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Phenotypic variation of the hemiparasite *Pedicularis densiflora*

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ABSTRACT

Phenotypic variation allows plants to survive in a wide variety of environments and has been a topic of study for many decades. However, relatively little is known about the effect of biotic and abiotic factors on the phenotypic variation of parasitic plants. In this study we examined how biotic factors related to host-parasite interactions affect the phenotype of *Pedicularis densiflora.* We surveyed four sites of serpentine chaparral habitat at McLaughlin Natural Reserve in Lower Lake, CA. We found that a higher number of neighboring woody plants was correlated with greener leaves on *P. densiflora*. Additionally, we discovered that recent burn sites fostered redder, smaller *P. densiflora* that were less likely to be flowering than those in old burn sites. We propose that these phenotypic differences are due to the increased stress from sun exposure that follows a clearing of vegetation and canopy cover. We suggest that vegetation surrounding *P. densiflora* may facilitate its growth and reproductive success by providing crucial cover, as marked by greener, larger individuals that were more likely to be flowering in these areas.

Keywords: Pedicularis densiflora, leaf coloration, plant parasitism, phenotypic variation, anthocyanins, sunlight, serpentine, chaparral, fire, parasite-host interactions

INTRODUCTION

Phenotypic variation results from the interaction between the genotype of an organism and its environment (Fordyce 2006). While phenotypic variation is ubiquitous in all organisms it is most exhibited in plants (Alpert and Simms 2002). Both biotic and abiotic factors can trigger phenotypic variation. Among biotic factors are species interactions, such as those between parasitic plants and their hosts (Fordyce 2006). Previous studies have explored the effects of host plants on the phenotypic variation of parasitic plants. However, this research has largely focused on parasitic plants of the Orobanchaceae family as agricultural pests (Joel *et al.* 2013, Li *et al.* 2012). Yet, the members of the Orobanchaceae family that exist almost exclusively in natural communities remain understudied (Joel 2013, Li *et al.* 2012, and Pennings and Callaway 2002).

Pedicularis densiflora (hereafter referred to as *Pedicularis*), a member of Orobanchaceae, is a perennial plant native to California chaparral, pine forests, and oak woodlands. It is one of several *Pedicularis* species that are hemiparasitic, which means it can photosynthesize on its own, but given the opportunity it will facultatively parasitize a host plant. There is some evidence that this species may be able to survive without a host in highly favorable soil and climate conditions, but in general this species requires a host to mature beyond the seedling stage and reach reproductive maturity (Li *et al.* 2012, 2013, Sprague 1962a). Under favorable conditions, *Pedicularis* will use root-like structures called haustoria to extract water and nutrients from organic material in the soil. However, in unfavorable conditions *Pedicularis* will use haustoria to channel those essentials from a host plant (Li *et al.* 2012, 2013, Sprague 1962a). *Pedicularis* can parasitize multiple hosts simultaneously, though it is restricted to woody trees and shrubs including *Adenostoma fasciculatum* (chamise), *Pinus coulteri* (coulter pine), *Pinus sabiniana* (gray pine), *Arbutus menziesii* (madrone), *Arctostaphylos* (manzanitas), and *Quercus* (oaks) (Joel *et al.* 2013, Sprague 1962a). This range of possible hosts may allow *Pedicularis* to persist in various habitats throughout California, making phenotypic variation in this species more likely (Dai *et al.* 2017, Li *et al.* 2013, Sprague 1962a, 1962b). Research is limited, however, one study revealed that the flowers of certain *Pedicularis* species exhibit phenotypic changes when transplanted from higher elevations to lower elevations (Dai 2017). Another study showed that the coloration of *Pedicularis* flowers of populations in different environments matched the coloration of their respective species of pollinating hummingbirds. (Monfils *et al.* 2007, Sprague 1962b). These studies show phenotypic variation between populations, but variation is also present within a population.

Pedicularis can vary in leaf color from almost entirely green to almost entirely red. There is evidence that red leaf coloration can provide plants with protection from sun exposure and herbivory (Dominy *et al.* 2002, Neil and Gould 2003). However, little is known of the effect of host-parasite interactions on this color variation in parasitic plants. In this study we examined how leaf coloration in *Pedicularis* is affected by biotic factors related to its parasitic interactions. Additionally, we asked what biotic and abiotic factors affect other variations in size, flowering status, and density of individuals of this species. All of our study sites occurred in serpentine habitat, which is nutrient deficient, high in heavy metals, and has poor water retention (O'Dell *et al.* 2006). We therefore assumed that all individuals sampled were actively parasitizing. We hypothesized that the color, size, flowering, and density of *Pedicularis* would be affected by the properties of potential hosts.

