# *Leptosiphon* and flower color: investigating color acquisition in Polemoniaceae using phylogenetics

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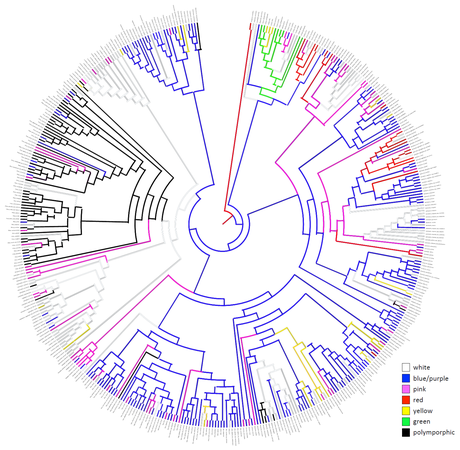
Transitions in flower color are asymmetric over evolutionary time, with some transitions more favorable than others. Several selective pressures and mechanisms involving pollinators often drive these color changes, while other forces such as genetic drift and pleiotropic effects may drive others. In this project, we investigated transitions in flower color in *Leptosiphon* (Polemoniaceae). *Leptosiphon* comprises 38 species and has a center of distribution in California. Based on our initial analyses, it appears that flower color (blue/purple, pink, and yellow) has been gained in many independent transitions from a white-flowered ancestor. A well-supported species-level phylogeny will allow for better mapping of floral characteristics. To refine phylogenetic relationships, plant material was obtained from field, herbaria, and greenhouse collections for 197 accessions, representing all 38 species, plus *Phlox* as an outgroup. We selected 96 samples, including all species and each color morph for a targeted gene capture protocol using MYbaits, a procedure that allows custom design of baits. Probe sequences were created through a reciprocal blast using four transcriptomes (two species of *Phlox*, *Fouqueria macdouglaii*, and *Ternstroemia gymanthera*) and the *Arabidopsis* genome. One hundred probes were created, with markers with large introns discarded. After gene capture, samples were multiplexed and sequenced with Illumina. Reads were assembled, and phylogenetic analyses were conducted on concatenated and individual gene data sets. The resolved phylogeny will be used to determine the number of transitions in flower color in *Leptosiphon* and the directions in which these changes have occurred.

## Background

Polemoniaceae contain approximately 383 species in 26 genera (Johnson et al. 2008). The center of diversity of the family is western North America, with many genera also extending into South America (Grant 1959). This group has been of historical interest to botanists due to its great diversity in flower color, flower size, and overall morphology. Pollinator studies across angiosperms have shown that flower color and pollinators are often correlated, although this view is not universally held. Some studies indicate that mutation may be the source of flower color change rather than selective pressure from specific pollinators (Waser and Price 1981; Schemske and Bierzychudek 2001; Strauss and Whittall 2006; Rausher 2008).



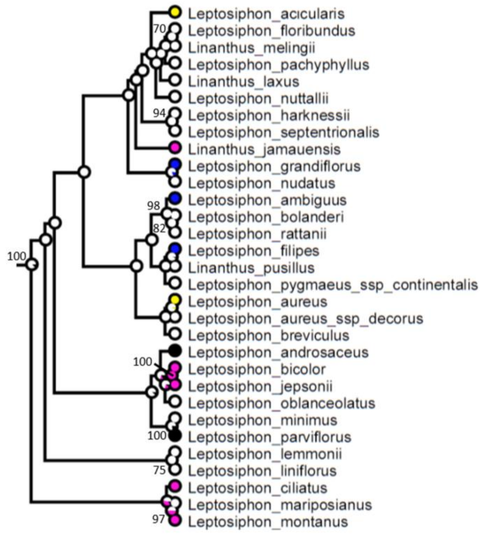

Phylogenetic reconstructions and ancestral character mapping of the family Polemoniaceae suggest a white-flowered ancestor. Based on the whole family analysis, two genera exhibit a white-flowered ancestor that gave rise to extant species with a range of flower colors. *Leptosiphon* and *Linanthus* serve as two evolutionarily independent groups to study the evolution of flower color, specifically the gain of flower color. The purpose of this study is to establish a highly supported phylogenetic reconstruction for both genera. Creating a more resolved phylogeny will allow for a robust foundation onto which flower color will be mapped, showing transitions between species and potentially transitions within some species through analysis of multiple accessions per species.

