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UNIVERSITY OF CALIFORNIA
SANTA CRUZ

**ADAPTIVE DIVERGENCE AND SPECIATION IN THE CALIFORNIA
SERPENTINE FLORA**

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

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September 2019

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ABSTRACT

Adaptive divergence and speciation in the California serpentine flora

Shelley A. Sianta

Charles Darwin and Alfred Russel Wallace emphasized the role of adaptive divergence among populations in initiating speciation, and studying the ways in which natural selection causes reproductive isolation among populations is an active field of research. Yet local adaptation to different environments does not always lead to speciation, as evidenced by species occupying a broad range of habitats. The overall goal of this dissertation research was to improve our understanding of how speciation occurs following adaptive divergence, and why it sometimes does not. My study system was the flora associated with naturally-toxic serpentine soils in California, wherein divergence across soil boundaries is accompanied by strong selection, leading to the evolution of both ecologically-variable species (serpentine tolerators) and the evolution of new, ecologically-specialized species (serpentine endemics). I use an experimental comparative design and a population-level phylogenomic approach to understand factors that promote speciation via adaptive divergence in this system. In my first chapter, I tested the hypothesis that serpentine endemics adapt to more harsh serpentine habitats or more divergent habitats relative to their progenitor populations than serpentine tolerators. I quantified soil chemistry data and the percent of bare ground in the habitats of 8 serpentine endemic species, 9 serpentine tolerator species, and in a paired nonserpentine taxon for each of the 17 serpentine species.

I found that serpentine endemics occur in barer serpentine habitats with lower soil calcium levels than serpentine tolerators. There was no difference in the degree of habitat divergence between tolerator serpentine-nonserpentine pairs and endemic serpentine-nonserpentine pairs. In my second and third chapters, I set up a multi-year greenhouse reciprocal transplant experiment with all 17 serpentine-nonserpentine sister taxa pairs using field-collected seed and soil. I included an additional treatment where I grew members of each taxa pair in the pair's nonserpentine soil with a standardized competitor, in order to measure the competitive ability of serpentine endemics vs. tolerators. In my second chapter, I quantified timing to first flower and phenological isolation in all pairs to answer the question of whether plasticity in flowering time shifts promotes or constrains speciation. I found that endemic and tolerator sister taxa pairs did not differ in the magnitude of phenological isolation, nor in the degree to which flowering time shifts were plastic versus genetically-based, suggesting that phenological isolation evolves early in the speciation process. Instead the magnitude of flowering time shifts between paired serpentine and nonserpentine sisters were partially explained by how different the pair's soils were. In my third chapter, I quantified fitness trade-offs, habitat isolation and competitive ability of all pairs to test Arthur Kruckeberg's long-standing hypothesis that a trade-off between serpentine adaptation and competitive ability promote the evolution of serpentine endemics but not serpentine tolerators. I found that, indeed, serpentine endemics were on average worse competitors than serpentine tolerators. I also found that there is

more divergence in competitive ability in endemic pairs than tolerator pairs, suggesting that a greater trade-off between serpentine adaptation and competitive ability has occurred in the endemic lineages. Lastly, I revisited a hypothesized case of budding speciation in the triad of species *Clarkia franciscana*, *C. rubicunda* and *C. amoena*. *Clarkia franciscana* was hypothesized to be a derivative species of *C. rubicunda*, as it is a very small-ranged serpentine endemic species, with its range subsumed by the range of the more ecologically-diverse *C. rubicunda*. I used population-level sampling and phylogenomic techniques to determine if there was evidence for progenitor-derivative speciation in this group. I found that there was not, and instead all three species formed well-supported monophyletic groups. However, there was a lot of gene tree discordance regarding the relationship of the three species, suggesting that they evolved simultaneously and rapidly. Instead of being a recently evolved serpentine endemic, as was hypothesized, *C. franciscana* was likely once a more widespread species that became restricted to serpentine over time. Taken together, the results from this dissertation are a unique insight into factors that promote progress towards speciation - from the establishment of edaphic ecotypes to the evolution of edaphic endemics.

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STATEMENT OF CONTRIBUTION

The text of this dissertation includes reprints of the following previously published material: Chapter one: Sianta, S.A. and K.M. Kay. 2019. Adaptation and divergence in edaphic specialists and generalists: serpentine soil endemics in the California flora occur in barer serpentine habitats with lower soil calcium levels than serpentine tolerators. *American Journal of Botany* 106(5): 1-14. The co-author listed in this publication directed and supervised the research which forms the basis for the dissertation.

GENERAL INTRODUCTION

Speciation is a process fundamental to the origin of biodiversity. The process of speciation can be studied as the way in which reproductive isolation evolves between taxa (Mayr, 1942). Over the decades, studies have demonstrated the complex ways in which natural selection and geographic isolation contribute to the evolution of different components of reproductive isolation (Ramsey et al., 2003; Coyne and Orr, 2004; Kay, 2006; Yost et al., 2012; Anacker and Strauss, 2014). From the myriad case studies that have quantified reproductive isolation among closely related plant species, we know that multiple forms of reproductive isolation contribute to speciation and that ecologically-based prezygotic barriers tend to be stronger than post-zygotic isolation (Lowry et al., 2008). Ecological divergence between taxa correlates with the strength of reproductive barriers between taxa in a wide range of organisms (Funk et al., 2006), and adaptive divergence has been championed as a strong factor in speciation (Schluter, 2001; Sobel et al., 2010). However, adaptive divergence among populations does not always lead to speciation, as evidenced by species composed of ecotypic differentiation (Clausen et al., 1948; Nosil et al., 2009). The goal of this dissertation is to understand factors that promote speciation via adaptive divergence and to understand the geographic mode in which speciation occurs via adaptive divergence. My study system is the serpentine flora of California, wherein edaphic divergence is often accompanied by strong selection, leading to the evolution of both ecologically-variable species and the evolution of new

ecologically-specialized species. I use an experimental comparative design to test the roles that habitat divergence, phenological isolation, and fitness trade-offs have on speciation in this system. I then take a population-level phylogenomic approach to understand the geographic mode of speciation in the rare serpentine endemic, *Clarkia franciscana*.

Chapter one— Adaptation and divergence in edaphic specialists and generalists: serpentine soil endemics in the California flora occur in barer serpentine habitats with lower soil calcium levels than serpentine tolerators

Regions of the world with a complexity of harsh edaphic substrates, such as serpentine soils, often have high species richness, with many edaphic endemics – i.e., species that only occur on a particular soil type (Cowling et al., 1994; Anacker, 2011; Baldwin, 2014; Moore et al., 2014). Adaptation to harsh edaphic substrates has repeatedly led to the evolution of both edaphic specialists and edaphic generalists. For example, adaptation to naturally-toxic serpentine soils in California has occurred across 39 plant families and has led to the evolution of serpentine endemic species (occurring only on serpentine) and serpentine tolerator species (occurring both on and off of serpentine) (Anacker, 2011). Serpentine soils are chemically characterized by low calcium, high magnesium (and low Ca:Mg ratios), low macronutrients and high heavy metals (Brady et al., 2005). However, serpentine habitats are varied, ranging from steep, rocky serpentine barrens, to serpentine chaparral, serpentine seeps and even productive serpentine

grasslands (Proctor, 1971; Yost et al., 2012; Kay et al., 2018). These habitats vary in the density of plant species, water availability, and soil chemistry; they likely mediate selection in different ways. Adaptation to harsher serpentine habitats could come with larger fitness costs when serpentine seeds disperse off of serpentine. Given that gene flow among populations requires dispersal to each other's habitats, I predicted that tolerator serpentine populations, which are connected to nonserpentine populations via gene flow, should occur in more benign serpentine habitats than endemic serpentine populations. However, it may be that adapting to more different, instead of harsher, habitats is what drives speciation and the evolution of endemism (Nosil et al., 2009). Thus, I predicted that endemic serpentine populations should occur in more different habitats relative to their nonserpentine sister species than serpentine and nonserpentine populations of tolerator species.

In Chapter 1, I quantified two features of serpentine habitats that mediate habitat harshness and to which adaptation could result in large fitness trade-offs in alternative environments: soil chemistry and microhabitat bareness. Microhabitat bareness is the percent of bare, non-vegetated ground in the neighborhood of a focal plant, and is a composite measure of multiple selective factors. Bare habitats could be rocky and have low water holding capacity, have high disturbance regimes, or have high herbivore pressure (Strauss and Cacho, 2013; Cacho and Strauss, 2014). I quantified both soil chemistry and microhabitat bareness in one population of 9 serpentine tolerator species and 8 serpentine endemic species. I

found that serpentine endemics occur, on average, in twice as bare serpentine habitats with 25% less soil calcium than serpentine tolerators. I also quantified soil chemistry and microhabitat bareness in a nonserpentine sister taxon for each of the 9 serpentine tolerator taxa and 8 serpentine endemic taxa. I found no statistical differences in the degree of habitat divergence between endemic and tolerator sister taxa pairs in individual soil chemistry variables nor in multivariate soil chemistry. There was a nonsignificant trend that endemic sister taxa pairs had more divergence in bare ground than tolerator sister taxa pairs. These results suggest that a factor promoting the evolution of serpentine endemism vs tolerance is the extremity of the habitat to which populations adapt. Adapting to more barren serpentine habitats or serpentine soils with extremely low soil Ca may trade-off with traits, such as competitive ability, that cause serpentine endemics to be relatively unfit in productive nonserpentine habitats.

Chapter two—Across the speciation continuum: genetically-based flowering time shifts evolve early in speciation following adaptation to serpentine soils

Reproductive barriers are often thought to evolve as a byproduct of natural selection, wherein adaptation to a new habitat results in selection on traits that also confer assortative mating (Coyne and Orr, 2004). Phenological isolation, or isolation due to differences in mating schedules, is one such component of reproductive isolation that can evolve as a byproduct because the onset of mating is often associated with environmental cues (Rathcke and Lacey, 1985). In plants,

phenological isolation is mediated through flowering time – both the time of flowering onset and flowering duration. Flowering times are known to have a genetic basis in plants, and yet can also be plastic in response to stressful environments (Levin, 2009; Anderson et al., 2012; Sheth and Angert, 2016). Levin (2009) argued that when a plant population colonizes a marginal habitat, plastic shifts in flowering time can act to protect the newly established population from the swamping effects of gene flow from source populations. Plastic shifts in flowering time prevent gene flow that occurs through pollen transfer between adjacent populations. However, if flowering time shifts are plastic and seeds disperse between populations in different habitats, then migrant individuals will have similar flowering schedules as the local plants, thus eroding the reproductive barrier. Shifts in flowering times between serpentine and nonserpentine populations are often noted in serpentine tolerators and endemics (Rajakaruna, 2004; Wright et al., 2006; Kay et al., 2011), and it is hypothesized that serpentine plants flower earlier than nonserpentine relatives as a mechanism to avoid the drought-inducing conditions of rocky serpentine substrates (Schmitt, 1983; Brady et al., 2005; Dittmar and Schemske, 2017). However, it is unknown if the strength of flowering time shifts and the degree to which they are genetically-based differs between endemics and tolerators.

In Chapter 2, I tested the hypothesis that speciation of serpentine endemics is more likely when serpentine adaptation is accompanied by larger flowering time shifts and more genetically-based (vs plastic) flowering time shifts. I grew

the same 9 serpentine tolerator sister taxa pairs and 8 serpentine endemic sister taxa pairs from Chapter 1 in a greenhouse-based reciprocal transplant experiment in field-collected soil. I collected soil and seed from the two populations comprising each of the 17 sister taxa pairs and planted seeds from each population into each soil type. I measured the number of days in between germination and first flower for roughly 30 plants per source population/soil combination, and I took weekly censuses to construct flowering time curves for each source population/soil combination. I found that genetically-based flowering time shifts were common among the sister taxa pairs. In contrast to most documented serpentine systems, I found that the majority of serpentine taxa flowered later than their nonserpentine sister taxon. Plasticity did act to increase the magnitude of flowering time shifts between sister taxa when each taxon was grown in its home soil in 7/17 sister taxa pairs, but the magnitude of plasticity did not differ between endemic and tolerator sister taxa pairs. While there was variation among all of the pairs in their degree of phenological isolation, there was no difference on average between endemic and tolerator sister taxa pairs. Taken together, these results suggest the genetically based flowering time shifts evolve early following adaptation to serpentine but are not strong enough to complete speciation, nor do they seem to become stronger with time since divergence. My results yield support for Levin's original argument that flowering time shifts following adaptation to a novel habitat can partially isolate newly-colonized populations from maladaptive gene flow for source populations. This partial isolation may

allow marginal populations to establish in and adapt to novel conditions.

However, in contrast to Levin's (2009) prediction, shifts in flowering time are accomplished through selection on flowering times and not solely through plastic responses of flowering times to marginal habitats.

Chapter three—Trade-offs between serpentine adaptation and competitive ability are associated with the evolution of serpentine endemic species but not through habitat isolation

Adaptive divergence can also directly lead to reproductive isolation when adaptation to a particular habitat comes with fitness trade-offs in alternative habitats (Nosil et al., 2005; Sobel et al., 2010). Within a habitat, fitness trade-offs can lead to migrant individuals being selected against relative to local individuals, decreasing gene flow through a reduction in mating opportunities. This form of reproductive isolation is known as habitat isolation, or immigrant inviability (Coyne and Orr, 2004; Nosil et al., 2005). In serpentine systems, habitat isolation is thought to play a key role in speciation (Kay et al., 2011). Within both serpentine endemic species and serpentine tolerator species, nonserpentine sister taxa often do not possess the alleles required for development on serpentine soils, leading to strong habitat isolation when nonserpentine seeds migrate into serpentine soils. However, the mechanisms by which habitat isolation may be achieved when serpentine seeds disperse to nonserpentine habitats is less obvious. Classic work by Kruckeberg (1951, 1967) demonstrated that serpentine seeds

have equal, if not higher, fitness in nonserpentine soil relative to serpentine soil, suggesting that serpentine taxa don't require the peculiar chemistry of serpentine. Instead, Kruckeberg (1951) hypothesized that a direct trade-off between serpentine adaptation and competitive ability prevented serpentine endemics from successfully establishing in nonserpentine habitats. Kruckeberg's hypothesis, known as the competitive trade-off hypothesis, became the paradigm for explaining serpentine restriction in endemic species and yet, until this point, had not been explicitly tested among multiple serpentine tolerator and endemic species. As well as currently restricting serpentine endemics to serpentine soils, trade-offs between competitive ability and serpentine adaptation could promote habitat isolation if serpentine taxa have low fitness in productive nonserpentine environments relative to their nonserpentine sister taxa.

For Chapter 3, I added an additional competition treatment to the greenhouse reciprocal transplant experiment described in Chapter 2. I tested the hypothesis that stronger trade-offs between serpentine adaptation and competitive ability lead to stronger habitat isolation in endemic sister taxa pairs than tolerator sister taxa pairs, thus facilitating speciation of endemics. The competition treatment involved growing all serpentine and nonserpentine taxa in their pair's respective nonserpentine soil with a competitor, *Bromus carinatus*. I measured fitness of all 2300 plants in the experiment and calculated competitive ability for every serpentine and nonserpentine taxa by comparing fitness in the taxon's nonserpentine soil with and without competition. I found that endemic serpentine

taxa are indeed worse competitors than tolerator serpentine taxa, and that there is more divergence in competitive ability within endemic sister taxa pairs than in tolerator sister taxa pairs. These results suggest that adaptation to serpentine in endemic lineages has come with a larger cost in competitive ability than it has in tolerator lineages. I found that on average endemic sister taxa pairs have higher degrees of divergence in ITS sequences than tolerator pairs, so it may also be the endemic lineages have continued to lose competitive ability over time. I did not find evidence that serpentine endemics have lower relative fitness in our recreated nonserpentine habitat (i.e., nonserpentine soil and one *B. carinatus* individual) than serpentine tolerators. This result suggests that habitat isolation via immigrant inviability in nonserpentine habitats is not stronger in the endemic pairs than in the tolerator pairs. Thus, stronger trade-offs between serpentine adaptation and competitive ability does not lead stronger immigrant inviability per se. However, given that the nuances of the natural competitive environments (e.g., density, community composition of competitors and limiting resources) varies among the taxa used in this study and was not reflected in our standardized competition treatment, our habitat isolation results here may not reflect what would happen in field conditions. Regardless, when combined with spatial isolation, the trade-off between serpentine adaptation and competitive ability in endemics lineages could lead to reproductive isolation by preventing incipient endemic taxa from successfully dispersing through the nonserpentine matrix that separates them from their progenitor populations.

Chapter four—Phylogenomic analysis resolves a controversial case of putative progenitor-derivative speciation for the serpentine endemic Clarkia franciscana

One of the major distinctions made in speciation research is the geographic mode by which new species evolve. While allopatric speciation via vicariance is a well-accepted and uncontroversial model of speciation (Coyne and Orr, 2004), budding speciation (Mayr, 1954; Lewis, 1962; Grant, 1981), whereby small marginal populations diverge from within a species, has less empirical support. Comparative analyses that regress geographic range characteristics of sister taxa against time since divergence in some clades are consistent with geographic predictions of budding speciation (Barraclough and Vogler, 2000; Malay and Paulay, 2010; Claremont et al., 2012; Anacker and Strauss, 2014; Grossenbacher et al., 2014), but post-speciation changes in geographic ranges can make inferences about budding speciation misleading (Losos and Glor, 2003). Budding speciation leaves a temporary phylogenetic signal of the derivative species being nested within the progenitor species that can provide some of the most definitive evidence for budding speciation (Rieseberg and Brouillet, 1994; Crawford, 2010). For example, populations of the serpentine endemic species *Layia discoidea* are monophyletic and nested within *Layia glandulosa*, its progenitor species (Baldwin, 2005).

In Chapter 4, I revisited a classic case of hypothesized budding speciation in the western North American genus *Clarkia*. In the 1950s-1970s Harlan Lewis

and collaborators published a suite of influential papers about the prominence of rapid and recent budding speciation in *Clarkia* (Lewis, 1953, 1962; Lewis and Roberts, 1956; Lewis and Raven, 1958; Bartholomew et al., 1973; Gottlieb, 1973, 1974). A combination of expansion into marginal xeric habitats, chromosomal rearrangements and inbreeding were the mechanisms used to explain the predominance of pairs of species that had the geographic range characteristics of budding speciation, but lacked strong morphological differentiation (Lewis and Raven, 1958; Lewis, 1962). One of their classic examples of budding speciation came from a triad of species. *Clarkia franciscana*, a hypothesized serpentine neoendemic, was thought to have evolved from within *C. rubicunda*, which in turn was hypothesized to be a derivative species of *C. amoena*. Lewis and Raven (1958) inferred these evolutionary relationships based on a combination of geographic range characteristics, shifts in mating system, and chromosomal rearrangements. Here, I used phylogenomic techniques to resolve the evolutionary history of these three species. Using hundreds of loci was important because if the species evolved very recently and/or very rapidly, I expected high levels of gene discordance and any one gene tree may not give the correct species tree topology. I found strong support for monophyly of each species, which rejects the hypothesis of budding speciation. High levels of gene discordance at the node that groups *C. franciscana* and *C. amoena* as sister taxa indicates that the three species speciated from one another nearly simultaneously. The degree of genetic differentiation between *C. franciscana* populations indicates that instead of being

a serpentine neoendemic, *C. franciscana* was once more widespread and underwent the process of biotype depletion (i.e., extinction of populations on nonserpentine), resulting in a serpentine paleoendemic species. Our results provide another cautionary tale for using the current geographic ranges of species as evidence of the mode of speciation.

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Chapter 1

Adaptation and divergence in edaphic specialists and generalists: serpentine soil endemics in the California flora occur in barer serpentine habitats with lower soil calcium levels than serpentine tolerators.

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ABSTRACT

• **Premise of the study:** Adaptation to harsh edaphic substrates has repeatedly led to the evolution of edaphic specialists and generalists. Yet, it is unclear what factors promote specialization versus generalization. Here, we search for habitat use patterns associated with serpentine endemics (specialists) and serpentine tolerators (generalists) to indirectly test the hypothesis that trade-offs associated with serpentine adaptation promote specialization. We predict that 1) endemics have adapted to chemically harsher and more bare serpentine habitats than tolerators, and 2) edaphic endemics show more habitat divergence from their sister species than tolerators do among on- and off-serpentine populations.

