1

Introduction

Shape analysis plays an important role in many kinds of biological studies. A variety of biological processes produce differences in shape between individuals or their parts, such as disease or injury, ontogenetic development, adaptation to local geographic factors, or long-term evolutionary diversification. Differences in shape may signal different functional roles played by the same parts, different responses to the same selective pressures (or differences in the selective pressures themselves), as well as differences in processes of growth and morphogenesis. Shape analysis is one approach to understanding those diverse causes of variation and morphological transformation.

Frequently, differences in shape are adequately summarized by comparing the observed shapes to more familiar objects such as circles, kidneys or letters of the alphabet (or even, in the case of the Lower Peninsula of Michigan, a right-handed mitten). Organisms, or their parts, are then characterized as being more or less circular, reniform or C-shaped (or mitten-like). Such comparisons can be extremely valuable because they help us to visualize unfamiliar organisms, or focus attention on biologically meaningful components of shape. However, they can also be vague, inaccurate or even misleading, especially when the shapes are complex and do not closely resemble familiar icons. Even under the best of circumstances, we still cannot say precisely how much more circular, reniform, or C-shaped (or mitten-like) one shape is than another. When we need that precision, we turn to measurement.

Morphometrics is simply a quantitative way of addressing the shape comparisons that have always interested biologists. This may not seem to be the case because conventional morphological approaches typical of the qualitative literature and traditional morphometric studies appear to produce quite different kinds of results. The qualitative studies produce pictures or detailed descriptions (in which analogies figure prominently), and the morphometric studies usually produce tables with disembodied lists of numbers. Those numbers seem so highly abstract that we cannot readily visualize them as descriptors of shape differences, and the language of morphometrics is also highly abstract and mathematical. As a result, morphometrics has seemed closer to statistics or algebra than to morphology. In one sense that perception is entirely accurate: morphometrics *is* a branch of mathematical shape analysis. The ways we extract information from morphometric

Geometric Morphometrics for Biologists
ISBN 0–12–77846–08

data involve mathematical operations rather than concepts rooted in biological intuition or classical morphology. Indeed, the pioneering work in modern geometric morphometrics (the focus of this book) had nothing at all to do with organismal morphology; the goal was to answer a question about the alignment of megalithic "standing stones" like Stonehenge (Kendall, 1977; Kendall and Kendall, 1980). Nevertheless, morphometrics can be a branch of morphology as much as it is a branch of statistics.

This is the case when the tools of shape analysis are turned to organismal shapes, and when those tools allow us to illustrate and explain shape differences that have been mathematically analyzed. The tools of geometric shape analysis have a tremendous advantage when it comes to these purposes: not only does this method offer precise and accurate description, but also it serves the equally important purposes of visualization, interpretation and communication of results. Geometric morphometrics allows us to visualize differences among complex shapes with nearly the same facility as we can visualize differences among circles, kidneys and letters of the alphabet (and mittens).

In emphasizing the biological component of morphometrics, we do not discount the significance of its mathematical component. Mathematics provides the models used to analyze data, including the general linear models exploited in statistical analyses, and the models underlying exploratory methods (such as principal components analysis). Additionally, mathematics provides a theory of measurement that we use to obtain data in the first place. It may not be obvious that a theory governs measurement, because very little (if any) theory underlay traditional measurement approaches. Asked the question "What are you measuring?", we could give many answers based on our biological motivation for measurement – such as (1) "Functionally important characters;" (2) "Systematically important characters;" (3) "Developmentally important characters;" or, more generally, (4) "Size and shape." However, if asked "What do you mean by 'character' and how is that related, mathematically or conceptually, to what you are measuring?", or even if just asked "What do you mean by 'size and shape'?", we could not provide theoretically coherent answers. A great deal of experience and tacit knowledge went into devising measurement schemes, but they had very little to do with a general theory of measurement. It was almost as if each study devised its own approach to measurement according to the particular biological questions at hand. There was no general theory of shape, nor were there specialized analytic methods adapted to the characteristics of shape data.

The remarkable progress in morphometrics over the past decade resulted largely from precisely defining "shape," then pursuing the mathematical implications of that definition. The most fundamental change has been in measurement theory. Below we offer a critical overview of the recent history of measurement theory, presenting it first in terms of exemplary data sets and then in more theoretical terms, emphasizing the core of the theory underlying geometric morphometrics – the definition of shape. We conclude the conceptual part of this Introduction with a brief discussion of methods of data analysis. The rest of the Introduction is concerned with the organization of this book, and available software and other resources for carrying out morphometric analyses.

A critical overview of measurement theory

Traditionally, morphometric data have been measurements of length, depth and width, such as those shown in Figure 1.1, which is based on a scheme presented in a classic

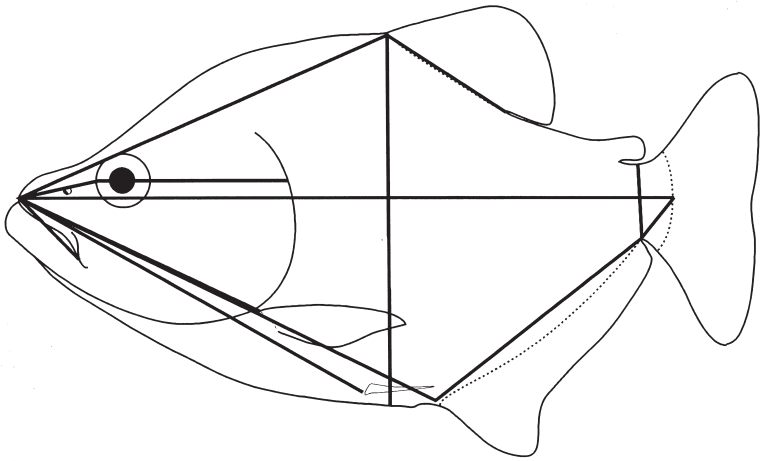

Figure 1.1 Traditional morphometric measurements of external body form of a teleost, adapted from the scheme in Lagler et al., 1962.

ichthyology text (Lagler et al., 1962). Such a data set contains relatively little information about shape, and some of that information is fairly ambiguous. These kinds of data sets contain less information than they appear to hold because many of the measurements overlap or run in similar directions. Several of the measurements radiate from a single point, so their values cannot be completely independent (which also means that any error in locating that point affects all of these measurements). Such a data set also contains less information than could have been collected with the same effort, because some directions are measured redundantly, and many of these measurements overlap. For example, there are multiple measurements of length along the anteroposterior body axis and most of them cross some part of the head, whereas there are only two measurements along the dorsoventral axis, and only two others that are measurements of post-cranial dimensions. In addition, the overlap of the measurements complicates the problem of describing localized shape differences like changes in the position of the dorsal fin relative to the back of the head. Also missing from this type of measurement scheme is information about the spatial relationships among measurements. That information might be given in the descriptions of the measured line segment, but it is not captured in the list of observed values of those lengths, which are the data that are actually available for analysis. Finally, the measurements in this scheme may not sample homologous features of the organism. Body depth can be measured by a line extending between two well-defined points (e.g. the anterior base of the dorsal fin to the anterior base of the anal fin), but it can also be measured wherever the body is deepest, yielding a measurement of "greatest body depth" wherever that occurs. This measurement of depth might not be comparable anatomically from species to species, or even from specimen to specimen, so it provides almost no useful information. When all of the limitations of the traditional measurement scheme are considered, it is apparent that the number of measurements greatly overestimates the amount of shape information that is collected.

