Do serpentine specialists have a weakened competitive ability and higher stress-tolerant traits than serpentine generalists?

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Abstract

Stressful edaphic environments contain unique plant communities that provide insight into ecological specialization and speciation. Serpentine soil is one such soil that harbors both endemic species, which are restricted to serpentine soils, and tolerator species, which are capable of growing on and off serpentine soil. However, it is unknown why some species evolve to become endemics versus tolerators. We hypothesized that adaptation to serpentine comes with a larger trade-off with competitive ability for endemics than tolerators, preventing endemics from expanding into productive nonserpentine habitats. To test this hypothesis, we conducted a common garden glasshouse experiment with eight serpentine endemic species and nine serpentine tolerator species. We quantified the competitive ability of all species by comparing fitness of plants grown with and without a single grass competitor. Our measure of fitness was above ground biomass collected 60 days after germination. We quantified two functional traits - water use efficiency (WUE) and root:shoot ratios - to see if endemics and tolerators on average differed in the traits that evolve following serpentine adaptation, and to test whether higher WUE or lower root:shoot ratios are associated with lower competitive ability. We found no difference between endemic and tolerator species in their competitive ability, root:shoot ratios, or WUE. Although, in the presence of a competitor, endemic populations had a decreased water use efficiency. Our results indicate that serpentine endemics are not restricted to serpentine by competition and that the functional traits we measured are not associated with a loss of competitive ability. We also find that serpentine endemics may experience more water stress in competitive environments, which could be a part of their restriction to serpentine outcrops. We anticipate that this work can be used to understand how serpentine endemics interact with competitors in the field and how their functional traits play a role in adaptation to different aspects of serpentine habitats.

Introduction

Stressful edaphic environments contain unique plant communities that provide insight into ecological specialization and speciation. Stressful edaphic substrates have a variety of characteristics such as macronutrient deficiency, drought-inducing characteristics and in some cases high heavy metal concentrations that can limit the establishment of plants (Rajakaruna, 2018). However, plant species have adapted to these stressful environments. Adaptation has led to the evolution of both specialists and

generalists. Different plant species have evolved a variety of traits to deal with harsh edaphic substrates, such as high water use efficiency (WUE), slower growth rates, high root to shoot biomass, earlier phenology, and hyperaccumulation(Brady, Kruckeberg, & Jr., 2005; Harrison & Rajakaruna, 2011). These complex systems provide a rare opportunity into how plants respond to highly stressful systems and why certain species speciate via edaphic divergence, while others do not.

California's state rock, serpentine, is an ultramafic rock that comprises 1% of California's soil surface, yet the soil derived of it contains 10% of California's endemic flora (Safford, Viers, & Harrison, 2005). Serpentine soils are known for having high concentrations of heavy metals (Zn, Ni, Cr, Co), low concentrations of essential nutrients (N,P,K) and low Ca:Mg ratios (Brady et al., 2005). While serpentine soils are derived from the same parent rock material, there are differences in the physical edaphic characteristics across serpentine habitats, from bare, rocky slopes to grasslands with well-developed soils (Brady et al., 2005, Sianta & Kay, 2019). Serpentine barrens are notably some of the harshest habitats mainly in part due to the lack of fine particles and organic matter within the soil, therefore exposing plants to drought like conditions (Brady et al., 2005). This variation in habitat can have a dramatic effect on productivity. Serpentine grasslands have higher productivity while serpentine barrens are often devoid of vegetation (Sianta & Kay, 2019).

Despite the challenges of serpentine, multiple plant species have adapted to serpentine soils. Tolerators are species that are capable of occurring on and off serpentine soil, while endemics are species that are restricted only to serpentine soil (Brady et al., 2005). This divergence within genera (endemics) and species (tolerators) across edaphic gradients creates an interesting system to understand how edaphic divergence affects speciation. Endemics are believed to have originated from a non-serpentine progenitor species that colonized serpentine habitats and eventually adapted, creating distinct taxa (Cacho & Strauss, 2014). With endemism and tolerance evolving across many plant families, it brings about the question as to why certain plants are restricted to serpentine while others are capable of occuring on and off serpentine.

The evolution of endemics might be more likely than the evolution of tolerators when local adaptation to serpentine comes at a large cost. While species may have a large distribution, populations can be divided by adaptation to local characteristics. However, the populations adapted to a stressful environment may not be able to survive outside their local environment because traits that are advantageous in stressful environments aren't advantageous in competitive environments (Grime, 1977). These tradeoffs may be the reason we find some species restricted to edaphically stressful environments. If an individual moves from one site to the other, it may have low fitness, resulting in mortality of the migrant population. Habitat isolation is driven by selection against non-locally-adapted individuals. Under this hypothesis, we would expect

endemics to have a larger trade-off between serpentine adaptation and competitive ability.

Arthur Kruckeberg conducted a common garden and greenhouse study that showed serpentine endemics are capable of growing on non-serpentine soil in controlled greenhouse environments (Brady et al., 2005). Kruckeberg hypothesized that endemics are restricted from productive nonserpentine habitats because adaptation to harsh serpentine soils involves the evolution of stress-related functional traits that trade-off with competitive ability (Brady et al., 2005). The functional traits that are associated with this trade-off are largely unknown. Based off Kruckeberg's hypothesis we quantified the competitive ability and two functional traits of serpentine endemics and tolerators.

In our study, we use a common garden greenhouse experiment to quantify competitive ability and two functional traits across multiple serpentine tolerator and endemic species. We quantified functional traits thought to be associated with serpentine adaptation that could be linked to a trade-off with competitive ability. We quantified root to shoot ratios because development of larger root systems in endemic species is hypothesized to facilitate growth in low-nutrient soils(Sambatti & Rice, 2007). We also quantified water use efficiency (WUE) because endemics tend to occur in habitats with low water holding capacity as well as other drought-inducing characteristics such as greater exposure to UV rays, and high surface soil temperatures (Cacho & Strauss, 2014; Sambatti & Rice, 2006; Sianta & Kay, 2019). We predict that serpentine endemic species will have a reduced competitive ability, higher WUE, and higher root:shoot ratios compared to the tolerator populations. We paired every serpentine population in our experiment with a closely related nonserpentine population(hereafter, "sister taxa"). The purpose of this comparison was to investigate the evolution of these traits in association with transitions to serpentine soils.

