Quantitative Evaluation of the Floral Shape Variation in Sinningia Speciosa Domestication

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Written for presentation at the

2013 ASABE Annual International Meeting

Sponsored by ASABE

Kansas City, Missouri

July 21 – 24, 2013

Abstract. *Floral shape variation is of substantial interests to botanists and evolutionary biologists. This study quantifies shape variation in Darwin's Gloxinia (Sinningia speciosa) using image processing and geometric morphometric methods. Darwin's Gloxinia has two shape forms – bilateral symmetric (zygomorphic) wild type and radially symmetric (actinomorphic) ornamental breeding (peloria). It is characterized by its easy crossing within its species; hence, is selected to be the study object in this research. In this work, the wild type Darwin's Gloxinia was crossed with the peloric one. Face and side views of flowers of second-generation hybrids photographed using a regular digital camera. Image processing algorithms were applied to isolate the flowers from the background, and to identify landmarks, i.e., the characteristic points on the flowers. Generalized Procrustes analysis was applied to the landmarks to define floral shapes by excluding their size, rotation, or transformation information. The variation in floral shapes was then investigated by principal component analysis. It was found that the first few principal components captured most structure variations. It is also shown that the proposed approach can adequately capture the shape variation and symmetry in Darwin's Gloxinia.*

Keywords. *Floral shape variation, Geometric morphometrics, Principal component analysis, Generalized Procrustes analysis*

Introduction

This work aimed to study floral shape variation in Darwin's Gloxinia, *Sinningia speciosa*. Darwin's Gloxinia is trumpet-shaped with a narrow tube and flared petals (see Fig. 1). It is a species with incredible range of floral shape variation (Zaitlin, 2012). The natural varieties and peloric cultivars can show very different appearances. In this study, an actinomorphic (radially symmetric) cultivar (Fig. 1(a)) was crossed with a zygomorphic (bilaterally symmetric) form (Fig. 1(b)) (Hsu et al., 2009). The second generation (F2) population showed segregation in floral symmetry, providing an opportunity to investigate floral shape variation.

Figure 1. Accession 'Carangola' (a) face and (b) side-view, and cultivar 'Peridots Darth Vaders' (c) face and (d) side-view

The shape of a flower can be represented by a set of characteristic coordinate points, also referred to as landmarks, along the flower's contour (Adams et al., 2004; Klingenberg, 2010). These landmarks are classified into two categories – primary and secondary landmarks (Zelditch et al. 2004). The primary landmarks are readily recognizable points, such as petals or sepals intersections. The secondary landmarks are equally spaced points between 2 conjunctive primary landmarks. Given the landmarks, shape variation can be conveniently defined, quantified, and visualized statistically.

Geometric morphometrics is a collection of landmark-based techniques that examine the properties of shapes (Adams, Rohlf, and Slice 2004). It has been applied to study the shape variation in petals (Cui et al., 2010; Dalayap et al., 2011; Feng et al., 2009; Gomez et al., 2006). Gomez et al. (2006) performed general Procrustes analysis (GPA) to determine the petal shape variation, and principal component analysis (PCA) to capture the major trends of variation in a wild population of *Erysimum mediohispanicum*. Feng et al. (2009) quantified shape variation of petals and leaves to investigate the major components of variation among *Antirrhinum* species. Cui et al. (2010) further revealed that petal shape was controlled by the gene specific to floral symmetry development in *Antirrhinum majus*.

The objective of this study was to quantify floral shape variation among F2 specimens of Darwin's Gloxinia. In the work, face and side views of the flowers were taken. A program was developed to semi-automatically identify and collect landmark coordinates from the floral images. After that, Procrustes superimposition was carried out to eliminate variance irrelevant to shape, such as variance of translation, orientation, and scaling, from the landmark dataset. PCA was then applied to determine the covariance of variation. It was shown the first principal component (PC) represented 19.2% and 54.4% of variation for the face-view and side-view images, respectively. The results of this study could potentially be extended to reveal the genotype-phenotype relationship of flowers in future studies.