The classical measurement scheme can be greatly improved, without altering its basic mathematical framework, by the box truss (Figure 1.2) – a scheme developed by Bookstein and colleagues (Strauss and Bookstein, 1982; Bookstein et al., 1985). This set of

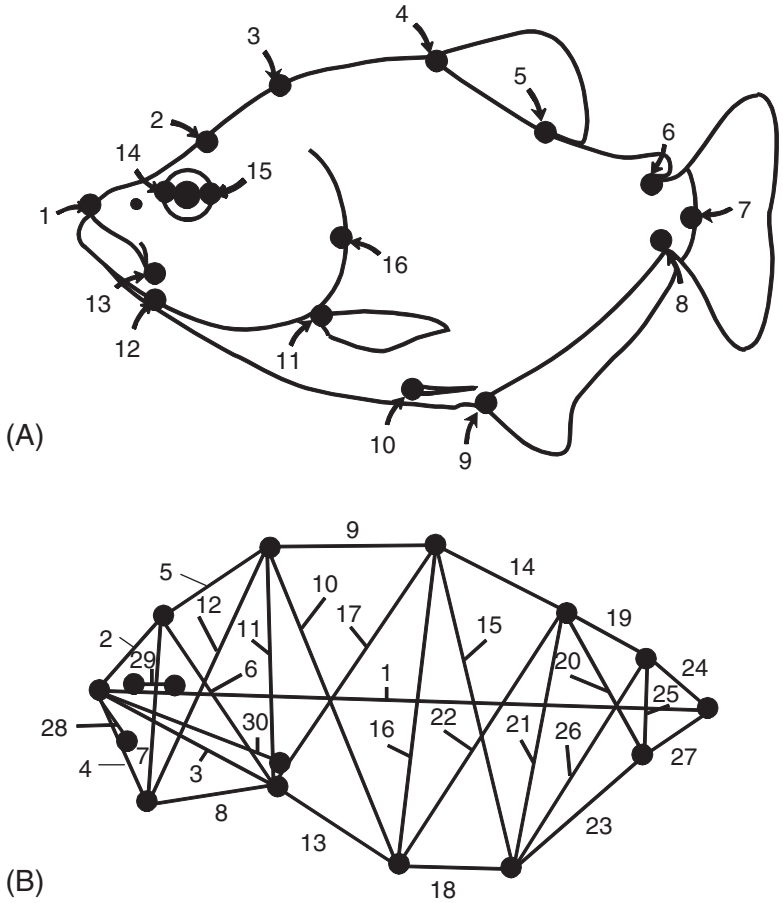

Figure 1.2 Truss measurement scheme of external body form of a teleost: (A) well-defined endpoints of measurements; (B) a selection of 30 lengths, arranged in a truss.

measurements samples more directions of the organism and the measurements are more evenly spaced; the set also contains many short measurements. Additionally, the endpoints of all of the measurements are biologically homologous anatomical loci – landmarks. Although these features make the truss a clear improvement over the classical measurement scheme, this approach still produces a list of numbers (values of segment lengths), with all the attendant problems of visualization and communication.

One problem shared by the two measurement schemes is that neither collects all of the information that could be collected. The truss scheme shown in Figure 1.2 contains 30 measurements, but this is only a fraction of the 120 that could be taken among the same 16 landmarks (Figure 1.3). Of course, many of the 120 are redundant, and several of them span large regions of the organism. We would also need extraordinarily large samples before we could perform the necessarily mathematical manipulations or perform valid tests of hypothesis. In addition, the results would be incredibly difficult to interpret because there would be 120 pieces of information (e.g. regression coefficients, principal component loadings) for each specimen, for each trend or difference. We might be tempted

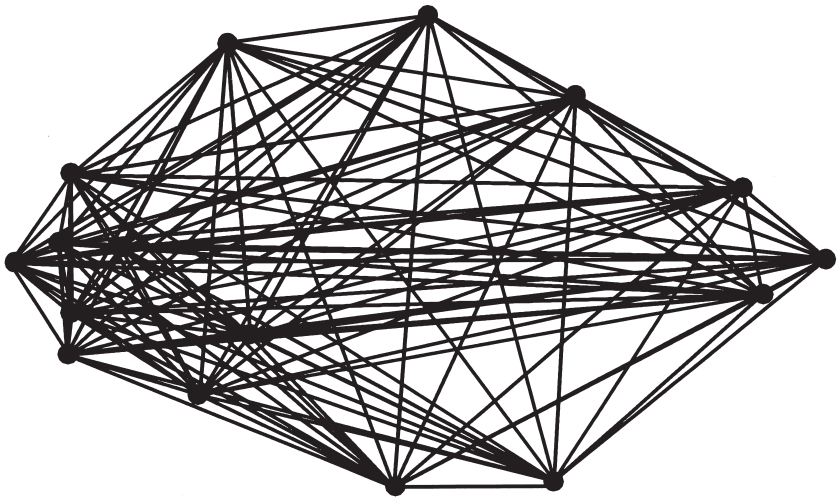

Figure 1.3 All 120 measurements between endpoints defined by the 16 landmarks of Figure 1.2.

to cull the 120 measurements to those that seem most likely to be informative, but until we have done the analysis we cannot know which to cull without altering the results. Clearly, we need another way to get the same shape information as the 120 measurements, but without the excessive redundancy.

Another problem that the truss shares with more traditional schemes is that it measures size rather than shape – each length is the magnitude of a dimension, a measure of size. This does not mean that the data include no information about shape – they do – but that information is contained in the ratios among the lengths, and it can be surprisingly difficult to separate information about shape from size. Some studies have analyzed ratios directly, but ratios pose serious statistical problems (debated by Atchley et al., 1976; Corruccini, 1977; Albrecht, 1978; Atchley and Anderson, 1978; Hills, 1978; Dodson, 1978). The more usual approach is to construct shape variables from linear combinations of length measurements, such as Principal Component (PC) loadings. Here, one component, usually the first (PC1), is interpreted as a measure of size, and all the others are interpreted as measures of shape. However, PC1 includes information about both shape and size, as do all the other PCs. The raw measurements include information about both shape and size, and so do their linear combinations.

Not only are the methods of separating size from shape problematic; the *idea* of size and shape has been one of the most controversial subjects in traditional morphometrics. One reason for this controversy is the multiplicity of definitions of size (and also of shape), several of which are articulated by Bookstein (1989). Virtually any approach to effecting this separation can be disputed on the grounds that the notion of "size" that is separated from "shape" is not really "size." Another reason for the controversy is that some workers argue that no such separation is biologically reasonable (see, for example, the discussion of studies of heterochrony based on growth models in Klingenberg, 1998). However, even if we accept the argument that size and shape are intimately linked by biological processes, we still want to know more about their relationship than the mere fact of its existence.

Extracting the relationship between size and shape from a set of measurements can be especially difficult when the organisms span a broad size range. When some organisms are 20 mm long and others are 250 mm, *all* measurements will differ in length. Even if shape is not much influenced by a ten-fold change in size, all measurements will still be correlated with size; quantifying this fact is merely restating the obvious. In fact, we should expect size to be the dominant explanation for the variance in traditional morphometrics because these measurements *are* measurements of size. Instead, we should be concerned about the possibility that the variance in shape is not fully explained by the variance in size, but is simply overwhelmed by it. For instance, in analyses of ontogenetic series of two species of piranha (one being the running example throughout this chapter), we find that 99.4% of the variance is explained by the PC1 in both species. This suggests that there is nothing else to explain in either species, because it is hard to imagine that the remaining 0.6% is anything but noise. And yet, we do not actually know what proportion of shape variation is explained by size; nor do we know whether different proportions or patterns of shape change are explained by size in these species.