| | |
|---|---|
| *Leptosiphon acicularis* | Yellow |
| *Leptosiphon ambiguus* | Purple |
| *Leptosiphon androsaceus* | Purple, pink and white |
| *Leptosiphon androsaceus ssp luteolus* | Yellow |
| *Leptosiphon androsaceus ssp micranthus* | Yellow and white |
| *Leptosiphon aureus ssp aureus* | Yellow |
| *Leptosiphon aureus ssp decorus* | Blue, purple and white |
| *Leptosiphon bicolor* | Pink, purple and white |
| *Leptosiphon bolanderi* | Pink, purple and white |
| *Leptosiphon breviculus* | Pink and white |
| *Leptosiphon ciliatus* | Pink and white |
| *Leptosiphon croceus* | Yellow |
| *Leptosiphon filipes* | Purple |
| *Leptosiphon floribundus ssp floribundus* | White |
| *Leptosiphon floribundus ssp glaber* | White |
| *Leptosiphon floribundus ssp hallii* | N/A |
| *Leptosiphon grandiflorus* | Purple and white |
| *Leptosiphon harknessii* | White |
| *Leptosiphon jamauensis* | Pink and purple |
| *Leptosiphon jepsonii* | White |
| *Leptosiphon latisectus* | Pink and white |
| *Leptosiphon lemmonii* | Yellow and white |
| *Leptosiphon liniflorus* | Pink, purple and white |
| *Leptosiphon longitubus* | White |
| *Leptosiphon mariposanus* | N/A |
| *Leptosiphon montanus* | Pink, purple and white |
| *Leptosiphon nudatus* | Pink |
| *Leptosiphon nuttallii* | White |
| *Leptosiphon oblanceolatus* | White |
| *Leptosiphon pachyphyllus* | White |
| *Leptosiphon parviflorus* | Pink, purple and white |
| *Leptosiphon pusillus* | N/A |
| *Leptosiphon pygmaeus* | Purple |
| *Leptosiphon pygmaeus ssp contintentalis* | White |

| | |
|---|---|
| *Leptosiphon pygmaeus ssp pygmaeus* | White |
| *Leptosiphon rattanii* | Purple and white |
| *Leptosiphon septentrionalis* | Purple and white |
| *Leptosiphon serrulatus* | White |
| *Linanthus arenicola* | Yellow |
| *Linanthus bakeri* | Purple |
| *Linanthus bellus* | Pink and purple |
| *Linanthus bigelovii* | Purple and white |
| *Linanthus breviculus* | Pink |
| *Linanthus caespitosus* | White |
| *Linanthus californicus* | Pink and purple |
| *Linanthus campanulatus* | White |
| *Linanthus concinnus* | White |
| *Linanthus demissus* | White |
| *Linanthus dianthiflorus* | Pink, purple and white |
| *Linanthus dichotomus* | Pink and white |
| *Linanthus dylanae* | N/A |
| *Linanthus filiformis* | Yellow |
| *Linanthus inyoensis* | White |
| *Linanthus jaegeri* | White |
| *Linanthus jonesii* | Yellow and white |
| *Linanthus killipii* | White |
| *Linanthus laxus* | White |
| *Linanthus maculatus* | White |
| *Linanthus melingii* | White |
| *Linanthus orcuttii* | Pink and white |
| *Linanthus parryae* | Yellow, purple and white |
| *Linanthus parviflorus* | White |
| *Linanthus pungens* | Pink, purple and white |
| *Linanthus uncialis* | White |
| *Linanthus veatchii* | Yellow and white |
| *Linanthus viscidiensis* | White |
| *Linanthus watsonii* | White |

Shown here are all the species in both *Leptosiphon* and *Linanthus* that were used for this study, along with the color morphs collected for each species.

