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Quantitative analysis of floral symmetry and tube dilation in an F_2 cross of *Sinningia speciosa*

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ABSTRACT

Shape variation within a flower breeding line is a topic of considerable interest to horticulturalists. *Sinningia speciosa* (Florist's Gloxinia) is a species that presents diversified floral shapes. This study aimed to quantitatively assess floral shape variation in an F_2 cross of *S. speciosa* between a zygomorphic wild variety and an actinomorphic peloric cultivar via geometric morphometrics. The result indicated symmetric variation and tube dilation accounted for the major variance of floral shape changes. We further tested whether these shape variations can be correlated to any inherited genetic variation. ANOVA analysis detected candidate *CYCLOIDEA* (*SsCYC*) marker allele that showed strong associations with variations of corolla symmetry and tube dilation. This study demonstrated that using geometric morphometrics might considerably enhance the detection of phenotypic and genetic association on complex floral shape variation.

1. Introduction

Sinningia speciosa, also known as Florist's Gloxinia, is a tropical Brazilian species of the flowering plant family Gesneriaceae. The species is widely used as an ornamental indoor plant after domestication because of its considerable variation in floral shape and long-lasting bloom (Borochov and Shahar, 1989; Citerne and Cronk, 1999; Zaitlin, 2012). Wild varieties of *S. speciosa*, "Carangola," produce zygomorphic (bilaterally symmetrical) flowers with nodding corolla and narrow corolla tube throats (Fig. 1A and B). By contrast, its peloric cultivars "Peridots Darth Vaders" develop actinomorphic (radially symmetrical) flowers with erect corolla and open corolla tubes (Fig. 1C and D). When these 2 varieties are crossed, the individuals in the F_2 population vary in floral size and shape. These F_2 flowers thus provide excellent material for the study of floral shape variation in a breeding line.

Corolla shape must be captured and quantified with high precision to accurately determine its variation. Typically, corolla images are captured and then the floral contours are subject to geometric morphometrics (GM; Adams et al., 2004; Bookstein, 1991; Pavlinov, 2001) for quantitative shape analysis. Curve-based and landmark-based approaches are the two most widely used GM methods. Curve-based GM presents petal outlines by using elliptic Fourier descriptors (Kuhl and Giardina, 1982). In previous studies, morphological assessments of flowers have been conducted using these curve-based methods (Kawabata et al., 2011; Kawabata et al., 2009; Yoshioka et al., 2006; Yoshioka et al., 2007). By contrast, landmark-based approaches describe floral shape by using a set of characteristic points, referred to as landmarks, selected along the corolla contour (Adams et al., 2004; Klingenberg, 2010). Analysis is then conducted on the landmarks to examine the morphological disparity among flowers (Feng et al., 2009; Gómez et al., 2006; Savriama et al., 2012; Shipunov et al., 2005). Typically, landmark-based GM methods are applied for analyzing the shape variation of the entire flowers (e.g., corolla symmetry and tube dilation).

Symmetry and tube dilation are 2 key morphological characteristics of flowers. They are significantly related to the commercial

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Fig. 1. (A) Face view and (B) side view of accession "Carangola", and (C) face view and (D) side view of accession "Peridots Darth Vaders".

value of the floral products (Huang and Yeh, 2009), and have received attention in the relevant literature (Citerne and Cronk, 1999; Endress, 1999; Harrison et al., 1999; Perret et al., 2001; Savriama et al., 2012; Savriama and Klingenberg, 2011). Therefore, defining the scores of symmetry and dilation can facilitate the study of variations in these 2 characteristics among the F_2 segregations.

This study aimed to quantify the floral shape variation in an intercross line from a zygomorphic accession and an actinomorphic cultivar of *S. speciosa*, and using landmark-based GM to capture major principal components that contribute the floral shape variation. Front or, "face," views and side views of the F_2 corollas were photographed, thereby enabling landmarks to be identified using image-processing algorithms. To further test whether these floral shape variation can be putatively correlated to floral trait candidate locus, we investigated the association between these shape variation and *S. speciosa CYCLOIDEA (SsCYC)* marker allele inheritance among F_2 individuals using ANOVA. Recent studies have indicated that a *CYCLOIDEA* (*CYC*) gene plays a crucial role in the floral development of many major angiosperm family species (Busch et al., 2012; Chapman et al., 2012; Citerne et al., 2006; Feng et al., 2006; Preston and Hileman, 2012; Wang et al., 2008).

2. Materials and methods

2.1. Flower samples

The accessions "Carangola" and "Peridots Darth Vaders" (Fig. 1) were supplied by the Dr. Cecilia Koo Botanic Conservation and Environmental Protection Center (Pingtung, Taiwan). These 2 parental accessions were crossed to breed the F_1 plants, and the F_2 population was generated by selfing an F_1 individual (Hsu and Wang, unpublished data, Kuo et al., 2013). The plants were grown in a greenhouse under natural lighting with 20% shade, at 22–28 °C with 70–80% humidity, in a soilless mix vermiculite, perlite, and peat moss with a ratio of 1:1:1 (V/V/V), respectively. Flowers with different numbers of petal lobes were incomparable, or non-homologous (Adams et al., 2004), in shape. Therefore, only plants with flowers comprising 5 petal lobes were included in this study. In addition, only flowers at the front of each inflorescence were selected to avoid capturing the abnormal patterns of terminal flowers (Rudall and Bateman, 2003) could be avoided. Thus, 2 flowers were acquired from each of the selected 73 F_2 plants, providing a total of 146 specimens. The floral images were taken between May and November, 2011.

