Doctoral Dissertation Proposal
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Biodiversity and Ecology of Monkeyflower Fungal Endophytes Across its Range

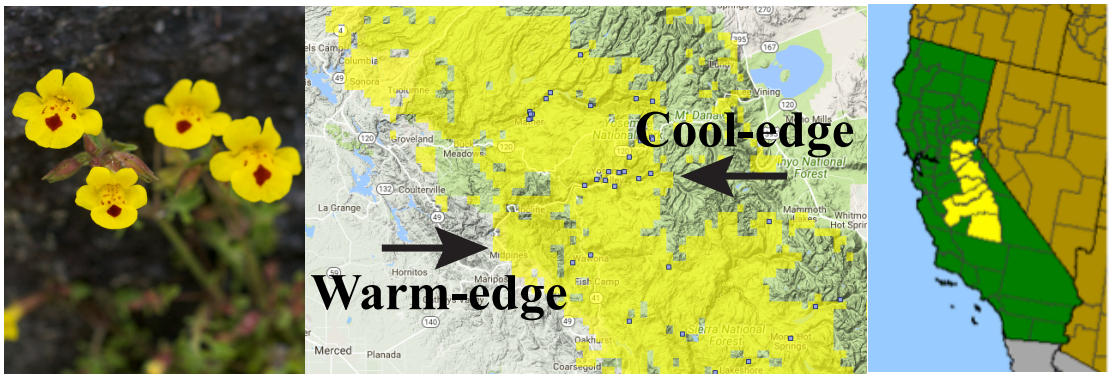

Figure 1: *Erythranthe laciniata* (left), part of its range from lower (warm-edge) to higher (cool-edge) elevations, and its entire range across the Sierra Nevada (right).

Overview

All plants have a community of asymptomatic fungi inhabiting their tissue, known as fungal endophytes. These fungi are increasingly recognized for their role in plant tolerance to stress, including heat (Redman *et al.* 2002, Rodriguez et al. 2008, Hubbard et al. 2014), drought (Rodriguez *et al.* 2009, Azad *et al.* 2016), and plant response to global climate change (Compant *et al.* 2010, Redman *et al.* 2011, Kivlin *et al.* 2013, Slaughter *et al.* 2018). The severity and rate of climate change are unprecedented and is already having major consequences for life on Earth (Diffenbaugh & Field 2013, Kemp *et al.* 2015, Trenberth 2018). Plants are particularly susceptible to changes in their climate environment (Thomas *et al.* 2004), and in order for plants to persist, they must adapt (Nicotra *et al.* 2010), rapidly shift species ranges (Chen *et al.* 2011, Valladares *et al.* 2014, Lenoir & Svenning 2015), or tolerate the stress (Berg *et al.* 2010, Bita & Gerats 2013). Biotic interactions have been known to factor into persistence for some time (Prestidge *et al.* 1982). Increasing evidence suggests that microbes are an extension of plant host phenotype and can ultimately help them adapt in response to stress from changing environments (Compant *et al.* 2010, Hirt 2012, Lau *et al.* 2017, Bang *et al.* 2018). Additionally, stressful conditions may select for distinct fungal endophyte taxa with specific functions (Lemanceau *et al.* 2017). Plants with populations spanning a steep elevation gradient experience different stresses at their ranges: warmer temperatures at low elevations and cooler temperatures in high elevations. Several large-scale studies on biotic interactions affecting native species ranges have been conducted (Van der Putten *et al.* 2010, Dawson *et al.* 2011, Wiens 2011, HilleRisLambers *et al.* 2013, Wisz *et al.* 2013), however few explore the effects of fungal endophytes across species ranges (Rodriguez *et al.* 2009, Herrera Paredes & Lebais 2016), especially one with a distinct elevation-climate gradient. Moreover, the biodiversity and ecological assemblage of fungal endophytes across species ranges experiencing a steep elevation-climate gradient is a novel system to study fungal diversity in relation to climate. I propose to address this gap by characterizing fungal community composition and assembly of native endophytes from plants experiencing climate stress at the edges of their species range and test the effect of stress on these communities.

