Euclidean Distance Matrix Analysis: Confidence Intervals for Form and Growth Differences

SUBHASH LELE AND JOAN T. RICHTSMEIER
Department of Biostatistics, School of Hygiene and Public Health (S.L.), and Department of Cell Biology and Anatomy, School of Medicine (J.T.R.), The Johns Hokins University, Baltimore, Maryland 21205

KEY WORDS Bootstrap, Craniofacial morphology, Estimation of form, Landmark data, Morphometrics, Sexual dimorphism

ABSTRACT Analysis of biological forms using landmark data has received substantial attention recently. Much of the statistical work in this area has concentrated on the estimation of average form, average form difference, and average growth difference. From the statistical, as well as the scientific point of view, it is important that any estimate of a scientifically relevant quantity be accompanied by a statement regarding its accuracy. Such a statement is contained in a confidence interval. The purpose of this paper is to provide a method to obtain confidence intervals for form difference and growth difference estimators. The estimators are based on Euclidean distance matrix analysis. The confidence intervals are calculated using the model independent bootstrap method. We illustrate the method by using three examples: morphological differences between samples of craniofacial patients and normal controls using two dimensional data from head X-rays, sexual dimorphism of craniofacial morphology in *Cebus apella,* and sexual dimorphism of facial growth in *Cebus apella* using three-dimensional data.

Much research is currently being devoted to the analysis of biological forms using landmark data. Some of these methods have been reviewed recently (Bookstein, 1991; Rohlf, 1991; Richtsmeier et al., 1992; Rohlf and Marcus, 1993). Until recently there were two broad classes of approaches to the analysis of landmark data: (1) superimposition-based approaches (e.g., Bookstein, 1986; Rohlf and Slice, 1990; Goodall, 1991), and (2) deformation-based approaches (e.g., Lew and Lewis, 1977; Bookstein, 1989, 1991). An alternative approach based on the Euclidean distance matrix representation of form was recently introduced (Lele, 1991).

Lele (1991) discusses the merits and demerits of these approaches. He shows that superimposition methods involve an arbitrary choice of fitting criteria that can affect scientific interpretations and conclusions. Deformation-based approaches involve the arbitrary choice of a smoothing or homology function that can also affect the scientific conclusions. Lele (1991) argues for the use of the Euclidean distance matrix representation of form in the analysis of landmark data.

Euclidean distance matrix analysis of form difference has been developed in various publications (Lele and Richtsmeier, 1991, 1992; Richtsmeier and Lele, 1990). Richtsmeier et al. (1993) and Richtsmeier and Lele (1993) extend this method to the analysis of growth. Most recently, Lele (1993) has further developed the statistical procedures for estimation of average form, form difference and growth difference based on the Euclidean distance matrix represen-

Received December 8, 1992; accepted January 11, 1995.

Address reprint requests to Dr. Subhash Lele, Department of Biostatistics, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205.

tation. Lele (1993) demonstrates that these distance-based estimators possess important statistical properties, namely those of consistency and asymptotic normality. It is also shown that these estimators are fairly robust against model misspecification, handle missing data easily and are extremely simple to compute. Lele (1993) provides a detailed evaluation of the statistical properties of superimposition-based methods and evaluates these properties in terms of the implications for the results of statistical analyses.

Most of the statistical work discussed by Lele (1993) concentrates on the estimation of various quantities such as average form, form difference, and testing of the null hypothesis of equality of shapes. From a statistical, as well as a scientific point of view, it is important that any estimate of a scientifically important quantity carry with it a statement about its variability. This is because an estimate with large variance is generally less useful than an estimate with small variance. A method for obtaining a confidence interval associated with an estimate is a powerful tool (see Kowalski, 1993 for a comment).

The purpose of this paper is to provide a methodology for calculating confidence intervals for the difference in forms and growth patterns as defined in Euclidean Distance Matrix Analysis (EDMA). The methodology is based on the Bootstrap technique (Efron, 1981) and is model robust in nature. Consequently, detailed statistical models which may not be biologically justifiable (Lele and Richtsmeier, 1990) are not assumed.

EUCLIDEAN DISTANCE MATRIX ANALYSIS: A BRIEF REVIEW

In this section, we provide background information by briefly describing the basic concepts and definitions involved in EDMA of landmark coordinate data. For details see Lele (1991) and Lele and Richtsmeier (1991).

Definition of form and the role of maximal invariant

The form of an object is considered to be that characteristic which remains invariant under translation, rotation and reflection. Lele (1991) argues that analysis of form should begin with a representation that is invariant under these operations. (Some methods for the analysis of landmark coordinate data consider reflection as part of shape, e.g., Goodall, 1991.)

Consider a biological object represented by K landmarks in D dimensions. Such a biological object can be represented by a $K \times D$ matrix of landmark coordinates with D equal to 2 or 3. Let S denote the space of all $K \times D$ matrices. This space S is equivalent to the space of all objects represented by K landmarks which belong to a D dimensional Euclidean space.

Let F(.) be a function defined on this space such that for forms A and A^* in space S, $F(A) = F(A^*)$ if and only if A^* is a rotated, reflected and/or translated version of A; i.e., if and only if $A^* = A\Gamma + t$ where Γ is a $D \times D$ orthogonal matrix and t is a $K \times D$ matrix of identical rows representing rotation, reflection and translation or any combination of these operations. A function with the characteristics of $F(\cdot)$ is called a maximal invariant on the space S under the group of rotation, reflection and translation transformations.

A representation of landmark data that is coordinate system free and is invariant under translation, rotation, and reflection, is the Euclidean Distance Matrix consisting of all interlandmark distances. An object A, with K landmarks, is represented by the Euclidean Distance Matrix:

$$FM(A) = \begin{bmatrix} 0 & d(1,2) & d(1,3) & \cdots & d(1,K) \\ d(2,1) & 0 & d(2,3) & \cdots & d(2,K) \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ d(K,1) & d(K,2) & d(K,3) & \cdots & 0 \end{bmatrix}$$

where d(i,j) denotes the Euclidean distance between landmarks i and j. We write this matrix as:

$$FM(A) = [FM_{ij}(A)]$$
$$where \quad i,j = 1, 2, 3, \ldots, K.$$

We call this matrix a form matrix (FM) since it retains all the relevant information about the form of an object as summarized by landmark coordinates.

