Can mycobiont selection change the demography of a rare orchid by affecting phenological progression?

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Introduction

The angiosperm family Orchidaceae has the largest number of rare taxa with limited distributions due to their specialized ecological requirements including mycorrhizal symbiosis. Understanding their ecological niche requirements that could drive plant distributions and population persistence, especially under the changing climatic conditions, is fundamental for both plant ecology and conservation. Given the vital role of mycorrhizas in an orchid life cycle (1), spatial and temporal mycorrhizal dynamics exhibited by orchid taxa can help explain their demography and population dynamics.

Questions and Hypotheses

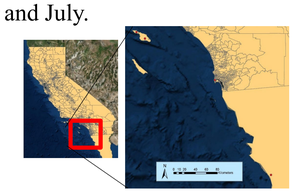
1) Do the mycorrhizal partners change through the phenological development of the species?
2) Is there evidence for the relationship between dependence on a specific fungal partner and population demography of the plant species?

Hypotheses: A rare orchid species is a specialist in its mycorrhizal fungal interactions. Variation in mycorrhizal fungal associations through phenological stages are linked to the demography of a population.

Materials and Methods

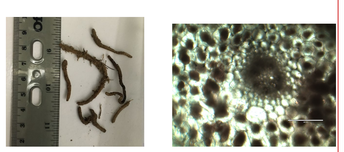
Study species: We used a perennial orchid species *Platanthera (Piperia) cooperi* (2) which occurs in the coastal scrub habitat within the California Floristic Province as our model species; it grows actively between November and July.

Sampling sites – two in Point Loma (PLF, PLE), San Diego County and two on Santa Catalina Island (SCE, SCW) , Los Angles County, CA

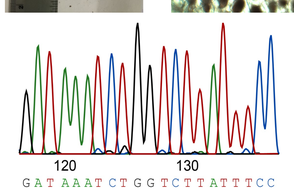


Roots sampled from seedlings (S) (a), vegetative (V) (b) and reproductive (F) (c) plants

Peloton inspection, DNA extraction, and fungal DNA barcoding of the nuclear ribosomal ITS (nrITS) locus



Sanger sequencing, bioinformatics analysis with MEGA and biostatistical analysis in R



Results

- We generated 190 sequences from roots of 83 individuals; 155 sequences belonged to the Tulasnellaceae, and 35 were to the Ceratobasidiaceae. Altogether, twenty-one operational taxonomic units (OTUs) were recovered, of which eight belonged to Tulasnellaceae (Fig. 1) and 13 belonged to Ceratobasidiaceae (Fig. 2).
- Kruskal Wallis test revealed similar OTU alpha diversity across sites, phenological stages, and years ($p>0.05$).

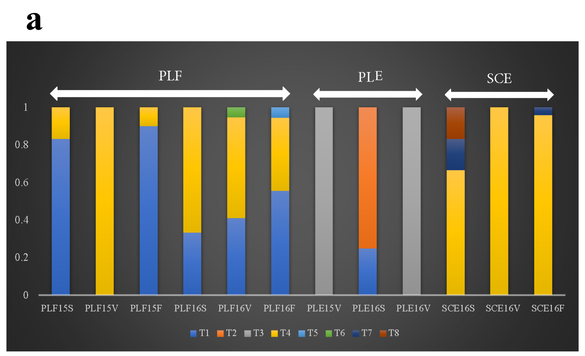


Figure 1. Tulasnellaceae Operational Taxonomic Units (OTUs). a) The relative abundance of Tulasnellaceae OTUs identified from roots of seedling (S), vegetative (V), and flowering (F) plants. Roots were collected in February from seedling and vegetative plants, and in April from flowering plants in both 2015 (15) and 2016 (16). b) Phylogenetic placement of Tulasnellaceae OTUs associated with roots of *Platanthera cooperi* in a phylogram generated by using MrBayes with 1mil generations and mid-point rooting. The clade credibility scores are provided for each clade.

- Tulasnellaceae OTUs were observed at PLF, PLE and SCE sites; T1 and T4 were associated with flowering plants and were abundant at PLF and SCE sites (Fig. 1a). PLE site displayed unique OMF community in the roots of the orchid.
- Tulasnellaceae did not show complete spatial segregation across sites (Fig. 1b).