Background

The presence of microorganisms living inside plants was first discovered over 200 years ago by German botanist Heinrich Friedrich Link (1809). The term endophyte ("endo" meaning inside and "phyte" meaning plant) was coined by de Barry (1866) and later characterized by Victor Gallipe (1887) as fungi or bacteria with beneficial associations to their host. More recently, the term endophyte has been clarified to convey the asymptomatic nature of this interaction with its

host (Wilson 1995). It has since been thought that all plants harbor symbiotic endophytes in natural ecosystems (Petrini 1996). Research on endophytes has been on the rise due to its important role in directly benefiting plant growth and diversity (Hardoim *et al.* 2015, Afkhami & Strauss 2016), ameliorating the effect of stress on host plants (Khan *et al.* 2011, Chadha *et al.* 2015, David *et al.* 2018), and assisting plants in obtaining nutrients from water deficient environments (Vázquez-de-Aldana *et al.* 2013). Plants depend on seed dispersal and physiological adaptations to mitigate the impacts of drought (Bartels *et al.* 2005) however; they may not be acting alone (Rodriguez & Redman 2008). Some plants are known to select for specific fungi from the surrounding soil (Urbina *et al.* 2018), nevertheless the specificity of this recruitment and the role stress induced by climate has on the assemblage of fungal communities represents a novel system of research in plant-fungal interactions.

Plant tissues have multiple internal compartments and provide spatially and temporally diverse microbial habitats that can support specific communities of fungal endophytes (Sun & Guo 2012). Although diversity and abundance of fungi can be a significant facet to an ecosystem and environmental studies (Saikkonen *et al.* 1998, Hibbett *et al.* 2016, Blackwell & Vega 2018), the vast majority of research examines plant-fungal interactions in agricultural systems. In order to understand how these interactions occur in nature, it is important to study the structure, assembly, and biodiversity of native fungal endophytes from natural environments. Assembly or the way communities are shaped, is a well-studied community ecology process (Hutchinson 1957, MacArthur & MacArthur 1961, Ricklefs 1987, Vellend 2010) that can inform how functional traits of communities are distributed across space (McGill *et al.* 2006), however assembly has been less explored in microorganisms due to their high-dispersal capacity. The main assumption is that microbes are deterministically assembled through filtering by habitat characteristics, but this idea has been argued to be too simplistic (Hanson *et al.* 2012, Morrison-Whittle & Goddard 2015, Stegen *et al*. 2013). Originally, endophytes were thought to enter hosts aerially through the leaves and stems (Carroll 1896). This was later edited to suggest that some endophytes are latent pathogens living inside their host at all times (Petrini 1991). More recent studies suggest endophyte filtering by abiotic conditions (Zimmerman & Vitousek 2012), while others have found biotic interaction to be the main cause of fungal endophyte assemblage (Harrison *et al.* 2018). Most only occupy a small portion of tissue (Lodge et al. 1996) and are transmitted to their host by air, rain, or animals (Rodriguez 2009, Frank *et al.* 2017). They can be transmitted vertically through seeds (Corbin *et al.* 2017) or horizontally through the soil, stomata, or from pollinators (Saikkonen *et al.* 1998). Furthermore, since fungi vary dramatically in their resource allocation, there are often distinct differences in fungal community structure between plant compartments, above-ground (shoot) and below-ground (root) (Taylor & Bruns 1996). In some cases, plant compartments and biogeography are the main drivers in community composition (Coleman-Derr *et al.* 2016). In other circumstances, endophytes can have host preferences and demonstrate levels of specificity (Cannon & Simmons 2002). Endophytes can also switch ecological roles from latent pathogens to mutualisms (Carroll 1988). Mutualisms can range from wide breadth generalist partnerships (Batstone *et al.* 2018) to specific associations where endophytes may have evolved to adapt to host traits (Hiruma *et al.* 2018). Some studies have demonstrated that fungal endophyte infection or host interaction can also be affected by nutrient availability (Cheplick *et al.* 1989, Tall & Meyling 2018), mostly in response to varying accessibility to organic nitrogen (Newsham et al. 2011). There is an incredible amount of variation in the literature regarding fungal endophyte structure, assembly, and transmission and to understand the ecological role of fungal communities these processes need to be unpacked.

Plant root systems are able to attract and select microbial communities endowed with plant growth promoting traits to persist through water scarcity events (Marasco *et al.* 2012). Contrarily, precipitation has been linked to microbial community diversity (Giauque & Hawkes 2013), indicating that drought could affect the structure of endophytes. Drought has been heavily studied in the context of plant-fungal interactions with the vast majority suggesting that these

associations help reconcile the effects of drought on the plant host, for example conferring physiological protection to plants when water is scarce (Hahn *et al.* 2008) and stimulating plant growth (Marulanda & Azcón 2009). The use of culture-dependent and next-generation sequencing has been instrumental in identifying the role of endophytes in stress response from drought (Sun *et al.* 2010, Singh *et al.* 2011). Sequencing technology has been used to assess fungal diversity and abundance from particular plant compartments (Bills & Polishook 1994, Sun & Guo 2012), transmission routes, the effect of fungal composition on the host, and the effect of treatments or environment type on the fungal communities (Arnold *et al.* 2007, Dissanayake *et al.* 2018). Although much of the literature investigates plant-fungal interactions in the context of drought in agricultural systems, this proposal aims at studying these interactions over an entire species range impacted by the effects of rising temperatures in a natural ecosystem.

