

# 1

## *Introduction*

### **Morphometrics is the study of covariances of biological form.**

The objects of morphometric study are not the forms themselves, but rather their associations, causes, and effects. This book treats the theory and practice of such studies in their most important special case, the application to **landmark data**. Most of the text introduces and exemplifies a diversity of modern geometric tools that increase the sensitivity and specificity of biological explanations of form. In this introductory chapter I tersely present the four main design principles of these tools and lead the reader through a typical example to demonstrate their interplay. The example is followed immediately by a second introduction aimed at the reader more conversant with statistics than with biology. The chapter closes with a brief sketch of the organization of the rest of the book.

#### 1.1. FOUR PRINCIPLES

Many logical and methodological themes recur throughout this book. I have collected them here in the guise of “principles,” tenets the meanings and implications of which will echo again and again in the theorems, constructions, and examples to follow. The principles appear together in this section and the next and then go their separate ways throughout the next six chapters. Chapter 8 draws them together again to serve as a framework for the summary of the techniques recommended here as “routine.” Their limits, also mentioned many times in Chapters 2 through 7, will suggest the closing speculations of Chapter 8 on new tools for the next edition of this tool kit.

## 1.1. Principles

### 1.1.1. First Principle: landmark locations

In many biological and biomedical investigations, the most effective way to analyze the forms of whole biological organs or organisms is by recording geometric locations of **landmark points**. These are loci that have names (“bridge of the nose,” “tip of the chin”) as well as Cartesian coordinates. The names are intended to imply true homology (biological correspondence) from form to form. That is, landmark points not only have their own locations but also have the “same” locations in every other form of the study and in the average of all the forms of a data set (see Section 5.4).<sup>1</sup> An explanation of findings based on covariances of landmark locations usually will be phrased as an argument either that particular processes “push landmarks around,” “push them apart,” “deform substructures,” and so forth, during part or all of ontogeny, or else that evolution has been induced to arrange similar changes over phylogeny.

Chapter 3 is devoted to the lore of landmarks: theory, practical hints, and introduction of the five extended examples that underlie most of the worked analyses later in this book. Section 2.4 will briefly touch on other styles of data concerning biological form, such as textures or outlines. In general, these other styles sustain explanations not in terms of relative displacements (often there is nothing to point to as undergoing “displacement”) but only using different semantics of much weaker force: bulges form or do not (but how does one know the locations are comparable?); cells cluster linearly or circularly (but how can we describe the locations, orientations, or directions of the clusters?). An example later in the book will indicate how information from curving outlines can be analyzed effectively once the landmarks are dealt with.

### 1.1.2. Second Principle: shape coordinates

Measurement of the shapes of configurations of landmark locations reduces to multiple vectors of **shape coordinates**. These come in pairs that represent the shape of one triangle of landmarks in a manner completely independent of size. That is, the study of covariances of landmark configurations *begins* when the configurations are represented by multiple triangles. This is equally true in two dimensions or in three. For two-dimensional data the triangulation can be managed by locating all other landmarks with respect to two in particular that are held fixed at points with coordinates (0, 0) and (1, 0) of an abstract “common digitizing plane.”

<sup>1</sup> The organization of this book is lexicographic by chapters, sections, subsections, and sub-subsections. Thus Section 5.4 is the fourth section of Chapter 5. Figures, tables, and equations are numbered serially within each *section*. Thus Table 3.4.2 is the second table in Section 3.4, and Figure 7.3.5a is panel *a* of the fifth figure in Section 7.3.

That triangles are sufficient to represent landmark data for the purpose of studying their associations of shape is the subject of Chapter 5. I am not claiming that the shape coordinates are likewise sufficient for *reporting* the findings they generate; the form of these reports is the subject of the Fourth Principle. The reduction of landmark configurations to sets of shape coordinates is the most pictorial of a few statistically equivalent ways (see Section 5.6) to circumvent all further arbitrary choices of coordinate systems or of “variables” once a selection (itself unavoidably arbitrary) of landmarks has been made.