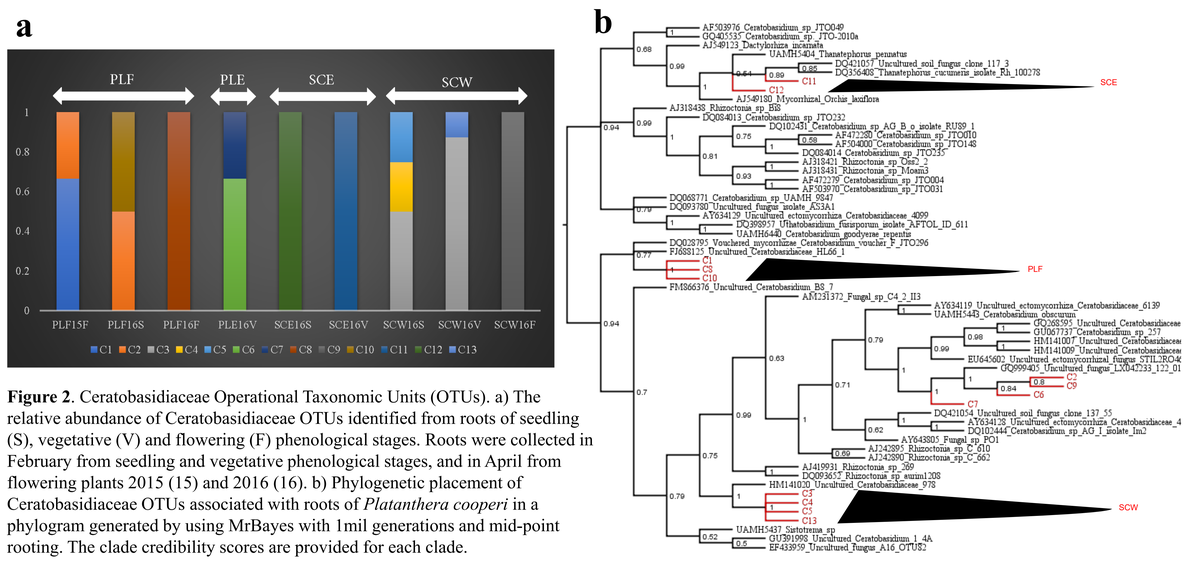


Figure 2. Ceratobasidiaceae Operational Taxonomic Units (OTUs). a) The relative abundance of Ceratobasidiaceae OTUs identified from roots of seedling (S), vegetative (V) and flowering (F) phenological stages. Roots were collected in February from seedling and vegetative phenological stages, and in April from flowering plants 2015 (15) and 2016 (16). b) Phylogenetic placement of Ceratobasidiaceae OTUs associated with roots of *Platanthera cooperi* in a phylogram generated by using MrBayes with 1mil generations and mid-point rooting. The clade credibility scores are provided for each clade.

- Ceratobasidiaceae OTUs were observed at PLF, PLE, SCE and SCW sites; each site displayed unique OMF communities within orchid roots (Fig. 2a).
- Ceratobasidiaceae OTUs exhibited higher spatial segregation in comparison to Tulasnellaceae (Fig. 2b).

- PERMANOVA based on unweighted Bray-Curtis distances revealed differences in OTU richness across sites, phenological stages, and their interaction ($p<0.05$), though OTU richness across years was similar (Fig. 3).

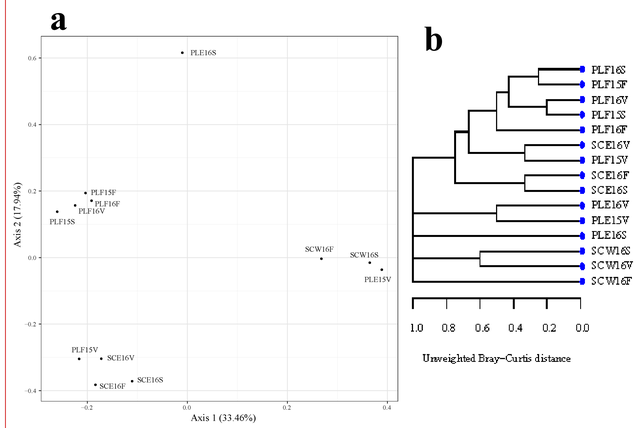


Figure 3. Clustering of *Platanthera cooperi* sampling sites based on OTU richness. a) Principal Co-ordinate analyses, and b) hierarchical clustering based on unweighted Bray-Curtis dissimilarity index clustered PLF and SCE sites together, and these two sites segregated from both PLE and SCW.

Conclusions

- Our data suggest that *P. cooperi* is specific toward few narrow clades of mycorrhizal fungi, and that its populations with different demographies display distinct mycorrhizal communities.
- Cluster analyses (Fig. 3b) explain population demography because among the four sampled populations only PLF and SCE hosted flowering plants, and OTUs found in flowering plants at these sites were not recovered from seedling or vegetative plants sampled at PLE and SCW.

Acknowledgements

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Literature Cited

1. Rasmussen, H. N. (1995). Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press.
2. CNPS, Rare Plant Program. 2015. Inventory of Rare and Endangered Plants (online edition, v8-02). California Native Plant Society, Sacramento, CA.