# 4

# Methodological Issues in the Description of Forms

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## 4.1 Introduction

There are many situations in which biologists wish to compare morphology. These include studies of normal and pathological variation, growth, and evolution. Each presents its own morphometric challenges. In the study of the shapes of populations of cells, for example, there may be a lack of unequivocally definable equivalent landmarks on which to base comparative measurements. In this case the investigator seeks morphometric methods that show little dependence on landmark identification. In contrast, studies of variation in skull shape might be based on landmarks that are equivalent between individuals in an evolutionary, developmental, or functional sense. Thus, comparative data may be based upon the relative locations of these landmarks rather than upon the shape or curvature of the outline itself.

In undertaking a morphometric study, one chooses, then, between methods that describe forms in terms of landmarks or interlandmark distances, and those that describe form with little or no reference to landmarks. There are, however, a large number of other issues involved; these are, to a degree, dependent on the problem at hand and the particular questions being addressed. For instance, in choosing landmarks, questions may arise concerning homology, the sampling of form, and the types of measurements to be taken. Many alternative strategies are available and choosing between them requires some knowledge, not only of the biology, but also of the morphometric issues concerning each approach.

Modern desktop computers, digitizing tablets, frame grabbers, and 3-D digitizing equipment allow many "new" types of data to be gathered. Therefore, it becomes important to consider how each of these data types may be compared between specimens, the limitations of each possible approach, and the extent to which the use of different types of data might affect the outcomes of morphometric investigations.

This chapter will review a number of approaches to the quantitative description of form, consider the differences between landmark-based and landmark-independent approaches, and examine the issue of homology in relation to landmarks and to boundary representations of forms with few or no landmarks. A large part of this review will be devoted to Fourier analysis, since this is the theme of this book. It is hoped that the discussion of Fourier analysis against a background of other morphometric approaches will be useful in providing context and perspective.

Landmark-dependent approaches are considered first, and methods which are less dependent on landmarks are discussed later. This is to some extent an artificial classification since landmark identification plays a part in the majority of strategies that can be applied to form description. A brief description of each technique is followed by a consideration of the theoretical and practical problems associated with it. This chapter will provide a critical overview of currently available tools for form description.

## 4.2 Landmark-based approaches to form analysis

### *4.2.1 Interlandmark distances*

A classical application of landmarks to the study of biological forms comes from craniometry, in which landmarks may be of two kinds; anatomical (e.g., tips of prominences, sutural junctions) or extremal (e.g., the most dorsal or superior point). Examples of such landmarks, and measurements taken between them, are given by Brothwell and Trevor (1964) and Martin (1928). Interlandmark distances, and indices constructed from them, may be used in phenetics.[1] Classically, statistical analysis of interlandmark distances proceeds through univariate and multivariate analysis (Sneath and Sokal, 1973). Such distances, however, can be gap-coded (i.e., gaps in the statistical distribution of metrical characters between Operational Taxonomic Units (OTUs) are identified and coded as different character states) and used in cladistic[2] analyses.

#### *4.2.1.1 Euclidean distance matrix analysis*

Euclidean Distance Matrix Analysis (EDMA) refers to the comparison of forms based on matrices of Euclidean distances between equivalent landmarks (Lele and Richtsmeier, 1991). This procedure extends the classical approach to the com-

[1] Editor's note: Phenetics refers to a classification based on morphological similarity, without consideration of evolutionary relationships. An Operational Taxonomic Unit (OTU) has been defined as a collection of objects (specimens in biology), each member of which is described with a set of measurements, which becomes a dataset. Thus, OTUs are the lowest-ranking taxa in studies of variation. A set of OTUs is built in order to arrange the specimens in a hierarchical manner (see Sneath and Sokal, 1973).

[2] Editor's note: Cladistics implies an evolutionary classification based on the succession of splittings through which an organism has passed during its divergence from an ancestor.

parison of OTUs through the comparison of interlandmark distances. A series of equivalent landmarks is identified on each OTU to be compared. The distances between these landmarks are determined, and for each OTU a matrix of these interlandmark distances is produced (a form matrix). The geometric relations of all landmarks are preserved in the form matrix, since it contains all interlandmark distances. Form difference matrices (containing the ratios of corresponding distances between OTUs) are then calculated between single OTUs or between the average form matrices of populations of OTUs. The magnitudes of the ratios in the form distance matrix can be used to assess differences and to identify landmarks whose locations vary between forms. Statistically, the behavior of these ratios is complicated, but statistical inference can be approached through bootstrapping (Lele and Richtsmeier, 1991).

#### *4.2.1.2 Criticisms of the use of interlandmark distances*

The use of interlandmark distances or indices as the basis of morphological description has been criticized on several counts. First, the way in which interlandmark distances are commonly collected is such that no attempt is made to systematically describe the relative locations of landmarks, one to another. The result is a collection of measurements that may fail to describe the full 3-D disposition of landmarks as well as over-sample some regions at the expense of others. This is a criticism of study design rather than of the use of interlandmark distances *per se*; it does not apply when interlandmark distances are collected systematically, as in EDMA. Second, as Bookstein (1978) has pointed out, extremal landmarks (i.e., ones that occupy extreme limits of objects with respect to a particular line or plane; e.g., the most dorsal, most superior) are entirely orientation dependent. It is wise to avoid such landmarks. Third, no information relating to the curvature of form between landmarks is preserved; this criticism applies not only to interlandmark distances but to all landmark-based studies, and this issue will be considered later in this chapter.

A further criticism relates to the difficulties of visualization of shape differences. Typically, univariate and multivariate analyses are undertaken to investigate patterns of morphological variation. The results of such studies are often presented as plots of OTUs on canonical axes or principal components (PCs), or as a matrix of inter-OTU distances. These approaches lead to precise mathematical descriptions of patterns of covariance between (often disconnected) variables, but they do not, in themselves, produce a simple, readily interpretable, spatially integrated map of the size and shape differences under study. As such, their contribution to a ready understanding of the complex differences in size and shape between OTUs is limited.

However, it is possible to work backwards from eigenvectors to reconstruct the interlandmark distances of an OTU with any given set of PC scores. If these dis-

tances have been carefully measured, it is then possible to reconstruct the original landmarks in arbitrary registration and so to proceed to the visualization techniques that utilize coordinates, as outlined later in this chapter.

One advantage of the study of interlandmark distances over the study of landmark coordinates is that, in contrast to coordinates, these distances are independent of reference frame and registration (the way in which coordinates from different OTUs are "superimposed" on each other).

### *4.2.2 Co-ordinates and geometry*

Use has also been made of Cartesian coordinate data in phenetic studies (e.g., Creel and Preuschoft, 1971; Corruccini, 1988). Rather than through interlandmark distances, specimens are described in terms of the $x$, $y$, and possibly $z$, coordinates of a set of landmarks. Coordinate data allow a description of morphology in which landmarks can be readily related one to another. As such, the description of form is complete (in the sense that all landmark locations are fully defined). Coordinates, like linear distances, are amenable to multivariate statistical analysis. Before carrying out such analyses, however, it is necessary to register ("superimpose") coordinates from different OTUs within the same reference frame.

With coordinate data, the particular patterns of between-OTU variation represented by a particular principal component or canonical axis will be entirely dependent on the way in which the OTUs have been registered (scaled, reflected, rotated, and translated to "register" or "superimpose" the data within the same reference frame) with respect to each other (see Bookstein, 1978). Thus, the perceived displacement of any particular landmark from one shape to another depends upon this registration. Different registrations will produce different impressions of the shape transformations, and regions close to registration points (if registration is undertaken using such points) will appear to change less than those more distant.

The methods of Procrustes analysis (reviewed by Rohlf, 1990a; Goodall, 1991) register forms by minimizing the "fit" (e.g., the mean square distance between landmarks on each OTU). Differences between objects after Procrustes fitting can be expressed in terms of Procrustes distances. Each OTU is represented as a point in Kendall's shape space, which is isometric with a sphere of radius 0.5 when an object is defined in terms of three landmarks in two dimensions (Kendall, 1984). When more landmarks or dimensions are used the shape space becomes more complicated. The Procrustes distance coefficient between any two OTUs is non-Euclidean and can be thought of as the closest great circle distance between them (Kendall, 1984). For practical purposes, statistical analyses (such as principal components) of Procrustes-fitted data are generally carried out in the tangent plane to

shape space (which, as long as variations are small, adequately approximates the curving surface of Kendall's shape space). In this case, the distances between OTUs are treated as if they are Euclidean, and normal statistical assumptions are made.

It is worth reiterating that statistical studies of coordinate data rely on registration and scaling, whereas studies of interlandmark distances are independent of registration. This difference in approach allows the investigator to confirm or modify the conclusions drawn by using one set of techniques (e.g., Procrustes analysis) in the light of studies using the other (e.g., EDMA). It can be argued that such confirmatory analyses should form an important part of all morphometric studies since all techniques suffer to a certain extent from their own peculiar constraints and limitations.