Materials and Methods

Flower samples

Flower samples were developed by recombinant inbreeding (Kover and Mott, 2012) two species of *S. speciosa*. In the process, the first generation (F1) was bred by intercrossing two parents, "Carangola" and cultivar "Peridots Darth Vaders" (Fig. 1). The F2 was developed by single selfing of one F1 plant. Three hundred and twenty F2 plants were randomly selected and raised. All plants grew in a greenhouse under natural lighting with 20% shading, at 22–28°C with 70–80% humidity.

Flowers of F2 plants with more or less than 5 petal lobes are not similar to each other, i.e., inhomologous, and therefore and were excluded in this study (Adams et al., 2004). In addition, only flowers of at the front of an inflorescence were subjected to image acquisition to prevent the abnormal floral patterns of terminal flowers (Rudall and Bateman, 2003). As a result, 73 F2 plants were selected, and 2 flowers were sampled from each plant.

Image acquisition

The face and side views of each flower sample were acquired. The specimens were first pinned on a black board. Their images were captured with Canon® SD1000 digital camera and at a size of 1600 by 1200 pixels. The face-view images were captured with the camera confronting the plane of unfolded petal lobes (see Fig. 1(a) and (c)). The side-view images were taken with the camera confronting the dorsiventral plane of the flowers (see Figs. 1(b) and (d)). All images were acquired at the stage when the corolla was fully unfolded, and the stamen and stigma had stopped growing.

Floral landmark identification

Image processing algorithms were applied to the flower samples for landmark identification. The algorithms were implemented with a program written with Qt Creator (Nokia®) and OpenCV (Intel®). Figure 2 shows the flowchart for landmark identification.
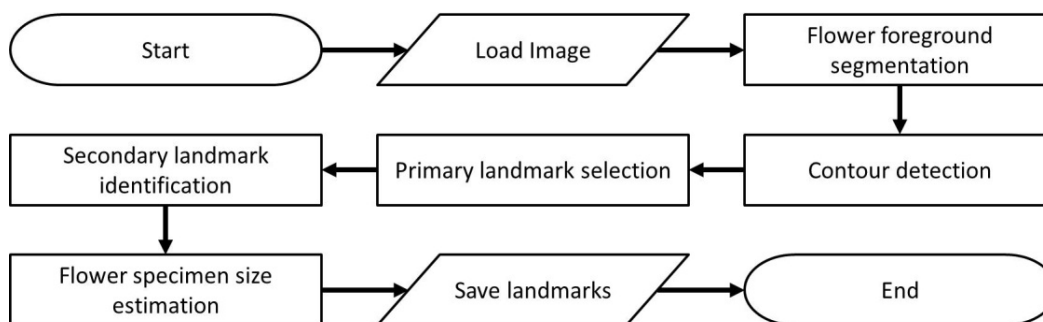

Figure 2. Flowchart of flower landmark identification

The foreground of flowers was segmented using the grabCut algorithm (Rother et al., 2004) after manually assigning a region of interest enclosing the flower object. The background image could be obtained by extracting the original image from the foreground image. Contours of the flower foreground image was determined using the Suzuki85 algorithm (Suzuki and Abe, 1985). The results were binary images that contained only the pixels of the contour lines. Note that, in the face-view images, lobe contours usually overlap at the intersection of 2 lobes (see Figs. 1(a) and (c)) and concealed the lobes at the back. To solve this problem, it was assumed that the overlapped lobe contour sections were symmetric. The contour of a lobe in front was mirror-mapped to replace the undetected section of the back-lobe contour.

