

Pollination biology and hybridization among Tomatillo (*Physalis*) Species in the
Southwestern Region of North America

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ABSTRACT

Physalis, the tomatillo genus, has 90 species that are globally distributed with its center of diversity in North America. Despite its economic importance, relatively little is known about the natural history of this genus, and in particular, its pollination biology. My proposal aims to better understand the evolution of the group by characterizing its reproductive biology and quantifying hybridization among species. This project will sequence multiple loci in the nuclear genome to better understand the genetic structure and diversity within species populations. This study focuses on four targeted taxa: the domesticated, *P. philadelphica*, and wild relatives' *P. crassifolia*, *P. purpurea*, and *P. heterophylla*. These species were chosen because of their economic importance, geographical distribution, and potential for gene flow among the species. The sample sites range from Texas to California to Northwestern Mexico. This research will contribute to greater knowledge of *Physalis*, as well as add resources to herbaria and entomology collections that will help inform future work done in this system.

INTRODUCTION. Pollinators play a crucial role in plant fitness with their ability to disperse pollen throughout a population and among different species living in the same area. Understanding the pollinators' role in this process is the first step to determine the mechanism in place for hybridization. This study focuses on the dispersal and diversity of genetic material in the tomatillo and other species in the genus *Physalis*. *Physalis*' center of diversity is in North America with roughly 90 native species of which two have been domesticated, and several cultivated species (Waterfall, 1951; Whitson and Manos, 2005; Estrada-Loera, 1996). Within *Physalis* there has been very few pollination studies, (Sullivan, 1984; Chaura-Mellizo, 2012), and the extent of gene flow among *Physalis* domesticated and wild species has never been studied and is unknown.

This study uses the tomatillo genus, *Physalis*, as a study system for understanding the effects that pollination has on gene flow among crop-wild species. The intensity of gene flow among species is shaped by several different factors: evolutionary distance, mating systems, and geography. Artificial crossing studies in *Physalis* have demonstrated that hybridization among several different species is possible, but may be rare (Menzel 1951; Hinton, 1970; Sullivan, 2013). For example, Menzel (1951) conducted 164 different crosses among *Physalis* species, and 50 of the species pairs resulted in seed set. The in the 1970s, Hinton, working with two sympatric species (*P. virginiana* and *P. heterophylla*), suggested that interspecific directional hybridization has occurred. Despite these reports of hybridization among some *Physalis*, Sullivan (1985) found no crossability among three sympatric species growing in



Fig. 1: Above are pictures of the plants that will be studied. A) *P. crassifolia* B) *P. philadelphica* C) *P. purpurea* D) *P. heterophylla*

Oklahoma. Taken together, these studies suggest that hybridization is possible and is perhaps common among some pairs of taxa, while other pairs are reproductively isolated.

While previous studies have focused exclusively on wild species, the present study will examine both wild and domesticated taxa and patterns of gene flow among them. I will use four species in the genus, *P. philadelphica*, *P. crassifolia*, *P. purpurea*, and *P. heterophylla* (**Fig. 1**) because of their geographical distribution and thus the potential for gene flow. Phylogenetic studies suggest that these species fall in different subclades of *Physalis*, (Whitson and Manos, 2005; Zamora-Tavares et al., 2016), which should increase the power to distinguish gene flow from incomplete lineage sorting (ILC). I will investigate patterns of hybridization among wild and cultivated *Physalis* species by making field collections in regions of sympatry and allopatry. From May to July, I will travel to ten different sites that span from Texas to California to Northern Mexico. Occurrence location was aggregated from SIENet (online herbarium specimen database).

APPROACH

POLLINATION ECOLOGY. Understanding gene flow starts with understanding the reproductive mechanisms in the species. Very few pollination studies have been done in *Physalis*. In total, floral visitors that affect hybridization in *Physalis* have been documented for only one of the 90 North American species (Sullivan, 1984), but did not include any of the domesticated taxa. In the field, I will 1) test pollen viability; 2) document pollinator activity and effectiveness; and 3) test for potential genetic barriers. I will check pollen viability by staining the pollen and then later view it under a microscope. This will test if the pollen is capable of fertilizing other individuals in the population. To better understand the role that pollinators have in the facilitation of gene flow, I will observe the plants they visit, document their behavior while they visit a flower, remove the pollen from the bee to determine which plants they have collected pollen from, and collect the stigma that the bee lands on to determine if the bee deposits pollen. I will identify the pollen from the bee's body in the lab to determine which *Physalis* species it came from using reference slides I created this semester. The combination of all these tests will indicate whether or not the visitors collected pollen from different species and deposited it on another plant species. If the visitors are depositing different plant species' pollen on a different plant species' stigma, I will conclude that hybridization could occur. I will also collect these visitors to identify, mount, and deposit specimens in the museum collection at CU-Boulder with help from the collection managers.

Next, I will use controlled crosses to test each species for self-compatibility and to determine the level of the cross compatibility among species. This will be done using the method of Baek et. al. (2015) that quantifies pollen growth in style tissue to detect pollen rejection (incompatibility). If the pollen tube does not grow all the way down the style to the ovary, I will conclude that there is a genetic barrier that prohibits growth. In the field, I will collect styles after being manually pollinated to determine if there is a difference among species and populations. During the field trip, I will collect plant vouchers to add to the University of Colorado-Boulder Herbarium collections, seeds to grow in the greenhouses at CU, and silica-dried leaf material for a more extensive testing with the uses of molecular methods.

