A Revolution in Morphometrics

F. James Rohlf and Leslie F. Marcus

We are now in the midst of a revolution in morphometric methodology. The new approaches are more effective in capturing information about the shape of an organism and result in more powerful statistical procedures for testing for differences in shape. They are also more effective in enabling a researcher to visualize differences in shape and in suggesting simple traditional measurements that could be used in future studies. In this review we emphasize applications to exploratory studies in taxonomy and evolution.

The field of morphometrics is concerned with methods for the description and statistical analysis of shape variation within and among samples of organisms and of the analysis of shape change as a result of growth, experimental treatment or evolution. Morphometric methods are needed whenever one needs to describe and to compare shapes of organisms or of particular structures. The samples may represent geographic localities, developmental stages, genetic effects, environmental effects, etc.

Traditional morphometrics

The approach now referred to as traditional morphometrics[1,2] or multivariate morphometrics[3] is only a few decades old. It is characterized by the application of multivariate statistical methods to sets of variables. The variables usually correspond to various measured distances on an organism. The measurements are usually lengths and widths of structures and distances between certain landmarks. Sometimes angles and ratios are used. Applications have frequently been concerned with allometry (change in shape as a function of size) and size correction (to enable the study of shape differences among samples of organisms adjusted to a common size). The results are mostly expressed numerically and graphically in terms of linear combinations of the measured variables. Examples of the techniques used are principal component analysis, canonical variate analysis, discriminant functions and generalized distances[1,4].

It is not possible to recover the shape of the original form from the usual data matrices of distance measurements, even as an abstract representation. The overall form is neither really archived nor used in the analysis. An investigator may know, for example, that several measurements share a common landmark, but this information is not used in the multivariate analyses. As a result the analyses cannot be expected to be as powerful as they could be if that information were taken into account.

The new morphometrics

The following points characterize the new approach:

(1) Data are recorded to capture the geometry of the structure being studied. This is in the form of two-dimensional (2-D) or three-dimensional (3-D) coordinates of morphological landmark points. The coordinates are much more useful than traditional measurements, and, of course, the usual distance measurements can be computed from the coordinates. One can check their adequacy in covering the structures of interest by a visual evaluation of a graphical display of the landmarks. Emphasis is given to recording homologous landmarks, since this allows a more complete biological interpretation of the results. Rather than just reporting that the shape has changed, one can report that certain structures have moved relative to others. When it is not possible to find such landmarks, one is forced to use pseudo-landmarks – points located at ends of structures, points at extremes of curvature of the outline of a structure, or arbitrary points along an outline. If one is interested in the overall outline or surface of a structure (or of just parts of a structure between landmarks in 2-D or a surface in 3-D), then this can be captured by a sequence of digitized points along the outline or over a surface. Such approaches have been used for many years.

(2) The geometrical relationships among the landmarks are not inherent in the raw coordinates themselves. The relationship among the points is captured by fitting an appropriate function to them in 2- or 3-D. The estimates of the parameters of the fitted function can then be used as variables in standard univariate and multivariate statistical analyses.

For landmark data, Bookstein[5–7] has recently proposed the use of thin-plate spline functions to fit the differences in the positions of landmarks in one organism relative to their positions in another. The term 'thin-plate spline' comes from a model of the deformation of a thin metal sheet. The use of this spline does not imply that biological tissue behaves like metal sheets (just as the use of Fourier analysis does not imply that outlines behave as vibrating strings). It is simply a convenient function that is able to express the differences in two configurations of landmarks as a continuous deformation. One can also transform arbitrary grid lines, outline contours and any other points describing the image that are not used in the computation of the transformation. These properties enable the automatic construction of transformation grids such as those associated with D'Arcy Thompson[8].