2.2. Image acquisition

The flowers were pinned to a blackboard and photographed using a digital camera (SD1000, Canon) to obtain images of 1600 by 1200 pixels in dimension. The face- and side-view images were captured with the camera facing the plane of unfolded petal lobes (Fig. 1A and C) and the dorsiventral plane of the flowers (Fig. 1B and D), respectively. All images were acquired during full bloom when the corolla had unfolded completely and the stamen and stigma had stopped growing.

2.3. Landmark identification

Floral landmarks are categorized as being either primary or secondary (Zelditch et al., 2004). Primary landmarks are readily recognizable points, such as the intersections between petals or sepals, whereas secondary landmarks are equally spaced points between 2 conjunctive primary landmarks. Five primary landmarks were defined for both the face-view and side-view images. The primary landmarks were defined as the intersection of 2 consecutive petal contours. Fig. 2 shows the landmarks and their assigned numbers. In the face-view images, the primary landmarks were labeled as 1, 7, 13, 19, and 25, starting from the dorsal lobe and proceeding clockwise (Fig. 2A). In the side-view images, the primary landmarks were the intersections of the sepal and tube, and the 2 consecutive petals. These were assigned to the numbers 1, 7, 8, 9, and 15 (Fig. 2B). The secondary landmarks were 5 equally spaced points between 2 conjunctive primary landmarks. Consequently, 30 face-view and 15 side-view landmarks were collected for each flower.

The landmarks were identified semi-automatically using image-processing algorithms (Rother et al., 2004; Suzuki, 1985). These algorithms were implemented by a program written in C++ using Qt Creator (Nokia) and OpenCV (Intel). The landmark identification

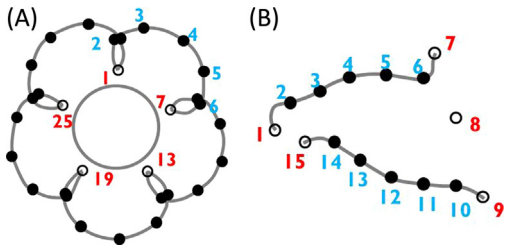

Fig. 2. (A) Face-view and (B) side-view primary (red) and secondary (cyan) landmarks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

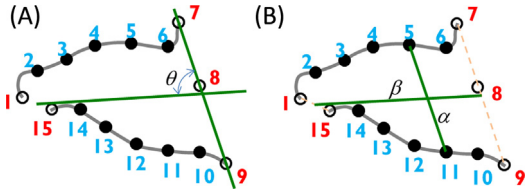

Fig. 3. Illustration of (A) symmetry and (B) dilation scores.

involved 4 steps: foreground segmentation, contour line detection, primary landmark selection, and secondary landmark determination. The semi-automatic process improves the accuracy of landmark identification. The details of the program are reported in (Kuo et al., 2013).

2.4. Shape variation analysis

Landmark-based GM was performed to evaluate floral shape variation. In this study, the "shape" was defined as the form that is unaffected by changes in the translation, scale, or rotation of the images. Landmark-based GM involves 2 processes: generalized Procrustes analysis (GPA) and principal component analysis (PCA). GPA (Gower, 1975; Rohlf and Slice, 1990) was performed to eliminate between-flower variation that was irrelevant to shape from the landmarks. The resulting data were then subjected to PCA. The first few principal components (PCs) derived from the PCA accounted for most of the between-flower variance, and could be used to summarize the floral shape variation with little loss of information. GM was performed using MATLAB (MathWorks Inc.).

2.5. Symmetry and dilation scores

The scores were defined to quantify degrees of symmetry and tube dilation of the flowers depicted in the side-view images. For the symmetry score, 2 lines were first obtained for each image, including a line regressed with Landmarks 7, 8, and 9; another line linking the middle point of Landmarks 7 and 9, and the middle point of Landmarks 1 and 15 (Fig. 3A). The symmetry score s was defined as the sine value of the angle θ between the 2 lines:

$$s = \sin(\theta). \quad (1)$$

According to this definition, a flower is perfectly actinomorphic when the angle θ is 90° and the symmetry score is 1. For the dilation score, 2 segments were obtained for each image. α was defined as the segment between Landmarks 5 and 11, and β was defined as the segment between the middle point of Landmarks 7 and 9, and the middle point between Landmarks 1 and 15 (Fig. 3B). The dilation score d was defined as the length of α divided by the length of β:

$$d = \frac{|\alpha|}{|\beta|}, \quad (2)$$

where the absolute symbol denotes the length of a segment. These 2 scores are unitless. The score calculation was performed using MATLAB (MathWorks Inc.).

2.6. Association of shape with marker inherited variation

Since shape variations among these F_2 flowers could potentially be attributed to their inherited genetic variation, we used analysis of variance (ANOVA) performed in MATLAB (MathWorks Inc.) to test for possible association between shape and one candidate marker *SsCYC*.

