

Host specificity of hemiparasitic *Castilleja* and its influence on plant community diversity.

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Abstract

Hemiparasitic plants rely on their hosts for some of their nutritional requirements, but are still able to photosynthesize and absorb water and nutrients through their roots. Parasitic plants have been shown to impact plant community diversity by reducing dominant species' competitive ability, thus allowing for the growth and establishment of secondary, less competitive species. *Castilleja* is a hemiparasitic genus in the family Orobanchaceae. It forms root haustorial connections with hosts underground. Near The Rocky Mountain Biological Laboratory, there are six common, naturally occurring species of *Castilleja*. The diversity and specificity of host species of local *Castilleja* are unknown due to the difficult nature of observing belowground interactions. In this study, we aim to determine the most likely hosts of *Castilleja*, assess host specificity, and measure *Castilleja*'s impact on plant diversity and productivity in various Rocky Mountain ecosystems. We found local *Castilleja* species to be generalists which likely parasitize the most abundant species in the community and that *Castilleja* presence is positively correlated with plant community diversity and richness, but not evenness.

Introduction

Parasitic plants are a broad, diverse, and widespread group of angiosperms (Westwood, 2010). They are globally ubiquitous and can be found in abundance across all terrestrial biomes and ecosystems (Westwood, 2010; Tesitel et al., 2015). They display a broad range of life history strategies, life forms, and morphologies (Westwood, 2010). Because of their parasitic adaptations, they are unique in their ability to survive and thrive while simultaneously lacking or underutilizing many of the necessary physiological functions possessed by the rest of the autotrophic plant kingdom, such as photosynthetic ability (Press & Phoenix, 2005). Root parasites reduce the biomass, health, and fitness of their hosts by directly uptaking water and nutrients from the vascular tissue of host plants (Ameloot et al., 2005; Press & Phoenix, 2005). They access the hosts' xylem and phloem using haustoria, specialized attachment organs on their roots that form haustorial connections with host plants underground (Kuijt, 1969; Tesitel, 2016).

Holoparasites are parasitic plants that are completely reliant on their hosts for all carbon, nutrient, and water requirements (Press, 1998). Hemiparasites, however, are partly parasitic plants that retain absorptive roots and are capable of carbon fixation through photosynthesis

(Tesitel, 2016), but still need to draw some of their resources from their hosts because they have low nutrient-use efficiencies (Ameloot et al., 2005). Hemiparasites are an understudied group of organisms that could potentially exert strong influence upon their communities. While many are generalists with broad host ranges, they are known to behave as specialists and show higher preference for nitrogen-rich hosts, such as grasses and legumes (Gibson and Watkinson, 1989; Press, 1998; Press and Phoenix, 2005). Depending on a species' host specificity, they may alter community structure and competitive relations among surrounding plants by preferentially targeting dominant or invasive species, which may reduce competitive pressure among hosts and non-host plants and indirectly allowing for the establishment of secondary species (Gibson and Watkinson 1991; Press and Phoenix, 2004; Hartley et al., 2015; McKibben and Henning, 2018; Hodzic et al., 2022). The subsequent alteration of plant richness and evenness may have significant indirect effects on the overall health and biodiversity of ecosystems.

There is a growing body of evidence to support the argument that hemiparasitic plants enhance ecosystem productivity in a number of ways. As found by Hartley et al., (2015), the hemiparasite, *Rhinanthus minor* (Orobanchaceae), was strongly associated with increased abundance of higher trophic organisms and displayed some amount of bottom-up control in grassland communities. Many consider parasitic plants to be keystone species due to their ability to alter community structures and diversity (Hartley et al., 2015; Tesitel et al., 2021).

For this study, we focus on the root hemiparasitic genus *Castilleja*, also known as Indian Paintbrush due to its vibrantly colored bracts. *Castilleja* contains over 200 species of herbaceous annuals and perennials (Tank et al., 2009), all being polyploid (Olmstead and Tank, 2008). It belongs to the family Orobanchaceae, which is made up almost entirely of holoparasitic and hemiparasitic plants and contains over 2,000 species. In Gunnison County, there are six common species (Consortium of Southern Rocky Mountain Herbaria, 2023). In this study, we observed various species of *Castilleja in situ* and investigated their impact on community diversity. We investigate *Castilleja's* host specificity and determine the most-probable hosts of *Castilleja* around The Rocky Mountain Biological Laboratory, Colorado. **The primary goals of this study are to gain an understanding of what species are being parasitized by *Castilleja* and to see what impact, if any, the presence of *Castilleja* has on richness and evenness.** To accomplish this task we utilize diversity analysis and plant species cover data. After potential hosts have been identified, it is our plan to confirm host species through haustoria presence.