Since FM(A) is symmetric with diagonal elements zero, one can equivalently use a vector consisting of all the off-diagonal, upper entries of the matrix to represent FM(A), or the form of the object A. The form of an object with K landmarks then is uniquely represented by a vector of K(K − 1)/2 distances between all possible pairs of landmarks. Equivalently, an object with K landmarks is represented by a point in L = K (K − 1)/2 dimensional Euclidean space. This space is called the form space (Lele, 1991). Form space is a subset of the L-dimensional Euclidean space as shown in Richtsmeier and Lele (1993).

Form difference matrix

Suppose we want to compare the forms of two objects, A and B, each with K landmarks. Following the ideas presented above, the forms of these two objects correspond to two points in an L-dimensional Euclidean space. If the forms are identical, these two points are indistinct. If the two forms are similar (i.e., their shapes are the same) then these two points lie on a ray going through the origin. If neither of these conditions are true, then we say that the forms are different. There are several ways to describe this difference. An obvious description is the vector difference F(B) − F(A) where subtraction is done elementwise (i.e., for each individual linear distance). This representation defines the absolute difference between forms. Alternatively, the changed morphology relative to the initial morphology can be studied. To do this we have proposed the use of the form difference matrix (FDM; Lele, 1991; Lele and Richtsmeier, 1991):

$$FDM(B,A) = \frac{FM_{ij}(B)}{FM_{ij}(A)}$$

$$\text{where} \quad i,j = 1,2,\cdots,K$$

where the ratios of corresponding linear distances from two forms are calculated.

FDMs contain all the relevant information (as represented by the landmarks collected) regarding morphological differences between two forms (or samples of forms). Differences in form can reflect a simple difference in scaling of two forms (i.e., only in size), or a combination of difference in size and shape. Previous work (Lele and Richtsmeier, 1992) has provided a method for localizing differences between forms using the EDMA.

Growth difference matrix

When the forms being compared are specimens from an ontogenetic series, comparison of the forms reflects the changes in geometry that occur due to growth. For our purposes, growth is defined as a process that acts to change a form through time from configuration A1 at time t_1 to configuration A2 at time t_2. These two forms A1 and A2 correspond to two distinct, but comparable points in form space, provided they are represented by the same number of landmarks. Absolute growth, the difference between A1 and A2 resulting from the growth process, can be represented by the vector that joins these two points in the form space, namely by $(FM(A2) - FM(A1))$. Our research orientation focuses on the study of relative growth, or the change due to growth in an organism (A2) as compared to its previous morphology (A1).

To study relative growth we use the growth matrix (GM) defined as:

$$GM_{ij}(A1,A2) = \frac{FM_{ij}(A2)}{FM_{ij}(A1)}$$

$$\text{where} \quad i,j = 1,2,\cdots,K.$$

where the older form is in the numerator and the division is conducted elementwise. This vector of $K(K-1)/2$ ratios reflects the intuitive definition of relative growth.

When comparing growth patterns, the following questions are of interest: are two growth patterns the same? If they are different, can we localize the differences? We develop a method for comparing growth patterns based on the concept of relative growth. We formulate the comparison of growth patterns in terms of four form matrices FM(A1), FM(A2), FM(B1) and FM(B2) where A and B are two distinct organisms, and 1 and 2 represent the ages of the organisms. Differences in relative growth are defined by the comparison of two growth matrices, one for growth of object A1 into object A2 (GM(A1,A2)), and one for growth of object B1 into object B2 (GM(B1,B2)). Elementwise comparison of these growth matrices is accomplished by the growth difference matrix (GDM) given by:

$$GDM(A1,A2;B1,B2) = \frac{GM_{ij}(B1,B2)}{GM_{ij}(A1,A2)}$$

$$\text{where} \quad i,j = 1,2,\cdots,K.$$

For more details, see Richtsmeier and Lele (1993).

STATISTICS OF FORM AND GROWTH

This section discusses estimation of form, form difference and growth difference matrices using landmark data and gives a bootstrap algorithm for calculating confidence intervals for these quantities. The mathematical details of the estimation procedures are discussed by Lele (1993). Here we provide only those details necessary for programming of these algorithms. For those who prefer, our programs are available when requests are accompanied by 2 diskettes (3-1/2 inch, HD-DS) formatted for IBM compatible PCs. Statistical procedures for testing equality of shapes and of growth patterns are detailed in Lele and Richtsmeier (1991) and Richtsmeier and Lele (1993); they will not be discussed here.

Estimation of average form matrix

In practice, when one compares forms, only a sample of individuals from each population is available for study. The first concern then, is estimation of average form. Lele and Richtsmeier (1991) suggested two different methods for the estimation of mean form difference and Lele (1993) proposed a third method. Method 1 described in Lele and Richtsmeier (1991) is based on Generalized Procrustes Analysis (Goodall, 1991). In view of Lele (1993), we do not recommend this method. Method 2 described in the same paper is statistically correct and adequate if one is interested in estimating the form difference matrix (FDM). This method does not need the assumption of Gaussian errors. However, the method does require the assumption of equality of covariance matrices for the two populations. The method for estimation of the average form matrix (FM) described in Lele (1993) can be used for obtaining both the average form matrix (FM) for a single population and also the average form difference matrix (FDM) or the average growth difference matrix (GDM). Although the formulae in Lele (1993) are derived under the assumption of Gaussian perturbations, they depend only on the first two moments (mean and variance) of the perturbations and not on the complete specification of the distribution. This method does not require equality or covariance matrices as required by Method 2. Here we adopt the method proposed by Lele (1993) because it provides estimators that are shown to possess good statistical properties, are fairly robust against model misspecification, and are easy to compute.

Let $A_1, A_2, A_3, \ldots, A_n$ be landmark coordinate matrices for n individuals from population A. Let $e_{ij,k}$ denote the squaredEuclidean distance between landmarks i and j for the k-th individual. Let:

$$\bar{e}_{ij} = n^{-1} \sum_{k=1}^{n} e_{ij,k} \qquad (1)$$

$$s^2(e_{ij}) = n^{-1} \sum_{k=1}^{n} (e_{ij,k} - \bar{e}_{ij})^2 \qquad (2)$$

be the sample mean and sample variance for the squared distance between landmarks i and j calculated for the n individuals. If the objects under consideration are two dimensional, then calculate:

$$\delta_{ij} = (\bar{e}_{ij} - s^2(e_{ij}))^{0.25}. \qquad (3)$$

If the objects under consideration are three-dimensional, then use the formula:

$$\delta_{ij} = (\bar{e}_{ij} - 1.5 \times s^2(e_{ij}))^{0.25}. \qquad (4)$$

(In general, the quantities in parentheses are not guaranteed to be non-negative for any given sample. However, they can be negative only if the variance of a linear distance is larger than its mean. This, in turn, can happen if landmarks flip their positions with high probability. This is a highly unlikely event in real life.) The estimate of the average form matrix for population A using this sample is given by:

$$FM(A) = (\delta_{ij})_{i,j} = 1,2,\cdot\cdot\cdot,K. \quad (5)$$

Similarly, the average form matrix for the sample $B_1, B_2, B_3, \ldots, B_m$ is calculated from population B and is called FM(B). The estimate of the form difference matrix between these two populations is given by:

$$FDM(B,A) = \frac{FM(B)}{FM(A)}$$

where the division is conducted elementwise. Growth matrices and growth difference matrices are calculated using the estimates of the average form matrices from the four samples and the definitions given in the previous section.