### *4.2.3 Visualization and graphical representation of shape differences using landmark data*

#### *4.2.3.1 Transformation grids*

In contrast to multivariate analysis, an alternative strategy for comparing coordinate representations of form is to describe "shape changes" or "shape differences" (both are commonly used) as a deformation that smoothly rearranges the configuration of landmarks as a whole. The best-known representation of such a deformation is in the form of a "transformation grid" (Thompson, 1917) in which the differences in morphology between OTUs are described through distortions of a rectangular grid.

There have been several attempts to produce mathematically defined, reproducible visualizations of shape transformations. For practical reasons these are commonly restricted to 2-D ($x$ and $y$ coordinates). Earlier attempts (e.g., De Coster, 1939; Moorees and Lebret, 1962) tended to suffer from problems associated with the registration of one form on another, and with the extrapolation of the shape differences indicated by differences in landmark location to the spaces between them (Bookstein, 1978; Sneath, 1967).

One approach (Sneath, 1967) is to register landmark configurations from two forms using least squares and to model the displacements of landmarks between the first (base form) and second (target form) in both the $x$ and $y$ directions using pairs (one for $x$ and one for $y$) of linear, quadratic, and cubic power surfaces (trend analysis). These surfaces are used to displace the nodes of a square grid in both $x$ and $y$ (see below; thin plate spline) producing a distorted (transformation) grid reminiscent of those hand drawn by Thompson (1917).

A very similar approach was introduced to morphometrics by Bookstein (1989). A pair of surfaces is defined as in Figure 4.1a. The $x$ and $y$ coordinates of the base form are represented in $x$ and $y$, while the $x$ coordinate, and then the $y$ co-

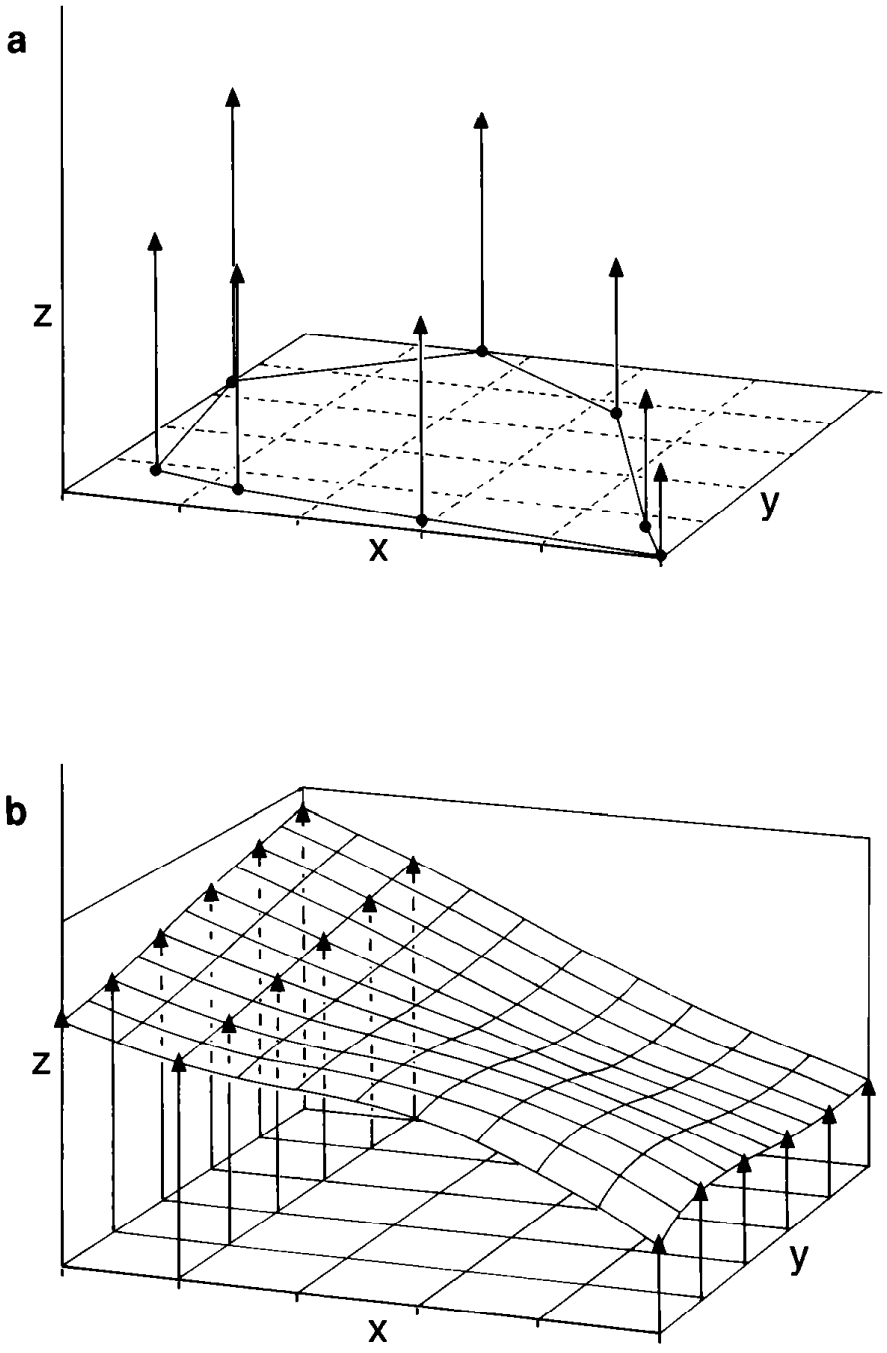


Fig. 4.1 The construction of a Cartesian transformation grid using thin plate splines. In (a) the coordinates of landmarks taken from an ape skull are plotted in $x$ and $y$. The $z$ axis represents the $x$ coordinates of corresponding landmarks on the target form (not shown). In (b) a thin plate spline is used to fit a surface to the $z$ coordinates shown in (a). A grid drawn in the coordinate system of the original form is shown in the plane of the $x$ and $y$ axes. Arrows connecting the nodes of the grid to the surface indicate the degree to which the nodes of the square starting grid are displaced in $x$ in the transformation grid. For clarity, only some of the arrows are shown connecting the nodes with the surface. Displacements in $y$ are treated in the same way; thus, a pair of thin plate splines (one in $x$ and one in $y$) are used to draw a Cartesian transformation grid.

ordinate, of the target form are each plotted in $z$. In this way two surfaces are defined (i.e., two diagrams like Figure 4.1a); one illustrating the changes in $x$ and the other in $y$, between the base and the target forms. These surfaces are then fitted by a pair of thin plate splines (one for $x$ and one for $y$). The thin plate spline is a sensible choice since it minimizes the "bending energy" required to take the first form into the second. The pair of thin plate splines sends points in the first form to points in the second in such a way that landmarks are mapped exactly to landmarks and other points are mapped smoothly in between.

A Cartesian transformation grid can be constructed using the pair of thin plate splines. The nodes of a square grid in the coordinate system of the base form are repositioned first in $x$, by displacing them in $x$ according to the height ($z$) of the surface defined by the thin plate spline (as in Figure 4.1b) and then in $y$, according to the surface defined by the thin plate spline for $y$. Thus, by applying the same pair of splines to displace the nodes of a square grid, they are shifted in $x$ and $y$ and the resultant deformed grid is known as a transformation grid (Figure 4.2a) that is visually very close to those derived by Thompson (1917). The perceived mapping does not depend on the particular coordinate systems of the figures, making this is a registration-free method for visualizing the shape differences between two OTUs.

This approach can be criticized since the interpretation of the observed transformation may owe as much to the starting grid geometry as it does to the biological reality of the shape change (Bookstein, 1978:94). Note also that the interpolant function between the grids (the thin plate spline) is not the only possible choice. That the thin plate spline minimizes "bending energy" is, however, intuitively appealing.

Besides producing a transformation grid, the method of thin plate splines can also be extended to examine the affine and non-affine components of shape difference and to explore variation among populations of OTUs. Thus, it is possible to "decompose" shape differences between Procrustes- registered OTUs into their affine and non-affine components (the partial warps) and to apply principal components to OTU partial warps in order to investigate shape variation among OTUs (relative warps). These refinements are beyond the scope of this chapter and the interested reader is referred to Rohlf and Bookstein (1990) and Reyment (1991).

#### *4.2.3.2 Finite element analysis*

In biology, finite element analysis, originally devised for describing the effects of stresses on engineering materials, has been adapted to the task of characterizing shape changes. In this approach, shape differences are described in terms of the directions and magnitudes of the principal strains in the transformation of one form to another. These methods are also "registration free" since they provide in-

formation about the "stretching" of elements rather than the movement of landmarks relative to the coordinate system.