We hypothesized that the *Castilleja spp.* surrounding Rocky Mountain Biological Laboratory would have generalist tendencies, but gravitate towards a small number of ideal hosts that we would record in high frequencies around *Castilleja*. Our null hypothesis is that *Castilleja* would show no host preference and simply parasitize the most abundant and easily accessible species in the community. Parasitic plants typically decrease the health, fitness, and biomass of dominant species within a given community, which enable the establishment of secondary species (Paine, 1969; KcKibben and Henning, 2018). Hence, we expected that plots containing *Castilleja* would have greater richness and evenness than plots without.

Methods

Study sites

We collected data for three species of *Castilleja* which flower perennially during different periods of the summer months at various elevations and habitats near the Rocky Mountain Biological Laboratory (RMBL), which is located at 2,895 m elevation in the West Elk Mountains of Colorado. *Castilleja chromosa* occurs about 18 miles south of RMBL down valley near Almont and flowers throughout the month of June. The site is a sagebrush community with direct sun, dry, rocky sediment, and desert plants (elevation 2,673 m). *Castilleja linariifolia* occurs from down valley up to the elevation of the lab from late June to July. Our two *C. linariifolia* sites were up on the slope of a hill near the Deer Creek trail (elevation 3,016 m), just southeast of RMBL. It also received direct sun, with greener vegetation than Almont. *Castilleja septentrionalis* is common and can be found at various elevations in the area. We studied two populations— one near Avery Picnic (elevation 2,939 m) and one near Emerald Lake (elevation 3,191 m), both north of RMBL.

Host determination and host specificity sampling

At the *C. chromosa* and *C. septentrionalis* study sites, we located a *Castilleja* population and laid out a fifty meter transect stretching from a point at least five meters from the trail or road to the other side of the population. Next, we used a random number generator to travel short, random distances along the transect and select the nearest *Castilleja* plant from each random point to sample. Each *Castilleja* plot must have been located within a two meter range of our random point. We then centered a quarter-meter² quadrat around the focal *Castilleja* and recorded the species growing in close proximity, within 10 cm (Gibson and Watkinson, 1989) to the *Castilleja*. We repeated this process twenty times per study site in order to have twenty randomized, independent data points. At our *C. linariifolia* sites, the *Castilleja* in the populations were much more sparse. We found it more effective to sample every *Castilleja* that we encountered along the transect. When faced with denser clumps of *Castilleja*, we used a random number generator to select a subset of the clump to sample.

Next year, we will excavate a small subset of ideal *Castilleja* specimens and their potential hosts to cross examine and look for haustorial connections. We will remove the plants using a small trowel and store in a plastic bag in the refrigerator until they are ready for analysis. We will soak the roots in water for 24 hours before carefully washing away the soil and observing haustoria under a dissecting microscope.

Community diversity sampling

Subsequent to host sampling, we used paired plots to draw comparisons between plots containing *Castilleja* and plots that do not. At each random point, after identifying possible hosts, we recorded counts for every species present within the quadrat, visually estimated percent cover for bare ground and all of the species, including *Castilleja*, and took an overhead photo of the plot. If we were unable to identify a plant in the field, we took a voucher specimen for later identification. After we finished with each *Castilleja* plot, we placed an adjacent paired control plot that contained no *Castilleja*, no less than 25 cm away from the nearest *Castilleja*, and no greater than two meters away from the transect line. Similarly to our *Castilleja* plots, we recorded each species occurring within the quadrat and percent cover. We repeated this process for 20 paired plots, giving us 40 randomized independent data points per research site and 80 per *Castilleja* species. Due to time constraints, we excluded *C. chromosa* from this portion of the study,

Statistical analysis

We gauged potential hosts by examining the relative frequency of each species growing near *Castilleja* using frequency distributions generated in Excel (Microsoft Corporation, 2018). We determined which species occur most frequently in close proximity to *Castilleja* and created count-based frequency distribution graphs to compare species incidence. We considered something more likely to be a host if it was frequently found within 10 cm of *Castilleja*. If it was found more frequently in association with *Castilleja* than would be expected by chance alone, then we consider it even more likely to be a host species. To reject the null hypothesis, there must be evidence that possible hosts are not just more abundant overall, and that *Castilleja* was actually showing a tendency towards co-occurring with those species.