The *SsCYC* gene was polymorphic between the parental lines, zygomorphic wild variety and actinomorphic peloria (Citerne and Cronk, 1999; Citerne et al., 2000). The actinomorphic peloria allele of *SsCYC* contains a single nucleotide deletion between TCP and R domain at codon 201 Lysine position with one adenine dropped out for the codon AAA which results in a frame shift yielding a truncated protein (Citerne et al., 2000). We also identified another SNP located within the *Ear*I recognition site of zygomorphic wild variety (data not shown). We thus applied co-dominant Cleaved Amplified Polymorphic Sequences (CAPS) marker analysis (Konieczny and Ausubel, 1993) based on these polymorphic sites to discriminate *SsCYC* marker allele for genotyping of 73 F_2 plants. In that, part of *SsCYC* about 1060 bp in length were first PCR amplified with a combination of primer FPI (5′-TTC CCA ATT CAT CAT AYC TTC GTC CT-3′) and F*S (5′-TTT TGA AGT TTT CAG TTT TCA GAT AAT TGC T-3′). The amplicon from zygomorphic wild variety can then be digested by restriction enzyme *Ear*I into two fragments of 447 and 613 bp in size, while the amplicon from the actinomorphic peloria remain uncut. Since *SsCYC* of actinomorphic peloria produce truncated protein, we denoted the allele of zygomorphic wild variety as *C* and that of actinomorphic peloria as *c*.

3. Results

3.1. Landmark identification

Floral landmarks were identified using the procedure described in the Materials and Methods section. Fig. 4 shows the original image, foreground image, floral contour, primary landmarks, and all the landmarks.

3.2. Areal size and contour length

Floral areas and contours (Fig. 4) were quantified. The actual areal sizes and contour lengths of the flowers were then calculated based on a ruler placed in the background when the images were taken (Fig. 1). Fig. 5 shows histograms of the areal sizes (unit: cm^2) and contour lengths (unit: cm), color-coded by genotype. The numbers of flowers in genotypes *C*/*C*, *C*/*c*, and *c*/*c* were 56, 72, and 18, respectively. The mean face-view areal size and contour length were 22.7 cm^2 and 23.9 cm, respectively. The mean side-view areal size and contour length were 12.8 cm^2 and 18.4 cm, respectively. No significant difference was observed in the areal size and contour length among the flowers of different *SsCYC* marker alleles.

3.3. Shape variation

GM was performed on the identified landmarks. The contribution of each PC to the total variance was calculated. The first 2 PCs of the face-view images, referred to as F-PC1 and F-PC2, accounted for 19.2% and 16.0% of the total variance, respectively. The first 2 PCs of the side-view images, referred to as S-PC1 and S-PC2, accounted for 54.5% and 13.5%, of the total variance, respectively.

Fig. 6 shows the degree of floral shape variation caused by changes in the first 2 PCs. In the process of visualizing the

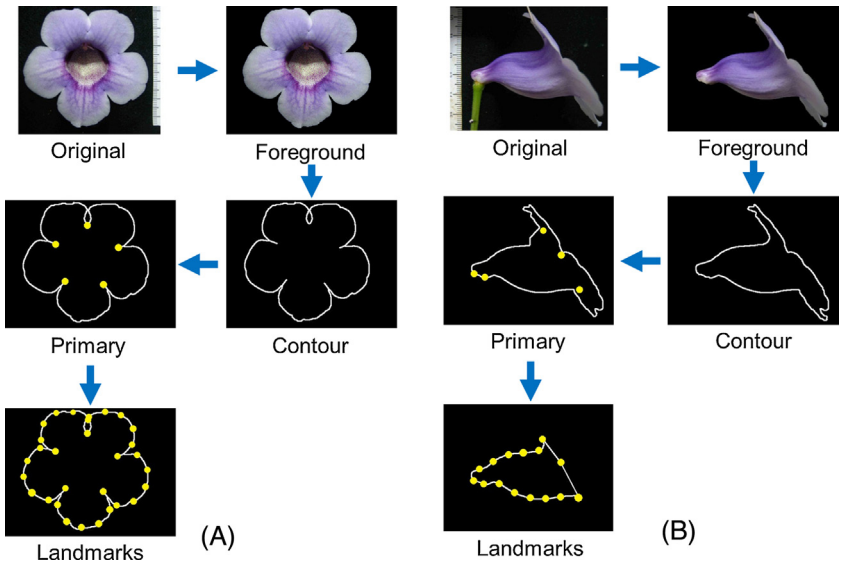

Fig. 4. Landmark identification process of the (A) face-view and (B) side-view images.

floral shape variation, the mean and standard deviation of the PCs were calculated. Landmarks were then obtained using inverse PCA, with the value of a particular PC being equal to the mean value plus or minus the value of 2 standard deviations, while the values of the other PCs were maintained at the mean values. Floral contours were then reconstructed based on the resulting landmarks. The contours were demonstrated using thin-plate spline approach (Rohlf and Slice, 1990) to reveal the degree of variation in the transformation of one mean shape to another. The mean floral contours are illustrated in gray. The contours of the mean plus or minus 2 standard deviations are illustrated in red. The blue arrows depict the direction and degree of transformation from one shape to another at the landmarks.