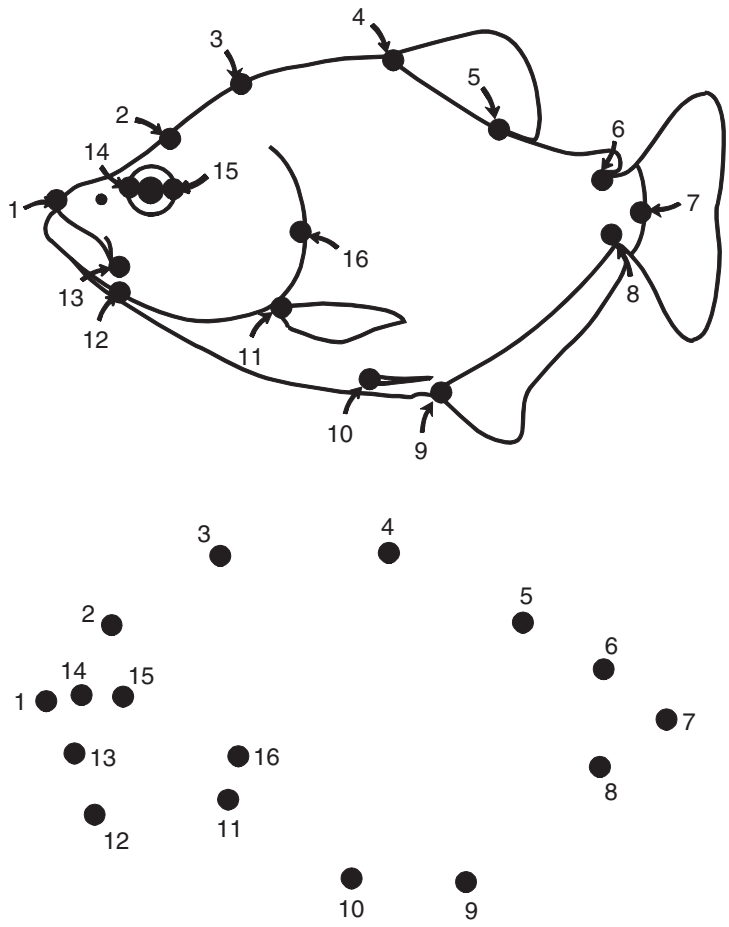

Figure 1.4 The 16 landmarks, stripped of the line segments connecting them.

One other serious limitation of traditional morphometrics is that the measurements convey no information about their geometric structure. If we strip off the line segments connecting the landmarks in Figure 1.3 and just look at the position of the landmarks on the page (Figure 1.4), we can see that some are close to each other (e.g. 12 and 13) and others are far apart (e.g. 1 and 7); some are dorsal (3 and 5), others are more posterior (6–8). That information about relative positions, which is so important to morphologists, is contained in the coordinates of the landmarks but not in the list of distances among them – not even in the comprehensive list of 120 measurements. In fact, the list of 32 coordinates contains all of this positional information in addition to all of the information contained in the 120 distances (the distances can be reconstructed from the coordinates if the units of the coordinate system are known). More importantly, simple algebraic manipulations allow us to partition the information captured by the coordinates into components of size and shape (and to strip off irrelevant information like the position and orientation of the specimen). Afterward, we have slightly fewer than 32 shape variables (because information about size, position and orientation has been separated from information about shape), but we still have the information about the geometric structure of our landmarks that was captured when we digitized the specimens, and we have the information that is present in the full list of 120 measurements without the redundancy. Consequently, we do not need to cull the data in advance of the analysis, and so we do not lose any information we might have had prior to that culling. In addition, partitioning the morphological variation into components of size and shape means that variance in size does not overwhelm variance in shape even when the variance in size is relatively large. In the two species mentioned above (in which PC1 accounts for 99.4% of the variance), size explains 71% of the variance in shape in one species, but only 21.7% in the other.

An important advantage of analyzing landmark coordinates is that it is relatively easy to draw informative pictures to illustrate results. In Figure 1.5, the shape changes that occur during the ontogeny of one species of piranha are shown as vectors of relative landmark displacement and as a deformed grid interpolating among those vectors. In both representations, it is quite clear that the middle of the body becomes relatively deeper while the postanal region becomes relatively short, especially the caudal peduncle (between landmarks 6 and 7). Both pictures also show that the posterodorsal region of the head (above and behind the eye) becomes relatively longer and deeper while other regions of the head become relatively shorter. (We emphasize that these are *relative* changes, because the piranha becomes *absolutely* larger in every dimension and region mentioned.)

It is possible to present traditional morphometric results in graphic form by placing the numbers on the organisms, as in Figure 1.6. This, like Figure 1.5, shows that the middle of the body grows faster and becomes deeper than the rest of the animal. The limitation of this representation (and of the analysis) is exemplified by the difficulty of interpreting the large coefficient (1.23) of the posterior, dorsal head length – it is not clear whether the head is just elongating rapidly, or if it is mainly deepening, or if it is both elongating and deepening. We also cannot tell if the pre- and postorbital head size increases at the same rate, because the measurement scheme does not include distances from the eye to other landmarks. None of these ambiguities arose from the geometric analysis of the landmark coordinates; the figure illustrating that result showed the information needed to understand the ontogenetic changes in these specific regions. This ability to extract and communicate information about the spatial localization of morphological variation

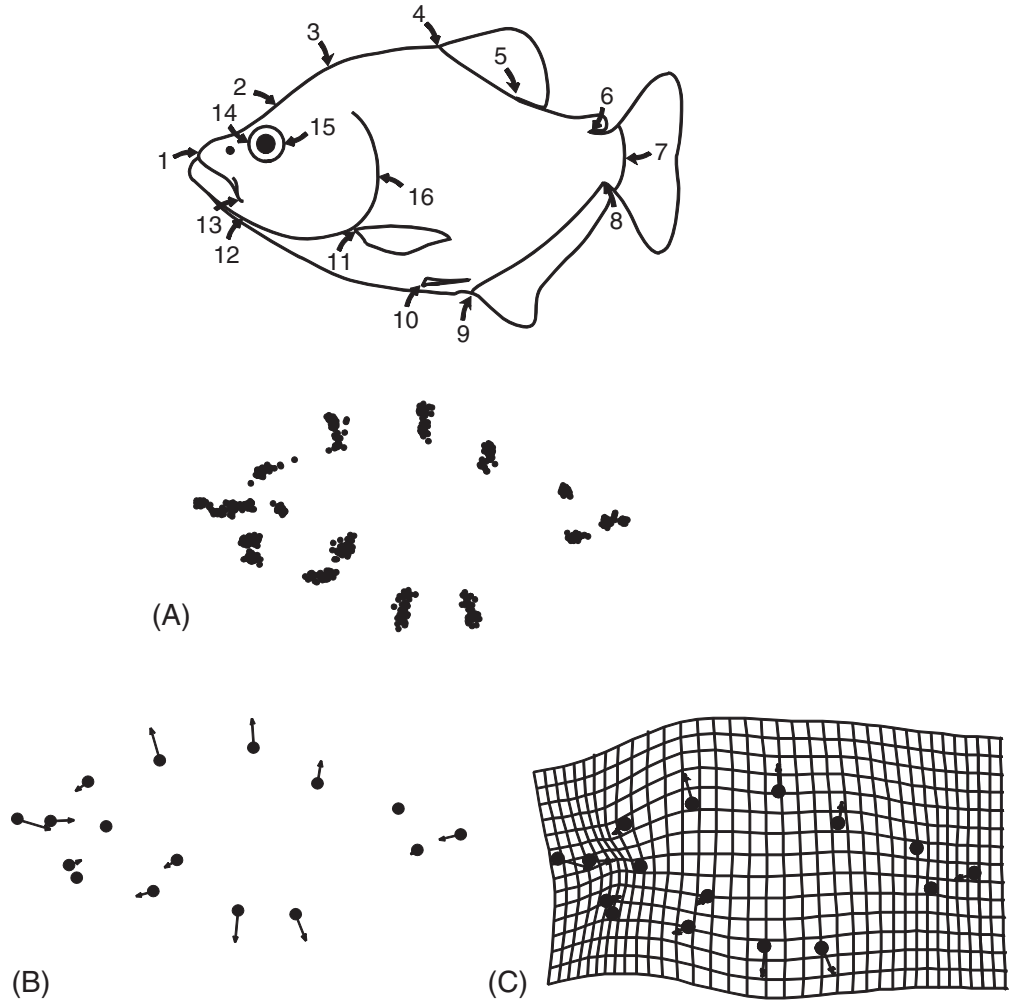

Figure 1.5 Ontogenetic shape change depicted in two visual styles. (A) Landmarks of all specimens; (B) vectors of relative landmark displacement; (C) deformed grid.