METHODS

2.1 Site Description

We conducted our research at McLaughlin Natural Reserve in Lower Lake, California from February 22 to 28, 2021. McLaughlin Reserve is 7,000 acres of oak woodlands, grasslands, and serpentine and non-serpentine chaparral. Serpentine soil covers a large portion of McLaughlin Reserve and is characterized by its lack of nutrients and large presence of heavy metals. Most plants found on serpentine soil are endemic and able to survive in its unique chemical and nutrient makeup, creating non-traditional vegetation and landscape on the reserve (O'Dell *et al.* 2006). McLaughlin Reserve was also disturbed by two recent fires, the 2015 Jerusalem Fire and the 2020 SCU Lightning Complex fires which cleared much of the established flora.

We targeted sites where *Pedicularis* was present and measured the following characteristics of individual plants: leaf color, flowering status, height, leaf length, distance to the nearest woody neighbor (NWN), the diameter and species of that neighbor, the number of woody plant neighbors, the density of other *Pedicularis* around the individual, and what year the site was burned. We selected four sites where *Pedicularis* was known to be present on the reserve (Paul Aigner, pers comm, 2021). Sites were named as follows: Grid (38.827821, -122.352432), Mid (38.855941, -122.395974), Cypress (38.855772, -122.406191), and Quarry Valley (38.865172,-122.426675). The Grid and Quarry Valley sites burned in 2020, and the Mid and Cypress sites burned in 2015. The Grid site was east facing, the Mid site was west facing, the Cypress site was east facing, and the Quarry Valley site was south and west facing. We used a compass and two landmarks to establish parallel belt transects. The transects were 4 meters wide and 10 meters apart running east to west. We surveyed the first individual on the transect line then moved at least 3 meters past the individual to establish independence from the previous sample. After 3 meters we surveyed the next individual we encountered. The end of the transect was marked by an absence of individuals or inaccessibility.

2.2 Sampling

For each *Pedicularis* individual selected, we determined the color of the foliage by comparing the lowest whorl of leaves to a predetermined color gradient from green to red (Fig. 1). We observed whether the plant was flowering or not. Individuals with flower petals or flower buds were considered to be flowering. To determine height, we measured the distance from the base of the stem at the soil level to the highest point of each individual's foliage without touching the individual. For individuals that were flowering, we measured from the base of the stem at the soil level to the point below the lowest flowering structure. To measure leaf length, we straightened the longest leaf from the bottom whorl of leaves and measured from the tip of the leaf to where the petiole connected to the stem.

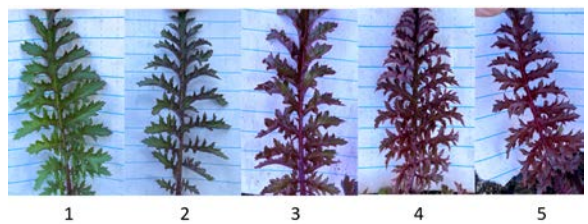

Figure 1: *Pedicularis* leaf color scale. Color scale was created using preselected photos of *Pedicularis* lower leaves that ranged from green to red. Color scale ranged from 1 to 5; 1 represented leaves that were almost completely green, with only a little red appearing on the tips. 3 represented leaves that were half green, half red. 5 represented leaves that had no green at all. We categorized the color of each *Pedicularis* sampled by comparing its lowermost whorl of leaves to this scale.

For each individual we also noted characteristics of the surrounding environment. We measured the distance from the individual *Pedicularis* to its nearest living woody neighbor (NWN) that was at least 30 cm tall. If a neighbor was burned to the ground, but had new growth at its base, we considered it to be alive and to be the NWN. We assumed NWN to be representative of parasitic interaction since many members of the Orobanchaceae family need close proximity to a host in order to germinate (Malcolm 1966). We measured the diameter of the base of the NWN. If the NWN was a single stalk of a larger clustered individual, we also measured the diameter of the largest stalk in the cluster. We identified the species of the NWN. If the NWN was burned, we used its new growth to identify it. Additionally, we counted the number of woody plant neighbors within a 1 m radius of the selected *Pedicularis*. For the number of woody neighbors we also only considered individuals that were alive and at least 30 cm tall. We considered clusters of one species that appeared to originate from the same roots to be one individual. We calculated the density of *Pedicularis* by counting the number of *Pedicularis* plants within a square meter of the individual. Lastly, we noted the aspect of the slope of the site, and the burn year of the site.

