Knockdown of floral symmetry in *Fedia graciliflora*

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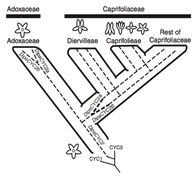
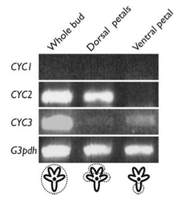
Introduction

Shifts between radial symmetry (actinomorphy) and bilateral symmetry (zygomorphy) in flowers have occurred multiple times independently within angiosperms. These morphological shifts are commonly associated with increased pollinator specialization and speciation rates.

Evidence from the studies across angiosperms indicate that the *CYCLOIDEA* (*CYC*)-like genes, which belong to the TCP gene family, play a role in patterning bilaterally symmetrical flowers. Three core eudicot clades of *CYC*-like genes have been identified: CYC1, CYC2 and CYC3, with *CYC2*-like genes being the most involved in dorsal specialization.



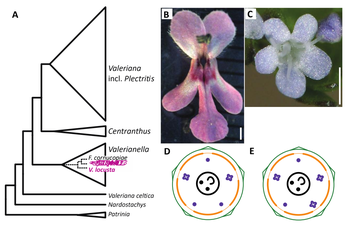
Howarth et al. 2011, Annals of Botany



Howarth and Donoghue 2006, PNAS

Previous work in the lab has suggested that bilateral symmetry is regulated by dorsoventral gradient of expression. To further investigate the *CYC*-like genes and the regulation of bilateral symmetry we used the non-model plant, *Fedia graciliflora* (Caprifoliaceae). *Fedia* species have strongly bilaterally symmetrical, pink flowers with morphologically distinct dorsal (upper), lateral, and ventral (lower) petals and only two functional stamen. *Fedia* has evolved within the *Valerianella*, a group with white, pseudo-radially symmetric flowers with three functional stamen, providing a model for studying the evolution of strong bilateral symmetry.

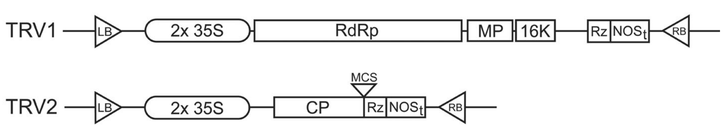
In order to examine the function of CYC-like genes in *F. graciliflora*, we used Virus-induced gene silencing (VIGS), a technique that utilizes the RNAi pathway to downregulate protein coding genes in non model plants. Around the divergence of the bilaterally symmetrical Caprifoliaceae from the radially symmetrical Adoxaceae, duplications occurred in CYC2 and CYC3, and therefore, *F. graciliflora* contain two paralogs of each of these genes. In this study, VIGS was used to study morphological differences in floral symmetry by knocking down each of these separately: *FgCYC2A*, *FgCYC2B*, *FgCYC3A*, and *FgCYC3B*.



Phylogeny and morphology of Valerianaceae. (A) Phylogenetic diagram of Valerianaceae showing *Fedia* within *Valerianella*. (B-E) Flower pictures and diagrams of *F. graciliflora* (B, D) and *V. locusta* (C, E). Scale bars represent 1 mm (B, C). The floral diagrams sepals (green), petals (orange), stamens (brown; dots indicate staminodes), and gynoecium (black; dots indicate non-fertile locules).

Experimental Strategy & Material

VIGS Constructs



Schematic of TRV1 and 2 constructs: LB, left border; RB, right border; RdRp, RNA-dependant RNA polymerase; MP, movement protein; 16 K, 16 Kd protein; Rz, self-cleaving ribozyme; NOSt, NOS terminator; CP, coat protein; MCS, multiple cloning site. (From, Gould and Kramer 2007)

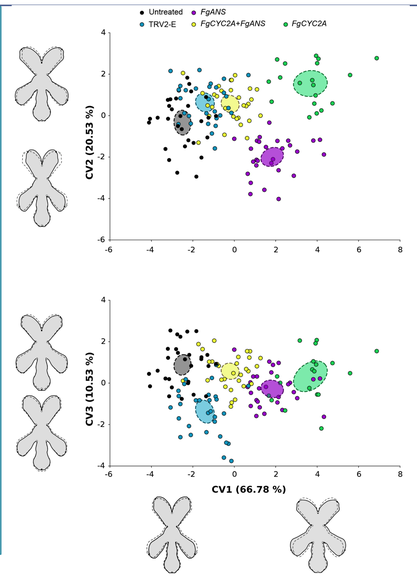
Tobacco rattle virus (TRV) expression vectors are used to carry inserts, in this case fragments of *FgCYC2A*, *FgCYC2B*, *FgCYC3A*, or *FgCYC3B*, which are ligated to the vector in the MCS. The TRV constructs are then Introduced to *Agrobacterium,* and cultured before inoculated into the leaves of the plants.

The down-regulated plants will be assayed using geometric morphometrics combined with tools of multivariate statistical shape analysis to quantify shape changes. Ten landmarks will be collected from the intersection between the primary and secondary veins with the petal margin or at the junction of the petal bases. Previous work in our lab has demonstrated the utility of this protocol to measure changes of shape (Berger et al. 2017).