(its magnitude, position and spatial extent on the organism) is among the more important benefits of geometric morphometrics.

Geometric morphometrics does not solve all of the problems confronting traditional methods, and one remaining problem becomes evident when we try to examine the changes in head profile over the piranha's ontogeny (Figure 1.7). We can see that the average slope on either side of landmark 2 must get steeper, but we cannot tell whether the profile becomes more S-shaped, C-shaped or any other shape. This uncertainty arises because the three landmarks provide no better a sample of the curve's shape than do the line segments connecting them. Clearly, any solution of this problem will require analysis of points on the curve that are not landmarks (Figure 1.8). Methods for analyzing curves are being developed and used (we discuss them in Chapter 15), so this limitation of geometric morphometrics will likely prove transitory.

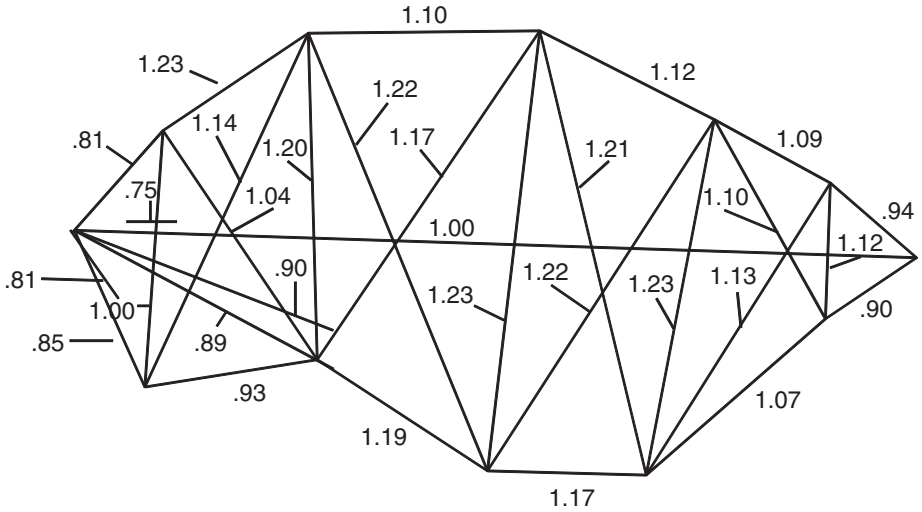

Figure 1.6 Allometric coefficients of traditional morphometric measurements, plotted on the organism.

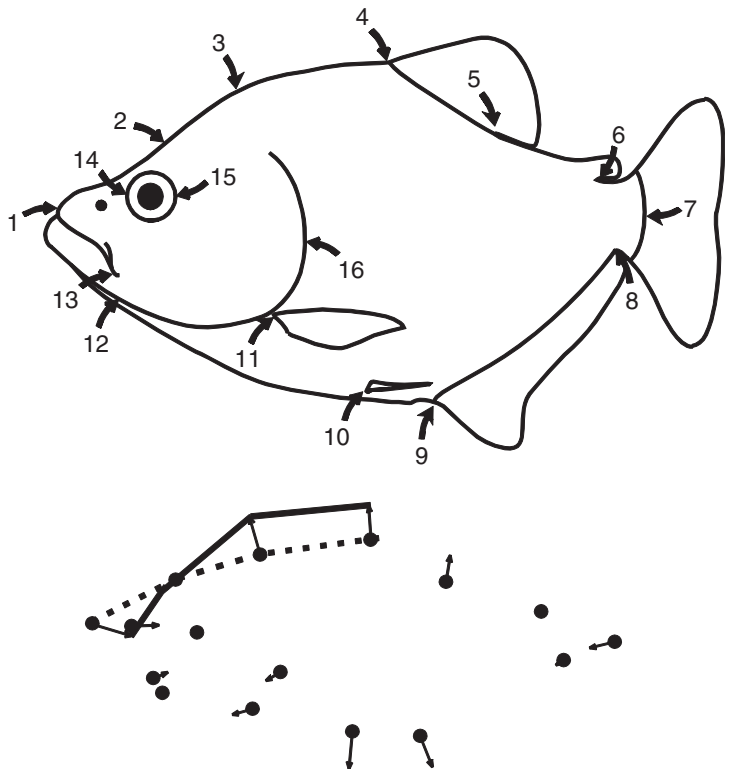

Figure 1.7 Ontogenetic change in head profile as implied by changes in the orientation of straight lines drawn between landmarks of the head.

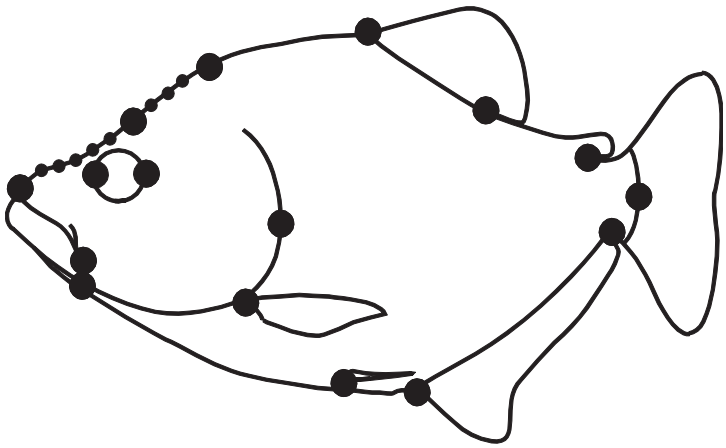

Figure 1.8 Additional points on the head profile, which are not landmarks.

Geometric morphometrics may also appear to have a limitation that does not confront traditional methods: the restriction to two-dimensional data. The reality is that mathematical theory poses no obstacles to analysis of three-dimensional shapes. Instead, the obstacles lie in other constraints restricting biologists to two-dimensional data, notably (1) the cost of the equipment for obtaining three-dimensional coordinates (which is also time-consuming to use) and (2) the difficulty of depicting the results on static, two-dimensional media like the pages of a journal. Traditional morphometric studies need not face these obstacles because, if the equipment required for three-dimensional digitizing is exorbitant (in time or money), specimens can always be measured with calipers. However, in using calipers we do not collect three-dimensional coordinates, so this approach sidesteps rather than solves the problem. The difficulty of depicting results on a two-dimensional page does not arise when results are tables of numbers, which is another case of sidestepping rather than solving the problem.

Geometric analyses of landmark coordinates do solve many of the problems confronting traditional methods of measurement. Those that remain involve analyses of curves with few or no landmarks, and the illustration of three-dimensional results. Without denying that these are real issues, we can still obtain a great deal of information about shape and size from geometric studies.

Shape and size

The rapid progress in geometric morphometrics has resulted largely from having a coherent mathematical theory of shape, which requires articulating a precise definition of the concept. Like the definition of any word, that of "shape" is entirely a matter of semantics. However, semantics is not trivial. We cannot have a coherent mathematical theory of an undefined concept; the definition of shape is the foundation for a mathematical theory of shape. Whether that theory applies to our biological questions depends on whether it captures what we mean by shape. Thus it is important to understand the concept of shape underlying geometric morphometrics, and also, because the concept of size is so closely

related to that of shape, we cannot fully understand one without understanding the other and also how they are related to each other.