2.3 Statistical Analysis

All statistical analyses were conducted using JMP statistical software v15. We used t-tests, χ^2 tests, ANOVA as well as simple linear and logistic regressions to examine how environmental factors affect *Pedicularis's* color, size, flowering status, and density. Tables 1 and 2 outline the specific environmental factors measured with their corresponding tests.

RESULTS

3.1 Color

We found that taller plants with longer leaves were greener (Table 1). Plants with flowers were also greener than plants without flowers (Table 1). In addition, plants with more woody neighbors were greener (Fig. 2). *Pedicularis* from areas that had burned in 2015 were greener, and those from areas that burned in 2020 were redder (Table 1, Fig. 3). Plants from the Mid site were greener than those from Quarry Valley and Grid (Table 1). The color of plants from the Cypress site did not differ from the color of plants from any of the other sites (Fig. 4). There was no effect of distance to NWN, species of NWN, diameter of NWN, largest diameter of NWN in the cluster, aspect, or conspecific density on color (Table 1).

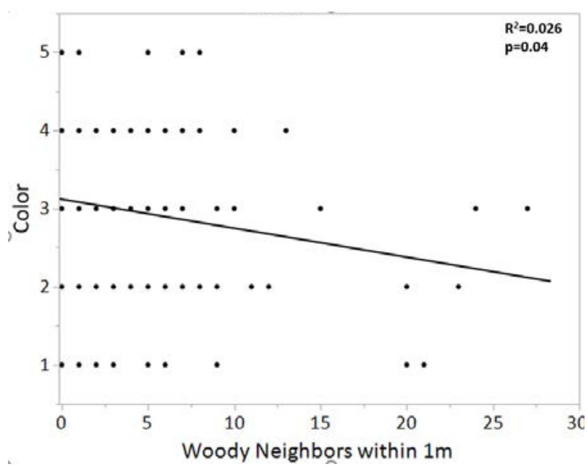

Figure 2: Effect of number of woody neighbors on *Pedicularis* leaf color. *Pedicularis* with more woody neighbors were greener. The number of woody neighbors consisted of all woody plants within one meter of the *Pedicularis* specimen that were taller than 30 cm or had a larger burned stump that was resprouting. Clumps of sprouts were counted as one individual. See Figure 1 for color scale.

Table 1: Effect Tests on and Analysis Methods on Continuous Response Variables:

Effects	DF	F	Analysis Method
Color			
Flowering	1	20.581957***	t-test
Leaf Length	1	37.8758***	simple linear regression
Height	1	23.6972***	simple linear regression
Distance to NWN	1	1.5439	simple linear regression
Species of NWN	9	0.4082	ANOVA
Number of WN	1	4.1592*	simple linear regression
Diameter of NWN	1	0.00465	simple linear regression
Largest Diameter of NWN Clump	1	1.5439	simple linear regression
Aspect	2	2.0279	ANOVA
Burn Year	1	7.3328*	t-test
Site	3	3.7904*	ANOVA
Density	1	0.9481	simple linear regression
Height			
Color	1	23.6972***	simple linear regression
Flowering	1	19.7599***	t-test
Leaf Length	1	25.39094***	simple linear regression
Distance to NWN	1	0.0193	simple linear regression
Species of NWN	9	0.8292	ANOVA
Number of WN	1	2.0933	simple linear regression
Diameter of NWN	1	0.08	simple linear regression
Largest Diameter of NWN Clump	1	3.5827	simple linear regression
Aspect	2	1.4324	ANOVA
Burn Year	1	0.9973	t-test
Site	3	3.3671*	ANOVA
Density	1	0.0342	simple linear regression
Leaf Length			
Color	1	37.858***	simple linear regression
Flowering	1	6.2751*	t-test
Height	1	253.9094***	simple linear regression
Distance to NWN	1	0.1732	simple linear regression
Species of NWN	9	0.3285	ANOVA
Number of WN	1	1.5904	simple linear regression
Diameter of NWN	1	1.544	simple linear regression
Largest Diameter of NWN Clump	1	2.6105	simple linear regression
Aspect	2	0.0248	ANOVA
Burn Year	1	1.1746	t-test
Site	3	1.3366	ANOVA
Density	1	0.0634	simple linear regression
Density			
Flowering	1	1.8783	t-test
Color	1	0.9481	simple linear regression
Leaf Length	1	0.0634	simple linear regression
Height	1	0.0342	simple linear regression
Distance to NWN	1	0.9609	simple linear regression
Species of NWN	9	0.4524	ANOVA
Woody Neighbor Density	1	2.9967	simple linear regression
Diameter of NWN	1	0.6486	simple linear regression
Largest Diameter of NWN Clump	1	0.4517	simple linear regression
Near Pine	1	0.2096	t-test
Aspect	2	2.9578	ANOVA
Burn Year	1	0.1854	t-test
Site	3	2.0206	ANOVA