• **Methods:** We selected 8 serpentine endemic and 9 serpentine tolerator species representing independent adaptation to serpentine. We characterized soil chemistry and microhabitat bareness from one serpentine taxon of each species and from a paired nonserpentine sister taxon, resulting in 8 endemic and 9 tolerator sister taxa pairs.

• **Key results:** We find endemic serpentine taxa occur in serpentine habitats averaging twice as much bare ground as tolerator serpentine taxa and 25% less soil calcium, a limiting macronutrient in serpentine soils. We do not find strong evidence that habitat divergence between sister taxa of endemic pairs is greater than between sister taxa of tolerator pairs.

• **Conclusions:** These results suggest serpentine endemism is associated with adaptation to chemically harsher and more bare serpentine habitats. It may be that

this adaptation trades off with competitive ability, which would support the longstanding, but rarely tested, competitive trade-off hypothesis.

INTRODUCTION

Edaphic, or soil, factors are important selective agents for plants, causing trait evolution, adaptive population divergence, and speciation (McNeilly, 1968; Kruckeberg, 1986; Macnair and Gardner, 1998; Rajakaruna, 2004; Antonovics, 2006; Escudero et al., 2015). Regions around the world that have a complexity of edaphic substrates typically exhibit high species richness (Cowling et al., 1994; Anacker, 2011; Schnitzler et al., 2011; Molina-Venegas et al., 2013; Baldwin, 2014; Moore et al., 2014), with many edaphic endemics, or species that are restricted to atypical edaphic conditions. Substrates associated with edaphic endemics tend to be chemically or physically harsh environments, such as gypsum, serpentine, granite, quartz, heavy clay, and even mine tailings. Strong selection imposed by these edaphic habitats is implicated in the speciation of edaphic endemics from progenitor species (Stebbins and Major, 1965; Caisse and Antonovics, 1978; Kruckeberg, 1986; Baldwin, 2005; Kay et al., 2011; Anacker and Strauss, 2014). However, adaptation to harsh substrates can also result in edaphic generalists, which we broadly define here as species with populations occurring on multiple soil types (Sexton et al., 2017). We see the repeated evolution of endemics and generalists across diverse edaphic systems, and yet it is still unclear why species evolve to become edaphic endemics versus generalists.

Soils derived from ultramafic serpentinite rocks are an example of harsh edaphic habitats that harbor both endemic species and generalist species. Worldwide, serpentine habitats exhibit high endemism relative to their area. For example, 9%, 27% and 50% of California's, Cuba's and New Caledonia's endemic species, respectively, are endemic to serpentine substrates, despite the fact that serpentine covers only 1%, 7% and 29% of each region's total area, respectively (Anacker, 2011). More frequently, though, adaptation to serpentine leads to species that occupy both serpentine and non-serpentine substrates, hereafter called serpentine tolerator species (Anacker et al., 2011; Harrison and Rajakaruna, 2011). Serpentine tolerator species have been shown to comprise either locally adapted soil ecotypes or individuals that can tolerate both serpentine and nonserpentine soils (Kruckeberg, 1967; Wright, Stanton, et al., 2006; Branco, 2009; Baythavong and Stanton, 2010). In either case, establishment on serpentine requires mechanisms to deal with the potentially lethal chemical conditions of serpentine soils (Brady et al., 2005; Kazakou et al., 2008; Palm and Van Volkenburgh, 2014), such as high levels of Mg, low Ca/Mg ratios, low macronutrient concentrations, and high heavy metal (Ni, Cr, Co) concentrations. However, serpentine habitats vary in their degree of weathering and severity – they can range from rocky, steep serpentine barrens to serpentine chaparral, serpentine seeps and even productive serpentine grasslands. The chemical challenges of serpentine soils can vary both within and among serpentine habitats (Proctor, 1971; Proctor and Woodell, 1971; Baythavong, 2011; Yost et al., 2012;

Kay et al., 2018). It is not known, however, if serpentine endemic and tolerator species differ in the chemical harshness of the serpentine habitats in which they occur.

One explanation for the evolution of serpentine endemism is that fitness trade-offs associated with adaptation to serpentine prevent endemics from expanding their ranges beyond serpentine substrates. In his influential study on ecotypic variation in serpentine species, Kruckeberg (1951) found that serpentine taxa often don't require the peculiar chemistry of serpentine substrates, but have equal or higher fitness when planted in pots with non-serpentine soil. Kruckeberg hypothesized that competition prevents the spread of serpentine endemics into more productive non-serpentine habitats because serpentine tolerance traits directly trade off with competitive ability. A strong fitness trade-off could block gene flow between soil ecotypes through selection against migrants, effectively isolating endemic lineages from their progenitor populations. Although this trade-off hypothesis is the main paradigm for the restriction of serpentine endemics (Kruckeberg, 1951; Rune, 1953; Whittaker et al., 1954; Stebbins and Major, 1965; Proctor and Woodell, 1971; Rajakaruna, 2017), direct evidence for trade-offs between serpentine adaptation and competitive ability is insubstantial (but see Anacker et al. 2011 for macroevolutionary evidence). It follows that if trade-offs between serpentine adaptation and competitive ability promotes the evolution of serpentine endemics, we predict weak to no trade-offs in tolerator species, depending on the degree of local adaptation within tolerator species. It also

follows that if endemic species are generally less competitive than serpentine populations of tolerator species, we predict endemics will be found in less competitive serpentine habitats than serpentine populations of tolerators. Yet, these predictions have not been tested across multiple replicate serpentine-adapted plant taxa.

Adaptation to two aspects of serpentine habitats may cause a trade-off with competitive ability – the soil environment and the degree of microhabitat bareness. Adaptation to stressful serpentine soil chemistry selects for traits, such as intrinsically slow growth rates, high root:shoot ratios or low stature, that may be disadvantageous in a more competitive environment (Grime, 1977; Sambatti and Rice, 2007; Kay et al., 2011; Fernandez-Going et al., 2012). Additionally, mechanisms that deal with detoxification of the high magnesium and heavy metals in serpentine can be energetically costly (Brady et al., 2005; Kazakou et al., 2008; Palm and Van Volkenburgh, 2014). Studies have shown that there are multiple physiological mechanisms that species use to tolerate the low nutrient levels and high toxicity of serpentine soils (O’Dell and Rajakaruna, 2011; Palm and Van Volkenburgh, 2014). Different costs associated with different serpentine tolerance mechanisms may affect whether serpentine adaptation leads to true generalist species, tolerators composed of locally adapted populations, or endemic species.

Limitations imposed by adaptation to bare microhabitats may also trade off with competitive ability. Microhabitat bareness is defined as the amount of

ground devoid of vegetation in the neighborhood of a plant. Multiple potential factors likely mediate selection in bare areas and cause trade-offs with competitive ability, such as greater apparency to herbivores, greater soil surface temperatures and UV radiation, greater disturbance regimes, greater rockiness, or lower water availability (Cacho and Strauss 2014, and references therein). Trade-offs between adaptation to these selective agents and competitive ability could come from resource allocation trade-offs (e.g., trade-offs between defense and growth; Coley et al., 2005; Fine et al., 2006), or life history trade-offs (Grime, 1977). A prior study in *Streptanthus*, a genus dominated by serpentine-affiliated species, found that a population's average microhabitat bareness was inversely correlated with its competitive ability (Cacho and Strauss, 2014). This result suggests either that adaptation to bare microhabitats selects for low competitive ability, or that species found in bare microhabitats are those that are competitively excluded from habitats with higher plant densities. These two causes aren't mutually exclusive; for example, the latter can cause a plant population to occur in relatively bare habitats, and then further selection in bare habitats may result in a greater reduction in competitive ability. Given that there is substantial variation among serpentine habitats in microhabitat bareness, we expect to find serpentine endemics in more bare serpentine habitats than serpentine tolerators.

Alternatively, other factors, such as the time since divergence and the extent of spatial isolation, may better explain why lineages evolve to become endemics instead of tolerators. For example, the evolution of endemism may take

more time than the evolution of tolerance (Kay et al., 2011), or serpentine tolerators may represent a stage towards the evolution of endemism (Kruckeberg, 1986). Dispersal to more geographically distant serpentine habitats also may favor the evolution of endemism because of limited gene flow from off-serpentine populations (Kay et al., 2011). It is likely that these various factors are not mutually exclusive, but contribute in different proportions to what causes endemism over tolerance in different lineages.

We search for overarching patterns between evolutionarily independent endemic and tolerator lineages in order to better understand why edaphic divergence causes lineages to evolve into serpentine endemics or tolerators. We use replicated instances of serpentine soil adaptation across multiple families in the California flora to choose sister taxa pairs that have all undergone edaphic divergence but vary in whether that divergence is associated with serpentine endemism or serpentine tolerances (Figure 1.1A). We first ask whether endemic serpentine taxa occur in chemically harsher and/or more bare serpentine habitats than serpentine tolerators (i.e., as in Figure 1.1B). If adaptation to certain types of serpentine soils or bare microhabitats comes with stronger competitive ability trade-offs and if stronger trade-offs promote the evolution of serpentine endemics, then we predict endemics will occur in harsher serpentine habitats than tolerators. We also test the hypothesis that there is more habitat divergence between sister taxa of endemic pairs than sister taxa of tolerator pairs (i.e., as in Figure 1.1C). If

true, this greater habitat divergence could limit gene flow by selecting against migrants, promoting the isolation of endemic species.

MATERIALS AND METHODS

Study system—To assess whether serpentine endemics live in harsher serpentine habitats than serpentine tolerators, we chose one serpentine population from 8 endemic species and 9 tolerator species from which to characterize the habitat (Table 1.1; we hereafter use population and taxon interchangeably). To choose populations and species, we first generated a list of annual serpentine plants representing independent origins of serpentine tolerance or endemism that occur in the North, Central or South Coast Ranges of California. We generated this initial list using tables of serpentine affinity scores from Safford et al (2005), phylogenetic relationships and serpentine status of 23 genera generated by Anacker et al. (2011), and supplemental phylogenies for genera not included in Anacker et al. (2011) study (see Table 1.1 for species-specific citations). We chose to survey only annual taxa for more straight-forward metrics of fitness in subsequent transplant experiments.

We narrowed our list of serpentine taxa by searching for locally abundant populations in serpentine habitats at the UC McLaughlin Reserve in the North Coast Range, at Mt. Tamalpais in Marin Co., at serpentine grasslands in the West San Francisco Bay Area, in the Mt. Diablo Range, and in the iconic serpentine

barrens of New Idria in southern San Benito Co. Locality information for all taxa is provided in Table 1.1. The majority of our collections occurred at the UC McLaughlin Reserve, which spans a heterogeneous edaphic landscape with different kinds of serpentine and non-serpentine habitats. We chose serpentine species that were easy to access and had a nonserpentine sister taxon nearby to where we found the serpentine taxon (see below). When we found multiple serpentine populations per species, we chose the population that was the easiest to access and had the largest population size. Our final list of serpentine taxa spanned six plant families and nine genera.

In order to quantify habitat divergence within serpentine endemic and tolerator lineages with an evolutionarily relevant comparison, we compare the serpentine habitats of our serpentine taxa to non-serpentine habitats of putative sister taxa. For serpentine taxa of tolerator species we chose a non-serpentine population of the same species as the sister taxon. For serpentine taxa of endemic species we chose a non-serpentine population of the endemic's sister species as the sister taxon (Figure 1.1A). In all cases we selected our non-serpentine sister taxa by using occurrence data from CalFlora (<http://www.calflora.org>) to identify a non-serpentine taxon nearby its paired serpentine taxon in an effort to minimize differences in abiotic conditions other than soil chemistry and productivity, such as climate, between the sister taxa. However, due to restricted and allopatric ranges of sister taxa, the distance between our sister taxa varies (Table 1.1). We

use these metrics of geographic distance as covariates in our analyses of pairwise divergence (i.e., as in Figure 1.1C).

We use three of our nonserpentine sister taxa as the nonserpentine sister taxon in two pairs. For example, *Mimulus nudatus* is a serpentine endemic hypothesized to be derived from within *Mimulus guttatus* (Macnair and Gardner, 1998). We chose a *M. guttatus* nonserpentine population to serve as the nonserpentine sister taxon for *M. nudatus*. However, because *M. guttatus* is a serpentine tolerator itself, we also use the same nonserpentine population as the nonserpentine sister taxon for a serpentine taxon of *M. guttatus*. We use this same overlapping design for the *Collinsia greenei* - *C. sparsiflora* endemic pair and *C. sparsiflora* tolerator pair, and for the *Navarretia rosulata* – *N. heterodoxa* endemic pair and the *N. heterodoxa* tolerator pair. It is reasonable to assume that the endemic taxa (e.g., *M. nudatus*) evolved independently of the serpentine tolerator taxa (e.g., serpentine population of *M. guttatus*) from a similar nonserpentine ancestor (e.g., nonserpentine population of *M. guttatus*). Serpentine adaptation has been shown to evolve independently multiple times within tolerator species, e.g., within *Cerastium alpinum*, *Alyssum bertolonii*, the *Lasthenia californica* complex, *M. guttatus*, and *Arabidopsis lyrata* (Nyberg Berglund et al., 2001, 2004; Mengoni et al., 2003; Rajakaruna and Whitton, 2004; Turner et al., 2010; Selby, 2014; Selby and Willis, 2018). Likewise, independent origins of serpentine adaptation within tolerator species has led to the evolution of endemic species. For example, there are at least 3 local *Streptanthus* endemic

species hypothesized to be derived from the tolerator *S. glandulosa* (Kruckeberg, 1957), and there are two local/restricted *Mimulus* endemic species hypothesized to be derived from the tolerator *M. guttatus* (Macnair and Gardner, 1998).

Characterizing soil chemistry and texture—We characterized one pooled soil sample from each chosen population. The sample was pooled from five randomly chosen sub-locations within the plant population, each collected within the first 10 cm from the surface. We sent soils to the University of Maine Analytical Laboratory for chemical and texture analyses of the following: soil pH, calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K; neutral ammonium acetate extractions); calculated cation exchange capacity; electrical conductivity; nitrate (NO_3^-) and ammonium (NH_4^+) (KCl extraction); phosphorus (P), sulfur (S), boron (B) (modified Morgan extract, pH 4.8); micronutrients (zinc (Zn), manganese (Mn), iron (Fe), aluminum (Al), and copper (Cu)) and heavy metals (nickel (Ni), chromium (Cr), cobalt (Co; diethylenetriaminepentaacetic acid (DTPA) extraction); microbial activity (burst respiration method); and, particle size (percent clay, sand, and silt; determined by the hydrometer method, gravimetrically after wet sieving, and as the remainder in the sample, respectively).

Characterizing microhabitat bareness—We estimated percent bare ground within each population (Table 1.1) by centering a 25 cm x 25 cm quadrat

over 15 randomly selected individuals and using a point-intercept method to score each point for bare ground or vegetation. All but four taxa were sampled at 16 points per quadrat, and the rest were sampled at 28 points per quadrat. The variation in points sampled was due to a change in methodology. We explicitly incorporate this variation in points sampled per quadrat in our statistical models (see below). We did not have consistent sampling of microhabitat bareness for three populations: the *Navarretia rosulata* (endemic species) serpentine population, and both the serpentine and nonserpentine population of *N. heterodoxa* (tolerator species). Thus, these taxa are not included in the bare ground analyses, leaving the sample size at seven endemic taxa and eight tolerator taxa for the comparison of the serpentine taxa, and seven endemic pairs and eight tolerator pairs for the divergence in bare ground analyses.

Phylogenetic inference of serpentine taxa—We inferred phylogenetic relationships among our taxa so that our analyses could include an error term that accounts for the nonindependence of our data points due to relatedness (Felsenstein, 1985). We used ribosomal DNA, specifically the internal transcribed spacer 1 (ITS1), 5.8S rDNA subunit, and ITS2 sequences, to infer a phylogeny. We grew seeds collected from each population and extracted DNA with a modified Chelex extraction as in Yost et al., 2012. We amplified the ITS1, 5.8S rDNA and ITS2 regions with the ITS5 and ITS4 primers described in Baldwin (1992). PCR reactions consisted of 6.25 μ L GoTaq Colorless Master Mix

(Promega, Madison, Wisconsin, USA), 0.75 μ L each of the ITS5 and ITS4 primers, 1 μ L of DNA, and 3.75 μ L of water. The PCR program ran at 94°C for 1 min, followed by 25 cycles of 1 min at 94°C, 0.75 min at 49°C, and 0.75 min at 72°C, and finished with 72°C for 7 min. We cleaned PCR products with EXOSaP-IT (Affymetrix, Santa Clara, California, USA) and sent samples to the UC Berkeley Sequencing Facilities for Sanger Sequencing. We aligned sequences using MUSCLE (Edgar, 2004) in the Mesquite platform (Maddison and Maddison, 2018). We inferred Bayesian trees on the concatenated ITS1, 5.8S, and ITS2 sequences using the default settings in MrBayes (Ronquist et al., 2011), except that we used a GTR substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites. We enforced topological constraints in the MrBayes trees based on known relationships of the genera from the Angiosperm Phylogeny Website (Stevens, 2017). We ran four MCMC chains each for 200,000 cycles and discarded the first 50% of trees. Trace plots indicated that the chains mixed well and the potential scale reduction factor approached 1. We inputted the MrBayes 50% majority rule consensus tree (.con.tre file) to R using the read_annotated command in the R package phylotate v1.2. We ultrametricized our trees with the Grafen method (Grafen, 1989) using the compute.brlen function in ape v5.2 with power = 1 as in Mitchell et al. (2015). Because our bare ground analyses don't include two of the serpentine taxa (*Navarretia rosulata* (E) and *N. heterodoxa* (T)), we used the drop.tip function in ape v5.2 for the tree used in the bare ground analyses.

Habitat analyses—

Differences between endemics and tolerators in serpentine soil

harshness—We first asked whether endemic serpentine taxa occur in harsher serpentine soils than tolerator serpentine taxa (i.e., as in Figure 1.1B). We a priori parsed our soil variables down to just variables that are thought to be particularly challenging aspects of serpentine soils: Ca, Mg, Ca:Mg ratios, macronutrients (N in ammonium form, P, K), heavy metals (Ni, Cr, and Co), and texture (percent sand, silt and clay). All soil variables were log-transformed for normality. We included texture variables in an attempt to capture variation in the physical differences among serpentine soils. Coarser soils will have lower water-holding capacities and impose more drought-like, stressful conditions on the plants that grow there. We used individual phylogenetic generalized least squares (PGLS) models to test whether there was a difference between the endemic and tolerator serpentine taxa in each of the 12 soil variables. The PGLS models were implemented with the `gls` function in the package `nlme` v3.1-137. The correlation structure was made with the `corBrownian` function in `ape` v5.1, using the ultrametricized phylogenetic tree. We used a sequential Bonferroni correction to adjust p-values for multiple comparisons.

Differences between endemics and tolerators in serpentine microhabitat

bareness—Next, we asked whether endemic serpentine taxa occur in barer serpentine microhabitats than tolerator serpentine taxa (i.e., as in Figure 1.1B).

We constructed the following hierarchical Bayesian model that incorporates both the phylogenetic non-independence of data as well as within-population sampling variation:

$$y_{ij} \sim \text{binomial}(n, \phi_j), \quad (\text{Eq. 1})$$

$$\phi_j \sim \text{beta}(\alpha_j, \beta_j), \quad (\text{Eq. 2a})$$

$$\alpha_j = \frac{\mu_j^2 - \mu_j^3 - \mu_j \sigma^2}{\sigma^2}, \quad (\text{Eq. 2b})$$

$$\beta_j = \frac{\mu_j - 2\mu_j^2 + \mu_j^3 - \sigma^2 + \mu_j \sigma^2}{\sigma^2}, \quad (\text{Eq. 2c})$$

$$\mu_j = \text{inverse logit}(\beta_1 x_j + \beta_{0j}), \quad (\text{Eq. 3})$$

$$\beta_0 \sim \mathcal{N}(0, \Sigma) \quad (\text{Eq. 4})$$

Each observation y_{ij} is the number of points within quadrat i of taxon j that were recorded as bare ground. The term y_{ij} has a binomial probability distribution, where n represents the number of total points sampled for bare ground in quadrat i of taxon j , and ϕ_j is the probability of encountering bare ground in the taxon j 's habitat (Eq. 1). ϕ_j is interpreted as the “true” proportion of

bare ground within taxon j , inferred from the variation among all quadrats taken within taxon j . ϕ_j has a beta distribution (Eq. 2a), where the α_j and β_j parameters are calculated using moment matching from the mean (μ_j) and variance (σ^2) of the distribution (Eq. 2b, 2c). Modeling our observations, y_{ij} , as a random variable described by a binomial distribution incorporates sampling error, i.e., error that is due to the fact we only sampled a subset of the possible point space within each quadrat. Modeling our “true” probability of bare ground parameter, ϕ_j , as a beta distribution incorporates process error, i.e., error that is due to the fact that our deterministic model (Eq. 3) doesn’t include all parameters that influence the mean proportion bare ground within each taxon.

