**Introduction**

*Platanthera leucophaea* (Eastern Prairie fringed orchid or EPFO, Figure 1) is currently listed as a U.S. federally threatened species (U.S. Fish and Wildlife Service, 1989). Conservation efforts for recovery have included habitat restoration, hand pollination, population augmentation, and reintroduction (Lah, 2003). Despite these efforts, seedling establishment leading to self-sustaining populations in situ have yet to be verified (Bowles et al. 2005; Zettler and Piskin, 2011).

The levels of genetic diversity of the fungal associates in EPFO populations are unknown. The 2016 EPFO Five Year Review (USFWS, 2016) stated that understanding fungal diversity across prairie sites is important for knowing what strains can be released between sites. Proper identification of the relevant fungi will also prove useful for lab experiments on seed germination. The purpose of this research was to examine fungal diversity across populations of EPFO in Illinois and Wisconsin using molecular markers (ITS and CETH primers, Figure 2) for the fungal ITS sequences, these two groups are quite diverse (Nei’s PI ~ 8.22).

**Methods**

Fungi has been isolated from root samples collected between 2018 and 2019 from four EPFO sites in Illinois (the Nachusa Grasslands) and seven sites in Wisconsin and have been maintained in our lab at SIUE (Figure 3). Fungal DNA isolations were done with the DNA Power PRO soil kit from Qiagen after cultures were grown in potato dextrose broth. PCR ITS reactions were done with the Extract-N-Amp Plant PCR Kit from Sigma Aldrich and products were sequenced by the U of I Core sequencing Facility, Champaign Urbana. Sequence alignments were filtered to exclude regions that typically only included sequence gaps. Nucleotide diversity statistics, including Nei’s pi, were calculated using the PopGenome package in R. The seqinr package was used to calculate the distance matrix between isolates, which was used to generate the heatmap and dendrogram (Figure 4). The adegenet and adegenet packages were used to visualize the multivariate positions with the most genetic variation (Figure 5) and to conduct a principal component analysis (PCA; Figure 6).

**Results**

<table>
<thead>
<tr>
<th>Nucleotide Diversity</th>
<th>Nei’s PI value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>2.75</td>
</tr>
<tr>
<td>Within Illinois/Iowa populations</td>
<td>1.44</td>
</tr>
<tr>
<td>Within Wisconsin populations</td>
<td>14.57</td>
</tr>
<tr>
<td>Between group diversity</td>
<td>8.22</td>
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</tbody>
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Table 1. Overall nucleotide diversity for this ITS region is low. Fungal sequence diversity within combined Illinois/Iowa sequences (Nei’s PI 1.444) was much lower than within Wisconsin populations (Nei’s PI 14.57). In comparisons among Illinois/Iowa versus Wisconsin fungal ITS sequences, these two groups are quite diverse (Nei’s PI ~ 8.22).

**Discussion**

Previous research has determined that the mycorrhizal fungus *Ceratobasidium* may be favored over other orchid fungi, such as *Tulasnella* because it has genes that can utilize both nitrate as well as ammonium, while *Tulasnella* only utilizes ammonium (Fochi 2017). The ability of *Ceratobasidium* to utilize more forms of nitrates could facilitate orchid survival in more environments.

Why are these fungi so genetically similar over long range geographic areas and sometimes very diverse, even within the same collection location? The Illinois/Iowa fungal populations all exhibit high genetic similarity, and the similarity may be explained by high nutrient levels at these sites and the differences in the ability of the fungi to tolerate high nitrate environments (Beyrle et al. 1995). Orchid seed germination is inhibited by high nitrate levels, which may be an adaptation to occupy nutrient poor areas and reduce competition with other species (Figura et al. 2021). Figure 6 (2021) hypothesize that the genetic similarity in orchid mycorrhizal strains is due to the select genotypes that can survive in higher nitrate environments and are able to form the symbiotic relationships necessary for seed germination. Nutrient poor sites would select for more fungal diversity in order to utilize as many nutrient sources as possible. The mechanisms behind the selective survival remain unknown. The higher fungal diversity seen in the Wisconsin populations and the Illinois Helm road site may reflect more nutrient poor sites where more root mycobionts are needed for orchids to access more nutrients. In terms of conservation an understanding of nutrient levels (nitrates, potassium, etc.) at sites may help in identifying fungi that are capable of seed germination at higher nutrient levels and could be mixed with seed sources to more successfully augment population numbers.

**Future Work**

We would like to examine fungal diversity across a larger range of the species, including more fungal isolates from Wisconsin and from Michigan and Ohio. Fungal diversity has not yet been extensively surveyed in Michigan and Ohio and fungal collected now will provide a record of what was beneath the soil in light of climate change.

**References**