To do this, we used data from our plot pairs to compare the frequency distribution of all plants in the community versus plants associated with *Castilleja*, which we refer to as “nearest neighbors” (NN). We looked at the frequency that each species co-occur as NNs to *Castilleja* versus the frequency that they occurred in the rest of the population. If they have relatively the same distribution, there is no host specificity. If they occurred much more frequently as a NN than they occur overall, this suggests host specificity. We then took the incidence of each species in each quadrat and made a linear regression of NN frequency vs overall frequency.

All diversity analyses were conducted in Rstudio (v4.1.2; R Core Team, 2023) using the Vegan package (v2.6-4; Oksanen, et al., 2022). We used a generalized linear model and an analysis of variance (ANOVA) to compare the mean species richness, evenness, and overall diversity in our *Castilleja* versus non-*Castilleja* plots. We used a linear regression analysis with pair, site, and treatment as independent variables. We quantify diversity using the Shannon Diversity Index. For evenness and diversity, we utilized vegetative cover data instead of count data because we found it to be more consistent and reliable. We used count data for richness because it was a

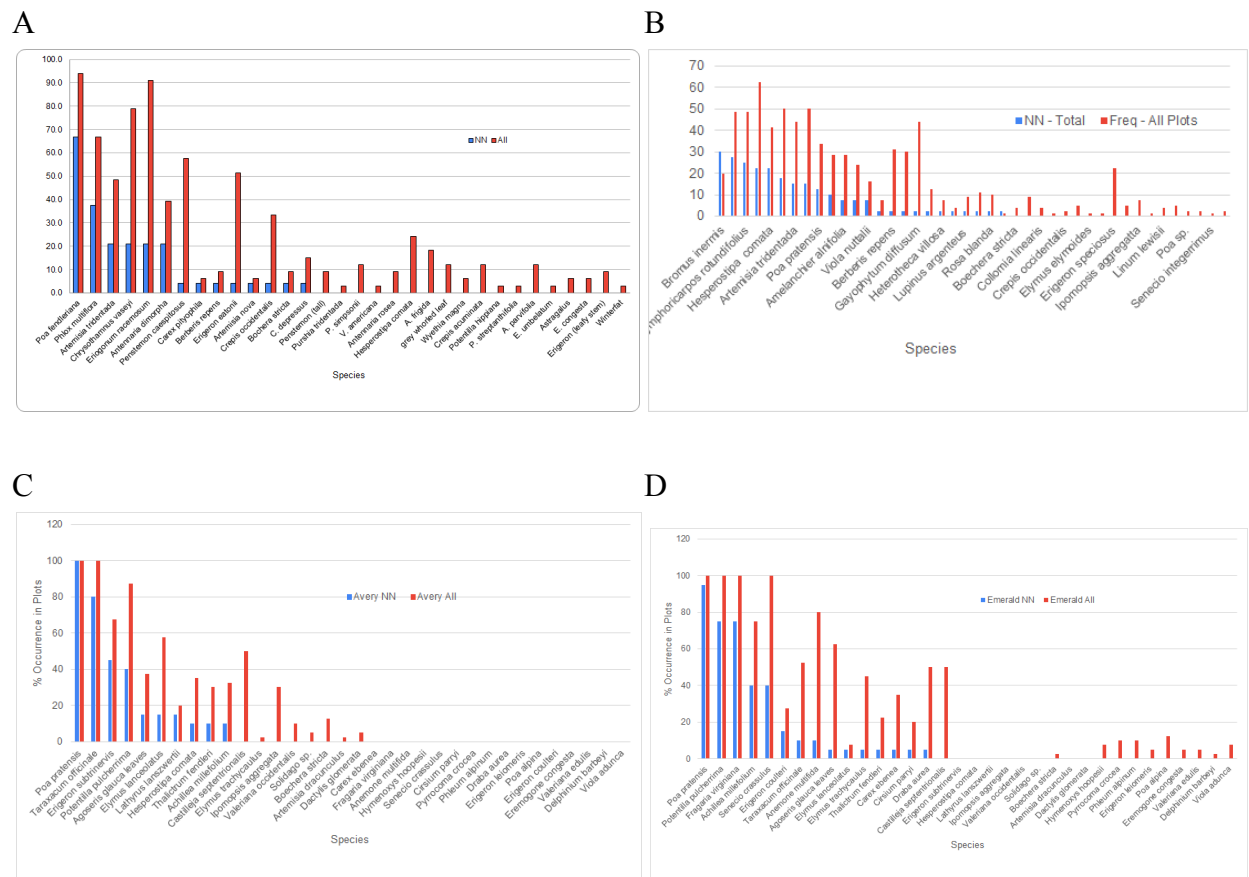
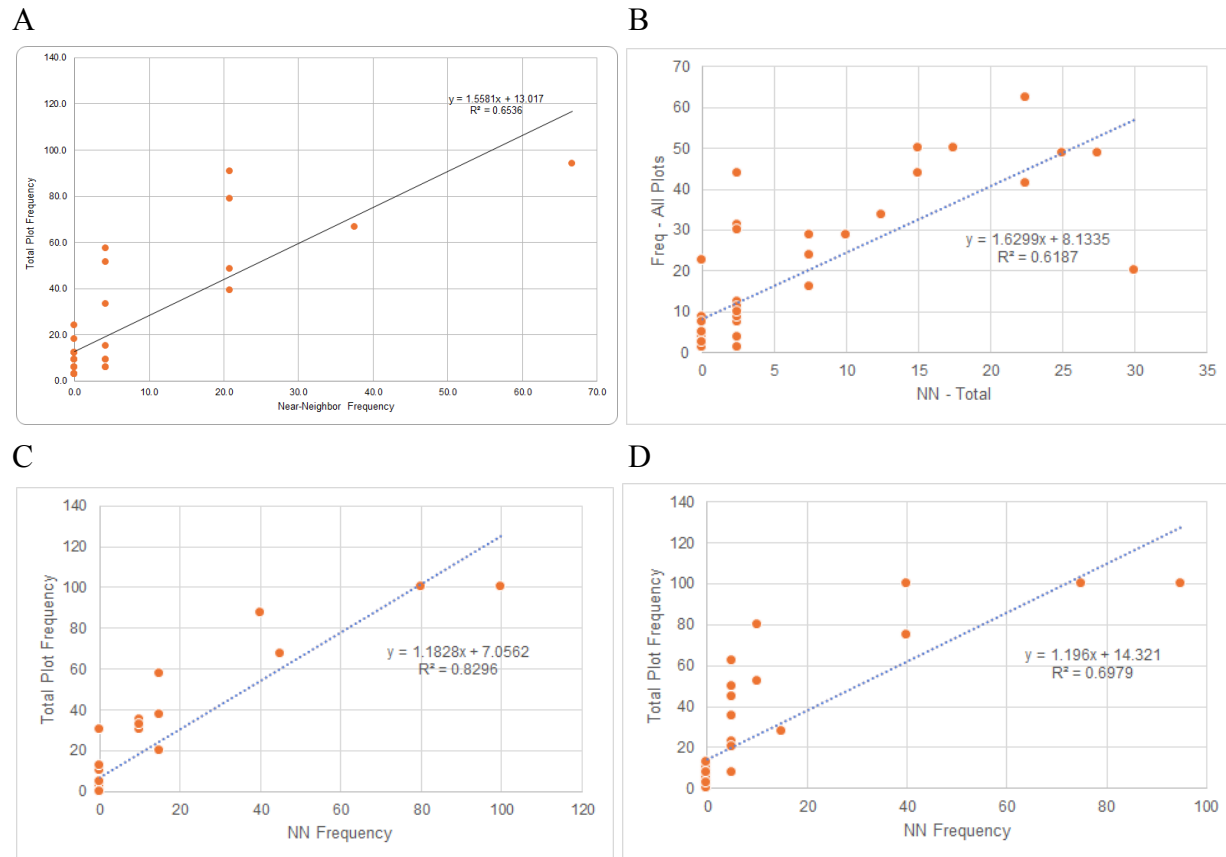


Fig. 2. Regression of total population frequency on near neighbor frequency of species occurring with *Castilleja*. (A) *C. chromosa* at Almont site. (B) *C. linariifolia* at Deer Creek sites. (C) *C. septentrionalis* at Avery Picnic site. (D) *C. septentrionalis* at Emerald Lake site. Points below and to the right of the line represent species that co-occur with *Castilleja* more frequently than expected by chance.



