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Research Article

Visualizations in geometric morphometrics: how to read and how to make graphs showing shape changes

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This is also an opportunity to thank Larry Leamy for many discussions on geometric morphometrics, back in the late nineties and early noughties, which considerably helped me to develop the ideas that I have finally spelled out in this paper. It was during these discussions that we coined the expression "lollipop graph", but I do not recall whether Larry or I first came up with it.

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Abstract

An important aspect of geometric morphometrics, since its beginnings, has been the visualization of shape changes. A range of methods has been developed with advances in the theory of statistical shape analysis and new possibilities in computer graphics. Most approaches are based either on relative shifts of landmark positions in starting and target shapes after superimposition or on D'Arcy Thompson's idea of transformation grids. Both approaches are in wide use in current morphometrics, and both have their distinctive advantages and shortcomings. This paper discusses the assumptions and some caveats of both approaches. The paper also offers some recommendations for authors of geometric morphometric studies.

Introduction

The visualization of shape changes is at the very core of geometric morphometrics. Indeed, one of the key advantages of geometric morphometrics is that shape differences can be visualized directly as illustrations or computer animations. Accordingly, at the time of the "revolution" in morphometrics, when geometric morphometrics was established as a discipline, the ease of visualization was used as an important argument in favour of geometric morphometrics by comparison with "traditional" morphometrics (Rohlf and Marcus, 1993). Since then, the success of geometric morphometrics is substantially due to the fact that the various methods of visualization can communicate even complex morphological changes much more effectively than the tables of coefficients that result from traditional morphometric analyses. Above all, these visualizations provide information on morphological changes in their immediate anatomical context. Visualizing shape changes remains an important tool for understanding morphological variation, as geometric morphometrics is used to address an increasingly varied range of questions about evolution and development of organisms (Klingenberg, 2010).

A number of different kinds of visualizations for shapes and shape changes have been widely used in geometric morphometrics. They are based mainly on two principles: visualization of shape change by showing the relative displacements of corresponding landmarks in different shapes or by showing the deformation of a regular grid, an outline or a surface that is interpolated from the shape change. These two approaches can also be used in combination. Advances in computer graphics have made it easier to produce appealing illustrations of shape changes in two or three dimensions with both these approaches, and computer animation holds further potential.

All methods for visualising shape changes are based on particular lines of reasoning that makers and viewers of such graphics need to understand and follow, and which occasionally give rise to misunderstandings and controversy. To avoid those problems, it is also important to consider the language in which shape changes and their visualizations are described in the morphometric literature. Despite their crucial importance for geometric morphometrics, the principles and implicit assumptions of the methods for visualizing shape changes have not been discussed in any detail.

This paper explores the conceptual basis and implicit assumptions of visualization through landmark displacements and through graphs based on deformation, such as transformation grids and warped 3D surfaces. I show that both types of visualization have problems that authors and readers of morphometric studies need to keep in mind when making and interpreting visualizations of shape changes. My aim is not to recommend or to condemn a particular approach, but rather to compare and contrast the different logical and visual bases of the approaches. I hope that this will help morphometricians, on the one hand, to interpret

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published illustrations and descriptions of shape changes correctly and, on the other hand, to produce visualizations and to describe them in a manner that will communicate morphometric results to readers without misunderstandings.

Shape and shape changes: two key concepts in geometric morphometrics

Shape is defined technically as all the geometric features of an object except for its size, position and orientation (e.g. Dryden and Mardia 1998). This definition may seem abstract and complicated, but in fact it refers to exactly the sort of information we are using when we interpret what appears on a photograph. Imagine you are looking at a picture of a friend. You easily recognize your friend in the picture even though the picture is much smaller than your friend and despite the fact that you may be looking at the picture far away from your friend or from where the picture was taken. The fact that size and position of the picture are "wrong" does not affect your ability to recognize the person in the image (and, in the first place, to see that it is a person). You might even be able to interpret the photograph if you hold it upside down or at some other angle: orientation also is not essential for our ability to recognize people in images. The main source of information for interpreting images is the shape of the objects they contain, but we tend to discount information on size, position and orientation. So, whenever we are looking at a picture, we are intuitively and unconsciously applying the technical definition of shape.

Because of this definition of shape, we can display the shapes of objects without worrying about their size, position and orientation for the purposes of visualization in morphometric analyses. This is true both for diagrams showing individual shapes and visualizations that display a combination of two or more shapes to show the differences between them – but of course, the resulting diagrams may look very different. Because size, position and orientation are not part of shape, they can be varied freely without affecting the shape information. It does not matter how big a visualization of a shape appears on the page or screen, where exactly it appears and what its orientation is. Therefore, size and location can be chosen so that diagrams fit together conveniently and meet the requirements of a journal format or screen size. Orientation is best chosen to follow anatomical convention for the structure and organisms under study (e.g. the convention that the dorsal side is up and the ventral side is down).

Kendall's shape space is a key component of geometric morphometrics (Bookstein, 1996a; Small, 1996; Dryden and Mardia, 1998; Kendall et al., 1999). Each possible shape (for a given number of landmarks and dimensionality) corresponds to a single point in the shape space and every point in shape space corresponds to a particular shape. Because shape spaces are curved, non-Euclidean spaces, it is advantageous to use a local approximation by a linear, Euclidean tangent space that touches the shape space at the location of the average shape in the sample. This approximation is the same as the one that is used when the curved surface of the Earth is approximated by a flat map of a particular region. For limited ranges of shape variation, as they are usually used in biological studies even at large taxonomic scale, this approximation tends to be very good (e.g. Marcus et al. 2000). Just as in the shape space, each point in the shape tangent space corresponds to a particular shape, and each shape that is sufficiently close to the average shape in the sample (the shape at the tangent point) corresponds to a point in the shape tangent space. Because of this one-to-one correspondence, it is possible to reconstruct the physical shape of an object corresponding to each point in the shape space or tangent space. Therefore, it is possible to go back and forth between the abstract results of statistical analyses in the shape tangent space and the actual shapes of objects. This back-and-forth is the key to visualization in geometric morphometrics. Visualization is possible not just for the particular specimens included in a morphometric study, but for any point in the shape space or shape tangent space (to be precise: any point in the tangent space that is a projection of a point in shape space).

Shape changes are equally fundamental in geometric morphometrics as the concept of shape itself, and it is shape changes that are normally the subject of visualization. Shape changes are among the results of many statistical analyses, such as principal components, regression, partial least squares and others. The concept of shape change is closely related to the concept of shape difference. A difference between two shapes implies that the two shapes are not the same, but it is not directed – there is no designated start or end. In contrast, a shape change involves a directed difference from a starting shape to a target shape. An example of a shape difference is sex dimorphism, which may be studied as the shape change from male to female or from female to male (Gidaszewski et al., 2009; Astúa, 2010). Examples of shape changes are ontogenetic changes associated with growth and development, where there is a clear directionality from younger to older organisms, or historical changes from earlier to later time (Drake and Klingenberg, 2008; Weisensee and Jantz, 2011). Whereas a shape corresponds to a single point in shape space or shape tangent space, a shape change is the movement from the point representing the starting point to the point representing the target shape. This means that it is a vector that has a direction and a magnitude (or length; Klingenberg and Monteiro 2005).