"Homogeneous" finite element methods (e.g., Bookstein, 1978; Moss et al., 1987) work under the assumption that shape changes (strains) are uniformly distributed throughout each element (are homogeneous). This is not necessarily true of biological forms in which an element may span diverse tissues; consequently, this simplifying assumption of homogeneity may have an effect on the biological interpretation of results. Elements may be of different shapes but the simplest possible ones are triangles whose apexes are equivalent landmarks between two forms. The shape transformation between homologous elements can be described by the major and minor axes of the ellipse obtained by deforming one triangle, together with its inscribed circle, into the other (Bookstein, 1978). The directions of these axes (principal strains) indicate the directions of maximum and minimum shape change, and their magnitudes indicate the relative measures of these changes (Figure 4.2b). It is noteworthy that from the biometrician's perspective, the principal strains are invariant to element registration and that they relate the uniform deformation of each element in the base form to the equivalent element in the target form.

"Nonhomogeneous" finite element methods, on the other hand, do not make the assumption of homogeneity; they use more complex elements (i.e., cubes rather than triangles), and allow the computation of local deformations around landmarks. The Finite Element Scaling Method (FESA; e.g., Lew and Lewis, 1977) is nonhomogeneous and has been widely applied in studies of craniofacial growth and sexual dimorphism (Cheverud and Richtsmeier, 1986; Richtsmeier, 1986; 1989).

Although the selection of landmarks and finite elements is largely arbitrary, the interpretation of shape changes in particular anatomical regions may differ according to element design (Zienkiewicz, 1971; Cheverud and Richtsmeier, 1986). For instance, thin triangles will tend to "amplify" small shape changes. In order to minimize these effects, O'Higgins and Dryden (1993) have recently proposed the use of the Delauney triangulation (see Green and Sibson, 1977).

#### *4.2.3.3 Biorthogonal grids*

Bookstein's (1978) solution to the problem of element design is to compute the deformation of many elements interpolated over the interior of the forms under study and to derive a smooth map of shape changes over these elements. He calls this method "biorthogonal grids." O'Higgins and Dryden (1993) constructed such grids using the thin plate spline as the interpolant. A small triangle was transformed between OTUs using the pair of thin plate splines, and its principal strains were calculated. Moving off a short distance in the direction of the first principal

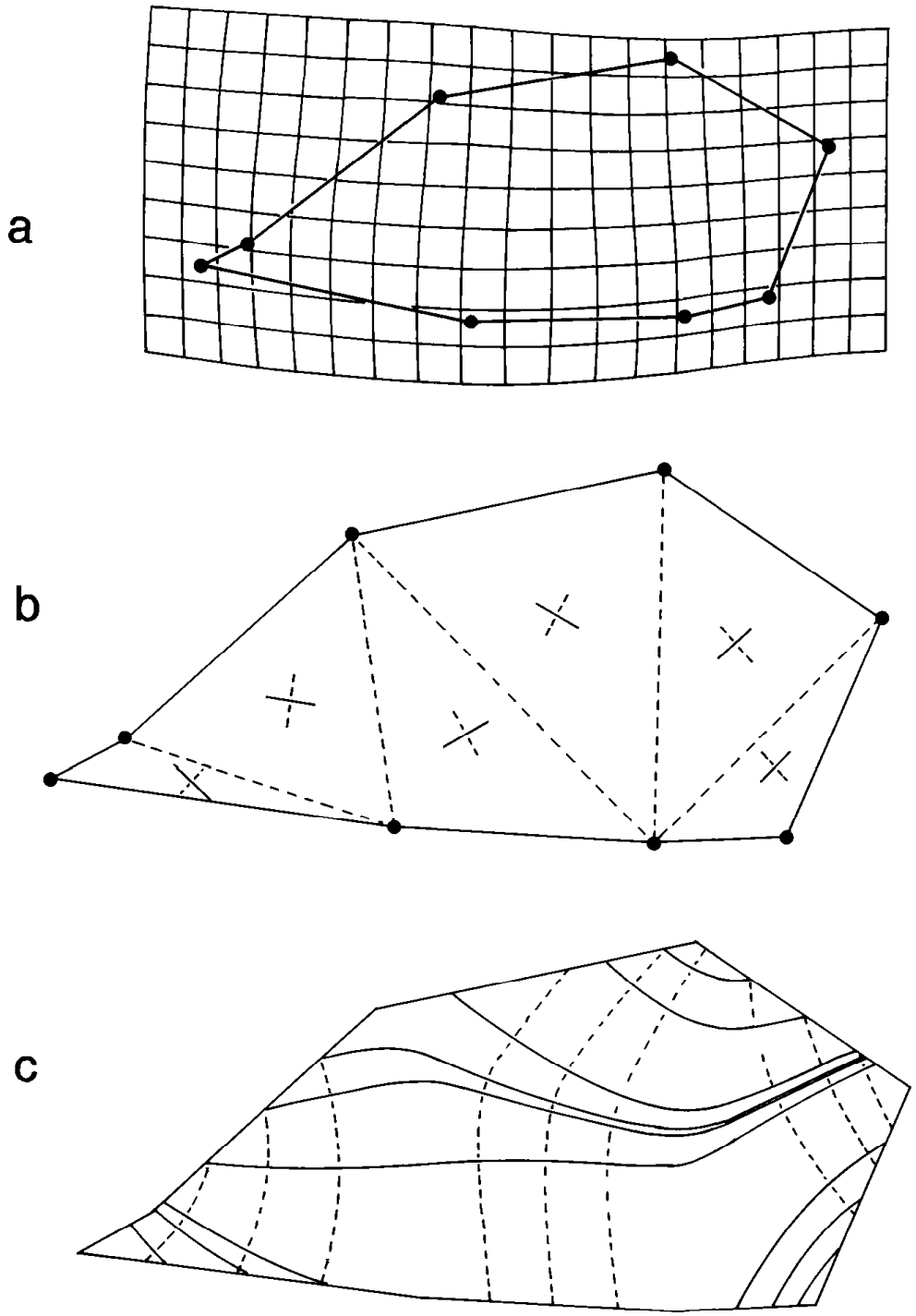


Fig. 4.2 The transformation from female to male gorilla crania: (a) Cartesian transformation grid; (b) finite elements analysis; the crosses indicate the directions of the major (———) and minor (——) principal strains; the limbs of the crosses are drawn to scale indicating the magnitudes of these strains; (c) biorthogonal grid; curves showing major (———) and minor (——) principal strains. Magnitudes are omitted for clarity.

strain, a second triangle was transformed and its strains calculated. The strains were smoothly joined in a diagram and the process was repeated for the second (minor) principal strain. The result is a biorthogonal grid (see Figure 4.2c).

Using this procedure, the vagaries of element design are largely side-stepped since the whole interior of the forms is taken to smoothly deform between them and the matching of internal and boundary "homologies" is taken to conform to a smooth mapping of the homologous landmarks. Note, however, that the choice of homology mapping function (e.g., the thin plate spline) is not unique.

These approaches (Cartesian transformation grids, finite elements, and biorthogonal grids) for the visualization and geometric study of landmark data offer interesting possibilities for the spatially integrated, graphical analysis of form differences. All of these methods should be employed, however, with due regard to their limitations. Cartesian transformation grids derived using thin plate splines (Bookstein, 1989) suffer from a potential problem in that starting grid geometry may influence the interpretation of shape transformation (Bookstein, 1978). Additionally, it should be noted that a different interpolant applied to the same data would produce different transformation grids. These considerations aside, Cartesian transformation grids, like finite element methods and biorthogonal grids, result in reproducible, mathematically defined graphical descriptions of shape change independent of registration. All of these approaches may, however, produce different results when different landmarks are selected (a little-studied issue) and element design will influence the outcome of finite element analyses.

It is important to appreciate that these methods do not attempt to model the biological mechanisms of shape transformation (e.g., growth processes); rather, they produce geometric or graphical descriptions of shape differences and transformations. As such they allow conclusions to be drawn about patterns, but not about the mechanisms that underlie the transformation or difference. Moreover, in the study of these patterns, the limitations of each method should be clearly borne in mind, and it would seem useful to consider the results from several approaches simultaneously to provide a check on interpretation.

### *4.2.4 General issues relating to the use of landmark-based data*

Recent developments in methods for the study of landmark-based data offer interesting possibilities for the analysis of form differences. There are, however, a number of issues relating to the use of landmark-based data that deserve consideration.

If landmark data are to provide the basis for comparison of forms, it is important that the landmarks be, in some way, equivalent between OTUs. The term "ho-

mology" is often applied in this context, although the usual usage of this term is to refer to components (organs, parts, characters) of organisms rather than landmarks on these structures.