Bootstrap confidence intervals for form difference

There are two ways to obtain confidence intervals for any estimator. One is based on the exact statistical distribution of the estimator calculated under some model assumptions. The other approach is model independent, meaning that very little is assumed about the underlying statistical model.

To obtain model independent confidence intervals we use the Bootstrap procedure (Efron, 1981; for a user-friendly discussion of Bootstrap, see Efron and Tibshirani, 1986). Model independent confidence intervals are, in general, more desirable than likelihood or Monte Carlo model-based confidence intervals because these latter estimates tend to be extremely sensitive to deviations from the underlying model (Huber, 1972). Monte Carlo methods are preferable if one has reasonable confidence in the correctness of the underlying model. Bickel and Freedman (1981) provide mathematical and statistical justification of the validity of bootstrap confidence intervals under fairly general conditions.

Algorithm for calculating confidence intervals for form difference using a bootstrap procedure. Let $A_1, A_2, A_3, \ldots, A_n$ and $B_1, B_2, B_3, \ldots, B_m$ be the landmark coordinate matrices for individuals from the two populations A and B.

Step 1: Obtain a simple random sample with replacement of size n from sample A and of size m from sample B.

Step 2: Calculate the average form difference matrix, FDM(B,A), for the samples obtained in step 1 using equations 1, 2, 3, and 5 if the objects are two-dimensional objects, and equations 1, 2, 4 and 5 if the objects are three dimensional.

Step 3: Repeat steps 1 and 2 C times, where C is sufficiently large, say between 300 and 1,000.

A collection of bootstrap samples obtained in this way has $K(K - 1)/2$ rows and C columns in matrix. Each column is a form difference matrix obtained at the end of step 2 and each row represents C form difference ratios for a linear distance between a specified pair of landmarks. To obtain a confidence interval for each linear distance, the ratios in each row are sorted in increasing order. If a 90% confidence interval is sought, then the first 5% and the last 5% of the total entries in this sorted row are deleted. The minimum and maximum entries remaining in that row constitute the lower and upper confidence limits for that particular linear distance. This is done separately for each row to obtain a confidence interval for each linear distance ratio.

Bootstrap confidence intervals for growth difference. This algorithm is similar to the one described above but involves four populations, namely A1, A2, B1, and B2. In this case, we compare growth patterns for two populations A and B. A1 represents the population for the first age category for population A and A2 represents the second age category for the same population. The populations B1 and B2 represent the age-matched categories for population B. We are interested in calculating the confidence interval for the growth difference matrix that compares growth in populations A and B as

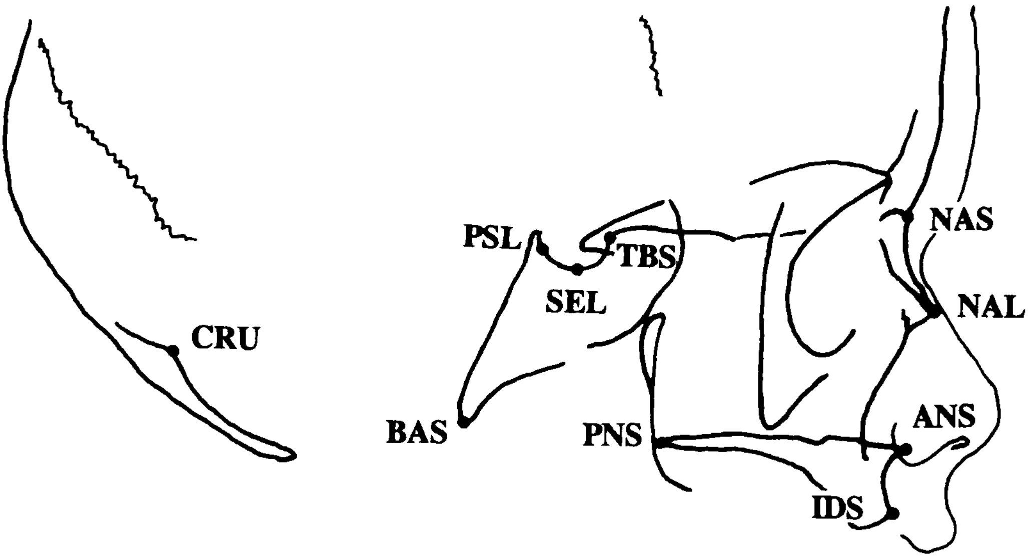

Fig. 1. Osseous landmarks located on the tracing of a head X-ray in two dimensions. All landmarks are located on the sagittal plane. Outline of the skin surface is shown for orientation. Landmark abbreviations are as follows: NAS, nasion; NAL, nasale; ANS, anterior nasal spine; IDS, intradentale superior; PNS, posterior nasal spine; TBS, tuberculum sella; SEL, sella; PSL, posterior sella; BAS, basion; CRU, cruciate eminence. Details of data collection and further definition of the landmarks can be found in Richtsmeier (1987).

defined in the previous section. The sample sizes are assumed to be of size n, m, p and q for A1, A2, B1, and B2, respectively.

Step 1: Obtain a simple random sample with replacement of size n from population A1, of size m from population A2, of size p from population B2 and of size q from the population B2.

Step 2: Calculate the average growth difference matrix, GDM(A1, A2: B1, B2), for the samples obtained in step 1. This is obtained by: (1) first calculating the average form matrix for each age category namely, FM(A1), FM(A2), FM(B1), FM(B2), within each population separately as sescribed in the Estimation of Average Form Matrix Section; (2) calculating the average growth matrices namely GM(A1,A2), GM(B1,B2); and (3) calculating average growth difference matrix GDM(A1,A2;B1,B2) using the definitions given in the Growth Difference Matrix Section.

Step 3: Repeat steps 1 and 2 C number of times where C is sufficiently large, say between 300 and 1,000.