The primary landmarks were manually assigned by "mouse-clicks" in a graphic user interface (GUI) developed in the program. Five landmarks were chosen for both the face-view and side-view images, respectively. In the face-view images, the landmarks were assigned starting from dorsal lobe and proceeding clockwise, labeled 1, 7, 13, 19, and 25. In the side-view images, the landmarks were assigned from the intersection points of the sepals and tube, labeled 1, 7, 8, 9, and 15. Figure 3 shows the primary landmarks and their assigned numbers.
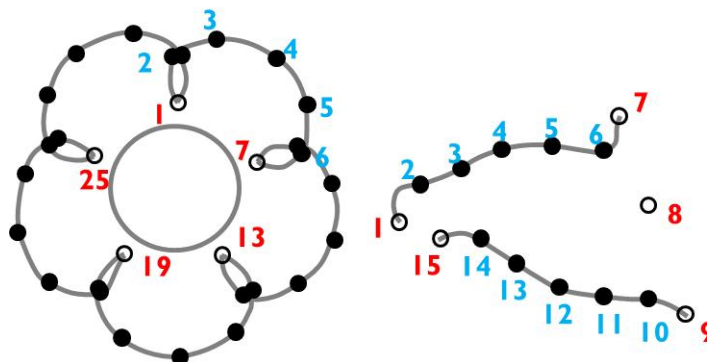
The secondary landmarks were identified automatically by the procedure below. In the face-view images, the complete flower contour was partitioned into lobe contour sections using the primary landmarks as separation points. Each lobe section was then divided into 6 equally long segments. The endpoints of the segments were defined as the secondary landmarks. Here a total of 5 secondary landmarks was chosen for each lobe because it had been shown that 5 points were adequate for describing the lobe shape (Dryden and Mardia, 1998). In the side-view image, the up and down flower contour sections between primary landmark 1 and 7, 9 and 15 were respectively divided into six equally long segments. The endpoints of the segments were defined as the secondary landmarks. Figure 3 shows the secondary landmarks and their assigned numbers. A total of 35 secondary landmarks, including 25 in the face-view and 10 in side-view images, respectively, was collected for each flower.

Flower size estimation

The images of the flower specimen were taken with a ruler placed vertically in the background. The ruler was isolated from the flower (see Fig. 1) and would be contained in the background images from the grabCut algorithm. The image was converted into binary for pixel calculation. A histogram showing the number of white pixels for each row was first generated for background image. The horizontal stripes in the histogram corresponded to the ruler ticks, representing one millimeter in length. The conversion ratio of the ruler ticks to image pixels—used for estimating floral specimen size—was calculated by averaging the number of pixels between two neighboring stripes throughout the histogram. The flower specimen size was then estimated based on the ratio.

Morphometrics

Morphometrics was applied to the landmark coordinates from image processing for evaluation of floral shape variation. Here "shape" is defined as the form that does not alter by translation, scale, or rotation. GPA (Gower, 1975; Rohlf and Slice, 1990) was performed to remove non-shape information from the landmark coordinates. In the method, the mean shape, i.e., average landmark coordinate, was calculated. The centroid of each individual landmark coordinate is translated to the mean shape. Then the translated landmark coordinate is scaled and rotated to minimize the deviation between it and the mean shape. This procedure is applied to each landmark coordinate recursively until no change occurs in the landmark coordinate. The final coordinates obtained are called GPA landmarks.

Principal component analysis

PCA was applied to the GPA landmarks for dimension reduction. The GPA landmarks of a flower are multiple measurements of its shape and are highly correlated. There exists a certain level of redundancy in the high-dimensional landmark vectors. Practically, the shape variation can be adequately represented using only a few significant variables. Here the PCA was performed to project the GPA landmarks into a set of orthogonal variables, namely principal components (PCs). The first few PCs account for most of the variation in the landmarks and can well summarize the variance in shape with little loss of information. The analyses of variation were then performed on only the first few PCs.