Methods

Study System: We selected one population from 8 serpentine endemic species and 9 tolerator species that represent independent origins of serpentine adaptation. Sampling serpentine endemic and tolerator populations across multiple families yields broader insights into serpentine adaptation. To create sister taxa pairs, we paired each serpentine population with a closely related nonserpentine population. The eight serpentine endemic species were paired with a nonserpentine population from their sister species. The nine tolerator serpentine populations were paired with a nonserpentine population from within the same species. The plants used in the experiment were collected from various serpentine and adjacent non-serpentine sites from the years 2015-2018 (Appendix A for full population and species information). The selected populations span six different plant families and nine different genera, including *Erythranthe* (Phrymeaceae), *Plantago, Collinsia* and *Collomia* (Plantaginaceae), *Clarkia*

and *Camissonia* (Onagraceae), *Trifolium* (Fabaceae), *Navarretia* (Polemoniaceae) and *Layia* (Asteraceae).

Greenhouse Experiment: We quantified the competitive ability, root-shoot ratios and WUE of all species in a greenhouse common garden experiment. We grew all populations with and without a standardized competitor in potting soil. We chose *Bromus carinatus* as our competitor because it is an annual grass that has a wide distribution occurring over most serpentine sites across California (Calflora). Our study was conducted at the UCSC Coastal Biology greenhouses from September 2018 to January 2019. We grew out all of our plants in a Promix HP-based soil mix in the following ratios: 7 parts Promix HP (Premier Horticulture Ltd.), 2 parts washed sand (RediGro Horticultural Grade), and 3 parts perlite (Therm-o-rock). We used Stuewe & Sons conetainers as our containers with a cotton ball placed in the bottom of each pot to eliminate soil loss.

For each population we sowed 30 pots per treatment. Each treatment consisted of one individual growing either with or without a *B. carinatus* competitor. We planted three seeds of *B. carinatus* and the selected populations into each pot. Pots were placed into a germination chamber with conditions set to induce stratification of seeds (4℃, 70% relative humidity, no light, daily misting with DI water). Each pair was left in the stratification chamber until radicles began to emerge, about two weeks after initial planting, although the time to germination varied across species. We recorded germination day for all seedlings as the day of radicle emergence. After most of the individuals within a pair germinated, they were topped with soil and placed into a seedling establishment chamber with optimal "spring" conditions (21℃, 50% relative humidity, 12 hour day length) where they received daily DI water from a water can. As seedlings of the selected populations and *B. carinatus* began to emerge, they were thinned out to one individual per pot.

All seedlings spent 20 days in the seedling establishment growth chamber and were moved into the greenhouse in randomly assigned positions. The greenhouse was set to regulate temperature(15.5℃) and provide supplemental light to ensure lighting for 12 hours per day. In order to mimic drought-like conditions, we watered every four days or when plants started to show symptoms of drought stress (i.e wilting).

Measurements of competitive ability, root:shoot ratios, and WUE: We used total above-ground biomass at 60 days as a metric of fitness. After a total of 60 days from germination, all above-ground biomass was harvested, placed into labeled envelopes, and put in a drying oven at (55℃ for 21 days). We used fitness to calculate competitive ability for each population as the log response ratio (lnRR). A positive value of the log response ratio indicates a facilitative effect of a competitor, where a negative value indicates a negative effect of competition.

$$\text{lnRR} = \ln\left(\frac{\text{biomass of plant growing with competition}}{\text{average biomass of same seed source growing without competition}}\right)$$

Upon completion of harvesting above-ground biomass, a subset of five individuals from each non-competition treatment were selected for root harvesting. Individuals were removed from their pots, soaked and rinsed free of all soil particulate, leaving bare roots. Samples were then placed into a drying oven (55℃ for 21 days) and placed into a sealed container with silica. Our samples were weighed roughly 20 days after coming out of the dryer. We divided the below ground biomass by above-ground biomass to calculate root:shoot ratios for the subsamples within each population.

$$\frac{(Below\ ground\ biomass)}{(Above\ ground\ biomass)} = R{:}S\ Ratio$$

We used carbon isotope discrimination to quantify intrinsic WUE in a subset of individuals in all populations. We predicted that serpentine endemics will be more drought tolerant than serpentine tolerators, thus having a higher WUE. The most recently fully expanded leaves were collected before plants began to flower in order to get an accurate representation of carbon discrimination in the vegetative state. We collected leaves from 5 individuals per treatment. Tissue was dried in the same way as above-ground biomass. We placed ~1mg of tissue in Costech 5x9 Capsules for carbon isotope testing in a Continuous Flow Elemental Analyzer at UCSC's Stable Isotope Laboratory. δ13C, which indicates carbon isotope discrimination, was used as a metric for understanding water use efficiency, with more negative values indicating a lower WUE. Average δ13C values were calculated for each population and used to compare the difference between endemic and tolerator pairs.

Data Analysis
Competitive ability: We first asked whether there was an effect of competition by *B. carinatus* on each population we tested. We used a one-sample t-test to test if the mean log response ratio (lnRR) of each population was different from zero. Next, we asked whether endemic serpentine populations were worse competitors than tolerator serpentine populations by using a phylogenetic generalized least squares (PGLS) model on the lnRRs of all the serpentine populations in R-studio (Version 1.1463).

