Geometric Morphometrics: Recent Applications to the Study of Evolution and Development

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Abstract

The field of morphometrics is developing quickly and recent advances allow for geometric techniques to be applied easily to many zoological problems. This article briefly introduces geometric morphometric techniques and then reviews selected areas where those techniques have been applied to questions of general interest. This article is relevant to non-specialists looking for an entry into geometric morphometric methods and for ideas of how to incorporate them into the study of variation within and between species, the measurement of developmental stability, the role of development in shaping evolution, and the special problem of measuring the shape of fossil specimens that are deformed from their original shape.

Keywords: geometric morphometrics; shape coordinates; landmark data; Procrustes

Introduction

The geometric morphometric revolution has added to the sophistication of quantitative biological shape analysis, while at the same time making it easier to collect and analyze data to answer questions about shape of the phenotype (Bookstein et al., 1985; Bookstein, 1991; Marcus et al., 1996; Dryden & Mardia, 1998; MacLeod & Forey, 2001; Zelditch et al., 2004). Shape is the geometric information of an object after removing location, orientation and scale (Kendall, 1977) – many zoological research questions concern shape: To what species does an osteological specimen belong? Is sexual dimorphism in skull morphology related to sex-specific food procurement roles? Are phenotypic differences that arise during speciation due to genomic reorganization or to simple allometric growth shifts? These questions and others are best addressed with statistical tests, which often require a sophisticated quantitative representation of the phenotype that
captures the functional, genetic, or developmental attributes that are biologically important for the hypothesis being tested. Geometric morphometrics is particularly suited to test such hypotheses because of the sophistication with which it can be used to measure phenotypic shape.

Use of geometric morphometrics has increased rapidly in the biological sciences over the past decade – A keyword search with the phrase “geometric morphometrics” in Google Scholar reveals an increase in publications per year from roughly 50 in 1998 to approximately 225 in 2007. Here we review the variety of some of those applications in zoology, especially studies that address differences within and between species, developmental stability, the role of development in shaping evolution, and problems arising in addressing these issues in the fossil record. Because the zoological geometric morphometric literature is increasingly vast, we concentrate our review on papers published in the past three years, which only scratches the surface of most topics, but we attempt to refer readers back to seminal papers on each topic. Interested readers are encouraged to consult other recent reviews of geometric morphometrics (Rohlf, 1990; Rohlf & Marcus, 1993; Bookstein, 1996; O’Higgins, 2000; MacLeod, 2002; Richtsmeier, Deleon & Lele, 2002; Adams, Rohlf & Slice, 2004; Slice, 2007; Polly, 2008a). Our review is intentionally non-technical and will be of most interest to those who are not already familiar with geometric morphometrics and who are looking for an entry into the literature of how it can be applied to address zoological problems.

Geometric Morphometric Techniques

‘Geometric morphometrics’ is the analysis of Cartesian geometric coordinates of morphological structures rather than linear, areal, or volumetric variables. Geometric morphometrics is often hailed as a ‘revolution’ in morphometrics (e.g., Adams et al. 2004); in some ways the focus on geometric coordinates has been a revolution, but precise delineations of the field from so-called ‘traditional’ morphometrics become murky because a range of techniques exist that grade between analysis of Cartesian coordinate variables and size variables, some of which, such as EDMA, are introduced below. Indeed, many of the underlying mathematical algorithms used in geometric morphometric analysis are identical to those that have been used in morphometrics for many decades. Arguably the most distinctive feature of most geometric morphometrics is that results can usually be reported using the same geometric coordinates as a pictorial representation of the morphological structure itself. A heterogeneous set of tools are combined under the term geometric morphometrics. Tools are available to analyze landmark points, curves, outlines (in either two- or three-dimensions), and three-dimensional surfaces. Details of how the geometry of objects is mathematically represented differ from one method to another, but all geometric morphometric methods use the Cartesian coordinates of comparable features on the objects as their variables. Most methods transform the landmarks, curves, or outlines of objects into ‘shape coordinates’, which are new variables that can be analyzed with the full range of ordinary multivariate statistical procedures, while others transform them into a matrix of inter-landmark distances similar in many ways to older kinds of morphometric analysis. Often the shape coordinates are principal component scores
derived from Procrustes superimposition, but other types also exist. In this section we present a brief overview of typical methods with references for those readers interested in learning more.