Results and Discussions

Landmark identification

The flower landmarks were identified using the procedure described in the Material and Methods section. Figure 4 shows the original, foreground, contour images, as well as the landmarks. For each flower, 30 landmarks were obtained from the face-view image, and 15 from the side-view image.
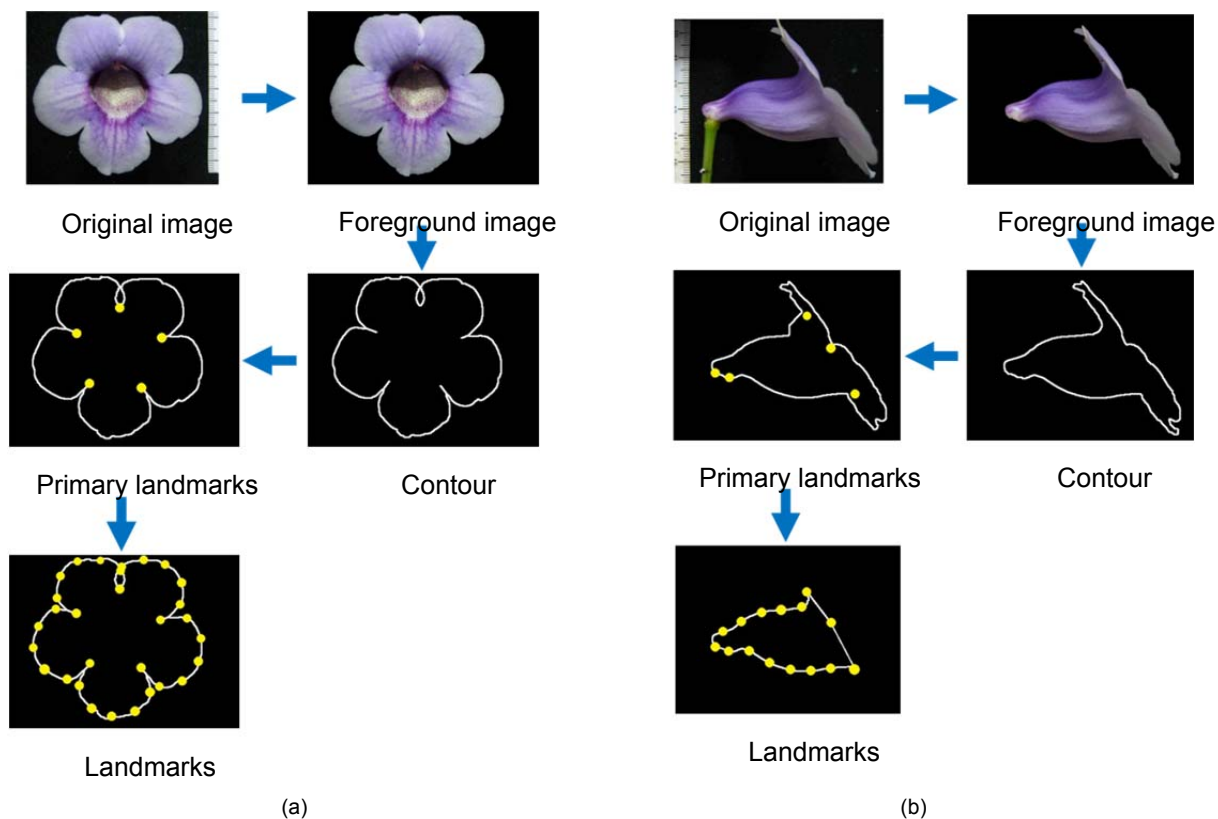
Figure 4. Landmark identification of the (a) face-view and (b) side-view images

General Procrustes analysis

GPA was carried out on the landmarks. Figure 5 shows the face-view and side-view landmarks before and after GPA. It can be shown that the scaling, translation, or rotation effects from the original images are minimized.
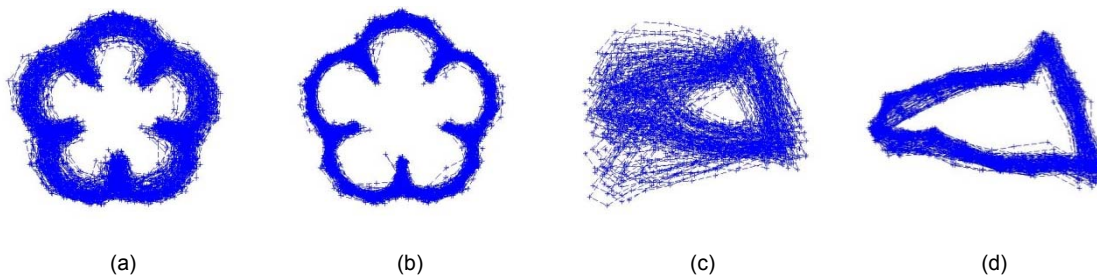