In order to investigate if adaptation to serpentine is accompanied by a greater loss in competitive ability we calculated the divergence between nonserpentine and serpentine populations by subtracting the mean lnRR of each serpentine population from that of its paired nonserpentine population. We predicted that endemics would have greater divergence in competitive ability than tolerators. We used a PGLS to test

our prediction that adaptation to serpentine comes at a greater loss in competitive ability in endemics versus tolerators.

Functional Traits:
Root:Shoot Ratios: To test the hypothesis that serpentine endemic populations have a higher root:shoot ratio because they have evolved in harsher edaphic conditions than tolerators, we used a PGLS model to test for a difference in R:S ratio divergence.

$$Divergence\ R{:}S \\ = (Mean\ R{:}S\ ratio\ nonserpentine\ pop.) \\ - (Mean\ R{:}S\ ratio\ serpentine\ pop.)$$

Water Use Efficiency: We hypothesized that serpentine endemics have an increased WUE compared to serpentine tolerators due to their adaptation to drought-inducing environments. We compared the mean WUE of each serpentine population using a PGLS model.

Divergence between nonserpentine and serpentine populations of each pair were calculated to investigate if WUE evolved following adaptation to serpentine. The mean of each population was calculated and the mean WUE of serpentine populations were subtracted from the mean WUE of nonserpentine populations. Differences between endemic and tolerator pairs in their degree of WUE divergence was tested with a PGLS model.

$$Divergence\ =\ (Mean\ WUE\ of\ each\ nonserpentine\ population) \\ - (mean\ WUE\ of\ each\ serpentine\ population)$$

To investigate if functional traits are associated with a trade-off with competitive ability, we compared the results from our competitive ability analysis, root to shoot ratios, and water use efficiency. To test our hypothesis that a reduced competitive ability is associated with a trade-off conferring greater WUE we used the mean WUE and lnRR of each population and used a PGLS to regress WUE on lnRR. We ran this analysis with all populations and with just serpentine populations. Root:shoot ratios of each population were compared to lnRR, analyzing the results with a PGLS.

Results
Competitive ability

Bromus carinatus, the grass competitor, had a negative effect on plant fitness for all but five populations: the serpentine and nonserpentine populations of *Navarretia pubescens* (tolerator, T), *Mimulus nudatus* (endemic, E), *Navarretia jepsonii* (E), and the nonserpentine sister taxon of *Layia discoidea* (E) (Table 2).

There was not a significant difference in competitive ability between serpentine populations of endemic and tolerator species (t=0.4915, DF = 15, P=0.1029; Fig. 1). In two of the 17 pairs there was a significant difference in competitive ability between the serpentine and nonserpentine sister taxa (Table 3). The two pairs were the *Mimulus nudatus - M. guttatus* and the *Clarkia gracilis subsp. tracyi - C. gracilis subsp. albicaulis* endemic pairs. In each case the serpentine taxon had a lower competitive ability than the nonserpentine taxon. However, there was no difference between endemics and tolerator pairs in the degree of divergence in competitive ability (t=-1.7522, DF=15, P=0.1033; Fig. 2).

Functional Traits

The root:shoot ratios of serpentine endemic populations did not differ from that of serpentine tolerator populations (t=-0.1299, DF= 16, P=0.8984; Fig. 3). Divergence in root:shoot ratios between serpentine and nonserpentine populations of each taxa pair was not significantly different between endemic and tolerator populations (t=-0.2505, DF= 16, P=0.8058; Fig. 4). We compared WUE's of our taxa pairs in both the no-competition and competition treatments. In the no-competition treatments, there was no difference in WUE between the serpentine populations of endemic and tolerator pairs (t=1.2877, DF=15, P=0.2203; Fig. 5). Divergence in WUE between the serpentine and nonserpentine populations of each taxa pair was not significantly different between endemic and tolerator populations (t=0.4358, DF=13, P=0.5458; Fig. 6). In the competition treatments, serpentine endemic populations showed a decreased WUE relative to tolerators (t=2.3857, DF=14, P=0.0344; Fig. 7). In the competition treatment, tolerators showed on average an increase in WUE while endemics' WUE remained constant. The divergence in WUE between nonserpentine and serpentine populations showed no difference between endemic and tolerator populations (t=-0.7473, DF=14, P=0.0820; Fig. 8).

There was no relationship between a population's average WUE and its competitive ability in the noncompetition treatment (t=0.7939, DF=14, P=0.4427)(Fig. 9), nor in in the competition treatment (t=0.7063, DF=13, P=0.4947; Fig. 10). There was no relationship between competitive ability and root:shoot ratios (t=0.3969, DF=11, P=0.7006; Fig. 11).

Discussion

With soil being one of the most influential factors in determining plant communities, edaphically stressful habitats are a fantastic model system for understanding adaptation and speciation. The suite of characteristics influencing the speciation of serpentine endemics brings about questions of which characteristics are resulting in restriction to serpentine habitats. Kruckeberg hypothesized a lack of competitive ability as being the main driver of restriction (Kruckeberg, 1951). This lack

of competitive ability is believed to be the result of a trade-off involving functional traits that have been developed to cope with stressful soils.

Serpentine endemics are believed to be restricted to serpentine outcrops due to a trade-off associated with adaptation to stressful serpentine habitats and competitive ability, which limits spread into nonserpentine habitats (Brady et al., 2005). High amounts of bare ground in serpentine habitats suggest competition is less important in these edaphically stressful habitats (Cacho & Strauss, 2014). It follows that serpentine endemics, which are found solely on serpentine outcrops, should have a reduced competitive ability compared to tolerators, which have populations on and off serpentine outcrops. We quantified the competitive ability of serpentine endemics and compared them to serpentine populations of tolerator taxa. Contrary to our predictions, serpentine endemic populations did not have a reduced competitive ability compared to serpentine tolerator populations. While serpentine endemics did not show a reduced competitive ability compared to tolerator populations, there was a lot of variation among populations in competitive ability.