A collection of bootstrap samples obtained this way has $K(K - 1)/2$ rows and C columns in matrix format. Each column is a growth difference matrix obtained at the end of step 2 and each row represents C growth difference ratios for a linear distance between a specified pair of landmarks. To obtain a confidence interval for growth differences for each linear distance, the ratios in each row are sorted in increasing order. If a 90% confidence interval is sought, then the first 5% and the last 5% of the total entries in this sorted row are deleted. The minimum and maximum entries remaining in that row constitute the lower and upper confidence limits for that particular linear distance. This is done separately for each row to obtain a confidence interval for growth difference for each linear distances ratio.

Interpretation of the confidence intervals

If an interval for a particular linear distance contains the value of 1, there is probably no difference in form (or growth) for that linear dimension. Confidence intervals that

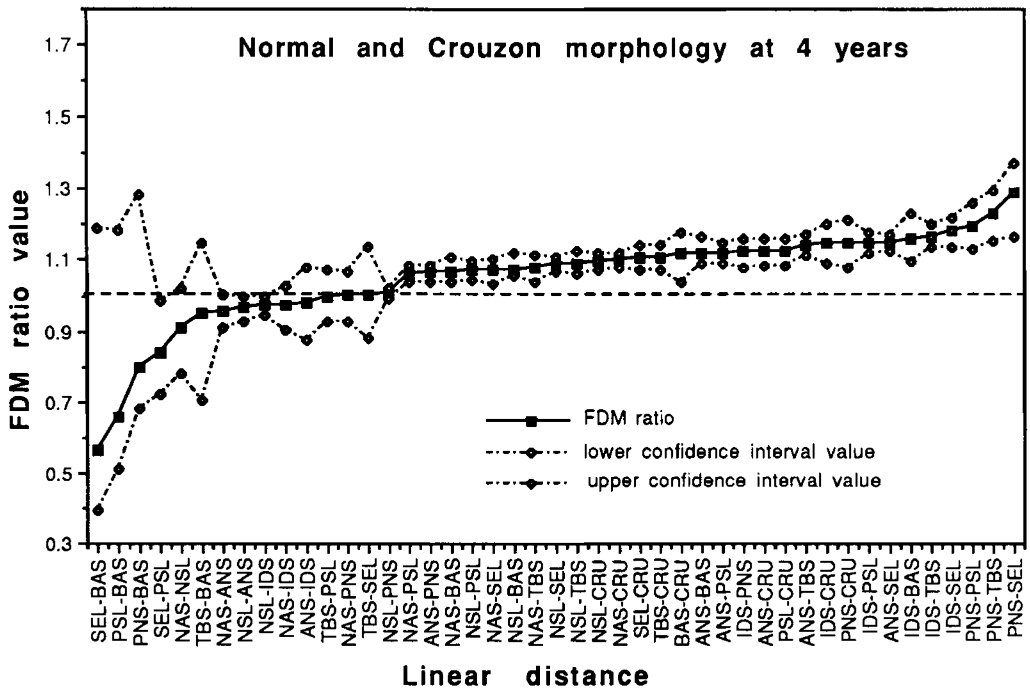

Fig. 2. Values of the form difference matrix (FDM) for the comparison of normal and Crouzon craniofacial form at age four years, and the 90% confidence intervals ($\alpha = 0.10$) for each linear distance. Individual values equal to 1 and confidence intervals that contain the value 1 indicate similarity between the samples for a particular linear distance. This and the following figure do not depict confidence bands for the difference in form between two populations. Confidence intervals are calculated separately for each linear distance (see text for discussion).

do not contain the value 1 indicate that a local form or growth difference involves that particular linear distance. Linear distances grouped on the basis of anatomical, functional or developmental criteria can be considered as units when reviewing confidence interval results. If all confidence intervals within an FDM or GDM contain a constant, then the difference in growth or form may be due to scaling.

EXAMPLES

To demonstrate the use of the methodology just introduced, we present three simple examples. All of our examples focus on biological problems related to morphology. These examples represent suggestions for use of our methodology. Potential applications are not limited to the types of research problems described by our examples.

Example 1. Normal and Crouzon morphological comparison: Two-dimensional data from X-rays

Abnormal craniofacial morphology is a phenotypic consequence of many human genetic disorders. Humans affected with Crouzon syndrome have premature closure of some craniofacial sutures, maxillary hypoplasia, occular proptosis due to shallow orbits and frontal bossing (see Kreiborg, 1981; Cohen, 1986; Kreiborg et al., 1993 for further information). The facial features typical of Crouzon syndrome are striking and are usually corrected surgically during infancy with diverse results. Exact morphological measurements can be used in surgical planning and postoperative evaluation of these children.

In this example, we compare the morphology of normal children and those diagnosed

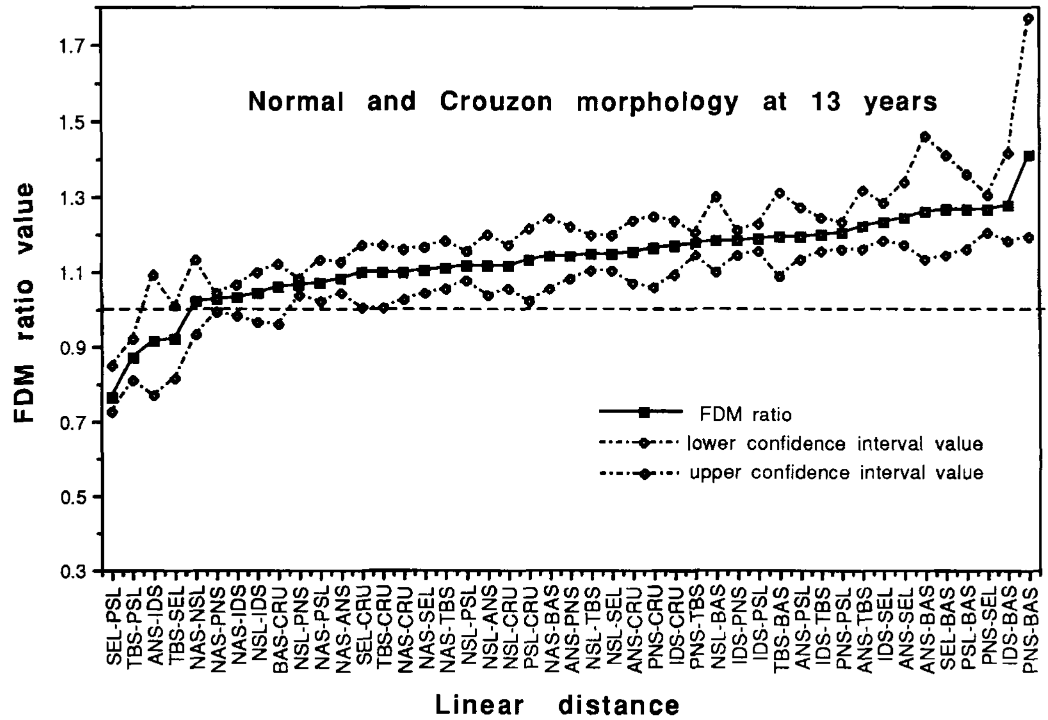

Fig. 3. Values of the form difference matrix (FDM) for the comparison of normal and Crouzon craniofacial form at age thirteen years and the 90% confidence intervals (α = 0.10) for each linear distance.