(a)   (b)   (c)   (d)

Figure 5. The face-view landmarks (a) before and (b) after GPA; the side-view landmarks (c) before and (d) after GPA

Principal component analysis

PCA was conducted on the face-view and side-view GPA landmarks, respectively. Figure 6 shows the percentage of total variance of the PCs for the (a) face-view and (b) side-view images, respectively. It is shown that the most of the variance in floral shape variation can be explained by the first few PCs. In fact, the first two PCs (PC1 and PC2) of the face-view images account for 19.2% and 16.0% of the total variance. PC1 and PC2 of the side-view images account for 54.5% and 13.5% of the total variance, respectively.
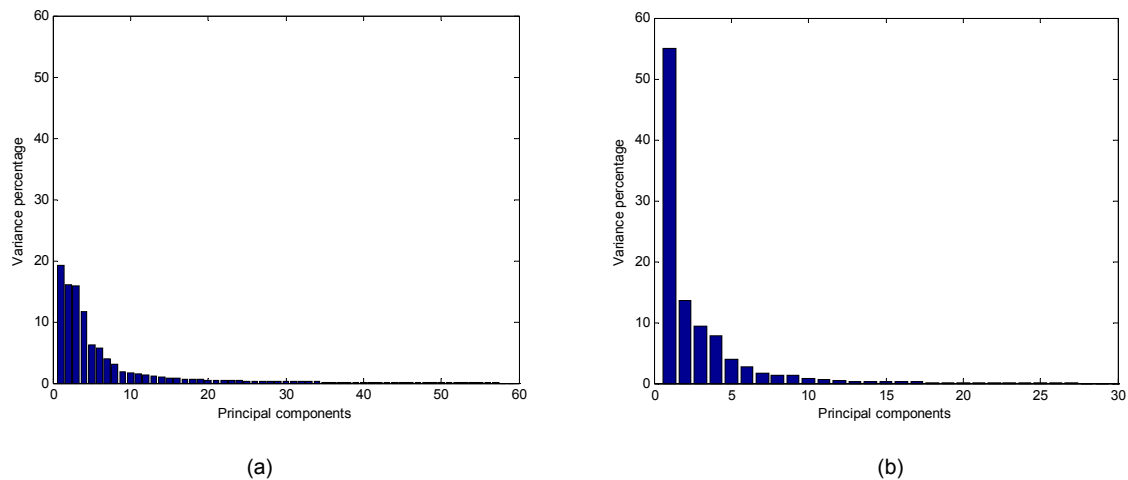
(a)   (b)

Figure 6. Variance percentage of each principal component in (a) face-view and (b) side-view images

Figure 7 shows the shapes of flowers with 3 PC1 values. In the face-view images, PC1 corresponds to the degree of overlap between the lobes. Higher degree of overlap occurs at larger PC1 values. In the side-view images, the PC1 corresponds to the degree of dorsiventral asymmetry (Giurfa et al., 1999). Higher degree of asymmetry occurs at larger PC1 values.
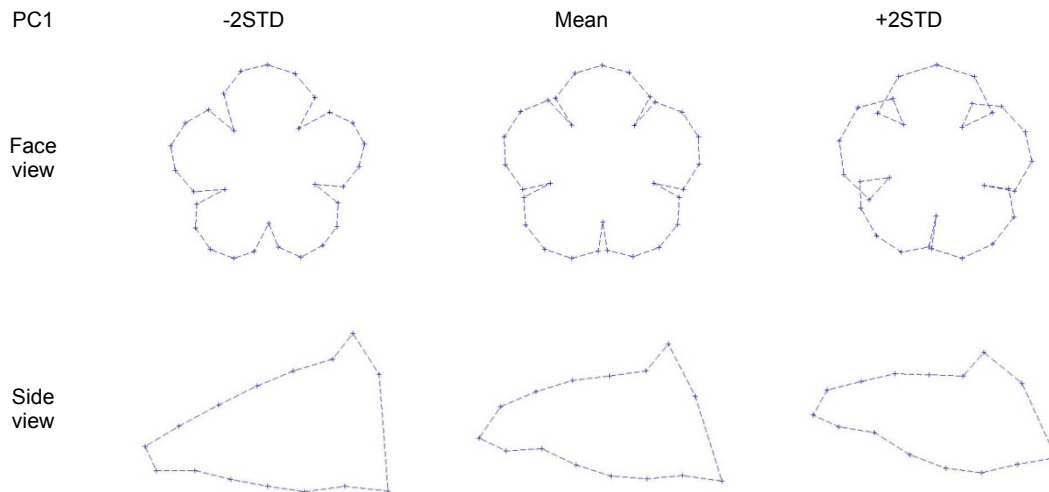