Fig. 6 shows that the variations in F-PC1 and F-PC2 primarily correspond to the degree of overlap between lobes, and shape changes in the lateral and ventral lobes. The variations in S-PC1 and S-PC2 primarily correspond to the degree of dorsiventral asymmetry and the degree of tube dilation. The variation in dorsiventral

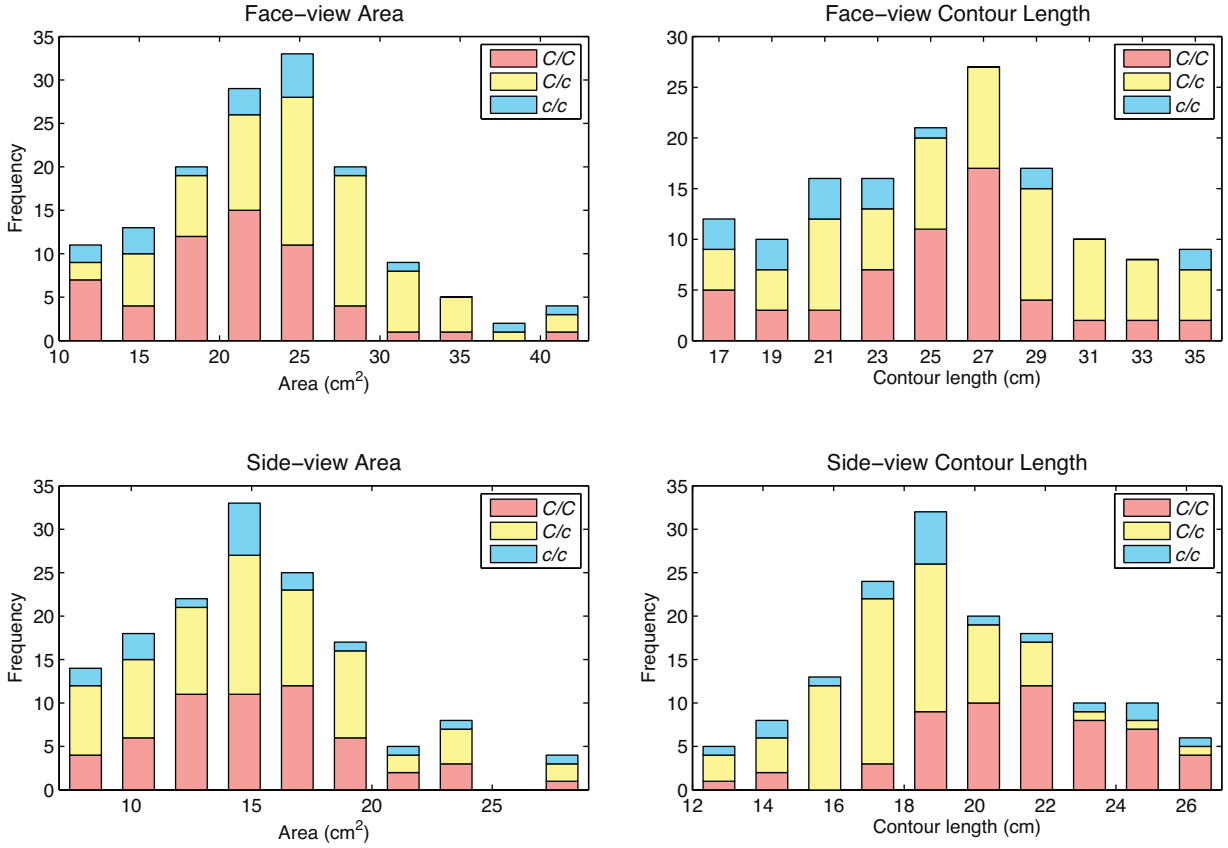

Fig. 5. Histograms of areal size and contour length.

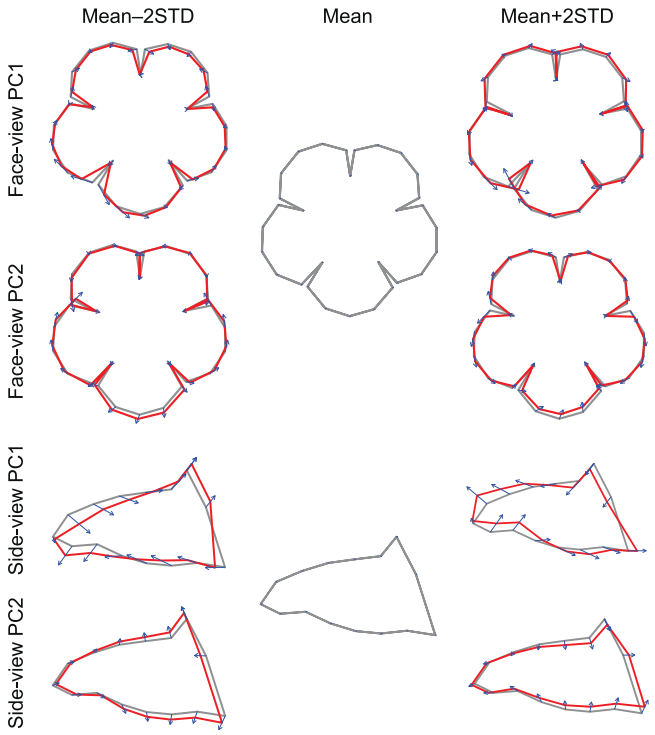

Fig. 6. Effect of principal components to floral shape variation.

asymmetry and tube dilation is considerable, thereby justifying the need to define and discuss the symmetry and dilation of the corolla further.

3.4. Principal component distribution and association with SsCYC inheritance

Fig. 7 shows PC1-PC2 scatterplots and PC histograms color-coded by genotype. The results of conducting the Kolmogorov–Smirnov test indicated that F-PC1, F-PC2, and S-PC2 were normally distributed (p value = 0.2953, 0.7434, and 0.6895). However, S-PC1 was not normally distributed (p value < 0.0001). The flowers of genotype *c*/*c* were associated with small S-PC1 values, and the flowers of genotype *C*/*C* were associated with large S-PC1 values. The S-PC1 values between the flowers of genotypes *c*/*c* and *C*/*C* were readily distinguishable (red and blue marks in Fig. 7B).