An especially important feature of this transformation is that one can easily separate those changes due to differences in size, translation, rotation and uniform shape change (affine transformation) and those describing purely inhomogeneous changes (non-affine or local deformations). The purely inhomogeneous part can be further split into principal warps – geometrically orthogonal components corresponding to deformations at different geometric scales (analogous to different powers in polynomial curve fitting or harmonics in Fourier analysis). A recent study suggests that the relative contributions of these components can be used as taxonomic characters[9]. Figure 1 shows an example of their use as variables in a multivariate analysis of 13 cranial landmarks in Talpidae (Mammalia, Insectivora)[10]. Figure 2 shows the landmarks used and an example of how one can visualize variation along a canonical axis by the use of thin-plate splines.

The coordinates of points around an outline (or some simple transformation of them) are usually approximated by a weighted sum

James Rohlf is at the Dept of Ecology and Evolution, State University of New York, Stony Brook, NY 11794-5245, USA; Leslie Marcus is at the Dept of Biology, Queens College of CUNY, Flushing, NY 11367, USA.

of sine and cosine terms corresponding to various types of Fourier analysis[11,12]. Elliptic Fourier analysis[13] is now commonly used for complex shapes. Those studies in which only the harmonic amplitudes (the sum of the squares of the coefficients of the sine and cosine terms) are used should not be considered as examples of geometric morphometrics since the ability to capture and reconstruct the original outlines is lost when the information on phase angle (the starting points for each sine and cosine function) is discarded[14].

(3) Rather than having to decide beforehand exactly which variables should be measured, the analyses are designed to indicate directions of maximum variation and hence may suggest which conventional variables one should emphasize in verbal descriptions of the results. For example, Fig. 2 suggests that a character such as the ratio of distances between landmarks 11, 12 and 13 would be useful. Furthermore, this guides the choice of variables to measure and use in future studies to efficiently capture the most important patterns of variation.

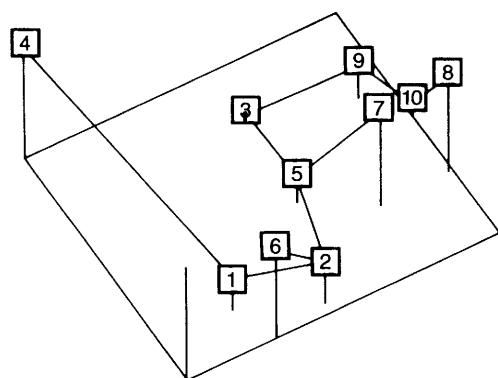(4) Displays of the results of the analyses are emphasized, using

Fig. 1. Three-dimensional ordination of ten samples of moles based on a canonical variate analysis of partial warp scores to show differences in nonuniform shape (79% of the among-sample relative to within-sample variance is accounted for). A minimum spanning tree based on generalized distances has been superimposed to show near neighbor relationships. Original data are coordinates of 13 landmarks from the skull of seven fossorial species from the family Talpidae[10]. Male and female samples are pooled since a MANOVA showed no significant differences between sexes nor of an interaction between sex and sample (P>0.9). Sample codes: 1 and 2, *Talpa romana*; 3, *Mogera latouchei*; 4, *Parascalops brewerii*; 5, *T. occidentalis*; 6, *T. stankovicki*; 7, *T. caeca*; 8–10, *T. europaea*. Computations and plot produced by TPSRW and NTSYSpc programs.

differences or changes that can be shown on pictorial representations of the organisms studied. The displays are expressed in terms of distances in the 2- or 3-D space of the organism, rather than distances in multidimensional vector spaces (although such distances are used in the computations and the tests of significance). It is easier to visualize and interpret the results from these displays than from tables of numerical coefficients.

There are now several alternative approaches that fit our rubric 'new morphometrics'. All of these can analyse shape variation in a sample of organisms or compare shape differences among two or more samples. Below, we summarize some of the characteristics of these methods and speculate on the future direction of the field. We expect and look forward to continued vigorous discussions on the relative merits of the different approaches.

