

Patterns of gene expression associated with floral scent composition in *Oenothera harringtonii* (Onagraceae)

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Introduction

Linalool is a monoterpene alcohol commonly found in floral fragrance that acts as an attractant to a wide variety of pollinators, herbivores, and parasites (Raguso and Pichersky, 1999). As part of a larger monoterpene biosynthesis pathway, geranyl pyrophosphate (GPP) is converted to S-linalool by the enzyme linalool synthase (Cseke et al., 1998). The expression of linalool synthase in the evening primrose family, Onagraceae, has been characterized in the genus *Clarkia*, but little is known about the process in other members of this diverse family. Species of *Clarkia* have unscented flowers, with the exception of *Clarkia breweri*, which emits a floral fragrance rich in linalool (Raguso and Pichersky, 1995). Dudareva et al. (1996) found that the gene that encodes linalool synthase was expressed in high levels in *C. breweri* petals and stigmata, but at very low levels in the stigmata of its unscented progenitor, *C. concinna*. This suggests that the production of linalool in floral scent is regulated through transcription rather than, for example, specific variants of the linalool synthase gene.

In contrast to *Clarkia*, the Arkansas Valley Evening Primrose, *Oenothera harringtonii*, varies intraspecifically for the presence of linalool (Skogen, pers. comm.). Typically, populations in the southeastern part of its range produce very little linalool (if at all), while populations in the northwestern part of its range produce a significant amount (Skogen, pers. comm.). The goal of this study is to determine to what extent the variation in linalool production of *O. harringtonii* has a genetic basis. First, the heritability of linalool and other floral traits will be determined; then, differences in gene expression between individuals and floral organs producing linalool and those not producing linalool will be measured. As part of a larger project that will characterize specific genes involved in linalool synthesis, this study will provide gene expression information that will help determine its role relative to polymorphisms that may be associated with the process.



Figure 1: An *O. harringtonii* flower.

Methods

All plants for this project were obtained from crosses conducted within and between *O. harringtonii* populations that produce linalool and those that do not. To determine the relationship between floral scent, stage-specific gene expression, and floral morphology, I collected fragrance and extracted RNA from 11 plants (Table 1). RNA was extracted from the first flower after floral fragrance was collected, as well as bud and leaf tissue. For all other plants, fragrance and floral morphology was collected as flowers bloomed. In addition, RNA was extracted from an *O. harringtonii* seedling, which provided a reference transcriptome for the species to aid in analysis.

Fragrance Collection

- Newly opened flowers were enclosed in a plastic bag with a filter attached to a vacuum pump.
- Pumps were operated for one hour then eluted with hexane.
- Scent samples will be analyzed using a gas chromatography mass spectrometer.

Floral Morphology

- Corolla diameter, floral flare, filament length, hypanthium length, style length, amount of nectar, and sucrose equivalent were measured.
- These morphological traits will be compared to those of the parental generation in order to determine the level of heritability.

RNA Extraction

- For each plant in Table 1, RNA was extracted from the first flower, a bud 24-48 hours prior to opening, and leaf tissue. The flower was dissected into stamens, petals, stigma/style, and hypanthium. All parts were frozen in liquid nitrogen immediately after excision from the plant.
- Extraction used the Sigma-Aldrich Spectrum Plant Total RNA Mini Kit. RNA concentration was assayed using a Qubit 2.0 Fluorometer.

Transcriptome Sequencing and Assembly

- RNA was extracted from a seedling of *O. harringtonii* and sequenced at BGI-Shenzhen using the Illumina HiSeq 2000 system with 100 base pair reads.
- Trinity was used to assemble the sequence reads into contigs that represent mRNA transcripts present in the RNA pool. Functional annotation of these transcripts provides gene identification. This set of annotated transcripts will be used as a template for measuring relative expression from the stage-specific gene expression study described above.

Cross	Parental Linalool	Flower	Bud	Leaf
I	Linalool? x Linalool+	✓	✓	✓
J	Linalool+ x Linalool?	✓	✓	✓
K	Linalool+ x Linalool?	✓	✓	✓
L	Linalool- x Linalool-	✓		
R	Linalool? x Linalool+	✓	✓	✓
S	Linalool+ x Linalool?			
T	Linalool+ x Linalool?	✓	✓	✓
AI	Linalool- x Linalool-			
AJ	Linalool- x Linalool?			
AL	Linalool? x Linalool-	✓	✓	✓
AN	Linalool- x Linalool?	✓		

Table 1: RNA extraction checklist.

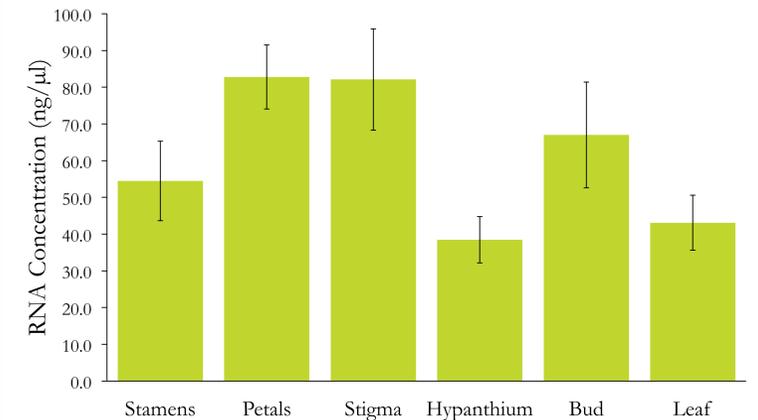


Figure 2: Mean RNA concentration for extractions. If concentration was too high to read on the Qubit, the maximum readable value was used.

Future Directions

- Immediate future work will focus on the completion of RNA extractions and scent collection for the plants in Table 1. Fragrance collection and floral morphology will also continue as the *O. harringtonii* plants continue to flower during the fall.
- Once the presence or absence of linalool is confirmed for each plant, RNA samples will be sequenced and relative expression levels between each plant and each floral organ will be determined.

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Results and Discussion

RNA extractions with a green checkmark in Table 2 have been completed. The mean RNA concentration of each tissue type is shown in Figure 2. Petals, stigma/styles, and buds had the most extracted RNA, followed by stamens, leaves, and hypanthiums.

Sequencing the extracted RNA of an *O. harringtonii* seedling yielded 871822 reads. These reads were assembled via Trinity into 57815 contigs, which represent the total number of mRNA transcripts present. Of these contigs, 20860 were able to be annotated with functional information.

References

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