Evol Biol (2008) 35:186–190
DOI 10.1007/s11692-008-9029-4

COMMENTARY

Novelty and "Homology-free" Morphometrics: What's in a Name?

Christian Peter Klingenberg

Received: 23 June 2008 / Accepted: 25 June 2008 / Published online: 16 August 2008

The field of morphometrics has developed fast over the last two decades. After a "revolution" that established a new "synthesis" in morphometric methodology about 15 years ago (Rohlf and Marcus 1993; Bookstein 1996), the focus has recently shifted to applying this methodology to various biological problems. In his review, Polly (2008) singles out quantitative genetics for special mention. In this area, morphometric methods have been applied in the context of analyses that base genetic inference on resemblance among relatives (Klingenberg and Leamy 2001; Myers et al. 2006) and analyses of natural selection (Gómez et al. 2006), which both use the additive genetic covariance matrix, or **G** matrix, as a central quantity, as well as QTL studies that aim to identify the effects of single loci (Liu et al. 1996; Klingenberg et al. 2001). All these studies share a common approach in that they use the variables derived from a morphometric space to characterize shape. These variables are then used as the data in the context of the classical multivariate methods of quantitative genetics, as they have been available for 20 years or more (e.g., Lande 1979; Lande and Arnold 1983; Lynch and Walsh 1998). None of this is inherently revolutionary, as it is mainly a question of "plugging in" a new type of data into established methods (perhaps with minor changes of the algebra). One might be tempted to say that the most surprising thing is that it took morphometricians so long to apply shape data in these contexts.

The article of Polly (2008) discusses a further body of literature, which is concerned with the developmental origins of evolutionary novelties (e.g., Oster et al. 1988; Alberch 1989; Salazar-Ciudad et al. 2003). This kind of study focuses on developmental and morphological variation at a scale where the local approximation by the linear models that underlie quantitative genetics is no longer satisfactory. Instead of small steps in phenotypic space, evolution of novelty can occur by large leaps. As a consequence, morphological changes are not necessarily just minor rearrangements of a constant set of morphological features, but entirely novel features can arise. Because standard morphometric methodology requires a strict correspondence of the landmarks or measured distances among all taxa under study, it presents considerable difficulties for analyzing variation of this sort. Therefore, Polly (2008) suggests that novelty is best analyzed by what he calls "homology-free" characterizations of the phenotype. These characterizations include analyses of outlines or surfaces that do not require the user to establish an explicit correspondence of structures as it is required, for instance, for analyses of morphological landmarks. These "homology-free" approaches easily can accommodate even drastic changes of shape, where no apparent correspondence of shapes is maintained. Accordingly, these approaches appear to be very suitable for studying morphological novelty (Polly 2008).

In this paper, I raise two caveats to this conclusion. First, I point out that for some types of novelty, such as those where novel parts arise by duplication of existing parts, it may well be possible to include them in the standard morphometric analyses using landmarks. This requires an explicit interpretation of the developmental and anatomical change that underlies the novelty, and will thus not always be feasible. Second, I examine whether the "homology-free" methods recommended by Polly (2008) really are free of assumptions about the correspondence of parts. My survey finds that all these methods are making such

C. P. Klingenberg (✉)
Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK
e-mail: cpk@manchester.ac.uk

assumptions in some way, and therefore all critically depend on some sense of homology.

Shape Spaces for Landmark Data and Novelty

By far the most thoroughly understood shape spaces are those for landmark data, because a large body of theory exists about them (Dryden and Mardia 1998; Kendall et al. 1999). The starting point of a study is the total set of landmarks that correspond in a one-to-one manner among all the objects to be included in an analysis. For biological studies, this correspondence is based on criteria derived from comparative anatomy, and is therefore often referred to as homology (e.g., Polly 2008). In comparative evolutionary studies, this correspondence is indeed the same as biological homology, and the use of the word is therefore justified; in other contexts, such as functional morphology, other criteria for correspondence may be preferable, and therefore the word "homology" is better avoided. But for all applications of the landmark methodology, an explicit definition of the correspondence of landmarks is the core of this approach.