Confirmed hosts of *Castilleja* from haustorial analysis

This portion of the project will be completed in a follow up study. So far, we have confirmed the presence of haustoria of *C. septentrionalis* on *Poa pratensis*.

Castilleja presence and community diversity

We found that the presence of *Castilleja* is associated with higher plant richness in all of our *Castilleja* sites (Fig. 3a & 3b). There was no significant change in plant evenness in response to *Castilleja* presence (Figure 4a & 4b). Overall, *Castilleja* presence has a significant positive correlation with diversity, as quantified by the Shannon Diversity Index (Figure 5a & 5b).

Fig. 3a. Richness in *Castilleja* vs. non-*Castilleja* plots at Deer Creek. *Castilleja linariifolia* plots on average had significantly higher richness (N=80, M=7.62, SD=1.86, $p < 0.001$) than non-*Castilleja* plots (N=80, M=5.82, SD=1.87, $p < 0.0001$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).

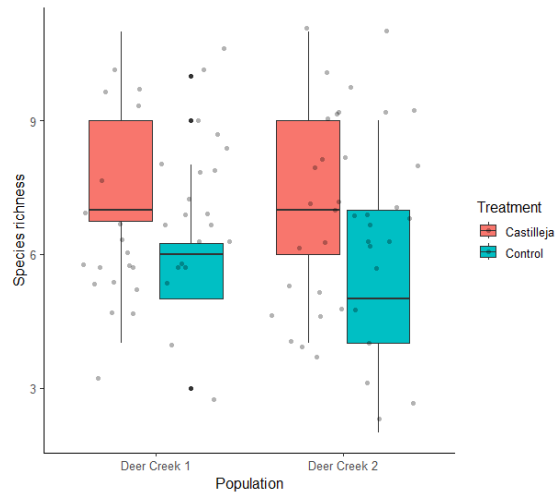


Fig. 3b. Richness in *Castilleja* vs. non-*Castilleja* plots at Avery Picnic and Emerald Lake. *Castilleja septentrionalis* plots on average had significantly higher richness (N=80, M=9.22, SD=2.44, $p < 0.001$) than non-*Castilleja* plots (N=80, M=7.53, SD=2.16, $p < 0.0001$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).

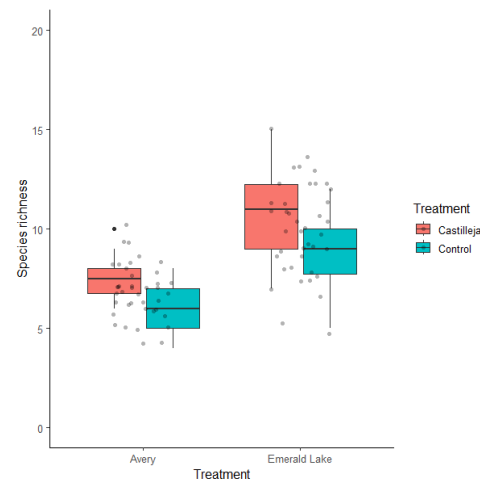


Fig. 4a. Evenness in *Castilleja* vs. non-*Castilleja* plots at Deer Creek. *Castilleja linariifolia* plots were not significantly different (N=80, M=0.76, SD=0.13, $p > 0.05$) from non-*Castilleja* plots (N=80, M=0.72, SD=0.15, $p > 0.05$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).