The most common variety of geometric morphometrics uses landmark points that are aligned with one another using Procrustes superimposition (Sneath, 1967; Gower, 1975; Siegel & Benson, 1982; Rohlf & Slice, 1990). Because geometric morphometric variables are Cartesian coordinates, a common coordinate system must be used to specify landmark locations on all objects – Procrustes superimposition rotates, translates and scales landmarks so that they have the same orientation and size (Rohlf & Slice, 1990) – the removal of differences in orientation, position, and size allows pure shape to be analyzed. Procrustes superimposition has the potentially undesirable effect of spreading variance in shape as equally as possible across all landmarks as a result of the least-squares algorithm, meaning that it is impossible to determine which, if any, landmark is more variable in position than another without an *a priori* model of shape variance for the structure being studied.

Euclidean distance matrix analysis (EDMA) is an alternative to superimposition methods, developed specifically to localize shape variance at particular landmarks to test for differences among objects at particular landmarks rather than overall differences in shape among objects (Lele, 1991; Richtsmeier & Lele, 1993; Richtsmeier *et al.*, 2002). EDMA uses as its data the linear distances among landmarks, which do not depend on Procrustes superimposition and which, therefore retain information about variation in specific landmark points, which is critical for some kinds of studies. A downside to EDMA is the results are not amenable to being illustrated as deformations of the original morphological shape as they are with Procrustes-based landmark analysis.

Several methods exist for the analysis of curves and outlines. Outlines can be analyzed using semi-landmarks, which are the points that fall at defined intervals along a curve between two landmarks (Bookstein, 1997a, 1997b). Semi-landmarks can be analyzed with Procrustes superimposition like ordinary landmarks. Another outline method is perhaps the oldest type of geometric morphometrics – Fourier analysis (Younker & Erlich, 1977). Fourier methods use sine and cosine harmonic functions to describe the positions of outline coordinates. Fourier analysis can be applied to two-dimensional outlines (Younker & Erlich, 1977; Ferson, Rohlf & Koehn, 1985) or three-dimensional closed surfaces (Styner *et al.*, 2006; McPeek *et al.*, 2008). Eigenshape is a third method for the analysis of outlines or curves (Lohmann, 1983; MacLeod & Rose, 1993; MacLeod, 1999). In eigenshape, the coordinate points of an outline or curve are converted to a *phi* function, which is a list of the angles from one point to the next one in the series. Outline methods have been criticized because their individual coordinate points are not each biologically homologous (Zelditch, Fink & Swiderski, 1995), but this issue is important only in cases where a one-to-one mapping between individual variables and biological homology is required. In most cases the outline objects themselves will be either biologically homologous or functionally analogous; if what is important is how those objects differ in their overall shape, then homology of individual coordinate points is a red-herring. Extended eigenshape is a method developed specifically to address the homology issue by allowing the ends of outline segments to be ‘pinned’ at biologically homologous points (MacLeod, 1999).
Geometric methods for the analysis of 3D surfaces is a developing area – to date, three approaches have been tried. The first uses a combination of landmarks and semilandmarks to represent the surface of a 3D object (Mitteröcker, Gunz & Bookstein, 2005; Wiley et al., 2005). Selected landmarks are Procrustes-superimposed and the remaining surface points are transformed along with them for pictorial ‘morphing’. Eigensurface is a second approach that reduces surface scan data to a grid of hundreds or thousands of surface points that are all included in the analysis as semi-landmarks (MacLeod, 2008; Polly, 2008b; Polly & MacLeod, 2008). Spherical Fourier harmonic analysis, SPHARM, is a third approach that works on the same principal as the 2D outline Fourier method described above (Styner et al., 2006; McPeek et al., 2008).

Once shape coordinates, which are a reduced number of variables that collectively describe the variation in the original shapes, have been derived using one of these methods, any type of multivariate statistical analysis can be performed (Bookstein, 1991; Dryden and Mardia, 1998; Zelditch et al., 2004). The most common sorts of analysis are principal components analysis (PCA), which is simply a method for ordinating specimens along the major axis of shape variation; multivariate analysis of variance (MANOVA) or Hotelling’s $T^2$ test, which are multivariate tests for differences in group means; canonical variates analysis (CVA) and discriminant function analysis (DFA), which find the features that best differentiate between known groups and which classify unknown objects to one of those groups; multivariate regression, which tests shape variation for association with continuous variables; two-block partial least squares (PLS), which tests for associations between two shape data sets or between shape and a set of quantitative variables; and cluster analysis, which groups objects based on similarity of shape and includes old methods like UPGMA tree construction or newer methods like $k$ means clustering or maximum-likelihood tree building.