Shape

In geometric morphometrics, shape is defined as "all the geometric information that remains when location, scale and rotational effects are filtered out from an object" (Kendall, 1977). The earliest work that depends on this definition of shape began the analysis with the coordinates of points; consequently, the "objects" are sets of those coordinates – i.e. configurations of landmarks, such as that shown in Figure 1.4. An important implication of Kendall's definition is that removing the differences between configurations that are attributable to differences in location, scale and orientation leaves only differences in shape. These operations and their consequences are illustrated in Figure 1.9. In Figure 1.9A there are two configurations, side by side. This difference in location has no bearing on their shape difference, so in Figure 1.9B both have been translated to the same location. The two configurations still differ in scale, which also has no bearing on their shape difference, so in Figure 1.9C they are converted to the same scale. The two configurations still differ in orientation (their long axes are about 45° apart), which also has no bearing on their shape differences, so in Figure 1.9D they are rotated to an alignment that leaves only the shape differences. After removing all the differences that are not shape differences, and provided that this is done in a way that does not alter shape, we are left with only the shape differences. We can now use the coordinates of the final configurations (Figure 1.9D) to analyze these shape differences.

Representing an organism solely by a configuration of landmarks leaves out some aspects of what we might normally mean by shape, such as curvature. Curvature *is* a feature of an object that remains after filtering out location, scale and rotational effects, but it is not necessarily captured effectively by the coordinates of a set of landmarks. Because curvature fits the broad definition of shape, we can anticipate eventually having a theory of shape analysis that applies to the shapes of curves and is consistent with the theory that applies to configurations of landmarks.

Size

Kendall's definition of shape mentions scale as one of the effects to be removed to extract differences in shape between two configurations. The implication of this statement is that scale provides a definition of size that is independent of the definition of shape. The concept underlying geometric scale is quite simple, and may be intuitively obvious by visual inspection – in Figure 1.9A the landmarks are generally further apart in one configuration than in the other, which is what we would expect when a configuration is larger. Before computing geometric scale, we need to determine the location of the center of the form (its "centroid") and calculate the distance between each landmark and the centroid. Figure 1.10 shows the location of the centroid and the segments connecting the landmarks to the centroid for one of the piranhas we have been discussing. Now we compute geometric scale by calculating the square of each of those distances, summing all the squared distances, and then taking the square root of that sum. This quantity is called "centroid size."

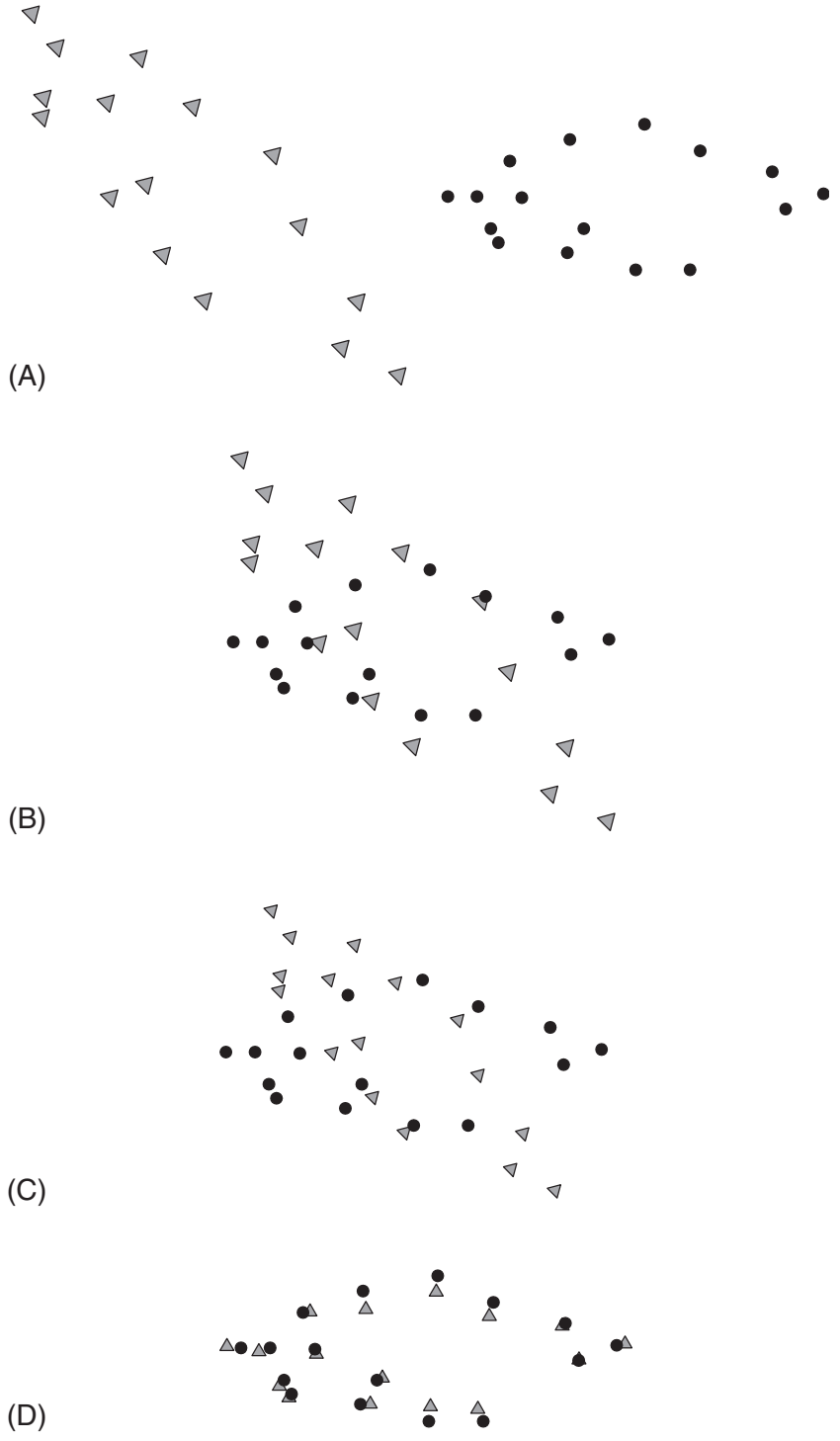

Figure 1.9 Removing variation due to differences in position, scale and orientation. (A) Two original configurations; (B) after removing differences in location; (C) after removing differences in scale; (D) after removing differences in orientation, leaving only differences in shape.

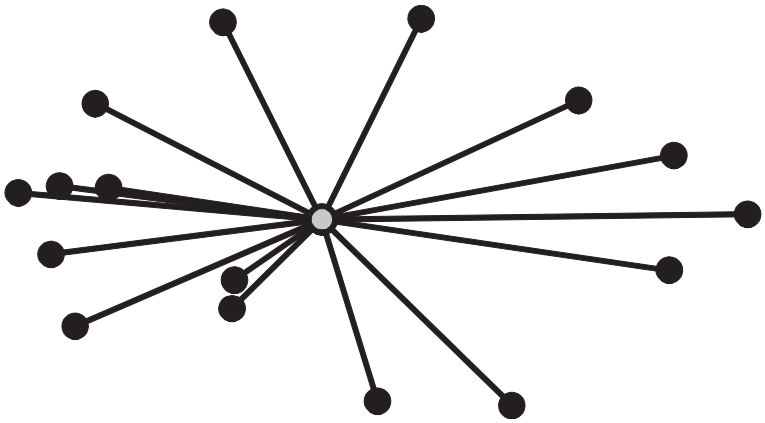

Figure 1.10 A visual representation of centroid size as computed for 16 landmarks on a piranha. The open circle is the centroid; the segments connecting the centroid to the landmarks represent the distances used to compute centroid size.

Centroid size is the one measure of size that is *mathematically* independent of shape. Empirically, centroid size may often be correlated with shape because larger organisms are usually shaped differently than smaller ones. The fact that we have measured shape and size separately does not mean that we lose any information about the relationship between them, any more than measuring shape and age separately bars us from analyzing their relationship. We can easily evaluate the empirical relationship between shape and size using those conventional statistical methods that can be applied to both size and shape data.