Relative warp analysis

Perhaps the most exciting new method is relative warp analysis. This analysis finds the thin-plate spline transformations that map a reference configuration of landmarks (usually the mean of a sample) onto each specimen. The parameters of these transformations can be used as variables in conventional multivariate statistical analyses (principal component analysis, canonical vector analysis, etc.). This is because the new variables are simply weighted linear combinations of the deviations of the specimens from the reference. Under the null hypothesis of no shape variation, the scatter of each specimen's landmark positions should deviate like digitizing error from the position in the reference. Thus, these new variables will have multivariate normal distributions if the deviations at each landmark are normally distributed.

An important aspect of the use of such variables is that one can express the results of statistical analyses in terms of displays of thin-plate splines and show, as in Fig. 2, the effect of the first canonical variate as a deformation of the average specimen – rather than having to examine lists of numerical coefficients as in a traditional

morphometric study. In Bookstein's original method, the principal warps corresponding to large-scale changes are given greater weights, which seems appropriate in growth studies[5]. These weights may be adjusted by a single parameter. Rohlf[15], based on a suggestion by Bookstein[7], has investigated setting this parameter to zero for taxonomic and other exploratory studies, where one has no *a priori* expectation that important deformations will occur at particular scales.

Figure 3 shows an example of an analysis of allometry. Both uniform and nonuniform components of shape were regressed on size and then visualized by transforming the reference configuration of landmarks (the average rat) into that predicted for the smallest and the largest rats (which match the observed very well since $R^2 = 0.94$). One can see that most of the shape change during growth is uniform.

Superimposition methods

Superimposition methods (also called Procrustes methods) are based on the simple idea of overlaying the images of two or more specimens so that their homologous landmarks match as closely as possible according to an optimality criterion. One then reports any difference in shape in terms of residuals, usually shown graphically as displacement vectors at each landmark. Several different criteria for fitting are available. The resistant fit[16–18] approach usually yields a more satisfactory alignment when shape change is mostly limited to a small proportion of the landmarks.

There have also been important developments in the statistical analysis of the properties of superimposition methods[19]. One can also align the specimens with differences due to uniform shape change (affine transformations) taken into consideration. This technique is effective in showing relative levels of variation at different landmarks. On the other hand, it is difficult to show covariation in displacements at different landmarks, which is one of the strong points of relative warp analysis.

Euclidean distance matrix analysis

Lele[20] has proposed Euclidean distance matrix analysis, which is

based on an examination of ratios of distances between all pairs of landmarks in two specimens. If they are all the same then the two organisms must have the same shape and the constant ratio gives the difference in size. While the technique can show the existence of statistically significant shape changes, the results are expressed in the form of lists of distances that are unusually larger or smaller in one specimen than in another. These lists are not very easy to visualize in terms of changes in shape of organisms. The fact that the method is coordinate-free, and therefore invariant to translation and rotation since it operates only on distance, has been given great emphasis[21]. However, statistics based on thin-plate splines, Procrustes analyses, shape coordinates, etc., all have this same property, so the supposed advantage of coordinate-free methods is unclear. The concern seems to be that there is a possibility of bias due to the use of any particular coordinate system – even if it is used only for displaying the results of a statistical analysis.

Finite element scaling analysis

Finite element scaling analysis[22,23] is another useful method. In this technique the landmarks are connected so as to form small closed regions (often triangles in 2-D and tetrahedra in 3-D). Each region is then compared to its corresponding region in a second organism by computing the affine transformations that map one into the other. The results are usually displayed in terms of strain crosses that indicate the directions of the principal strains and their magnitudes within each element, or are averaged in some way and then shown as strains at each landmark. This shows how two organisms differ in shape. However, a problem with this approach is that the division of the organism into regions is not unique. While different divisions are mathematically equivalent for showing overall differences in shape, the strain crosses can be rather different. This means that one can obtain different values for summary statistics over the regions, such that the average

angle of the principal strain or the variance in its angle depends upon how the organism is divided into elements. Thus, it is difficult to recognize global effects such as uniform shape change. Bookstein[7] describes these and other problems that limit the usefulness of this technique for the statistical analysis of shape.

