

Strange things can happen when organisms form tight interactions, defined as symbioses. Fungi and algae pair to form lichens, protists and bacteria can team up with termites so the insect can break down wood. One of the more inexplicable symbioses involves orchids and fungi. Other plants give beneficial root fungi sugar they made from photosynthesis and receive limiting nutrients like nitrogen. Orchids however, require sugar from fungi so their seeds can grow and develop. What do the fungi get in return? Why do these fungi participate in this symbiosis? These questions motivate my research program.

I am an evolutionary biologist who uses molecular and computational approaches to study the organisms involved in the orchid mycorrhizal symbiosis. I began my PhD by studying the evolutionary relationships of orchids and a phenomenon called whole genome duplication (WGD) also known as polyploidy. Over the course of that study, I teamed up with Dr. Lawrence Zettler to combine genomics approaches with his wonderful fungal library at Illinois College. We formed an international team and I led the writing of a successful grant to the Department of Energy's Joint Genome Institute (JGI) to explore the evolutionary relationships between Dr. Zettler's fungal isolates and to find patterns that may indicate why they form a symbiosis with orchids. The results of these studies will form the remainder of my dissertation. I envision my future research program as an extension of the work I've started; I want to learn more about the biology of these fungi and explore their genomes more in-depth. I see this system as a long-term study focus that will take many different approaches and an influx of new ideas which makes it perfect for curious and driven undergraduate researchers.

### **Past work**

I studied orchid evolution because I wanted to understand how we get such unusual traits like orchids' highly modified flowers. The phenomenon of whole genome duplication (WGD) where every gene in the genome gets copied, has been implicated as a source of evolutionary innovation in many different plant families including grasses and asters so I wanted to see if this pattern held for orchids as well. With Dr. Michael McKain (University of Alabama), we used transcriptome data to infer phylogenetic relationships and investigate the incidence of WGD in orchids. We confirmed relationships in previous publications and we did find strong evidence for a whole genome duplication event that all orchids share.

This project resulted in a paper (Unruh et al., 2018) and the genes used for our phylogenetic analyses are now used to examine orchid evolution in other genera by a joint group of scientists at the University of Georgia and the Atlanta Botanical Garden. Scientists at Kew are also interested in the applications of this work. I was asked by Dr. Mike Fay to present at the 7<sup>th</sup> International Orchid Conservation Congress this summer 2019 in London.

### **Current work**

The more I learned about the role that fungi play in the life cycle of orchids the more I realized I had to study these organisms. Though my training up to that point had been with plants, I took the initiative to attend and present at conferences specializing in fungi and mycorrhizal interactions such as the International Conference on Mycorrhiza and the Fungal Genetics Meeting. There I met and began collaborating with researchers like Dr. Jason Stajich (University of California - Riverside) to study the genomics of these fungi. The aims of my current projects were inspired by a study looking at a different plant/fungal relationship (Kohler et al., 2015). In

Kohler et al.'s (2015) study, they examined the genomes of ectomycorrhizal fungi to look for a pattern to explain why this niche has evolved multiple times. Across the evolutionary tree, many of these fungi independently lost the same types of genes which indicated a "tool-kit" of sorts. I wanted to find if there is such a genomic signature for orchid mycorrhizal fungi.

#### *Phylogenetics of orchid fungi*

To form the base of any comparative work, I needed to identify the evolutionary relationships between these strains. For this study, I extracted DNA from all the isolates that were viable from Dr. Zettler's collection at the time of sampling, about 35 strains in all. Once the sequencing comes back from JGI, I will use these data to generate a multi-locus nuclear phylogeny using a pipeline I've modified from Jason Stajich ([https://github.com/stajichlab/PHYling\\_unified](https://github.com/stajichlab/PHYling_unified)).