Pre-Darwinian homology was determined through the correspondence of parts in their relative position (Owen, 1847). A homologue was seen as the same organ in different animals under every variety of form and function. This correspondence was taken to reflect the *Bauplan* of ideal types and does not refer to evolution at all. Homologous structures in this sense were defined on the basis of their correspondences and relations.

After Darwin (1859), homology was reinterpreted as reflecting the structure of ancestral types; evolution was taken as the explanation of sameness. Indeed, to Darwin, homology was another component of the evidence for evolution; homologous structures between species are such because they derive from a common structure in a common ancestor.

Sneath and Sokal (1973) have pointed out the inherent circularity in the identification of homology (using its post-Darwinian definition), since this identification depends on knowledge of evolutionary history, and knowledge of evolutionary history depends on the study of homologous features. In order to develop a pragmatic solution to this "homology problem," Sneath and Sokal (1973:77–82) loosely describe homology as composed of "compositional correspondence" (implying a qualitative resemblance) and "structural correspondence" (referring to a spatial arrangement of parts, as in Owen's definition). This leads them to the approach they term "operational homology." Thus, two characters are operationally homologous if they are "very much alike in general and particular." In so doing, they posit homology based on criteria of similarity, and leave the testing of homologies to repeated finer detail analyses. It was hoped that further analyses will lead to further support or refutation of operational homologies. This operational approach allows the taxonomist to "get started" in a study, since homologies are identified without reference to phylogenetic reconstruction.

Cladistic approaches to phylogenetic reconstruction have pointed to a method for the testing of operational homologies in an evolutionary sense. Eldredge and Cracraft (1980:36) in viewing homology from a cladistic perspective indicate that the solution to the "homology problem" rests with the concept of synapomorphy (the sharing of derived character states). "*Homologous similarities are inferred inherited similarities that define subsets of organisms at some hierarchical level within a universal set of organisms*" (italics in original). Thus, they suggest that the "test" for homology is not similarity, but the congruence of other hypothesized synapomorphies in defining sets of a cladogram. Postulated (operational) homologies are used to construct a cladogram in which (if it is taken to be a true reflection of phylogeny) congruent characters are attributed to synapomorphy or

"true homology." Noncongruent characters (homoplasies) are taken as reflecting convergence and parallelism. One problem with this cladistic approach is that, in practice, a cladistic analysis may result in the identification of several equally parsimonious cladograms. In this case some operational homologies are supported by some analyses and not by others, and so it is not possible to unequivocally confirm evolutionary homology.

This cladistic approach to homology, like the approach of "operational homology" in phenetics, relies on the testing of hypothesized homology through iterative analysis and reanalysis. However, unlike the operational approach, the cladistic testing of homology utilizes hypothesized evolutionary relationships and, as such, is truer to Darwin's, rather than Owen's, definition. This is not to say that an operational approach to the identification of homology is unnecessary for the practice of cladistics; rather, it is "useful, even necessary, to organize data as putative homologies, which are either corroborated or refuted by the cladogram that best fits the data" (Nelson, 1994).

The question arises as to how the concept of evolutionary homology can be applied to landmarks as opposed to characters or organs. Landmarks might be identified as operationally homologous through the identification of corresponding local relations (after Owen). Such operationally homologous landmarks can and do adequately serve as the basis for phenetic studies, and may form the basis of phylogenetic reconstructions using characters derived from sets of landmarks. Thus, individual landmarks serve as the basis for the quantification of characters whose homology might be tested through subsequent phylogenetic analyses. In practice, an operational approach to landmark homology is adopted in the early stages of both phenetic and cladistic analyses, and the problem of Darwinian homology appears to have little impact; operationally homologous landmarks are, in general, readily recognized and adequately serve as the basis for evolutionary studies (of variation, phylogeny, and biogeography) among closely related OTUs.

Another problem relating to landmark equivalence may be encountered in developmental studies. A landmark defined as the junction between three bones in the skull may be taken to be equivalent to a similarly defined landmark on another skull or on the same skull at a different time (e.g., a radiographic study). Developmental variation may, however, result in differences in the derivation of the exact parts of the three defining bones that meet to form the landmark. Likewise, local growth phenomena (e.g., bony remodeling, shifting muscle insertions) influence the derivation of landmarks at tips of prominences or in pits. As such, landmarks that *appear* equivalent in terms of their local relations need not necessarily reflect the locations of homologous material. Thus, in what sense, if any, can such landmarks be considered homologous?

Wagner (1994) has recently addressed this issue. He notes that, despite the fact

that during growth bony material is likely to be completely replaced, structural identity is maintained. This maintenance of identity requires the action of "morphostatic" mechanisms, and although landmarks may not be equivalent in the sense of being located on homologous material, they may be equivalent in terms of the continuity of these morphostatic mechanisms. Developmental equivalence between landmarks may therefore be considered to equate to homology in the sense of "correspondence caused by continuity of information" (van Valen, 1982).

These considerations open up a possible role for landmark-based descriptions of form in understanding ontogenetic processes. In the case of the skull, for instance, displacements of landmarks during ontogeny result from underlying processes such as sutural growth or bony remodeling. Therefore, the combination of morphometric data with data on these processes (e.g., remodeling activity) might offer new insights into the ontogeny of shape transformation. An example of such a study is provided by the work of O'Higgins and Dryden (1992), in which cortical remodeling maps are combined with transformation grids in an attempt to examine the integration of facial bone displacements with cortical remodeling in the mangabey.

The issue of equivalence between landmarks arises in yet another circumstance. For example, in biomechanical studies, functional equivalence may be more important than either evolutionary or developmental homology. In that case, the ontogenetic or phylogenetic equivalence of landmarks on bat and bird wings is of less concern than their functional equivalence in considering the biomechanical basis of flight in these species. In comparing the functional morphology of such structures, landmarks defining, for instance, the extremes of lever arms or the locations of muscle insertions, may be selected on the basis of their functional equivalence.

Besides the problems inherent in identifying equivalent landmarks on OTUs, there are a number of other issues that surround their use. Landmark-based methods leave the form between landmarks unsampled. A problem is presented where no landmarks can be readily identified in a particular anatomical region because of a lack of surface features (e.g., on the smooth bones of the vault). In this case it is possible to interpolate landmarks according to the locations of observed (operationally homologous) landmarks and surface curvature. It is doubtful, however, that such landmarks (more appropriately termed pseudo landmarks) can be considered homologous between OTUs, in either a developmental or evolutionary sense, since their location relates to mathematical, as well as biological, constraints. This consideration is important in studies that may use several constructed landmarks (see Section 4.2).

A further issue arises in the context of different types of landmarks, since, by their nature, some can be readily located (e.g., a sutural junction), whereas oth-

ers can only be approximately identified (e.g., the tip of a prominence). Practical considerations, therefore, play a role in limiting the number of landmarks that can be usefully included in a morphometric study.

Thus, a number of issues surround the choice and use of landmark data as the basis of form description. Despite some of these constraints, landmarks continue to provide an important basis for the analysis of form and offer one important advantage over morphometric approaches that use few or no landmarks; it is possible to investigate variations between OTUs in terms of "homologous" regions. For example, it would be impossible to consider differences in the facial skeleton between two apes unless the location of the face relative to rest of the skull were defined on each, for which, some landmarks defining the locations of skull components are required.

Comparison of the disposition of equivalent landmarks between OTUs is a good way of describing changes in the "homology map." In some studies, however, there may be difficulties in defining equivalent, or indeed, any landmarks (e.g., cell shape, shell shape). In these circumstances, and in the case where comparisons are sought based on the form of outlines between definable landmarks, it may be necessary to turn to alternative morphometric strategies.

### 4.3 Forms with reduced landmark dependency

In certain circumstances the biometrician may be faced with the challenge of examining shape variations between OTUs lacking sufficient readily identifiable, equivalent, landmarks. Examples are found in studies of cells, leaves, insect wings, ostracods, and so on. Alternatively, although landmarks may be readily identified, it is possible that outlines of regions between these landmarks are the focus of study. Such an example is presented by the cranial vault and the curvature of vault bones between landmarks.

In each of these situations the investigator may justifiably seek morphometric strategies that show little or no dependence on landmark identification. Most such work has been restricted to the analysis of 2-D outlines, although many of the available approaches are extensible to 3-D.

The outlines of objects can be traced from photographs, or projected using a digitizing tablet, or derived from video images using readily available image analysis software. Details of such an apparatus are given in Johnson et al., (1985) and an algorithm for tracing the outline of an object is given by Rohlf (1990b). Points are sequentially read from the outline at determined intervals and stored as a series of $x$ and $y$ coordinates. If general measurements such as the perimeter (or enclosed area) are sought, these can be derived directly from raw digitizer output using standard software.