with Crouzon syndrome. A normal sample of children at 4 years of age (N = 20) is compared to a sample of age-matched Crouzon syndrome children (N = 4), and we compare a sample of normal children at age 13 (N = 19) to an age-matched sample of Crouzon patients (N = 5). None of the Crouzon cases had undergone surgical correction. Two-dimensional coordinate locations of ten osseous landmarks located on x-rays of craniofacial patients diagnosed with Crouzon syndrome and on X-rays of normal controls serve as the raw data (Fig. 1). Data sources and data collection techniques can be found in Richtsmeier (1987). In this example, we use the normal sample as the base population and the Crouzon sample as the comparative population in the calculation of the FDM.

Formal statistical testing for shape difference between these two samples using procedures developed by Lele and Richtsmeier (1991) indicates that the Crouzon and normal craniofacial form defined by two-dimensional coordinate locations of osseous landmarks are statistically distinct at 4 years of age. The confidence intervals calculated for the linear distances indicate a distinct pattern between those linear distances that are smaller than normal and those that are larger than normal in the Crouzon population (Fig. 2). For those distances that are larger in the Crouzon population (ratios less than 1), the spread of the confidence intervals is extreme, suggesting a large degree of variability within the samples for these linear distances. The linear distances exhibiting the widest confidence intervals share basion as an endpoint, suggesting that the location of basion is highly variable. As the ratios for specific linear distances in the FDM approach the value of 1, the confidence intervals narrow (Fig. 2). Those linear distances with an associated confidence interval that includes 1 reflect similarities between the normal and Crouzon samples in

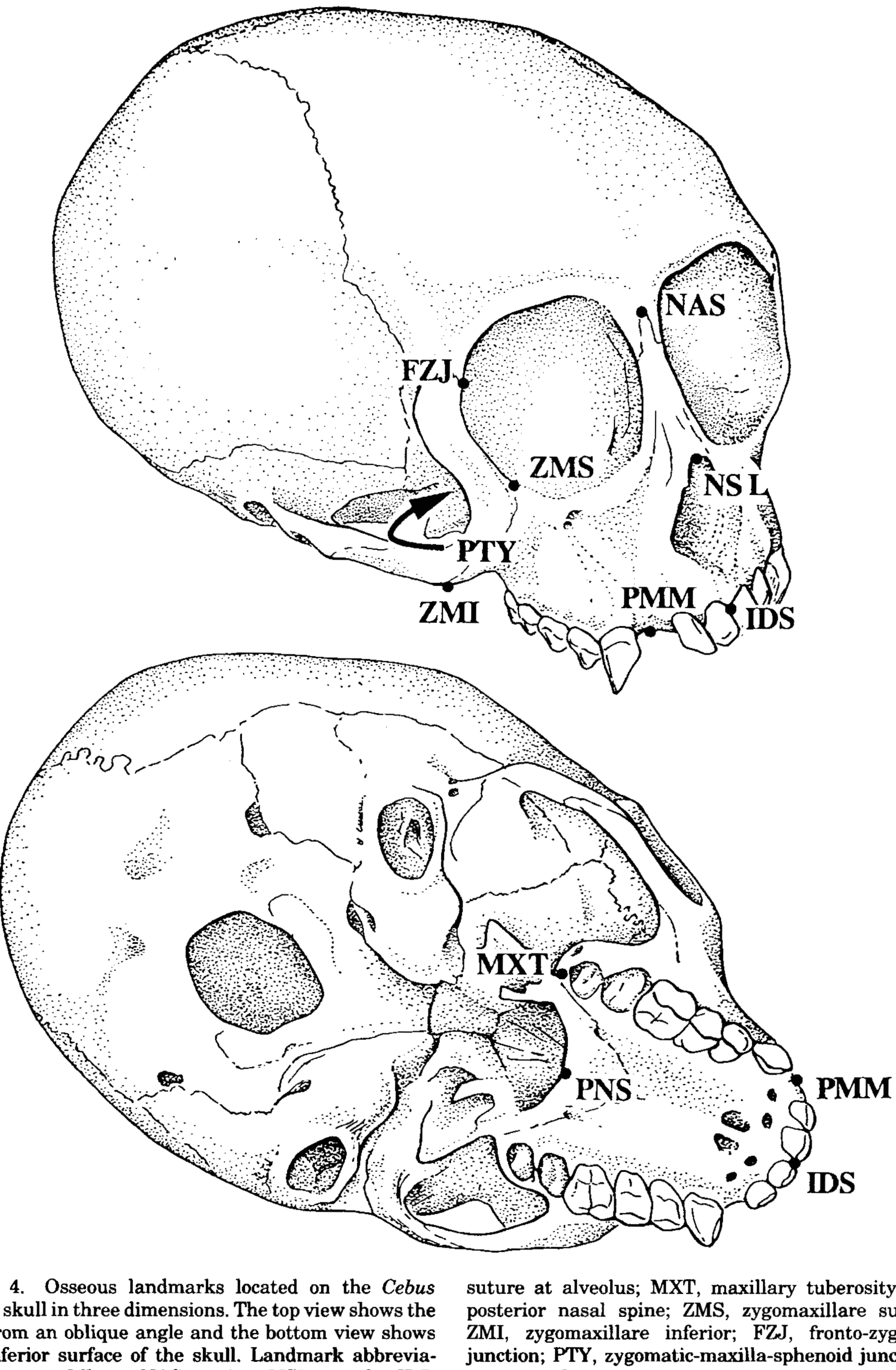

Fig. 4. Osseous landmarks located on the *Cebus apella* skull in three dimensions. The top view shows the face from an oblique angle and the bottom view shows the inferior surface of the skull. Landmark abbreviations are as follows: NAS, nasion; NSL, nasale; IDS, intradentale superior; PMM, premaxillary-maxillary suture at alveolus; MXT, maxillary tuberosity; PNS, posterior nasal spine; ZMS, zygomaxillare superior; ZMI, zygomaxillare inferior; FZJ, fronto-zygomatic junction; PTY, zygomatic-maxilla-sphenoid junction at pterygo-palatine fossa.