The shape space contains all possible shapes that can be defined by variation in the relative positions of a set of landmarks. There are different shape spaces for different numbers of landmarks and for different numbers of dimensions (usually two or three dimensions). These shape spaces also include so-called degenerate shapes where two or more landmarks occupy exactly the same point. The only exception is the extreme degenerate case where *all* landmarks are in exactly the same point; this single case is excluded from consideration as a shape (in practice, this is not really a limitation—even our intuitive notion of shape requires some spatial expanse). Every shape has its specific place in the shape space, and the relative arrangement of shapes can be used to define distances between them, average shapes for sets of shapes, or other statistical properties (Dryden and Mardia 1998; Kendall et al. 1999). For practical studies, a small neighborhood around the mean shape in the sample can be approximated by a tangent space, which allows subsequent analyses to use the conventional methods of multivariate statistics (Dryden and Mardia 1998). In the vast majority of biological data sets, the range of shape variation is small enough for the tangent space to be a good approximation of the shape space, even for large-scale comparisons (e.g., skull shape variation in mammals Marcus et al. 2000).

Despite the requirement for rigorous one-to-one correspondence, the landmark approach can accommodate certain kinds of qualitative novelties. When novelties arise by bifurcation in a conserved developmental process (e.g. Oster et al. 1988) so that there is branching or duplications of structures, one can argue that the landmarks of the simple ancestral structure correspond to the landmarks on both the duplicated structures (Fig. 1a). Accordingly, a morphometric analysis can proceed by including the total set of landmarks including all duplicated ones (in the example of Fig. 1a, this is the set A1, A2, B1 and B2). For the simple configurations, the same point location is recorded for the duplicates of a single landmark (for the left diagram in Fig. 1a, the location of point A is recorded for both A1 and A2, the location of point B for B1 and B2). Clearly, this approach requires very specific information about the nature of the novelty and the correspondence of duplicated landmarks.

The logic of this method is similar to the reasoning that can be used if landmarks are missing because of an evolutionary loss of structures (Fig. 1b). In this case, it is often helpful to consider a transformation series, as it has traditionally been done in comparative anatomy. The transformation series for the example of Fig. 1b, from left

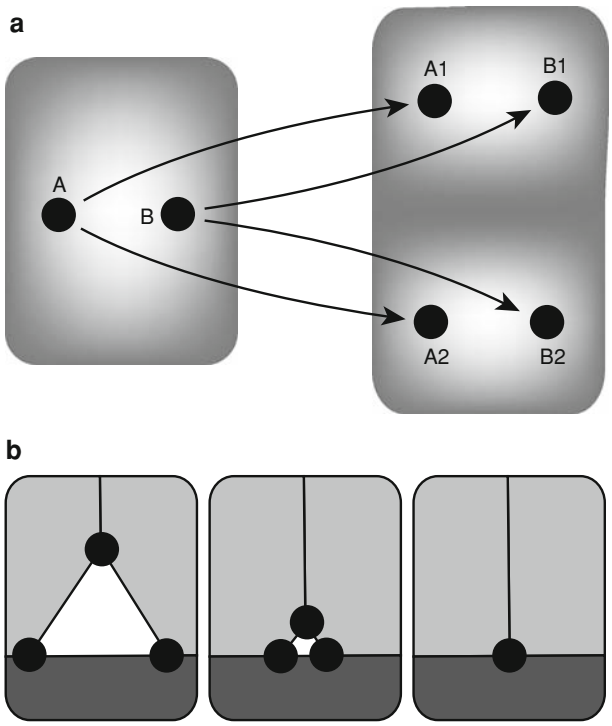

Fig. 1 Novelty and loss of structures in morphometric studies using landmarks. (**a**) Novelty by duplication of landmarks. A bifurcation event in a developmental process leads to a duplication of a part of a structure (light region) and the corresponding landmarks (black dots). Each one of the landmarks in the diagram to the left therefore corresponds to *two* landmarks in the diagram to the right (arrows). (**b**) A transformation series for a structure (white triangle) that is reduced and then vanishes (from left to right). In the diagram to the right, where the white triangle is lost completely, the three landmarks surrounding the triangle all fall on the same point. If read from the right to the left, this sequence can be interpreted as the origin of a novelty

