Three-dimensional geometric morphometrics for studying floral shape variation

Van der Niet, T; Zollikofer, C P E; Ponce de León, M S; Johnson, S D; Linder, H P

Postprint available at:
http://www.zora.uzh.ch

Posted at the Zurich Open Repository and Archive, University of Zurich.
http://www.zora.uzh.ch

Originally published at:
Three-dimensional geometric morphometrics for studying floral shape variation

Abstract

Variation in floral shape is of major interest to evolutionary and pollination biologists, plant systematists and developmental geneticists. Quantifying this variation has been difficult due to the three-dimensional (3D) complexity of angiosperm flowers. By combining 3D geometric representations of flowers obtained by micro-computed tomography scanning with geometric morphometric methods, well established in zoology and anthropology, floral shape variation can be analyzed quantitatively, allowing for powerful interpretation and visualization of the resulting patterns of variation.
Three-dimensional geometric morphometrics for studying floral shape variation

Timotheüs van der Niet1,2, Christoph P.E. Zollikofe3, Marcia S. Ponce de León3, Steven D. Johnson1 and H. Peter Linder2

Variation in floral shape is of major interest to evolutionary and pollination biologists, plant systematists and developmental geneticists. Quantifying this variation has been difficult due to the three-dimensional (3D) complexity of angiosperm flowers. By combining 3D geometric representations of flowers obtained by micro-computed tomography scanning with geometric morphometric methods, well established in zoology and anthropology, floral shape variation can be analyzed quantitatively, allowing for powerful interpretation and visualization of the resulting patterns of variation.

The need for a revolution in morphometric studies of angiosperm flowers

The origin and functional consequences of variation in the shape of biological organisms has intrigued biologists for centuries [2]. The description and analysis of shape differences was traditionally done through multivariate morphometrics based on measured distances [3]. The development of geometric morphometrics (GM) [4], based on the spatial relationships between anatomical landmarks representing homologous biological structures, revolutionized the study of biological shape variation. The main advantage of GM over traditional multivariate morphometrics is that, in addition to the shapes of the organs, the geometric relationships among the organs are also quantified, allowing for powerful interpretation and visualization of the results, which was previously impossible [4,5]. The number of studies applying this method has increased exponentially during the past few decades [5] and GM is now frequently applied to questions regarding ontogeny, evolutionary ecology, systematics and developmental genetics [6]. The application of GM has, however, been largely restricted to zoological and anthropological studies [6,7].

Variation in shape of angiosperm flowers is extensive [8] and relevant to a wide variety of botanical disciplines. GM methods, despite their huge potential, are rarely applied, however, seriously hampering progress in understanding causes and consequences of floral shape variation. Here, we explain a potential reason for this conundrum, suggest a major step forward by linking three-dimensional (3D) imaging of flowers to GM and highlight the potential of this novel application.

Current methods for analyzing floral shape variation

Most botanical morphometric studies still use traditional multivariate analyses of measured distances [9]. Some studies have applied GM analyses to study variation in shape of leaves [10,11], seeds [12] or isolated floral organs, mainly the corolla [13–15]. Without exception, these studies focus on plant features which can probably be adequately represented in two dimensions. Entire flowers, however, have complex geometries which require 3D representations to capture the full information implicit in these structures. The absence of 3D GM botanical studies cannot be explained by analytical limitations of 3D landmark data, as this is straightforward [5]. It is probably due to technical limitations of acquiring 3D data from flowers because the tissue is too soft to collect coordinate data using 3D contact digitizers and structurally too complex to use laser surface scanners.

