Establishing the functional role & adaptive value of pollen color variation in *Geranium maculatum*

Introduction: Over the past 130 million years, flowering plants have evolved a variety of visual and chemical cues that mediate species' interactions. The diversity of color phenotypes in flowers has been the subject of many ecological studies and the biosynthesis and regulation of the main compounds responsible for pigmentation is well understood[1,2]. These compounds are well-known for their role in pollinator attraction and additionally have many important biological functions that have been described (e.g., allelopathy, lignification, protection from UV radiation)[3]. However, despite the significance of pigmentation in plant growth, development, and reproduction, the role of pollen color remains unclear.

About 75% of flowering plant species have yellow or white colored pollen, though pollen may also be pigmented red, blue, purple, or black[4,5]. The composition of specialized metabolites that occur in pollen across several taxa (e.g., phenolic compounds, alkaloids, terpenoids) have also been described[6]. Several of these compounds are known to be important for pollen development, pollen germination, pollen tube growth, and protection from abiotic stress (i.e., temperature, UV)[7].

Recent work from Dr. Shu-Mei Chang's lab at the University of Georgia, Athens (UGA), has discovered pollen color polymorphism in wild geranium, *Geranium maculatum*. Field observations along the Appalachian Mountain region show that purple and yellow pollen color morphs persist in different ratios along an elevational cline[8]. Though pollen color polymorphism has been observed in other plant species, the ecological function and adaptive value of this trait is still unknown[9-12]. Therefore, I propose to examine the geographic distribution patterns of pollen color morphs, characterize their phenotypes, and evaluate the functional role of this trait in an ecological context (Fig. 1).

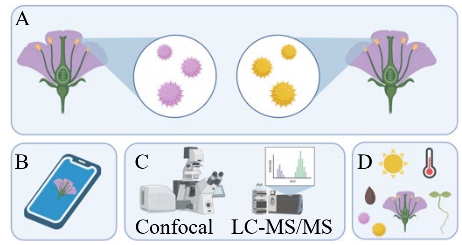

Figure 1. Experimental Design. (A) Purple & yellow pollen color morphs. (B) iNaturalist distribution data by community scientists. (C) Confocal microscopy & LC-MS/MS characterization of pollen phenotypes. (D) Reproductive trait evaluation under abiotic stress.

Aim 1: Determine the distribution of pollen color by integrating field surveys and community science

In native *G. maculatum* populations, dark pollen color morphs have been observed at higher elevations[8]; a trend that has not been observed in other plant species[9-12]. iNaturalist is an online platform and smartphone application that allows anyone to record observations in nature. To date, there are over 13,000 records of *G. maculatum* and over 1,600 individuals that have made observations across the US. To determine if there is a correlation between elevation and pollen color, I will use this data to analyze the occurrence of purple and yellow pollen color morphs in a logistic generalized linear model with latitude and elevation predictors, as described by Austen et al[12]. I expect to see a positive correlation between elevation and pollen color intensity along an elevation gradient based on previous field observations.

Furthermore, I will geo-reference 20 populations of *G. maculatum* along an elevation gradient to serve as collection sites for my study. To supplement my own collection material, I will advertise this study on the Ecological Society of America listserv and on social media pages of native plant societies to recruit community scientists. I will then create collection kits with an overview of the project and a guide to propagule collection to mail to each individual, who will harvest from their respective site and mail their completed kits to UGA. Clear instructions will be provided to each collector on how to image the population and gather one representative specimen to allow confirmation.

Aim 2: Characterize reproductive traits and pollen phenotypes of *G. maculatum* populations

Pollen features that vary among pollen color morphs can have a significant impact on male fitness of a plant[13]. Thus, I will examine the morphological and biochemical characteristics associated with pollen color. I will propagate *G. maculatum* from rhizomes from Aim 1 in the greenhouse at UGA and generate an F2 population that segregates in pollen color. Upon flowering, reproductive traits (e.g., flower number, flower size, flower color, pollen color, seed set) for each plant will be described. I will collect and image pollen by confocal microscopy at the UGA Biomedical Microscopy Core (BMC) for characterization of pollen features (e.g., size, surface ornamentation). Then, I will utilize the Proteomics and Mass Spectrometry facility at UGA to quantify specialized metabolite accumulation in anther tissue and pollen by liquid chromatography tandem mass spectrometry (LC-MS/MS). Specialized metabolites have previously been characterized for *G. maculatum* pollen and it was found that the metabolite profile differed significantly among four collection sites[6]. I will further characterize the correlation between pollen color intensity and metabolite profile to identify compounds that are important for pollen performance in Aim 3.