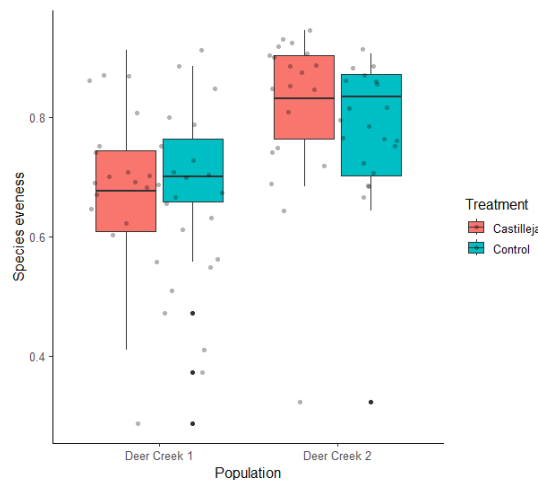


Fig. 4b. Evenness in *Castilleja* vs. non-*Castilleja* plots at Avery Picnic and Emerald Lake. *Castilleja septentrionalis* plots were not significantly different (N=80, M=0.86, SD=0.07, $p > 0.05$) from non-*Castilleja* plots (N=80, M=0.84, SD=0.06, $p > 0.05$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).

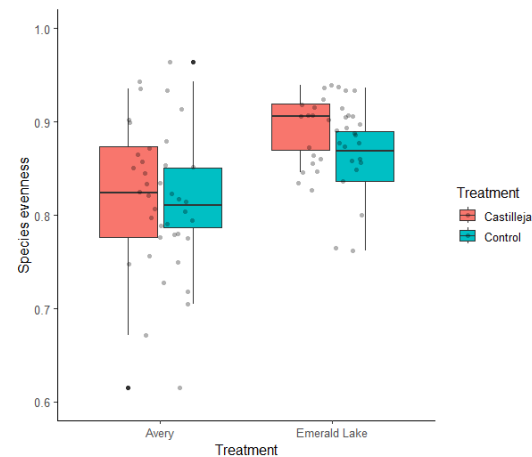


Fig. 5a. Overall diversity in *Castilleja* vs. non-*Castilleja* plots at Deer Creek. *Castilleja linariifolia* plots are significantly more diverse ($N=80$, $M=1.52$, $SD=0.36$, $p < 0.05$) than non-*Castilleja* plots ($N=80$, $M=1.26$, $SD=0.41$, $p < 0.05$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).

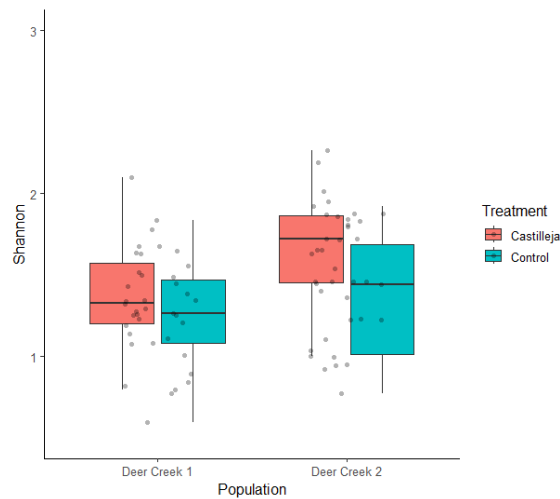
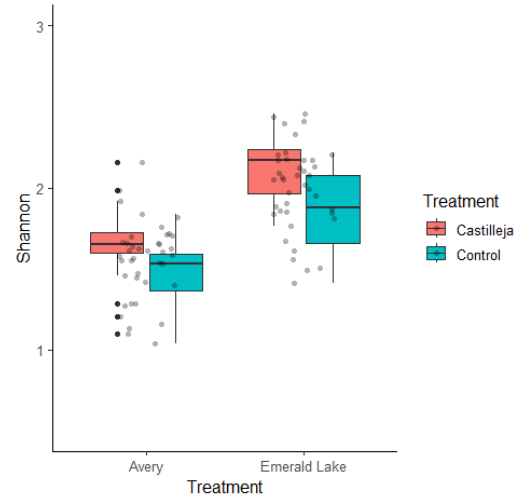


Fig. 5b. Overall diversity in *Castilleja* vs. non-*Castilleja* plots at Avery Picnic and Emerald Lake. *Castilleja septentrionalis* plots are significantly more diverse ($N=80$, $M=1.89$, $SD=0.34$) than non-*Castilleja* plots ($N=80$, $M=1.67$, $SD=0.30$, $p < 0.0001$). Red = *Castilleja* presence and blue = *Castilleja* absence (control). The median value is indicated by the midline, with the 25th and 75th percentiles signified by the upper and lower hinges. The whiskers extend from the hinges to the highest and lowest values, no further than 1.5x the interquartile range (IQR).