3D geometric morphometrics of flowers

Micro-computed tomography (microCT) is a non-invasive, X-ray-based technique frequently used for the acquisition and visual rendering of digital volume data from small objects. MicroCT imaging of floral material was described in 2003 [16], but this technique has not yet been linked to GM. Here, we demonstrate that combining these two approaches offers huge potential for future applications in plant biology. As an example of how microCT and GM can result in a major step forward in understanding patterns of floral shape variation, we applied microCT scanning to orchid flowers (see supplementary material online), which are considered some of the most structurally complex among the angiosperms. We found that the resulting 3D geometric representation of the flowers matched real flowers very closely (Figure 1). We visualized these geometric representations and used them for the sampling of 3D landmark coordinate data [17].

It has been suggested that the number of homologous landmarks typically available for botanical studies is low [18]. This matter could well apply to structures such as leaves [10], whose often complex outlines are typically
analyzed with landmark-free methods such as elliptic Fourier analysis [19]. However, flowers are characterized by a highly conservative bauplan [20], and at the taxonomic level of typical morphometric studies, such as intrageneric comparisons, there will be little doubt about homology between floral structures. In addition, it is possible to use so-called semilandmarks, which are located along curves or surfaces between unambiguous homologous landmarks [7]. For the orchids shown here, this resulted in the identification of 40 landmarks (26 homologous and 14 semilandmarks; Table S1 in the supplementary material online) which seemed adequate to quantify floral form accurately. Relatively simple flowers, with few highly differentiated organs, might require fewer landmarks for shape quantification.

A sample of landmark configurations (each of which represents a specimen) can be analyzed using standard multivariate GM methods (see supplementary material online). The form of each specimen is decomposed into statistically independent measures of size and shape. After removing differences in position and orientation between size-normalized specimens by generalized Procrustes analysis (GPA) [7], shape differences between individuals can be analyzed with standard techniques of multivariate analysis such as principal components analysis (PCA) and visualized accordingly (Figure 2).

Potential applications of geometric morphometrics to floral studies

In general, any botanical study which relies on a detailed quantification of floral shape might benefit from implementing 3D GM. Below, we highlight how and to which subdisciplines this might apply most strongly, although this is by no means an exhaustive overview.

Floral shape variation often reflects differences in plant pollination systems, particularly for plant species with specialized plant–pollinator interactions [21]. For single traits, a link between variation in floral shape and plant fitness through pollinator behavior has been shown [22]. Comparative studies have resulted in qualitative descriptions of these shape differences [23] and multivariate analyses of measurements of floral traits have, with varying degrees of success, shown that plant species can cluster in phenotype space according to general pollinator classes [9,24]. However, in comparison to these traditional approaches, GM methods have the potential to provide additional and more accurate insights into the associations between floral shape variation and pollination biology. The typically large number of 3D landmarks used in GM studies, compared to the number of distance measurements available in traditional methods, provides a more detailed level of quantification of floral shape. This can strongly enhance studies of floral integration, which rely on accurate multivariate quantification of floral shape [25]. One of the major advances of GM over traditional methods is that it establishes direct and mutual links between the statistical patterns of shape variation in multivariate shape space (as evaluated by means of GPA followed by standard techniques of multivariate analysis such as PCA) and the actual patterns of floral shape variation in physical space (as represented by the original landmark data). Visualization of patterns of variation in either space thereby allows for a detailed identification of the spatial rearrangement or similarity of floral parts in relation to differences in pollination systems (Figure 2). The link between the arrangement of species in multivariate shape space and their actual floral shape can also be used to predict adaptive optima in floral shape [26]. Species typically cluster in a multivariate analysis according to their pollinator [9,24], possibly representing local optima, whereas unoccupied space might represent maladapted shapes. This can be tested by creating artificial hybrids with intermediate shape, which can be included in a GM analysis. The fitness of hybrid shapes can subsequently be measured in field experiments [27]. Establishing adaptive surfaces along shape gradients might be more readily applied to botanical studies compared to zoological studies, as hybrids are more easily available for plants and fitness can be more easily quantified. Finally, the separation of size and shape as geometrically orthogonal entities is inherent to GM. In traditional morphometric methods, size and shape are often conflated, resulting in overestimation of allometric
effects (covariation between size and shape) [28]. GM methods have great potential to detect patterns of allometry in floral forms, as well as departures from allometric trajectories which can be used to identify floral gigantism [29].