In the context of geometric morphometrics, shape changes are part of the results of most multivariate analyses. Many multivariate procedures, including principal component or partial least squares analysis, provide a system of new coordinate axes that have particular properties (maximum variance, maximum covariance with other features, etc.). Because these axes are in the shape tangent space, their directions can be interpreted as features of shape variation – together with a magnitude and a sign ("up" or "down" direction along the axis), each axis can specify a shape change (Dryden and Mardia, 1998; Klingenberg and McIntyre, 1998; Klingenberg and Zaklan, 2000; Klingenberg et al., 2003b; Drake and Klingenberg, 2010). If the analysis is conducted in the space of Procrustes coordinates (projected to tangent space), the coefficients from the multivariate analysis (eigenvector for principal components, singular vector for partial least squares, etc.) can be directly used, with an appropriate choice of scaling, for visualizing the shape change. Similarly, multivariate regression of shape on some other variable yields a vector of regression coefficients that indicates the change of shape per unit of change in the independent variable (e.g., the shape change for an increase of size by one unit or per unit of time, etc.; Monteiro 1999; Drake and Klingenberg 2008; Rodríguez-Mendoza et al. 2011; Weisensee and Jantz 2011). For regression, the scaling of the shape change is defined because the regression analysis provides the amount of shape change expected per unit of change in the predictor variable. Other multivariate analyses yield results that can be visualized as shape changes in similar ways.

Shape changes always need to be visualized in conjunction with a shape. In order to interpret the change in shape, we need to interpret the relative displacements of landmarks in the context of their overall arrangement. This is partly due to our perception, which requires a shape as the context for making sense of a shape change. Yet, human perception is not the only reason for this. Whereas, in principle, it is straightforward to think of "transplanting" the same shape change vector from its original context to any other point in the same shape space, this does not necessarily make sense. Even though analyses of *Drosophila* wings and mouse mandibles may both use the same shape space for 15 landmarks in two dimensions (e.g. Klingenberg et al. 2003b; Breuker et al. 2006; Klingenberg 2009), it does not mean that a shape change taken from one of these systems and applied to the other has any biological meaning. Thus, shape changes are only interpretable in the context of the structure for which they were found and in conjunction with the shape of that structure. In addition, there is a slightly more subtle limitation: shape changes need to be expressed in the same coordinate system as the shapes from which they are computed, so that the alignment of the coordinate system of landmarks with the anatomical axes of the structure is consistently the same. This is usually not a problem if all shape changes are derived from the same Procrustes superimposition and visualizations are produced using starting and target shapes with the the same Procrustes alignment – this ensures that the shape change is aligned correctly in relation to the starting shape used in the visualization (only once the visualization has been produced, it can be freely rotated, translated and scaled and as a whole).

Shape changes can vary in their magnitudes and directions. Because some biological phenomena are associated with relatively small shape variation, even biologically important shape differences can be subtle. Examples include deformations of skulls under mechanical loading (O'Higgins et al., 2011; O'Higgins and Milne, this issue), effects of quantitative trait loci (Klingenberg et al., 2001; Workman et al., 2002; Klingenberg et al., 2004), or directional asymmetry (Klingenberg et al., 1998, 2010), but the same applies to many studies of shape variation within populations because there is often relatively little variation at this level. To make such small shape changes better visible to viewers, visualizations often show them with exaggerated magnitudes. Choosing by how much to exaggerate a shape change is a compromise: the exaggerated shape change should be big enough to be clearly visible, but not so big that it results in major distortions. Of course, viewers need to be alerted, for instance in a figure legend, that the shape change has been exaggerated.

There are several ways of visualizing shape changes, which all have their advantages and shortcomings. Therefore, they need to be used and interpreted with appropriate caution. In the rest of this paper, I will discuss different options for visualization. I hope this discussion will be useful both for investigators who are facing choices of how to present results from their geometric morphometric analyses and for readers of morphometric studies who need to know how to interpret such graphs.

Landmark displacements and Procrustes superimposition

Because shape is defined as all the geometric features of an object except its size, position and orientation, one way to analyze the variation of shape in a data set is to remove the variation of size, position and orientation in some way. The remaining variation then concerns shape only. For data consisting of landmark coordinates, the variation of shape, position and orientation is most often removed with a Procrustes superimposition that achieves a best fit of landmark configurations according to a least-squares criterion (Sneath, 1967; Gower, 1975; Rohlf and Slice, 1990; Goodall, 1991; Dryden and Mardia, 1998). The least-squares Procrustes superimposition is of fundamental importance in geometric morphometrics because it is providing the link to the theory of Kendall's shape spaces (Small, 1996; Dryden and Mardia, 1998; Kendall et al., 1999), which provides a sound mathematical foundation for empirical studies of shape variation.

The generalized Procrustes superimposition, which is used for extracting shape information from samples of multiple landmark configurations, is an iterative procedure that fits each configuration to the mean shape in the sample as closely as possible. Variation in size is removed by scaling each configuration so that is has centroid size 1.0 (centroid size is a measure of size that quantifies the spread of landmarks around their centroid, or centre of gravity). Variation in position is removed by shifting the configurations so that they share the same centre of gravity. The remainder of the procedure deals with rotations to find an optimal orientation for each configuration. The procedure then aligns all configurations in the dataset to one particular configuration, for instance the first one, using least-squares Procrustes fitting so that the sum of squared distances between corresponding landmarks of each configuration and the common target configuration is minimal. After this step, an average shape is computed by averaging landmark positions (and rescaling to ensure that the centroid size of the average is exactly 1.0). In the next iteration, this average shape is used as the new target configuration and every configuration is fitted to it. A new average is then calculated, and the procedure is repeated until the average no longer changes (changes are usually negligible after two or three rounds).

As a result of this procedure, every configuration in the sample is optimally aligned to the average configuration. Also, unless the shape variation in the sample is unusually large, every configuration is nearly optimally aligned to every other configuration in the sample. Because the configurations are aligned so that size, position and orientation are kept constant according to the criterion for the least-squares fit, the remaining variation in landmark positions is due to variation of shape. Accordingly, the relative displacements of the landmarks from the mean configuration to any particular configuration, or the relative displacements from one shape to another shape nearby in shape space, can be used to assess the corresponding shape difference. These relative displacements provide a visualization of the shape change by showing how the landmarks are rearranged against each other when the non-shape components of variation, size position and orientation, are held constant with the Procrustes superimposition.

Note that I have referred consistently to relative displacements of landmarks. Because all the landmarks are included in the Procrustes superimposition and jointly determine the alignment of each configuration in relation to the mean shape, variation in the position of each landmark after superimposition is relative to the positions of all other landmarks. This interpretation of shape changes as relative shifts of landmarks against one another is central for all types of visualizations based on Procrustes fits.