Figure 7. Effect of first principal component (PC1) in face-view and side-view images

Figure 8 shows the histograms of PC1 values in the face-view and side-view images. For the face-view images, most of the samples are located on the right and are associated with larger PC1 values, indicating that the lobes of the most flowers overlap substantially. For the side-view images, the samples can be clustered into two groups with a threshold near 80, indicating that a flower sample can only resemble one of the parents in terms of dorsiventral asymmetry.
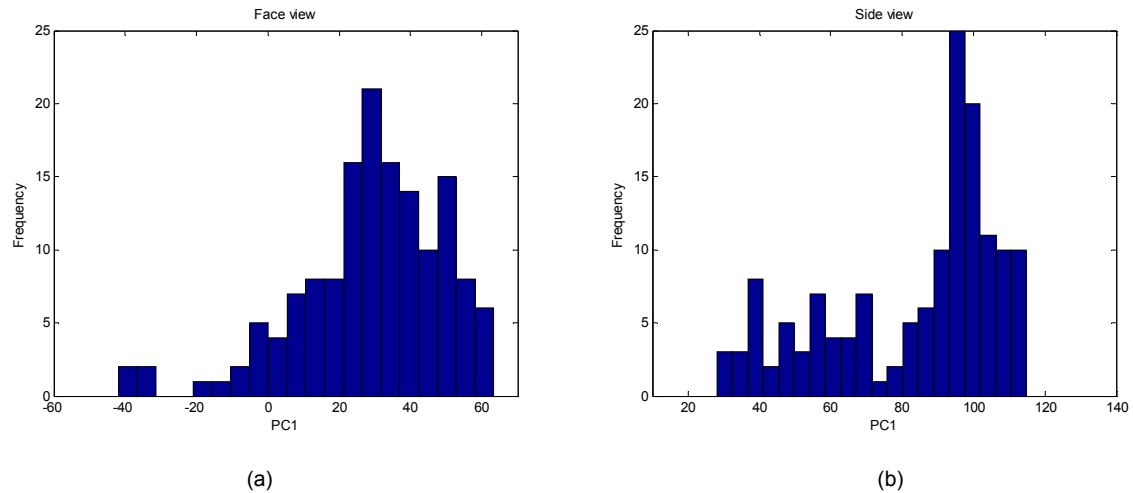
Figure 8. The histogram of the PC1 values in the (a) face-view and (b) side-view images

Conclusion

Floral shape variation was studied in the F2 population of an *S. speciosa* hybrid obtained by crossing wild-type and peloria forms of the plant. A semi-automatic procedure of image processing was developed to identify floral landmarks. The landmarks were then used for analyzing shape variation by morphometrics. GPA showed that PC1 of face-view and side-view images accounted for 19.2% and 54.5%, respectively, of floral shape variation in relation to the roundness of petal lobe, overlap of lobes, lobe configuration, dorsiventral asymmetry of the floral tube, openness, and the form of the nectar chamber. The floral shape variation was successfully quantified in the current study using a newly developed method. The proposed approach can be applied in the examination of floral genotype-phenotype association in future work.

Acknowledgements

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