Discussion

Castilleja presence is associated with increased richness and overall diversity

We found that overall, the presence of *Castilleja* is associated with a significant increase in richness and overall diversity. These findings align with those of previous studies that have studied hemiparasitic plants and their impacts on community composition (Gibson and Watkinson 1991; Press and Phoenix, 2004; Hartley et al., 2015; McKibben and Henning, 2018; Hodzic et al. 2022). However, we did not see a relationship between *Castilleja* presence and evenness, which is in contrast to past literature. McKibben and Henning (2018) found that *Castilleja* presence was associated with a 5% increase in evenness. In the future, there should be further analysis of evenness and how % cover may be changing for individual species. It would be especially beneficial to analyze how the % cover of dominant species is impacted. We found multiple outliers that were either occurring with *Castilleja* much more or much less than expected. The least plausible hosts were *Gayophytum diffusum* and *Galium bifolium*, which are both annuals. Despite the strong correlation that we have found between *Castilleja* presence and diversity, this merely implies causation, not confirms it.

Our three *Castilleja* species sites follow an elevational temperature and moisture gradient. *C. chromosa* sites were the hottest, driest, and the least diverse with 38 recorded species occurring in our plots. *C. septentrionalis* sites were cooler, moister, and had the highest species richness (44 species). Despite these large differences in abiotic factors, we saw the same patterns in *Castilleja* presence and diversity. This suggests that if *Castilleja* causes increased diversity, then its magnitude of influence may be independent of abiotic factors (McKibben and Henning, 2018). Overall, we found that different *Castilleja* species and study sites produced varying results. Out of our six sites, Emerald Lake had the strongest association between *Castilleja* presence and increase in diversity, while the first Deer Creek site had the weakest.

***Castilleja* host specificity**

With the exception of a couple outliers, few species were found to occur in higher frequency with *Castilleja* than expected by chance. This suggests low host specificity and generalist tendencies. *Castilleja* appears to occur most frequently with the most abundant species in the community, which supports the idea that parasitizing dominant species may influence community composition. As we saw in our species incidence figures, the most dominant species at each site are in the family Poaceae (*Poa fendleriana*, *Bromus inermis*, *Poa pratensis*). *Castilleja* may also show preference towards them due to the apparent high fitness of grasses in their communities. Gibson & Watkinson (1989) found that *Rhinanthus minor*, a hemiparasite in the United Kingdom, is also most frequently associated with grasses. They predict that this is due to the high probability of finding a suitable host when the host species is already very successful in the community and has extensive root systems. So far, we have confirmed the presence of haustoria of *C. septentrionalis* on *Poa pratensis*. However, grasses are only a part of the diverse array of the dominant plant species in our sites. Alpine meadows, which make up the majority of our study sites, are characteristic for having a high abundance of forbs in comparison to previous studies. Among grasses, we also found a high occurrence of forbs, such as *Phlox multiflora* (Polemoniaceae) in *C. chromosa* sites, *Eriogonum umbellatum* (Polygonaceae) in *C. linariifolia* sites, and *Potentilla pulcherrima* (Rosaceae) and *Taraxacum officinale* (Asteraceae), both of which in *C. septentrionalis* sites. We observed species of other functional groups that were highly abundant as well, like *Symphoricarpos rotundifolius* (Caprifoliaceae), a shrub, and *Carex obtusata* (Cyperaceae), a sedge. Both of which are from our *C. linariifolia* sites. It is important that our predicted hosts be confirmed in the future using haustorial analysis.

Conclusion

Overall, we observed *Castilleja* presence to be associated with increased richness and diversity and not associated with evenness. From our nearest neighbor analysis, we saw that *Castilleja* is a generalist with little host specificity. However, it does show preference towards select hosts. Future haustorial analysis will be used to substantiate the existence of a host-parasite relationship between *Castilleja* and the predicted host species. This is part of a larger project that aims to

explain the relationship between *Castilleja* presence and diversity. Subsequent *Castilleja* research could be done to further investigate indirect methods of identifying hosts and observing the complex, mystifying world of belowground plant interactions.

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