Scatter of Procrustes-superimposed samples

At the beginning of most shape analyses, investigators want to get an overview of their data and the variation they contain. In geometric morphometrics, this is complicated by the fact that the raw coordinate data contain variation in the position and orientation of objects that is of no biological relevance (it simply reflects the variable positioning of specimens relative to the camera or other equipment used for obtaining images or landmark coordinates). The simplest way of viewing the data without these extraneous components of variation is to keep them constant by plotting the scatter of landmark locations after a Procrustes superimposition (Fig. 1). Similar representations are also available for three-dimensional data. This type of presentation of data is rather popular, both in textbooks (Dryden and Mardia, 1998; Monteiro and dos Reis, 1999; Claude, 2008; Weber and Bookstein, 2011; Zelditch et al., 2012) and in research papers (e.g. Bookstein et al. 1999; Robinson et al. 2001; Dworkin and Gibson 2006; Bruner et al. 2010; Webster 2011). It

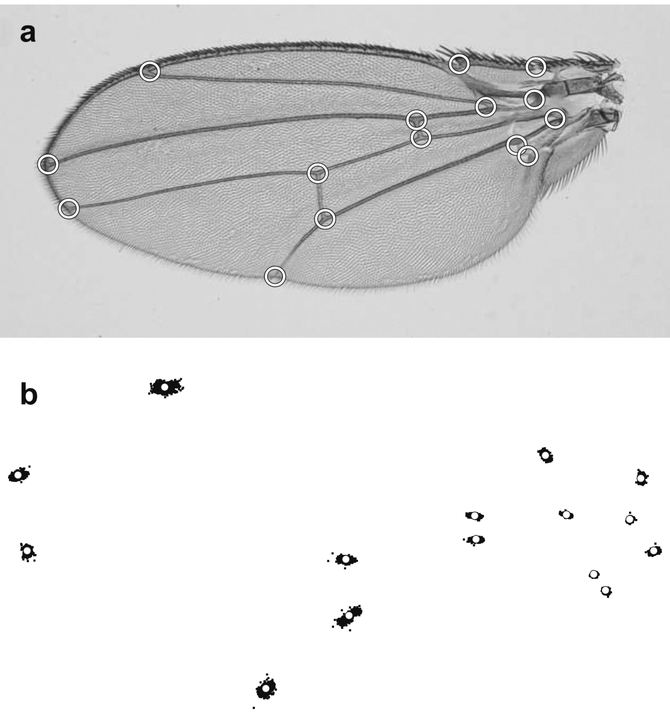

Figure 1 – Procrustes fit for the example of *Drosophila* wings. (a) The set of 15 landmarks used in the example (modified from Breuker et al. 2006). The dataset includes 834 wings of male and female *Drosophila melanogaster*, either wild type (Oregon-R strain) or heterozygous for the *spalt-major*^{1} mutation. (b) The landmark configurations of all wings in the dataset after Procrustes superimposition. For each landmark, the white circle indicates the location of the landmark for the average shape and the black dots indicate the locations for individual wings.

is therefore useful to discuss briefly what graphs of this kind can show and what they inevitably hide.

The example in Fig. 1b shows the scatter of landmark positions around the average shape after Procrustes superimposition of a sample of fly wings. It appears that the spread of positions for some of the landmarks in the distal part of the wing is considerably greater than for some of the landmarks at the wing base (but there are differences among the landmarks even within these regions). There is some justification for this impression, but interpreting the difference between the amounts of variation at different landmarks is complicated by the fact that variation present at any one landmark depends on all other landmarks. Both the amount of variation at other landmarks and their spacing in relation to each other can affect the variability of the position of a given landmark (readers can verify this by conducting a thought experiment or computer simulation in which they drastically increase variation at one landmark or move its average location far from the others and examine the consequence for the Procrustes superimposition). Therefore, the amounts of variation of positions of individual landmarks are not attributable to those landmarks on their own, but they result from the superimposition of the entire configurations.

The scatters of variation of several landmarks show a clear directionality, which, for some landmarks, lines up with anatomical features such as the wing veins in the *Drosophila* example (Fig. 1). This directionality indicates that shape variation is organized in specific ways so that it is concentrated mainly in certain directions, whereas other directions have less variation. In other words, shape variation appears to be integrated (e.g. Klingenberg 2008a). Although some patterns are clearly apparent, the scatter of landmark positions after the Procrustes fit shows only a part of the covariance structure in the data. What can be seen is the covariation between the x and y coordinates at each landmark. By contrast, the patterns of covariation among landmarks do not appear at all in the plot. To assess and display those patterns of integration, fully multivariate methods of analysis are required.

Plots or three-dimensional displays of landmark positions after Procrustes superimposition are often used as preliminary scans for differences among groups of observations in the data, for instance different species or age groups. Because the Procrustes fit does not take into account a possible group structure in the data, there is no guarantee that group differences will be visible in the plot even if they do exist. If such differences are very large, they may be visible, but even then this is by no means certain. For any purpose beyond a preliminary look at the data, fully multivariate analyses in the shape tangent space should be used. These analyses take into account the complete structure of the data, including the covariation among landmarks. For examining group structure in the data, for instance, there are analyses that specifically focus on differences between groups, such as discriminant analysis or canonical variate analysis (e.g. Rohlf et al. 1996; Duarte et al. 2000; Klingenberg et al. 2003a; Weinberg et al. 2009; Weisensee and Jantz 2011; Florio et al. 2012; Klingenberg et al. 2012). Analyses in the shape tangent space also produce graphical outputs that characterize group differences in optimal ways, because they can use all the information about shape variation, even if it does not appear in a display of Procrustes-aligned landmark positions.

Plots of landmark positions after Procrustes superimposition are useful for a first, informal assessment of the data. They cannot show the covariation among landmarks and therefore hide a fundamental aspect of the variation in morphometric data. Therefore, they are not suitable for a thorough and complete examination of the variation in the data. Even preliminary analyses such as the search for outliers in the data should use the complete information about variation in the landmark data and thus should be conducted in shape tangent space, not just by inspecting displays of superimposed landmark configurations. My recommendation is not to use this kind of graphs for formal presentation or publication.

Why shape changes should not be attributed to particular landmarks

The situation where variation is extremely localized to a small region of the landmark configuration, or even a single landmark, relative to the remaining landmarks is widely known as the "Pinocchio effect" and has been extensively discussed in the morphometrics literature (e.g. Siegel and Benson 1982; Chapman 1990; Rohlf and Slice 1990; Walker 2000). These discussions have pointed out that the least-squares Procrustes superimposition tends to spread variation from landmarks with greater variation to landmarks with less variation. This has been illustrated with examples and simulations where variation is concentrated in a single landmark versus all the others, which are constant in relation to reach other. Chapman (1990) provides the fictitious example of Pinocchio's nose, where the tip of the nose varies relative to all other landmarks of the head, and he demonstrates the example of a starfish with a single amputated and regenerating arm that is contrasted with the intact starfish before amputation, so that the tip of the amputated arm varies relative to all other landmarks of the starfish. These examples are used to argue that the superimposition by the least-squares Procrustes superimposition is in some way incorrect because the variation is "spread around" from the one variable landmark to all others. For instance,Chapman (1990, p. 260 f.) directly contrasted the Procrustes superimposition to a resistant-fit superimposition that he considered to be correct.