The expected value of ϕ_j (i.e., μ_j) is estimated from a deterministic model with a fixed effect (β_1) for whether the taxon is a serpentine endemic or serpentine tolerator (x_j) and a random phylogenetic effect (β_{0j}) (Lynch, 1991; Mitchell et al., 2015). Because μ_j describes the proportion of bare ground in taxon j (with values between 0 and 1), we took the inverse logit of our deterministic model. The random phylogenetic effect, β_0 , is estimated based on taxon identity and the phylogenetic relationship among taxa. β_0 is sampled from a multivariate normal distribution with a mean of 0 and variance proportional to Σ , which is the inverse of the coancestry matrix, G , of our taxa. We calculated G from the ultrametric phylogeny with the `v cv()` function from `ape` v5.1., as in Mitchell et al., (2011). The β_1 prior was sampled from a normal distribution with mean of 0 and a variance sampled from a uniform distribution with bounds (0,100). The prior we

used on σ^2 , the deterministic model error, was a uniform distribution with the bounds (0, 0.25). The bounds on the σ^2 prior were calculated such that σ^2 values would yield α and β parameters with the correct support (i.e., α and $\beta > 0$).

Here we are specifically interested in the posterior distribution of β_1 , which indicates the extent to which endemic serpentine taxa and tolerator serpentine taxa differ in their microhabitat bareness. If the β_1 parameter is greater than zero, then endemics occur in barer serpentine habitats than tolerators. We estimated the percent of the β_1 posterior distribution that is greater than 0 with the empirical cumulative distribution function in the R stats package v3.5. We implemented the model in JAGS v4-6, running the model on three chains over 60,000 MCMC generations, discarding the first 10,000 as burn-in. We combined the non-burn-in MCMC generations for each parameter into one vector, yielding posterior samples of 150,000 points. Gelman and Rubin's (1992) convergence diagnostic was equal to or less than 1.02 for all parameters, indicating satisfactory convergence.

Divergence in the soil environment between endemic and tolerator sister taxa—We quantify pairwise divergence (i.e., as in Figure 1.1C) in the soil environment in two ways. We first calculated pairwise divergence in the 12 individual harshness soil variables. Within each pair we divided the serpentine taxon's soil value by the nonserpentine taxon's value, and tested for differences between the proportional pairwise divergence in endemic pairs and tolerator pairs with individual PGLS models that include geographic distance between sister taxa

as a covariate. We used a sequential Bonferroni correction to adjust p-values for multiple tests across soil variables.

Second, because soil elements may be correlated, we also calculated a multivariate view of soil divergence between the serpentine and non-serpentine taxa of each pair using principal components analysis. All soil variables were centered to zero and scaled to have a unit variance. We inputted the 25 soil variables from all populations in the PCA and calculated Euclidean distances between the serpentine and nonserpentine sister taxa of each pair in 25-dimensional space. We tested whether the Euclidian distances separating sister taxa of endemic pairs is greater than the Euclidian distances separating sister taxa of tolerator pairs with a PGLS model that includes geographic distance between sister taxa as a covariate.

Divergence in microhabitat bareness between endemic and tolerator sister taxa—Lastly, we ask whether sister taxa of endemic pairs have more divergence in microhabitat bareness than sister taxa of pairs (i.e., as in Figure 1.1C). We constructed two hierarchical Bayesian models to 1) estimate the magnitude of divergence in bare ground within each one of our taxa pairs, and 2) test whether there is an effect of a pair being an endemic or tolerator pair after controlling for the phylogenetic relatedness of the pairs. In the first model estimate ϕ_j , which we interpret as the “true” proportion of bare ground within taxon j (Eq. 1). For each pair, k , we subtracted the ϕ_j value of the nonserpentine taxon from the ϕ_j value of the serpentine taxon (Eq. 5). We calculated pairwise

divergence in this direction because we predict that adaptation to serpentine is associated with adaptation to barer microhabitats than in nonserpentine habitats. We also calculated the mean pairwise divergence for all endemic pairs and all tolerator pairs, where m is a binary variable corresponding to whether a pair is an endemic or tolerator pair (Eq. 6).

$$pairwise.divergence_k = \phi_{kS} - \phi_{kNS} \quad (\text{Eq. 5})$$

$$pair.type.mean_m = \frac{\sum_{k=1}^{K_m} pairwise.divergence_k}{K_m} \quad (\text{Eq. 6})$$

We calculated the mean and variance of the posterior distributions of each pair's pairwise divergence (Eq. 7 and Eq. 8, respectively). The mean of the posterior distribution is the most probable estimate of the pairwise divergence and the variance of the posterior distribution reflects error due to our sampling method. We input both of these values into a second hierarchical Bayesian model that incorporates our original sampling error (Eq. 9), a deterministic model to test for the effects of endemism and tolerance on pairwise divergence (Eq. 11), and error associated with factors not captured in our deterministic model (Eq. 10):

$$PD_k = \text{mean}(pairwise.divergence_k) \quad (\text{Eq. 7})$$

$$var.PD_k = \text{variance}(pairwise.divergence_k) \quad (\text{Eq. 8})$$

$$PD_k \sim \text{normal}(\mu_k, \text{var. } PD_k) \quad (\text{Eq. 9})$$

$$\mu_k \sim \text{normal}(\alpha_k, \sigma^2) \quad (\text{Eq. 10})$$

$$\alpha_k = \beta_1 x_k + \beta_0 + \beta_2 z_k \quad (\text{Eq. 11})$$

The deterministic model (Eq. 11) is effectively the same as the deterministic model used to test for differences in habitat bareness between just the serpentine taxa of endemics and tolerators, except that it includes geographic distance between sister taxa (z_k) as a covariate. The β_1 coefficient quantifies the effect of a pair being an endemic or tolerator (x_k) on the expected pairwise divergence in habitat bareness (α_k). The β_1 coefficient was sampled from a normal distribution with a mean of 0 and a variance sampled from a uniform distribution with bounds (0,100). The variable intercept, β_0 , was estimated based on the phylogenetic relatedness of the pairs and was calculated here the same as in the previous model (i.e, Eq. 4).

We were specifically interested in the posterior distribution of β_1 . If the β_1 coefficient is greater than zero, endemic pairs have more pairwise divergence of habitat bareness in the expected direction than tolerator pairs (i.e., ϕ of the serpentine taxon is greater than the ϕ of the nonserpentine taxon). We implemented the first model in JAGS v4-6, running the model on three chains for

20,000 MCMC generations and discarding the first 10,000 as burn-in. We combined the remaining samples from all three chains, yielding posterior samples of 30,000 points. The Gelman and Rubin's (1992) convergence diagnostic was 1 for all parameters, indicating satisfactory convergence. We implemented the second model in the same fashion, although we ran the three chains for 200,000 MCMC generations, and discarded the first 100,000 as burn-in. The Gelman and Rubin's (1992) convergence diagnostic indicated satisfactory convergence.

Correlations between habitat variables— Lastly, we test for correlations between the habitat variables used in the above analyses, specifically between microhabitat bareness and the individual soil variables. We subset the data to test for correlations among habitat variables from just serpentine taxa, from just nonserpentine taxa, and then from all taxa. We use the `rcorr` function in R to calculate Pearson's r and asymptotic p-values between median ϕ_j values and soil variables from taxon j . We adjust p-values for multiple comparisons using a sequential Bonferroni correction.

RESULTS

Do serpentine endemics occur in chemically harsher serpentine soils than serpentine tolerators?—We find that endemic taxa occur on serpentine soils with an average of 25% less Ca than tolerators ((PGLS; $F_{1,14} = 17.45$, $P = 0.002$); Figure 1.2). However, there were no other statistically significant differences

between endemics and tolerators in any of the other soil harshness variables tested after correcting for multiple comparisons (Table 1.2).

Do serpentine endemics occur in barer serpentine microhabitats than serpentine tolerators?— When we compare the posterior distributions of ϕ_j , the estimated proportion of bare ground for taxon j , we find that endemic taxa are found in significantly barer serpentine microhabitats than tolerator taxa, although there is substantial variation among the taxa (Figure 1.3; Appendix S1 and S2; see Supplemental Data with this article). The β_1 parameter from our deterministic model is the extent to which endemics and tolerators differ in serpentine microhabitat bareness. 96.7% of the posterior distribution of β_1 is greater than zero, which we interpret as support that our endemic serpentine taxa occur in barer serpentine habitats than the tolerator serpentine taxa. Because we used an inverse logit transformation on the deterministic model, we interpret the value of the β_1 coefficient in terms of the odds of encountering bare ground over vegetated ground. The median value of the posterior distribution of β_1 is 0.76, and $e^{0.76} = 2.14$; thus, endemics have 2.14 times the probability of occurring in bare serpentine microsites compared to tolerators.

Do serpentine endemic sister taxa pairs have more divergence in soil chemistry than serpentine tolerator sister taxa pairs?—Pairwise divergence between sister taxa of endemic pairs and sister taxa of tolerator pairs does not

differ in the twelve soil harshness variables (Appendix S3, Appendix S4). The PCA of soil chemistry and texture of all populations used in this study show clustering of serpentine and non-serpentine taxa, respectively (Figure 1.4A). The first five principal components explained over 75% of the variation in the dataset, with Mn, Fe and pH loading the strongest on PC1 and Ca, Na and S loading the strongest on PC2 (Appendix S5). There is substantial variation among endemic and tolerator pairs in their Euclidean distance across 25-dimensional space (2-D distances indicated by lines in Figure 1.4A). The soil distances of the serpentine tolerator pairs did not differ from the soil distances of the serpentine endemic pairs, nor was there an effect of geographic distance between sister taxa pairs on soil distances (Figure 1.4B; PGLS, pair type $F_{1,14} = 0.475$, pair type $P = 0.502$, geographic distance $F_{1,14} = 1.603$, geographic distance $P = 0.226$).

Do endemic sister taxa pairs have more divergence in microhabitat bareness than tolerator sister taxa pairs?—Although there is substantial variation among our pairs in the amount of pairwise divergence in bare ground (Figure 1.5, Appendix S6, Appendix S7), the endemic pairs have a higher average pairwise divergence than the tolerator pairs (Figure 1.5, diamond points). All but one of the pairwise divergence posterior distributions of endemic sister taxa pairs are greater than 0, meaning that the serpentine taxon is in a barer microhabitat than the nonserpentine sister taxon. Three of the eight tolerator sister taxa pairs have pairwise divergence posterior distributions that are greater than zero, while

three of the tolerator pairs' posterior distributions overlap zero, indicating there is little to no divergence in microhabitat bareness between the sister taxa, and the remaining two tolerator pairs have posterior distributions that are less than zero, indicating that the nonserpentine taxon's habitat is barer than the paired serpentine taxon's habitat. Our deterministic model, which incorporates the phylogenetic relatedness among the pairs and the geographic distance between sister taxa pairs, indicates that there is an 83% chance that endemic sister taxa pairs have greater divergence in microhabitat bareness than tolerator pairs (i.e., 83% of the β_1 posterior distribution is greater than zero). The 95% credible intervals of the β_1 coefficient posterior distribution overlap with zero (lower and upper: -0.299, 0.866). The median value of the distribution is 0.27 which means that endemic serpentine taxa occur in, on average, 27% barer microhabitats relative to their nonserpentine sister taxon than tolerator serpentine taxa. There is no effect of geographic distance in this model – the β_2 posterior distribution is centered around zero (lower and upper 95% credible intervals: -0.026, 0.017).

Is microhabitat bareness correlated with soil variables?— Surprisingly, we find little correlation between microhabitat bareness and soil chemistry or texture variables (Appendix S8). In both the analyses with just serpentine taxa and all taxa, there are no significant correlations between microhabitat bareness and the soil variables after adjusting for multiple comparisons. In the analysis with

just nonserpentine taxa, microhabitat bareness is only significantly correlated with soil potassium (Pearson's $r = -0.77$).

DISCUSSION

Much of the rationale used to explain the evolution of habitat specialization is the existence of fitness trade-offs between habitat types (Futuyma and Moreno, 1988), and edaphic endemism is no exception (Rajakaruna, 2017). The primary hypothesis explaining the apparent specialization of serpentine endemics to serpentine substrates is a trade-off between serpentine tolerance and competitive ability that excludes endemic taxa from more productive nonserpentine areas. We characterized the habitats of 8 serpentine endemic sister taxa pairs and 9 serpentine tolerator sister taxa pairs to test for patterns of serpentine habitat use consistent with predictions from the trade-off hypothesis. We ask whether endemic serpentine taxa occur in more bare and chemically harsher serpentine habitats than tolerator serpentine taxa. We also ask whether endemic sister taxa pairs have undergone more habitat divergence than tolerator sister taxa pairs, because larger degrees of habitat divergence can drive larger fitness trade-offs and adaptive divergence, and contribute more to reproductive isolation (Funk et al., 2006). Below we highlight our main findings and discuss the implications for causes and consequences of serpentine endemism.

Our first main finding is that the endemic and tolerator serpentine taxa used in this study differ only in soil Ca out of the twelve soil harshness variables tested. On average, endemic serpentine taxa occurred in serpentine soils with 25% less Ca than the tolerator serpentine taxa, although the ranges were overlapping between the groups. For example, some endemic serpentine taxa had relatively high soil Ca levels (e.g., *Camissonia benetensis*, *Collomia diversifolia*, *Clarkia gracilis* ssp. *tracyi*) and some tolerator serpentine taxa had relatively low soil Ca levels (e.g., *Collinsia sparsiflora*, *Plantago erecta*, and *Collinsia heterophylla*). Interestingly, a t-test comparing serpentine soil Ca levels between endemics and tolerators does not show a significant difference (analysis not shown). The differences between the PGLS and t-test results indicate that the Ca levels of closely related endemic and tolerator species vary more than expected based on their relatedness.

It is important to note that soil Ca levels may not actually reflect the Ca tolerance range of an individual plant. For example, a species may be able to tolerate lower Ca levels than levels in the soil it occupies. Studies that link foliar and soil nutrient concentrations (e.g., Verboom et al., 2017) or experimentally test the lower Ca tolerance limits of the taxa are needed to understand whether the differences in soil Ca we see here translate to biologically meaningful differences. However, the differences in soil Ca are intriguing, given that Ca deficiency is often cited as the harshest chemical challenge in serpentine soils (Loew and May, 1901; Vlamis and Jenny, 1948; Kruckeberg, 1954; Walker et al., 1955) due to the

essential role Ca plays in cell signaling and cell wall formation (Brady et al., 2005; Palm and Van Volkenburgh, 2014). Nutrient amendment studies have highlighted Ca as the limiting factor affecting survival and growth of multiple agricultural and native species in serpentine soils (Walker, 1948; Vlamis, 1949; Kruckeberg, 1954; O'Dell and Claassen, 2006). Conversely, some serpentine adapted taxa show no growth response to increased Ca amendment in serpentine soils – likely due to the ability to regulate their internal Ca levels (Walker, 1948; Kruckeberg, 1954; O'Dell et al., 2006).

Despite finding endemic serpentine taxa occur in serpentine habitats with lower soil Ca, we do not find strong evidence that the amount of divergence in soil Ca between sister taxa is higher in endemic versus tolerator pairs. Pairwise divergence in soil Ca was marginally significant between endemic and tolerator pairs ($p = 0.07$), with tolerator sister taxa pairs having on average less divergence in Ca than endemic pairs. Because endemics occur in serpentine soils with less Ca, endemics may have evolved from nonserpentine taxa that were preadapted to low soil Ca. However, we do not find evidence that the soil Ca levels of endemic nonserpentine sister taxa are lower than those of tolerator nonserpentine sister taxa (results not shown). Interestingly, a study that used phylogenetic methods to reconstruct soil chemistry and serpentine use found no signal that preadaptation to low Ca levels facilitates shifts to serpentine in *Streptanthus* sensu lato (Cacho and Strauss, 2014), although they did not separate out shifts leading to tolerance versus endemism.

Our second main finding is that endemic serpentine taxa tend to occur in barer serpentine microhabitats than tolerator serpentine taxa. The most bare serpentine habitats (i.e., > 70% bareness) are occupied by serpentine endemics while the least bare areas (i.e., < 30% bareness) are occupied by serpentine tolerators, but there are both endemic and tolerator taxa in moderately bare habitats. For example, *Clarkia breweri* occurs on the barest serpentine habitat of all of the tolerator species (60% bareness), but this may reflect preadaptation, as the nonserpentine population also occurs in a bare, highly disturbed habitat – a pattern seen in the genus *Streptanthus* sensu lato (Cacho and Strauss, 2014).

Plants in bare microhabitats may be preadapted for, or subsequently adapt to, multiple non-mutually exclusive selective agents (Cacho and Strauss, 2014). Low plant densities can indicate a lack of facilitative interactions, greater plant apparency and herbivore pressure (Endara and Coley, 2011; Strauss and Cacho, 2013), greater UV radiation (Baskin and Baskin, 1988), and greater levels of disturbance (Rogers and Schumm, 1991). Bare areas also tend to be rocky habitats with low water holding capacity that impose drought-like conditions on resident plants (Baskin and Baskin, 1988; Rajakaruna et al., 2003; Brady et al., 2005; Cacho and Strauss, 2014; Kay et al., 2018). Although we didn't quantify the rockiness of our soils, personal observations in the field support a correlation between bareness and soil rockiness. The association of narrow ecological endemics and rocky, bare habitats has been documented in other parts of the world, e.g., in the stone plant family (Aizoaceae) of the Cape Floristic Province

(Ellis and Weis, 2006; Ellis et al., 2006), and in 20 congeneric pairs of taxa, spanning 17 angiosperm families, in the French Mediterranean region (Lavergne et al., 2004). Interestingly, we find that habitat bareness does not correlate with any of the soil chemistry or fine-texture variables we measured, indicating that features other than the soil chemistry *per se*, such as soil rockiness, contribute to the lack of vegetation in bare areas. It may be that adaptation to drought, or any of these other selective pressures, contributes to trade-offs in competitive ability instead of adaptation to serpentine soil chemistry. For example, shifts to earlier flowering times are common in serpentine plants (Rajakaruna, 2004; Wright, Davies, et al., 2006; Kay et al., 2011; Dittmar and Schemske, 2017) and are hypothesized to evolve as a mechanism to escape drought in rocky serpentine habitats (Brady et al., 2005; Ferris and Willis, 2018), but earlier flowering may come with a trade-off in growth that would be disadvantageous in a competitive environment. A QTL mapping study between *Microseris douglasii* (serpentine tolerator) and *M. bigelovii* (non-tolerator) found that earlier flowering and less leaf production mapped to the same QTL (Gailing et al., 2004), indicating a genetic basis for a trade-off that connects performance in drought and competitive environments.

Evidence for a trade-off between adaptation to bare microhabitats and competitive ability was found in the genus *Streptanthus* (Cacho and Strauss, 2014). If there is a similar relationship between microhabitat bareness and competitive ability in the taxa used in this study, then our results suggest that

endemic serpentine taxa have lower competitive abilities than tolerator serpentine taxa, a hypothesis that we are now testing with experimental studies of competitive ability *per se*. Nevertheless, that we find endemics occur in barer serpentine habitats than tolerators is an intriguing result, given that the competitive trade-off hypothesis is the main paradigm for the restriction of serpentine endemics, and yet there isn't much evidence to support a trade-off between serpentine tolerance and competitive ability. In particular, the few other studies that have compared the competitive abilities of serpentine endemics and tolerators have either inconclusive sample sizes (e.g., Powell and Knight, 2009) or have found inconsistent differences between endemics and tolerators in neighbor removal effects on fitness (e.g., Fernandez-Going and Harrison, 2013).