Aim 3: Evaluate trait-correlated tolerance to abiotic stress

To evaluate the pollen performance of the F2 individuals, I will measure pollen viability, germination, and siring success under different conditions. I will expose different colored pollen to a gradient of temperature and light intensity treatments (mimicking field conditions), and assay for pollen viability and germination *in vitro*, where pollen germination rates and pollen tube length will be recorded. Additionally, I will observe the siring success of each pollen color morph *in vivo*.

I will then conduct common garden experiments to evaluate fitness in field conditions. Replicate F2 populations that contain identical genotypes (by splitting rhizomes) will be planted in common garden plots at the Highlands Biological Station in North Carolina (high elevation) and the UGA State Botanical Garden (low elevation). Floral/reproductive traits (as described in Aim 2) and transplant survival will be recorded for each population over the span of 2 years. I hypothesize that dark pollen individuals will have greater reproductive success in high elevation due to specialized metabolites that confer abiotic stress tolerance. I expect that light pollen individuals will have decreased transplant survival but compensate to some extent by producing more flowers with higher seed set; a maternal reproductive strategy described by Koski et al. in *Campanula americana*[13]. I will also collect the same subset of individuals from each garden for metabolite analysis (as described in Aim 2) to determine whether there is a genetic-by-environment (GxE) effect on their profiles.

Intellectual Merit*:* My previous research experience in floral development, pollen biology, biochemistry, and molecular biology makes me uniquely positioned to lead this interdisciplinary and community-science supported endeavor. Under the guidance of Dr. Chang, an expert in plant ecology and plant mating systems, I will expand our limited understanding of the role of pollen color polymorphism as a strategy for reproductive success. Additionally, previous graduate students in the Chang group have led community-science research endeavors using iNaturalist, making this an ideal environment to build upon this infrastructure. In taking advantage of the biological research stations available to graduate students at UGA, this study will be the first of its kind to evaluate pollen color morph-dependent fitness in an ecological context. Moreover, coupling biochemistry and ecology approaches, I will generate a comprehensive understanding of the role that specialized metabolites play in pollen germination and reproductive success – information that has implications in both agricultural production and native plant conservation.

Broader impacts: I foresee my graduate research as a vehicle for mobilizing community scientists, providing educational opportunities to underserved communities, and improving diversity in academia. I will work closely with K-12 instructors to develop plant biology lessons with field work and family engagement components to create community awareness of these relevant topics. Students and their families will be given demos on how to use the iNaturalist platform to encourage outdoor activity and participation in community science. In collaboration with Max Barnhart, a graduate student in the Integrated Plant Sciences program who is the lead PI on an American Society of Plant Biology (ASPB) science communication grant, I will create and distribute a zine to provide an overview of my work to the general public and the community scientists involved. I will also share my experience with the scientific community by developing a workshop entitled "Incorporating Community Science Into Your Research Program" for the annual ASPB conference. During this workshop, I will discuss the various platforms available for building community science projects, demonstrate how to navigate and utilize these platforms, and lead an exercise on brainstorming ways to engage community scientists in your research.

References: [1]Rausher MD. *Int. J. Plant Sci*. 2008. [2]Grotewold E. *Annu. Rev. Plant Biol.* 2006. [3]Jiang et al. *Plants*. 2016. [4]Lunau K. *Plant Syst. Evol.* 1995. [5] Miller et al. *Optics & Laser Tech*. 2011. [6]Palmer-Young et al. *Ecol. Monogr*. 2018. [7]Muhlemann et al. *PNAS*. 2018. [8]Udell-Perez R. Field Obs. [9]Jorgensen & Andersson. *New Phytol.* 2005. [10] Koski & Galloway. *New Phytol*. 2018. [11]Wang et al. *Evolution*. 2018. [12]Austen et al. *Ecology*. 2019. [13]Koski et al. *J Evol. Biol.* 2020.