Methods of data analysis

Replacing the distances of traditional morphometrics with landmark coordinates does not force us to sacrifice conventional statistical analyses of shape. We can ask all the questions we have ever asked. Such questions often comprise two parts, the first of which Bookstein (1991) termed the "existential question": *is* there an effect on shape? We answer that by determining the probability that the association between variables is no greater than could have arisen by chance. The second question, "*what* is the effect?", calls for a description. In the ontogenetic series of piranhas discussed earlier, we can analyze the relationship between shape and size by computing the centroid size of each configuration of landmarks, and then computing the configurations of landmarks from which differences in position, scale and rotational effects have all been removed. These new configurations, shown in Figure 1.11A, represent the shapes of all the specimens. To answer the first question about the existence of an effect, we regress shape on centroid size using multivariate regression in which "shape" is the dependent variable and "centroid size" (or its logarithm) is the independent variable. For this example, we can conclusively reject the null hypothesis of no effect at $p < 1 \times 10^{-5}$ (we can also determine that 71% of the shape variation is explained by size). To answer the second question about the description of the effect, we present the pictures showing relative landmark displacement (Figure 1.11B) or the deformed grid computed by interpolation (Figure 1.11C).

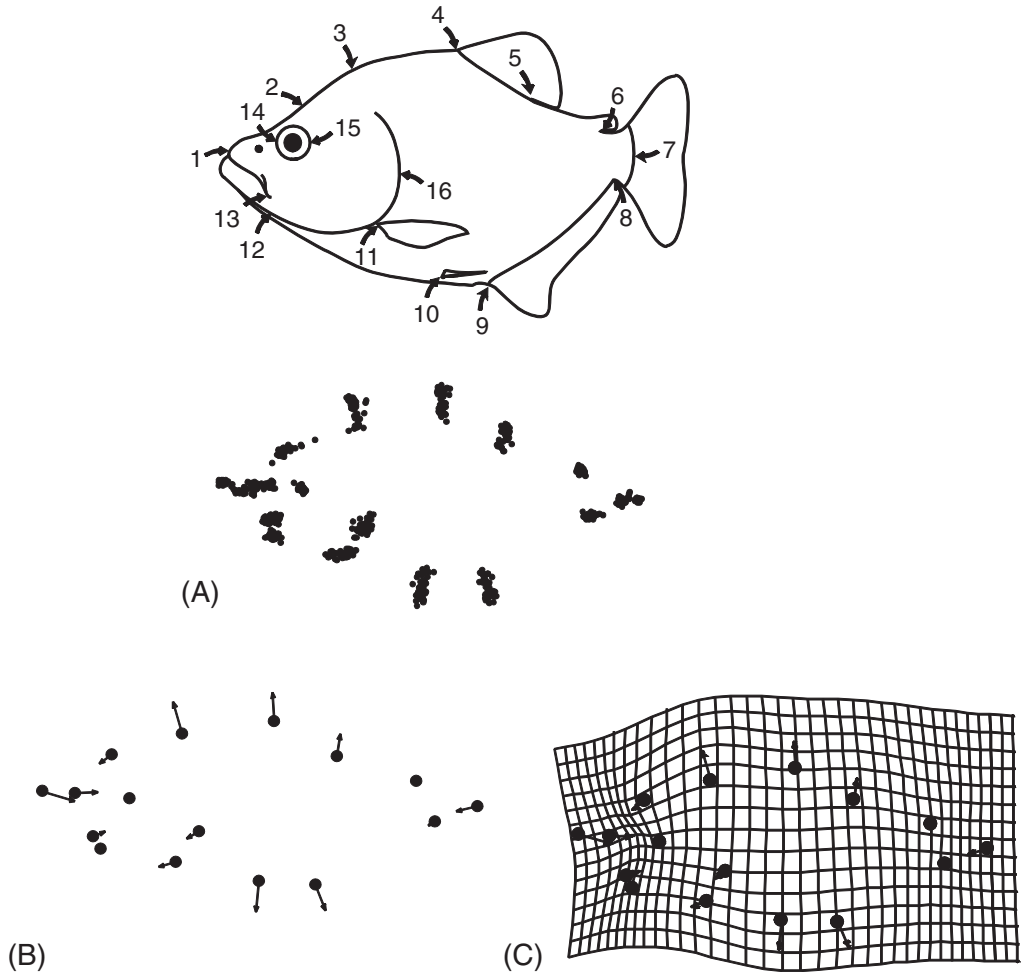

Figure 1.11 Analyzing the impact of size on shape by multivariate regression. (A) Configurations of landmarks from which differences in position, scale and orientation have been removed; (B) the covariance between size and shape depicted by vectors of relative landmark displacements; (C) the covariance between size and shape depicted by a deformed grid.

Replacing distances with coordinates also does not require us to abandon familiar ordination methods, such as principal components analysis and canonical variates analysis. These are methods that are used frequently to explore patterns in the data; their results include scatter plots of specimens that describe patterns of variation among individuals or differentiation among groups. These patterns often provide hints about the causes of variation or differentiation; hints that are reinforced by the accompanying graphics of the dimensions along which specimens most vary (Figure 1.12) or groups most differ (Figure 1.13).

The one important distinction between analyses of geometric shape data and those of conventional morphometric data is that all analyses of landmark configurations are necessarily multivariate. By definition, shape is a feature of the whole configuration of landmarks. Even the simplest shape, a triangle, cannot be analyzed univariately; more

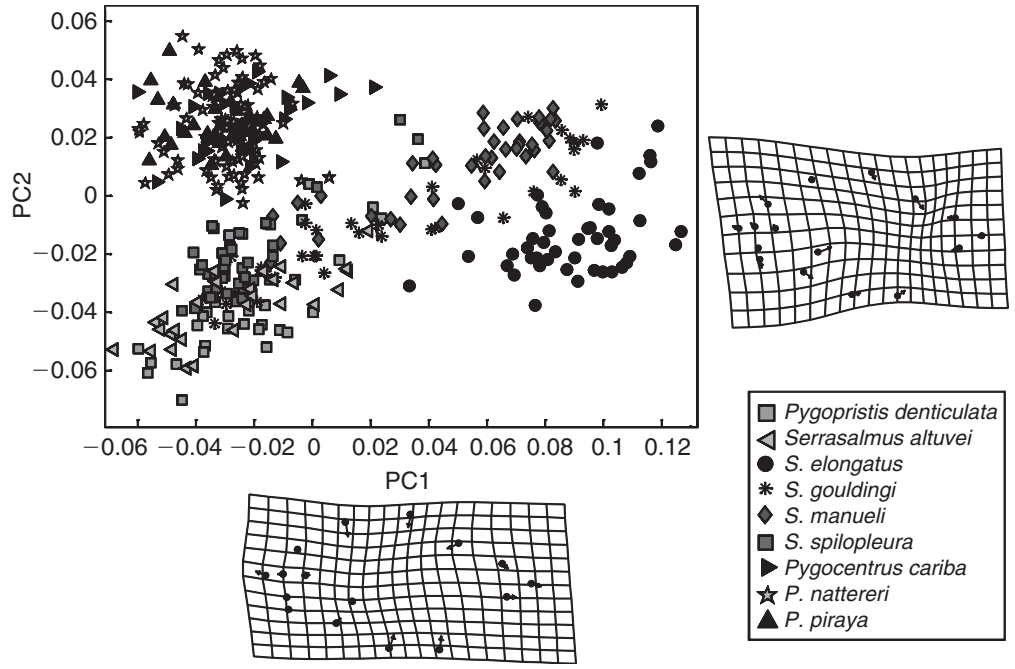

Figure 1.12 Principal components analysis of piranha body shape.