Similar to our results of pairwise divergence in soil Ca, we find a marginal trend that sister taxa of endemic pairs have more divergence in microhabitat bareness than sister taxa of tolerator pairs, with the serpentine taxon being in more bare microhabitats than the nonserpentine taxon. The probability that endemic sister taxa pairs have more divergence in bare ground than tolerator sister taxa pairs is 0.8, and the average effect size is 0.27 (i.e., divergence in percent bare ground between sister taxa increases by an added 27% in endemic pairs relative to tolerator pairs). When we run the same deterministic model without the phylogenetic correction, the median value of the pair type effect is the same (0.27) but there is stronger evidence for a significant effect of pair type (0.93 probability; results not shown). In contrast to the divergence in soil Ca results, the

discrepancy between models of divergence in bareness with and without the phylogenetic correction indicate that there is some phylogenetic signal in the extent to which lineages diverge in microhabitat bareness. For example, the four Onagrad species (*Clarkia breweri*, *C. concinna*, *C. gracilis ssp. tracyi*, and *Camissonia benitensis*) all show little to no divergence in habitat bareness.

Small degrees of divergence in microhabitat bareness suggests that, in some lineages, preadaptation to bare ground may facilitate transitions to serpentine soils, regardless of whether that leads to endemism or tolerance. Phylogenetic evidence revealed that preadaptation to bare ground facilitates shifts in the genus *Streptanthus* sensu lato (Cacho and Strauss, 2014). Another empirical study of paired endemic and widespread congeners in *Centaurea* and *Arenaria* found that the two species within each pair both occurred in rocky, open habitats and had similar competitive abilities (Imbert et al., 2012), indicating some level of preadaptation to rocky, open habitats was involved in the evolution of the endemic species. When preadaptation to bare habitats facilitates shifts to serpentine, we expect fitness trade-offs between adaptation to bare habitats and competitive ability to play a small role in the evolution of endemic species. It may be that other factors such as spatial isolation play an important role in the isolation of endemic species from their progenitors (e.g., in the *Streptanthus glandulosus* complex; Kruckeberg, 1957; Mayer et al., 1994; Mayer and Soltis, 1999).

In contrast, some pairs had very high levels of divergence in microhabitat bareness. Interestingly, the pair with the most divergence in bare ground is the

Layia discoidea (endemic) – *L. glandulosa* (non-tolerator) pair, which is one of our best examples of budding speciation (Crawford, 2010). *Layia discoidea* is phylogenetically nested within *L. glandulosa*, and most closely related to spatially proximal *L. glandulosa* populations that occur on relatively harsher soil substrates than other *L. glandulosa* populations (Baldwin, 2005). Because the *L. glandulosa* population we chose is from the populations closely related to *L. discoidea*, our data show microhabitat divergence was an important factor in speciation of *L. discoidea*.

A common feature of all of our results, whether comparing just serpentine taxa (i.e., as in Figure 1.1B) or pairwise divergence (i.e., as in Figure 1.1C) and whether comparing microhabitat bareness or soil chemistry, is that there is variation among endemic and tolerator taxa. We highlight three reasons for the variation among endemic and tolerator pairs. First, this variation could in part be due to the wide swath of angiosperm phylogenetic diversity that our taxa span – from families in the Rosids to families in the Asterids. Different lineages may be doing different things, although we account for that statistically with our phylogenetic corrections. Second, the variation among pairs may reflect our design of sampling one population per taxon. This sampling scheme assumes the variation in habitat features within a taxon is less than that between taxa, but this may not be the case for taxa with large ranges. Third, all of our analyses test for differences in endemics and tolerators, and there are multiple ways in which plants species can be serpentine tolerators. Tolerator species can be composed of

highly locally adapted populations or of highly plastic individuals that can live both on and off serpentine (Sexton et al., 2017), and variation along this spectrum is certainly seen in serpentine tolerator species (Kruckeberg, 1951, 1967; Wright, Stanton, et al., 2006; Branco, 2009; Baythavong and Stanton, 2010; Kay et al., 2011). We would predict that tolerators comprised of locally adapted populations would be intermediate to endemics and tolerators comprised of plastic individuals in terms of their habitat harshness and/or habitat divergence measures. Evidence from the literature supports this hypothesis for *C. sparsiflora* and *M. guttatus*, two of the tolerator pairs that show relatively high divergence in bare ground and strong local adaptation (Wright, Stanton, et al., 2006; Selby and Willis, 2018). Thus, grouping the tolerator species as we have done here likely makes our results conservative. Our pairwise divergence results that are marginally significant may have shown a more definitive trend if we were able to split our tolerator species. Our on-going work will quantify the degree of local adaptation in all of these species, and confirm whether locally adapted tolerator species actually do differ from endemics in costs associated with serpentine adaptation.

CONCLUSIONS

The serpentine endemics in this study generally occur in more bare serpentine habitats with lower Ca than serpentine tolerators. Serpentine endemism and tolerance have evolved independently across 39 plant families in California and in

at least 105 plant families worldwide. Given the divergent phylogenetic histories of serpentine plants and that there are multiple physiological pathways to serpentine tolerance, it comes as no surprise that we find variation among our serpentine tolerators and serpentine endemic taxa in the types of serpentine habitats in which they occur and in the relative habitat divergence that accompanies serpentine adaptation. With this expected variation, it is notable that we uncover general differences in the habitats of serpentine endemics and serpentine tolerators. Our sampling scheme focused on serpentine flora in primarily one region of California, and future work is needed to see these patterns are consistent across the whole California serpentine flora, the worldwide serpentine flora and other types of edaphic specialists. Furthermore, a central paradigm of ecological specialization is that adaptation comes with fitness trade-offs in alternate environments, although there is mixed empirical evidence to support this prediction. Our results suggest that some combination of constraints associated with adaptation to low soil Ca and/or bare microhabitats contributes to the apparent specialization of serpentine endemics. On-going experimental work with the serpentine and nonserpentine taxa used in this study will make the connections between microhabitat bareness, competitive ability and fitness trade-offs.

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Table 1.1: Serpentine (S) and nonserpentine (NS) taxa of nine tolerator pairs and eight endemic pairs used in this study. Species codes are provided for subsequent figures. The three nonserpentine taxa for which we use as the nonserpentine comparison for two pairs (i.e., *Collinsia sparsifolia*, *Navarretia heterodoxa*, *Mimulus guttatus*) are listed twice in the table – once with the respective tolerator taxa and once with the respective endemic taxa.

^aCitations used to determine serpentine status and sister taxa relationships:

¹Personal observation, ²Safford et al., 2005, ³Macnair and Gardner, 1998, ⁴Anacker et al., 2011, ⁵Spencer and Porter, 1997, ⁶Baldwin et al., 2012, ⁷Gottlieb and Weeden, 1979, ⁸Green, 2010, ⁹Baldwin, 2005, ¹⁰Baldwin et al., 2011, ¹¹Dick et al., 2014

^bCollection locations: ¹Lake Co: UC McLaughlin Reserve, ²Stanislaus Co: Del Puerto Canyon, ³Napa Co: UC McLaughlin Reserve, ⁴San Mateo Co: Edgewood County Park, ⁵Napa Co: Foote Botanical Preserve, ⁶Butte Co: Horncut, ⁷Marin Co: Carson Ridge, ⁸Napa Co: Foote Botanical Preserve, ⁹Butte Co: Paradise, ¹⁰Lake Co: Cobb Mountain, ¹¹San Benito Co: Clear Creak Mgt. Area, ¹²Napa Co: Knoxville Wildlife Reserve

Pair type ^a	Population origin ^b	Species	Latitude	Longitude	Species Code	Distance (km)
T ¹	S ¹	<i>Clarkia concinna</i>	38.82403	-122.35441	CACO	1.85
	NS ¹	<i>Clarkia concinna</i>	38.86488	-122.37175		
T ²	S ²	<i>Clarkia breweri</i>	37.41108	-121.43060	CABR	3.03
	NS ²	<i>Clarkia breweri</i>	37.42246	-121.34293		
T ²	S ¹	<i>Plantago erecta</i>	38.86189	-122.41630	PLER	1.75
	NS ³	<i>Plantago erecta</i>	38.85107	-122.36597		
Tr ³	S ¹	<i>Mimulus guttatus</i>	38.85797	-122.40932	MGUT	6.17
	NS ⁴	<i>Mimulus guttatus</i>	38.75227	-122.28473		
T ²	S ¹	<i>Collinsia sparsiflora</i>	38.86065	-122.41167	COSP	2.48
	NS ³	<i>Collinsia sparsiflora</i>	38.83809	-122.34328		
T ⁴	S ²	<i>Collinsia heterophylla</i>	37.41087	-121.43031	COHT	2.23
	NS ²	<i>Collinsia heterophylla</i>	37.42264	-121.36665		
T ²	S ¹	<i>Trifolium wildenovii</i>	38.85826	-122.40930	TWIL	1.57
	NS ³	<i>Trifolium wildenovii</i>	38.84920	-122.36364	D	
T ²	S ¹	<i>Navarretia pubescens</i>	38.85821	-122.40356	NAPB	1.44
	NS ³	<i>Navarretia pubescens</i>	38.84825	-122.36241		
T ¹	S ⁵	<i>Navarretia heterodoxa</i>	37.46160	-122.28256	NAHX	37.76
	NS ⁶	<i>Navarretia heterodoxa</i>	38.33951	-122.22940		
E ^{2,4,5}	S ¹	<i>Navarretia jepsonii</i>	38.85993	-122.41154	NAJP	37.58
	NS ⁷	<i>Navarretia heterandra</i>	39.36490	-121.49095	NAHN	
E ^{2,5,6}	S ⁸	<i>Navarretia rosulata</i>	37.96745	-122.62773	NARS	20.87
	NS ⁶	<i>Navarretia heterodoxa</i>	38.33951	-122.22940	NAHX	
E ^{2,7}	S ¹	<i>C. gracilis ssp tracyi</i>	38.86001	-122.41724	CAGT	52.05
	NS ¹⁰	<i>C. gracilis ssp albicaulis</i>	39.90193	-121.61798	CAGA	
E ^{2,8}	S ¹	<i>Collomia diversifolia</i>	38.86873	-122.38710	CLDV	11.06
	NS ¹¹	<i>Collomia heterophylla</i>	38.80779	-122.70823	CLHT	
E ⁹	S ¹²	<i>Layia discoidea</i>	36.37538	-120.73183	LADI	6.19
	NS ¹²	<i>Layia glandulosa</i>	36.27188	-120.61047	LAGL	
E ^{2,3}	S ¹	<i>Mimulus nudatus</i>	38.85968	-122.41819	MNUD	6.4
	NS ⁴	<i>Mimulus guttatus</i>	38.75227	-122.28473	MGUT	
E ^{2,10}	S ³	<i>Collinsia greenei</i>	38.84411	-122.37722	COGR	1.17
	NS ³	<i>Collinsia sparsiflora</i>	38.83809	-122.34328	COSP	
E ^{2,11}	S ¹²	<i>Camissonia benitensis</i>	36.35913	-120.76188	CABE	4.58
	NS ¹²	<i>Camissonia strigulosa</i>	36.32100	-120.63835	CAST	

Table 1.2: Endemic serpentine taxa occur in serpentine soils with lower Ca levels than tolerator serpentine taxa. Results from phylogenetic generalized least squares models for the 12 soil harshness variables. The β_1 coefficient indicates the effect of tolerance compared to endemism on the log-transformed variables. All F-statistics are drawn from a $F_{1,15}$ distribution. P-values in bold are those with a significant effect of endemism or tolerance after sequential Bonferroni corrections.

Variable type	Soil variable	β_1	F statistic	P-value
<i>Calcium and magnesium</i>	Ca:Mg	0.720	6.172	0.025
	Mg	-0.222	0.628	0.440
	Ca	0.498	13.346	0.002
<i>Macronutrients</i>	N	0.390	2.482	0.136
	P	0.081	0.253	0.623
	K	0.370	1.444	0.248
<i>Heavy metals</i>	Ni	0.455	1.108	0.309
	Cr	0.260	3.063	0.100
	Co	0.746	6.989	0.018
<i>Texture</i>	% Sand	-0.111	0.660	0.429
	% Silt	0.083	0.279	0.605
	% Clay	0.262	1.461	0.246

Figure 1.1: Conceptual diagram of the experimental design (A) and two comparisons used throughout the analyses (B, C). Our experimental design (A) consists of sister taxa pairs. Each pair contains a taxon from serpentine soil and a taxon from nonserpentine soil, but the pairs differ in whether they are endemic or tolerator pairs. Half of our analyses compare habitat features (soil chemistry and microhabitat bareness) between the endemic serpentine taxa and tolerator serpentine taxa (B). The other half of our analyses compare pairwise divergence in habitat features between sister taxa of endemic pairs and sister taxa of tolerator pairs (C).

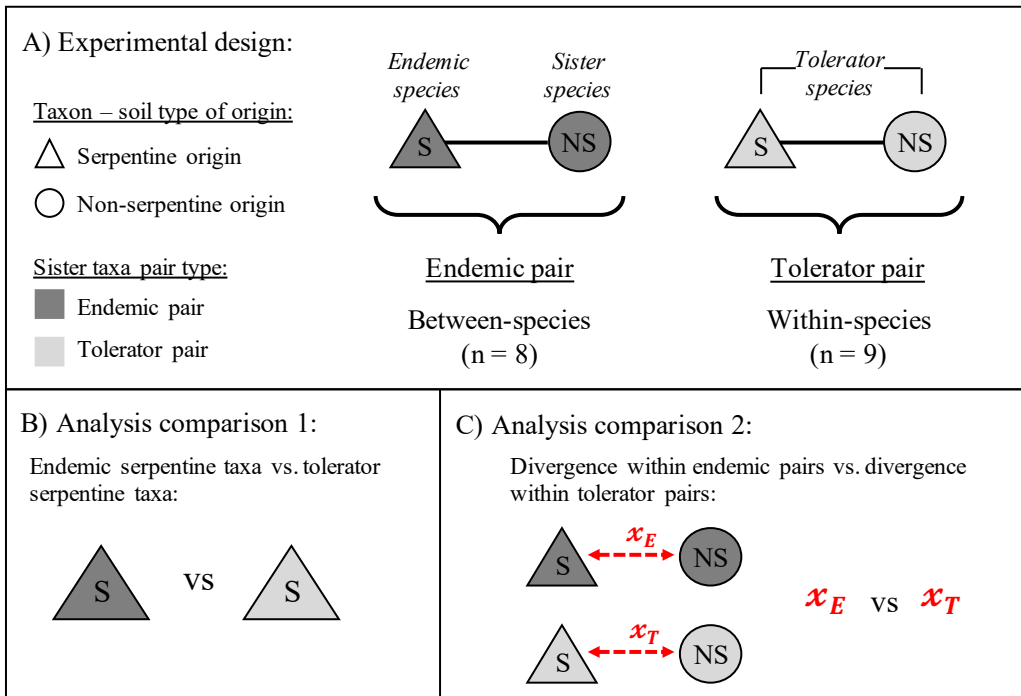


Figure 1.2: Differences in soil harshness variables between endemic and tolerator serpentine taxa. The lower and upper edges of the boxplots show the first and third quartiles, respectively, and points indicate data points that are farther than 1.5 times the interquartile range. Only Ca is statistically different between endemics and tolerators (*).

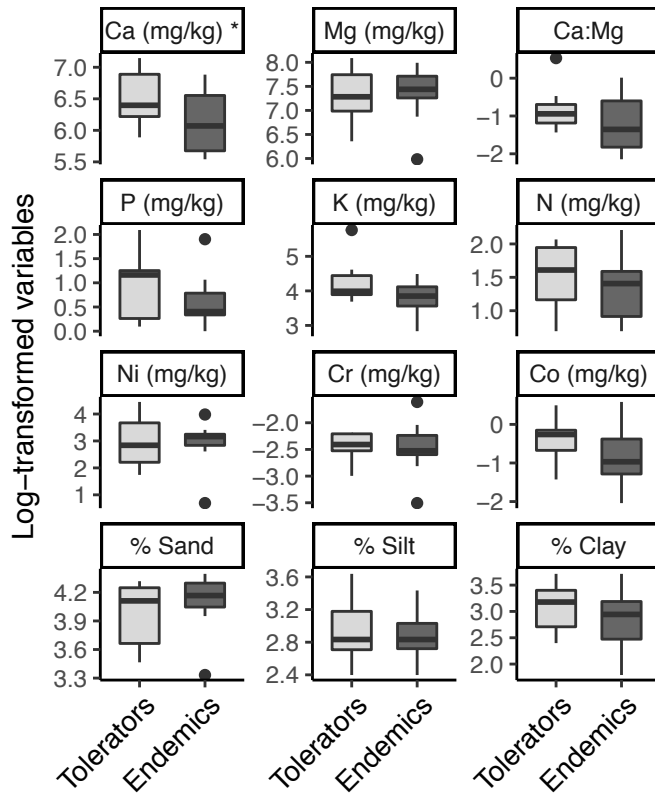


Figure 1.3: Endemic serpentine taxa occur in barer serpentine habitats than tolerator serpentine taxa, in a model that incorporates phylogenetic relatedness. Lines show the individual posterior distributions of the ϕ parameter, i.e., the estimated proportion of bare ground, for the 8 tolerator serpentine taxa and 7 endemic serpentine taxa.

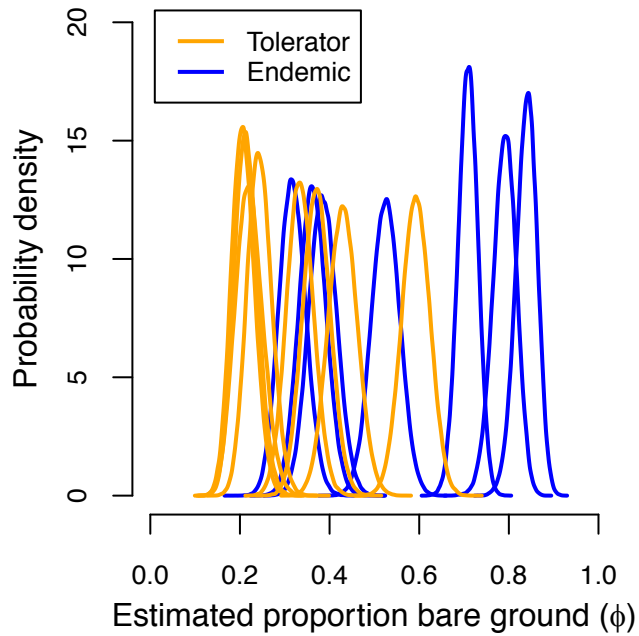


Figure 1.4: Divergence in the multivariate soil environment. A) The principal component analysis that includes all soil variables. Each point is one taxon. Shapes differentiate the soil the taxon is from and colors indicate whether the taxon is part of an endemic or tolerator pair. Solid lines connect members of endemic pairs and dashed lines connect members of tolerator pairs. B) Box plots showing the variation in Euclidean distances of the endemic and tolerator pairs. There is no difference in the degree of multivariate soil divergence between the two pair types.