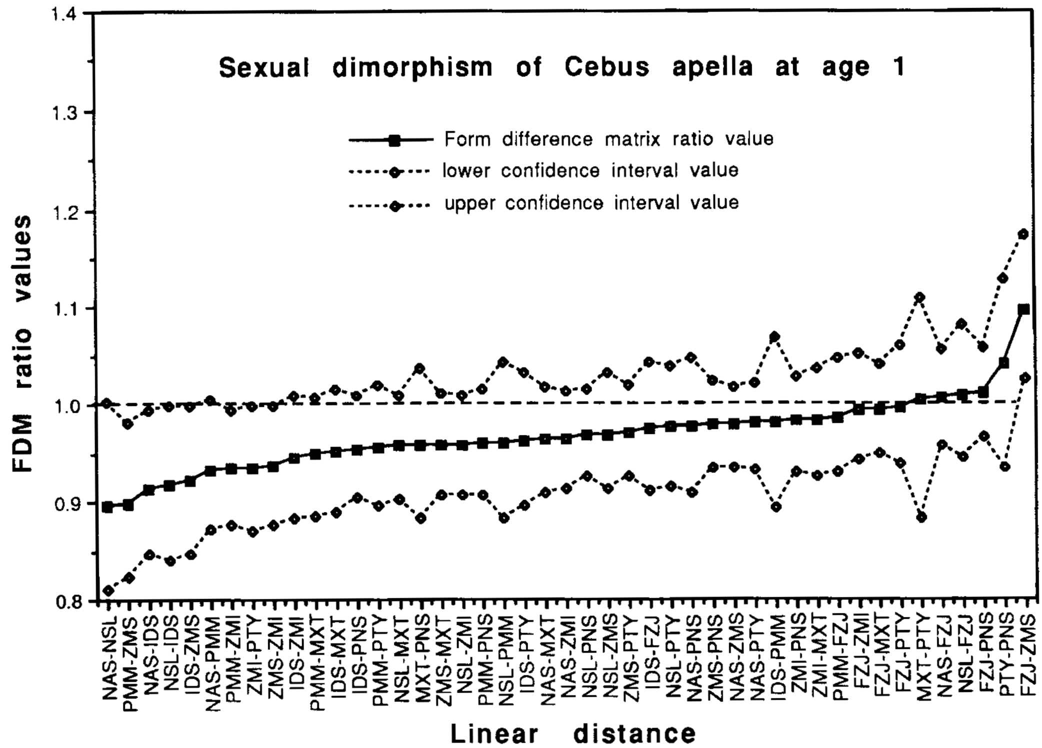

Fig. 5. Values of the form difference matrix (FDM) for the comparison of immature male and female *Cebus apella* faces and the 90% confidence intervals ($\alpha = 0.10$) for each linear distance. Individual values equal to 1 and confidence intervals for a linear distance that contain the value 1 indicate similarity between the samples for this particular linear distance.

the overall height of the face, anteroposterior dimension of sella turcica, and the oblique distance from the posterior hard palate to the nasal aperture. The 90% confidence intervals for all other linear distances indicate morphological difference between the normal and Crouzon samples at age 4.

Comparison of the same populations at age 13 shows very few linear distances that are smaller in the normal population (ratios less than 1; Fig. 3). Four of the linear distances exhibiting ratios close to 1 and the associated confidence intervals that include the value 1 for this age group were among those showing intergroup similarity at age 4. This suggests a growth pattern for these linear distances (NAS-IDS, ANS-IDS, NAS-PNS, TBS-SEL) that maintains similarity of local features between the groups. The majority (80%) of the ratios computed for the linear distances are greater than 1 however, and exhibit confidence intervals that exclude the value 1. These results demonstrate basic differences in the morphology between the adolescent normal individuals in our sample and those affected with Crouzon syndrome.

Example 2. Sexual dimorphism of *Cebus apella* facial morphology: Three-dimensional data from dry skulls

Three-dimensional coordinates of 10 landmarks located on the right side and midline of the facial skeleton of *C. apella* were used in analysis (Fig. 4). Details regarding aging and sexing of skeletons and data collection procedures can be found in Corner and Richtsmeier (1991). Male and female samples of immature (some or all deciduous teeth present; male N = 11, female N = 3) and

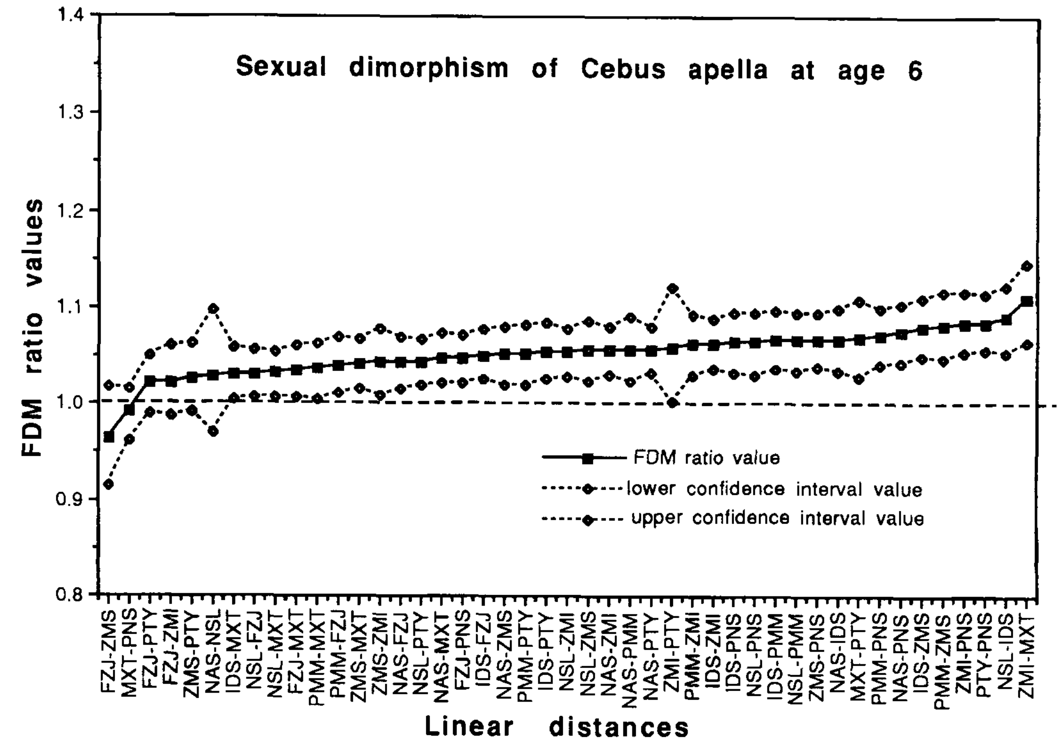

Fig. 6. Values of the form difference matrix (FDM) for the comparison of adult male and female Cebus apella skulls and the 90% confidence intervals (α = 0.10) for each linear distance.

adult (all permanent teeth in place; male N = 34, female N = 38) skulls were compared to determine the degree of sexual dimorphism for these two developmental age groupings using methods described above. In this example, the male data are always the numerator and the female data are always the denominator.

