The Genetics and Biochemistry of Pollen Color Variation in *Geranium*

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***ABSTRACT.*** Flower color polymorphism has been documented across the angiosperm and is known to be driven by both pollinator and non-pollinator interactions. This color variation is due to biochemical differences in the type and quantity of pigment produced in tissues. While petal color variation has received much attention, much less is known about color variation in the primary sexual organs – anthers and stigmas. I aim to investigate the genetics and biochemistry of anther color variation in the allotetraploid plant system, *Geranium maculatum*, which exhibits anther & pollen color polymorphism across an elevational cline*.* I will use tandem LC-MS profiling to characterize pigment profiles between color morphs and integrate these results with RNA-sequencing to identify candidate structural/regulatory genes involved in anther color dimorphism in *G. maculatum*. This work will provide insight into the evolution of this ecologically relevant, yet understudied trait, and shed light on the effects of polyploidization on the Anthocyanin Biosynthetic Pathway (ABP).

A picture containing piece, dessert, eaten

Description automatically generated***INTRODUCTION.***Flower color has been the focus of numerous studies due to the striking variation seen across the angiosperms (Rausher, 2008). While the true number of species with flower color polymorphism is unknown, within-species color variation has been documented across the angiosperms. This phenotypic variation has been an important model to test different the evolutionary drivers maintaining trait variation in natural populations (e.g. pollinator preference, abiotic environment heterogeneity, genetic drift, etc.) (Sapir et al., 2021).

Figure 1. Purple and yellow pollen anther/pollen color morph in *Geranium maculatum.*

Ecological, genetic, and evolutionary research on flower color polymorphism has largely focused on large, conspicuous petal organs, however, much less is known about color variation in primary sexual organs – anthers and stigmas. Color variation of these organs has been shown to impact pollinator visitation (Ison et al., 2019), seed production (Jorgensen et al., 2006), and thermal tolerance (Koski & Galloway, 2018). To our knowledge, anther color polymorphism has been studied in only a small number of species (e.g., *Nigella degenii* (Jorgensen et al., 2006)*, Linum pubescens* (Wolfe, 2001)*, Erythronium Americanum* (Austen et al., 2018)*, Campanula americana* (Koski & Galloway, 2018), *Epimedium pubescens* (Xiao-Yue et al., 2018)).

Recent work from our group has discovered anther & pollen color polymorphism in allotetraploid *Geranium maculatum*, which has not been previously described in the literature (Figure 1)*.*Field surveys across the Southeast US revealed that Wild Geranium populations have higher frequencies of purple anther individuals in high elevations compared to lower elevations. Furthermore, cold temperatures, high precipitation, and high UV-B radiation favor populations with high purple frequencies(Perez-Udell, 2022), suggesting that non-pollinator agents played a role in maintaining pollen anther & pollen variation in *G. maculatum.*My dissertation aims to uncover the genetic mechanism this color variation using a multi-omics approach.

***RESEARCH OBJECTIVE.*** Anthocyanins are the most common, responsible for orange, red, purple and blue coloration. Further, the Anthocyanin Biosynthetic Pathway (ABP) is highly conserved across flowering plants and key *structural genes* encoding enzymes which synthesize pigments and *regulatory genes* which control transcription have been characterized (Grotewold, 2006). Thus, much insight can be gained from biochemical analysis of these compounds in plant tissues. I aim to 1) biochemically characterize the differences in pigment profiles between *G. maculatum* anther color morphs and 2) integrate this metabolomics data with RNA-sequencing to identify candidate structural and regulatory genes contributing to anther color dimorphism.

***METHODS***

*BIOCHEMICAL ANALYSIS.* To identify and quantify concentrations of anthocyanins and associated compounds, I will collect floral and vegetative tissues (stigma, stamen, petal, leaf) from both color morphs (4 organs x 2 morphs x 3 biological replicates) from *G. maculatum* plants grown in thegreenhouses at the University of Georgia in Athens, GA, USA. Samples will be lyophilized and sent to the UGA Plant Metabolomics Laboratory for metabolite extraction and Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) profiling.