## Preliminary Results




Maximum-likelihood reconstruction of flower color in *Leptosiphon* and *Linanthus*. Pie charts show the likelihoods for each flower color for each taxon. Colored circles represent colors as people see them with the five possible states being white, yellow, pink, purple/blue, and polymorphic.

Based on initial analyses, a strongly supported hypothesis is that the ancestor of both genera had white flowers. Due to the low bootstrap values for most of the species relationships within the tree, sampling was increased to include more accessions for each species and each color morph for those that commonly exhibit multiple colors in different populations. Doing so will provide a statistically better supported tree on which floral color can be mapped. From the family level analysis, with the current nomenclature *Leptosiphon* and *Linanthus* are not monophyletic.

## Materials and Methods

### *Plant material*

- Plant material was obtained from various herbarium samples across the United States, field collections in California including the Mojave Desert, and freshly sampled species growing in the greenhouse at the University of Florida.
- 38 species of *Leptosiphon* with an outgroup of *Phlox* and 30 species of *Linanthus* with an outgroup of *Gymnosteris* were represented in the 299 accessions, along with each color morph seen within the individual taxa. DNA from these accessions was obtained through a CTAB procedure, totaling 186 samples.

## Materials and Methods

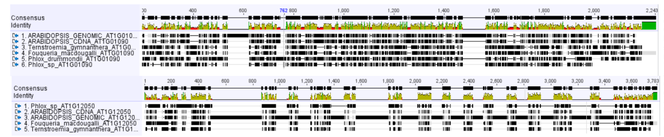
### *Probe creation*

- One hundred nuclear genes were selected to conduct this analysis. Transcriptomes for four species (*Phlox drummondii*, *Phlox sp.*, *Fouqueria macdouglaii*, and *Ternstroemia gymanthera)* were taken from oneKP for use in the creation of probes from nuclear gene sequences. These genes were selected through a reciprocal blast in which we compared these sequences against the *Arabidopsis* genome. This was done through the use of the MarkerMiner pipeline which targeted single-copy nuclear genes within the transcriptomes. Genes that occurred in combination with any of the four mentioned species were considered for probe creation. The breakdowns are shown below:

| | |
|---|---|
| Present in one *Phlox*, *Fouqueria*, and *Ternstroemia* | 47 |
| Present in both *Phlox* and *Fouqueria* | 10 |
| Present in all four | 21 |
| Present in both *Phlox* species | 8 |
| Present in *Phlox sp.* and *Fouqueria* | 12 |
| Present in *Phlox drummondii* and *Fouqueria* | 2 |

For more information on the pipeline used in this project, visit Grant Godden's talk entitled MarkerMiner 1.0: A New Pipeline and Tool for Phylogenetic Marker Development Using Angiosperm Transcriptomes.

- Using the program Geneious, the pool was narrowed down upon investigation of base pair sequences and intron and exon lengths.
- The one hundred genes were selected across the five *Arabidopsis* chromosomes to minimize any chromosome bias.



Shown here are two genes displayed on the program Geneious. The top gene has large exons and small introns, shown by the genomic DNA from the Arabidopsis genome. This gene was chosen for the analysis due to the criteria mentioned above. The lower gene has small, sporadic exons and introns larger than 300 base pairs across, therefore it was discarded and not used for the study.

### *Library preparation and sequence capture*

- Illumina library preparation was conducted by RapidGenomics with 192 unique barcodes
- Gene sequences were captured using custom MYbaits probes and probe capture following the manufacturer's protocol.
- Hybridization between the baits and DNA ran for 36 hours at 65 degrees Celsius
- Once hybridization was complete, targeted DNA segments were removed using magnetic beads, and nonspecific DNA was washed away.
- Targeted genomic DNA will be sequenced on a single Illumina NextSeq run

### *Phylogenetic analysis*

- Gene trees will be created for each sequence.
- A concatenated data matrix approach will be conducted for all available genes.

## Acknowledgments and References

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