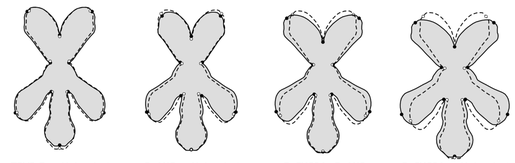
Results

Previous research showed that down-regulation of *FgCYC2A* and *FgANS* (*Anthocyanidin Synthase*) resulted in flowers in different shape morphospace. *FgCYC2A* down-regulation resulted in the most shift in dorsal petals, with them being more splayed (shifting ventrally).

Figure shows canonical variate analysis (CVA) plots of *F. graciliflora* flower shape data. CVA is corrected for within-group allometry with mock-treated controls (TRV2-E) (N = 30), FgANS (N = 28), FgCYC2A + FgANS (N = 30), and FgCYC2A (N = 19) knockdown treatments compared to untreated (N = 29). 95% confidence ellipses of means for each group is represented (filled ellipses with dashed contours). The outline drawings of flowers show shape changes associated with each CV from the overall average shape (dotted outline and open circles) for CV1 scores of −3 and +4, for CV2 scores of −3 and +2, and for CV3 scores of −2 and +2 (solid black outline with grey background and solid black circles). Note that these outline drawings are an interpolated form of presentation from the actual landmarks based on the thin-plate spline technique that makes it easier to visualise shape changes. This means that the relevant information in this CVA is from the positions of the landmarks, not from the outline drawings (From Berger et al. 2017).



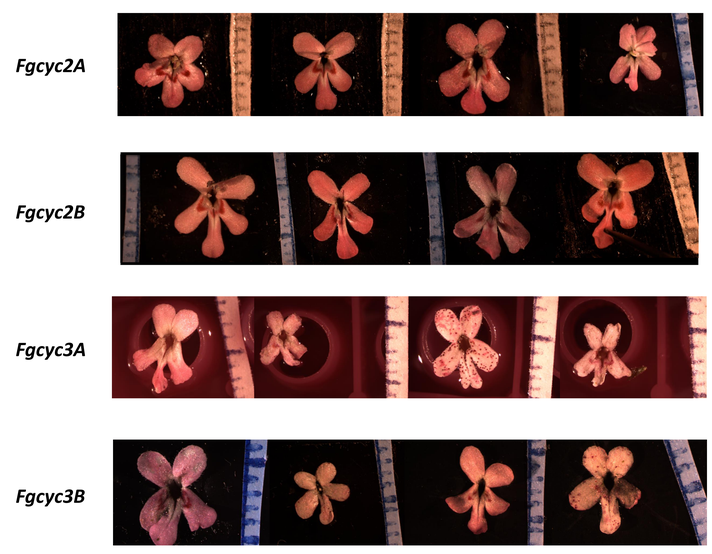
The outline drawings of flowers show the shape change deviations from the untreated average shape (dotted outline and open circles) for each treatment (solid black outline with grey background and solid black circles) for a Procrustes distance of +0.1. Note that these outline drawings are an interpolated form of presentation from the actual landmarks based on the thin-plate spline technique that makes it easier to visualise shape changes. This means that the relevant information is from the positions of the landmarks, not from the outline drawings (Berger et al. 2017).



TRV2-E vs Untreated | *FgANS* vs Untreated | *FgCYC2A+FgANS* vs Untreated | *FgCYC2A* vs Untreated

CYC knockdowns compared to wildtype

We have down-regulated each of the four CYC paralogs from at least ten plants. From each plant, between five and 25 flowers have been photographed with a stereomicroscope in triplicate. A subset of four flowers are shown in the figure below. Lines on ruler represent 1 mm. Wildtype flower is shown in upper left. Floral morphology in F. graciliflora is highly variable with frequent increases in petal number, changes in folding of the lateral petals, and changes in size. This makes geometric morphometrics analyses instrumental in pinpointing each paralogs effect on shape. Our previous analyses on *Fgcyc2A* knockdowns show a shift to wider, shorter flowers, with dorsal petals that are more splayed (Berger et al. 2017). These changes make the flowers appear more radial, which is similar to what has been characterized other core eudicots. Loss-of-function of CYC2 paralogs across core eudicots have a ventralizing effect on flowers, while CYC3 paralogs remain largely uncharacterized. We outline the general patterns for each paralog in the conclusions.



Conclusion

- VIGS treated flowers were morphologically distinct from the wildtype. Floral pigment and stamen number (either gain or loss) was also affected.
- *FgCYC2A* knockdowns have shorter, wider dorsal petals that are more splayed
- *FgCYC2B* knockdowns have dorsal petals that are even more splayed and longer than those of *FgCYC2A*
- Most flowers of *FgCYC3A* knockdowns were only 4-5 mm long, much smaller than the typical 8-10 mm of wildtype plants. Petals were thin and often transparent. The ventral petal was often shortened or not properly developed. Dorsal petals did not appear to splay away from each other.
- *FgCYC3B* knockdowns were the most variable. Many flowers often had dorsal and lateral petals of similar length, and with longer ventral petals making them resemble "elephant faces."

Further Experiments

- We aim to place landmarks on flowers from each knockdown for geometric morphometric and CVA analyses to quantify the precise changes in shape that result from down-regulation of each paralog.
- We aim to compare expression levels of the each CYC gene copy in each knockdown via qPCR. For example, how the expression levels of *Fgcyc2B*, *Fgcyc3A*, and *Fgcyc3B* are affected in the *Fgcyc2A* knockdown using qPCR to measure the expression levels.
- We also aim to examine how other transcription factors are affected when Fgcyc is down-regulated.

References

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Acknowledgements

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