By evaluating the accumulation of anthocyanins and associated compounds in reproductive and vegetative organs, I will identify the primary pigment responsible for color dimorphism and outline the ABP in Wild Geranium. Additionally, I will test relationships between anthocyanin contents in each plant organ with Pearson correlations and a Bonferroni adjustment for multiple comparisons (Rice, 1989).

While the loss of function of a gene encoding an enzyme important to pigment biosynthesis can cause a shift from purple to yellow, *I hypothesize that this phenotype is caused by a tissue-specific regulatory mechanism in anthers that may have evolved after polyploidization*. If the ABP regulatory mechanism is shared across floral organs (null hypothesis), I expect to see a correlation between ABP product type and abundance across organ types. Alternatively, if the pathway is regulated in a tissue-specific manner, I expect to see a non-significant correlation across organ types.

*RNA-SEQ.* To evaluate differences in gene expression across floral organs and identify candidate structural/regulatory genes involved in anther color shift in *G. maculatum*, I will use a comparative transcriptomics approach. Petals, stamens, stigmas, and leaves (4 organs x 2 morphs x 4 biological replicates) will be collected from purple and yellow anther color morphs at three developmental stages: bud, opening, and anthesis. RNA-Seq libraries will be prepared for paired-end sequencing via Illumina NextSeq 500 to generate 150 bp reads. I will align reads to a draft genome assembly and generate a list of differentially expressed genes between morphs that are important for color variation.

***SIGNIFICANCE****.* Interestingly, a shift to yellow anthers in several North American *Geranium* species (unpublished data) appears to be associated with an allotetraplodization event ~4.5 ma (Marcussen & Meseguer, 2017). Thus, investigating the genetic mechanism behind this color variation in *G. maculatum*, an allotetraploid plant species, will shed light on how hybridization affects ABP regulation and gene expression.Further, our group aims to leverage the color variation and polymorphism seen across *Geranium* to conduct comparative analyses to understand the ecological implications behind sexual organ pigmentation in plants. A recent meta-analysis on anthocyanin levels in response to abiotic stresses showed that low temperature and UV radiation increased anthocyanin content in plants (Yan et al., 2022). Thus, this work will build on the evidence for the role of anthocyanins in photoprotection in natural plant populations.

References

Austen, E. J., Lin, S.-Y., & Forrest, J. R. K. (2018). On the ecological significance of pollen color: A case study in American trout lily (Erythronium americanum ). *Ecology*, *99*(4), 926–937.

Grotewold, E. (2006). The Genetics and Biochemistry of Floral Pigments. *Annual Review of Plant Biology*, *57*(1), 761–780.

Ison, J. L., Tuan, E. S. L., Koski, M. H., Whalen, J. S., & Galloway, L. F. (2019). Role of pollinator preference in the maintenance of pollen colour variation. *Annals of Botany*, *123*(6), 951–960.

Jorgensen, T. H., Petanidou, T., & Andersson, S. (2006). The potential for selection on pollen colour dimorphisms in Nigella degenii: Morph-specific differences in pollinator visitation, fertilisation success and siring ability. *Evolutionary Ecology*, *20*(4), 291–306.

Koski, M. H., & Galloway, L. F. (2018). Geographic variation in pollen color is associated with temperature stress. *The New Phytologist*, *218*(1), 370–379. MEDLINE with Full Text.

Marcussen, T., & Meseguer, A. S. (2017). Species-level phylogeny, fruit evolution and diversification history of Geranium (Geraniaceae). *Molecular Phylogenetics and Evolution*, *110*, 134–149.

Perez-Udell, R. A. (2022). *GEOGRAPHIC VARIATION IN FLORAL PIGMENTATION OF US NATIVE HERB, GERANIUM MACULATUM, AS EVALUATED BY TRADITIONAL AND CITIZEN SCIENCE APPROACHES*. [Dissertation, University of Georgia]

Rausher, M. D. (2008). Evolutionary Transitions in Floral Color. *International Journal of Plant Sciences*, *169*(1), 7–21.