Figure 1 shows some of the basic illustrative capabilities of geometric morphometrics. In this example, a snake vertebra in anterior view has been represented with 15 two-dimensional landmarks (Fig. 1a). This landmark system was applied to five specimens each of three North American Pitviper species (*Agkistrodon piscivorus*, *Crotalus adamanteus*, and *Sistrurus catenatus*), the landmarks were Procrustes superimposed, and a principal components analysis was performed. A scatterplot of the first two principal components shows that vertebral shape separates the species completely (Fig. 1b). The differences in shape of the species *C. adamanteus* and *A. piscivorus* are illustrated graphically using thin-plate spline deformations (Fig. 1c). Thin-plate splines are graphic deformations of a square grid based on the difference in position of individual landmarks between one shape and another. In this example, *C. adamanteus* is used as the reference (Fig. 1c, bottom) and it is deformed into the target shape of *A. piscivorus*. The bending of the grid lines emphasizes which landmarks are displaced in the target relative to the reference. *A. piscivorus* vertebrae have relatively shorter lateral processes compared to *C. adamanteus*.

Geometric morphometric analysis can be performed in mathematical statistical packages such as MATLAB©, Mathematica©, or R, but most beginning users are likely to prefer purpose-specific packages for particular methods or types of data. A comprehensive list of such software, much of it provided free by authors, is maintained by F. James Rohlf on the morphometrics website at SUNY Stony Brook (http://life.bio.sunysb.edu/morph/).
Current Topics

Organisms may be similar or differ in shape due to age, sex, geographic location, phylogenetic relationship, disease, or preservation. Geometric morphometrics can identify shape differences and help explore the causes of variation within and between individuals. In this section we briefly review selected research areas where geometric morphometrics have been used to address topical questions about intraspecific variation, development, morphological integration, and retrodeformation of fossil specimens.

**Within-species variation, phylogeography, and sexual dimorphism**

Geographic variation within species is a long-studied phenomenon that is receiving renewed attention with the advent of molecular phylogeography. Many zoologist are now interested in phenotypic adaptation to local environments and phylogeographic differentiation, both genetic and phenotypic, that leads to speciation. Methods for studying clines, assessing correlations between phenotype and environment, and measuring between-population differentiation using allele frequencies, phenotypic measurements, and molecular markers are expanding (e.g., Endler, 1977; Weir & Cockerham, 1984; Avise, 2000) and are now being combined with geometric morphometrics to study these phenomena in complicated morphological structures, such as teeth, mandibles, and skulls.

For example, Cardini, Jansson & Elton (2007) made particularly effective use of geometric visualization in their study of ecogeographical and clinal variation in the skulls of the vervet monkey, *Cercopithecus aethiops*. They found that rainfall patterns were closely related to variation in skull shape, with shorter faces being found in areas with lower annual precipitation. These authors then used trend surface analysis (Legendre & Legendre, 1998) to extract the aspects of shape that were most closely associated with a geographic cline. The resulting geographic model was then combined with climatic variables, including mean annual precipitation, to partition shape variance into non-environmental spatial, spatially structure environmental, non-spatial environmental, and unexplained components. The variation in skull shape associated with the geographic cline was visualized using 3D wireframe models.

Gaubert *et al.* (2005) used geometric morphometrics as part of an integrative approach to identifying hybrid zones and hybrid individuals in group of genets (*Genetta* spp., Carnivora, Mammalia) which contained nominal species of uncertain biological status. Molecular sequence data, discrete morphological traits, and geometric morphometrics were used to determine the amount of genetic and phenotypic differentiation among populations and to determine whether some populations might not be hybrid intermediates between otherwise distinct groups. Using geometric morphometrics in combination with canonical variates analysis, they found that the morphological data were nearly as good as the molecular sequence data for identifying hybrid individuals, concluding that one nominal species, *G. mossambica*, which had been described by Matschie in 1902, was really a hybrid individual crossed between *G. maculata* and *G. angolensis*.