Mimulus nudatus, a serpentine endemic, was the most negatively affected by competition. Potential reasons for this are likely because *M. nudatus* has been found to have functional traits that may result in decreased competitive ability, such as earlier flowering and reduced leaf size (Harrison & Rajakaruna, 2011)*.* These traits likely contributed to the results we found because *M. nudatus* had some of the lowest biomass production of all of the populations grown and went to flower earlier than most of the populations grown in the experiment (personal observation). This is interesting in that *M. nudatus* has been shown to be more drought tolerant and occur in drier serpentine microsites than *M. guttatus* (Hughes, Bachmann, Smirnoff, & Macnair, 2001). Adaptation to these drought inducing conditions might trade-off with competitive ability.

While a weakened competitive ability may not be a limiting factor in a majority of the serpentine taxa, it plays a role in some. With all of the functional traits that serpentine plants have evolved to cope with serpentine habitats, it is likely certain taxa have independently evolved different mechanisms (Brady et al. 2005). It is possible that there are a suite of factors playing into the restriction of serpentine endemics to edaphically stressful islands. Competitive ability may contribute to this suite in many of these plants as can be seen with the significant increase in the WUE of serpentine tolerators in the competition treatment. This implies that tolerators may be more capable of increasing water use efficiency in competitive environments, whereas endemics remained with a lower water use efficiency. Endemics may be less capable of adjusting water use strategies in competitive environments, and thus may be worse competitors over the course of their lifetime.

The study that preceded this and inspired it's inception is in large part very similar. Sianta (2019), used the same species and grew them with and without the same competitor, but in field-collected soil. The results from her study found that endemics were poorer competitors than tolerators, in contrast to our results here. This

could be attributed to our collection of biomass after 60 days rather then following through to senescence where we could observe floral characteristics and ovule production. When plants are small there is less competition for limiting resources in the pot, but as they get larger there is more competition. It is possible that we would see differences in traits such as phenological differences, flower production and fruit and seed development. In the future it would be valuable to look into the way that controlling and inducing drought affects the phenology and growth of endemics compared to tolerators.

Root to shoot ratios were not significantly different between endemic and tolerator populations which was different from our expectation of endemics having greater root:shoot ratios. A previous study in *Helianthus exilis* showed that this serpentine endemic had greater root to shoot ratios than its nonserpentine sister taxa (Sambatti & Rice, 2007). *Helianthus exilis* has been noted as having a greater investment in root development earlier in its life cycle (Sambatti & Rice, 2006, 2007). We may not have seen a difference in root:shoot ratios because we may not have been collecting data at the proper life history stage. Another factor may be that greater root development is commonly associated with growing in macronutrient deprived soils (Brady et al., 2005). We provided an environment that was ideal for the growth of all of our populations and therefore may not have seen a difference in root development.

Our results may not indicate that there is a difference in competitive ability between endemics and tolerators but we gain valuable insight into the way that serpentine endemics and tolerators perform in neutral soil environments with the presence of competition. While competitive ability may not clearly be the defining factor in the restriction of serpentine endemics, it certainly plays a role in edaphic restriction of some serpentine endemic taxa. If a loss in competitive ability is not driving speciation of endemics it is possible that endemics could have lost or developed reduced functional traits. Speciation is a complex subject and being able to investigate these serpentine habitats provides a tangible means for understanding how and why plants have evolved edaphic specialization while others have not.

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Table 1: Full list of serpentine endemic and tolerator species used in study.

Pair type	Family	Species Abbreviation	Species	Population origin	Location
Tolerator	Onagraceae	CACO	*Clarkia concinna*	Serpentine	Lake Co: UC McLaughlin Reserve
			Clarkia concinna	Nonserpentine	Lake Co: UC McLaughlin Reserve
Tolerator	Onagraceae	CABR	*Clarkia breweri*	Serpentine	Stanislaus Co: Del Puerto Canyon
			Clarkia breweri	Nonserpentine	Stanislaus Co: Del Puerto Canyon
Tolerator	Plantaginaceae	PLER	*Plantago erecta*	Serpentine	Lake Co: UC McLaughlin Reserve
			Plantago erecta	Nonserpentine	Napa Co: UC McLaughlin Reserve
Tolerator	Plantaginaceae	COSP	*Collinsia sparsiflora*	Serpentine	Lake Co: UC McLaughlin Reserve
			Collinsia sparsiflora	Nonserpentine	Napa Co: UC McLaughlin Reserve
Tolerator	Fabaceae	TWILD	*Trifolium wildenovii*	Serpentine	Lake Co: UC McLaughlin Reserve
			Trifolium wildenovii	Nonserpentine	Napa Co: UC McLaughlin Reserve
Tolerator	Polemoniaceae	NAPB	*Navarretia pubescens*	Serpentine	Lake Co: UC McLaughlin Reserve
			Navarretia pubescens	Nonserpentine	Napa Co: UC McLaughlin Reserve
Tolerator	Phyrmaceae	MGUT	*Mimulus guttatus*	Serpentine	Lake Co: UC McLaughlin Reserve
			Mimulus guttatus	Nonserpentine	Napa Co: UC McLaughlin Reserve
Tolerator	Plantaginaceae	COHT	*Collinsia heterophylla*	Serpentine	Stanislaus Co: Del Puerto Canyon
			Collinsia heterophylla	Nonserpentine	Stanislaus Co: Del Puerto Canyon
Tolerator	Polemoniaceae	NAHX	*Navarretia heterodoxa*	Serpentine	San Mateo Co: Edgewood County Park
			Navarretia heterodoxa	Nonserpentine	Napa Co: Foote Botanical Preserve
Endemic	Polemoniaceae	NAJP	*Navarretia jepsonii*	Serpentine	Lake Co: UC McLaughlin Reserve
		NAHN	*Navarretia heterandra*	Nonserpentine	Butte Co: Horncut
Endemic	Polemoniaceae	NARS	*Navarretia rosulata*	Serpentine	Marin: Carson Ridge
		NAHX	*Navarretia heterodoxa*	Nonserpentine	Napa Co: Foote Botanical Preserve
Endemic	Onagraceae	CAGT	*Clarkia gracilis subsp tracyi*	Serpentine	Lake Co: UC McLaughlin Reserve