Rice, W. R. (1989). Analyzing Tables of Statistical Tests. *Evolution*, *43*(1), 223.

Sapir, Y., Gallagher, M. K., & Senden, E. (2021). What Maintains Flower Colour Variation within Populations? *Trends in Ecology & Evolution*, *36*(6), 507–519.

van der Kooi, C. J., Dyer, A. G., Kevan, P. G., & Lunau, K. (2019). Functional significance of the optical properties of flowers for visual signalling. *Annals of Botany*, *123*(2), 263–276.

Wolfe, L. M. (2001). Associations among Multiple Floral Polymorphisms in Linum pubescens (Linaceae), a Heterostylous Plant. *International Journal of Plant Sciences*, *162*(2), 335–342.

Xiao-Yue, W., Qiu-Mei, Q., Bo, W., Yun-Xiang, L., & Shuang-Quan, H. (2018). Discovery of androecium color polymorphism in Epimedium pubescens with habitat preference of anther/pollen color in the genus. *Journal of Plant Ecology*, *11*(4), 533–541.

Yan, W., Li, J., Lin, X., Wang, L., Yang, X., Xia, X., Zhang, Y., Yang, S., Li, H., Deng, X., & Ke, Q. (2022). Changes in plant anthocyanin levels in response to abiotic stresses: A meta-analysis. *Plant Biotechnology Reports*, *16*(5), 497–508.

**BUDGET**

This grant would be used to help cover the costs of library preparation and RNA-sequencing at the Georgia Genomics and Bioinformatics Core (GGBC).

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| *Item* | *Cost* |
| NGS Library Preparation (32 samples/$112 each) | $3584 |
| Library Pooling up to 25-48 samples | $105 |
| NextSeq 500 Mid Output Flow Cell | $2,566 |
| **Total Expenses** | **$6255** |

Biographical Sketch

**Summer Blanco**

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**A. Education**PhD Student, Plant Biology, University of Georgia (UGA) Aug. 2021 – PresentSuccess in Graduate Education Program, Michigan State University Aug. 2020 – June 2021B.S. in Biology, California State Polytechnic University, Pomona Sept. 2015 – May 2020

**B. Selected Awards and Fellowships**UGA Plant Biology Palfrey Graduate Student Research Grant Feb. 2023  
UGA Plant Center–Plant Metabolomics Doctoral Dissertation Improvement Award Dec. 2022

American Association of Hispanics in Higher Education Graduate Fellowship Sept. 2022-Present

UGA Presidential Fellowship Sept. 2021– Present  
National Science Foundation Graduate Research Fellowship Sept. 2021– Present

**C. Presentations  
Blanco, S.**, Rentsch, J., & Leebens-Mack, J. (2022). Comparative transcriptomics of Venus flytraps during various stages of prey capture & digestion. *Agavoideae Conference and Bioinformatics Workshop.* (Mexico City, Mexico)

**Blanco, S.**, Chang, S. (2022). The Colors of Pollen: Using citizen science to survey anther color variation in *Geranium*. *Botany 2022.* (Anchorage, AK, USA).

**D. Selected Service and Outreach**Instructor Certification, Software Carpentries February 2023

Athens Science Café, *VP of events* May 2022 – Present  
Plant Biology Graduate Student Association, *Outreach chair* May 2022 – Present  
UGA Integrated Plant Science Diversity Preview, *Inaugural organizer* Aug. 2022 – Dec. 2022   
Center for the Integration of Research, Teaching, & Learning Network May 2022

*Research Mentor Certification*Scientific Research and Education Network (SciREN) January 2022

*K-12 Lesson Planning Participant*Cientifico Latino Graduate School Mentorship Initiative, *Mentor* Aug. 2021 – Dec. 2021