Such examples have considerable intuitive appeal and appear immediately convincing. We might ask, however, how we know that it is the tip of Pinocchio's nose that is moving anteriorly and not the remainder of his head moving posteriorly. Of course, the actual change is occurring in the tissue of the nose between the landmarks, not in the landmarks themselves. Therefore, the question which landmarks are responsible for a shape change misses the point: the tip of the nose moving forward (with the remainder of the head remaining in the same position) or the remainder of the head moving backward (with the tip of the nose remaining constant) are both describing the consequences of the lengthening of the nose. Both descriptions of the change are equivalent, and there is no obvious criterion for choosing one over the other. Curiously, however, we find that the description with just the tip of the nose moving seems quite natural, whereas the description with the remainder of the head moving away from the tip of the nose sounds distinctly odd. Apparently, we intuitively apply some criterion of parsimony when evaluating changes in the arrangements of landmarks, which makes us prefer descriptions that involve changes in as few landmarks as possible.

This bias in our perception makes us favour one way to characterize the change, as one landmark moving alone, over the equivalent one, as all other landmarks moving the opposite way, even though both are equally accurate and both descriptions equally miss the point that the change actually originates between the landmarks. Because this intuitive bias appears to be quite strong, we need to be aware of it and consider how it affects the ways in which we visualize and interpret shape changes.

Above all, we should resist the temptation to attribute shape changes to particular landmarks. Shape changes involve shifts in the positions of landmarks relative to other landmarks. This relative nature of landmark movements introduces a certain ambiguity in how a given shape change can be visualized. For any shape change, there are multiple ways of visualizing it (Fig. 2).

Because it is the same shape change, all these visualizations are equivalent, even though they may appear different. What differs between them is how the starting and target configurations are scaled, translated and rotated relative to each other. The differences are in the size, position and orientation of the two configurations, and therefore do not affect their shapes. Fig. 2 provides an example of this: the three panels show three ways in which the same two shapes can be compared. The landmark configurations after a Procrustes fit show differences in the positions of all landmarks (Fig. 2a). For the specific shapes used in this example, it is possible to change the sizes and positions so that the two configurations coincide in four of the five landmarks (Fig. 2b).

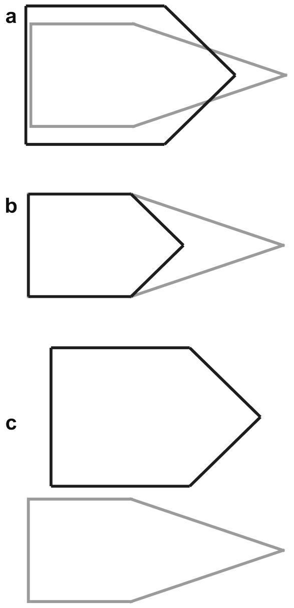

Figure 2 – Three equivalent ways of comparing two shapes. (a) Procrustes fit of the two configurations. (b) Superimposition so that four points match between the two configurations. (c) The two configurations drawn next to each other, in arbitrary positions. Note that the difference between the three ways of presenting the same shape change is in the size and position of the configurations (the orientation happens to be the same in all these three examples, but might also vary).

The Procrustes superimposition is optimal in the sense that it minimizes the sum of squared distances between corresponding landmarks (and also for various theoretical reasons), whereas the second type of superimposition minimizes the number of landmarks that differ in their positions (note that it is not always possible to obtain a superimposition for which more than two landmarks coincide in their positions). Finally, the configurations can be drawn side by side (Fig. 2c). This is in some ways the least problematic of the possible comparisons of shapes, but if the shape difference is subtle, it may be hard to see the difference.

Recall that all three displays are equivalent because the same two shapes are compared in each of them, so the shape change is the same. Yet, the absolute displacements of individual landmarks are quite different. What is constant are the relative displacements: in all three comparisons, it is clear that the landmark on the right protrudes further relative to the other four landmarks in the grey shape than in the black shape. But note that this change cannot be attributed to this landmark by itself, because the change might just as well be assigned to the other four landmarks, or to some change of all five landmarks in the configuration. When interpreting visualizations of shape changes, it is important for viewers to keep in mind this inherently relative nature of landmark displacements.

Arrows, lollipops and other visualizations based on landmark displacements

Various types of graphs visualize shape changes with landmark displacements after some kind of superimposition, most often the Procrustes superimposition. The simplest of these graphs indicate shifts of landmarks positions by lines or arrows, for instance in the "lollipop" graph, where the landmark positions of the starting shape are indicated by dots (the "candy" of the lollipop) and the shifts of landmarks to the target shape are represented as lines (the lollipop stick; Fig. 3a). Visualizations with arrows or lollipops are suitable in two or three dimensions (but they are somewhat cumbersome in the 3D context). Visualizations of this type were used from the beginning of geometric morphometrics (e.g., Siegel and Benson 1982; Chapman 1990; Rohlf and Marcus 1993), but more recently have been replaced mostly by more sophisticated visualization techniques that include more anatomical details.

Visualizations that contain only the shifts of landmark positions (Fig. 3a) are difficult to read because they do not provide any information that can help the viewer to relate those shifts of landmarks to the underlying anatomical structure. To provide that additional information and to make the graphs appear as more realistic representations of the structures under study, different ways are available to indicate the morphological context of the landmarks. One option is to use a pair of superimposed wireframe graphs that connect the landmarks with straight lines for the starting and target shapes (Fig. 3b; e.g. Siegel and Benson 1982; Baylac and Penin 1998; Marcus 1998; Frost et al. 2003; Willmore et al. 2005; Drake and Klingenberg 2008; Weisensee and Jantz 2011) or by superimposed outline drawings that are warped using the thin-plate spline according to the information from the landmarks (see below; Fig. 3c).

All these types of graphs show shifts of the landmarks from the starting to the target shape. It is important for viewers to keep in mind that these shifts, at any landmark, are relative to the shifts at all the other landmarks. No matter what graphical means are used to indicate anatomical context, this relative nature of landmark movements is shared by all visualizations based on superimpositions. Also, viewers need to remember that different registrations of the same starting and target shapes might result in displays with fundamentally different appearance.