Problems to be solved

Traditional morphometric methods employing multivariate analyses of selected distance measurements are not 'wrong'. There is nothing wrong with multivariate methodologies as such (in fact they are essential for the statistical analyses of the variables generated by the new methods); the approach is just not nearly as
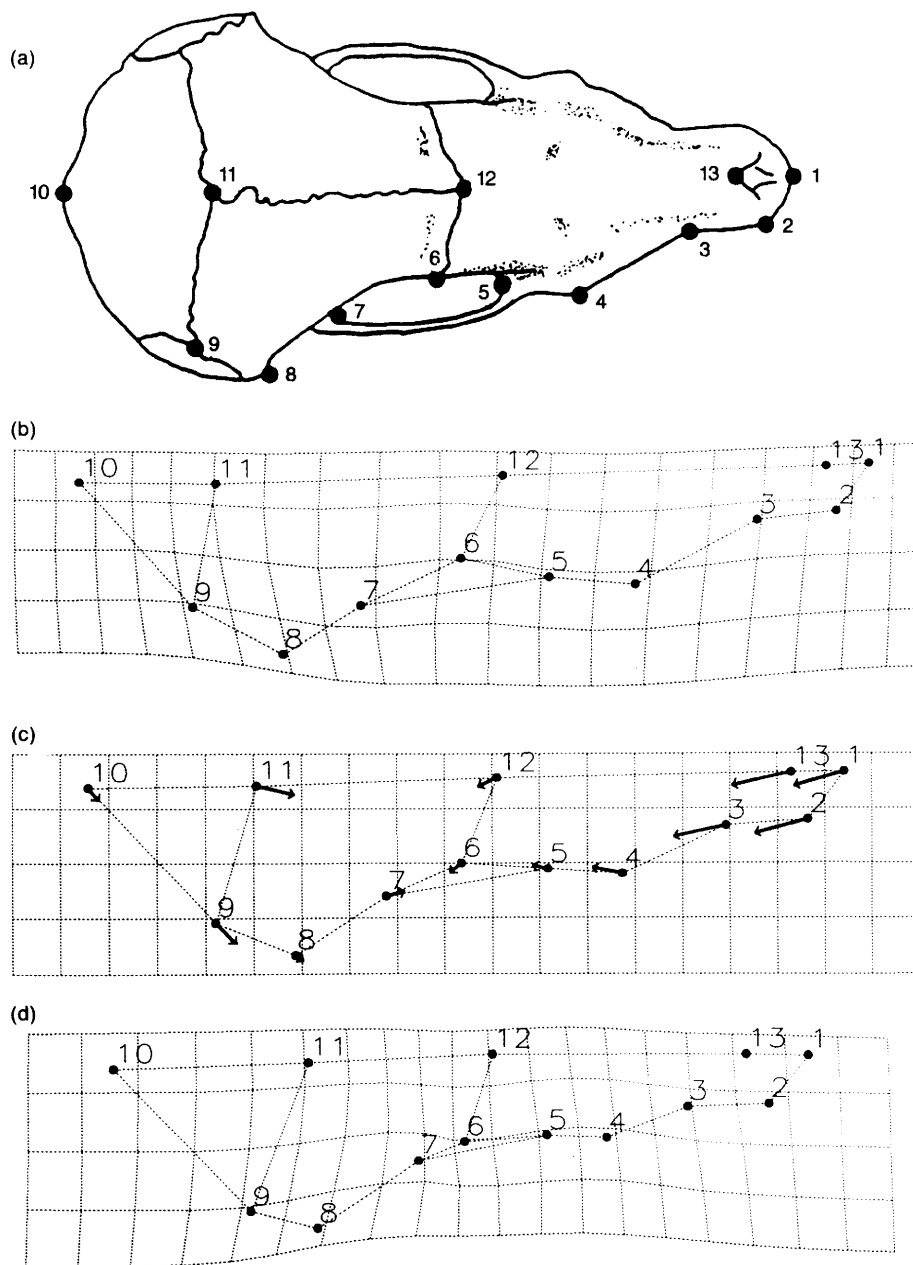
Fig. 2. Visualization of the component of nonuniform shape change corresponding to the first canonical variate shown in Fig. 1. (a) shows the locations of the landmarks on a dorsal view of the skull. (b) and (d) correspond to an extrapolation beyond the left and right ends of the first canonical vector axis (this exaggeration was necessary in order to make the differences visible). (c) shows the average positions of the landmarks with vectors pointing in the direction of positive changes along the axis. Plots produced using the TPSREGR program.

