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

Hao-Chun Hsu[a,1], Chia-Ying Chen[e,1], Tzu-Kuei Lee[c], Li-Kang Weng[c], Der-Ming Yeh[d], Ta-Te Lin[c], Chun-Neng Wang[a,b,*], Yan-Fu Kuo[c,**]

[a] *Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei 106, Taiwan, ROC*
[b] *Department of Life Science, National Taiwan University, Taipei 106, Taiwan, ROC*
[c] *Department of Bio-Industrial Mechatronics Engineering, National Taiwan University, Taipei 106, Taiwan, ROC*
[d] *Department of Horticulture and Landscape Architecture, National Taiwan University, Taipei 106, Taiwan, ROC*
[e] *Department of Life Science, National Taiwan Normal University, Taipei 116, Taiwan, ROC*

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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.

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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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* Corresponding author at: National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan, ROC. Tel.: +886 2 33664537; fax: +886 2 23673374.
** Corresponding author at: National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan, ROC. Tel.: +886 2 33665329; fax: +886 2 23627620.
E-mail addresses: leafy@ntu.edu.tw (C.-N. Wang), ykuo@ntu.edu.tw, yanfukuo@gmail.com (Y.-F. Kuo).

[1] These authors contributed equally to this work.

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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

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