#### 1.1.4. Answers

#### 1.1.3. Third Principle: the form of questions

All the main styles of biometric investigation can be realized upon landmark data by submitting the shape coordinates to multivariate statistical analyses. Although the analyses may be nearly standard in form, the questions to be asked need not be standard at all. They may refer to individual diagnoses, individual forecasts of future form, planning of individual treatment, detection or description of group differences, effects of growth or age or size difference upon form, covariances of outside factors with form, ecophenotypy, covariances of form with its nonmorphometric consequences (such as function or survival), treatment or selection effects upon growth or form, detections of patterns of systematic variability of form at diverse geometric scales, or any of a number of other possibilities.

Most of these questions can be posed in two semantically distinct variants: not only “*Is there (statistical evidence for) a covariance between the landmark data and (the putative factor or covariate)?*” but also “*What is the nature of the covariance between the landmark data and (the putative factor or covariate)?*” These interrogative forms exclude the questions typical of earlier applications of morphometrics in numerical taxonomy. Questions about “similarity” or “common ancestry” are not easily phrased in terms of covariances and have no particular meaning in the context of landmark data.

#### 1.1.4. Fourth Principle: the form of answers

The most unusual of this book’s themes is the form I recommend and exemplify for reporting answers to these morphometric questions. Findings usually are best represented not in conventional statistical tabulations but instead via geometric diagrams superimposed over a picture or drawing of a typical form. Ordinarily, a single data analysis will exploit several different diagrams *each of which corresponds to one part of the covariance signaled by a single statistical analysis*. The “parts” are size change and shape change, or size variation and shape variation; the shape part is further subdivided at a diversity of geometric scales. The diagrams graphi-

## 1.2. *The phenytoin face*

cally express formulas for particular “variables” – distances and ratios – aligned with the pattern of covariances or effects uncovered by one of these parts of the analysis.

For instance, an effect on the shape of a single triangle of landmarks may be drawn as a vector attached to any one of its vertices; the same effect usually can be drawn as an ellipse – a tensor – the axes of which refer to ordinary distance measures. The two reports have identical multivariate statistics but correspond to rather different sorts of biological explanations or metaphors. A size effect can be incorporated in the picture of the ellipse, but that is graphically separate from the depiction of the same shape change as displacement; which will be the more suggestive of biological insight will depend on the phenomenon under study. The application of either of these diagrams may be to any single triangle or instead to an “average triangle” – the *uniform component* of shape difference or shape variation, to be introduced in the next section. Likewise, effects on configurations of more than three landmarks might be reported as “displacements,” “growth gradients,” or “deformations” that are, in turn, either “global” or “local,” and in which size change is variously a parameter or a covariate. In fact, in landmark-based morphometrics, the exploration of multiple interpretations of overlapping findings is the model for good reporting.

### 1.2. A TYPICAL EXAMPLE: THE “PHENYTOIN FACE”

#### 1.2.1. A study of prenatal exposure to phenytoin

Dr. Lewis B. Holmes, M.D., director of the Embryology–Teratology Unit at Massachusetts General Hospital, has kindly permitted me to reproduce an example drawn from his long-term study of phenytoin effects. Phenytoin is an anticonvulsant (specifically, antiepileptic) agent often prescribed for adults in the form of a maintenance dosage. Recently it has been suspected of being teratogenic to fetuses when administered through the mother. In a manner typical of explorations in teratology, this suspicion was founded on a psychological, not a biological, finding: A trained dysmorphologist often can classify children as “exposed” or not – that is, can characterize certain children as having “the face” (of phenytoin) – without being able to report in words or diagrams exactly what it is that generates that recognition. (A similar “feeling” led to the discovery of Fetal Alcohol Syndrome, the most prevalent avoidable birth defect.)

Once a dysmorphologist spots a reliable similarity among faces in association with a common insult, it becomes possible to mount a sustained investigation of dose–response relationships, covariances between morphological and functional aspects of teratogen-

esis, and the like. Holmes's project, of which this example is a small part, is designed in this spirit: The facies corresponding to prenatal damage will be used as proxy for effective dose in studies of neurologic sequelae. One of the principal running examples of this book, treated recurrently in Chapters 5 through 7, is the report of a prospective study involving the morphological consequences of prenatal exposure to moderate levels of alcohol. Here, too, the larger intention is to use the face as proxy for measures of specific brain damage.