In the comparison of male and female facial morphology at developmental age 1, the 90% confidence intervals for nearly all (93%) of the linear distances include the value 1, demonstrating that the lengths of few linear distances are different between the sexes at this time (Fig. 5). Those linear distances that are significantly different between the sexes at developmental age 1 suggest a much larger superoinferior dimension of the zygomatic bone (FZJ-ZMS) in male *C. apella* and a comparatively shorter snout in the males (PMM-ZMS, NAS-IDS, NSL-IDS, IDS-ZMS, PMM-ZMI). Although the face of the female developmental age 1 *C. apella* is generally larger than the male as demonstrated by the majority of FDM ratios being less than 1, the confidence intervals and our statistical tests (*P* value = 0.30) show the male and female facial skeletons to be similar in overall form.

The male *C. apella* face is generally larger than the female face at developmental age 6, as evidenced by most FDM ratios being greater than 1 (Fig. 6). The 90% confidence intervals for only 6 (13%) of the linear distances contain the value 1 (Fig. 6). Dimensions that are not significantly different between male and female adults describe certain dimensions of the orbital rim and zygomatic bone (FZJ-ZMS, FZJ-PTY, FZJ-ZMI, ZMS-PTY), length of the nasal bones (NAS-NSL) and width of the posterior palate (MXT-PNS). The FDM suggests that sexual dimorphism of the adult *C. apella* facial skeleton is not simply a matter of scale: the shape of the adult male facial skeleton is statistically different from that of the fe-

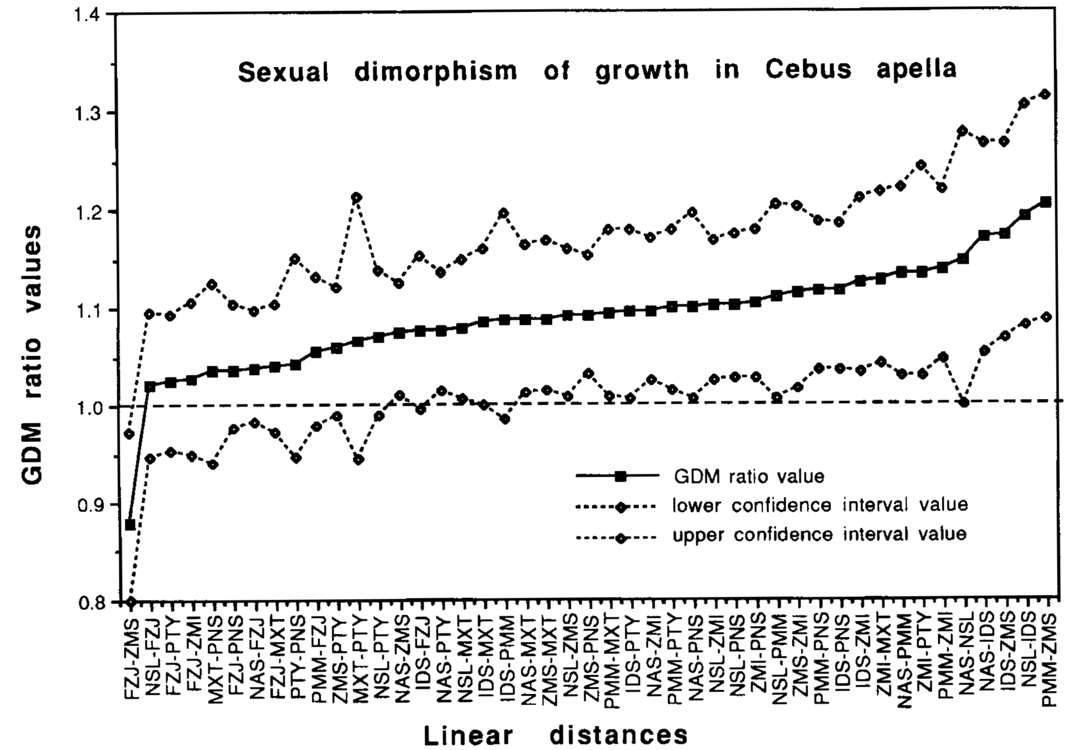

Fig. 7. Values of the growth difference matrix (GDM) for the comparison of facial growth in male and female *Cebus apella* and the 90% confidence intervals ($\alpha = 0.10$) for each linear distance.

male. Our statistical test (using methods proposed by Lele and Richtsmeier, 1991) supports the hypothesis that the shape of male and female adult faces are different.

Example 3. Sexual dimorphism of facial growth in *C. apella*: Three-dimensional data

In this example, we compare the male *C. apella* growth pattern from developmental age 1 to 6 to the female growth pattern for the same developmental age interval by calculating a Growth Difference Matrix and associated 90% confidence interval. Results of this analysis represent confidence intervals for sexual dimorphism of growth for all linear distances considered. For each sex, data from the older age group are the numerators while data from the younger age group are the denominators. When computing the growth difference matrix, the male form difference matrix is the numerator and the female form difference matrix is the denominator.

The 90% confidence intervals for approximately 28% of the linear distances contain the value 1 (Fig. 7) indicating that shape change due to growth is similar between the sexes for these linear distances. All of the linear distances that show similarity in growth pattern between the sexes are located on the more posterior portion of the face and do not include the anterior muzzle or alveolus (PMM-FZJ being the single, notable exception). Those linear distances that show the largest degree of sexual dimorphism of growth (ratios furthest from 1) describe dimensions among the more anteriorly placed facial landmarks (PMM, NSL, IDS) and between those facial landmarks and landmarks located on the zygomatic and interorbital regions (ZMI, ZMS, NAS).

These results suggest that the anterior muzzle and translation of the muzzle from the orbital region show the greatest degree of growth dimorphism. Growth patterns of the upper face, the posterior palate, and facial features located posterior and inferior to the orbits are similar between male and female *C. apella*.