Notes: NWN = nearest woody neighbor   *p<.05, **p<.005, ***p<.001

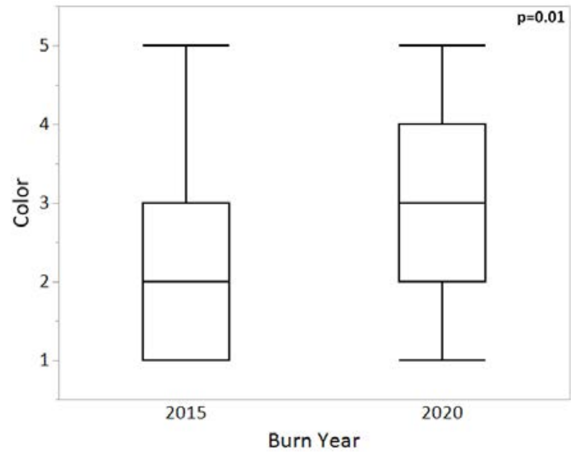

Figure 3: Effect of fire history on *Pedicularis* leaf color. The average color of *Pedicularis* was greener in sites that burned in 2015 (Grid and Quarry Valley) than in sites that burned in 2020 (Mid and Cypress). The study was conducted in February 2021. Sites that had burned in 2020 were still scorched and devoid of canopy. Sites that had not burned since 2015 had a largely re-established chaparral ecosystem with woody shrubs providing canopy cover at varying degrees of regrowth. See Figure 1 for color scale.

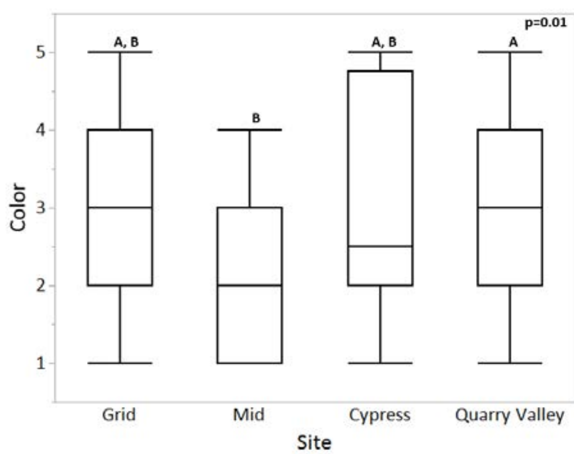

Figure 4: Effect of study site on *Pedicularis* leaf color. This study was conducted in four sites across Mclaughlin Reserve. The leaf color of *Pedicularis* in Mid site (38.855941,-122.395974) was greener than the leaf color of *Pedicularis* in Grid (38.827821,-122.352432) or Cypress (38.855772,-122.406191). The leaf color of individuals in Quarry Valley (38.865172,-122.426675) did not differ from the other sites. See Figure 1 for color scale.

3.2 Plant Size

Plant height and leaf length were positively correlated with each other (Table 1). Plants that were taller had longer leaves, were greener and were more likely to be flowering (Table 1). Height also varied with site, such that plants from Mid were on average taller than those from Grid, but Cypress and Quarry Valley did not differ notably from either one (Fig. 5). Aspect, burn year, density, distance to NWN, diameter of NWN, largest diameter of NWN clump, number of woody neighbors, and species of NWN did not show any effect on height or leaf length.

