgery is as a shape descriptor. We use various linear and angular measurements to achieve a concise and comprehensive description of the craniofacial pattern. However, conventional cephalometric methods have certain inherent problems regarding their applicability as shape measures. They provide only a partial and localized description of shape because of the 2D projection of a 3D object. They are also confounded by our biases regarding the reference structures (cranial base, Frankfort horizontal, or others) because of the difficulty in identifying specific landmarks (Hixon, 1956; Bjork and Solow, 1962; Savara et al., 1966; Gravely and Benzies, 1974; Mitgaard et al., 1974). At the beginning of this new century, we are in a position to return to a full three-dimensional analysis by using the helical technique in computed tomography scanner. Our results indicate that data collected from 3D reconstructions made with CT scans are internally consistent, precise, and accurate when compared to those collected from Microscribe. This results from the great evolution in medical imaging, since the spiral CT scanner allows rapid acquisition of a volume data set and the reconstruction of images at any plane without magnification errors caused by geometric distortions. Since the landmarks chosen in this study are sutural (except Maxillare, Basion, and Opisthion), they are easily located both on Microscribe and on new spiral CTs. This increases the precision of landmark definition and the reproducibility of landmark location.

In this study of five modern human groups, 136 crania have been digitized and the coordinates have been processed using generalized Procrustes analysis (GPA), which superimposes the landmark coordinates configurations of the specimens and scales them for size, so that the differences they exhibit are due to shape (Rohlf, 1990; Slice, 1996). The selection of the morphometric method that should be used in a study seems to be very important. As the purpose of this study was to estimate the mean shape in the sample and study its variability among five populations, it is important to use methods that yield unbiased estimates and are as close as possible to the true shape. It has been shown that Procrustes estimates show no evidence of bias and are the most accurate among the other morphometric methods (Rohlf, 2000). Thus, Procrustes-based methods seem to be the most appropriate approach for this study.

After calculating the mean shape by Procrustes superimposition, we have studied the variability of shape using PCA, a statistical technique for reducing the number of variables when a significant correlation between the variables is present. A significant disadvantage of PCA is that the resulting components, because they are derived statistically, do not necessarily have a clear biological interpretation. Usually, only the first, or the first few, can be described satisfactorily. The application of PCA in this sample results in 14 PCs explaining approximately 70% of the total shape variability. The first principal component, which accounts for 16.3% of the total sample variability, is mostly influenced by the relative flatness of the upper face, the relative prognathism of the maxilla, the width and the length of the face, as well as the length and the relative inclination of the palate. While examining the two extremes associated with this first component, we conclude that a flat upper face is relatively associated with a short and large face and a prognate maxilla. This results in a more anterior incisal foramen (IF), which leads to a longer palate inclined anterosuperiorly (the extreme shape represented in blue color in Fig. 7). All these changes are relative because no information about the orientation of the specimens could be provided with Procrustes superimposition. However, the width of the maxilla (RZM-LZM) and the palate (RP-LP) do not show a great variability (Fig. 7a). This may be the common invariant feature responsible for the alignment of the teeth in all specimens. This may correspond to functional patterns related to masticatory constraints manifested by the important interproximal and occlusal dental wear in all specimens. It would be of major interest to compare this model of craniofacial form to that of living subjects with crowded dentitions in order to test the difference in size and shape in the masticatory and alveolar regions as well as in the entire facial skeleton. Previous studies on nonhuman primates have demonstrated that specific regions of the face are differentially affected by masticatory strains, the effect being higher in the lower face and lower in the middle and upper face (Hylander et al., 1991; Hylander and Johnson, 1992). This reflects the importance of using sutural landmarks in this study, which allow a delimitation of particular regions of the skull associated with both a particular growth pattern and a specific functional requirement in order to analyze them independently (theory of functional craniology) (González-José et al., 2005).

Our discriminant analysis has not shown great differences among the five regional groups. The European group is the most distinctive and had the least percentage of misclassifications (26.4%), followed by the American group (28.5%) and the African group (38.7%). The pattern of shape differences among these groups should be corroborated by the analysis of paired groups in order to examine closely the shape differences along each component and to make additional comparisons of the relative positions of recording points.

Size accounts for a certain percentage of the facial morphological variability. The main allometric pattern involves a downward maxillary development, a forward development of the palate, and the growth of the midsagittal upper facial areas as size increases. Nevertheless, there is no evidence of size differences between the five groups, and it may be assumed that the allometric process is shared by all the populations. A similar conclusion on facial size differences has already been proposed on the basis of geometric morphometrics (Bruner and Manzi, 2004) and traditional morphometrics (Howells, 1973, 1989).

Finally, the sample used in this study may be considered as a sample of ideal or normal subjects for two reasons: it represents the general population from all over the world, and it includes specimens with aligned teeth and without dental anomalies of form, number, volume, or position. Therefore, the results obtained on the present sample could be used as a baseline data for clinical applications (orthodontics and maxillofacial surgery) and future studies (comparison between this craniofacial model of modern humans and that of other sapiens or nonsapiens hominids). The reference model statistics will be given in a future publication.

This study confirms the high precision of measurements performed on CT scans and the importance of geometric morphometrics, which provides an accurate characterization of the overall craniofacial shape and its variation within the entire population. Using geometric morphometrics combined with CT scan allows a three-dimensional analysis without returning to the original crania.

The future directions would be to compare, on the one hand, this 3D modern human craniofacial model to that of living subjects with crowded dentitions and, on the other hand, to that of fossil skulls that were already CT scanned. By analyzing the differences in the craniofacial size and shape, we would better understand the respective functional adaptations and plastic response to mechanical stress.

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