to right, shows the progressive reduction and loss of the white triangle. The series shows that the landmarks first move closer together, and ultimately end up in the same point when the white triangle has disappeared completely. Comparative morphometric studies at a large phylogenetic scale encounter situations like this fairly regularly (e.g., for the nasal bone in a series from hyrax through the manatee to the dugong). This type of reasoning also can be used to address novelty in morphometric studies: simply read the transformation series in Fig. 1b from the right to the left side, in which case the white triangle is interpreted as a novelty.

Overall, the methods of landmark morphometrics can address qualitative novelty by using partly degenerate configurations of landmarks (i.e., configurations in which more than one landmark is in the same location) for the forms that do not possess the novelty. This approach clearly cannot handle all morphological innovations, but it should be feasible and useful for a broad range of novelties.

"Homology-free" Characterization: Free of What?

A key point in the review of Polly (2008) is that the limitations of landmark-based methods to characterize novelty apply less to morphometric methods using outlines or surfaces because those methods are "homology-free". By "homology-free", Polly means that this class of descriptors has no fixed link between the data that are recorded (points on an outline or surface) and the anatomical structures they represent. Therefore, the appearance or disappearance of new features in the outline or surface can easily be captured by these methods, and thus analyzing this kind of evolutionary novelties poses no problem.

The fact that these methods do not require the user to identify the features that are potential novelties does not mean, however, that these approaches avoid assumptions about the correspondence between the forms under study. These assumptions concern the correspondence of the points sampled from the outlines or surfaces. Because the variables describing the shapes are derived from these points, an assumption of correspondence among the sampled points is built into the procedure. The precise nature of this assumption differs among the various methods.

The assumption of correspondence among sampled points is clearest for the analysis of semilandmarks (Bookstein 1997; Hammond et al. 2004; Gunz et al. 2005). Semilandmarks are points sampled along a smooth outline or surface that defines the boundary of the structure under study. Each semilandmark provides information about the structure in the direction perpendicular to the boundary, but no information in the direction of the boundary itself. The idea is therefore to slide the points along the boundary so that they match each other optimally in some sense, and then to treat them as landmarks. Criteria for sliding to an optimal agreement differ (e.g., minimizing Procrustes distances or bending energy) and the choice of different criteria can produce differences in the results (Perez et al. 2006). Treating the points as landmarks implies the full set of assumptions about one-to-one correspondence of points, even though an algorithm for sliding is substituted for the information from comparative anatomy that guides conventional landmark analyses.

For eigenshape analysis, at least one landmark is usually included as a starting point for the outline, and multiple landmarks may be used in extended eigenshape analysis (Lohmann and Schweitzer 1990; MacLeod 1999). In addition, the analysis implicitly assumes that the points along the outline correspond among all the specimens, because the changes of direction of the curve at these points are used to define the variables of the analysis.

For Fourier analysis, things are less clear-cut than for eigenshape analysis, because the approach is based on functions fitted to the data points, rather than directly on the data points themselves. For some types of Fourier analysis, however, it has been shown that the results are identical to those of eigenshape analysis (up to a rigid rotation; Rohlf 1986), from which it follows that the same assumptions concerning the homology of points must also apply. If only a reduced number of harmonics are included to achieve a smoothing of the contour, this relationship is no longer a simple identity, although it remains as a background in the analysis (the smoothing does not alter the fundamental nature of the data). Assumptions about correspondence also "creep in" through the standardization for the size and orientation of the outline using the best-fitting ellipse (Ferson et al. 1985) or through alignment by specific landmarks (e.g., Frieß and Baylac 2003). Analyses of three-dimensional surfaces by methods related to Fourier analysis (McPeek et al. 2008) or by entirely different methods (Plyusnin et al. 2008) also include steps for alignment and standardization that make implicit assumptions about homologies of the structure.