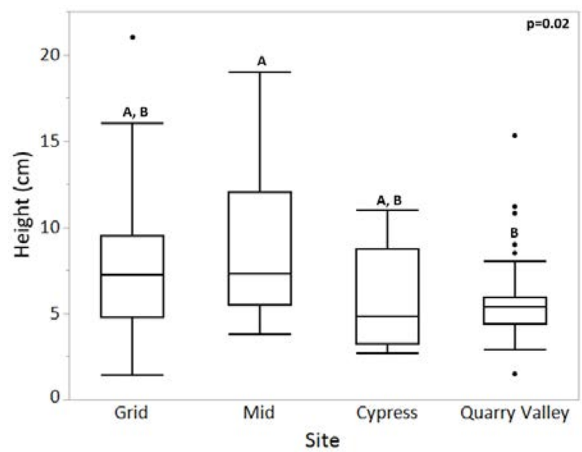

Figure 5: Effects of study site on *Pedicularis* height. *Pedicularis* were taller in Mid site (38.855941,-122.395974) than in Quarry Valley (38.865172,-122.426675). The height of individuals in Cypress (38.855772,-122.406191) and Grid (38.827821,-122.352432) did not differ significantly. Grid and Quarry Valley had both burned in the recent 2020 fire, while Mid and Cypress had not burned since 2015, and thus had more regrowth.

3.3 Flowering

Plants that were flowering were greener, taller, and had longer leaves (Table 2). Flowering was more prevalent on sites with an east or west aspect than south (Table 2). Flowers were also more likely to be present in sites that burned in 2015 than 2020 (Table 2).

Table 2: Effect Tests and Analysis Method on Flowering of *P. densiflora*:

Effect	DF	X^2	Analysis Method
Flowering			
Color	1	17.74995***	logistic regression
Leaf Length	1	6.39324*	logistic regression
Height	1	20.2558***	logistic regression
Distance to NWN	1	0.18	logistic regression
Species of NWN	9	6.4562	chi-squared test
Woody Neighbor Density	1	0.2657	logistic regression
Diameter of NWN	1	1.1644	logistic regression
Largest Diameter of NWN Clump		0.01971	logistic regression
Aspect	2	5.9964*	chi-squared test
Burn Year	1	4.4421*	chi-squared test
Site	3	15.3147**	chi-squared test
Density	1	1.9507	logistic regression

Note: NWN = nearest woody neighbor *p<.05, **p<.005, ***p<.001

3.4 Other Characteristics

We found no correlation between any of the factors examined on the density of *P. densiflora* (Table 1).

DISCUSSION

We hypothesized that parasite-host interactions would be a main factor in the phenotypic variation of *Pedicularis*. However, the only correlation we observed between potential hosts and *Pedicularis* phenotype was that individuals with more woody neighbors were greener (Table 1). We did not find an effect of the number of woody neighbors on height, leaf length, flowering, or density. Nor did we find any effects of nearest woody neighbor species, diameter, or proximity on any of our phenotypic measurements. Phenotypic variation is a complex interaction of environment and genetics. It is challenging to differentiate which factors contribute to phenotypic variation. It appears that parasitic interactions did not have a large influence on phenotype. However, finer scale measurements such as radioactive tracers described by Ljunggren (1967) would yield more accurate assessments of parasite-host relationships and might reveal effects on phenotype. Based on our results, it is likely that abiotic factors have more of an effect on *Pedicularis* phenotype.

Sunlight may be one such abiotic factor. The red coloration in *Pedicularis* is caused by the pigment anthocyanin, which can protect plants from sun damage (Neil and Gould 2003). Individuals were greener, larger, and more likely to be flowering in sites that had burned in 2015. In the 2015 sites, the chaparral shrubs had five years to regrow foliage and as such provided more canopy cover than the recently scorched 2020 burn sites. *Pedicularis* is usually found near woody plant hosts so the increased sun exposure after the burn may have acted as a stressor, causing it to be smaller, bloom less, and produce photoprotective anthocyanins (Sprague 1962a). The lower incidence of flowering on a southern aspect further supports that sunlight is likely a stressor, as southern aspects receive more direct sunlight in the northern hemisphere. This could also explain why plants with more woody neighbors are greener (Figure 2). Woody neighbors may not only be serving as hosts to *Pedicularis*, but may also be facilitating its growth by providing cover from the sun. Future experiments that manipulate light exposure could provide insight on the extent to which sunlight affects *Pedicularis* phenotype.

Ultimately, phenotypic variation in plants is a complex phenomenon, and it is difficult to discern exactly what factors influence it.

Variables may be numerous and often interact in intricate ways. While our study focused specifically on *Pedicularis*, the phenotypic flexibility highlighted in this study is a hallmark of the plant kingdom as a whole.

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