### 1.2.2. Data

#### 1.2.2. The data: sample, photographs, landmarks

For preliminary analysis, Holmes selected a sample of 45 older children, all of whom appeared to have "the face" of phenytoin. Of these, 31 had been exposed to phenytoin *in utero* in typical dosages. (Thirteen of these children had also been exposed to phenobarbital, but there appear to have been no additional effects of that exposure upon the data we explored.) Each of the 45 was photographed from the front in a manner that, because ours was a no-budget pilot study, was uncontrolled. Not much attention was paid to issues of head position, and no information about absolute scale is available (camera-to-subject distance was not monitored, nor is there a ruler visible in the photographs). Thus we can study only shape, not size, for these faces. The landmarks selected for this project numbered 16 on each frontal photograph. Although they were digitized directly from the photographs, it is simplest to display them schematically in outline drawings of "typical" forms from the two groups (Figure 1.2.1).

For the analysis here, four landmarks from the midline and six pairs of lateral landmarks were located by pinholes through actual prints and digitized at a conventional digitizing tablet to the nearest 0.1 millimeter. This is in accordance with the First Principle, which suggests the thoughtful reduction of pictorial data to named landmark locations as the first step in any analysis of organic form above the tissue level. These landmarks are discussed further, along with other facial landmarks, in Section 3.4.4;

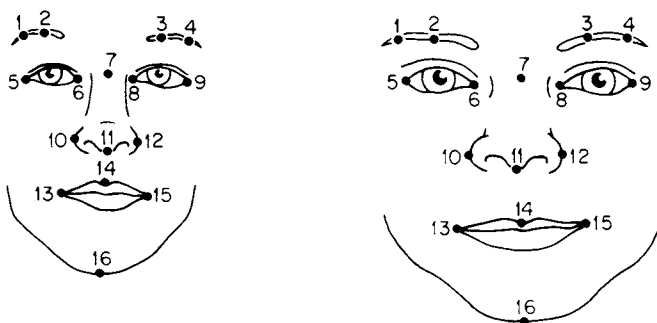


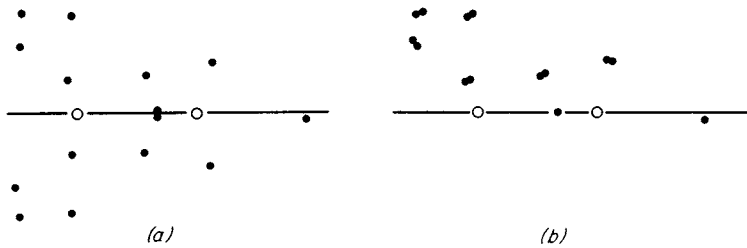
Figure 1.2.1. Typical frontal forms from the phenytoin face study. Tracings of typical unexposed (left) and exposed (right) subjects have been altered between landmarks to conceal subjects' identities. The 16 landmarks of the morphometric analysis are indicated by serial numbers (see Table 1.2.1).

Table 1.2.1. *Landmarks for the phenytoin study*

Landmark name	Number(s)	Description
<i>Midline landmarks</i>		
Nasal bridge	7	Bridge of the nose
Columella	11	Bottom of the columella where it springs from the upper lip
Upper vermilion	14	Midpoint of Cupid's bow, upper vermilion border
Chin	16	Lowest midline point of symphysis
<i>Bilateral landmarks</i>		
Eyebrow central	2, 3	Intersection of the eyebrow curve with a vertical line through the midpoint of the pupil
Eyebrow lateral	1, 4	Intersection of the eyebrow curve with a vertical line through exocanthion
Exocanthion	5, 9	Lateral intersection of upper and lower eyelids
Endocanthion	6, 8	Medial intersection of upper and lower eyelids
Nasal ala	10, 12	Most lateral points on alar curvature
Lip commissure	13, 15	Lateral intersection of upper and lower vermilion borders; also known as Cheilon

see also Farkas (1981). Holmes adapted this particular set of landmarks from Clarren et al. (1987).