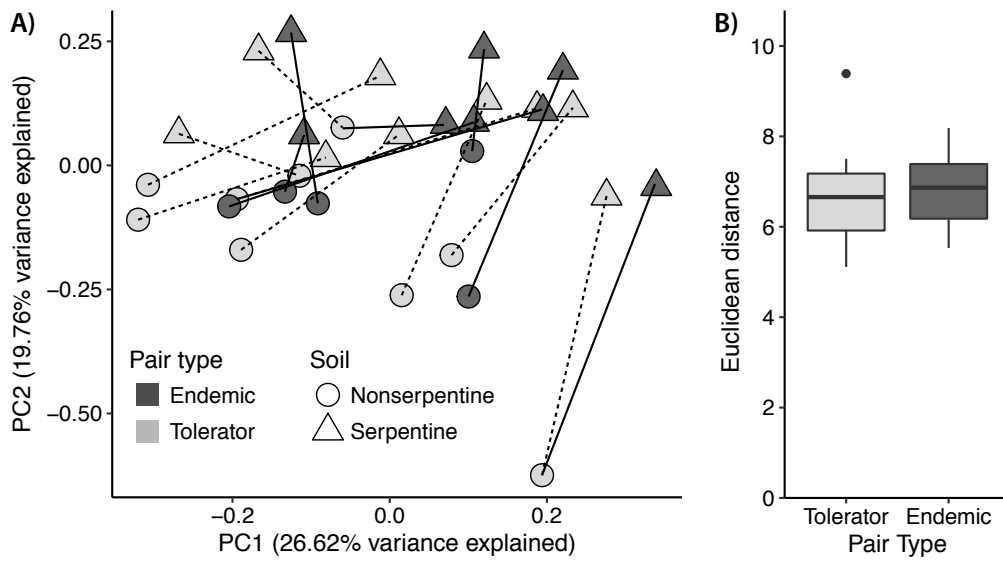
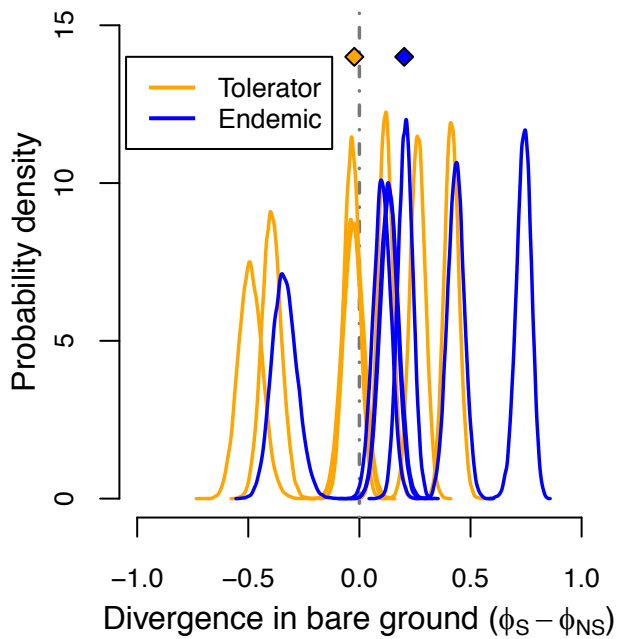
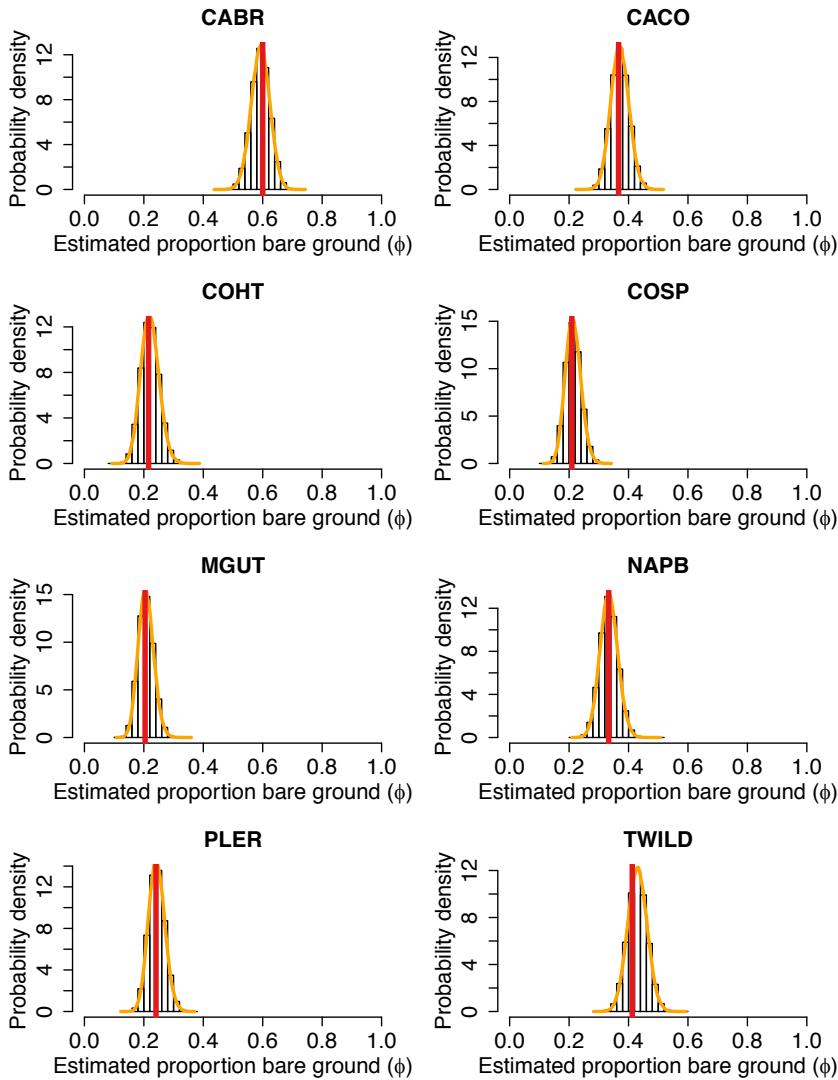


Figure 1.5: Endemic sister taxa pairs tend to have more pairwise divergence in bare ground (i.e., $\phi_S - \phi_{NS}$) than tolerator sister taxa pairs. A model that incorporates relatedness among pairs indicates there is a probability of 0.83 that endemic sister taxa pairs have more divergence in bare ground than tolerator sister taxa pairs. Curves are posterior distributions of estimated pairwise divergence in bare ground for the 8 serpentine tolerator pairs and 7 serpentine endemic pairs. The vertical dashed line indicates a difference in bare ground between serpentine and nonserpentine sister taxa of 0. The two diamonds are means of posterior distributions of the mean pairwise divergence among endemic pairs (blue) and tolerator pairs (orange).

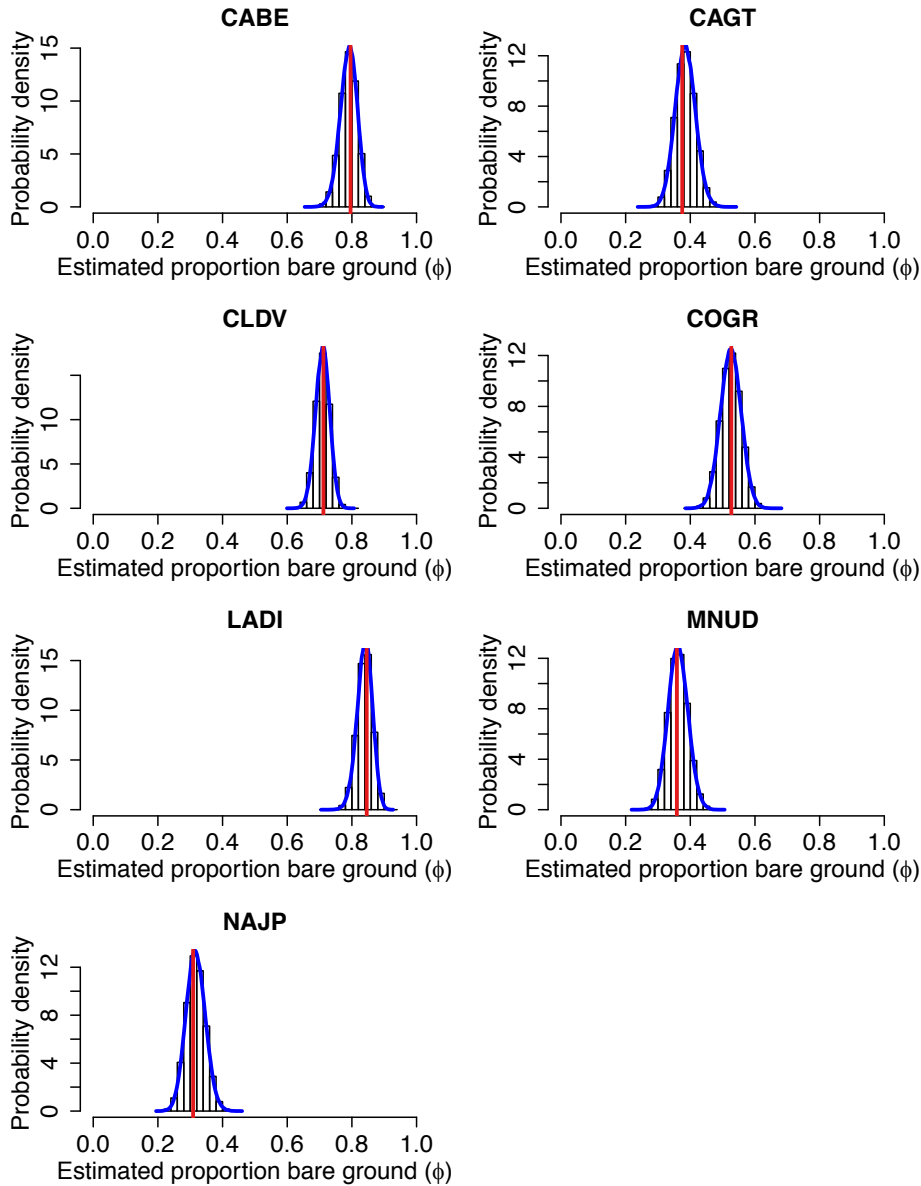


APPENDIX

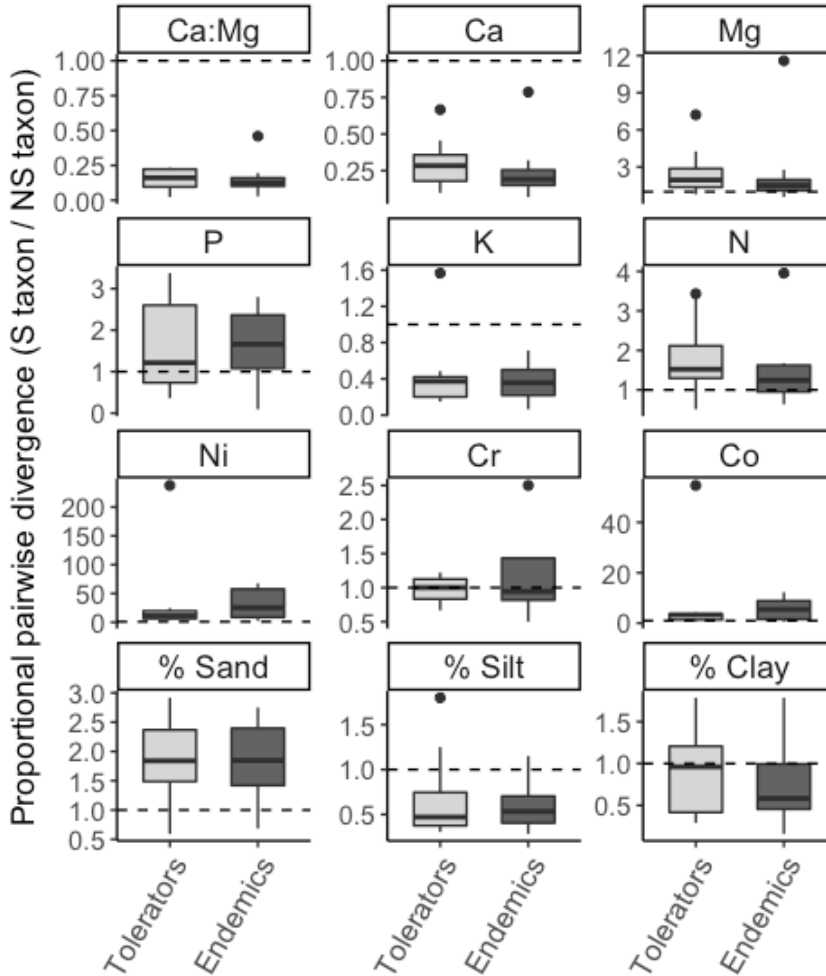
Appendix S1: Individual posterior distributions of estimated proportion bare ground, ϕ , for every tolerator serpentine taxon. The posterior distribution is represented by the histogram and orange probability density function. The red line indicates the empirical mean habitat bareness that is calculated from the proportion bare ground in each of the 15 quadrats.



Appendix S2: Individual posterior distributions of estimated proportion bare ground, ϕ , for every endemic serpentine taxon. The posterior distribution is represented by the histogram and blue density curve. The red line indicates the empirical mean habitat bareness that is calculated as the proportion bare ground in each of the 15 quadrats.



Appendix S3: Endemic and tolerator pairs do not differ in proportional pairwise divergence in individual soil harshness variables. Pairwise differences are calculated by dividing the serpentine taxon soil value by the nonserpentine sister taxon soil value. The dashed line in each graph is at 1. The greater the absolute distance from the dotted line, the more divergence between the two populations. The lower and upper edges of the boxplots show the first and third quartiles, respectively, and points indicate data points that are farther than 1.5 times the interquartile range.



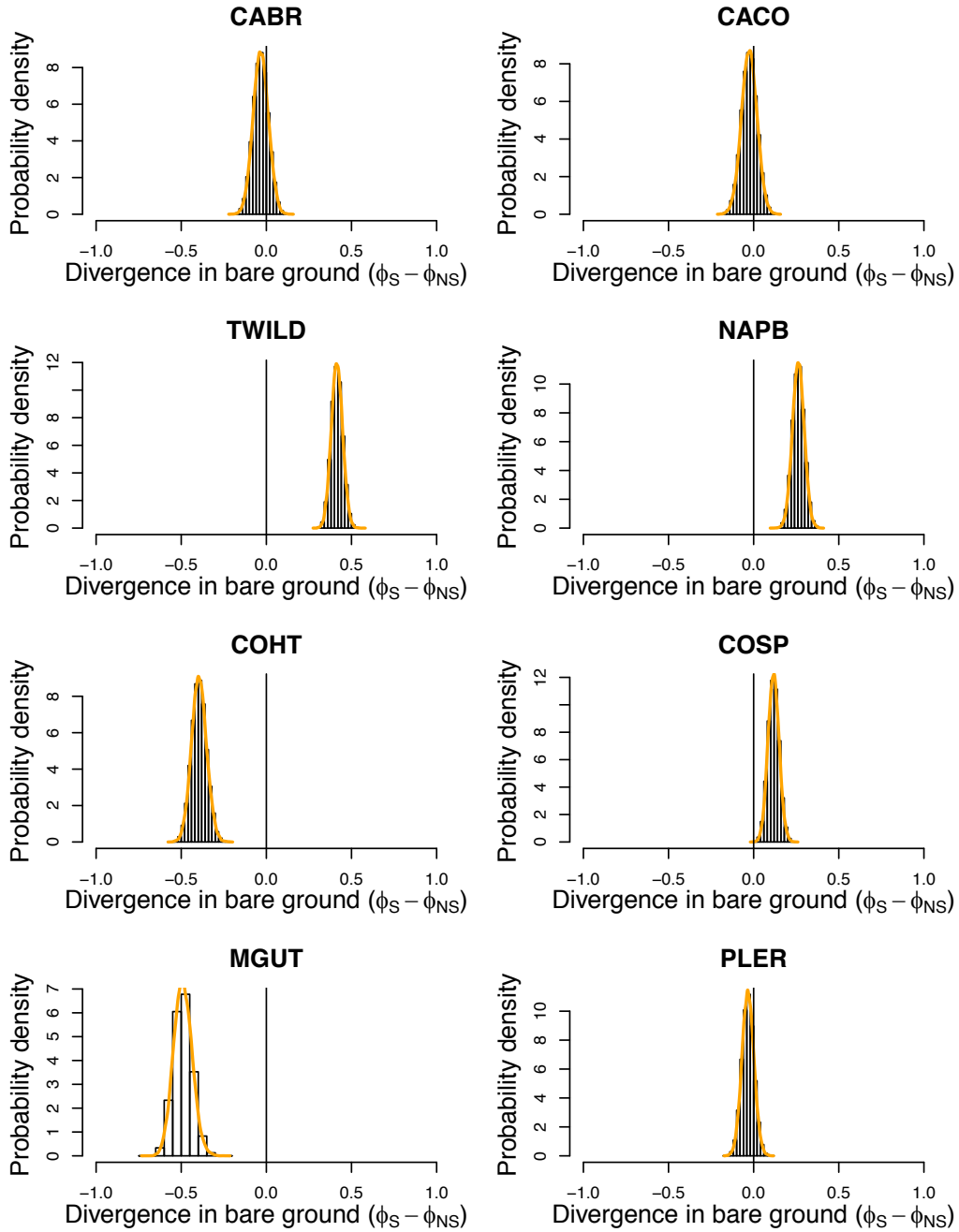
Appendix S4: Phylogenetic generalized least squares model results of proportional pairwise divergence in 12 soil harshness variables indicate no differences between endemic and tolerator sister taxa pairs. Geographic distance between each sister taxa pair is used as a covariate. All F-statistics are drawn from a $F_{1,15}$ distribution. P-values are for the pair type effect. Sequential Bonferroni corrections were used to account for multiple comparisons.

Variable type	Soil variable	Pair type		Geographic distance	
		F statistic	P-value	F statistic	P-value
<i>Calcium and magnesium</i>	Ca:Mg	0.389	0.642	0.440	0.518
	Mg	0.138	0.666	0.002	0.969
	Ca	3.701	0.079	0.145	0.709
<i>Macronutrients</i>	N	6.170	0.022	0.478	0.501
	P	0.019	0.852	0.071	0.794
	K	0.280	0.628	0.013	0.912
<i>Heavy metals</i>	Ni	3.864	0.028	6.457	0.024
	Cr	0.135	0.768	0.083	0.778
	Co	4.689	0.024	3.865	0.069
<i>Texture</i>	% Sand	0.055	0.654	1.248	0.283
	% Silt	1.245	0.180	2.461	0.139
	% Clay	0.223	0.644	1.625	0.223

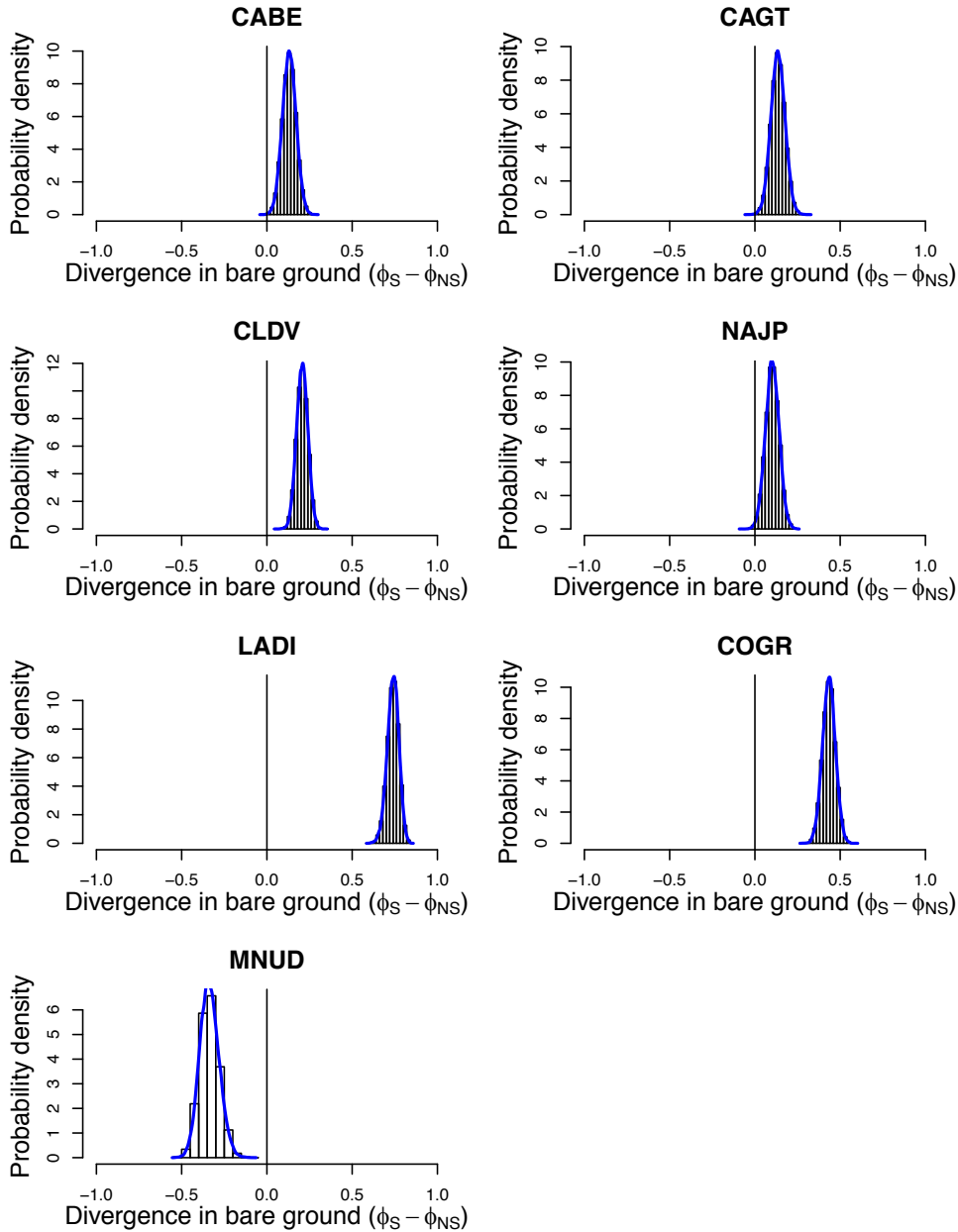
Appendix S5: Loading matrix of soil variation among all of the serpentine and nonserpentine taxa for the first five principle components, which explain over 75% of the variation in the dataset.