### *4.3.1 Outlines and the enclosed area within them*

There are several landmark-independent methods available for the description of forms in terms of their outlines and the area enclosed within them.

#### *4.3.1.1 Shape factors*

Shape descriptors that are invariant to differences in OTU position and orientation (i.e., non-registration-dependent quantities) are desireable. Examples are area, perimeter, maximum length, and so on. A very simple measure of shape is given by the aspect ratio:

$$F_1 = \frac{\max\ length}{\max\ breadth}, \tag{4.1}$$

where the *max breadth* is 90° to the *max length*. Given the area, $A$, and the perimeter, $P$, of an object, two further quantities can be readily calculated:

$$F_2 = \frac{4\pi A}{P^2}, \tag{4.2}$$

$$F_3 = \frac{P - \sqrt{P^2 - 4\pi A}}{P + \sqrt{P^2 - 4\pi A}}. \tag{4.3}$$

$F_1$ provides a measure of elongation and $F_2$ and $F_3$ are measures of the undulation of the outline relative to a circle (in which $P^2 = 4\pi$ A).

Note that quite different outlines can have similar values for one or more, of these simple measures, and it is advisable to consider all three simultaneously. Examples of their use in biology are studies of cell shape (e.g., Young et al., 1974) and cranial form in the primates (O'Higgins, 1989).

#### *4.3.1.2 Moments*

Sometimes a form may be specified as a collection of interior points. In the case of digitized images, the positions of interior points are specified by the $x$ and $y$ coordinates of pixels, the distribution of which can be used to describe a form.

For a single variable, for example, the $x$ locations of pixels, $m_p$, the $p$th moment of $x$ is given by:

$$m_p = \sum(x^p) = \int_{-\infty}^{\infty} x^p f(x)dx. \tag{4.4}$$

Thus, in a binary image, the zero order moment is the number of pixels enclosed by the outline. The first moment is the mean of $x$, the second its variance, and so on.

For a 2-D distribution along arbitrary axes $x$ and $y$, the moment of the order $(p+q)$ is defined by:

$$m_{pq} = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} x^p y^q f(x,y) dx\, dy. \tag{4.5}$$

This series of moments uniquely describes an image and can, therefore, be used to reconstruct it.

As described above, the moments are dependent on position and orientation and, as such, are of little use in taking measures of shape that will allow comparisons between forms differing in registration. Central moments, which are translation-independent, can be calculated by referring the $x$s and $y$s to the centroid. Hu (1962) has further described 2-D moment invariants, which show size, rotation, and contrast invariance.

Moments represent a "landmark-free" method of form analysis and as such have been applied in situations where landmark identification is difficult (e.g., cell biology; Dunn and Brown, 1986). Rohlf (1990b) comments that there is evidence to indicate that moments perform well in classification but that, in his own experience, there have been problems in their use due to lack of statistical independence between moment invariants, and their sensitivity to rounding errors.

#### *4.3.1.3 Skeletons and medial axis transforms*

Blum (1967) has introduced a very different approach to describing shapes. The shape is defined by a symmetric axis or skeleton that consists of all points within a form that do not have a unique nearest boundary point upon the shape. Associated with each point on the symmetric axis is a width function defining the distance to any of the set of equally distant nearest boundary points. The "grassfire model" (Blum, 1973) makes comprehension easier. The shape is characterized as an area of dry grass. If it is fired simultaneously all around the edge, it will burn toward the interior. If an even rate of burning is assumed, the points at which the fire meets itself comprise the points defining the skeleton; the time taken to reach these points is the function.[3] The skeletal pair (axis and function) exhaustively describe as well as allow for the complete reconstruction of the form, independent of landmarks.

Straney (1990) considers Blum's and alternative approaches to skeletonization including Bookstein's (1979) variant; the line skeleton. Bookstein's differs from the symmetric axis by being composed of line segments rather than line segments

[3] Editor's note: The medial axis transform can also be visualized as a series of concentrically-overlapping circles that touch the outline in a tangential or orthogonal fashion. The skeleton is then defined as the locus of all of the centers of these circles, which are equidistant from all borders of the outline.

and parabolic arcs. Additionally, the width function associated with Bookstein's (1979) line skeleton is not necessarily symmetrically located within the form, and so is not single-valued as that of Blum (1973).

Line skeletons and symmetric axes have been applied to studies of mandibular growth (Bookstein, 1979; Webber and Blum, 1979). The results of these studies indicate that the branch points and angles between skeletal branches may be similar between OTUs. It has therefore been suggested that the branch points may serve as useful landmarks for morphometric analysis. Such an approach, in which branch points are taken as equivalent landmarks between OTUs, was followed by Straney (1990) in a study of the evolution of the baculum of rats.

The use of skeletons of images as the basis for the identification of operationally homologous landmarks, therefore, represents a strategy for the comparison of forms with limited external landmarks. It should be noted, however, that different skeletonization algorithms and subtle differences in outline form may result in skeletons with quite different topologies. Also, the identification of such operational homologies may not be necessarily supported from a developmental or evolutionary perspective.

### *4.3.2 Boundaries*

The three approaches outlined above, shape factors, moments, and skeletons, characterize form in terms of an outline and the area enclosed within it (note: gray scale extensions of these methods are possible with pixel data). More commonly, forms are studied in terms of the boundary alone.

Raw data from a digitizing tablet, or a video digitizer, generally consist of a stream of unevenly spaced $x$ and $y$ coordinates describing the boundary of each OTU. These cannot be directly compared between OTUs because of differences in landmark number and spacing, and in the registration of objects.

#### *4.3.2.1 Pseudo landmarks*

Several strategies exist for comparing OTU outlines. One approach is to divide the outline of each OTU into segments, each of which can be imagined as being delimited by a *pseudo* landmark. Such pseudo landmarks are operationally, but not necessarily biologically, equivalent.

The matching of pseudo landmarks between OTUs is relatively simple if one biologically equivalent landmark can be identified on each OTU, since all others can be counted sequentially from it. If no biologically equivalent landmarks can be identified, then matching can be achieved through Procrustes analysis. Such a maneuver will simultaneously "match" pseudo landmarks and register outlines with respect to each other.

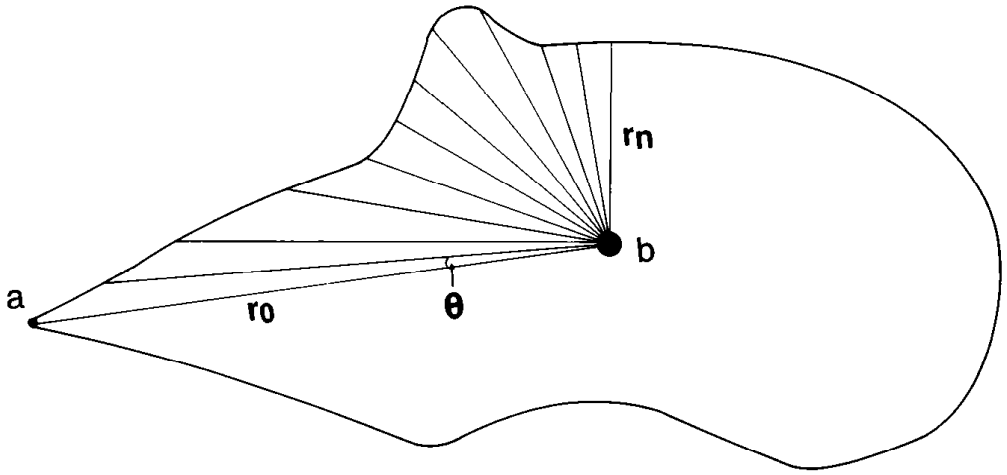


Fig. 4.3 Polar coordinates ($r_0$-$r_n$) of boundary points calculated from starting point (a) and centroid (b).

Pseudo landmarks, once identified, may form the basis of morphometric studies using any of the methods for the study of landmark data outlined earlier in this chapter. It must be borne in mind, however, that pseudo landmarks are unlikely to be equivalent in either an evolutionary or developmental sense.

### *4.3.2.2 Polar coordinates*

Rather than divide the outline into equally spaced segments with pseudo landmarks at each segmental junction, it is possible to transform the outline data into polar coordinates centered on the objects themselves. The boundary of a convex shape is reexpressed in terms of the lengths of radii spaced at equiangular intervals. On each shape an origin (center) is defined for the polar series together with a starting point on the outline from which the series will be deemed to begin (Figure 4.3).

As an example, Yasui (1986) used polar coordinates to study shape variation in Japanese crania. He registered polar representations of outlines with respect to each other by rotating them about their centroid and determining a criterion of best fit.[4] This is similar to the approach of O'Higgins et al., (1986) and is a form of Procrustes superimposition (Goodall and Bose, 1987).

