

Combining benchwork and bioinformatics to reconstruct the evolutionary history of *CUP-SHAPED COTYLEDON* in honeysuckles and relatives

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INTRODUCTION

Context

Fusion among adjacent parts is a phenomena that occurs throughout flowering plants. *CUP-SHAPED COTYLEDON*, *CUC*, a member of the *NAC* Subfamily 1a transcription factors, and has been shown to affect organ boundary formation.^{1,2} Honeysuckles, or *Lonicera* (Caprifoliaceae, Dipsacales), are known for fusing petals into long tubes and also exhibit fusion among ovaries, bracts and leaves. Variation in fusion across 160 species of *Lonicera* make them an excellent system to investigate the evolution of fusion.

Goals

- Recover *CUC* from the phylogenetic diversity of Dipsacales, using PCR and cloning; sample target species in which no genomic data is available.
- Reconstruct a gene tree for *NAC* Subfamily 1a and *CUC* using both direct sequences gained in this study and data extracted from available genomic resources.

DIPSACALES SPECIES SAMPLED



Figure 1. Representatives of four clades of Dipsacales sampled in this study. Collections and observations of these species were made from the living collections at the Arnold Arboretum of Harvard University

KEY FINDINGS

We recovered 15 *CUC* and one NAC100 sequence from a total of 38 species sampled across the Dipsacales. *CUC3* was not successfully amplified or sequenced.

Focusing on the evolution of *CUC* across Dipsacales, we recovered a duplication of *CUC1/2* and one loss of *CUC3* specific to Caprifoliaceae (Fig. 2):

- Our inclusion of *Diervilla* and *Weigela*, which together form a clade sister to the rest of Caprifoliaceae, confirms the duplication of *CUC1/2* occurred prior to the diversification of Caprifoliaceae rather than within Caprifoliaceae.
- Our inability to amplify *CUC3* from Caprifoliaceae continues to support the loss of this gene that occurs throughout angiosperms.

Directly isolating and sequencing *CUC* was an important approach to include species for which no genomic data were available (e.g., *Diervilla*, *Dipelta*, and *Weigela*).