Soil Variable	PC1	PC2	PC3	PC4	PC5
Mn	-0.35417	0.03588	0.08502	-0.01664	0.05573
Fe	-0.31714	0.12095	-0.00307	0.18988	-0.02534
Soil pH	0.29139	-0.08308	0.18445	-0.21012	0.22158
Burst respiration	-0.27649	0.03523	0.05027	0.02347	0.30275
Percent sand	0.27179	0.25826	-0.02314	0.11963	0.10055
K	-0.26446	-0.18251	-0.11038	0.06607	0.20665
Cu	-0.26050	-0.06865	0.10671	-0.23286	-0.16613
Percent clay	-0.24706	-0.18883	0.14951	-0.21876	-0.22073
Zn	-0.23651	-0.01055	-0.13833	0.39107	0.15940
Percent silt	-0.22629	-0.25102	-0.09231	-0.01044	0.01952
N (NH ₄ ⁺)	-0.18641	0.22685	0.26026	0.04615	0.28890
Estimated CEC	-0.17853	-0.24401	0.16216	-0.30267	0.09129
Co	-0.17238	0.24478	0.28523	-0.03138	0.13511
B	0.16670	-0.27438	0.22654	0.14838	0.25997
P	0.15875	-0.13077	0.03282	-0.20608	0.47638
Cr	-0.15113	0.19768	-0.02773	0.08977	0.11389
Al	-0.14450	-0.06182	-0.24791	0.12394	-0.24180
Ca	-0.13519	-0.30233	-0.22397	-0.20307	0.15820
Ni	-0.08554	0.23463	0.29666	-0.07187	-0.04262
Electric conductivity	0.06983	-0.29207	0.27414	0.32488	0.01422
Mg	-0.05875	0.04110	0.42249	-0.15431	-0.06030
NO ₃ ⁻	-0.05140	0.03998	-0.06518	0.30620	0.32350
Na	0.04619	-0.31439	0.25298	0.24786	-0.16587
S	0.03226	-0.31027	0.22914	0.31853	-0.11357
Ca:Mg	0.00171	-0.18997	-0.28198	-0.17357	0.22094

Appendix S6: Individual posterior distributions of the pairwise divergence in bare ground (i.e., $\phi_S - \phi_{NS}$ for each pair) for all tolerator pairs. Plot titles indicate the serpentine taxon of each pair.



Appendix S7: Individual posterior distributions of the pairwise divergence in bare ground (i.e., $\phi_S - \phi_{NS}$ for each pair) for all endemic pairs. The plot titles indicate the serpentine taxon of each pair.



Appendix S8: Correlation coefficients between a taxon's estimated microhabitat bareness (ϕ_j) and soil characteristics. The correlations were analyzed with just taxa from serpentine habitats, with just taxa from nonserpentine habitats, and then with all of the taxa. We bold correlation coefficients with an absolute value greater than 0.5. Within each subset we adjusted significance levels of p-values; p-values in bold are still significant after correcting for multiple comparisons. ECEC = estimated cation exchange capacity.

Soil variable	<i>Serpentine taxa</i>		<i>Nonserpentine taxa</i>		<i>All taxa</i>	
	Pearson's r	p-value	Pearson's r	p-value	Pearson's r	p-value
Ca:Mg	0.508	0.053	0.428	0.145	0.209	0.287
Mg	-0.386	0.155	-0.310	0.302	-0.225	0.249
Ca	0.151	0.592	0.078	0.800	-0.124	0.529
N (NH4)	-0.022	0.937	-0.657	0.015	-0.215	0.272
N (NO3)	-0.313	0.256	-0.362	0.225	-0.302	0.118
P	-0.427	0.112	-0.088	0.775	-0.223	0.254
K	-0.234	0.402	-0.770	0.002	-0.536	0.003
S	-0.386	0.155	0.238	0.434	0.096	0.625
Na	-0.482	0.069	0.345	0.248	0.142	0.471
Al	-0.036	0.898	-0.117	0.703	-0.172	0.380
B	-0.415	0.124	0.336	0.261	-0.007	0.972
Mn	-0.015	0.959	-0.625	0.022	-0.405	0.032
Fe	0.130	0.645	-0.564	0.045	-0.245	0.208
Cu	-0.131	0.642	-0.430	0.143	-0.355	0.064
Zn	0.255	0.359	-0.563	0.045	-0.353	0.066
Ni	0.143	0.611	-0.682	0.010	0.158	0.421
Cr	0.562	0.029	-0.209	0.493	0.278	0.153
Co	0.073	0.795	-0.603	0.029	-0.032	0.870
% Silt	-0.308	0.263	-0.501	0.081	-0.420	0.026
% Sand	0.349	0.203	0.517	0.070	0.405	0.033
% Clay	-0.282	0.308	-0.061	0.844	-0.255	0.190
pH	-0.076	0.788	0.411	0.163	0.271	0.163
ECEC	-0.327	0.234	-0.209	0.493	-0.325	0.092
Electrical conductivity	-0.583	0.023	0.287	0.342	0.080	0.687
Microbial activity	-0.241	0.388	-0.420	0.153	-0.365	0.056

Chapter 2

Across the speciation continuum: genetically-based flowering time shifts evolve early in speciation following adaptation to serpentine soils

ABSTRACT

Speciation is the process by which reproductive isolation evolves between taxa, and understanding the relative importance of different reproductive isolating mechanisms remains an outstanding challenge in evolutionary biology. Studying a single component of reproductive isolation across taxa at different stages of divergence gives insight into its relative importance to speciation. Here, we study the evolution of phenological isolation, specifically shifts in flowering time, among sister taxa that have diverged according to a similar selective pressure but that differ in their progress towards speciation. We focus on 17 plant species that have adapted to chemically and physically harsh serpentine soils, but vary in whether that adaptation has led to population-level divergence or speciation. We pair a serpentine population within each species with a nonserpentine sister taxon to form sister taxa pairs that vary in their progress toward speciation. We use a greenhouse-based reciprocal transplant experiment in field soil to quantify how often flowering time shifts accompany adaptation to serpentine, when flowering time shifts evolve in the speciation process, and the extent to which genetic change versus plasticity contributes to phenological isolation. We find that genetically-based shifts in flowering onset in serpentine-adapted taxa are common, with the majority of serpentine taxa flowering later than their paired

nonserpentine sister taxa, in contrast to the current paradigm of serpentine taxa flowering early to escape drought conditions in rocky soil. Plasticity in serpentine flowering onset increases flowering onset shifts in 7 of the sister taxa pairs, but the degree of plasticity does not differ between divergent populations and sister species. The magnitude of flowering time shifts varies among all of the pairs, but on average the within-species sister taxa pairs have similar levels of phenological isolation as the between-species sister taxa pairs. Our results suggest that genetically-based changes in flowering time evolve early in the speciation process but are not strong enough to confer full reproductive isolation. Additional reproductive barriers such as spatial isolation and habitat isolation are likely important barriers that combine with phenological isolation to drive speciation of serpentine endemics.

INTRODUCTION

A major goal of speciation research is to understand the relative importance of different types of reproductive isolating mechanisms, both across taxa and at different time points during the speciation process (Sobel et al., 2010; Butlin et al., 2012; Christie and Strauss, 2018). Ecological divergence plays a large role in the origin of species (Darwin, 1859; Schluter, 2001; Sobel et al., 2010), evidenced by higher levels of ecologically-driven prezygotic isolation versus intrinsic postzygotic isolation in many systems (McKinnon and Rundle, 2002; Nosil, 2007; Lowry, Modliszewski, et al., 2008). Phenological isolation, or

reproductive isolation due to differences in mating phenology, has the potential to be an important barrier in speciation following ecological divergence because mating cues are often tied to environmental factors (Rathcke and Lacey, 1985) and shifts in mating phenology automatically confer assortative mating (Stam, 1983; Fox, 2003). However, mating phenology often has a plastic component (Nussey et al., 2007; Levin, 2009; Anderson et al., 2012; Porlier et al., 2012), and if so, its contribution to RI may be ephemeral when ecological conditions change. Understanding the importance of phenological isolation at different stages in the speciation continuum requires understanding how often, at what stage, and to what degree genetically-based changes in phenology evolve following ecological divergence.

In plants, temporal isolation is manifested through flowering times shifts, i.e., changes in the onset and/or duration of flowering. Artificial selection (Sheth and Angert, 2016), reciprocal transplant (Nagy, 1997; Eckhart et al., 2004; Etterson, 2004; Colautti and Barrett, 2010), and resurrection (Franks et al., 2007) experiments demonstrate that populations harbor genetic variation for flowering time and that divergent selection can cause rapid flowering time shifts, with stronger environmental differences hypothesized to lead to larger flowering time shifts (Levin, 2009). While multiple biotic (Elzinga et al., 2007; Devaux and Lande, 2009) and abiotic selective agents (Hall and Willis, 2006; Franks et al., 2007; Jordan et al., 2015) act on flowering time, the edaphic environment is a primary driver of flowering time shifts because of its intimate role in plant water

and nutrient uptake. Discrete edaphic boundaries can cause shifts in flowering time at small spatial scales (Wright et al., 2006; Dittmar and Schemske, 2017). Indeed, some of our best cases of potential parapatric speciation mediated by phenological isolation involves divergence across edaphic substrates (McNeilly and Antonovics, 1968; Savolainen et al., 2006). However, flowering time shifts also are seen among edaphic ecotypes within species (Rajakaruna, 2004; Kay et al., 2011), indicating that phenological isolation may play an important role early in the speciation process.

Whereas flowering time shifts may indicate genic adaptation, they may also result from plasticity. As plants colonize a new edaphic environment, water and nutrient limitation and differences in exposure can induce plasticity in flowering times (Levin, 2009; Franks, 2011; Jordan et al., 2015), although it may not always be adaptive. Flowering time plasticity may serve to reproductively isolate populations in novel habitats from source populations, allowing colonizers to establish and adapt to their environment (Kirkpatrick and Ravigne, 2002; Levin, 2009). Yet, isolation due to plasticity is ephemeral given a change in the ecological context. For example, if seeds disperse between habitats and migrants survive to flower, migrants will have similar flowering schedules as local plants (Figure 2.1). Thus, while plastic flowering shifts may help promote niche expansion and local adaptation, they may not confer enough reproductive isolation to promote speciation. Conversely, if strong selection against migrants accompanies ecological divergence, plasticity in flowering times may act

synergistically to isolate taxa. It is therefore important to determine the relative roles of plasticity versus genetic differentiation in flowering time at different stages of speciation.

Plants adapted to serpentine soils present an excellent opportunity to study the importance and evolution of phenological isolation following ecological divergence along the speciation continuum. Serpentine soils are chemically and physically harsh substrates, imposing strong divergent selection across sharp ecological gradients (Brady et al., 2005; Kay et al., 2011). Replicated adaptive divergence across serpentine and non-serpentine boundaries has occurred in 39 families within California (Anacker, 2011), and has led to the evolution of species with populations on and off serpentine (i.e., “tolerator” species) and species that only occur on serpentine (i.e., endemic species). Tolerators and endemics represent a range of divergence in response to similar ecological pressures, from population divergence to speciation (Kruckeberg, 1986), allowing us to examine phenological isolation across the speciation continuum. Moreover, shifts in flowering time are often noted in serpentine systems, with shifts to earlier flowering in annuals hypothesized as a way to escape the drought-inducing conditions of rocky serpentine soils (Schmitt, 1983; Brady et al., 2005; Dittmar and Schemske, 2017). However, it is unknown whether flowering time shifts commonly promote adaptive divergence or speciation.

Here, we take a comparative approach to understanding how often flowering time shifts evolve following edaphic divergence and how the strength

and permanence of phenological isolation may change along the speciation continuum. We hypothesize that strong, genetically-based flowering time shifts promote speciation of serpentine endemics, and thus predict that edaphic ecotypes within serpentine tolerators will experience weaker and more plastic flowering time shifts, and have less phenological isolation, than endemic species in comparison to their sister species. We select 17 sister taxa pairs that span a large range of eudicot angiosperm diversity, each of which comprises a serpentine and nonserpentine population. Half of the pairs consist of serpentine and nonserpentine populations from within the same tolerator species, representing ecological divergence that has not led to speciation. The other half of the pairs comprise a serpentine endemic population and a nonserpentine population from its sister species, representing ecological divergence followed by speciation. We use a greenhouse-based reciprocal transplant experiment in field-collected soil to quantify differences in both flowering onset and the full distribution of flowering times between sister taxa of endemic and tolerator pairs.

We first ask whether shifts to earlier flowering are common following adaptation to serpentine. We then compare the strength of flowering onset shifts in tolerator versus endemic sister taxa pairs to understand whether flowering onset shifts evolve earlier or later in the speciation process. We compare the magnitude of flowering onset shifts between members of each pair when they are grown in their home soils versus in a common soil environment to determine the degree to which flowering time shifts in serpentine taxa are plastic versus genetically-based.

To understand how plasticity in serpentine taxa may have evolved, we characterize plasticity in nonserpentine taxa as a proxy for ancestral plastic phenotypes within each pair. Given the stress of serpentine soils, plasticity in flowering time may be a maladaptive developmental response. We further explore whether plasticity in flowering onset is adaptive by measuring phenotypic selection on flowering onset. We test whether endemic pairs have greater phenological isolation than tolerator pairs, taking into account the full distribution of flowering times, both when sister taxa are in their home soils (where genetic differentiation and plasticity contribute to phenological isolation) and in a common nonserpentine soil (where just genetic differentiation contributes to phenological isolation). We're specifically interested in whether endemic pairs have more permanent phenological isolation across these two ecological contexts. Lastly, we use two quantitative metrics of ecological divergence – multivariate soil divergence and multivariate climatic divergence – to understand if flowering time shifts are greater upon adaptation to more divergent habitats.

METHODS

Study system and sister taxa pair selection— We chose 9 tolerator sister taxa pairs and 8 endemic sister taxa pairs that represent independent origins of serpentine adaptation (Table 2.1). Each taxa pair is comprised of one serpentine and one nonserpentine populations. To choose populations and species, we generated a list of annual serpentine tolerators and serpentine endemics that occur

in the coast ranges of California. We used a mix of serpentine affinity scores from Safford et al (2005), phylogenetic relationships and serpentine status of 23 genera generated by Anacker et al. (2011), and supplemental phylogenies for genera not included in Anacker et al. (2011) study (see Table 2.1 for species-specific citations) to generate the initial list. We searched for locally abundant populations in serpentine habitats at the UC McLaughlin Reserve, Mt. Tamalpais in Marin Co, serpentine grasslands in the west San Francisco Bay area, in the Mt. Diablo Range, and in the serpentine barrens of New Idria in southern San Benito Co. We chose serpentine taxa that were easy to access and had a nonserpentine sister taxon nearby to where we found the serpentine taxon. We searched for a spatially proximate non-serpentine sister taxon using CalFlora occurrence data to minimize environmental differences other than the edaphic habitat. However, due to allopatric distributions our sister taxa pairs vary in geographic distance (Table 2.1). When we found multiple populations per taxon, we chose the population that was the easiest to access and had the largest population size. Our final list of sister taxa pairs spans six plant families and nine genera.

We use three of our nonserpentine taxa as the nonserpentine sister taxon in two pairings each. For example, *Mimulus nudatus* is a serpentine endemic hypothesized to be derived from within *Mimulus guttatus* (Macnair and Gardner, 1998). We chose a *M. guttatus* nonserpentine population to serve as the nonserpentine sister taxon for *M. nudatus*. However, because *M. guttatus* is a serpentine tolerator itself, we also use the same nonserpentine population as the

nonserpentine sister taxon for a serpentine population of *M. guttatus*. We use this same overlapping design for the *Collinsia greenei* - *C. sparsiflora* endemic pair and *C. sparsiflora* tolerator pair, and for the *Navarretia rosulata* – *N. heterodoxa* endemic pair and the *N. heterodoxa* tolerator pair. Assuming serpentine adaptation evolved independently for the tolerant population and the endemic species, this is a powerful paired way to compare endemics and tolerators to a common reference taxon. Serpentine adaptation has been shown to evolve independently multiple times within tolerator species, e.g., within *Cerastium alpinum*, *Alyssum bertolonii*, the *Lasthenia californica* complex, *Mimulus guttatus*, and *Arabidopsis lyrata* (Nyberg Berglund et al., 2001, 2004; Mengoni et al., 2003; Rajakaruna and Whitton, 2004; Turner et al., 2010; Selby, 2014; Selby and Willis, 2018). Likewise, independent origins of serpentine adaptation within tolerator species has led to the evolution of endemic species. For example, there are at least 3 local *Streptanthus* endemic species hypothesized to be derived from the tolerator *Streptanthus glandulosus* (Kruckeberg, 1957), and there are two local/restricted *Mimulus* endemic species hypothesized to be derived from the tolerator *Mimulus guttatus* (Macnair and Gardner, 1998).

Seed and soil collections-- At each population, we collected seed from 30-40 maternal plants, i.e., “families”. Maternal plants were haphazardly selected throughout the population, avoiding seed from individuals within 1-2 meters of each other to maximize genetic diversity. Most of the plant taxa we chose have

gravity-dispersed seeds, and thus clusters of individuals are likely closely related.

Collected fruits were stored in coin envelopes and kept at 4 °C until planting.

We collected approximately 15 liters of soil from within the plant population to use in a greenhouse reciprocal transplant experiment. Soil was collected from within the top 20 cm and stored in open 4-liter plastic bags at room temperature. We discarded rocks larger than 3.5 cm in diameter, but otherwise retained the natural variation in particle size in the soil.

Greenhouse reciprocal transplant experiment – For each sister taxa pair, we set up a greenhouse reciprocal transplant experiment in field-collected soil. Soil from each population was homogenized and potted into 60 RayLeach Conetainers (3.8 x 21 cm), including all rocks that would fit. On average, 30 pots were sowed with seeds from the serpentine taxon and 30 pots were sowed with seeds from the nonserpentine taxon. Seed from each maternal family was potted into one pot per soil type. In each pot, we planted anywhere between 1-5 seeds to ensure that at least one would germinate. Seeds were sown on the surface and watered down into the soil. We put all pots into a stratification treatment (no light, 4° C, daily misting) in a growth chamber (Conviron model E15) to induce germination for a period of 2 weeks or until seeds started germinating, whichever came first. Pots were then moved to a seedling establishment growth chamber (Conviron model E15; 20° C day, 15° C night, 10-hour days, daily top-watering) for an average of 5.4 weeks. We planted seeds in early November to mimic the

cycle of most annual plants in a Mediterranean climate (cool, wet winters and hot, dry summers).

Four of the sister taxa pairs were germinated in petri dishes. The *Layia discoidea*-*L. glandulosa* pair were germinated on filter paper soaked with 150 ppm gibberellic acid because of their germination requirement for light (B. Baldwin, personal communication). The *Camissonia benitensis*-*C. strigulosa*, *Mimulus nudatus*-*M. guttatus*, and the *M. guttatus*-*M. guttatus* pairs were all germinated on filter paper soaked in DI water. The seeds of these species were all too small to distinguish in the pots, and thus it was difficult to distinguish the planted seeds from seeds that recruited naturally. All petri dishes were kept in the stratification growth chamber until germination. Once radicles emerged, seeds were transplanted into soil and put into the seedling establishment growth chamber.