powerful as it could be, since it is not able to take into account the geometrical relationships among the measurements – that pairs of measurements were made from a common landmark, or that a set of three measurements correspond to a triangle of landmarks on the organism.

There are limitations and problems to be solved in the new morphometrics:

(1) There is a need for an appropriate distance measure for summarizing differences between organisms, and for evaluating characters so that phenetic or cladistic taxonomic relationships can be estimated. Sneath and Sokal[24] point out that the available distances have standard errors that are a function of the number of variables analysed – not just of sample size as for traditional statistics. Bookstein[7] is much less optimistic about obtaining sufficiently large numbers of landmarks since their variation is not independent. Another problem is that there does not seem to be a unique way of defining morphometric distance and thus there is a degree of arbitrariness in any such results reported.

(2) Different interpolating functions can yield different numerical results and pictorial displays since they give different weight to data points. Superimposition analysis[17,18] uses an identity function which weights all points equally – but that is also an arbitrary choice. It is important to point out that many methods agree on the nature of the differences among a set of shapes, but not with respect to the relative amount of difference between different shapes.

(3) Much more work is needed on methods to capture surfaces and the texture of both outlines and surfaces – especially in combination with landmark data.

(4) An important potential area of application is in taxonomy and phylogenetic inference, where characters are currently coded in roughly ordered or unordered categories. But the problem is that there are different metrics for measuring the distance between different forms and it is unclear how to choose among them or how to generate the kinds of characters desired for phylogenetic inference methods. The statistics of shape distances (e.g. Procrustes distance) is a surprisingly complex subject[25,26]. One cannot assume they can safely be used as measures of taxonomic distance. Theoretical research in this area is very active at present.
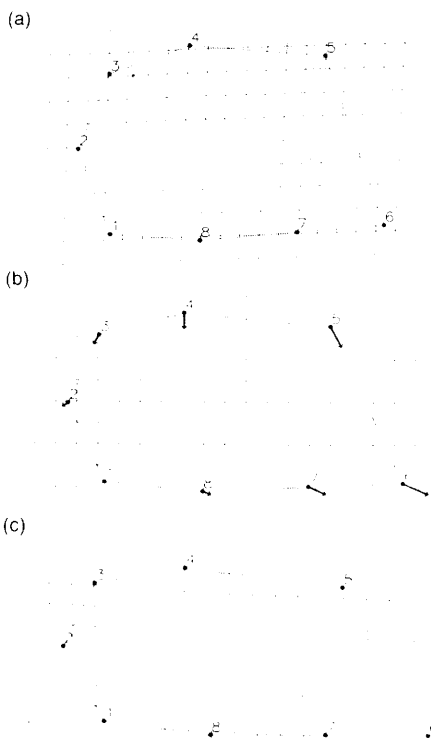
As in any scientific revolution, one cannot expect unanimity of opinion as to whose method is most effective. There is, however, a consensus among most workers that it is important to take geometry into account[21].

(a)

(b)

(c)

Fig. 3. Regression of both uniform and nonuniform components of shape on size. Rat calvarial growth data (ages 7–150 days, digitized by M. Moss from roentgenograms by H. Vilmann, published and illustrated Bookstein[7]). (a), (b) and (c) correspond to predicted shapes for rats at sizes corresponding to the youngest, the average and the oldest rats, respectively. The vectors in (b) show the direction of shape change from the average to the largest rats. Computations and plots produced using the TPSREGR program.

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