Displacements of surfaces in three dimensions

Over the past decade, several methods for analysing the shape of entire surfaces have been proposed that are based on superimposing the entire surface directly. In this context, shape changes are often visualized with a representation of the surface that is coloured according to a heat map that represents the distance of the surfaces at each point – for instance, "cold" colours for areas where the target shape recedes within the start-

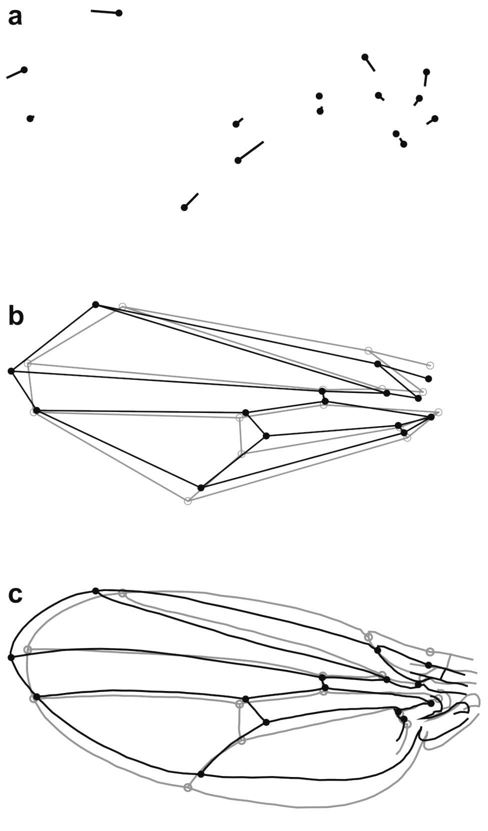

Figure 3 – Three ways of visualizing the same shape change that are based on the Procrustes superimposition. (a) A lollipop diagram, in which the positions of landmarks in the starting shape are shown as dots and the shifts of landmarks to the target shape are indicated by lines. (b) A wireframe graph with two wireframes: a gray one for the starting shape and a black one for the target shape. (c) A graph with a warped outline drawing (gray) for the starting shape and another one (black) for the target shape. The thin-plate spline was used, separately for the starting and target shapes, to warp the outline drawing to match the landmark configurations.

ing shape, and "warm" colours where the target shape bulges out of the starting shape (e.g., Zollikofer and Ponce de Léon 2002; Hammond et al. 2005; Kristensen et al. 2008; Claes et al. 2011). The displacements of surfaces are computed as the displacement of each point on one surface in relation to the nearest point on the other surface. These point-to-point relations can be interpreted as the equivalent of displacements between landmarks in approaches that use discrete landmark points.

In common with the visualization methods based on landmark displacements, the displacements of points on the surfaces, and thus the visualizations of those distances via heat-map colouration, depend critically on the specific superimposition that is used for aligning the surfaces. Different superimposition methods may produce markedly different results. This is particularly relevant for surfaces, because no standard method for aligning surfaces exists that is used universally. Above all, the "hot" and "cold" spots on the surface that correspond to the regions with greatest positive and negative displacements (one or the other surface is outside) are not local features, but depend on the entire surfaces. For instance, whether a part of the nose appears more prominent in one surface that the other not only depends on the nose in the two surfaces, but also on the remainder of the face or head that is included in the analysis. Even though there are no landmark displacements in this sort of study, the difficulties with interpreting this type of visualization are the same as with graphs based on landmark shifts.

Transformations

Visualizations based on the transformation grids of D'Arcy Thompson have played a special role in geometric morphometrics. The method was originally presented as part of an argument for the importance of mathematical and physical ideas for understanding animal forms (Thompson, 1961; Arthur, 2006). Even though the physical analogies have not have a substantial influence on contemporary understanding of growth and evolution, Thompson's appealing diagrams have captured the imagination of many biologists through most of the twentieth century, and efforts to develop a rigorously quantitative approach for making such diagrams contributed significantly to the development of geometric morphometrics (Sneath, 1967; Bookstein, 1978, 1989; Rohlf, 1993).

The idea is that changes of biological forms can be characterized by examining the transformations that have to be applied to the coordinate space in order to change one form into another. Imagine that you draw one form on a rubber sheet and stretch and compress it in different directions until the form drawn on the rubber sheet matches the other form exactly. Then the transformation that has been applied to the rubber sheet can be used to characterize the difference between the two forms. To make this transformation visible directly, a rectangular grid can be drawn on the rubber sheet before it is transformed. When the rubber sheet is stretched and compressed so that the two forms match, the originally rectangular grid is distorted and shows the nature of the transformation. In practice, of course, no rubber sheet is used, but the lines of the grid are fitted with the aid of morphological landmarks that are recognisable on both forms. Thompson fitted the lines of the grid by hand, but now this can be done computationally, using some interpolation method that guarantees that the relation of the grid lines to the landmark positions is correct. The available methods are based on different mathematical or statistical considerations (e.g. Dryden and Mardia 1998, chapter 10), but none of them has any biological basis. The method that is currently by far the most widely used is the thin-plate spline interpolation (Bookstein, 1989; Dryden and Mardia, 1998).

The thin-plate spline

The thin-plate spline is an interpolation technique that was brought into morphometrics as a flexible and mathematically rigorous implementation of D'Arcy Thompson's transformation grids (Bookstein, 1989). The thin-plate spline is a technique that guarantees that the corresponding points of the starting and target form appear precisely in corresponding positions in relation to the untransformed and transformed grids (something that is not guaranteed if the grids are drawn by hand or with some other computational approaches) and it provides the smoothest possible transformation for any pair of starting and target forms (in the sense of minimizing second derivatives). Much has been written in the morphometrics literature about the metaphor of an infinitely thin and infinitely large metal plate and about the related notion of bending energy, the measure of localized deformation. The key point for morphometric application is the property of the smoothest possible transformation, which also emerges from the consideration of deforming a metal plate because the metal plate will resist abrupt bending (a greater bending energy would be required). The thin-plate spline method has a number of desirable properties. For instance, unlike some earlier methods such as trend surface analysis (Sneath, 1967), the thin-plate spline always produces transformation grids where the changes diminish towards the margins, outside the region occupied by landmarks.

The thin-plate spline provides a one-to-one mapping not only between the landmarks of the starting and target forms, but also between every point of the plane or three-dimensional space in which the starting form is embedded and the plane or space of the target shape. It is this interpolation to the whole plane or space that provides the opportunity for using the thin-plate spline to draw transformation grids, as the mapping can be done for the points on the grid lines in the plane or space of the starting shape to obtain the transformed grids in the plane or space of the target shape.

In addition to its use as a tool for visualization, the thin-plate spline has also been used as a method to decompose shape changes into different geometric components: a component of uniform (or affine) shape change that is the same throughout the entire configuration and a non-uniform component of shape change that is localized to various degrees (Bookstein, 1989, 1991, 1996c; Rohlf and Bookstein, 2003). The only justification for this distinction was the suggestion that biomechanical effects on shape should be uniform (Bookstein, 1991, 1996b,c; Rohlf and Bookstein, 2003), but this idea has been discredited by numerous studies showing that biomechanical forces are highly non-uniform (e.g. Pierce et al. 2008; Fortuny et al. 2011; O'Higgins et al. 2011), which is unsurprising given the localized nature of joints, muscle insertions and similar structures involved in biomechanical function. The non-uniform component can be further broken down into partial warps, which are geometrically separate components of a shape change. Together, the uniform component and all partial warps provide a coordinate system for analysing shape variation, which has been widely used in geometric morphometrics (Rohlf, 1993). The complete set of partial warps and uniform components forms a coordinate system in shape tangent space that differs from the coordinate system of projected Procrustes coordinates only by a rotation (Rohlf, 1993, 1999). The results of multivariate analyses of the same data based on the two coordinate systems are therefore equivalent. Because of the considerable conceptual and computational complexity of the partial warps and uniform component, it is preferable to use Procrustes tangent coordinates as the basis for morphometric analyses, and to limit the use of the thin-plate spline as a tool for visualization.