After seedling establishment, all pots were moved to the greenhouse on the same day (within each experimental round, see below), where pots from all sister taxa pairs were randomized. Plants experienced the natural day lengths in the greenhouse, with supplemental lights turned on in between sunrise and sunset if the light intensity fell below 400 Wm². Pots were top-watered with DI water every 2-5 days for 16 weeks, and then reduced to watering pots every 8-10 days for 2.5 weeks, and in the 2018 experiment (see below) at a subsequent 15 and 6 day interval, before ultimately cutting the water off completely. The tapering of

the water regime was designed to mimic the precipitation cycle in California, where winter rains taper off in May.

Because of the large sample size of this experiment, we split the pairs into two experimental rounds. In the first year (2016-2017; “2017”) 5 tolerator pairs and 3 endemic pairs were grown and in the second year (2017-2018; “2018”) 4 tolerator pairs and 5 endemic pairs were grown (Table 2.1). Among the two years, there were some differences in growing conditions. For example, in the first experimental round, pairs spent an average of 23 days in the seedling establishment chamber, and in the second experimental round pairs spent an average of 50 days in the seedling establishment chamber. In the first experimental round, the greenhouse temperature controls were set to maintain day temperatures around 15° C and night temperatures around 18° C, whereas in the second experimental round, the controls got flipped and day temperatures were maintained around 21° C and night temperatures were maintained around 13° C. Because we grew a mix of endemic and tolerator pairs, any differences we see between endemic and tolerator pairs in the traits measured should not be driven by the variation in greenhouse temperatures across years. To statistically correct for the greenhouse variation among years, we use year as a covariate in analyses below.

For all plants that germinated and survived to flower, we quantified the number of days between germination and production of the first open flower as our measure of flowering onset. Because seeds were sown on the top of the soil

surface, we were able to characterize germination day as the day at which the radicle broke through the seed coat. Once individuals started flowering, we conducted weekly censuses of open flowers per plant to build flowering time distributions for each taxon in serpentine and nonserpentine soil. In the 2016-2017 greenhouse experiment, censuses occurred every 4-7 days. In the 2017-2018 greenhouse experiment, censuses occurred every 7-8 days. We did not hand-pollinate any of the plants in the greenhouse. Some of the taxa in the study readily self-pollinated, some underwent delayed self-pollination and some did not set any seed. This resulted in individual-flower lifespans and overall flower duration lasting longer in some species. Because phenological isolation incorporates flowering duration, we interpret our results in the light of these mating system differences (see Discussion).

Data analysis—

Are flowering onset shifts common following adaptation to serpentine?—

We characterized flowering onset shifts within each sister taxa pair by comparing flowering onset of each taxon in its home soil. We tested for differences in flowering onset between the sister taxa within each pair with a t-test, adjusting significance values across all taxa pairs to account for multiple comparisons. We qualitatively describe patterns in the direction of shifts in flowering onset.

Do endemic sister taxa pairs have greater flowering onset shifts than tolerator sister taxa pairs? —We asked whether endemic sister taxa pairs have greater shifts in flowering onset, regardless of direction, than tolerator sister taxa pairs. We modeled shifts in flowering onset within each sister taxa pair, and subsequently tested for differences between endemic and tolerator pairs while accounting for the phylogenetic relatedness among pairs using two hierarchical Bayesian models:

Model 1

$$y_{ijk} \sim \text{poisson}(\lambda_{jk}), \quad (\text{Eq. 1})$$

$$\text{onset.shift}_k = \text{abs}(\lambda_{S k} - \lambda_{NS k}) \quad (\text{Eq. 2})$$

Model 2

$$\text{mean}(\text{onset.shift}_k) \sim \text{gamma}(m(z_k, \sigma_{\text{sampling}}^2)) \quad (\text{Eq. 3})$$

$$z_k \sim \text{gamma}(m(\mu_k, \sigma_{\text{process}}^2)) \quad (\text{Eq. 4})$$

$$\log(\mu_k) = \beta_{0k} + \beta_1 \cdot \text{pair_type}_k + \beta_2 + \beta_3 \cdot \text{year}_k \quad (\text{Eq. 5})$$

In Model 1, the flowering onset of individual i of taxon j growing in its home soil of pair k is modeled as a random variable pulled from a Poisson distribution with a mean λ_{jk} (Eq. 1). The magnitude of the shift in flowering onset in pair k is taken as the absolute difference between the means of the Poisson distribution describing flowering time of serpentine seeds in their home soil ($\lambda_{S k}$) and nonserpentine seeds in their home soil ($\lambda_{NS k}$; Eq. 2). Eq. 2 is calculated at each MCMC generation and has a posterior distribution. The mean of that

posterior distribution, reflecting the most probable value of the magnitude of the flowering time shift is inputted into Model 2. We modeled the mean flowering onset shift per pair as a gamma distribution and used moment matching to calculate the mean and variance of that distribution from the shape and rate parameters (Eq. 3). The mean of the gamma distribution, z_k , represents the “true” mean flowering onset shift in pair k , and the variance term, $\sigma_{\text{sampling}}^2$, reflects variation in our “observed” estimate (i.e., $\text{mean}(\text{onset.shift}_k)$) due to sampling effects. The “true” mean is then modeled as a separate gamma distribution (Eq. 4) described by a mean parameter, μ_k , that is derived from a process model (Eq. 5) and a variance term, $\sigma_{\text{process}}^2$, that reflects variation due to effects not included in our process model. Equation 5 can be thought of as the expected value from a generalized mixed model, and $\sigma_{\text{process}}^2$ is analogous to the model error.

The process model incorporates a fixed intercept (β_2), a random intercept that accounts for the phylogenetic relatedness among pairs (β_0), a fixed effect for pair type (i.e., whether the sister taxa pair is an endemic or tolerator pair; β_1), and year the pair was grown in the greenhouse as a covariate (β_3). We were primarily interested in the effect of pair type on shifts in flowering onset. If β_1 is greater than zero, then endemic sister taxa pairs have larger flowering onset shifts than tolerator sister taxa pairs. We tested how much of the β_1 posterior distribution is greater than zero to assess significance of the pair type effect. The fixed intercept,

β_2 , reflects the average magnitude of flowering onset shifts in tolerator sister taxa pairs.

Are flowering onset shifts more genetically-based in endemic pairs than tolerator pairs?—To estimate the proportion of flowering time shifts due to plasticity versus genetic differentiation in serpentine taxa, we compared flowering onset shifts between sister taxa when each taxon was in its home soil to when each taxon was in the pair's nonserpentine soil. In a common nonserpentine soil, differences between the serpentine and nonserpentine sister taxa in flowering onset should be driven by genetic differentiation (and maternal effects). Conversely, differences in flowering onset between the serpentine taxon in the serpentine and nonserpentine soils reflect plasticity. For each sister taxa pair, we inputted standardized flowering onset data for three treatments – serpentine seeds in serpentine soil, serpentine seeds in nonserpentine soil, and nonserpentine seeds in nonserpentine soil – into an ANOVA, and extracted variance components for the effects of seed, soil and within treatment (i.e., residual) effects. The seed effect describes the difference in flowering onset between serpentine and nonserpentine seeds in the common nonserpentine soil, and thus represents the genetic effect. The soil effect describes the difference in flowering onset between serpentine seeds in nonserpentine and serpentine soil, and thus represents the plastic effect. We extracted the variance components from the above ANOVA analysis and used the proportion of non-residual variance contributed by the soil

effect to quantify the degree to which flowering time shifts are plastic, and the proportion of non-residual variance contributed by the seed effect to quantify the degree to which flowering time shifts are genetically-based. We use PGLS models with greenhouse year as a covariate to test whether the degree of plasticity and genetic differentiation in flowering time shifts differs between endemic and tolerator sister taxa pairs.

Has plasticity in flowering onset evolved following adaptation to serpentine and are plastic responses in an adaptive direction? —We compared the maternal family reaction norms of serpentine and nonserpentine taxa within each pair to determine if plasticity in flowering onset has evolved. We assume that the nonserpentine taxon represents the ancestral-like plasticity in flowering onset, and that differences in plasticity in flowering onset between nonserpentine and serpentine sister taxa are due to changes accompanying adaptation to serpentine. We calculate maternal family reaction norms by subtracting the flowering onset of the individual in nonserpentine soil from the flowering onset of its sibling in serpentine soil – positive reaction norm slopes indicate later flowering in serpentine soils. Within each pair we use t-tests to test for differences in the maternal family reaction norms, and adjust for multiple comparisons with a sequential Bonferroni correction.

To determine whether plasticity in flowering time is adaptive, we quantify linear selection gradients on flowering onset in serpentine soil by regressing total

flower production on flowering onset (Lande and Arnold, 1983). Because the serpentine and nonserpentine seeds have very different fecundities in serpentine soils in some of the pairs, we estimate selection on each taxon separately. Taxa with less than 5 individuals were excluded from the selection analysis. We standardize flowering onset (day of first flower) to a mean of zero and a standard deviation of 1. We use the `lm()` function in R to perform the regression.

Do endemic sister taxa pairs have stronger and more permanent phenological isolation following ecological change than tolerator sister taxa pairs?— We quantify phenological reproductive isolation between sister taxa in each pair when each is growing in its home soil and in the pair’s nonserpentine soil using a modified version of the Sobel and Chen (2014) equation for phenological isolation:

$$\text{Phenological isolation} = 1 - 2 \cdot \sum \left(\frac{C_i}{C_t} \cdot \frac{H_i}{C_i + H_i} \right) \quad (\text{Eq. 6})$$

Where C_i is the total number of conspecific flowers open on day i , C_t is the total number of conspecific flowers produced across the flowering season, and H_i is the total number of heterospecific flowers open on day i . The probability of heterospecific gene flow across the season (summation component) is a weighted average of the proportion of heterospecific flowers open on a given day, weighted by the proportion of total conspecific flowers open on that day. The temporal isolation metric ranges from 1 (when the probability of gene flow = 0), to 0 (when

the probability of gene flow = 0.5, representing random mating), and to -1 (when the probability of gene flow = 1, representing complete disassortative mating).

Temporal isolation, as calculated in Eq. 6, depends on differences in total abundance of con- and heterospecifics across the season, and thus incorporates isolation due to both factors that influence survival to flowering and flowering time shifts. In the field, the total abundance of con- and heterospecifics in the field varies based on selection against migrant individuals, resource availability, and seed germination. In our greenhouse experiment, the total abundance of con- and heterospecifics is limited by our sample size and mortality during the experiment. Because here we are interested in how flowering time shifts *per se* affect reproductive isolation, we use relative abundances instead of absolute abundances – thus assuming total con- and heterospecific abundances are equal. All C_t values equal 1 and the C_t and H_t values equal the percent of total flowers open on that day. In this way, the RI index describes the potential for reproductive isolation solely due to flowering time shifts. Under this modification, the RI index is bounded by 0 (total overlap in flowering schedule and random mating) and 1 (no overlap in flowering schedule and complete RI). We test whether endemic sister taxa pairs have higher temporal isolation than tolerator sister taxa pairs using a PGLS with greenhouse year as a covariate.

We use PGLS models to test for differences in phenological isolation between endemic and tolerator pairs, using greenhouse year as a covariate, in two ecological contexts: 1) when sister taxa are in their home soil and 2) when sister

taxa are in a common nonserpentine soil. We determine if phenological isolation is more permanent in endemic pairs than tolerator pairs by taking the difference of phenological isolation values obtained in these two ecological contexts. The first scenario simulates reproductive isolation between adjacent populations, where gene flow only occurs through pollen transfer between adults that have developed in their home soil. The second scenario simulates the case in which serpentine seeds migrate into their sister taxon's nonserpentine soil. We don't quantify temporal isolation when nonserpentine seeds migrate into serpentine soils because most nonserpentine taxa used in this study had high mortality in serpentine soil. We use a PGLS model to test whether phenological isolation is more permanent, i.e., there is less of a difference between ecological contexts, in endemic pairs than in tolerator pairs, including greenhouse year as a covariate.

Are flowering time shifts correlated with greater shifts in the edaphic and/or climatic environment?— Finally, up until this point we have treated the ecological contrast between members of each sister taxa as equal. However, there is variation within serpentine and nonserpentine substrates such that some pairs have undergone more divergent adaptation. Moreover, because some of our sister taxa were collected far apart from one another, other environmental factors, such as climate, may affect selection on flowering time. For every sister taxa pair, we quantify multivariate divergence in soil chemistry and texture of serpentine and nonserpentine populations (see Sianta and Kay (2019) for details). In brief, we

input 25 soil variables from all taxa into a principal component analysis and calculate Euclidean distances in 25-D space between each serpentine and nonserpentine sister taxa pair. We use a phylogenetic generalized least squares (PGLS) model to ask whether a pair's multivariate soil distance explains the magnitude of flowering onset shifts. We use the average value from the posterior distribution of flowering onset shifts for each pair from the Bayesian model above as the response variable in the PGLS. We include year grown in the greenhouse as a covariate.

We calculate distances in multivariate climatic space in a similar fashion to multivariate soil distances. We obtained climatic data for each population from the WorldClim bioclim dataset. We downloaded the bioclim data at the 2.5 minute degree resolution. We input all 19 bioclim variables into a principal components analysis and quantify Euclidean distance between members of each sister taxa pair. We test for a relationship between climatic distance and the magnitude of flowering onset shifts with a PGLS, including year grown in greenhouse as a covariate.

RESULTS

Do flowering onset shifts commonly accompany adaptation to serpentine?

We first test for the prevalence of flowering onset shifts among each of the 17 sister taxa pairs with each taxon grown in its home soil. We find that edaphic divergence is consistently associated with shifts in flowering onset – 16 of the 17

sister taxa pairs had significant differences in flowering onset (Table 2.2, Figure 2.2). The average difference in flowering onset ranges from 7 days in the *Clarkia breweri* tolerator pair to 39 days in the *Mimulus nudatus* – *M. guttatus* endemic pair. The average among all of the pairs is 20 days (S.E 2.35 days). Of the 16 pairs that showed significant differences in flowering onset, 12 pairs had the serpentine taxon flowering later than nonserpentine taxon. The four pairs in which the serpentine taxon flowered earlier were the *Navarretia heterodoxa* tolerator pair, the *Clarkia gracilis subsp. tracyi* – *C. gracilis subsp. albicaulis* endemic pair, the *Mimulus nudatus* – *M. guttatus* endemic pair, and the *N. rosulata* – *N. heterodoxa* endemic pair.

Do endemic pairs have larger flowering onset shifts than tolerator pairs?

There is a significant, but very small, difference in the magnitude of flowering onset shifts in endemic pairs relative to tolerator pairs. The posterior distribution of the β_1 coefficient, which indicates the effect of pair type, has a mean of 0.47 and a 95% credible interval of (-0.12, 1.09). 93.9% of the posterior distribution of β_1 is greater than zero, indicating flowering onset shifts are greater in endemics. The posterior distribution of the fixed intercept β_2 , which represents the average shift in flowering onset for tolerator pairs, has a mean of 2.58 and a 95% credible interval of (1.36, 3.83). Because we used a log-linear deterministic model, we exponentiate the coefficients to interpret their effect on shifts in flowering onset. The exponentiated β_2 coefficient is 13.15, which is interpreted as

the average magnitude of flowering onset shifts in tolerator pairs. The average change in absolute flowering onset in endemic pairs versus tolerator pairs is 0.60 days.

Do endemic serpentine taxa have more genetically-based flowering onset shifts than tolerator serpentine taxa?

For each pair, we compare flowering onset shifts when serpentine and nonserpentine sister taxa are in their home soils and in a common nonserpentine soil to quantify the proportion of genetic differentiation and plasticity that contributes to flowering onset shifts. (Figure 2.3A, Figure 2.4). Pairs vary in the proportion of plastic versus genetic differentiation that explains flowering time shifts (Figure 2.3B). All pairs showed a significant seed, i.e., genetic, effect, whereas only 7 of the 17 sister taxa pairs showed a significant soil, i.e., plastic, effect (Table 2.3). We find no difference between endemic and tolerator pairs in the relative contribution of plasticity to flowering onset shifts (PGLS, $t_{17,14}$ (pair type) = -0.887, p-value (pair type) = 0.390), nor the relative contribution of genetic differentiation to flowering onset shifts (PGLS, $t_{17,14}$ (pair type) = 0.887, p-value (pair type) = 0.390). There is a marginally significant effect of endemic pairs having higher absolute plasticity variance components than tolerator pairs (PGLS, $t_{17,14}$ (pair type) = 2.020, p-value (pair type) = 0.063).

Has plasticity in flowering onset evolved following adaptation to serpentine and are plastic responses in an adaptive direction?

We quantified maternal family reaction norms for both serpentine and nonserpentine taxa within each pair and compared the variation in maternal family reaction norms between sister taxa to determine if plasticity has evolved (Figure 2.5). Four pairs (*Navarretia heterodoxa*, *Camissonia benitensis*-*C. strigulosa*, *Collomia diversifolia*-*C. heterophylla*, and *N. rosulata*-*N. heterodoxa*) were not included in the analysis because only 1 or 0 maternal families survived in serpentine soil. *Collomia diversifolia*-*C. heterophylla* was the pair with 1 nonserpentine maternal family that survived in serpentine soil and it showed a very large plastic response to flower later in serpentine. Six of the pairs, 4 tolerator pairs and 2 endemic pairs, showed a significant decrease in plasticity in the serpentine taxon compared to the nonserpentine taxon, the latter of which flowered later in serpentine soil (Table 2.4). Two additional tolerator pairs, *Collinsia sparsiflora* and *Collinsia heterophylla*, showed a similar pattern in plasticity between the serpentine and nonserpentine taxon but did not have enough power to detect a difference due to mortality of nonserpentine seeds in serpentine soil.

We find overall support for selection for earlier flowering in serpentine soils at the taxon level among pairs (Figure 2.6, Table 2.5). As above, due to death of nonserpentine seeds in serpentine soil, we were not able to conduct selection analyses for the nonserpentine taxon of the *Mimulus guttatus*, *Collinsia*

sparsiflora, *N. heterodoxa*, *Camissonia benitensis*-*C. strigulosa*, *Collomia diversifolia*-*C. heterophylla* and *N. rosulata*-*N. heterodoxa* pairs. We find a significant effect of selection for earlier flowering in the serpentine taxon of the *M. guttatus*, *N. heterodoxa*, *N. pubescens*, *Clarkia gracilis* subsp. *tracyi*-*C. gracilis* subsp. *albicaulis*, *Collinsia greenei*-*C. sparsiflora*, *N. jepsonii*-*N. heterandra*, and *N. rosulata*-*N. heterodoxa* pairs. We find a significant effect of selection for earlier flowering in the nonserpentine taxon of the *N. pubescens*, *C. gracilis* subsp. *tracyi*-*C. gracilis* subsp. *albicaulis*, and *M. nudatus*-*M. guttatus* pairs.

Do endemic pairs have stronger and more permanent phenological isolation following an ecological change than tolerator pairs?—

We do not find a difference in phenological isolation between endemic and tolerator pairs when sister taxa are in their home soil (PGLS, $t = -0.64$, $p = 0.53$), nor when sister taxa are in the pair's nonserpentine soil (PGLS, $t = -0.31$, $p = 0.76$). In both ecological contexts, there is variation among sister taxa pairs, with some pairs having 60-80% of gene flow blocked by shifts in flowering time distributions. The remainder of pairs have low to moderate levels of phenological isolation (0 – 38% reduction in gene flow).

We took the difference in phenological isolation values between the two ecological contexts (sister taxa in their home soils and in a common nonserpentine soil) to estimate the degree of permanence of phenological isolation. We find a

marginally significant effect of endemic pairs having more permanent phenological isolation than tolerator pairs, (Figure 2.7; PGLS, $t = 2.16$, $p = 0.049$). This pattern is driven by the three tolerator pairs, *N. heterodoxa*, *N. pubescens* and *T. willdenovii*, that show large decreases in phenological isolation when sister taxa are in a common nonserpentine soil compared to their home habitats. All other taxa, except for the *Plantago erecta* pair, have small changes in phenological isolation.