RECOVERING CUC FROM DIPSACALES

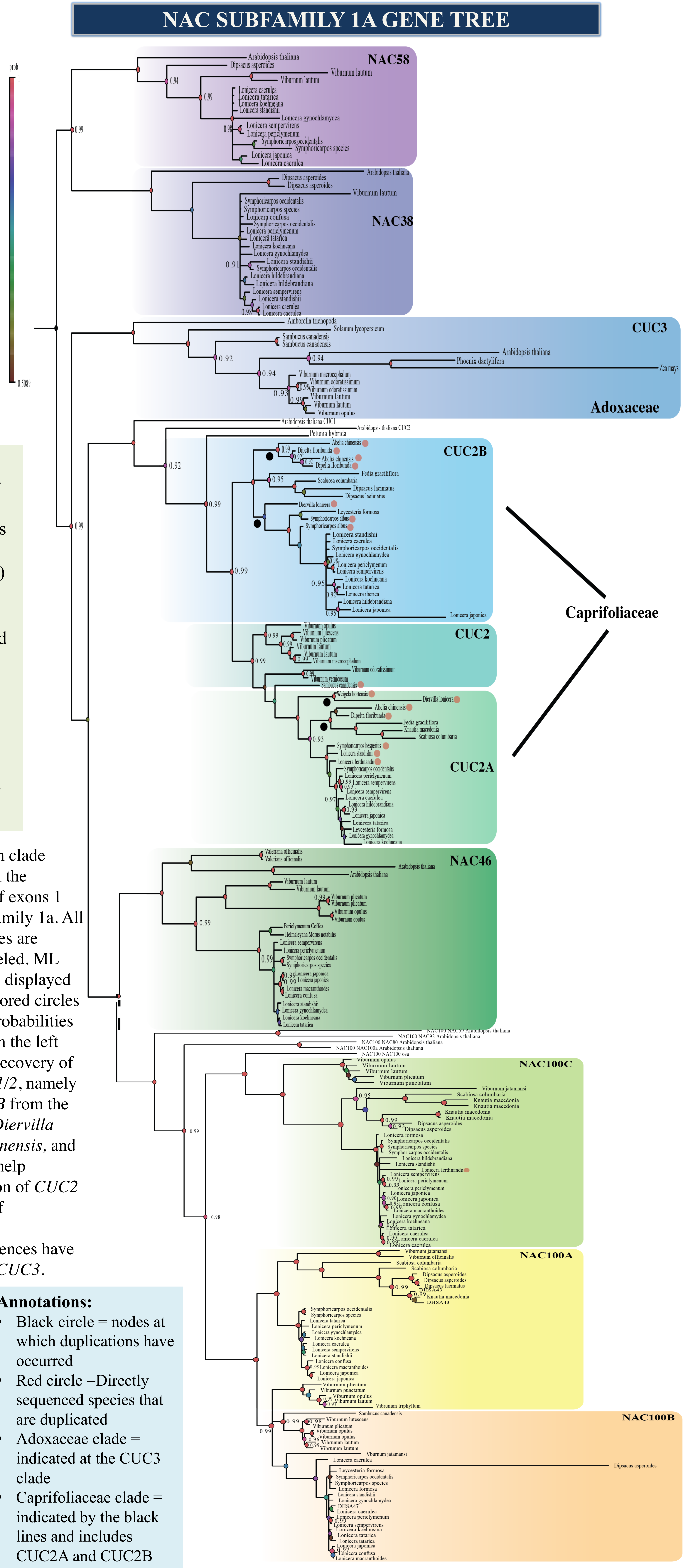
Sampling and Isolating CUC

- 38 species were selected across the Dipsacales for species that had no available genomic resources
- Degenerate primers were designed to isolate exons 1 and 2 of *CUC1/2* and *CUC3* based on reference genomes (e.g., *Arabidopsis*, *Petunia*, *Snapdragon*)
- CUC* specific primers were also created including all introns and exons of *CUC*
- Successful reactions near 500 bp were isolated and cloned
- Multiple clones per species were sequenced at the Yale Sequencing on the Hill Facility
- Sequences were assembled and manually edited using Geneious v.9³
- Assembled sequences were subject to a BLASTn⁴ against the *Arabidopsis thaliana* genome to verify and/or determine which *NAC* gene was recovered

Alignment and Gene Tree Reconstruction

- Sequences were combined with a larger dataset of *NAC* Subfamily 1a (Lee et al. unpublished) recovered from available genomic resources. From here, two datasets were formed; (1) *NAC* dataset with exons and (2) *CUC* dataset that included introns (not shown in this poster) were aligned in MUSCLE⁵ and MEGA⁸
- Phylogenetic analyses were conducted using MrBayes^{6,7} under a single partition with the GTR+I+G model for a million generations

Figure 3. Maximum clade credibility tree from the Bayesian analysis of exons 1 and 2 of *NAC* Subfamily 1a. All *NAC* and *CUC* clades are highlighted and labeled. ML bootstrap values are displayed at the nodes and colored circles indicate posterior probabilities following the key on the left side of the figure. Recovery of two copies of *CUC1/2*, namely *CUC2A* and *CUC2B* from the same accession of *Diervilla lonicera*, *Abelia chinensis*, and *Dipelta floribunda* help confirm a duplication of *CUC2* prior to the origin of Caprifoliaceae. No Caprifoliaceae sequences have been recovered for *CUC3*.



Annotations:

- Black circle = nodes at which duplications have occurred
- Red circle = Directly sequenced species that are duplicated
- Adoxaceae clade = indicated at the CUC3 clade
- Caprifoliaceae clade = indicated by the black lines and includes CUC2A and CUC2B