The presence of a strong association between floral shape and pollinator classes [9,21,23,24] (Figure 2) seems to be at odds with the results of a recent analysis [30]. Using relatively crude measures of floral syndromes, including categorical shape-related characters, they showed that floral phenotypes across plant communities worldwide rarely fall within discrete pollination syndrome clusters. It is, however, not unexpected that distantly related plant species which share a pollinator can be highly dissimilar in shape, as floral shape is likely to carry a signature of both contemporary natural selection (with pollinators as selective agents) and phylogenetic history. Therefore, as advocated by the authors [30], instead of trying to identify universal pollination syndromes, it might be more informative to take a phylogenetic approach and disentangle which components of floral shape variation and stasis are the result of phylogenetic inertia, and which are the result of selection by current pollinators. GM holds great promise for such an investigation, as it facilitates statistical and visual characterization of how floral shape changes along phylogenies and according to pollinators, and how floral subunits covary with each other and with these factors. Simultaneous phylogenetic optimization of both shape [31] and pollinators [24] can thereby result in unique insights into the major determinants of floral shape evolution.

In addition to the study of evolutionary and ecological determinants of floral shape, there is a rapidly growing interest in the identification of genes underlying complex floral morphology. With the development of financially and technically feasible molecular methods for studying this, the current limitation is the ability to accurately describe the floral phenotype [11]. Developmental genetics will therefore perhaps benefit most from applying GM due to the improved resolution of shape differences [15]. The recent rise of new plant model systems for genetic studies, such as Aquilegia [32], for which plant–pollinator interactions are relatively well understood in a phylogenetic framework [24], might benefit from a thorough analysis of floral shape, especially given the spatially complex structures of Aquilegia flowers.

What are the prospects and limitations of volume data acquisition and 3D GM applied to flowers? Owing to new developments in microCT scanning technology, high-resolution 3D data can now be obtained at the cellular level of plants (Stijn Dhondt et al., this issue). Using these nano-imaging techniques, it will be possible to extend 3D GM analysis to small-flowered plant families. One issue that cannot be resolved with conventional X-ray absorption tomography, however, is the possible lack of X-ray absorption difference between neighboring structures, for
example between a flower and an embedding medium used to preserve its 3D form, or between adjacent floral tissues. Here, advanced scanning technologies such as phase contrast synchrotron X-ray tomography (pcST) [33,34] can be applied (in analogy to phase contrast light microscopy, pcST enhances the contrast at the interface between two media of similar density). In some cases the application of GM analyses might be inappropriate, for example for flowers with delicate, loose floral parts, whose 3D geometry cannot be preserved with stabilizing and/or embedding media throughout the storage and scanning process. Another problem might be the identification of homologous landmarks for plant species which have variable floral organ numbers [35]. However, none of these limitations are unique to plant studies, rather they are challenges of GM in general. Despite these issues, 3D GM has played a major role in improving understanding of shape variation. Its application to plant sciences holds great promise in major role in improving understanding of shape variation.

Acknowledgements
We would like to acknowledge the Georges and Antoine-Claraz Schenkung for financial support for fieldwork, Robert Ineichen for help with the landmarking and www.plantweb.co.za for permission to use the photograph of Satyrium erectum. The editor and three anonymous reviewers are kindly acknowledged for providing useful comments on an earlier version of the manuscript. This research was supported by Swiss National Science Foundation Grant 31-66594.01 ‘Adaptive and rapid radiation in the Cape flora’.

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2010.05.005.

References
1360-1285/$ – see front matter © 2010 Elsevier Ltd. All rights reserved. doi:10.1016/j.tplants.2010.05.005 Trends in Plant Science 15 (2010) 423–426