On a palaeontological time scale, Wood *et al.* (2007) measured geometric shape change in the molar tooth crowns of the Paleocene-Eocene mammal *Ectocion*. They found that...
tooth size and shape appears to be constrained over the 3.5 million year period of the study, except that there was a sharp excursion in size that coincides with the global Eocene thermal maximum.

**Fluctuating Asymmetry and Other Studies of Development**

The phenotype of an organism does not always directly reflect its genotypic information because of perturbations in the intermediate developmental pathways. Animals exhibiting symmetry provide a special case for the study of the phenotype to genotype relationships because the developmental process happens in replication for each symmetric side under extremely similar environmental and identical genetic conditions. For example, extrinsic and intrinsic perturbations in the development of an organism can cause normally bilaterally symmetrical characters to develop asymmetrically (Van Valen, 1962). The resulting random or fluctuating asymmetry has been considered a proxy for environmental or genetic stress (Van Valen, 1962; Parsons, 1992). Geometric morphometrics is a particularly good way to study fluctuating asymmetry in that, the quantification of shape captures more subtle differences in asymmetry than traditional morphometric techniques (Klingenberg & Mcintyre, 1998; Zelditch et al., 2008). In this section we introduce a study that investigates developmental instability with fluctuating asymmetry and then follow up with a short discussion of other developmental complications influencing the phenotype.

Measures of fluctuating asymmetry could serve as an indicator of fitness if genetic stress, indicating low fitness, is caused by low genetic diversity and genetic diversity is negatively correlated with fluctuating asymmetry. White & Searle (2008) investigated whether measures of fluctuating asymmetry can serve as a morphological proxy for genetic diversity in island populations of the common shrew, Sorex araneus. They performed a geometric morphometric analysis to quantify the asymmetry between the left and right mandible and to explore the relationship between genetic diversity measured with microsatellite loci. White & Searle found only a weak relationship between genetic diversity and fluctuating asymmetry.

Other developmental complications, such as mutations in genes that are active in development, may be responsible for modified or deformed morphology. Combining geometric morphometrics and genomics in a study on dogs, Fondon & Garner (2007) hypothesized that simple morphologic modifications are caused by incremental mutations at developmental loci and used 3D craniofacial models of dog skulls to test this idea. They found evidence that tandem repeat length variation in the runt-related transcription factor 2 (Runx2) is correlated with variation in domestic dog facial morphology. Sears et al. (2007) also found evidence of a correlation between tandem repeat length in Runx2 and facial length, calculated from landmarks on 3D images when they examined 30 mammalian species, including dog. This result, combined with their examination of transcriptional activity, provides support for the hypothesis that mutations in Runx2 give a genetic mechanism for morphologic evolution (Sears et al., 2007).
Modularity and Morphological Integration

Geometric morphometrics has revitalized the study of morphological integration, which stems from the classic work of Olson & Miller (1958), who pioneered the use of statistical correlations to test hypotheses that certain characters were functionally or developmentally linked. Simply put, traits that are functionally integrated or whose morphogenesis is influenced by common developmental factors are expected to be “modularized”, or composed of traits that are intercorrelated to a greater extent with one another than with traits that do not belong to the same functional or developmental complex (see papers in Pigliucci & Preston, 2004 and Schlosser & Wagner, 2004 for an entry into this literature). Geometric morphometrics naturally complements this analytical framework because potentially integrated, developmentally and functionally complicated structures such as vertebrate skulls are readily quantified with landmarks and because the standard method of decomposing landmark variation into independent mathematical components is similar to the basic methodology used to identify modular suites of traits. Cranial integration has also been studied extensively with geometric morphometrics (see especially Cheverud 1995; Marroig, De Vivo & Cheverud, 2004). Goswami (2007) recently combined a morphometric assessment of modularity and a histological survey of the sequence of ossification in cranial bones to demonstrate that evolutionarily conserved modules in the adult are not significantly integrated in regard to the developmental formation of the bones making up the modules. González-José et al. (2008) explicitly combined the concept of morphological integration and phylogenetic inference in a study of hominid evolution. These authors used geometric morphometrics to identify intercorrelated modules in the hominid skull and then performed a phylogenetic analysis using each module as a separate “character” to avoid the pitfall of inflating support for some tree topologies through the inclusion of functionally or developmentally redundant characters. They found trees that better corresponded with established hypotheses of hominid relationships than would have been produced by continuous morphometric data had modular intercorrelations not been taken into account. Morphometric data and phylogeny reconstruction are controversial; interested readers are invited to consult the broader literature (see Caumul and Polly, 2005 for an entry point).