Following the Second Principle, the archiving of these landmark locations is quickly followed by a reduction to the descriptor space of shapes: their expression as shape coordinates to some convenient baseline. The appropriate formulas, such as equations (5.1.1), will be explained *in situ*. In this approach, two relatively reliable landmarks are fixed in position on the page (by a combination of scaling, translating, and rotating); the resulting locations of all the other landmarks express their positions "with respect to" that fixed pair. (However, the "size variable" that would have been associated with any of these shapes had the photographs preserved scale information is *not* the scale factor divided out in the course of this construction; see Section 5.5.) For nearly symmetric forms, it is better to choose a baseline along the axis of putative symmetry. Of the four landmarks along the midline (Table 1.2.1), the most reliable are the first and third, Nasal bridge (#7) and Upper vermilion (#14), and so that segment serves as our baseline for this first exemplary analysis (Figure 1.2.2a).



### 1.2.3. Questions

Figure 1.2.2. Construction of the hemiface (symmetrized frontal view). (a) Fourteen shape coordinates to a baseline from #7 to #14. (b) Reflection across a convenient midline, to be followed by averaging (not shown).

Each shape coordinate that results from this construction may be interpreted as a legitimate, though often rather odd-sounding, ratio of distances, that is, a conventional shape variable. The vertical shape coordinate (horizontal in Figure 1.2.3) is the more familiar; doubled, it represents the distance between the inner corners of the eyes in this coordinate system normalized to the “upper facial height” from Nasal bridge to Upper vermillion, and so it is actually the ratio of intercanthal distance to that version of upper facial height. The other coordinate in the same plot is the proportional height of the inner canthi upon that same upper facial baseline. Whereas these may seem to provide an odd and arbitrary foundation for any study of the corner of the eye, the theorems in Chapter 5 ought to convince you that they are sufficient. No set of shape measures of a landmark configuration can be more powerful than the corresponding set of shape coordinates for detecting or diagramming group differences or other covariates pertaining to “aspects of shape” not specified or restricted in advance of the analysis. That is, there is nothing to lose by proceeding via these coordinates, but a great deal to gain in terms of multiplicity of feature spaces, availability of alternate interpretations, and the like.

Although the data were digitized on both sides of the face, it is more efficient to analyze the landmarks in a symmetric form. Geometrically, one might imagine picking up the left side of the face, reflecting it over a “midline” to superimpose on the right, then averaging the sides. The effect of this operation is not much changed by small deviations in the actual midline locus chosen for this exercise. Following the Second Principle, we carry out the symmetrization using the shape coordinates just created. We “reflect” the left side over the midline that is used as the baseline (Figure 1.2.2*b*), then average the shape coordinates of left and reflected right landmarks. Algebraically, this is the same as averaging the coordinates after the second (anatomically horizontal) coordinate of one side has had its sign reversed.

### 1.2.3. Questions

The question asked of this sample of 45 symmetrized forms is exactly in accordance with the Third Principle. It deals with the covariance between form (landmark configuration) and an exoge-



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nous variable (exposure to phenytoin or not). Specifically, we inquire whether or not there are statistically significant differences between the group mean forms and, if so, which biological process(es) might account for these differences. Underlying this mode of explanation is the epidemiological/teratological supposition that those truly exposed were systematically affected by the prenatal insult, whereas those not truly exposed have come to resemble “the face” in unpatterned, haphazard ways.

#### 1.2.4. Findings

##### 1.2.4.1. A shift “at” Endocanthion. —

It is customary to submit a set of shape coordinates to one single multivariate test for some large general hypothesis (group difference, size or age effect, etc.) at issue. This proved unnecessary for these data, as the difference between the group means proved adequately significant for one of the shape-coordinate pairs by itself: Endocanthion (inner corner of the eye), as shown in the scatter in Figure 1.2.3. In the lateral coordinate of this plot, which is the ratio of endocanthal width to an “upper facial height,” the 16 highest values [relatively most hypertelorlic (widely set) eyes] pertain without exception to children who were, in fact, exposed. The difference in mean ratio is about 12%: 0.549 for the 14 unexposed children, 0.613 for the 31 exposed children. The appropriate  $T^2$ -test (not a  $t$ -test, in light of the full plane of directions of shape change from which we were free to choose; see Section