Species ranges are dynamic structures that are frequently in flux and their limits are caused by a variety of abiotic and biotic interactions (Brown 1996, Gatson 2003, Sexton *et al.* 2009). Plant ranges are influenced by environmental tolerance, seed dispersal, and biotic interactions (Peterson *et al.* 2011, Wisz *et al.* 2013, Pellissier *et al.* 2013), and rapidly shifting climate conditions have been causing redistributions of life on Earth (Pecl *et al.* 2017) at an unparalleled rate (Lawning & Polly 2011). Over generations, plants have been shifting toward higher latitude or elevation in response to warming lowlands conditions (Parmesan & Yohe 2003, Chen *et al.* 2011, Halbritter *et al.* 2018). Plant species with high vulnerability to climate change have narrow climate niches, may experience limitation to range expansion or migration, or are unable to adapt, as is often the case for mountain species that are restricted in their latitudinal range (Dullinger *et al.* 2012, Pereira *et al.* 2012). Present-day dynamics of distribution ranges, demography, and evolutionary processes that accompany species expansions have been increasingly studied (Thomas *et al.* 2001, Peischl *et al.* 2015), with particular attention to the range edges (Travis & Dytham 2004). Climate affects the genetic variation of species at the edge of their range, especially when the range expands over an environmental gradient (Sexton *et al.* 2016). When considering environmental gradients in climate studies, there is typically a warm limit in the lower part of species ranges deemed the "rear" or "trailing" edge as it is typically most vulnerable to eventual extinction and a cool limit at higher elevations deemed the "leading" edge where populations are expected to eventually colonize as ranges shift (Hampe & Petit 2005, Cahill *et al.* 2014). This phenomenon represents a natural experimental condition for studies aiming to understand the effects of climate on populations across a range.

In the *Origin of Species* (1859), Darwin suggested that abiotic forces set species range limits at high latitude, high elevation, and other abiotically stressful areas, while species interactions set range limits in apparently more benign regions. Only a few studies have begun to investigate the role fungal mutualisms have in shaping host ranges in response to stress (Afkhami *et al.* 2014), and there is a need to research fungal endophyte community shifts and abundance patterns across plant species ranges experiencing stress across a climate gradient. Therefore, I propose to address this gap by determining the biodiversity and structure of fungal endophyte communities from plant species across its range and experimentally test whether their presence alleviates stress or not. My ***central hypothesis*** is that native fungal endophytes are habitat-adapted and mediate climate-induced stress tolerance to their hosts. To further investigate the effect fungal endophytes have on plants experiencing stress I propose to isolate fungi cultured from plant tissue and inoculate plants in controlled treatments. I address my central hypothesis by pursuing the following ***overall aim***: *identify and characterize plant-fungal interactions across a species range with an elevation-climate gradient and manipulate them in a controlled environment to test their effect in stressed conditions*. My working hypothesis for this aim is that plant populations at the warm-edge of their range experience greater stress and will subsequently host different fungal endophytes compared to plants in the cool-edge of the range. These communities may have the potential to benefit plant populations at warm-edges by alleviating stress from drought or heat.

Plants adapting to novel stresses and experiencing limits to adaptation at the edge of their range make an ideal study system for identifying fungal species with mutualistic interactions that ameliorates abiotic stress. In order to explore these plant-fungal interactions empirically, it is important to study plants with well-known genomics, ecology and that can be easily collected and bred in a laboratory environment. Thus, the subject for this project is the annual self-fertilizing Cut-leaf Monkeyflower (*Erythranthe laciniata,* formerly *Mimulus laciniatus*), a species increasingly used to understand adaptation at species range limits due to its restricted mountain range and colonization potential (Sexton *et al.* 2016). This plant is an ideal system to study the scope of my overall aim for its ease of manipulation in the lab, the proximity of its range to lab facilities, availability of the plant genome, and its unique ecology and evolutionary patterns. Monkeyflowers are a hyperdiverse group of flowering plants that are a model system for studying changing climates across elevation ranges (Angert 2009, Sexton 2016). *Erythranthe laciniata* are native endemics to the Sierra Nevada and experience a distinct elevation-climate gradient with low elevation warm-edges susceptible to increasing temperatures and high elevation cool-edges (Fig. 1). Monkeyflowers are known for their ability to adapt to a variety of environments (Twyford & Friedman 2015). As a seep-adapted plant, *E. laciniata* grows from mossy patches in cracks and shelves of granite rock outcrops and acquire water seasonally from snowmelt runoff. Their flowering season takes place between March and July and their seeds (several hundred per flower) are easily accessible in the wild from dried capsules. This allows the plant and its fungal endophytes to be sampled several times throughout its season and for the collection and implementation of seeds in future experiments. This system provides a range-wide perspective in a climate-stressed context, giving it the potential to inform the complexity of interactions at range margins and how climate may be affecting these interactions. With this system in mind, I have identified the following specific aims:

Aim 1: Identify *E. laciniata* endophyte community shifts in response to drought.