Transformation grids

Transformation grids produced with the thin-plate spline (e.g. Fig. 4) are in such widespread use in geometric morphometrics that they have become one of its most familiar tools, and they are covered in detail in textbooks of geometric morphometrics (Bookstein, 1991; Monteiro and dos Reis, 1999; Zelditch et al., 2012) and statistical shape analysis (Dryden and Mardia, 1998). The immediate visual appeal of transformation grids reinforces this familiarity. But despite this familiarity, or perhaps because of it, there has not been very much discussion about transformation grids as a visualization tool. Here, I will present some caveats that should be taken into account when using and interpreting transformation grids.

A first and most fundamental point is to keep in mind that the grids are based on an analogy, but do not represent actual biological phenomena. The analogy of the rubber sheet is an interesting and intuitive way to think about biological shape change, but there is usually no direct equivalent to such a smooth elastic deformation in the biological

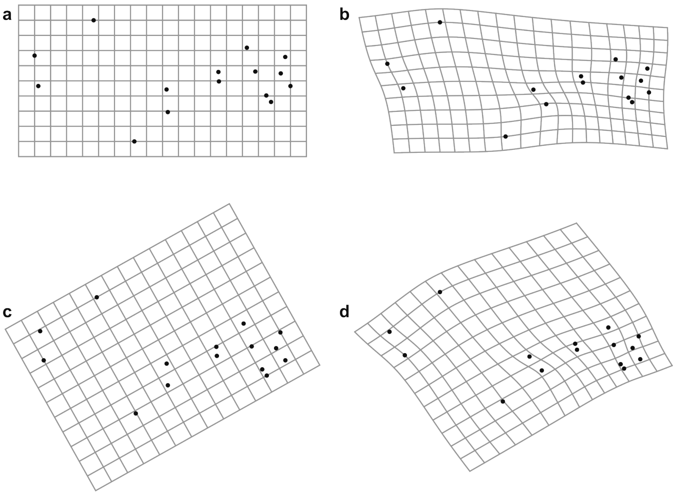

Figure 4 – Visualizations using the thin-plate spline, using the same shape change as in Fig. 3 (see also Fig. 1). (a) The starting shape, with grid lines aligned so that the horizontal grid lines approximately follow the anterior–posterior compartment boundary of the wing. (b) The target shape, aligned as in (a). (c) The starting shape, with the grid in a different alignment relative to the wing. (d) The target shape, with the grid aligned as in (c). Note that example of a shape change is not an extreme case of the effect of a change in how the grid is aligned to the structure.

processes that produce biological shapes and shape variation. Accordingly, the transformation grids do not depict a biological reality, but are an imagined aid for visualization. Unlike other visual aids such as arrows or lollipops, which indicate relative shifts of landmark positions, transformation grids render the space between the landmarks, precisely where no data are available. Literally, for that reason, transformation grids are pure fiction!

It is debatable whether the median (sagittal) plane is an exception to this line of reasoning. One might argue that the median plane is not just imagined, but reflects an anatomical reality. It is tangibly embodied in structures such as the nasal septum, and the midline also plays an important role in development. Accordingly, one might argue with some justification that the median plane is a real developmental and anatomical entity.

Other anatomical lines and planes, however, arbitrarily cut through various structures. These planes may be useful for establishing a frame of reference for alignment (e.g. the Frankfurt plane in human anatomy), but in general they have no biological significance beyond that. Viewers should keep this in mind when interpreting visualizations of shape changes using transformation grids. Some software packages allow users to specify arbitrary planes in visualizations of three-dimensional that are then warped with the thin-plate spline to visualize some shape change (e.g. O'Higgins and Jones 1998). In visualizations of shape changes that contain such warped surfaces, it is usually difficult to determine what their spatial positions and anatomical relations are, and it is therefore doubtful whether they contribute to the viewer's understanding of the shape change. Even if such warped surfaces provide the viewer with a better feeling of the transformation as interpolated by the thin-plate spline, it is an open question whether that reflects the actual shape change.

The algorithms used for computing transformation grids, such as the thin-plate spline, have no biological basis but are based exclusively on geometric or statistical criteria. The interpolation between landmarks is therefore problematic and cannot be expected to be biologically realistic. In the immediate vicinity of landmarks, the behaviour of the grids is driven by the nearby landmarks and therefore by the actual biological data. In regions that are devoid of landmarks, however, the influence of the biological information diminishes with increasing distance from the landmarks. Therefore, particularly in regions that are at some distance from the nearest landmarks, transformation grids need to be interpreted with caution.

Depending on the alignment of the rectangular grid relative to the anatomical features in the starting form (cf. Fig. 4a versus Fig. 4c), the visual appearance of transformation grids can differ even if the shape change is the same (Fig. 4b versus Fig. 4d). Also, the visual impression of a shape change depends on whether grid lines pass through the regions with the most accentuated localized deformations. Therefore, transformation grids that are shifted relative to the landmark configurations or that differ in the intervals between grid lines (i.e. the density of the grids) can make the same shape change look quite different. These effects are not always obvious to the viewer, particularly if the starting shape with the rectangular grid is omitted to save space, as it is often done in publications (imagine Fig. 4b,d without the comparison to Fig. 4a,c). Depending on the software that is used, these properties of the visualizations can be changed by the user. In general, it is a good idea to choose an alignment that has a clear anatomical meaning (e.g. the anterior–posterior direction or median line parallel to the grid lines). In some cases, this requires an active choice by the user, because the default options will not result in an anatomically meaningful alignment. For instance, if landmark configurations from half-skulls are aligned according to the major axes of the mean configuration, the grid lines usually will be at oblique angles to the median axis or plane, which is not an anatomically sensible arrangement. Software packages for geometric morphometrics have options for users to choose anatomically meaningful alignments and other important properties of visualizations (e.g. the number of horizontal and vertical grid lines), but users need to make active choices because the default options often are not the best choice for particular study situations.