When different methods are applied to the same outline data, different results may be obtained (e.g., Cannon and Manos 2001; Navarro et al. 2004). These differences partly originate from the way different methods treat the data, but they also partly result from differences in how they establish correspondences between points of the outlines that are used in the analysis. Although some relationships between methods do exist (Rohlf 1986), most of them are not transparent.

A simple thought experiment may be useful to illustrate the general importance of the homology relationship for morphometric studies. Imagine a smooth structure that has a bump either in the middle or two-thirds along its length.

Evolution from one of these conditions to the other can occur either as a shift of the bump or by the disappearance of one bump and the origin of the other as a novelty. The answer to this question will differ, depending on what is assumed about how points on the surface of the structure correspond to each other. If a method uses the location on the surface or outline as the defining criterion for correspondence, as all the methods based on equally spaced points do, the scenario of a shift is ruled out a priori (see also Gunz et al. 2005).

Overall, therefore, none of the "homology-free" methods are really free of assumptions about homology. Instead of making decisions about homology explicitly, based on the criteria of comparative anatomy, the user merely delegates them to various algorithms inherent in the methods.

Conclusions

This paper contains some good news and some bad news for investigators who want to use geometric morphometrics for studying morphological novelty. The good news is that landmark methods can be used to study some types of qualitative novelty, namely those that arose by duplication of existing structures. The investigator must explicitly specify the nature of the novelty as part of "coding" the correspondences of landmarks (e.g., coincident landmarks in taxa lacking the duplication), based on information from comparative anatomy and developmental biology. The bad news is that "homology-free" morphometric approaches are not free of assumptions about homology. Different assumptions about the correspondence among the points of outlines and surfaces are built into the algorithms that are used by these approaches. The decisions about correspondence of parts are merely shifted to the choice among the different methods, and the assumptions are substantially less transparent than they are in landmark-based approaches.

References

Alberch, P. (1989). The logic of monsters: Evidence for internal constraint in development and evolution. In: B. David, J.-L. Marchand, J. Chaline, & B. Laurin (Eds.), Ontogenèse et Évolution: Geobios (mémoire spécial) 12:21–57.

Bookstein, F. L. (1996). Biometrics, biomathematics and the morphometric synthesis. *Bulletin of Mathematical Biology, 58*, 313–365. doi:10.1007/BF02458311.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: Morphometrics of group differences in outline shape. *Medical Image Analysis, 1*, 225–243. doi:10.1016/S1361-8415(97)85012-8.

Cannon, C. H., & Manos, P. S. (2001). Combining and comparing morphometric shape descriptors with a molecular phylogeny: The case of fruit type evolution in Bornean *Lithocarpus* (Fagaceae). *Systematic Biology, 50*, 860–880. doi:10.1080/106351501753462849.

Dryden, I. L., & Mardia, K. V. (1998). *Statistical shape analysis* (p. xx + 347). Chichester: Wiley.

Ferson, S., Rohlf, F. J., & Koehn, R. K. (1985). Measuring shape variation of two-dimensional outlines. *Systematic Zoology, 34*, 59–68. doi:10.2307/2413345.

Frieß, M., & Baylac, M. (2003). Exploring artificial cranial deformation using elliptic Fourier analysis of Procrustes aligned outlines. *American Journal of Physical Anthropology, 122*, 11–22. doi:10.1002/ajpa.10286.

Gómez, J. M., Perfectti, F., & Camacho, J. P. M. (2006). Natural selection on *Erysimum mediohispanicum* flower shape: Insights into the evolution of zygomorphy. *American Naturalist, 168*, 531–545. doi:10.1086/507048.

Gunz, P., Mitteroecker, P., & Bookstein, F. L. (2005). Semilandmarks in three dimensions. In D. E. Slice (Ed.), *Modern morphometrics in physical anthropology* (pp. 73–98). New York: Kluwer Academic/Plenum Publishers.

Hammond, P., Hutton, T. J., Allanson, J. E., Campbell, L. E., Hennekam, R. C. M., Holden, S., et al. (2004). 3D analysis of facial morphology. *American Journal of Medical Genetics, 126A*, 339–348. doi:10.1002/ajmg.a.20665.