Significance and Innovation

Further understanding of how the structure of endophyte communities shift in response to drought and heat is a potentially important avenue for identifying significant biotic interactions that may play a role in stress response to climate change and perhaps predicting species distribution shifts (Van der Putten *et al.* 2010). By sampling the microbiome of *E. laciniata* in controlled and drought conditions it may be possible to demonstrate the role of drought in the composition and abundance of endophyte communities. As plants acquire endophytes either through recruitment, horizontally from the soil, or vertically from their seeds, different taxa will settle in different parts of the plant. As the plant experiences stress these compositions may shift, providing evidence for the effect of stress on the endophyte community structure. Fungi with increased abundance in stressed conditions may be associated with the plant stress response, though to test this would require experimental manipulated of isolates (Aim 3). To investigate if particular endophyte communities are shaped by stress this project aims to examine the fungal communities within plants with access to their native microbes in 1) drought conditions and 2) controlled watering conditions. This specific aim addresses the following questions: Does drought alter the composition and diversity of fungal communities? Does drought select for particular fungal taxa? Is there increased fungal diversity above-ground or below-ground tissue? To answer these questions, I will test the following hypotheses:

H.1.1: Drought alters the fungal endophyte composition and diversity in *E. laciniata.*

H.1.2: Fungal endophytes are more diverse in root tissues than in the shoot of the plant.

H.1.3: Particular fungal endophytes will be more abundant in drought conditions.

Research Approach

In 2017, native soil was collected from a central *E. laciniata* population in the Sierra Nevada. The soil was homogenized through 2 mm sterile sieves and then mixed in sterile bins. The soil was then potted in three 36-cell potting trays and seeds were sown from bred lines. Potted trays were cold stratified at 4 °C for two weeks to induce wintering conditions that trigger seed germination. Trays were then transferred to controlled growth chambers (PGC Flex, Conviron) and grown on 14-hour day cycles ranging from 23 °C to 10 °C for the day and night conditions, respectively. Plants were bottom-watered with sterile MilliQ™ (MilliporeSigma, Burlington, MA, USA) water every 1–2 days for 3–4 weeks. Trays were rotated occasionally to remove chamber position bias.

Once plants grew to maturity, they were randomly assigned to either a drought or control treatment group or served as pre-experiment samples. Pre-experiment plants were harvested, and drought and non-drought treatments were arranged in separated trays. For 2–3 weeks drought plants received no water while the control group experienced the prior watering routine. Harvested plants were separated into root and shoot compartments using a sterile razor, surface sterilized by sonication for 5 minutes (Lundberg *et al.* 2012), and stored in -80° C. To homogenize plant tissue for DNA extractions, samples were flash frozen with liquid nitrogen and ground into a powder using a hand drill and ceramic bits.

Extractions were conducted using PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). Extracted DNA was amplified using the ITS ribosomal RNA gene to acquire diverse fungal groups (White *et al.* 1990). Amplified DNA was tested for quality using NanoDrop™ (2000, ThermoFisher). Microbiome sequence data was generated at the Joint Genome Institute (JGI). Sequences were prepared for the Illumina MiSeq sequencing platform (San Diego, CA, USA) for ITS rRNA amplicon analysis with fungal primers ITS9F GAACGCAGCRAAIIGYGA and ITS4R TCCTCCGCTTATTGATATGC (Menkis *et al.* 2012, White *et al.* 1990), using 96 well plates as per the JGI protocol (Rivers 2016). All data from the sequencer were demultiplexed and filtered from contaminants. Microbial community composition was analyzed (Fig. 2) using Qiime2 bioinformatics platform (release 2018.6, https://qiime2.org). Currently, these data are being analyzed by comparing communities using Unifrac (Lozupone & Knight 2005), a distance metric used for comparing communities, and permutational multivariate analysis of variance (PERMANOVA) to statistically test for similarity (Anderson 2001).

Expected Outcomes

To determine if drought alters the fungal endophyte composition and diversity in *E. laciniata,* accepting H.1.1, and whether there is increased diversity in the root of the host, accepting H.1.2., I should find significantly different numbers of taxa between pre-experiment and drought treatment groups and between root and shoot. In order to conclude that particular fungal endophytes are more abundant in drought conditions, accepting H.1.3., I should find specific fungi that are more abundant in different treatment groups. Preliminary results indicate differences in fungal endophyte abundance between drought and pre-experiment treatments and differences in diversity between root and shoot compartments, however, statistical analysis is still being analyzed to confirm these assumptions (Fig. 2).