3.5. Association of symmetry and dilation scores with SsCYC inheritance

Fig. 8 illustrates the floral shapes for various S-PC1 values and their corresponding symmetry scores and angles, as well as histograms of the symmetry scores and angles. The angles of genotype *c*/*c* were generally larger than the angles of genotype *C*/*C*. ANOVA revealed that both the symmetry scores and angles of the 3 genotypes were significantly different (p-values < 0.0001). This indicates that the *SsCYC* inheritance associates to the variation in tube symmetry among F_2 individuals. The mean symmetry scores of the genotypes *C*/*C*, *C*/*c*, and *c*/*c* were 0.966, 0.959, and 0.996, respectively. The mean angles of the genotypes *C*/*C*, *C*/*c*, and *c*/*c* were 75.49°, 75.15°, and 86.97°, respectively.

Fig. 9 illustrates the flowers for various S-PC2 values and their corresponding dilation scores, and also shows a histogram of the dilation score. ANOVA revealed that the dilation scores of the 3 genotypes were significantly different (p value = 0.0029). This indicates that the *SsCYC* inheritance correlates to the tube dilation variation among F_2 individuals. The mean dilation scores of the genotypes *C*/*C*, *C*/*c*, and *c*/*c* were 0.545, 0.524, and 0.490, respectively.

4. Discussion

In this study, GM was conducted to evaluate shape variation in floral face-view and side-view images independently. However, flowers are essentially 3-dimensional (3D). Performing GM on 2-dimensional images separately may result in the loss of the correlation between the face-view and side-view images. A previous study has demonstrated the application of 3D GM on flowers (van der Niet et al., 2010). By using appropriate 3D image acquisition techniques, such as micro-computed tomography, 3D GM can be used in future studies to examine the floral morphology of *S. speciosa* more accurately and completely.

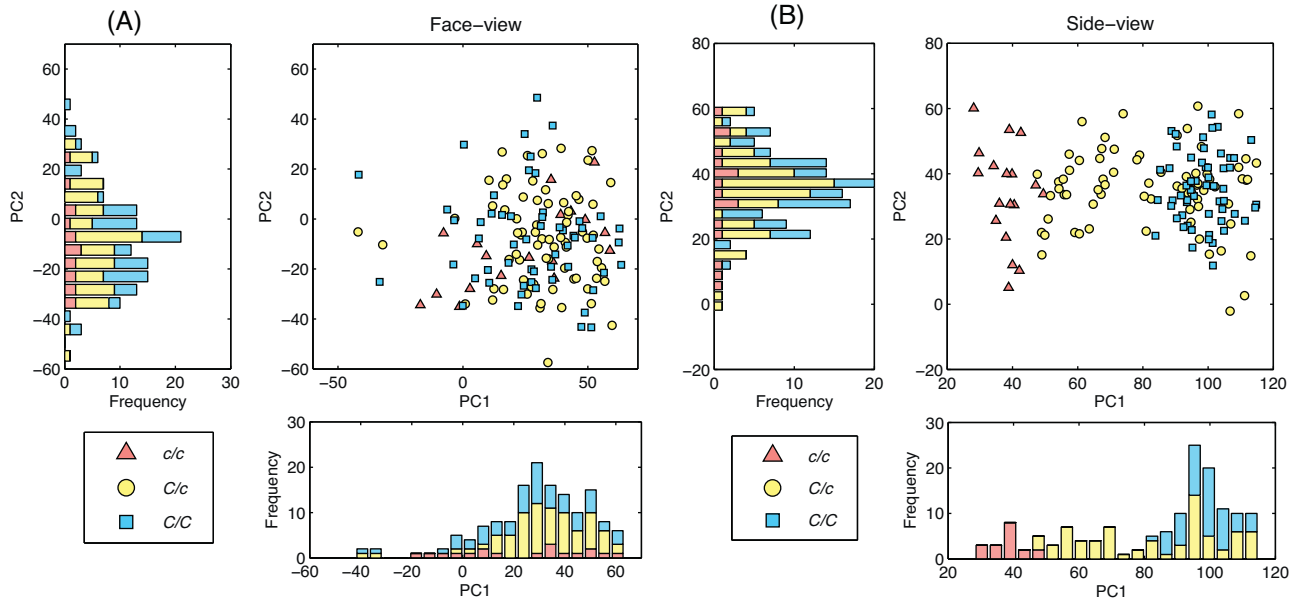

Fig. 7. (A) Face-view and (B) side-view PC1-PC2 scatterplots and PC histograms.