POPULATION GENETICS. Genetic testing is required to determine if gene flow among *Physalis* species occurs. This is because hybrids cannot be confidently identified from morphology, because they might resemble only one of the parents due to the dominance of that parent's alleles. I will extract DNA from leaf material using the CTAB method (Doyle and Doyle, 1987) and characterize genetic variation using targeted sequence capture of 100 loci across the nuclear genome. As a first pass, these data will include ten individuals of each species present at the each site. The collection sites will be spread through their distribution so a complete understanding of each species genetic variation across their range can be made. The reads will be initially cleaned with Trimmomatic (Bolger, 2014), then sequences will be assembled using bwa (Li, 2012), and aligned using SPAdes (Nurk, 2013). Phylogenetic analysis and tests of introgression will be conducted using *Tree Incongruence Checking in R* (TICR). (Stenz et. al. 2015). This program examines gene trees to determine if shared variation is due to incomplete lineage sorting (ILS) or introgression. By selecting distantly related *Physalis*, I aim to minimize the level of ILS and maximize the power to detect introgression.

SIGNIFICANCE. This initial study of pollination and hybridization within the *Physalis* system will contribute to a better understanding of the evolutionary history of *Physalis* species. This is the first step to understanding if there is or is not gene flow among species, which could help to resolve the taxonomic confusion in the genus. With an increased demand of tomatillos consumption in the US, (Labata, 2015) we want to preserve and protect their gene pool. We often rely variation from wild species for improving crops for things like drought tolerance or pest resistance, but first we must understand the basic evolution of the group. This project will determine the mechanisms for gene flow and deepen our understanding of the basic evolution of *Physalis*. This research will help conserve biodiversity during a time when there is an increased demand for tomatillos.

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BUDGET

I am applying for other grants to help with other cost for this research such as the population genetic approach and travel. This grant would be used for the needed supplies for the pollination component for the research and the ability to continue research in greenhouses during the school year.

Microscope slides: \$85.00
Microscope slide covers: \$15.00
10 ml Eppendorf tubes: \$30.00
Acetic acid: \$25.00
Pollination bags: \$50.00
Hydrogen peroxide: \$8
Coin envelopes: \$7
Greenhouse space at CU: \$280.00

Total: \$500.00

Last summer I loaded my car up and moved two states over to start a PhD program at the University of Colorado-Boulder in Stacey Smith's lab to start working in the genus *Physalis*. *Physalis* caught my interest because of its economic importance, containing the tomatillo, and its beautiful inflated calyx and dark maculates. Past researchers moved on to new questions and different research systems only after working in *Physalis* for a short time leaving a need for further in depth research in this group. I have been spending my first year talking with researchers who have worked with *Physalis*, and I spoke to a tomatillo breeder who has been breeding for over 20 years. This was surprising to me since the basic evolution of this group is lacking yet, he was tasked with job of improving the tomatillo through breeding. This same issue of people lacking basic knowledge of the crop, but still breeding it, happened with the tomato. Once more was known about the tomato species, *Solanum lycopersicum*, breeders were able to think about the species in a holistic way to improve their success. I believe that establishing this basic knowledge and understanding the evolution of *Physalis* will in the long run help tomatillo breeders.

This summer I will be doing field work in the Southwestern region of North America visiting different population sites of several different species to collect information about what plants co-occur, if the plants are self-compatible or unilaterally compatible, what pollinates them, and material for genetic comparisons. My ultimate goal is to better understand the confusing taxonomy by determining if interspecific gene flow occurs within the genus. My approaches will include understanding the mechanistic barriers of the pollination system and genetic work. This grant will help pay for the necessary materials need for the pollination component of this project.

Before graduate school, I attended Harris-Stowe State University in St. Louis, MO. During that time I knew I was interested in plant science, but I did not know in what aspect. I have spent time conducting research through the Missouri Botanical Garden REU program looking at edge effects in prairies with Danielle Hakke; Danforth Plant Science REU program, studying the effectors of a cassava plant pathogen in Dr. Rebecca Bart's lab; and working in Dr. Allison Miller's lab where I extracted DNA samples for a hybridization of native *Vitis* species project. Through these experiences I started to understand the many different aspects of plant science and that helped narrow down my focus to what I am working on today. During my time as an undergraduate, I was part of Botanical Society of America PLANTS program, which cemented my desire to go to graduate school and be a part of a larger science community.

The guidance of the PLANTS program made me understand how important a supportive science community is to the development of the field. Understanding the importance of supportive community, the next year I was a PLANTS peer mentor and I have continued to find ways to be engaged. This past year I was co-organizer of a monthly museum day that was focused around anthropogenic evolution to celebrate Darwin's birthday in the month of February. This event reached out to the community as a whole and attracted many families from the area. This type of work is important when doing science work, since it is easy for researchers to get wrapped up in their work, they can forget to share it to the general audience. These funds will help fund a project and also someone who is dedicated to engaging others in the plant science community.