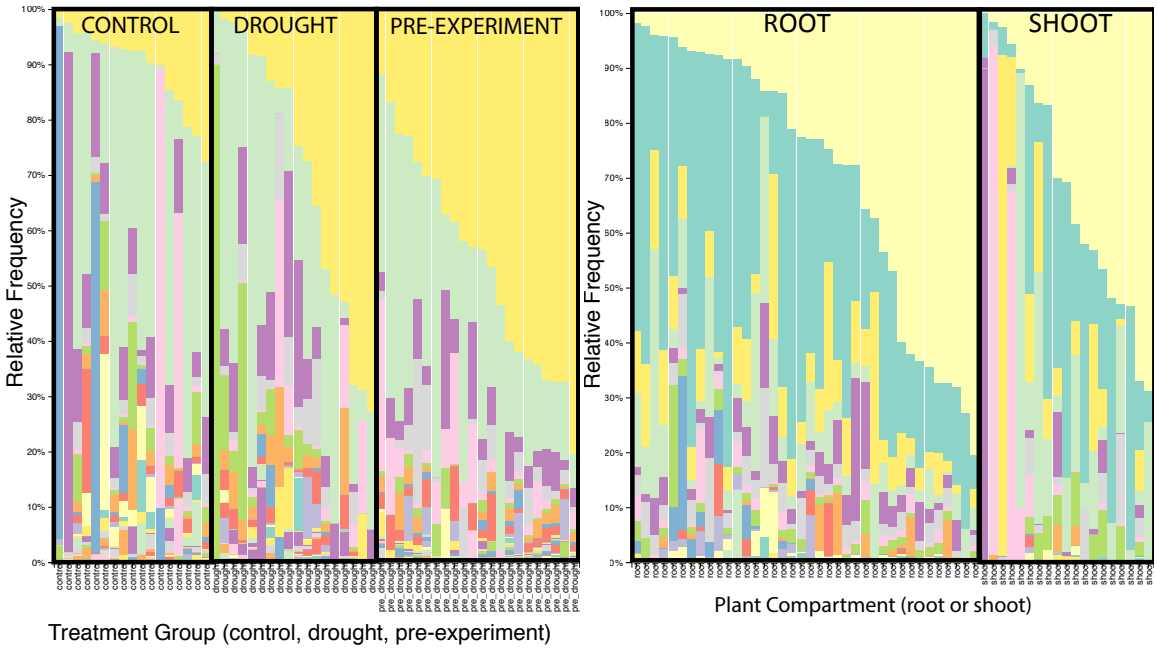

Figure 2: Preliminary results from Qiime2 analysis from Aim 1. Fungal endophyte taxa bar plots organized by treatment groups (left) and plant compartment (right). Each color reflects a different taxa group at the class level.

Aim 2: Characterize the fungal endophyte communities associated with *E. laciniata* across its range

Significance and Innovation

The edge of a species range is a stressful habitat for a wild plant and endophytes help plants cope with stress. One way they do this is by recruiting specific fungal taxa for the purpose of alleviating stress (Lemanceau *et al.* 2017, Redman *et al.* 2002, Ruppel *et al.* 2013), potentially leading to a higher abundance of beneficial interactions at range limits. In some cases, plant-endophyte interactions can cause some organisms to have a wider breadth of tolerance (Bruno *et al.* 2003) including variations in climatic niche (Afkhami *et al.* 2014), resulting in theoretically larger species ranges by expansion or stability in increasingly stressful environments, such as the warming edge of a species range. Although several studies have explored the potential for endophytes to affect species ranges (Van der Putten *et al.* 2010, Dawson *et al.* 2011, Wiens 2011, HilleRisLambers *et al.* 2013, Wisz *et al.* 2013), few explore the effects of the range locale and subsequent plant response from a climate gradient on fungal endophytes composition across species ranges.

To elucidate the structure of fungal endophyte communities I will assess several potential drivers including plant compartment, climate, soil fertility, and host species. The effect of soil fertility came about during growth experiments in Aim 1 when it was discovered that native soil was nutrient deficient, and nutrients needed to be added in order for plants to develop. This suggests that native soil from *E. laciniata* populations is nutrient limited, and may indicate some of the composition and diversity of fungal endophyte communities is associated with this locale. It is known that fungal endophyte may specifically confer growth promotion under nutrient deficient conditions (Ravel *et al.* 1997) suggesting that this system may host endophytes for this function. Through sampling in Aim 2, I hope to gain a range-wide understanding of nutrient availability within *E. laciniata* populations and observe if there are any relationships between soil fertility, plant fitness, and fungal endophyte composition. Additionally, to test whether endophytes are structured by their host I will elucidate specificity by sampling co-occurring

species from each population. My *motivating questions* here are: Are fungal communities distinct across the elevation-climate gradient or consistent? What structures fungal endophyte communities in *E. laciniata*: climate, soil fertility, plant compartment, or the host species? Do nutrients vary in concentrations across the range or are they significantly low in certain parts of the range? To answer these questions, I will test the following hypotheses:

H.2.1: Fungal endophyte community composition is distinct between range edge habitats.
H.2.2: Climate is the strongest determinate of fungal endophyte structure.
H.2.3: Soil fertility is significantly low across the species range.