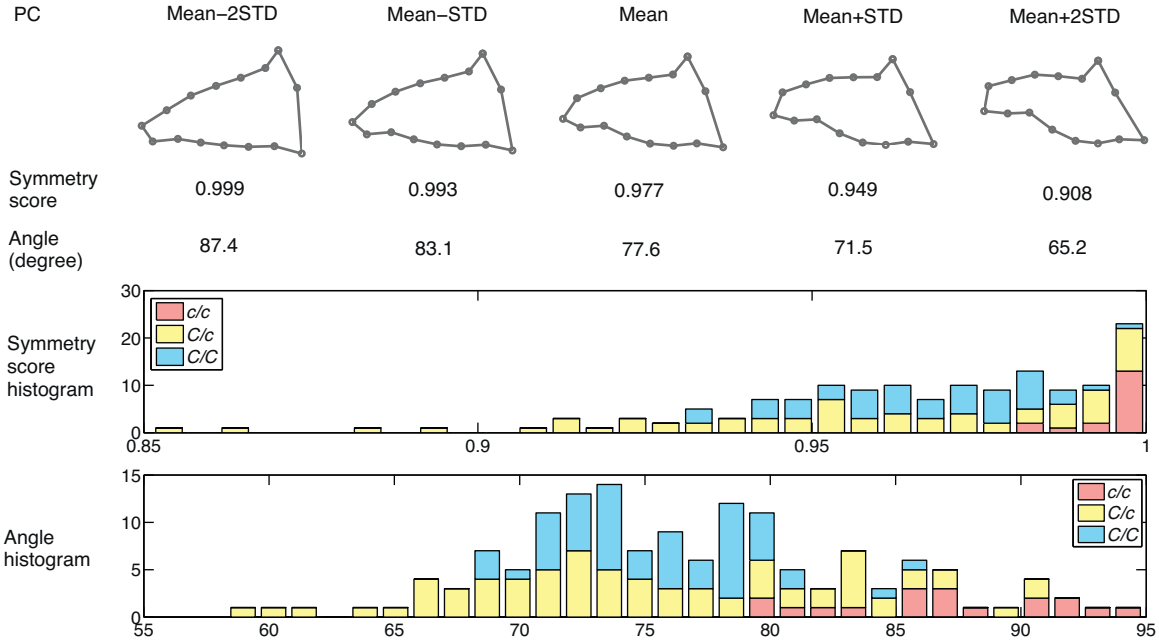

Fig. 8. Floral shapes of various symmetry scores, symmetry score histogram, and angle histogram.

The finding of associations of floral shape symmetric variation and tube dilation with *SsCYC* allelic inheritance implies either *SsCYC* or other genes tightly linked to *SsCYC* may have major roles in control floral shape. Further works on validating *SsCYC* function on flower phenotype via gene transformation analysis and detailed genetic analysis confirming the linkage between the allelic polymophism and the resulting phenotypes are necessary.

Phylogenetic analysis of *Cyc*-like genes in Gesneriaceae (*GCYC*) suggests that *GCYC* genes family has undergone duplication events from ancestral *GCYC* to *GCYC1* and *GCY2* (Citerne et al., 2000; Wang et al., 2004). It may be argued that *S. speciosa* might have several *CYC* copies as the cases found in other angiosperm species. However, all New World Gesneriaceae species including *S. speciosa* appear to inherit only one copy of *GCYC1* due to loss of *GCYC2* (Wang et al., 2004; Smith et al., 2004, 2006). By searching the *S. tuberosa* transcriptome data in 1KP project website (1000 plant transciptome http://onekp.com/project.html) and our unpublished transcriptome data on *S. speciosa*, there appears to have only single *SsCYC* copy. We thus regarded the *SsCYC* identified in this study as the single *CYC*-like gene that relates to floral symmetry in *S. speciosa*.

There are other well-known floral symmetry and shape genes such as *RADIALIS*, *DIVARICATA* and *MIXTA* may also contribute to affect the floral shape variations (Corley et al., 2005; Baumann et al., 2007). Relating these genes to GM-based petal outlines via candidate gene association or uncovering other new shape related genes via QTL mapping shall greatly extend our understanding on how genotypic combination of alleles affects floral shape variation

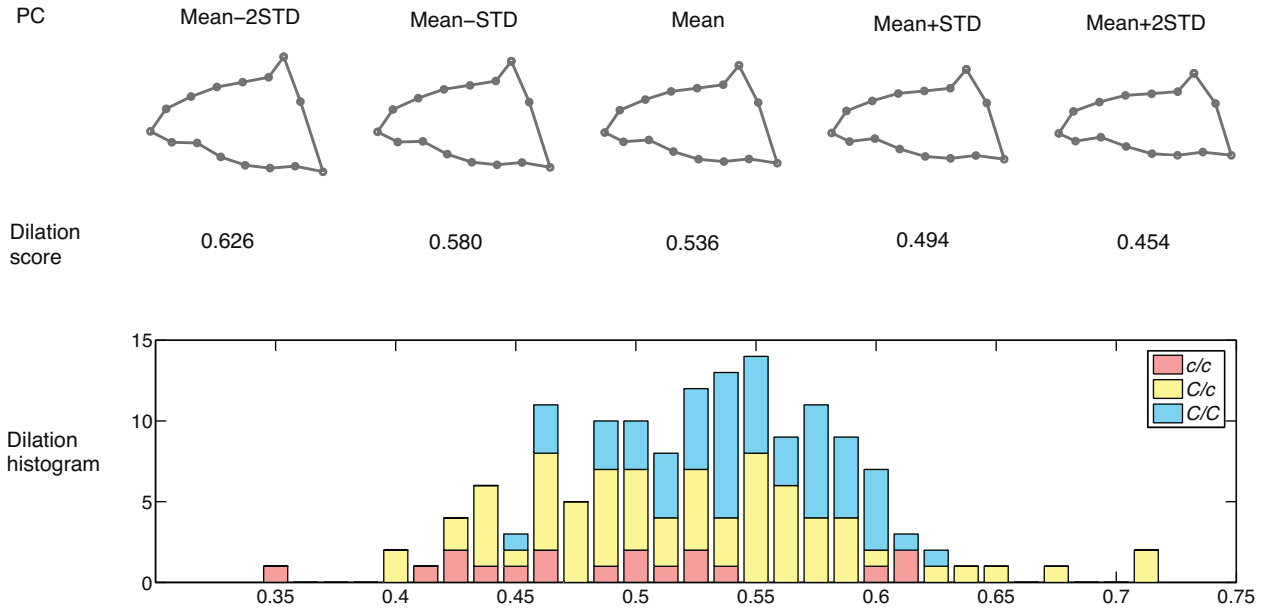

Fig. 9. Floral shapes of various dilation scores and dilation score histogram.