		CAGA	*Clarkia gracilis subsp albicaulis*	Nonserpentine	Butte Co: Paradise
Endemic	Polemoniaceae	CLDV	*Collomia diversiflora*	Serpentine	Lake Co: UC McLaughlin Reserve
		CLHT	*Collomia heterophylla*	Nonserpentine	Lake Co: Cobb Mountain
Endemic	Asteraceae	LADI	*Layia discoidea*	Serpentine	San Benito Co: Clear Creak Management Area
		LAGL	*Layia glandulosa*	Nonserpentine	San Benito Co: Clear Creak Management Area
Endemic	Phrymaceae	MNUD	*Mimulus nudatus*	Serpentine	Lake Co: UC McLaughlin Reserve
		MGUT	*Mimulus guttatus*	Nonserpentine	Napa Co: Knoxville Wildlife Reserve
Endemic	Plantaginaceae	COGR	*Collinsia greenei*	Serpentine	Napa Co: UC McLaughlin Reserve
		COSP	*Collinsia sparsiflora*	Nonserpentine	Napa Co: UC McLaughlin Reserve
Endemic	Onagraceae	CABE	*Camissonia benetensis*	Serpentine	San Benito Co: Clear Creak Management Area
		CAST	*Camissonia strigulosa*	Nonserpentine	San Benito Co: Clear Creak Management Area

Table 2: There was a significant negative effect of *Bromus carinatus* on all populations with the exception of a few. We used a one sample t-test to determine if the mean competitive ability (lnRR) of each population/taxon was different from zero. Red indicates a significant value, orange indicates a marginally significant value, black indicates no significance.

Pair Name	Genus sp.	Population	pair type	T	DF	P
CACO	*Clarkia concinna*	S	T	4.9911	14	0.0001977
CACO	*Clarkia concinna*	NS	T	2.4788	13	0.02767
CABR	*Clarkia breweri*	S	T	4.2557	13	0.0009372
CABR	*Clarkia breweri*	NS	T	3.7774	13	0.002304
PLER	*Plantago erecta*	S	T	NA	NA	NA
PLER	*Plantago erecta*	NS	T	2.7956	7	0.02669
TWILD	*Trifolium willdenovii*	S	T	4.8851	13	0.0002979
TWILD	*Trifolium willdenovii*	NS	T	4.9671	14	0.0002068
NAPB	*Navarretia pubescens*	S	T	1.8631	13	0.0852
NAPB	*Navarretia pubescens*	NS	T	1.6426	13	0.1244
COHT	*Collinsia heterophylla*	S	T	2.6251	6	0.03932
COHT	*Collinsia heterophylla*	NS	T	3.0009	8	0.01705
COSP	*Collinsia sparsiflora*	S	T	3.1675	6	0.01938
COSP	*Collinsia sparsiflora*	NS	T	7.6737	9	0.00003082
NAHX	*Navarretia heterodoxa*	S	T	6.8787	14	0.000007577
NAHX	*Navarretia heterodoxa*	NS	T	5.7181	14	0.00005314
MGUT	*Mimulus guttatus*	S	T	4.2262	13	0.00099
MGUT	*Mimulus guttatus*	NS	T	3.1675	13	0.007417
NAJP_NAHN	*Navarretia jepsonii*	S	E	2.4256	6	0.05147
NAJP_NAHN	*Navarretia heterandra*	NS	E	3.1401	11	0.009407
CAGT_CAGA	*Clarkia gracilis subsp. tracyi*	S	E	5.0781	14	0.0001684
CAGT_CAGA	*Clarkia gracilis subsp. albicaulis*	NS	E	2.4691	14	0.02703
CLDV_CLHT	*Collomia diversiflora*	S	E	3.0573	11	0.0109
CLDV_CLHT	*Collomia heterophylla*	NS	E	3.0377	10	0.01251

LADI_LAGL	*Layia discoidea*	S	E	2.4887	7	0.04168
LADI_LAGL	*Layia glandulosa*	NS	E	1.2968	5	0.2513
COGR_COSP	*Collinsia greenei*	S	E	3.4933	8	0.008159
COGR_COSP	*Collinsia sparsiflora*	NS	E	7.6737	9	0.00003082
NARS_NAHX	*Navarretia rosulata*	S	E	4.1985	6	0.005695
NARS_NAHX	*Navarretia heterodoxa*	NS	E	5.7181	14	0.00005314
MNUD_MGUT	*Mimulus nudatus*	S	E	8.3137	1	0.07621
MNUD_MGUT	Mimulus guttatus	NS	E	3.1675	13	0.007417