Research Approach

To test these hypotheses, I will characterize the fungal communities associated with *E. laciniata* across its range. Sampling will occur between March and July of 2019 and 2020, specifically targeting 2 warm-edge (avg. temp. 13.1 °C), 2 cool-edge (avg. temp 2.13 °C), and 2 central sites in the Sierra Nevada representing known climates from which plant fitness has been assessed (Fig. 3, Sexton *et al.* 2016). Plants will be collected at the budding, flowering, and fruiting stage of their annual lifecycle to account for variation in endophyte composition between life stages. In order to capture fungal endophytes *in situ* plants will be surface sterilized and flash frozen upon collection.

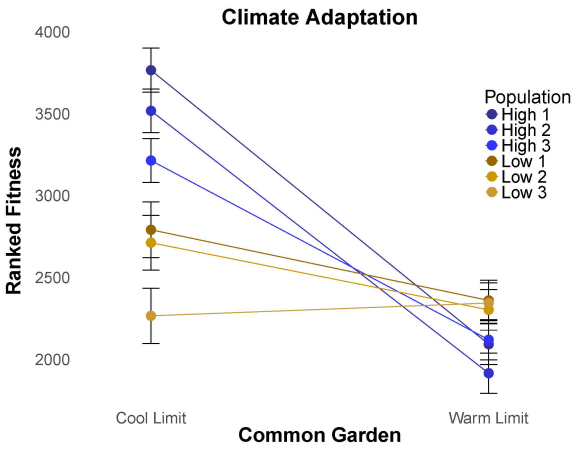

Figure 3: Crossing fitness reaction norms at cold and warm climates.

To test to what extent endophytes are assembled from the substrate, I will separate and sample compartments of the plant (roots and shoots) as well as the moss substrate. To test whether host species structures fungal endophyte composition co-occurring plants will be sampled from each site. I will collect 10 monkeyflower samples and 5 co-occurring species from each site, totaling 15 samples x 3 sites x 4 compartments (shoot, root, substrate, seed) x 2 range edges totaling 360 samples. In addition to molecular data, site-specific data will be taken including average temperature and precipitation from collection years. Lastly, environmental data will be taken including soil type, site soil fertility, nitrate, ammonium, and total soil organic nitrogen concentrations. For nutrient assessment, soil samples will be combined with KCl (2 M) in the field and left to separate over 24 hours. The supernatant, containing mobile nutrients such as ammonium and nitrate, will be analyzed with Latchet (Hach Co., Loveland, CO, USA).

Plant samples will be collected using a randomized stratified selection strategy. Samples will be a minimum of 5 meters apart from each other. Plants will be removed with roots intact. Samples will be surface sterilized in the field using 70% ethanol bath (30 s) followed by a 0.5% bleach (NaOCl) bath (60 s) followed by a second wash with 70% ethanol (30 s), followed by four rinses with sterile, autoclaved, water (Shultz *et al.* 1993, Greenfield *et al.* 2015). Plants will be patted dry with sterile wipes and separated into the shoot and root compartments. Once separated, samples will be flash-frozen in liquid nitrogen and stored on dry ice until transferred to -80 °C freezer. Some plant samples will be pressed in the field and kept intact to be submitted to the UC Merced herbarium for future work, while others will be collected for isolating fungal endophytes (Aim 3). The frozen tissue will be mechanically ground for downstream extraction, amplification,

and sequencing protocols similar to those taken in Aim 1. In this case, sequencing will occur on the Illumina MiSeq at UC Merced.