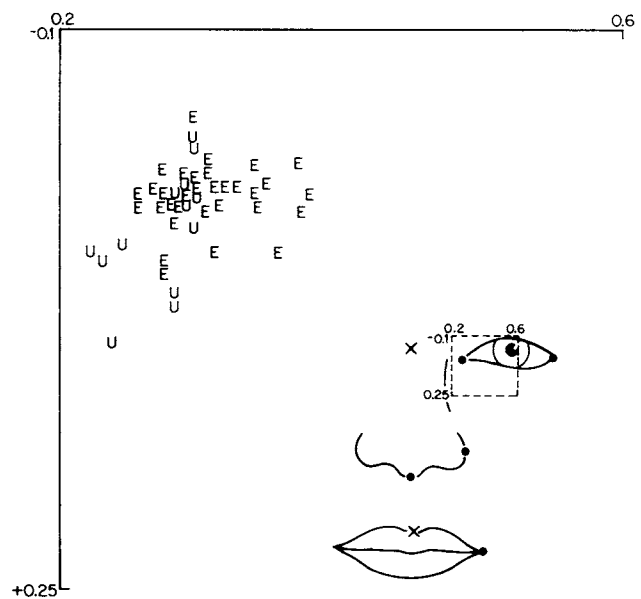


Figure 1.2.3. First finding of the phenytoin-face study. Scatter of shape coordinates for Endocanthion. Shape space has been rotated back to anatomical vertical. U, unexposed; E, exposed. (Recall that all cases appeared subjectively to have “the face” of phenytoin.)



6.5.1.3) is conventionally “significant” at about  $p \sim .007$ . These shape coordinates seem quite well behaved, without obvious sub-clusters or outliers.

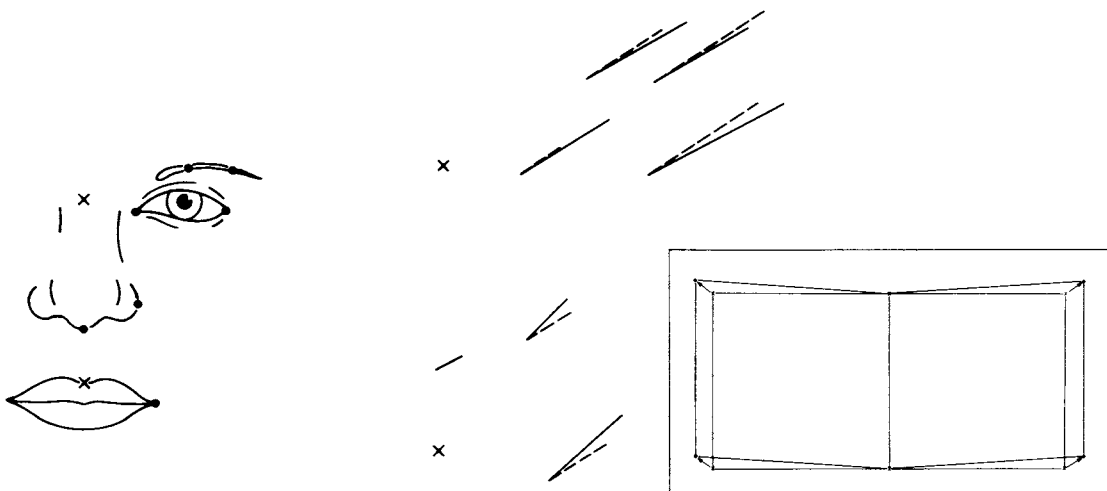
From this single figure, one cannot yet tell how to interpret the finding. Although extracted by manipulation of one set of shape coordinates, it actually expresses the change of shape of one entire triangle (Nasal bridge–Upper vermilion–Endocanthion). Furthermore, we cannot yet say whether the “effect” (the reconfiguration of landmarks within the criterion of having “the face”) is specific to this triangle or is instead distributed somehow over the whole photograph in a less focused fashion. That is, in accordance with the Fourth Principle, its “effect” must be construed wholly independently of arbitrary choices in the course of carrying out the statistical analysis. Reporting the difference, then, must wait until we have thoroughly explored all the other feature spaces that are relevant to a biological interpretation under the Fourth Principle.