Fig 1. The competitive abilities (lnRRs) of serpentine endemic populations were not significantly different from that of serpentine tolerator populations. The dashed line indicates no effect of competition. Negative values indicate that there was an effect of competition by *B. carinatus*, whereas positive values indicate a facilitative effect of *B. carinatus*.
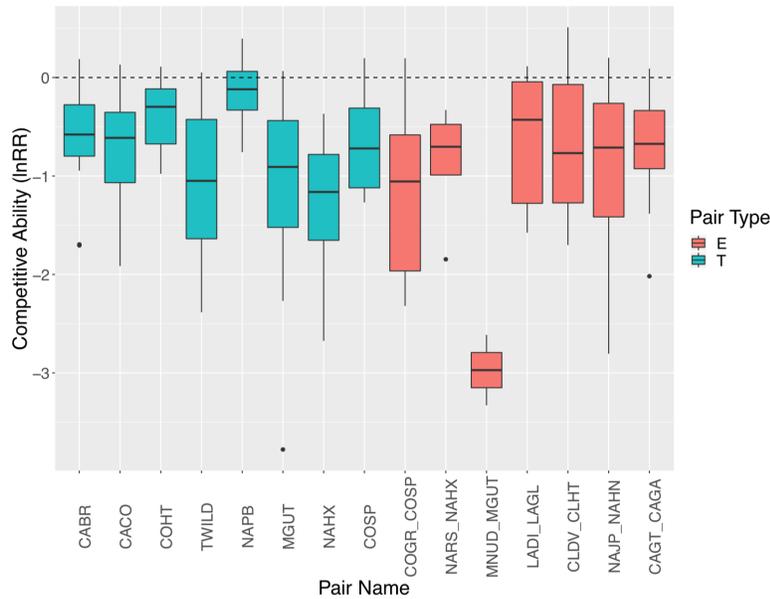
Fig. 2 There was no difference in divergence in competitive ability between endemic and tolerator pairs. The y-axis indicates the difference between nonserpentine and serpentine populations. The dashed line indicates that there was no difference in competitive ability between nonserpentine and serpentine populations. Data above the line indicates that the serpentine populations had a decreased competitive ability compared to the nonserpentine populations. A point below the line indicates that the nonserpentine populations had a decreased competitive ability compared to the serpentine populations.
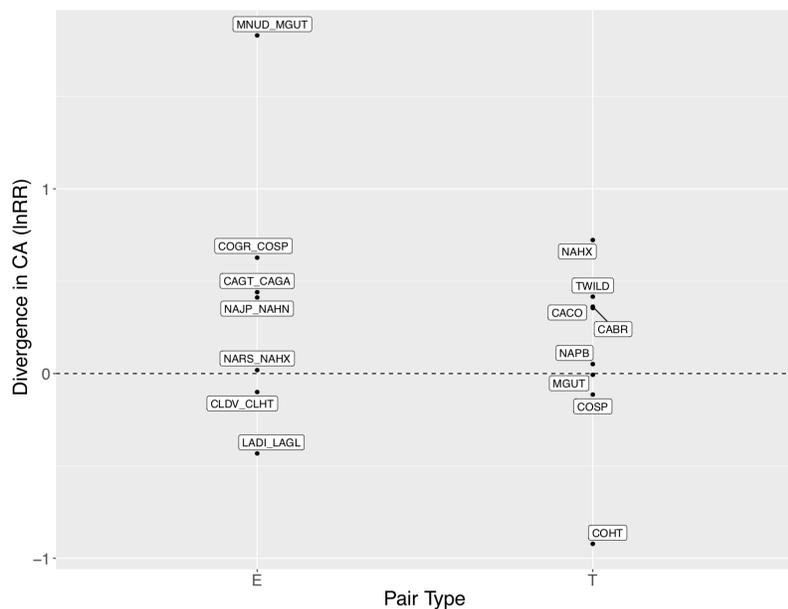
Table 3: There was a significant difference in competitive ability between nonserpentine and serpentine populations in only two pairs.

Pair Name	pair type	T	DF	P
CACO	T	1.5004	36.48	0.1454
CABR	T	0.1939	25.993	0.8477
PLER	T	NA	NA	NA
TWILD	T	1.7047	21.14	0.1029
NAPB	T	0.45792	24.553	0.651
COHT	T	-1.993	9.7915	0.07482
COSP	T	0.96437	6.9642	0.3671
NAHX	T	1.9498	26.316	0.06193
MGUT	T	-1.9933	9.7915	0.07482
NAJP_NAHN	E	0.9596	8.36	0.3642
CAGT_CAGA	E	2.53	26.21	0.01763
CLDV_CLHT	E	-0.2945	20.26	0.7711
LADI_LAGL	E	-0.5073	5.9541	0.6302
COGR_COSP	E	1.9896	8.5857	0.07938
NARS_NAHX	E	0.076452	12.368	0.9403
MNUD_MGUT	E	3.6058	3.76	0.02507