(Ehrenreich et al., 2007, 2009). Since floral shape variations are complex traits, our results have demonstrated the usefulness of using GM to adequately describe petal shapes (Gómez and Perfectti, 2010). Our approach will surely facilitate finding of corresponding floral shape phenotype-genotype associations in details if we further increase the F_2 mapping population sizes with segregating phenotypes and polymorphism in genes of interests in the future.

5. Conclusion

Floral shape variation in the F_2 intercross between the zygomorphic wild variety and actinomorphic peloria of *S. speciosa* was quantitatively determined. Side-view corolla symmetry and tube dilation were successfully uncovered by GM method as the major principal components that account for floral shape variations. The tight correlations of certain combinations of *SsCYC* alleles with corolla symmetry and tube dilation imply a putative major role of *SsCYC* on floral shape variations in *S. speciosa*.

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References

Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Ital. J. Zool. 71, 5–16.
Baumann, K., Perez-Rodriguez, M., Bradley, D., Venail, J., Bailey, P., Jin, H., Koes, R., Roberts, K., Martin, C., 2007. Control of cell and petal morphogenesis by R2R3 MYB transcription factors. Development 134, 1691–1701.
Bookstein, F.L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge.
Borochov, A., Shahar, T., 1989. Effects of mefluidide and paclobutrazol on growth, flowering and chilling response of gloxinia. Sci. Hortic. 39, 331–339.
Busch, A., Horn, S., Mühlhausen, A., Mummenhoff, K., Zachgo, S., 2012. Corolla monosymmetry: evolution of a morphological novelty in the Brassicaceae family. Mol. Biol. Evol. 29, 1241–1254.
Chapman, M.A., Tang, S., Draeger, D., Nambeesan, S., Shaffer, H., Barb, J.G., Knapp, S.J., Burke, J.M., 2012. Genetic analysis of floral symmetry in Van Gogh's sunflowers reveals independent recruitment of *CYCLOIDEA* genes in the Asteraceae. PLoS Genet. 8, e1002628.
Citerne, H.L., Cronk, Q.C.B., 1999. The origin of the peloric *Sinningia*. New Plantsman 6, 219–222.
Citerne, H.L., Möller, M., Cronk, Q.C.B., 2000. Diversity of *cycloidea*-like genes in Gesneriaceae in relation to floral symmetry. Ann. Bot. 86, 167–176.
Citerne, H.L., Pennington, R.T., Cronk, Q.C.B., 2006. An apparent reversal in floral symmetry in the legume *Cadia* is a homeotic transformation. Proc. Natl. Acad. Sci. U.S.A. 103, 12017–12020.
Corley, S.B., Carpenter, R., Copsey, L., Coen, E., 2005. Floral asymmetry involves an interplay between TCP and MYB transcription factors in *Antirrhinum*. Proc. Natl. Acad. Sci. U.S.A. 102, 5068–5073.
Ehrenreich, I.M., Hanzawa, Y., Chou, L., Roe, J.L., Kover, P.X., Purugganan, M.D., 2009. Candidate gene association mapping of Arabidopsis flowering time. Genetics 183, 325–335.
Ehrenreich, I.M., Stafford, P.A., Purugganan, M.D., 2007. The genetic architecture of shoot branching in *Arabidopsis thaliana*: a comparative assessment of candidate gene associations vs. quantitative trait locus mapping. Genetics 176, 1223–1236.
Endress, P.K., 1999. Symmetry in flowers: diversity and evolution. Int. J. Plant Sci. 160, S3–S23.
Feng, X., Wilson, Y., Bowers, J., Kennaway, R., Bangham, A., Hannah, A., Coen, E., Hudson, A., 2009. Evolution of allometry in *Antirrhinum*. Plant Cell Online 21, 2999–3007.
Feng, X., Zhao, Z., Tian, Z., Xu, S., Luo, Y., Cai, Z., Wang, Y., Yang, J., Wang, Z., Weng, L., Chen, J., Zheng, L., Guo, X., Luo, J., Sato, S., Tabata, S., Ma, W., Cao, X., Hu, X., Sun, C., Luo, D., 2006. Control of petal shape and floral zygomorphy in *Lotus japonicus*. Proc. Natl. Acad. Sci. U.S.A. 103, 4970–4975.
Gómez, J.M., Perfectti, F., Camacho, J.P.M., 2006. Natural selection on *Erysimum mediohispanicum* flower shape: Insights into the evolution of zygomorphy. Am. Nat. 168, 531–545.
Gómez, J.M., Perfectti, F., 2010. Evolution of complex traits: the case of *Erysimum* corolla shape. Int. J. Plant Sci. 171, 987–998.
Gower, J.C., 1975. Generalized procrustes analysis. Psychometrika 40, 33–51.
Harrison, C.J., Möller, M., Cronk, Q.C.B., 1999. Evolution and development of floral diversity in *Streptocarpus* and *Saintpaulia*. Ann. Bot. 84, 49–60.
Huang, L.C., Yeh, T.F., 2009. Floral consumption values for consumer groups with different purchase choices for flowers. HortTechnology 19, 563–571.