The line connecting the origin (center) to the starting point is the line to which all other polar coordinates are referred. If the origin and starting points are "homologous landmarks" (e.g., Lestrel, 1982), then all comparisons between polar coordinate pairs from outlines are, in effect, referred to these homologies. Again, it is for the investigator to decide whether, in the context of the particular study

[4] Editor's note: This is, in effect, a crosscorrelation procedure (Parnell and Lestrel, 1977).

and questions being addressed, successive polar coordinates can be considered equivalent between OTUs. Additionally, Bookstein (1978) has pointed out that errors in the location of the origin are expressed in the alteration of every value of the radial function in a complex and nonlinear way.[5]

#### *4.3.2.3 Curvatures*

Alternatively, the raw sequence of boundary $x$ and $y$ coordinates derived from a digitizing tablet or image analysis system can be subjected to direct analysis of outline curvature.

In certain circumstances, the investigator may wish to compare open curves (such as might be defined by a series of landmarks occupying some portion of an outline) between OTUs. If these can be expressed such that one coordinate (e.g., $y$) is a single-valued function of the other ($x$), then it is possible to fit a polynomial, cubic spline, or other function, to the sequence of $y$ coordinates (see Rohlf, 1990c).

Given a closed outline, that is, one in which the $y$ coordinates cannot be expressed as a single-valued function of $x$, different strategies need to be considered. One approach is to describe the outline in terms of the tangent angle at the points on the outline. These points may represent operational homologies, they may be spaced equidistantly around an outline, or they may represent the nodes of outlines divided into equal numbers of segments.

In the case in which the points are not operational homologies, tangent angles may be matched between outlines by relating them sequentially to an operationally homologous starting point on each form, or through a Procrustes superimposition.

A comparison of raw tangent angles is, however, sensitive to the orientation of forms, since the tangent angle is measured relative to some arbitrary line. This orientation-dependency can be eliminated by relating all tangent angles to the starting point. This is accomplished by calculating the difference in tangent angle between the start point, $\theta(0)$, and each outline point, $\theta(t)$, that is:

$$\phi(t) = \theta(t) - \theta(0), \tag{4.6}$$

in which $\phi(t)$ expresses the angular change between the tangent at a particular point on the outline and the starting point.

If the outline perimeter is scaled to a length of $2\pi$, and the distance along the outline between the starting point and the current point is $t$, then the angles, measured in radians, can be derived from a new function as:

$$\phi^*(t) = \phi(t) - t, \tag{4.7}$$

[5] Editor's note: This particular problem can be ameliorated by shifting the location of the center of the radii to the centroid and recomputing equiangular intervals between the radii.

which is equal to *0* for every point on the outline of a *circle* (for details see Zahn and Roskies, 1972). Thus, when applied to a plane closed curve, the magnitude and sign of $\phi^*(t)$ is related to the difference between the actual curvature and that of a circle. Note that $\phi^*(t)$ is invariant under translations, rotations, and changes in perimeter.

Rohlf and Archie (1984) applied the $\phi^*(t)$ function to the description of the shape of mosquito wings (Figure 4.4). They calculated $\phi^*(t)$ for 100 equally spaced values of $t$, subjected the $\phi^*(t)$s to Fourier analysis, and used the resulting harmonics as the basis for multivariate phenetic analyses. Lohmann has also used the $\phi^*(t)$ functions, in combination with principal components analysis, as the basis of his method of "eigenshape analysis" (reviewed in Lohmann and Schweizer, 1990).

Young et al., (1974) describe a related measure of shape based on the notion of "bending energy." A 2-D outline made out of a homogeneous material, if allowed to adopt its "free" form would assume the shape of a circle because this is the shape which minimizes the stored energy. To make more convoluted outlines requires the expenditure of work in the form of bending energy. The measure they describe creates equivalence classes of figures with equal "stored energy." The shape is divided into small regions and for each region the curvature, $K_n$, is defined as the change in direction per unit length. The total "bending energy" is given by the sum of $K_n^2$s over the whole outline.

They also describe a simple approach to the calculation of bending energy, directly computed from the chain code (directions from pixel to pixel) of an outline through difference codes (changes in direction from pixel to pixel). The calculated value of bending energy is invariant with respect to position and rotation but is affected by size as well as shape differences. This accords with the intuitive feeling that it takes more energy to bend a short length of material into a circle than a long one.

#### *4.3.2.4 Complexity*

In one sense, bending energy relates to the complexity of an outline, since highly convoluted outlines have larger values of bending energy than less-convoluted outlines of the same length.

Another approach to describing the complexity of form typical of biological materials is based upon the concept of fractal dimension (Mandelbrot, 1983). As a curve in a plane becomes increasingly more convoluted, it fills up more and more of that plane. A simple line has a dimension of one. A complex curve, to some degree, fills a plane, and so it can be considered to have a dimension greater than one; this is its fractal dimension. The fractal dimension can be employed as a summary measure of complexity.

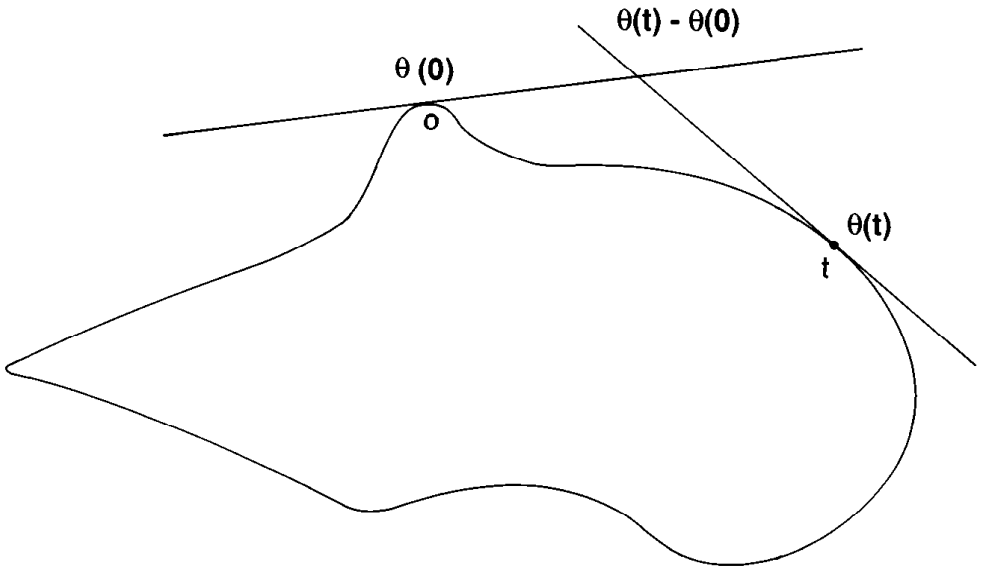


Fig. 4.4 Calculation of the tangent angle function from a skull outline. The boundary is scaled to length $2\pi$. $\phi^*(t) = \theta(t) - \theta(0) - t$ (see text).

If a line of length 1 is divided into $N$ equal parts, each equal in length, $r$, where $r = l/N$, then:

$$Nr = 1. \tag{4.8}$$

For two dimensions:

$$Nr^2 = 1, \tag{4.9}$$

where $r$ is being expressed in terms of a one-dimensional characteristic of area, that is, its "linear scale"; for example, the diameter of a circle, or the length of a side for a square. This leads to the general equation, which is:

$$Nr^D = 1, \tag{4.10}$$

where D is the fractal dimension.

D can be estimated by the relationship between the estimated length of an outline and the scale of measurement. Examples of the use of fractal dimension as a means of summarizing complexity in biological forms are provided by Reyment (1991:152) and by Katz and George (1985).

#### *4.3.2.5 Fourier analysis*

The methods considered above, in the context of the analysis of forms with few or no landmarks, can be divided into two groups. The first group of methods produces summary measures of form (e.g., shape factors, bending energy, fractal dimension), which can be compared directly. The second results in a re-expression

of the boundary information present in the original $x$ and $y$ coordinates (e.g., polar coordinates, tangent angle function). The latter ones offer one significant advantage; they provide largely registration- independent data (although they are dependent on the starting point and, in the case of polar coordinates, the centroid location). These new data may be compared using the techniques of multivariate analysis, or they may be subjected to a further transformation such as Fourier analysis; a transformation of data from the spatial to the frequency domain. This may be useful in understanding periodicity (but which rarely has a basis in the biological determinants of morphology), or in summarizing large datasets.