Transformation grids such as those in Fig. 4, with just the landmark positions and the grids, can be difficult to interpret because they do not provide anatomical information. This became particularly evident for me when I discovered that several transformation grids in a paper by a well-established group of investigators, published about a decade ago in a high-profile journal, are upside-down in relation to the other figures that explain the landmarks and other aspects of the study. The labels of the diagrams are printed correctly, which indicates that the figure in question was not just flipped upside-down by the printers, but the mistake happened to the authors and passed scrutiny by the reviewers, editors, and readers of the paper (the paper in question has been cited several dozen times, but it and its authors shall remain unnamed here). There has been no correction to the paper, nor was the mistake pointed out in a forum such as the Morphmet E-mail list, so that it seems the mistake has mostly gone unnoticed. If a fairly crude mistake such as upside-down transformation grids can go unnoticed even by the authors of a study, chances are that many readers will routinely overlook many of the more subtle anatomical features depicted in transformation grids.

Warped outlines or surfaces

The pitfalls of "bare" transformation grids can be avoided by providing additional information so that the viewer can easily see the anatomical context of the landmark configuration. This context can be provided by a drawing of the structure. Such drawings were an integral part of visualizations with transformation grids from the start, as D'Arcy Thompson (1961) always included a drawing of the structure with the transformation grid (the grids were derived from those drawings). The visualizations with bare grids only emerged later, when the grids were obtained by computational methods.

Another option is to use the techniques for warping transformation grids but to apply them to the drawing only, without the grid. Because the thin-plate spline interpolation works for every point of the image plane in the surroundings of the landmarks, it can be used not only to warp a rectangular grid, but just as well a drawing of the structure under study (Fig. 3c). The drawings can be simple outlines (e.g. Klingenberg and Leamy 2001) or quite detailed drawings (e.g. Rodríguez-Mendoza et al. 2011). Similarly, in three-dimensional applications, a scanned surface model can be warped (Fig. 5; e.g. Wiley et al. 2005).

For these visualizations with warped drawings or surface models, some of the same caveats apply as for transformation grids, but others do not. Because the information about shape change resides entirely in the landmarks, the interpolation between landmarks must be interpreted with caution, particularly in regions that are relatively far

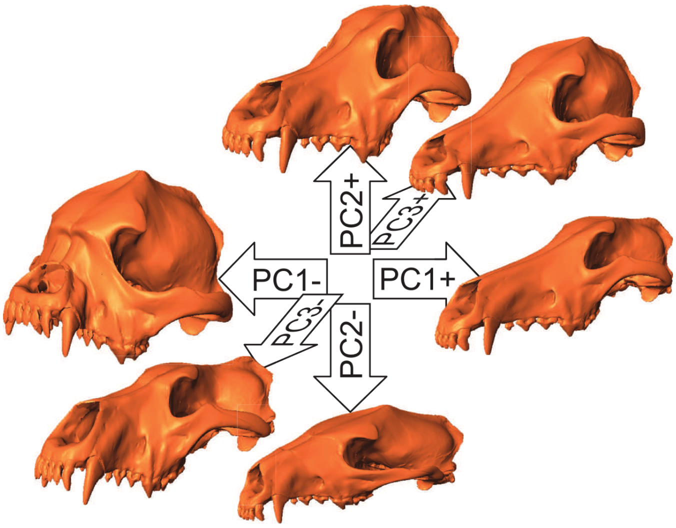

Figure 5 – Visualizations of shape changes with warped surfaces. A scanned surface of a wolf skull was warped with the thin-plate spline to the shapes near the extremes of the scores for the first three principal components of an analysis of domestic dogs and wild carnivorans (from Drake and Klingenberg 2010, Fig. 3D). This example shows shape changes at a fairly large scale, demonstrating that this method of visualization is fairly robust. Some of the changes are strongly reminiscent of actual extreme forms in the analysis (e.g. the contrast between dogs with short and elongate skulls), whereas other aspects are not realistic (e.g. the canine teeth, because no landmarks were digitized at the tips of the canines).

from the nearest landmarks (e.g. the posterior margin near the base of the wing in Fig. 3c). Because the drawing can be chosen to show all the anatomically relevant information, many of the ambiguities of the visualizations with "bare" transformation grids do not apply to warped drawings or surfaces. The interpretation of shape changes is therefore easier than for transformation grids. The main problem for authors is to choose an appropriate level of detail for the drawing that corresponds reasonably well to the coverage of the structure with landmarks. Also, investigators should avoid using drawings or surfaces that extend far beyond the landmarks (e.g. a drawing of a bird skull including the beak if no landmarks have been digitized on the beak).

In three dimensions, presentation of warped surfaces has become easier with advances in technology and standard file formats used for distribution. Even simple, "flat" images of warped surfaces can depict three-dimensional shape changes in an accessible way (e.g. Fig. 5), but new possibilities for distributing such visualizations are emerging that go beyond flat images. For instance, it is now possible to include entire three-dimensional surfaces within figures in published papers, so that readers, after downloading the respective PDF file from a journal web site, can rotate the structure under control of their computer's mouse (or similar control device) to view the warped surface from any direction (e.g. Drake 2011).

Discontinuities

A limitation of the transformation approach is that it fails in some circumstances, when the assumption of continuous deformation is violated. This assumption of continuity means that there is a one-to-one correspondence between every point in one of the structures under consideration and some particular point in every other structure under study. This correspondence applies both to the landmarks and to points between the landmarks (the difference is that the correspondence is not known for the latter, but is inferred through interpolation with an algorithm such as the thin-plate spline). Transformations can easily deal with groups of landmarks converging towards each other, as long as there is at least a small distance left between them, or with sets of landmarks diverging from each other or rotating relative to the remaining landmarks in the configurations. The transformation approach fails if continuous deformations are not sufficient to characterize a shape change – if multiple landmarks shift to the same point or, in reverse, if landmarks diverge from the same point and thus generate a "hole" in the transformation grid between them, or if portions of the landmark configuration "flip over" relative to the remainder so that the transformation grid folds over. All these problems disrupt the one-to-one correspondence of points in the planes (or 3D spaces) of the shapes under comparison, which is a fundamental assumption of the transformation approach.

Such changes are fairly widespread and cannot just be dismissed as rare exceptions (Klingenberg, 2008b; Oxnard and O'Higgins, 2009; Gómez-Robles et al., 2011). Examples are the disappearance of certain structures, such as the loss of the nasal bone in the dugong, structures that can bifurcate so that a single part in one organism corresponds to two parts in another, as it occurs for tooth cusps, or growth processes such as the closing of the fontanelle of many mammals, where the relations between adjacent cranial bones change and thus drastically affect the landmarks on those bones and on the sutures between them. Other examples of changes that violate the assumption of continuous change involve sets of landmarks that are defined in different ways: for instance, landmarks defined by the bones of the skull versus landmarks defined by the locations of muscle insertion. Because muscle insertions can shift relative to the structures of the skull, there can be drastic shifts in the relative arrangements of the respective sets of landmarks, which produce discontinuities that cannot be represented sensibly by the transformation approach (Oxnard and O'Higgins, 2009). Again, this is not a unique example, but similar problems can arise whenever different sets of landmarks can be displaced relative to each other (another example might be landmarks on butterfly wings that are defined by wing veins versus colour patterns).

