Research and Professional Works

Study System

The majority of my research has focused on the lycophyte genus *Isoëtes*, a group of cryptic, free-sporing, heterosporous plants. *Isoëtes* have a cosmopolitan distribution, and are found in mesic to aquatic habitats on every non-polar continent, as well as several oceanic islands. Despite being poorly known by non-botanists, this genus presents a fascinating avenue for research on many fronts. For example, they present an excellent system for molecular phylogenetic studies and integrative systematics. *Isoëtes* have very few obvious morphological traits by which they can be differentiated, and have a propensity to form inter-species hybrids and polyploids. This means that their taxonomy can be challenging even for people familiar with the plants. And their cryptic nature can lead to an under-description of their true species richness, which is something that can be addressed with molecular phylogenetics. Another avenue of potential research involves many of the features they have convergently evolved with seed plants, which opens the potential for evolutionary and functional studies at the morphological, anatomical, molecular and genetic level. And finally, because they are often found in patchy distributions on the landscapes in which they occur, they offer the potential for studying the genetics of naturally fragmented populations, including how such populations are affected by habitat loss.

Deep Springs College provides an exceptional opportunity to continue my *Isoëtes* research and to ultimately build an integrated picture of the systematic richness of the genus on the West Coast of North America. I am specifically interested in the West Coast taxa both because they are greatly understudied even within the context of *Isoëtes* and because my own research demonstrates that there is considerably more systematic work to be done in the region.

Personal Research

My Master's thesis at Claremont Graduate University focused on a morphological study of one of *Isoëtes*' more unusual features: the glossopodium, a small structure of unknown function that is found embedded in the upper surface of their leaves. In order to examine these structures, I researched several different approaches, including extracting them with acid, or using an x-ray computerized tomography to generate a 3D model of the structure. Ultimately, I went with a histological / differential staining approach, followed by creating 3D reconstructions from the scanned histological sections. These 3D constructs were compared with both elliptical Fourier analysis (EFA) and linear feature analysis of variance (ANOVA). While there were morphological differences between taxa, my study also showed that the leaf cells around the glossopodium appeared to be highly lignified, something hitherto not noted in the literature.

At UC Berkeley, my research focused more on trait evolution and on integrative systematics. My first project was an investigation into morphological simplification (reduction) in *Isoëtes* over evolutionary time. Synthesizing a literature review, historical descriptions of the different *Isoëtes* species, phylogenetic inference, and ancestral state reconstruction, I was able to demonstrate that there is indeed a trend towards morphological reduction in *Isoëtes*. This study also gave West Coast botanists a means by which to separate the two major lineages that occur in the region: one clade has corms with two lobes whereas the other clade has three lobes.

My major project dissertation project at UCB was a molecular / population genetic / morphological study of an endemic lineage of *Isoëtes*, which I call the Pacific Laurasian Clade in order to differentiate it from the more widespread "American Clade", which occurs across North and South America. This study involved collections from across California and Oregon, including locations in proximity to two of the three type localities for the taxa involved. These collections were used to generate a genome-wide low-copy nuclear dataset, along with a large dataset of morphological and ecological features organized by collection location. These datasets were analyzed using multi-locus phylogenetic inference and population genetics to infer if the current taxonomy does in fact reflect the evolutionary history and richness of the

West Coast plants. From the results of my research (Freund et al., 2025), there is strong evidence that the number of taxa in the West Coast endemic clade is at least eight, rather than the three currently recognized.

California Conservation Genomics Project

In addition to my core projects, I was a major contributor to the California Conservation Genomics Project on *Azolla*, a type of aquatic fern. Much of my work on the project was non-analytical, instead focusing on field work, logistics, and data generation. As with my work on *Isoëtes*, the *Azolla* project saw me make collections of the fern from across California. To find suitable populations to sample, I made heavy use of both herbarium and online resources, such as the iNaturalist platform, to identify areas where I could make collections. This broad-scale search required me to reach out to and work with multiple land holders (private, local, state and federal) and utilities to make my collections. Because *Azolla* is quite physically fragile, and prone to fragmenting when transported, I also developed a standardized protocol to safely keep the plants intact and alive on my collecting trips, which could last well over a week at a time. Once back in the lab, I was responsible for generating morphological data on the samples, which involved extensive microscope work, morphological data generation and literature review. The information gathered was primarily used to identify the species of *Azolla* that had been collected, since like *Isoëtes* they have few obvious morphological features. And most of the features they do have require the use of a microscope to positively identify. I was also responsible for overseeing the cleaning and preparation of the samples for deposition in the UC Herbarium, deep freeze archiving and DNA/RNA extraction. Thus far, there are at least three published papers where I am a contributing author that have resulted from this project.