Fourier analysis results in the decomposition of a periodic function (e.g., polar coordinates, $\phi^*(t)$) into a series of sinusoidal waves of differing frequencies, composed of phases and amplitudes which, when summed, can reproduce the original form. Fourier analysis has been applied to the measurement of biological shapes by a number of workers (e.g., Lu, 1965; Kaesler and Waters, 1972; Lestrel, 1982; Rohlf and Archie, 1984; Ferson et al., 1985). Briefly, a periodic function, f(t), can be approximated by:

$$F(t) = a_0 + \sum_{n=1}^{k} a_n \cos(nt) + \sum_{n=1}^{k} b_n \sin(nt), \tag{4.11}$$

where the $a_n$ are the cosine components, and the $b_n$ are the sine components. They describe the cosine and sine waves at a particular frequency, $n$ ($k$ is the maximum harmonic order of the calculated series). The single Fourier series will provide a fit to any smooth single-valued periodic function. It can be applied to both polar and curvature representations of an outline, and the resulting Fourier coefficients can be used to reconstruct that outline. The polar representation of the Fourier series can be written as:

$$r = F(\theta) = a_0 + \sum_{n=1}^{k} a_n \cos(n\theta) + \sum_{n=1}^{k} b_n \sin(n\theta). \tag{4.12}$$

In this form, the function, $F(\theta)$, describes the magnitudes of successive radii, $r$, at successive angular displacements, $\theta$.

Polar coordinates are only amenable to Fourier analysis when each radius intersects the outline at only one point. When the outline is more complex, it may be possible to calculate the Fourier series from $x$ and $y$ coordinates through the tangent angle formulation (Zahn and Roskies, 1972).

Alternatively, the $x$ and $y$ coordinates from an outline may be submitted to elliptic Fourier analysis (Kuhl and Giardina, 1982; Lestrel, 1989) in which Fourier series are separately fitted to $\Delta x$ and $\Delta y$ expressed as functions of cumulative chordal distance (see Chapter 2), or to dual-axis Fourier analysis in which the $x$ and $y$ coordinates are fitted directly (Moellering and Rayner, 1981).

There is also an alternative representation of the Fourier series; the amplitude-phase-lag representation:

$$r = F(\theta) = R_0 + \sum_{n=1}^{k} R_n \cos(n\theta + \phi_n). \tag{4.13}$$

The $\phi$s are known as the phase lag components; they contain all the rotational information (i.e., about "starting point"). As such, they register the waves of different frequencies with respect to each other in a way that allows reconstruction of the original outline. The phase lag components are readily calculated from the sine and cosine components considered earlier (Eq. 4.12) :

$$\phi_n = \tan^{-1}\left(\frac{b_n}{a_n}\right). \tag{4.14}$$

The amplitude components, $R_n$, are a measure of the contribution of each harmonic to the whole form. They contain no phase information, and so are independent of the boundary landmark chosen as the start of the polar series. They, too, are readily calculated from:

$$R_n = \sqrt{a_n^2 + b_n^2}. \tag{4.15}$$

This representation offers some advantages. In situations where dependency on the starting point definition is considered problematical, the amplitude components alone can be compared between shapes. It should be noted, however, that amplitude components alone do not uniquely specify a shape; different shapes may share the same amplitude components. In a biological situation it seems unlikely, however, that OTUs will differ in phase components alone. Thus, O'Higgins and Williams (1987) and O'Higgins (1989) have shown, in studies of cranial form in primates, that using amplitude components alone gives a similar pattern of between-species discrimination when compared to the combined amplitude/phase-lag spectrum. Nevertheless, the degree of between-OTU discrimination in the former analysis was reduced relative to that in the latter because of the lack of phase information.

## 4.4 Analysis of data and the reconstruction of form

In Section 4.3 a number of methods were considered by which Cartesian coordinate data representing a boundary can be used to provide measures of form. Some of these approaches (e.g., shape factors, bending energy, fractal dimension) result in simple summary measures of shape that can be readily compared between OTUs. Others result in a reexpression of the information contained within the original Cartesian representation in a way that is, to a greater or lesser degree, in-

dependent of the original registration. Thus, moments, medial axis transforms, polar coordinates, curvature functions, and Fourier series exhaustively describe, and can be used to reconstruct, an OTU. In this section some general aspects dealing with the statistical analysis and reconstruction of form will be considered, with an emphasis on the role of Fourier analysis. The section ends with some broad conclusions concerning shape analysis.

### *4.4.1 Size*

Many of the methods for shape description considered here result in data which are invariant with respect to size. This arises because the data are ratios, or angles, or are standardized with respect to some "size variable." Other methods, for example, landmarks, linear measurements, polar coordinates, and medial axes, preserve information about scale. It is important, therefore, to consider how to account for size differences and their consequences. The literature on scaling is large, and the reader is referred there for details of methods and approaches to scaling (e.g., Jungers, 1985; Schmidt-Nielsen, 1984). It is, however, worth raising some general points here.

Two specimens may differ not only in shape but also in size. At first this seems obvious and clear-cut, but there are semantic and mathematical difficulties in discussing size independent of shape in most circumstances. In the comparison between two objects of identical shape, the difference between any pair of homologous measurements, one from each OTU (e.g., lengths, widths, heights), will indicate the scaling required to make them identical. In most biological situations, however, OTUs will differ in shape. Consequently, intuition comes into play and the concept of size becomes less well defined. Sneath and Sokal (1973) ask, "which is bigger, a snake or a turtle?" The term "size" in this circumstance relates to the differences in scale over whole objects. As such, "size" might be best thought of as a vague term relating to the differences in magnitude of many dimensions.

Many different approaches have been taken in determining "size" differences between OTUs and for choosing suitable scaling variables between differently shaped OTUs. The choice of methods depends on the questions being addressed and on the investigator's concept of size. In biomechanical studies, a quantity such as body mass might be appropriate to scale measurements (e.g., Alexander, 1991). Alternatively, the length of a lever arm might be chosen, the choice of scaling variable being justified from an engineering perspective. In phenetic or cladistic studies, however, the problem is more difficult: is body mass the most appropriate choice? Different workers have used different "size variables." Some of these are external to the object under study. For instance, Wood (1976) used femur length as an estimate of body size, and determined the allometric relation-

ship between this and a number of cranial and other dimensions. Other workers have used "size variables" that are derived from the object itself. For instance Albrecht (1978) used three different measures of size: the greatest length of the skull, the geometric mean of the log transformed cranial variables, and an estimate of cranial volume, in a study of the craniofacial morphology of the Sulawesi macaques.

Considering the difficulties in providing an unequivocal size measure for differently shaped forms, it seems sensible to use some measure that describes the magnitudes of many variables. Examples of such measures include centroid size (Bookstein, 1978)—a measure of the deviation of landmarks from the centroid of a shape—and area (which relates to all boundary points).

Turning to Fourier analysis, it is often stated that the constant or zero-order cosine components ($a_0$s) are suitable size measures with which to scale OTUs described in terms of polar coordinates, since the $a_0$s are closely related to the area, and form a natural part of the Fourier series. Although scaling by the $a_0$ term is usually sensible and appropriate, it seems, from the considerations above, that in some circumstances another "size measure" may be more suitably applied to the polar coordinates themselves *prior* to Fourier analysis. In any case, the choice of a "size measure" in any one study needs to be undertaken in the knowledge that each may be different and should be based on the particular biomechanical, ontogenetic, or phylogenetic issue that is being addressed.

### *4.4.2 Data reduction and shape reconstruction*

Several of the methods (e.g., moments, medial axis transforms, polar coordinates, curvature functions, and Fourier series) described earlier result in exhaustive descriptions of individual OTUs and generate a large number of variables. As such, statistical comparisons are often best achieved through the use of the techniques of multivariate analysis. Principal components analysis (PCA) of the covariance or correlation matrices between OTUs may be carried out to investigate patterns of variation. In such analyses, each OTU may be described in terms of registered (Procrustes) Cartesian coordinates of outline points, polar coordinates of outline points, curvature functions, moments, or Fourier coefficients.

Alternatively, analyses might be undertaken to investigate patterns of variation between groups of organisms through Mahalanobis's distances, canonical axes or discriminant functions. In these circumstances the number of measurements (coordinates, moments, $\phi^*(t)$s, etc.) must be considerably less than the number of individuals included in the analysis. If large numbers of specimens are not available it becomes necessary to attempt to reduce the quantity of data from each. Many strategies are available to achieve this aim. These include the selection of

fewer data on the basis of what appears "sensible" from the biologist's perspective (a highly subjective exercise), selection on the basis of some measure of likely discriminating value (e.g., F-ratios), and data reduction by way of PCA.

It is also possible to apply the technique of Fourier analysis to the task of data reduction for statistical analysis, since good approximations of the original form can be achieved with relatively few Fourier components. One approach to selecting the number of Fourier components to be used is by means of the harmonic amplitudes ($R_n$s). These can be plotted against harmonic order to produce a power spectrum (Figure 4.6) that allows a rapid, objective, quantitative assessment of the contribution to the overall form of components of successive frequencies. Figure 4.5 illustrates a chimpanzee cranium reconstructed from increasing numbers of Fourier components via polar coordinates; a good approximation is achieved by relatively few of the lower-order harmonics in the series. This is because the form is generally globular and smooth; higher-order Fourier terms are required to describe finer, more jerky aspects of outline. Note that different boundary forms will be better summarized by different combinations of Fourier components.