Expected Outcomes

In order to determine if fungal endophyte community composition is distinct between range edge habitats, and accept H.2.1, I should find distinct taxa from each range habitat type. To conclude if the climate environment is the strongest driver of fungal endophyte community structure, and accept H.2.2, I should find the most differences in fungal endophyte composition from plant populations between the warm edge and cool edge of the species range, followed by plant compartment (Coleman-Derr *et al.* 2016), followed by host species. To determine if soil fertility is significantly low across the species range, and accept H.2.3., I should find significant deficiencies in nitrate, ammonium, and organic nitrogen across the species range

Aim 3: Test the effect of inoculated fungal isolates on range limit populations under drought conditions

Significance and Innovation

To determine if fungal endophytes mediate stress tolerance to their host, it is essential to provide empirical evidence of the beneficial effect of endophytes through experimental inoculation of isolated fungi. In addition, the transmission mechanism of fungal endophytes to the plant is an important area of research to understand how plants acquire fungal endophytes. Using soil and seed samples from Aim 2, I will be able to test transmission pathways between host plants and their substrate, a moss or spike moss, with its own fungal endophyte community that may confer beneficial functions to their hosts (Bragina *et al.* 2014). My *motivating questions* here are: How are fungal endophytes transmitted to their host? Do plants perform better when paired with their range-specific endophytes under stress?

H.3.1: The majority of fungal endophytes are horizontally acquired from soil and substrate.
H.3.2: Plants perform better with range-specific stress when paired with endophytes from their part of the range.

Research Approach

During sampling for Aim 2, additional plants will be selected and kept intact for isolating fungal endophytes. To estimate what proportion of fungal endophytes are seed-borne, and potentially vertically transferred, I will collect seeds at the end of the field season. All plant material for Aim 3 will be processed for isolation within 96 h of collection (Arnold & Lutzoni 2007). Plants will be collected in sterile plastic bags and kept on ice until transport to the lab. Once in the lab, all surface sterilization procedures will be conducted in the hood to prevent airborne contamination. Plant tissue will be washed with sterile water to remove epiphyllous debris (30 s), patted dry and surfaced sterilized in a stepwise protocol 1) bath in 70% ethanol for (10 s), 2) rinsed in sterile water (30 s), 3) bath in chlorine bleach (0.5% NaOCl; 60 s), 4) bath in 70% ethanol (10 s), and 5) rinse 4 × sterile water (1–2 min) (modified from Petrini & Dreyfuss 1981, Schulz *et al.* 1993). The tissue will then be patted dry and cut into compartments (root and shoot) and then into fragments (2 × 1 mm) and plated on Petri dishes containing 2% malt extract agar (MEA), which encourages growth from diverse fungal endophytes (Fröhlich & Hyde 1999, Arnold *et al.* 2003). Plates will be sealed and incubated at room temperature for 3–4 weeks and emergent fungi will be isolated into pure cultures. To isolate vertically transmitted endophytes from the seeds, surface sterilization procedure will remove outer microbes. Seeds will be surface sterilized using stepwise protocol including bleach baths and sterile water washes (Geisen *et al.* 2017) and then plated on Petri dishes. After 5–7 weeks, fungal isolates collected in Petri dishes will be categorized by shape and morphology and will be isolated into pure culture vouchers for

long-term storage. Isolates will also be sequenced following similar procedures to those outlined in Aim 1. Once isolated and identified, fungi can be added to sterilized water to create an inoculum spray that can be administered to soil and leaves in treatment groups in the growth chambers prepared similarly to treatments in Aim 1. To avoid random effects of invasive microorganisms, which are more likely to establish when native microbes are absent (Mallon *et al.* 2015, Wei *et al.* 2015) and potentially reduce endophyte transmission and subsequent composition, soil will be composed of a fraction of natural soil and sterile soil (Geisen *et al.* 2017), hereafter referred to as semi-sterile soil.

To empirically test for transmission pathways, I will set up a growth chamber experiment using fungal isolates from my culturing methods. Trays will be sown with seeds collected in the field with the following experimental set up: 1) sterile seeds and semi-sterile soil, 2) sterile seeds and inoculated soil, 3) surface sterilized fertile seeds and semi-sterile soil, and 4) surface sterilized fertile seeds and inoculated soil. Seeds will be both surface-sterilized and sterilized of seed-borne fungal endophytes using chloroform gas (Lindsey *et al.* 2017). Soil will be sterilized using an autoclave and then mixed with a fraction of native soil. Fungal isolates will be mixed in a sterile water-based media and sprayed into the soil. Plants will undergo both heat and water limiting treatments. Plant performance will be assessed using flower count as a proxy for plant fitness (Sexton *et al.* 2011). Finally, to test the final structure of fungal endophytes across compartments mature plants, seeds from the experiment, and the soil substrate will be sequenced following protocols outlined in Aim 1 an analyzed using methods outlined in Aim 2.