Fig. 3: There was no significant difference in root:shoot ratios between serpentine populations of endemic and tolerator populations. A R:S value of 1 indicates that the below ground root biomass was equal to the above ground biomass. An R:S value >1 indicates that there was more root biomass than shoot biomass. An R:S value <1 indicates that there was more shoot biomass than root biomass.
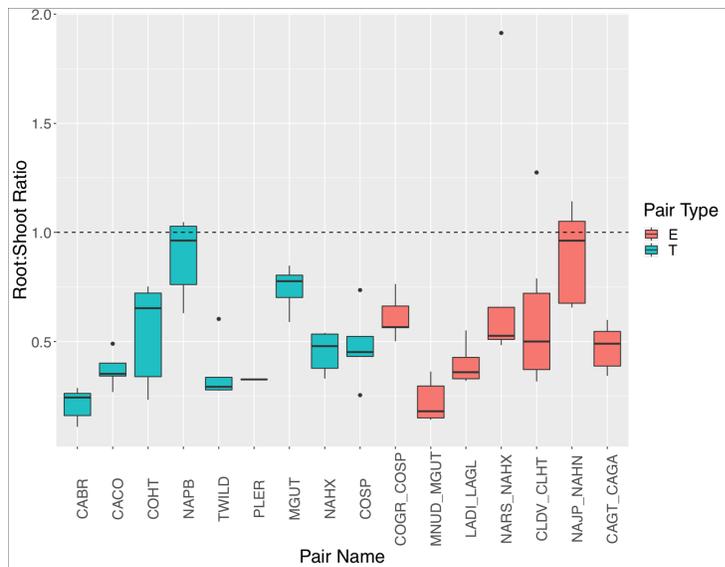
Fig. 4: The divergence between the root:shoot ratios of serpentine endemic and tolerator populations showed no significance. We calculated divergence in root:shoot ratios (y-axis) by subtracting the serpentine population's root:shoot ratio from the nonserpentine populations root:shoot ratio. A value at the dashed line indicates equal above and below ground biomass. A value below the dashed line indicates nonserpentine populations had greater root:shoot ratios. A value above the dashed line indicates serpentine populations had a greater root:shoot ratio.
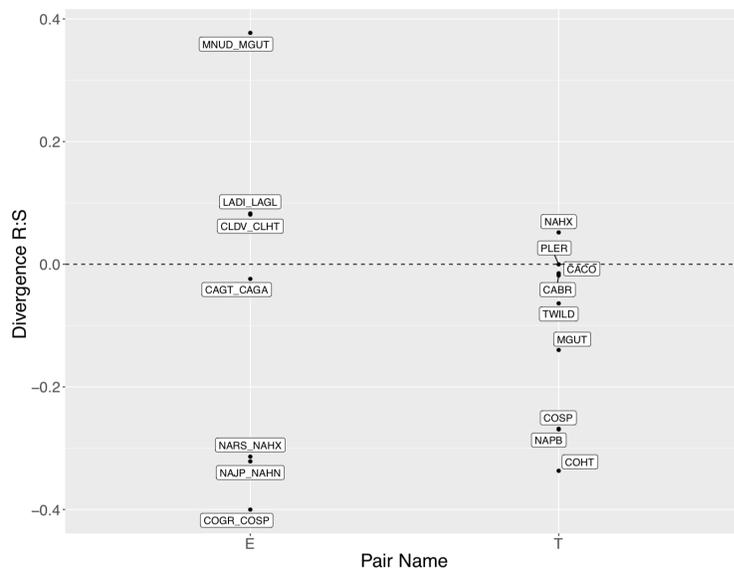
Fig. 5: Serpentine endemics in the non-competition treatment did not have a significant difference in WUE compared to serpentine tolerators. A lower (more negative) D13C value indicates lower water use efficiency, whereas a greater (less negative) D13C indicates an higher water use efficiency.
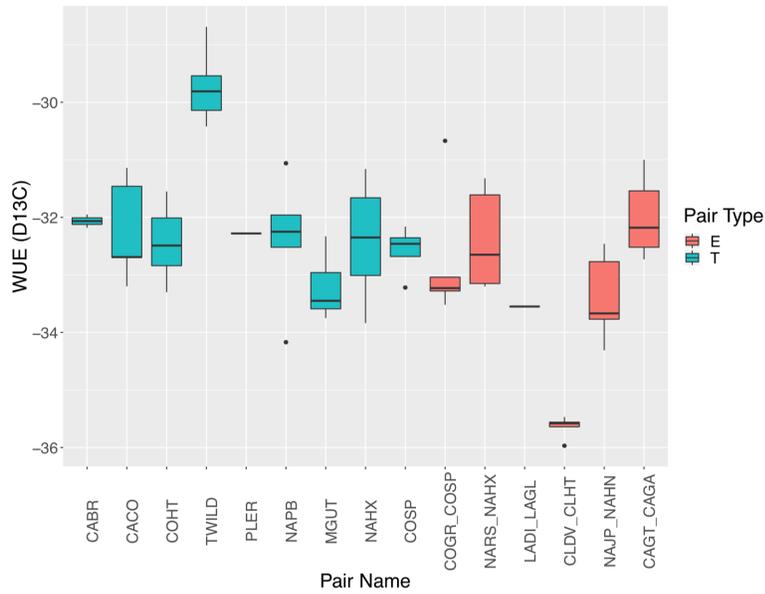
Fig. 6: Divergence in WUE between nonserpentine and serpentine populations of our endemic pairs in the non-competition treatment were not significantly different from our tolerator pairs. The y-axis shows the difference between nonserpentine and serpentine populations. Values above the zero line indicates nonserpentine populations with increased water use efficiency, whereas below the zero line indicates that serpentine populations have an increased water use efficiency.
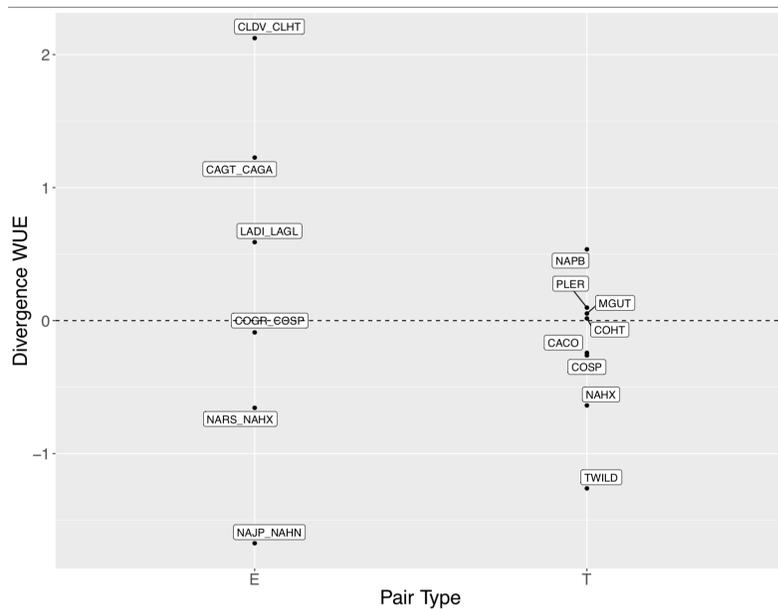