Kendall, D. G., Barden, D., Carne, T. K., & Le, H. (1999). *Shape and shape theory*. Chichester: Wiley.

Klingenberg, C. P., & Leamy, L. J. (2001). Quantitative genetics of geometric shape in the mouse mandible. *Evolution; International Journal of Organic Evolution, 55*, 2342–2352.

Klingenberg, C. P., Leamy, L. J., Routman, E. J., & Cheverud, J. M. (2001). Genetic architecture of mandible shape in mice: Effects of quantitative trait loci analyzed by geometric morphometrics. *Genetics, 157*, 785–802.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution; International Journal of Organic Evolution, 33*, 402–416. doi:10.2307/2407630.

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution; International Journal of Organic Evolution, 37*, 1210–1226. doi:10.2307/2408842.

Liu, J., Mercer, J. M., Stam, L. F., Gibson, G. C., Zeng, Z.-B., & Laurie, C. C. (1996). Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*. *Genetics, 142*, 1129–1145.

Lohmann, G. P., & Schweitzer, P. N. (1990). On eigenshape analysis. In F. J. Rohlf & F. L. Bookstein (Eds.), *Proceedings of the Michigan morphometrics workshop* (pp. 147–166). Ann Arbor, MI: University of Michigan Museum of Zoology.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer.

MacLeod, N. (1999). Generalizing and extending the eigenshape method of shape space visualization and analysis. *Paleobiology, 25*, 107–138.

Marcus, L. F., Hingst-Zaher, E., & Zaher, H. (2000). Application of landmark morphometrics to skulls representing the orders of living mammals. *Hystrix, 11*, 27–47.

McPeek, M. A., Shen, L., Torrey, J. Z., & Farid, H. (2008). The tempo and mode of three-dimensional morphological evolution in male reproductive structure. *American Naturalist, 171*, E158–E178. doi:10.1086/587076.

Myers, E. M., Janzen, F. J., Adams, D. C., & Tucker, J. K. (2006). Quantitative genetics of plastron shape in slider turtles (*Trachemys scripta*). *Evolution; International Journal of Organic Evolution, 60*, 563–572.

Navarro, N., Zatarain, X., & Montuire, S. (2004). Effects of morphometric descriptor changes on statistical classification

and morphospaces. *Biological Journal of the Linnean Society, 83*, 243–260. doi:10.1111/j.1095-8312.2004.00385.x.

Oster, G. F., Shubin, N., Murray, J. D., & Alberch, P. (1988). Evolution and morphogenetic rules: The shape of the vertebrate limb in ontogeny and phylogeny. *Evolution; International Journal of Organic Evolution, 45*, 862–884. doi:10.2307/2408905.

Perez, S. I., Bernal, V., & Gonzalez, P. N. (2006). Differences between sliding semi-landmark methods in geometric morohometrics, with an application to human craniofacial variation. *Journal of Anatomy, 208*, 769–784. doi:10.1111/j.1469-7580.2006.00576.x.

Plyusnin, I., Evans, A. R., Karme, A., Gionis, A., & Jernvall, J. (2008). Automated 3D phenotype analysis using data mining. *PLoS ONE, 3*, e1742.

Polly, P. D. (2008). Developmental dynamics and G-matrices: Can morphometric spaces be used to model phenotypic evolution? *Evolutionary Biology*. doi:10.1007/s11692-008-9020-0.

Rohlf, F. J. (1986). Relationships among eigenshape analysis, Fourier analysis, and analysis of coordinates. *Mathematical Geology, 18*, 845–854. doi:10.1007/BF00899747.

Rohlf, F. J., & Marcus, L. F. (1993). A revolution in morphometrics. *Trends in Ecology & Evolution, 8*, 129–132. doi:10.1016/0169-5347(93)90024-J.

Salazar-Ciudad, I., Jernvall, J., & Newman, S. A. (2003). Mechanisms of pattern formation in development and evolution. *Development, 130*, 2027–2037. doi:10.1242/dev.00425.