Kawabata, S., Nii, K., Yokoo, M., 2011. Three-dimensional formation of corolla shapes in relation to the developmental distortion of petals in *Eustoma grandiflorum*. Sci. Hortic. 132, 66–70.
Kawabata, S., Yokoo, M., Nii, K., 2009. Quantitative analysis of corolla shapes and petal contours in single-flower cultivars of lisianthus. Sci. Hortic. 121, 206–212.
Klingenberg, C.P., 2010. Evolution and development of shape: integrating quantitative approaches. Nat. Rev. Genet. 11, 623–635.
Konieczny, A., Ausubel, F.M., 1993. A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. 4, 403–410.
Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. Comput. Graphics Image Process. 18, 236–258.
Kuo, Y.F., Weng, L.K., Lee, T.K., Hsu, H.C., Lin, T.T., Wang, C.N., 2013. Quantitative evaluation of the floral shape variation in *Sinningia speciosa* domestication. Am. Soc. Agric. Biol. Eng. Annu. Int. Meet. 2013, 1933–1940.
Pavlinov, I.Y., 2009. Geometric morphometrics, a new analytical approach to comparison of digitized images. In: Information Technology in Biodiversity Research, Abstracts of the 2nd. International Symposium, pp. 41–90, St. Petersburg.
Perret, M., Chautems, A., Spichiger, R., Peixoto, M., Savolainen, V., 2001. Nectar sugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). Ann. Bot. 87, 267–273.
Preston, J.C., Hileman, L.C., 2012. Parallel evolution of TCP and B-class genes in Commelinaceae flower bilateral symmetry. EvoDevo 3, 1–14.
Rohlf, F.J., Slice, D., 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Biol. 39, 40–59.
Rother, C., Kolmogorov, V., Blake, A., 2004. GrabCut: interactive foreground extraction using iterated graph cuts. ACM Trans. Graphics 23, 309–314.
Rudall, P.J., Bateman, R.M., 2003. Evolutionary change in flowers and inflorescences: evidence from naturally occurring terata. Trends Plant Sci. 8, 76–82.
Savriama, Y., Gómez, J.M., Perfectti, F., Klingenberg, C.P., 2012. Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). New Phytol. 196, 945–954.
Savriama, Y., Klingenberg, C.P., 2011. Beyond bilateral symmetry: geometric morphometric methods for any type of symmetry. BMC Evol. Biol. 11, 280.
Shipunov, A.B., Fay, M.F., Pillon, Y., Bateman, R.M., Chase, M.W., 2005. Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia. Biol. J. Linnean Soc. 85, 1–12.
Smith, J.F., Hileman, L.C., Powell, M., Baum, D.A., 2004. Evolution of *GCYC*, a Gesneriaceae homolog of *CYCLOIDEA*, within subfamily Gesnerioideae (Gesneriaceae). Mol. Phylogenet. Evol. 31, 765–779.
Smith, J.F., Funke, M.M., Woo, V.L., 2006. A duplication of *gcyc* predates divergence within tribe Coronanthereae (Gesneriaceae): Phylogenetic analysis and evolution. Plant Syst. Evol. 261, 245–256.
Suzuki, S., 1985. Topological structural analysis of digitized binary images by border following. Comput. Vision Graphics Image Process. 30, 32–46.
van der Niet, T., Zollikofer, C.P., León, M.S., Johnson, S.D., Linder, H.P., 2010. Three-dimensional geometric morphometrics for studying floral shape variation. Trends Plant Sci. 15, 423–426.
Wang, C.N., Möller, M., Cronk, Q.C.B., 2004. Phylogenetic position of *Titanotrichum oldhamii* (Gesneriaceae) inferred from four different gene regions. Syst. Bot. 29, 407–418.
Wang, Z., Luo, Y., Li, X., Wang, L., Xu, S., Yang, J., Weng, L., Sato, S., Tabata, S., Ambrose, M., Rameau, C., Feng, X., Hu, X., Luo, D., 2008. Genetic control of floral zygomorphy in pea (*Pisum sativum* L.). Proc. Natl. Acad. Sci. U.S.A. 105, 10414–10419.
Yoshioka, Y., Iwata, H., Hase, N., Matsuura, S., Ohsawa, R., Ninomiya, S., 2006. Genetic combining ability of petal shape in garden Pansy (*Viola* × *wittrockiana* Gams) based on image analysis. Euphytica 151, 311–319.
Yoshioka, Y., Ohashi, K., Konuma, A., Iwata, H., Ohsawa, R., Ninomiya, S., 2007. Ability of bumblebees to discriminate differences in the shape of artificial flowers of *Primula sieboldii* (Primulaceae). Ann. Bot. 99, 1175–1182.
Zaitlin, D., 2012. Intraspecific diversity in *Sinningia speciosa* (Gesneriaceae: Sinningieae), and possible origins of the cultivated florist's gloxinia. AoB Plants 2012, pls039.
Zelditch, M., Swiderski, D., Sheets, D.H., Fink, W., 2004. Geometric Morphometrics for Biologists: A Primer. Elsevier Academic Press, Waltham, MA.