Fig. 7:Serpentine endemics in the competition treatment did have a significant difference in WUE compared to serpentine tolerators. A lower (more negative) D13C value indicates lower water use efficiency, whereas a greater (less negative) D13C indicates an higher water use efficiency.
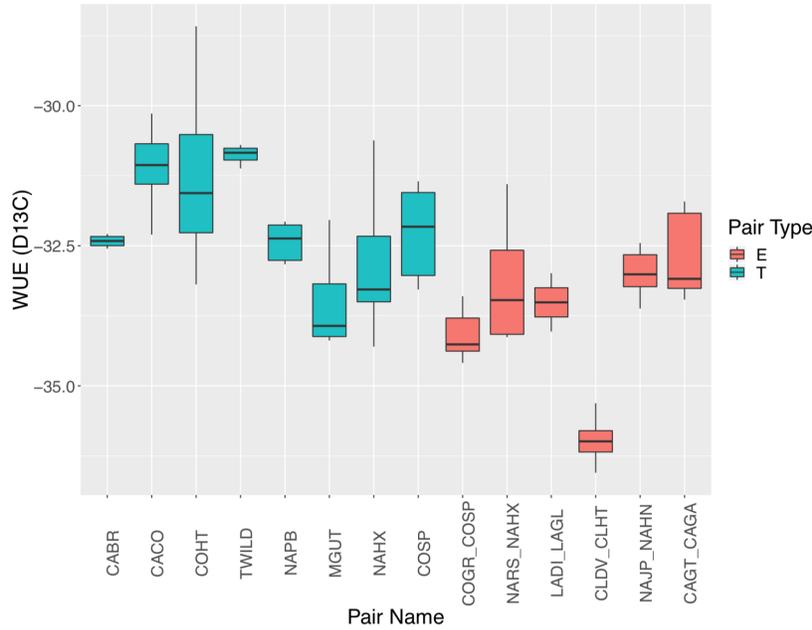
Fig. 8: Divergence in WUE within sister taxa pairs in the competition treatment was not different between endemic and tolerator pairs. We calculated divergence in WUE (y-axis) by subtracting the serpentine population's δ13C from the nonserpentine populations δ13C. Values above the zero line indicate nonserpentine populations with increased water use efficiency, whereas below the zero line indicates that serpentine populations have an increased water use efficiency.
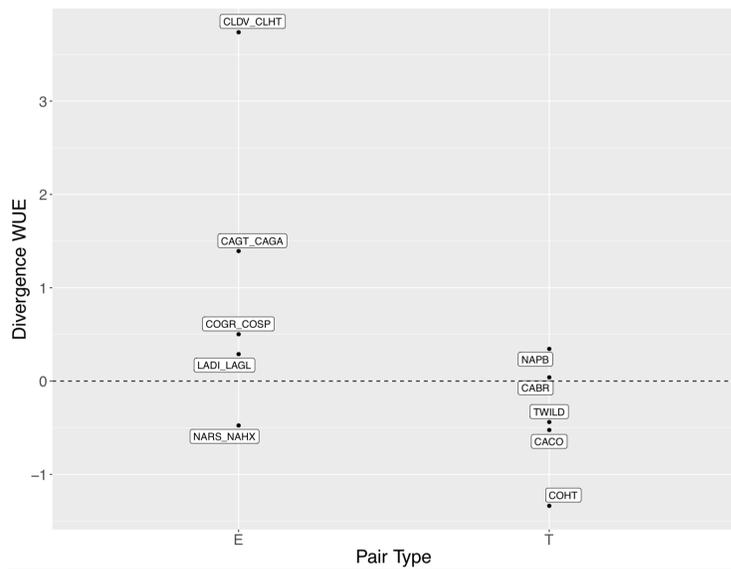

Fig. 9: There was no relationship between competitive ability and water use efficiency in the noncompetition treatment of our serpentine populations.

Fig. 10: There was no relationship between competitive ability and water use efficiency in the competition treatment of our serpentine populations.
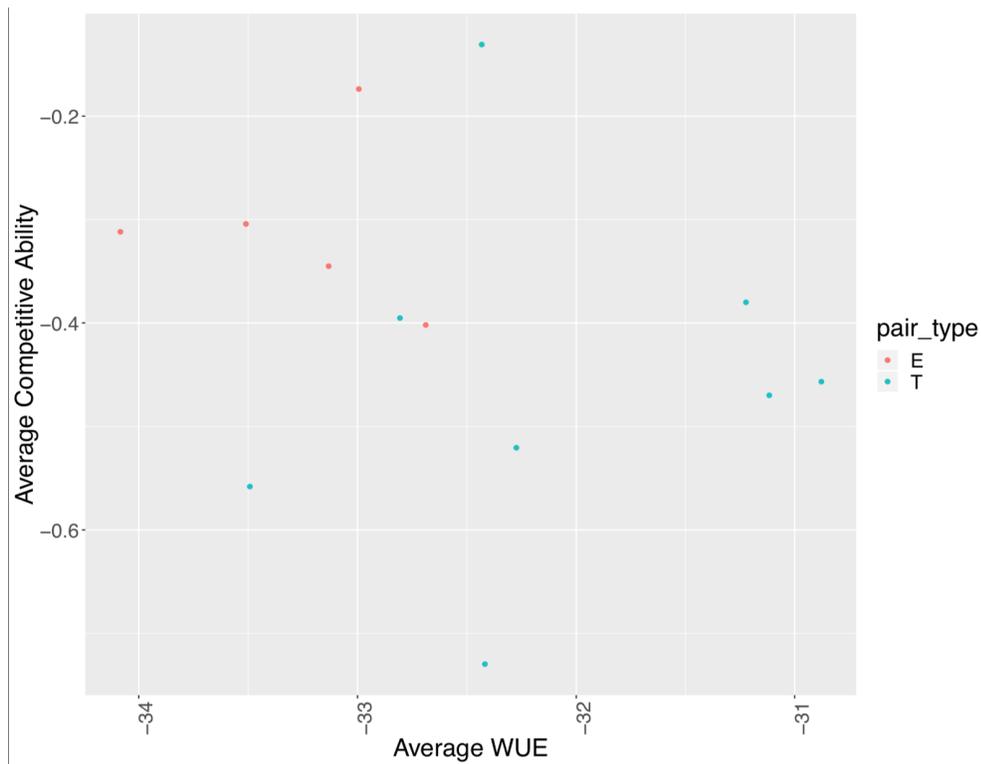

Fig. 11: There was no relationship between competitive ability and root:shoot ratios of our serpentine populations.