A closely related situation is the "switching" of positions among nearby landmarks. Zelditch et al. (2012, p. 30f.) mention this as a possible problem in morphometric studies,giving an example of a cranial foramen that can appear on either side of a suture. The consequence is an abrupt localized deformation in the region of the landmarks in question. Accordingly, deformation grids and related visualizations will show marked distortions in that region, for instance grids that are folding over, which violate the assumption of the transformation approach that there is an unambiguous one-to-one correspondence between each point in the two- or three dimensional space in which each specimen is recorded. This invalidates the interpolation step that is central to the transformation approach. If the landmarks that switch position are sufficiently close to each other, the resulting shape change may not be very large (as measured by Procrustes distance between the shapes).

All these cases pose no serious problem for geometric morphometric methods, provided that investigators use definitions of landmarks that take the situation into account. Landmarks can be displaced relative to each other in any way without problems for the Procrustes superimposition and the various multivariate methods used in geometric morphometrics. Problems only arise for the transformation approach as a tool for visualization of shape changes, because all these examples involve discontinuous changes that transformations cannot properly represent. Therefore, these problems can be circumvented by simply choosing another type of visualization.

Discussion

Although visualization of shape changes has been a central task of geometric morphometrics since its inception, there has been surprisingly little discussion of the approaches used for visualization. That different authors strongly favour different visualization methods is not just a matter of personal taste, but it indicates that these methods come with contrasting strengths and weaknesses. There are no "right" or "wrong" methods among the main approaches used for visualizations. Each method, however, comes with its own underlying ideas and conventions that have been established through repeated use and of which the viewer needs to be aware to interpret the resulting graphs correctly. Because these ideas and conventions tend to be so familiar and intuitive to every morphometrician, at least for the type of visualization that he or she prefers, they are rarely thought about or discussed explicitly. This paper has attempted to make the ideas and conventions explicit and compare them between the main approaches for visualization that are used in geometric morphometrics.

Making and interpreting visualizations are parts of a communication process. Authors and viewers of illustrations that visualize shape changes need to understand the visualization methods in order to communicate effectively and avoid misunderstandings. Here I sum up the main conclusions of this paper and make some recommendations.

Shifts of superimposed landmarks must not be interpreted one-by-one

In visualizations of shape changes based on shifts of superimposed landmarks, a shift is shown at every landmark, but it is important that these shifts are relative to all other landmarks. It is particularly important to keep in mind this relative nature of landmark shifts when describing shape changes and reading such descriptions – there are multiple ways of characterizing the same shape change. For instance, whether the tip of Pinocchio's nose moves anteriorly (relative to the rest of the head) or whether the rest of the head moves posteriorly (relative to the tip of the nose) does not make a difference to the shape change. Viewers need to keep in mind that the same shape change can produce different-looking patterns of landmark shifts, depending on the superimposition. Accordingly, equivalent visualizations of the same shape change might look quite different from each other and show the greatest shifts for different landmarks. Also, the same shape change might yield descriptions that sound very different because they involve different sets of landmarks (e.g. Pinocchio's nose versus the rest of the head) and thus might appear to support different biological interpretations.

A possible alternative to graphs of superimposed landmark configurations, which authors should consider seriously, is to display the starting and target shapes side by side. Exaggerating the shape change makes it clearly visible (finding the right factor for exaggeration is a matter of trial and error – the shape change should be clearly visible but not lead to gross distortions). This method avoids the ambiguity of the superimposition graphs but is usually very effective, particularly when used in combination with wireframes and similar visualization tools (e.g. O'Higgins and Jones 1998; Klingenberg et al. 2012).

Transformation grids are fiction

Despite their immediate visual appeal, it is important to keep in mind that transformation grids are merely a mathematical construct that provides a means for visualizing shape changes but do not represent a biological reality. Quite literally, these grids are fiction.

Nevertheless, with the appropriate caveats, transformation grids are a very effective tool for visualizing shape changes. The key point for viewers is to interpret them critically. In particular, the grids are likely to be unreliable guides to change in regions that are relatively far from the nearest landmarks – in these regions, the transformation is mostly resulting from the warping algorithm and not informed by biological data.

Authors should provide images of starting shapes with untransformed grids, so that viewers can understand the anatomical context of the grids (Fig. 4). For transformation grids that are used as part of three-dimensional visualizations, it is critical that authors provide detailed explanations of the position and anatomical relations of the plane used for the rectangular grid.

Using deformed outline drawings or surface models avoids some of the aspects that make transformation grids artificial, but it shares all the problems concerning the fact that the warping criteria are biologically arbitrary. Therefore, as with the grids, such visualizations need to be interpreted cautiously in regions that are relatively far from landmarks.

Despite these caveats, this method of visualization is probably the best one that is currently available because of its straightforward visual appeal and the direct biological relevance of all the elements in the graphs. To avoid the problems of superimposition outlined above, the warped outlines or surfaces are best shown side by side (Fig. 5).

Visualization as communication

In geometric morphometrics, visualization of shape changes is a key element in the exploration of data, formulating and testing of hypotheses and reporting of results to others. Just as scientific writing is best viewed as a form of communication between authors and readers, visualization is best considered as part of the same communication process. Accordingly, authors should aim to produce visualizations that are clear and easy to interpret, so that the viewers' task of reconstructing the meaning of the shape changes is straightforward and the risk of misunderstandings is minimal. In turn, viewers should be aware of the conventions and assumptions that are inherent to the various types of visualizations.

To make this communication effective, both authors and viewers of the visualizations should try to understand each other's perspective. Just as in technical writing (e.g. Gopen 2004), visualization is more effective if the author takes into account the viewers' expectations and general conventions. If an unusual type of visualization is necessary, authors should pay particular attention to provide sufficient explanations as text in publications or verbally for oral presentations. In particular, it is important to provide the information what the starting and target shapes are and whether the shape change is exaggerated (and if so, by how much). Explanations of shape changes should explicitly and consistently point out the relative nature of landmark shifts, and authors should not assume that readers will remember that landmark shifts are relative when reading a description. In turn, viewers should examine visualizations of shape changes carefully and also examine the accompanying explanations. Sometimes, an author's statement is not following these recommendations and does not really mean what it is literally saying, for instance, if the statement "landmark X is shifted dorsally" is meant as an abbreviation for something like "landmark X is shifted dorsally relative to other landmarks in the region" (but the exact meaning depends on the particular context). Readers need to anticipate and recognize the use of such shorthand or careless wording and grasp the correct interpretation that is behind them. In other words, authors should strive to be helpful to the viewers and readers of their visualizations and associate explanations, whereas readers should make the effort of examining the author's reasoning and logic.

The goal of visualizations is to communicate complex shape changes as a means for discovering and disseminating patterns of morphological variation in their full anatomical context. Sophisticated visualization techniques are not ends in themselves, but are means to support authors in sharing biological insights from their morphometric analyses with viewers. Used in this manner, the current methods for visualization of shape changes provide powerful means for communicating complex results in an intuitive and appealing manner, and future advances can continue to make important contributions to the development of geometric morphometrics.

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