

# Unraveling host genotype by environment effects on endophyte community structure across the geographic range of *Clarkia xantiana*

## Introduction

Fungal endophytes, a group of highly diverse, usually Ascomycetous fungi characterized by their asymptomatic occurrence in plant tissues, have been found inside every plant species examined thus far, and occur across all major land plant lineages<sup>1</sup>. Colonization by fungal endophytes can influence plant fitness, though subtly: often, plants show no observable phenotypic changes when inoculated, but under stress from abiotic or biotic factors, changes in plant traits like drought tolerance, thermal tolerance, and herbivore resistance may become notable<sup>2</sup>. These interactions are highly context dependent, with plant and endophyte species, environmental factors, spatial distribution, and plant-endophyte genotypes representing dramatic sources of variation. Given the role of endophytes in impacting plant phenotype under stress, endophyte community composition may be particularly important for mediating plant fitness at geographic range margins and across environmental gradients.

Few studies have explored genotype by environment interactions on endophyte community assembly in field experiments, and far fewer have explored these factors across the geographic range of a given plant species<sup>3</sup>. In my dissertation, I am investigating how plant species, host genotype, environment, and GxE interaction shape foliar endophyte communities across the native range of *Clarkia xantiana*. I am seeking funding to begin my field research on plant-endophyte interactions, in a large factorial transplant experiment exploring how endophyte community structure varies across the plant's geographic range, and the roles host genotype and environment play in shaping a plant's endophyte community.

## Background

*Clarkia xantiana* spp. *xantiana* (Onagraceae) (hereafter, *xantiana*), a Californian annual plant endemic to the southern Sierra Nevada foothills, is a prime system to understand microbial influences over geographic range. *Xantiana*'s natural history and population ecology are well characterized, and it occurs over an environmental gradient of elevation, temperature, and aridity, with a notably stark eastern range edge despite the absence of dispersal barriers<sup>4</sup>. In my dissertation I am examining the role of host genotype and environment in structuring *xantiana*'s endophyte communities across the plant's range, and exploring transmission and specialization within endophytes. Studies of other systems have revealed that endophytes can have notably large effects on individual plant fitness<sup>5</sup>, but their community and spatial structure across a host species' entire geographic distribution remains relatively unexplored.

## Host genotype by environment interaction

Very few studies have explored spatial distributions of endophyte species, leaving many questions regarding how their cryptic distributions may influence their hosts in overlapping areas. Experts in the field have called for studies exploring plant-endophyte interactions below the species level<sup>6</sup>, as several notable studies have shown that endophyte-plant interactions are influenced by endophyte genotype, host genotype, and the host plant's environment<sup>7</sup>. Ultimately, deciphering the relative contributions of host

genotype and environment is critical to understanding what drives endophyte community structure across a host's range, and subsequently, functional implications for their host plants. A primary goal of my research is to untangle the relative influence of host genotype and environment in shaping community structure of *xantiana* endophytes, and evaluate their levels of specificity to *xantiana* via their transmission strategies.

Untangling the influence of host genotype, environment, and the interaction between the two is complicated by the fact that endophytes may be either heritable from host parents or colonized horizontally from the environment<sup>8</sup>.

An understanding of endophyte transmission methods on the species level is critical to characterizing endophyte community structure across a plant's range, as vertically transmitted endophytes will likely be systemic, associated with local genotypic differentiation, and heritable across generations<sup>9</sup>, while horizontally transmitted endophytes infect host plants externally and may be environmentally ubiquitous in a given area<sup>10</sup>. In preparation for field transplant experiments and sampling, my previous work characterizing transmission methods of endophytes found in three species of *Clarkia* (*C. cylindrica*, *C. unguiculata*, *C. xantiana* ssp. *xantiana*) in one site will provide context for interpreting endophyte community assembly. **I am seeking funding to travel to and within my field sites, in order to collect and sample seeds and endophyte communities from existing *C. xantiana* populations, and initiate a large-scale factorial genotype-by-environment transplant experiment.**

### **Methodology**

Using environmental data from sensors placed at each population of *xantiana*, a principal component analysis will be performed to identify eight population transplant sites in order to appropriately capture the span of the environmental gradient across *xantiana*'s range. At each of the identified sites, leaf samples of mature plants will be collected to plate in culture, as well as seeds from mature *xantiana*. These seeds will be pooled by site (representing a locally adapted genotype), and planted in a fully factorial design with individuals from every site population represented at each transplant site, with a portion of the collected seeds saved for sterilization and culture to assess endophyte transmission mechanisms.

### **Analysis**

Since the design of the experiment is fully factorial, with all source population genotypes represented across an environmental gradient at all sites, I will use multivariate statistics, PERMANOVA, and maximum likelihood, to untangle the relative influence of population origin (genotype) and with environmental site variation in shaping endophyte community composition across *xantiana*'s range. Significant variation among source populations would indicate that populations have differentiated genotypically enough to create disparities in endophyte community structure (e.g. due to imposed plant sanctions on new endophyte colonization following a chitin-triggered immune response<sup>11</sup>) supporting a pattern seen in endophytes among other plants.<sup>12</sup> Differences within a source population among sites along an environmental gradient could indicate different pools of endophytes available for colonization at each site, suggesting a local environmental influence on community structuring across space.

## Significance

This project will provide one of the first manipulative field experiments to unravel the relative contributions of host genotype and environment to endophyte community structure across the range of a host. In concert with my upcoming dissertation work on how endophyte community structure varies within a site across a phylogeny of host species, this will provide detailed insight regarding how endophyte communities are structured at the level of the *xantiana* host genotype across its entire range and an environmental gradient.

## Literature Cited

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