Retrodeformation and Taphonomy

Deformed morphology is often a result of the process of fossilization, but deformed morphology can also be found in extant taxa from developmental irregularities or extrinsic impacts. It is important to consider the possible deformation of shape when studying morphology and one way to assess shape deformation is by using geometric morphometrics. Shape distortions can be identified using warp analyses and can then be attributed to taphonomic imprint, intraspecific variation, or interspecific variation. Shape modelling with landmarks is also a useful tool to study the effects of deformation or modification on morphologic structure. Once the effects of deformation are quantitatively described, they can be used to partition out the effects of deformation and obtain data with retrodeformed (pseudo-nondeformed) morphologic structures.
The usual cause of deformation in fossils is compaction, the distortion of morphologic structure due to the weight of the overlying sediment (Webster & Hughes, 1999). The effects of compaction on trilobite morphology in two species of Cambrian olenelloid trilobites were studied by Webster & Hughes. They compared landmarks on deformed and undeformed specimens to calculate the direction and extent of compaction on fossil specimens.

Most retrodeformation methods have been developed and applied to already deformed fossils, without any means to assess accuracy of retrodeformation analyses between nondeformed and retrodeformed data. Using geometric morphometric shape modelling of turtle plastra, Angielczyk & Sheets (2007) simulated deformation with a mathematical transformation applied to landmark data to obtain both nondeformed and deformed data. They applied three retrodeformation analyses to the deformed datasets and compared landmarks. Angielczyk & Sheets found the retrodeformation analyses reduce variation, improve the fit of models, and increase matrix correlations in morphologically deformed data, but do not accurately reconstruct the original shape. Although these retrodeformation analyses did not accurately reconstruct original shape, other retrodeformation analyses may. Angielczyk & Sheets advocated that in the development of new retrodeformation methods, shape modelling and simulated deformation should be used to assess the robustness of the method.

Conclusion

The most practical consequence of the geometric revolution follows from the technological revolution that has occurred over the past decade or two: digital data, especially photographs of animals in the field or of museum specimens, are easy to collect rapidly in large quantities. Geometric morphometrics allows for a very sophisticated multivariate analysis of such data. Despite its mathematical sophistication, geometric morphometric techniques are easy to master thanks to the availability of easy-to-use, quality software and to the intuitive nature of the visual methods for depicting the results. The technology of data collection continues to advance rapidly. Today three-dimensional scanners are increasingly common: laser scanners that rapidly record the surface of an object, CT scanners that image both surface and internal structures, these and other forms of scanning are becoming cheaper and more widely available. In many ways, existing coordinate-based geometric morphometric methods are not easily extendable to three-dimensional data, largely because of difficulties of mapping coordinates onto surfaces so that they are in at least geometrically, if not biologically, homologous positions. The few existing experiments (reviewed above) all have their strengths and weaknesses and further developments are needed to extend their generality. Such developments are likely to happen, allowing geometric morphometrics to be applied to even a greater number of problems with greater sophistication than it does now.
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Bibliography


Figure 1 Basic illustration of the capabilities of geometric morphometrics using North American Pitviper vertebrae. *Crotalus adamanteus* vertebra in anterior view is shown with 15 two-dimensional landmarks (a). Landmarks are represented by black filled circles. The dashed line crossing (a) and (c) identifies 2 homologous landmarks. A scatterplot of the first and second PC score shows the position in shape space of 15 samples from 3 species (*Agkistrodon piscivorus*, *C. adamanteus*, and *Sistrurus catenatus*) (b). A thin-plate spline deformation shows the differences in shape between *C. adamanteus* (c, bottom) and *A. piscivorus* (c, top). The arrow in (b) and (c) represent the transition in shape space from a position within the *C. adamanteus* cluster to a position within the *A. piscivorus* cluster.