#### 1.2.4.2. A uniform shear.—

The diagram in Figure 1.2.4, explained further in Section 7.2, is a serviceable exploratory display style for the pursuit of certain large-scale explanations. I have enlarged the relative displacements of all the intergroup mean shape coordinates 10-fold so as to highlight the deviations of these mean differences from a particularly simple geometry, the “linear” or “uniform” model. In the model, all shape change is by the same ratio of increase in length as a function of direction, regardless of location. In this application, as applied to symmetrized data, it represents a transformation of the two sides of the face outward and upward from

#### 1.2.4. Findings

Figure 1.2.4. Second finding of the phenytoin-face study: the intergroup displacements, enlarged 10-fold, between mean landmark locations to the upper facial base-line. (The origins of the vectors are not altered; they spring from the mean landmark locations for the unexposed group.) Solid lines: Observed mean landmark shifts. Dashed lines: Best-fitting uniform (linear) transformation between these means, under the “null” probability model explained in Section 7.2. Note the discrepancy between dashed and solid lines at Endocanthion: The intergroup divergence there is double what would be predicted from the others. Inset right: Sketch of the uniform transformation fitted by the dashed displacements, no longer multiplied 10-fold.



### 1.2. The phenytoin face

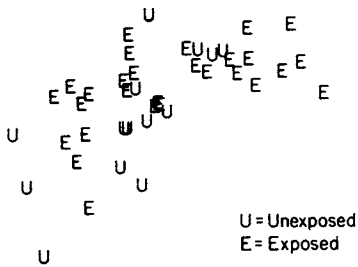


Figure 1.2.5. Third finding of the phenytoin-face study. The uniform component of shape variation, case by case, resembles that for Endocanthion alone (finding 1), but with greater group overlap.

the midline by parallel vectors all proportional to the original bilateral width landmark by landmark.

We can report the fit of this model by a quantity analogous to the “fraction of variance explained” in ordinary regression: 72% of the observed mean shift of shapes between the landmark configurations is summarized in one single global pattern, the change in width ratio by about 6% together with an upward cant of about half that extent. Most of the remaining 28% of this generalized sum of squares is the additional upward-outward expansion (amounting to 12%, not 6%) at Endocanthion.

#### 1.2.4.3. Scatter of a uniform component. —

Using equation (7.2.2) we can express this linear component of the group difference in a scatter having one point for every child, an “estimated linear component.” Information from Endocanthion has been *omitted* from this estimate. That scatter (Figure 1.2.5) resembles what we have already seen for the most sensitive landmark, Endocanthion, separately. The difference between the exposed group and the unexposed is aligned with the displacement from the vaguely elliptical scatter of U’s to the somewhat differently shaped ellipse of E’s. The separation is not perfect: some of the exposed children were not characterized by this upward-outward shear of the lateral landmarks. A similar observation applies to the scatter of the shape coordinates for Endocanthion alone (Figure 1.2.3). Apparently the dysmorphologist misidentified some nonhypertelorlic subjects as having having “the face” on the basis of a different feature or combination, but he was right in all sufficiently hypertelorlic cases. There is variability of expression of the effect within the “exposed” ellipse, but its coefficient of variation, in comparison with the mean difference from the “unexposed” group, seems under adequate control.

#### 1.2.4.4. Summary. —

There is a better way to explain this combination of features than “a linear term plus hypertelorism.” Looking again at Figure 1.2.4, we see that the four points of the orbital region are not changed much in shape. What we see might better be described as changes in the *positions* of those landmarks with respect to a baseline that is also changing its own relative scale. Visualizing this interpretation is much clearer with reference to a baseline on one of the rigid components of the rearrangement (see Section 7.4.4.1). At the left in Figure 1.2.6 is a display of the same group comparison (this time the changes multiplied by a factor of 5) to a baseline across the eye from inner to outer corner. The small changes in the positions of the eyebrow points can be ignored. Notice that Nasion appears to move relatively *downward*, the entire lip area