The selection of the lower-order Fourier components for statistical analysis of their harmonic amplitudes does not necessarily ensure optimal discrimination between OTUs. This is because information describing aspects of form that differ

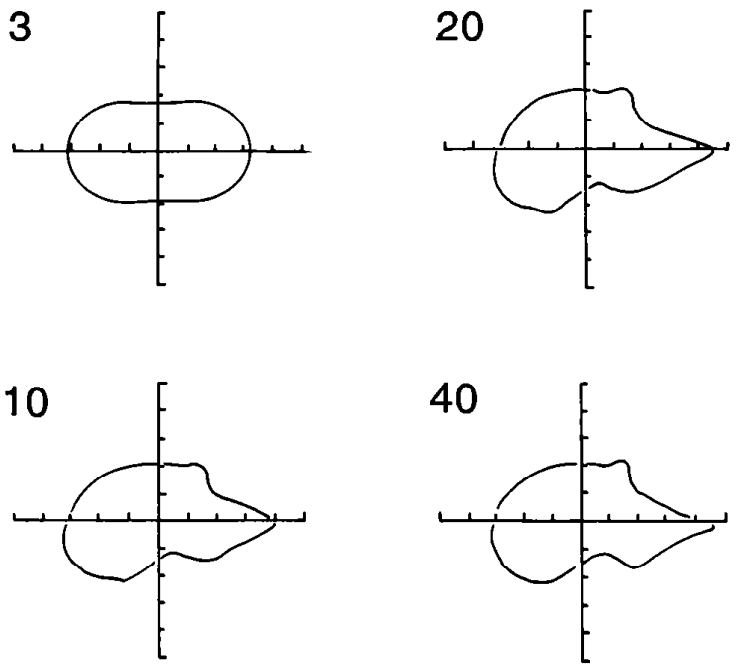


Fig. 4.5 A schematic illustrating the production of a power spectrum (see Fig. 4.6) for relatively simple, gently undulating forms. Most of the shape information is represented by the first few, low-order Fourier components.

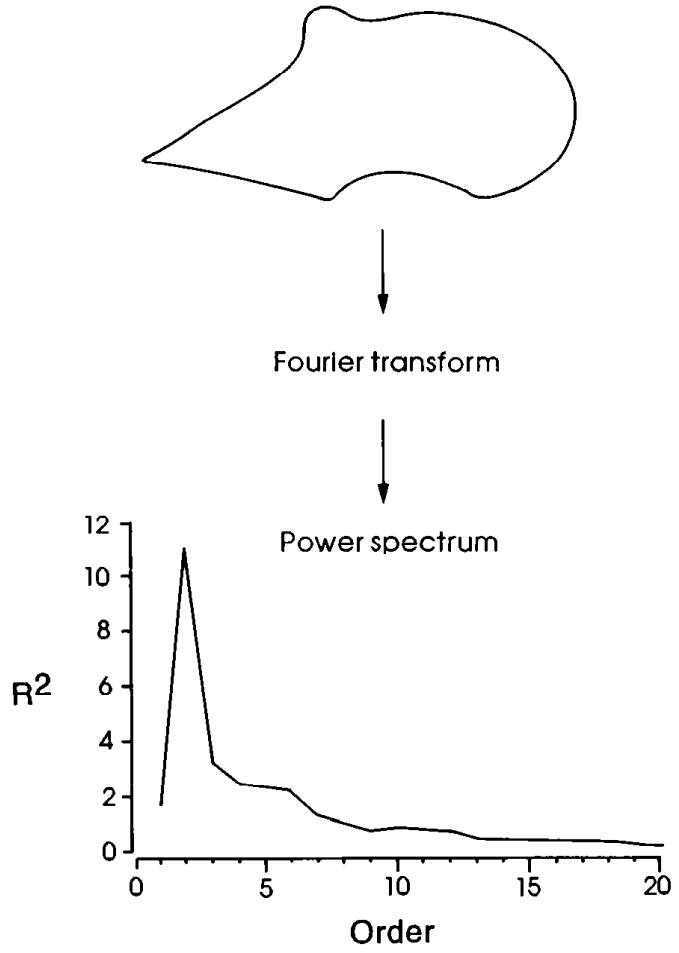


Fig. 4.6 A chimpanzee cranium reconstructed via polar coordinates from increasing numbers (indicated alongside each reconstruction) of Fourier components (see Fig. 4.5). A good representation is obtained with as few as twenty Fourier harmonics.

between OTUs may reside in some of the higher-order Fourier terms, omitted on the basis of their relatively small harmonic amplitudes. If discrimination is the objective of statistical analysis, then selection of Fourier components should be based on their discriminating ability rather than their overall contribution to the power spectrum. Thus, the selection of components on the basis of the ratio of between-group to pooled within-group variation (F-ratio) or through step-wise discriminant analysis may be more useful.

Thus, Fourier analysis presents a means of reducing the number of variables per OTU. It has, however, been criticized on a number of grounds. The Fourier series is essentially a spectral decomposition of a periodic function; data in the spatial domain are transformed into the frequency domain. In the process, all information regarding the relative locations of boundary points is referred to the

starting point, and even if this is taken to be homologous between forms, it is unlikely that individual Fourier coefficients can be considered homologous in an evolutionary or developmental sense. As such, the application of Fourier analysis in the study of biological variation has been the subject of debate.

Bookstein (1978) and Bookstein et al., (1982) have criticized Fourier analysis because the Fourier decomposition of a curvature function around an outline allows for one landmark only, the starting point. If the aim of the study is to examine differences in homology relationships, then homologies will not be discernible from the Fourier coefficients.[6] The description of the pattern of disposition of homologies is confused, not aided, by Fourier analysis.

Ehrlich et al., (1983) have replied to these criticisms. They rightly state that "examination of homologous skeletal features is only one of many approaches" to biomorphological studies. Their work on the foraminiferan *Globorotalia trucatulinoides* indicates that there is a "consistent angular relationship between the orientation of the second harmonic and the spiral side keel." With this noted, they indicate that it could be suggested that "the very fact that the radial Fourier series is locking onto homologous points is a good reason not to use the Fourier series; that is, given the relationship, why go through the complex calculations?" The justification they give is that "the possibility always exists, however, that the additional data needed to fully reconstruct the profile between homologous points may contain biologically interesting information."

Read and Lestrel (1986) provide an example in which measurements taken between homologous points fail to describe significant differences in morphology between structures because the measurements omit the boundary connecting the landmarks. They express agreement with the observation made by Bookstein et al., (1985:3) that "although no morphometric method can be wrong in all contexts, neither is any method universally applicable."

## 4.5 Concluding remarks

This chapter has been concerned with a survey of methods available for the description of biological forms. A number of issues have been raised concerning their use. These issues impact every aspect of shape analysis: the definition of landmarks, the identification of homologies, the strategies available when few homologies can be identified, the ways in which data might be compared between forms, and the consequences of data collection and transformation strategies on the perception of shape differences.

[6]Editor's note: Chapters 14 and 16 present a procedure that preserves homology withtin a boundary-outline context.

Clearly, there is no one approach or collection of approaches to shape analysis that is ideal in all circumstances. Fourier analysis, the subject of this volume, is just one of many techniques for shape analysis available to the modern investigator. In most biological circumstances, comparisons of individual harmonics between shapes will rarely provide readily interpretable information about basic biological processes such as ontogeny or phylogeny. This is because Fourier components are not necessarily homologous between OTUs in terms of the features they describe. In combination, however, the shape summaries that can be produced through Fourier analysis may be useful in the multivariate assessment of overall morphological differences arising because of underlying biological processes.

The morphometrician must choose strategies for shape description and comparison that are appropriate to the question at hand. Where several approaches appear equally applicable it seems sensible to apply a few in order to compare outcomes. Such a broad approach not only serves to provide a comparison of techniques, but also ensures that interpretations of results are widely based and so are less likely to be influenced by the foibles of one single technique. The morphometrician must be aware of the theoretical and practical limitations of each method and must apply this knowledge to each situation.

## 4.6 Acknowledgments

I should like to thank Pete Lestrel for organizing this volume and for inviting me to contribute to it. Ian Dryden, Lisa Wiffen, and John Kent of the Department of Statistics, University of Leeds have provided me with invaluable advice and help in understanding some of the methods outlined in this chapter. Martin Thompson of the Department of Anatomy and Human Biology, the University of Western Australia, kindly prepared the illustrations for this chapter.

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