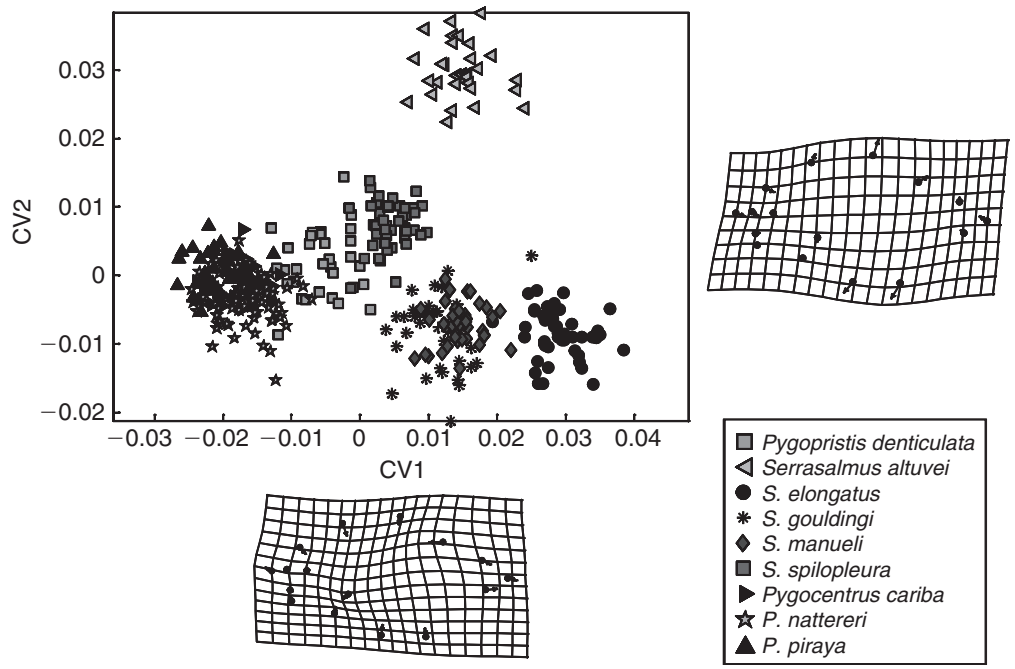

Figure 1.13 Canonical variates analysis of piranha body shape.

than one variable is needed to describe differences among triangles completely. We cannot simplify analyses (or interpretations) by partitioning the configurations of landmarks into subsets; subsets of landmarks are different shapes, not traits dissected from the whole. We cannot regress the coordinates of only one of the 16 piranha landmarks on size and consider the resulting coefficients to be a valid result about a part of the configuration of 16 landmarks. We cannot even regress the coordinates of 12 of the 16 landmarks on size, and consider the resulting 24 coefficients, taken together, to be a valid result about a part of the configuration. Because we have defined shape in terms of the whole configuration of landmarks, our analyses must be of that whole. However, this does not prevent us from subdividing an *organism* to analyze relationships between parts. For example, we could divide the piranha into the cranial and postcranial regions, and analyze the landmarks from each region as a separate configuration; we could then ask how the shapes of these two regions covary by analyzing the relationship between configurations. The requirement that *configurations* be analyzed multivariately and therefore as wholes does not force us to treat *organisms* as unitary wholes (although we may find out that they are).

Biological and statistical hypotheses

Few of the hypotheses of interest to biologists are as simple as the allometric hypothesis examined earlier. Only rarely can the more complex hypotheses be wrestled into the form of a statistical null hypothesis and its alternatives. The first difficulty is that the statistical null merely states that the factor of interest has no effect; this is the hypothesis we hope to reject in favor of the alternative hypothesis that the factor does have an effect. In this situation we have two hypotheses that are diametrically opposed, mutually exclusive. In contrast, many biological hypotheses are more complex, stating multiple alternative theories of causation, and these alternatives may not be mutually exclusive. Thus the real goal of many studies is to discriminate between expected effects, not to reject a hypothesis of no effect. Perhaps we are interested in the evolution of claw shape in crabs. We probably already know that claw shape has evolved; the more interesting (and difficult) question is whether the derived claw shape arose to enhance the ability to burrow into a muddy substrate or was intrinsically constrained by development (or both).

Another difficulty posed by realistic biological studies is that there may be other alternative hypotheses beyond the few we have chosen to test. For example, other explanations for the derived claw shape of the crabs might be an enhanced ability to block a burrow entrance or even to attract mates. We also might have several alternative theories about how development could constrain the evolution of claw shape.

Yet another obstacle to translating a biological hypothesis into a statistical one is that the complexity of the biological hypotheses rarely allows for adequate testing by any single method. To test whether the evolution of crab claw shape was intrinsically constrained by development, we must first determine whether development demonstrates any signs of constraint and then show that constraint could explain the evolution of claw shape. We should also show that the various adaptive hypotheses predict different evolutionary transformations than those specified by the developmental constraint hypothesis, so that we can rule out these biological alternative hypotheses.

In emphasizing the complexity of biological hypotheses we do not mean to say that they cannot be tested rigorously – they can be. However, doing so requires far more effort and creativity than testing the simple hypothesis that size affects shape. It also requires understanding what various analytic methods do, what their limits are, and how they are mathematically related. Far too often biologists use a limited array of techniques to analyze multivariate data, regardless of their questions. Throughout this book we emphasize the biological questions prompting a morphometric analysis, and underscore the applications of each method as we discuss them in turn. However, only after a variety of methods have been introduced (and mastered) can we begin to address questions of realistic biological complexity.

Organization of the book

We begin this book with a series of chapters covering the basics of shape data – what landmarks are and how to select them (Chapter 2), and how their coordinates are transformed into the shape variables that will be used in subsequent analyses (Chapters 3–6). The next section covers analytic methods: exploratory tools (Chapters 7 and 8) and more formal methods of hypothesis testing (Chapters 9–11). We then demonstrate the application of these methods to complex biological questions, which may require using multiple methods, both exploratory and hypothesis-testing (Chapters 12–13). The final two chapters cover issues that require continued development: Chapter 14 discusses the use of morphometric analysis in phylogenetic studies, and Chapter 15 covers some methodological topics on which there is still not complete consensus regarding either technical or graphical issues, but which are likely to yield promising new methods in the near future.

In presenting the basics of shape data, we follow the discussion of landmarks (Chapter 2) with a simple method of producing shape variables (Chapter 3) – namely the two-point registration that yields Bookstein's shape coordinates (Bookstein, 1986, 1991). These variables are easily understood, easily calculated by hand, and do not require an understanding of the general theory of shape. Presenting them first allows us to discuss a number of general issues (including the interpretation of results) before presenting the more abstract theory of shape analysis in Chapter 4. That theory provides the framework for generating (as well as analyzing) shape variables. After reviewing the basic theory, we return to the subject of shape variables in Chapter 5. Chapter 6 discusses the thin-plate spline, an interpolation function useful for depicting results by means of a deformed grid (as in Figures 1.11–1.13), and also for obtaining a set of shape variables that can be used in conventional multivariate analyses.

The second section of the book concerns methods for analyzing shape variables. In a sense, all these methods are used to produce the biologically interesting variables – the ones that covary with the biological factors of interest. Unlike the variables produced by the methods of the previous section, the variables produced by these analytic methods have a biological meaning. They answer such fundamental questions as "What impact does size have on shape?", or "By how much, and in what way, do these species differ in their ontogenies?", or "Do these populations vary along a single latitudinal gradient?", or even "What shape has the highest fitness in this population?" Each of these questions is answered in terms of a shape variable – the shape covariates of size or age, of latitude or

fitness, or of any other factor of interest. When we do not have any such factors in mind in advance of a study, we can explore the data algebraically, using the methods of matrix algebra to determine if any interesting patterns emerge (principal components analysis, PCA, is an example of this kind of algebraic exploration).

Because many biologists begin a study by exploring patterns in the data, the section on analytic methods begins with an overview of ordination methods (Chapter 7). These are useful for extracting simple patterns from complex multidimensional data because they provide a space of relatively low dimensionality, capturing most of the variation among specimens (PCA), or most of the differences among groups (canonical variates analysis, CVA). We explain the algebra underlying these methods, compare them, and discuss when each is appropriate in light of particular biological questions.