Expected Outcomes

In order for me to conclude that the majority of fungal endophytes are horizontally acquired from soil and substrate, and accept H.3.1., plants that are grown with inoculated soil and sterile seeds should have higher levels of species diversity than those grown without inoculated soil. To determine if plants perform better with range-specific stress when paired with endophytes from their range of origin, and accept H.3.2., plants inoculated with fungal endophytes isolated from their home site or environmentally similar sites will have a higher fitness (measured by the number of flowers) than those grown with foreign endophytes. More specifically, warm-edge populations paired with their native endophytes will demonstrate a higher tolerance for drought conditions. To confirm if endophyte composition is distinct, sequence results from plants, seeds, and soil substrate will be analyzed using similar methods to those outlined in Aim 2 and should result in significantly different communities structure between treatment groups.

Conclusions

By working through these outlined aims I may find that fungal endophytes are locally adapted to the range locale and may be structured by climate environment, the null hypothesis being that fungal endophytes are not different across the range and that plants may not need them to adapt to their environment. I will be able to confirm this by determining whether endophytes are horizontally transmitted from soil substrate. As a new system in fungal endophyte research, this will be the first attempt to characterize the structure, assembly, and transmission of endophytes within *E. laciniata,* an important model system in genetics, ecology, and evolution. Additionally, this proposal will investigate the effect of soil fertility across the *E. laciniata* range, a potentially informative ecological component yet to be described in this elevation gradient system. This project has the potential to determine if fungal endophytes are drought adapted, which would contribute to known ecological functions of endophytes in natural ecosystems. Most importantly if this project reveals that plants inoculated with endophytes from warmer populations confer drought tolerance it will be a significant contribution to plant-fungal ecology in the context of climate change and could benefit future efforts in restoration and conservation.

Dissertation Progress Timeline

Year One:

- Applied for new student fellowships and awards
- Awarded UC Merced QSB Recruitment Award
- Awarded School of Natural Science Dean's Relocation Award
- Developed growth chamber methodology
- Completed coursework for degree requirements
- Completed two pilot projects for Aim 1
- Discovered *E. laciniata* field soil is nutrient deficient
- Published 1st paper on monograph of *Marasmius* from Madagascar (master's thesis)
- Published 2nd paper reviewing evolution and ecology of niche breadth (a side project with Sexton)
- Published 3rd paper on *Tetrapyrgos* from Madagascar (a side project from my master's thesis)
- Published 4th paper reviewing transmission of bacterial endophytes (a side project with Frank)
- Participated in inaugural UCM NSF Interdisciplinary Computational Graduate Education Program
- Applied, received, and accepted summer internship with DOE Joint Genome Institute
- Served as a teaching assistant for BIO 001 and BIO/ESS 133
- Joined Center for Engaged Teaching and Learning (CETL) Teaching Internship Program

Year Two:

- Applied for NSF Plant Biotic Interactions (PBI) Grant (Lead Author: Sistrom)
- Completed growth and experimental setup for Aim 1
- Completed harvesting and sampling for Aim 1
- Complete data analysis for Aim 1
- Developed fungal culturing methods for Aim 2
- Developed field sampling protocols for Aim 2
- Develop extraction protocols for Aim 2
- Complete field surveys for Aim 2
- Served as a teaching assistant for BIO 001
- Awarded graduate student research (GSR) from Sexton
- Awarded California Native Plant Society Bristlecone Chapter Mary DeDecker Scholarship (side project sampling Eastern Sierra wildflower endophytes not mentioned here)
- Awarded Translational Mycology Grant from the Mycological Society of America
- Awarded Sonoma County Mycological Graduate Scholarship

Year Three:

- Awarded Southern California Edison Fellowship (Summer 2019)
- Take qualifying exam
- Verify extractions protocols on sampled materials for Aim 2
- Train on Latchet machine for nutrient analysis for Aim 2
- Practice preparing materials for sequencing for Aim 2
- Complete field sampling for Aim 2
- Sequence sampled populations for Aim 2
- Analyze sequence DNA in Qiime2 for Aim 2
- Verify fungal culturing on sampled materials for Aim 2 & 3
- Develop endophyte isolation and inoculation protocols for Aim 3
- Develop protocols to test transmission for Aim 3
- Publish 5th paper on monkeyflower adaptive response (side project Sexton)
- Publish 6th paper on drought experiment (Aim 1)
- Present research from Aim 1 at an international conference
- Apply for NSF (PBI) grant (Lead Author: Sistrom)
- Serve as the teaching assistant for BIO 120
- Develop and implement teaching research project

Year Four:

> Develop experimental manipulation protocols for Aim 3
> Complete inoculation experiments for Aim 3
> Publish 7th paper on monkeyflower microbiome across its range (Aim 2)
> Present research from Aim 2 at an international conference
> Serve as a teaching assistant
> Complete CETL Teaching Internship Program

Year Five (2021):

> Publish 8th paper from on experimental inoculation (Aim 3)
> Publish 9th paper from teaching research project
> Present research from Aim 3 at an international conference
> Write and defend the doctoral dissertation
> Serve as a teaching assistant

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