Even beyond my research contribution to the CCGP, the project itself represents a enormous and ambitious undertakings by the academic community for not just scholarly research, but for conservation efforts in the state. The project's stated goal is to provide "the most comprehensive multispecies genomic dataset" for the purpose of protecting and managing California's biodiversity in the face of climate change. The project spans thousands of taxa and has produced not only full genomes of these organisms, but interactive maps of local genetic variants, genetically diverse hotspots, and migrational corridors between populations.

Future Research

My passion remains focused on West Coast *Isoëtes*, which provides extensive research potential. First, there is still much work to be done on the systematics of the *Isoëtes* in the region. For example, the new taxa identified by my existing work need circumscriptions and formal publication. Furthermore, more sampling is needed in areas that I was unable to collect from due to time and budget constraints, including Washington state, Vancouver Island in British Columbia, and Baja California. There is also the matter of the American Clade species of *Isoëtes* found on the West Coast. My preliminary data suggests that, as in the Pacific Laurasian Clade, there is greater taxonomic diversity within the American Clade than is currently recognized. As many of the areas where *Isoëtes* occur are threatened by both climate change and habitat destruction, we desperately need good taxonomy if we are to preserve these plants and the ecosystems to which they belong.

In addition to the systematic work on the genus, I would be excited to investigate features that have convergently evolved in *Isoëtes* and seed plants. One such feature is crassulacean acid metabolism (CAM), a type of photosynthetic pathway that separates carbon fixation from the Calvin cycle. Unlike most CAM plants, which have evolved the pathway to preserve water in xeric environments, *Isoëtes* that posses CAM are aquatic, and appear to use the pathway to sequester carbon when other aquatic photosynthesizers are not active. While the potential candidate genes for this metabolic pathway have been identified, the question becomes has CAM arisen multiple times convergently within the genus, or might it be plesiomorphic

and later lost among certain lineages? Furthermore, it has been shown that CAM is not evenly expressed in *Isoëtes*. In fully aquatic plants, the pathway is always active, in most terrestrial species it is never active, and in amphibious species, it only becomes active once the plant is submerged. This variance in CAM expression raises the question of how this activation is controlled, what genes regulate it, if terrestrial species have lost the ability to turn it on, and if aquatic species have lost the ability to turn it off. While my own interest in CAM evolution in *Isoëtes* is strictly for the love of the genus, there are broader uses for this proposed method in evolutionary biology. By integrating ecology, phenotypes, genetics and phylogenetics in a broader evolutionary framework, we as evolutionary biologists can start digging even deeper into the when, how and even the potential why certain evolutionary patterns exist within a group. This comparative method can then be applied to other traits in other groups, which would deepen our understanding of the proceses that drive the evolution of life.

Conclusion

While the subject of my research is rather narrow, the number of facets of it I am interested in exploring and the questions I can ask about is quite broad. There is so much yet to be explored within *Isoëtes* from taxonomy, population genetics, to trait evolution. Further, the fact that these plants are so poorly understood gives me an opportunity to juxtapose my findings with what is known about the evolution of groups like the angiosperms, granting us further insight into the shared and divergent aspects of trait evolution within these two branches of the land plants. I want to bring a greater understanding of *Isoëtes* to the botanical community, as well as share my passion for these odd little plants with those around me. I adore *Isoëtes*, and botany more broadly, and hope to share this passion with my students, colleges and collaborators by continuing to delve into their evolutionary history.

References

Freund, F. D., Gates, D., Johnson, M. G., and Rothfels, C. J. (2025). Phylogenetics and population structure of the western north american endemic pacific laurasian clade of *Isoëtes*. *American Journal of Botany*, 112(4):e70030.