The next three chapters cover methods of statistical analysis. We begin with an overview of computer-based statistical methods, i.e. computer-intensive methods for constructing confidence intervals and/or hypothesis testing, such as bootstrapping and Monte Carlo simulations (Chapter 8). The next two chapters discuss the two broad classes of hypotheses that are conventionally tested statistically. Chapter 9 addresses hypotheses about the effects of an independent categorical variable – Hotelling's T^2-test, analysis of variance (ANOVA), and multivariate analysis of variance (MANOVA); Chapter 10 addresses hypotheses about the effect of a continuous variable on shape (regression). The final chapter in this section, Chapter 11, covers a method new to morphometric studies, one that analyzes the covariance between two blocks of variables, partial least squares analysis.

The third section covers applications of morphometric methods to realistically complex biological hypotheses, addressing more than just existential questions and requiring more of the answers than just descriptions. We begin with hypotheses that are often stated only in words, discuss framing them in the terms of more precise formal models, and then reframe these models into terms suited to statistical analysis. Once a hypothesis has been framed in the last set of terms, data analysis can proceed in a quite straightforward fashion, combining an array of techniques. As examples of complex biological questions we include those posed by studies of disparity and variance (Chapter 12), the analysis of relationships between ontogeny and phylogeny (Chapter 13), and also systematics (Chapter 14). The latter chapter represents a bridge between complex but tractable questions and subjects in need of additional tools.

The final chapter of this book (Chapter 15) briefly discusses two important areas in which a full set of tools have not been developed yet: (1) methods for analyzing three-dimensional coordinate data, and (2) methods for analyzing shapes of curves where no discrete anatomical loci can be found (by locating and analyzing points called "semi-landmarks"). Neither of these subjects is properly part of a primer that focuses on well-developed, uncontroversial methods, but both are important for biologists, and both are subjects of intensive ongoing work. In presenting these subjects we concentrate on the major points of departure (both conceptual and practical) from the primary subject of this book, the analysis of two-dimensional configurations of landmarks.

The terminology of statistical shape analysis can be daunting – there are many unfamiliar words and many terms differ by only a single letter or subscript. Thus we conclude this book with a glossary of terms, including general statistical terms (e.g. population, sample) and more specialized terms of shape analysis (e.g. Procrustes distance, partial warps).

Software and other resources

Geometric morphometrics studies require fairly specialized software, not so much to analyze the data as to depict the results graphically. Fortunately, the necessary software is readily and freely available. As Mac users will soon realize, virtually all the compiled software runs under Microsoft Windows.

At present, one major source of software is located at the SUNY Stony Brook website: http://life.bio.sunysb.edu/morph. Follow the link to **Software** (the rest of the links go to other valuable resources, including information about meetings, courses, and a directory of many people interested in morphometrics, with links to their webpages). We recommend that anyone planning a morphometric study downloads the videodigitizing program, **TPSDig**. Not only is this a well-designed and extremely useful program, but also many writers of morphometric software assume that is the one used for data collection, so the format in which it outputs the data (TPS format) has become the standard input format for several programs. There are other useful programs in the TPS series, but we generally do not provide detailed instructions for using them because we can neither anticipate nor control any changes in them.

Another major source of morphometric software is located at the website: http://www.canisius.edu/~sheets/morphsoft.html. This software, called the Integrated Morphometrics Programs (IMP), is written by one of us (HDS) and every method of analysis discussed in this book can be implemented by software in this series. There are three categories of software: (1) General Release; (2) Undocumented Software (which lacks manuals but the programs run and have been extensively used in research), and (3) Beta-Software (which has not been used in any serious research project, so may need considerable reworking before it is fully useful). There are some additional programs available that have been used in published research and so are made available; these can be found at the end of the "Update Information." At the end of most chapters of this book, we provide instructions for using the relevant software. These instructions are based on versions of the programs that have been frozen, so that you can run all the programs using these instructions. We do, however, anticipate upgrading the software; these upgrades will be available on the website and will (eventually) be documented. Major changes will be detailed in the "Update Information" on the bottom of the morphsoft webpage.

Running the IMP programs, which are written in Matlab (Mathworks, 2000) and compiled to run under Microsoft Windows, requires first installing a large package of software, **mglinstaller** (detailed instructions for installing it, and for installing other programs in the IMP series, are given below). Different versions of Matlab are often incompatible with each other (both upwardly and downwardly), so programs written in the future, using a newer version of Matlab, will require installation of a new version of **mglinstaller** (in a different directory).

Another important resource is the listserver Morphmet. It is useful to subscribe to this list, if only to be informed of new software and notified of any mathematical mistakes or bugs in the programs. Additionally the list is sometimes quite active, discussing topics of general interest, including conceptual issues like the meanings of size and shape, and practical issues like dealing with preservational artifacts. Some recent posts have also provided extensive bibliographies of morphometric studies of mollusks and fishes.

To subscribe to this list, send an email to majordomo@wfubmc.edu and include the following single line in the body of the message: subscribe morphmet.

Downloading and installing mglinstaller

Before you use any program in the IMP series, you need to download and run the self-expanding **mglinstaller** (megalo-installer). This will create the directories (folders) where the other IMP programs must be installed. To download **mglinstaller**, go to the IMP website (http://www2.canisius.edu/~sheets/morphsoft.html or www.biocollections.org), find **mglinstaller** and click on it. This is a very large file, so it may take a while to download. After the download is complete, you need to create the directory (folder) where you want **mglinstaller** to be expanded. We recommend you call this folder **Matlab6** so you can keep track of the version of Matlab used to write the software. Now expand **mglinstaller** in that directory. It will create a folder in **Matlab6** called **bin**, and a folder in **bin** called **win32**; it will also unpack a series of files needed to run the other IMP programs. The other programs are also packaged as self-expanding files. After you download them, they *must* be expanded into the folder **win32**. If they are not installed in **win32**, they will not run.

References

Albrecht, G. (1978). Some comments on the use of ratios. *Systematic Zoology*, **27**, 67–71.

Atchley, W. R. and Anderson, D. (1978). Ratios and the statistical analysis of biological data. *Systematic Zoology*, **27**, 71–78.

Atchley, W. R., Gaskins, C. T. and Anderson, D. (1976). Statistical properties of ratios. I. Empirical results. *Systematic Zoology*, **25**, 137–148.

Bookstein, F. L. (1986). Size and shape spaces for landmark data in two dimensions. *Statistical Science*, **1**, 181–242.

Bookstein, F. L. (1989). "Size and shape": A comment on semantics. *Systematic Zoology*, **38**, 173–190.

Bookstein, F. L. (1991). *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.

Bookstein, F. L., Chernoff, B., Elder, R. et al. (1985). *Morphometrics in Evolutionary Biology*. The Academy of Natural Sciences of Philadelphia.

Corruccini, R. S. (1977). Correlation properties of morphometric ratios. *Systematic Zoology*, **26**, 211–214.

Dodson, P. (1978). On the use of ratios in growth studies. *Systematic Zoology*, **27**, 62–67.

Hills, M. (1978). On ratios – a response to Atchley, Gaskins and Anderson. *Systematic Zoology*, **27**, 61–62.

Kendall, D. (1977). The diffusion of shape. *Advances in Applied Probability*, **9**, 428–430.

Kendall, D. G. and Kendall, W. S. (1980). Alignments in two-dimensional random sets of points. *Advances in Applied Probability*, **12**, 380–424.

Klingenberg, C. P. (1998). Heterochrony and allometry: the analysis of evolutionary change in ontogeny. *Biological Reviews*, **73**, 79–123.

Lagler, K. F., Bardach, J. E. and Miller, R. R. (1962). *Ichthyology*. John Wiley & Sons.

Strauss, R. E. and Bookstein, F. L. (1982). The truss – body form reconstructions in morphometrics. *Systematic Zoology*, **31**, 113–135.