#### *Comparative genomics of orchid fungi*

To identify any genomic signatures, I identified 15 isolates from Larry's collection, optimized high molecular weight DNA extraction protocols to generate enough DNA for reference genome sequencing, and sent in RNA samples to aid in the annotation process. These samples take a lot of time to process but once the genomes are assembled, annotated, and published on their repository, I will use methods like Kohler et al.'s (2015) study to explore the evolution of gene families identified in breakdown of plant matter such as CAZymes to see if gene expansion plays a role in why these fungi partner with orchids.

#### **Future research program**

The research program I envision running at Illinois College consists of three main areas:

1. Collaborate with Orchid Recovery Program
2. Measure fungal metabolic capabilities
3. Analyze fungal genomes

#### *Collaborate with the Orchid Recovery Program*

I want to continue to support the excellent work Dr. Zettler, Dr. Corey and their undergraduates already do in this area. My experience mentoring Luigi Erba and my interactions with orchid lab alumni have showed me how valuable their contributions are and how well this work prepares them to meet their goals.

#### *Measure fungal metabolic capabilities*

Fundamental questions remain unanswered: why do some fungi form symbioses with certain orchids? One determinant could be what the fungi are able to metabolize. More phenotypic data will increase our ability to model their metabolism and will be a good check against the sequencing data and lead to a better understanding of these orchid fungal relationships *in situ*. My approach to answer this question would be to grow fungal strains on minimal media with varying nutrients representing compounds containing nitrogen, phosphorus, and carbon. Students would measure growth over time or measure other traits of interest. The equipment needed for these experiments is minimal but would allow students to own an independent research project that builds our understanding of the biology of these fungi. I could then partner with other researchers at Illinois College and regional universities to study the chemistry or ecology of these fungi. These projects would suit students interested not only in biology or biochemistry, but disciplines like computer science or statistics.

*Analyze fungal genomes*

Beyond the analyses for my dissertation, I see many active areas of research possible with the fungal genomes I've generated or with any of the other publicly available data. One genome produced from my work has already been used in a broad study of methylation across the fungal kingdom (Bewick et al., 2019). Below are three possible projects though there are many avenues of research using these data:

1. **Search for evidence of bacterial endosymbionts.** In discussion with Dr. Melissa McCormick at the Smithsonian Environmental Research Center, bacteria may grow with or within the fungi that interact with orchids. Students could search the sequencing data to see if there is any evidence of bacterial genes and could then investigate if these bacteria are growing in co-culture. Identifying the presence of these genes would be straightforward but isolating bacteria would require persistence and more specialized equipment and expertise. I could see students working jointly with Dr. McCormick at SERC or doing an internship at the Smithsonian to further these projects.
2. **Examine gene metabolic clusters.** Fungal genomes often have clusters of genes. That is, the genes that are in the same pathway are close together on the chromosome. Students interested in metabolic pathways could design a project between me and my colleague Dr. Jennifer Wisecaver (Purdue University) who is an expert in this area (Wisecaver et al., 2014).
3. **Transposable elements.** Transposable elements (TEs) are found in all organisms and are a large percentage of many eukaryote genomes. However, many fungi have developed sophisticated machinery to suppress the spread of TEs, leading to a large amount of variation across the fungal kingdom. Students interested in studying the presence or variability of TEs in fungi could use approaches like Castanera et al (2016).

Many of the analyses mentioned in the above projects could be run on a laptop computer. For those projects that require more processing power, there are publicly available cloud services like CyVerse and XSEDE or we could collaborate with institutions that host a supercomputing cluster such as Purdue.

The orchid/fungal symbiosis is a rich area of study that has implications for orchid conservation and for other plant/fungal interactions. Every area of research would benefit from undergraduate involvement and the students would enhance their educational experience through first-hand knowledge of general molecular techniques—DNA extraction, PCR, as well as general bioinformatics skills like working on the command line and producing figures in R. I look forward to having students in my lab, including them as co-authors on publications, and bringing them to national conferences such as the Mycological Society of America or Botanical Society of America meetings.

**Citations**

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