DISCUSSION

Providing point estimates of scientifically interesting and important quantities is necessary but not sufficient. One should always provide a statement as to the accuracy of these point estimates. This is done by calculating a confidence interval associated with the point estimate. This paper provides a method for calculating model independent confidence intervals for form and growth difference estimates obtained from Euclidean distance matrix analysis of landmark data.

The following guidelines and cautions should be followed when constructing and interpreting these confidence intervals.

1. In general, for bootstrap confidence intervals, it is better to aim for a 90% confidence interval instead of the usual 95% confidence interval. Since the tails of the distribution of the estimator are estimated less precisely than the middle portion, a 95% confidence interval tends to be more unstable than a 90% or an 80% confidence interval. The general rule is, if the sample is small, one should aim for a smaller confidence level (known in formal statistical terms as smaller nominal coverage).

2. The confidence intervals reported by the program are *not* simultaneous confidence intervals for the whole form difference matrix, but are elementwise confidence intervals. They are confidence intervals for the corresponding linear distance only. Although it is possible to provide simultaneous confidence hyperellipsoids around the estimated mean form difference, interpretation of these hyperellipsoids might be problematic. Interpretation of the elementwise confidence intervals is straightforward. These confidence intervals can be used to localize the areas where forms or growth patterns are significantly different. These intervals are not to be used for testing the difference between two forms. For that purpose, the test proposed in Lele and Richtsmeier (1991) may be used. This method can be used in conjunction with the method proposed in Lele and Richtsmeier (1992) for the detection of influential landmarks. Given the amount of data usually available for such studies and the *non*-simultaneous nature of the confidence intervals provided in this paper, both methods should be viewed as "exploratory data analytic procedures." The method provided in this paper concentrates on individual linear distances whereas the method proposed in Lele and Richtsmeier (1992) tries to localize the differences to a set of landmarks.

ACKNOWLEDGMENTS

Part of this work was conducted while the first author was visiting the Department of Mathematical Sciences, Montana State University. Mr. Tao Wang wrote the computer programs. This work was supported by NSF grant DBS-9209083. We thank reviewers for their helpful comments. Figure 4 was drawn by Elaine Kasmer.

LITERATURE CITED

Bickel PJ, and Freedman DA (1981) Some asymptotic theory for the bootstrap. Ann. Stat. *9:*1196–1217.

Bookstein FL (1986) Size and shape spaces for landmark data. Stat. Sci. *1:*181–242.

Bookstein FL (1989) Principal warps: Thin plate splines and the decomposition of deformations. IEEE Trans. Pattern Anal. Mechine Intelligence *11:*567–85.

Bookstein FL (1991) Morphometric Tools for Landmark Data. London: Cambridge University Press.

Cheverud J, and Richtsmeier JT (1986) Finite element scaling applied to sexual dimorphism in Rhesus macaque (*Macaca mulatta*) facial growth. Syst. Zool. *35:*381–399.

Cohen MM, Jr. (1986) Craniosynostosis: Diagnosis, Evaluation and Management. New York: Raven Press.

Corner BD, and Richtsmeier JT (1991) Morphometric analysis of cranio facial growth in *Cebus apella*. Am. J. Phys. Anthropol. *84:*323–342.

Efron B (1981) The Jackknife, the Bootstrap and Other Resampling Plans. Philadelphia: SIAM.

Efron B, and Tibshirani R (1986) Bootstrap methods for standard errors, confidence intervals and other measures of statistical accuracy. Stat. Sci *1:*54–77.

Goodall CR (1991) Procrustes method in the analysis of shape (with discussion). J. Roy. Stat. Soc. Series B *53:*285–339.

Huber P (1972) Robust Statistics: A Review. Ann. Math. Stat. *43:*1041–1067.

Kowalski CJ (1993) Data analysis in craniofacial biology with special emphasis on longitudinal studies. Cleft Palate—Craniofacial J. *30:*111–120.

Kreiborg S (1981) Crouzon Syndrome: A clinical and roentgencephalometric study. Scand J Plast Reconstr Surg, Suppl. 18.

Kreiborg S, Marsh JL, Cohen MM, Jr, Liversage M, Pedersen H, Skovby F, Borgesen SE, and Vannier MW (1993) Comparative three-dimensional analysis of CT-scans of the calvaria and cranial base in Apert and Crouzon syndromes. J. Cranio-maxillo-facial Surg. *21:*181–188.

Lele S (1991) Some comments on coordinate free and scale invariant methods in morphometrics. Am. J. Phys. Anthropol. *85:*407–418.

Lele S (1993) Euclidean distance matrix analysis of landmark data: Estimation of mean form and mean form difference. Mathematical Geol. *25:*573–602.

Lele S, and Richtsmeier JT (1990) Statistical models in morphometrics: Are they realistic? Syst. Zool. *39:*60–69.

Lele S, and Richtsmeier JT (1991) Euclidean distance matrix analysis: A coordinate system free approach for comparing biological shapes using landmark data. Am. J. Phys. Anthropol. *86:*415–427.

Lele S, and Richtsmeier JT (1992) On comparing biological shapes: Detection of influential landmarks Am. J. Phys. Anthropol. *87:*49–65.

Lew WD, and Lewis JL (1977) An anthropometric scaling method with application to knee joint. J. Biomechanics *10:*171–184.

Richtsmeier JT (1987) A comparative study of normal, Crouzon and Apert craniofacial morphology using finite element scaling analysis. Am. J. Phys. Anthropol. *74:*473–493.

Richtsmeier JT, and Lele S (1990) Analysis of craniofacial growth in Crouzon syndrome using landmark data. J. Cranio. Genet. Dev. Biol. *10:*39–62.

Richtsmeier JT, and Lele S (1993) A coordinate free approach to the analysis of growth patterns: models and theoretical considerations. Biol. Rev. *68:*381–411.

Richtsmeier JT, Cheverud J, and Lele S (1992) Advances in anthropological morphometrics. Ann. Rev. Anthropol. *21:*231–253.

Richtsmeier JT, Cheverud J, Corner B, Danahey S, and Lele S (1993) Sexual dimorphism of ontogeny in the crab eating macaque (*Macaca fascicularis*). J. Human Evol. *25:*1–30.

Rohlf J (1991) Morphometrics. Ann. Rev. Ecol. Systematics *21:*299–316.

Rohlf FJ, and Marcus LF (1993) A revolution in morphometrics. Trends Ecol. Evol. *8:*129–132.

Rohlf FJ, and Slice D (1990) Methods for comparison of sets of landmarks. Syst. Zool. *39:*40–59.