Is the magnitude of flowering onset shifts explained by environmental differences?

We find that multivariate soil distance explains some of the variation in flowering time (PGLS, $t_{1,14}$ (soil distance) = 4.51, p -value (soil distance) = 0.051; PGLS without year as covariate, $p = 0.046$), such that sister taxa pairs with more divergent soil environments have larger flowering time shifts (Figure 2.8). However, we do not find any significant relationship between climatic distance and the absolute value of flowering time shifts (PGLS $t_{1,14}$ (climate distance) = 0.003, $p = 0.68$; Figure 2.9).

DISCUSSION

Independent replicates of ecological divergence in response to a common selective pressure provide an excellent opportunity to study the evolution of ecologically-driven reproductive isolation along the speciation continuum (Nosil et al., 2009; Rosenblum and Harmon, 2010; Faria et al., 2014). In this study we

focus on species that have adapted to chemically and physically harsh serpentine substrates but vary in whether that adaptation led to population-level divergence or speciation of serpentine endemics. We hypothesized that strong, genetically-based flowering onset shifts and phenological isolation would isolate serpentine endemics from their nonserpentine sister taxa, whereas weaker and plastic flowering onset shifts and phenological isolation would isolate serpentine and nonserpentine populations within the same tolerator species.

Surprisingly, we find no evidence of differences in the magnitude of flowering onset shifts, the degree to which shifts are genetically-based, nor the permanence of phenological isolation between endemic and tolerator sister taxa pairs. Both tolerator and endemic pairs exhibit flowering onset shifts and phenological isolation, suggesting that the evolution of phenological isolation following adaptation to serpentine evolves quickly, in the early stages of speciation. Other speciation studies have also found a role for flowering time shifts in isolating ecotypes within species (Antonovics, 2006; Lowry, Rockwood, et al., 2008; Briscoe Runquist et al., 2014; Peterson, 2015; Richards and Ortiz-barrientos, 2016). Given that multiple reproductive barriers isolate any two taxa (Lowry, Modliszewski, et al., 2008), it is likely that other barriers, such as spatial isolation and habitat isolation serve to push serpentine-adapted ecotypes along the speciation spectrum to become serpentine endemics (Kay et al., 2011).

However, flowering onset shifts and phenological isolation are common across the majority of sister taxa pairs, which suggests that flowering time shifts

are important in the establish of populations following colonization on serpentine and in the evolution of ecotypes. Levin (2009) argued the idea that flowering time shifts are a common factor promoting niche expansion. The automatic level of assortative mating conferred by shifts in flowering time (Fox, 2003) allows adaptation of newly established populations to serpentine substrates to occur without the hindrance of maladaptive gene flow (Lenormand, 2002). Levin's argument was predicated on the notion that plasticity in flowering time shifts would accompany adaptation to a novel and stressful environment. Here, we find that flowering time shifts in serpentine-adapted taxa relative to their nonserpentine sister taxa are primarily genetically-based, even for sister taxa pairs within tolerator species (Figure 2.3B). Yet for those pairs that do show a significant plastic variance component (*Navarretia heterodoxa*, *N. pubescens*, *Trifolium willdenovii*, *Collomia diversifolia*-*C. heterophylla*, *N. rosulata*-*N. heterodoxa*, and *Layia discoidea*-*L. glandulosa*), plasticity in the serpentine taxon acts in the same direction as the genetic differentiation between serpentine and nonserpentine sister taxa, increasing the flowering onset shift when each taxon is in its home soil. Thus, Levin's (2009) argument, that plasticity in flowering time increases flowering time shifts, is supported in some of our pairs.

In contrast to the paradigm that adaptation to serpentine soils is accompanied by shifts to *earlier* flowering, the majority of the serpentine taxa used here flower later in serpentine soils. Shifts to earlier flowering in serpentine plants was hypothesized to be a drought-avoidance mechanism in rocky serpentine habitats

(Schmitt, 1983; Hughes et al., 2001; Brady et al., 2005), a similar phenomenon to that seen in other rocky edaphic habitats (Ellis and Weis, 2006; Ferris and Willis, 2018). Indeed, some of the classic serpentine systems do show serpentine plants flowering earlier than relatives, both between species (Gardner and Macnair, 2000; Gailing et al., 2004; Selby et al., 2014) and between ecotypes within species (Proctor and Woodell, 1975; Rajakaruna, 2004; Sambatti and Rice, 2007; Kay et al., 2011; Selby et al., 2014; Dittmar and Schemske, 2017), with some studies making the explicit connection between drought avoidance and earlier flowering (Rajakaruna et al., 2003; Dittmar and Schemske, 2017). We were not able to quantify the rockiness of the soils from which we collected our seeds, nor was soil rockiness fully simulated in our potted greenhouse experiment. However, personal observations from the field confirm that many of the serpentine taxa occurred in rocky serpentine soils, and yet still flower later relative to their nonserpentine sister taxa. For example, *Layia discoidea* occurs in very rocky serpentine barrens and flowers later than its sister taxon *L. glandulosa* (Figure 2.2, Figure 2.4).

Our study adds to a growing body of evidence that shows serpentine plants flowering later than their nonserpentine relatives (Wright et al., 2006; Schneider, 2017). Given the low nutrient conditions and potential toxicity of serpentine soils, it is likely that later flowering is a developmental consequence of low-nutrient soils, with individuals having to grow for a longer time before they accumulate enough resources to flower. Flowering later could also be under selection so that

individuals can accumulate more resources and be more fecund, with the trade-off that individuals risk dying at the season's end before maturing all of their seeds (Stearns, 1976). However, we find no evidence for selection on later flowering in serpentine habitats, which suggests later flowering in serpentine is a developmental constraint. In contrast, most taxa show a trend of selection for earlier flowering. For the pairs that flowered the latest in the experiment (e.g., *N. heterodoxa*, *N. rosulata*-*N. heterodoxa*, and *N. jepsonii*-*N. heterandra*) the signal for selection on early flowering may be due to them flowering while the greenhouse watering schedule ended. However, these plants flower in late-spring and early-summer, once the natural drought has set in, and thus likely experience a similar drop-off in water availability in the field.

Comparing the maternal-level reaction norms in flowering onset between serpentine and nonserpentine sisters also yields insight into the pattern of later flowering we see in the serpentine taxa. Of the 14 pairs for which at least one individual from the nonserpentine taxon survived in serpentine soil, 12 of the nonserpentine taxon showed high plastic shifts to later flowering in serpentine soil (Figure 2.5). Ten of the sister taxa pairs have a reduction in plasticity in the serpentine taxon compared to the nonserpentine taxon, and if we assume that the plasticity in nonserpentine sister taxa represents ancestral-like levels of plasticity in the paired serpentine taxa, this result suggests that ancestral plasticity is selected against in serpentine taxa. Plastic responses can lead to genetic change and have been proposed as important drivers of phenotypic novelty and species

diversity (West-Eberhard, 1989, 2005). When plasticity is adaptive, the process of genetic assimilation fixes, and even further refines, an induced genotype (Crispo, 2008). Two pairs show a pattern consistent with genetic assimilation, where the nonserpentine taxon has plasticity for later flowering in serpentine substrates and the serpentine taxon has further genetic change for even later flowering (*Collinsia greenei*-*C. sparsiflora*, and *C. heterophylla*; Figure 2.10).

When plasticity is maladaptive, a process called genetic compensation (Grether, 2005) selects for genetic change that shifts an induced trait back towards the ancestral state. For example, if initial colonizers of serpentine soils flower very late due to developmental constraints but are still able to successfully reproduce and adapt to serpentine, they should be better able to acquire nutrients from and detoxify the heavy metals in serpentine soils over time. Once the developmental constraint of later flowering is overcome, selection can act to drive earlier flowering, until the right balance between size-at-flower and pre-maturation mortality is reached (Stearns, 1976). Six pairs show a pattern consistent with genetic compensation, wherein the nonserpentine taxon has plasticity for later flowering in serpentine substrates that exceeds the flowering onset of the serpentine taxon (i.e., the serpentine taxon undergoes genetic change that shifts flowering onset in the opposite direction; *Mimulus guttatus*, *Collinsia sparsiflora*, *Plantago erecta*, *Collomia diversifolia*-*C. heterophylla*, *Mimulus nudatus*-*M. guttatus*, and *Navarretia jepsonii*; Figure 2.10).

Plasticity in flowering onset of serpentine seeds generally translates to the permanence of phenological isolation when we compare sister taxa in their home soils versus in a common nonserpentine soil. We found a small, but significant, effect of endemic pairs having more permanent phenological isolation (i.e., less of a difference in phenological isolation between the two ecological contexts) than tolerator pairs. However, that trend is driven by three tolerator pairs, *Navarretia heterodoxa*, *N. pubescens* and *Trifolium willdenovii* that all showed a large decrease in phenological isolation when sister taxa were in the common nonserpentine soil. These three pairs also had high levels of plasticity in flowering onset shifts that lessen the degree of the shift in a common nonserpentine soil.

The magnitude of flowering onset shifts, *per se*, do not directly translate to the magnitude of phenological isolation (data not shown), and this is likely due to the fact that we didn't hand-pollinate any plants in the greenhouse, which affects the duration of flowering for taxa that don't readily self-pollinate. For example, the *Collinsia sparsiflora* pair has a very high temporal isolation value when each taxon is in its home soil (0.7), even though the flowering onset of the taxa only differs by 15 days. In contrast, the difference in flowering onset of the *Mimulus nudatus*-*M. guttatus* pair is 40 days, but the temporal isolation value for that pair is only 0.3. The nonserpentine taxon of the *C. sparsiflora* pair is highly self-fertilizing – individual flowers turn over quickly making them less likely to be detected in the census, and whole plants senesce quickly, which causes the nonserpentine taxon to have a tight, narrow flowering time distribution. In

contrast, *M. nudatus* and *M. guttatus* do not self-fertilize readily. Individual flowers persist on the *Mimulus* plants for longer periods of time, and plants produce more flowers and have open floral displays for longer. In the field, metrics of phenological isolation may be higher if natural pollination causes individuals to produce fewer flowers (more resources will be allocated to seed set instead of flower production), have higher turnover rates in flower number and senesce quicker. However, we don't predict that the differences in mating system among taxa, and their effects on phenological isolation, affect our result that tolerator and endemic pairs have similar levels of phenological isolation because self-fertilizing taxa are distributed across both groups.

Finally, we find that the degree of multivariate soil differences between serpentine and nonserpentine taxa somewhat predict the magnitude of flowering onset shifts. We didn't find any relationship between individual soil variables and the magnitude of flowering time shifts (data not shown), suggesting that it is differences in the overall soil environment that affect flowering time shifts. This result is consistent with the idea that larger degrees of ecological divergence will increase divergent selection and reproductive isolation (Nosil et al., 2009). While positive relationships between ecological divergence and reproductive isolation have been found (Funk et al., 2006), this is the first study we are aware of that connects edaphic divergence to the strength of flowering time shifts.

Conclusions:

Comparing the strength and degree of genetically-based differences in reproductive isolation across the speciation spectrum provides an excellent opportunity to understand the importance of forms of reproductive isolation at different stages of speciation. Genetically-based flowering time shifts generally evolve quickly following adaptation to serpentine soils, making phenological isolation an important form of reproductive isolation at early stages of speciation. Phenological isolation likely serves to promote the establishment of serpentine populations and provides enough reproductive isolation such that other forms of reproductive isolation can evolve between serpentine and nonserpentine populations. However, across all pairs, both endemics and tolerators, there is substantial variation in the strength of phenological isolation. The variation may reflect taxa pairs that continuously span the speciation spectrum, instead of representing two discrete groups. Alternatively, the variation may reflect the lineage-specific idiosyncrasies of how reproductive barriers evolve and how complete reproductive isolation is achieved. Future work in this field should move towards the “holy grail” synthesis of speciation research – having quantitative data on all forms of reproductive isolation acting between sister taxa that fall across the speciation spectrum (Sobel et al., 2010). A combination of studies like ours and case-specific studies that quantify total reproductive isolation brings us closer to this ideal.

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Table 2.1: Serpentine (S) and nonserpentine (NS) sister taxa pairs used in this study. The three nonserpentine taxa for which we use as the nonserpentine comparison for two pairs (i.e., *Collinsia sparsiflora*, *Navarretia heterodoxa*, *Mimulus guttatus*) are listed twice in the table – once with the respective tolerator taxa and once with the respective endemic taxa. Pair codes are given to refer to pairs in the subsequent tables and figures. Year refers to the year the pair was grown in the greenhouse reciprocal transplant experiment.

^aCitations used to determine serpentine status and sister taxa relationships:

¹Personal observation, ²Safford et al., 2005, ³Macnair and Gardner, 1998, ⁴Anacker et al., 2011, ⁵Spencer and Porter, 1997, ⁶Baldwin et al., 2012, ⁷Gottlieb and Weeden, 1979, ⁸Green, 2010, ⁹Baldwin, 2005, ¹⁰Baldwin et al., 2011, ¹¹Dick et al., 2014

^bCollection locations: ¹Lake Co: UC McLaughlin Reserve, ²Stanislaus Co: Del Puerto Canyon, ³Napa Co: UC McLaughlin Reserve, ⁴San Mateo Co: Edgewood County Park, ⁵Napa Co: Foote Botanical Preserve, ⁶Butte Co: Horncut, ⁷Marin Co: Carson Ridge, ⁸Napa Co: Foote Botanical Preserve, ⁹Butte Co: Paradise, ¹⁰Lake Co: Cobb Mountain, ¹¹San Benito Co: Clear Creak Mgt. Area, ¹²Napa Co: Knoxville Wildlife Reserv

Pair type ^a	Popul- ation origin ^b	Species	Pair Code	Year	Dist- ance (km)
Tolerator ¹	S ¹	<i>Clarkia concinna</i>	CACO	2017	1.85
	NS ¹	<i>Clarkia concinna</i>			
Tolerator ²	S ²	<i>Clarkia breweri</i>	CABR	2017	3.03
	NS ²	<i>Clarkia breweri</i>			
Tolerator ²	S ¹	<i>Plantago erecta</i>	PLER	2018	1.75
	NS ³	<i>Plantago erecta</i>			
Tolerator ³	S ¹	<i>Mimulus guttatus</i>	MGUT	2018	6.17
	NS ⁴	<i>Mimulus guttatus</i>			
Tolerator ²	S ¹	<i>Collinsia sparsiflora</i>	COSP	2017	2.48
	NS ³	<i>Collinsia sparsiflora</i>			
Tolerator ⁴	S ²	<i>Collinsia heterophylla</i>	COHT	2017	2.23
	NS ²	<i>Collinsia heterophylla</i>			
Tolerator ²	S ¹	<i>Trifolium willdenovii</i>	TWILD	2018	1.57
	NS ³	<i>Trifolium willdenovii</i>			
Tolerator ²	S ¹	<i>Navarretia pubescens</i>	NAPB	2017	1.44
	NS ³	<i>Navarretia pubescens</i>			
Tolerator ¹	S ⁵	<i>Navarretia heterodoxa</i>	NAHX	2018	37.76
	NS ⁶	<i>Navarretia heterodoxa</i>			
Endemic ^{2,4,5}	S ¹	<i>Navarretia jepsonii</i>	NAJP_NAHN	2018	37.58
	NS ⁷	<i>Navarretia heterandra</i>			
Endemic ^{2,5,6}	S ⁸	<i>Navarretia rosulate</i>	NARS_NAHN	2018	20.87
	NS ⁶	<i>Navarretia heterodoxa</i>			
Endemic ^{2,7}	S ¹	<i>Clarkia gracilis ssp. tracyi</i>	CAGT_CAGA	2018	52.05
	NS ¹⁰	<i>C. gracilis ssp. albicaulis</i>			
Endemic ^{2,8}	S ¹	<i>Collomia diversifolia</i>	CLDV_CLHT	2018	11.06
	NS ¹¹	<i>Collomia heterophylla</i>			
Endemic ⁹	S ¹²	<i>Layia discoidea</i>	LADI_LAGL	2017	6.19
	NS ¹²	<i>Layia glandulosa</i>			
Endemic ^{2,3}	S ¹	<i>Mimulus nudatus</i>	MNUD_MGU T	2018	6.4
	NS ⁴	<i>Mimulus guttatus</i>			
Endemic ^{2,10}	S ³	<i>Collinsia greenei</i>	COGR_COSP	2017	1.17
	NS ³	<i>Collinsia sparsiflora</i>			
Endemic ^{2,11}	S ¹²	<i>Camissonia benitensis</i>	CABE_CAST	2017	4.58
	NS ¹²	<i>Camissonia strigulosa</i>			

Table 2.2: Difference in flowering onset between sister taxa in their home soil. The difference was taken by subtracting the average days to first flower of nonserpentine seeds in nonserpentine soil from serpentine seeds in serpentine soil. Flowering differences in red indicate earlier flowering in serpentine soil, while flowering differences in black indicate later flowering in serpentine soil. All pairs, except *Clarkia breweri* (CABR) have significant differences after multiple comparisons.

*p < .05, ** p < .001, *** p < .0001

Pair name	Pair type	Mean days to first flower of NS taxon	Mean days to first flower of S taxon	Flowering onset difference (S taxon – NS taxon)	t	df
CACO	T	92.53	99.46	6.93*	-2.39	49.17
CABR	T	88.11	93.34	5.24	-1.84	54.98
TWILD	T	86.32	113.86	27.54***	-24.73	54.74
COSP	T	53.69	68.30	14.61***	-7.22	37.90
COHT	T	69.40	89.70	20.30***	-5.87	42.91
PLER	T	82.59	89.11	6.52**	-3.50	51.97
MGUT	T	116.27	134.47	18.20***	-5.30	38.29
NAHX	T	174.19	151.63	-22.56***	9.11	46.28
NAPB	T	102.79	127.00	24.21**	-4.23	30.94
CAGT_CAGA	E	140.63	119.42	-21.21***	9.18	47.49
CABE_CAST	E	83.64	107.40	23.76***	-5.17	26.81
COGR_COSP	E	53.69	90.68	36.99***	-15.29	29.77
MNUD_MGUT	E	116.27	77.26	-39.01***	13.47	38.42
CLDV_CLHT	E	92.50	107.58	15.08***	-6.71	45.43
NARS_NAHX	E	174.19	160.87	-13.32***	6.35	34.05
NAJP_NAHN	E	128.71	143.50	14.79**	-3.01	21.29
LADI_LAGL	E	84.73	112.50	27.77***	-7.74	45.65

Table 2.3: Summary of the effects of seed (genetic) and soil (plastic) on flowering time differences between serpentine seeds in their home soil and nonserpentine seed in their home soil.

Pair type	Pair name	Seed effect (genetic)		Soil effect (plastic)	
		F	p-value	F	p-value
Tolerator	MGUT	62.47	1.1E-10	0.03	0.858
Tolerator	CABR	5.30	2.4E-02	0.51	0.476
Tolerator	CACO	4.41	3.9E-02	0.81	0.372
Tolerator	COHT	48.23	2.7E-09	0.66	0.419
Tolerator	COSP	84.39	3.6E-13	0.06	0.800
Tolerator	NAHX	53.34	2.7E-10	37.14	4.6E-08
Tolerator	NAPB	28.49	7.9E-07	26.40	1.8E-06
Tolerator	PLER	21.04	1.7E-05	0.00	0.946
Tolerator	TWILD	390.11	6.5E-33	47.51	1.0E-09
Endemic	CABE_CAST	33.31	9.9E-07	2.03	0.162
Endemic	CAGT_CAGA	135.03	2.4E-18	0.66	0.419
Endemic	CLDV_CLHT	14.11	3.6E-04	41.15	1.5E-08
Endemic	COGR_COSP	416.35	7.0E-32	2.42	0.124
Endemic	LADI_LAGL	23.03	8.9E-06	32.27	2.9E-07
Endemic	MNUD_MGUT	299.01	3.3E-26	5.48	0.022
Endemic	NAJP_NAHN	7.40	9.6E-03	4.49	0.040
Endemic	NARS_NAHX	44.50	3.3E-09	1.73	0.192

Table 2.4: Within-pair t-tests between the maternal family reaction norm slopes of flowering time onset in nonserpentine versus serpentine soil. Reaction norms are calculated from subtracting family-level flowering onset in nonserpentine soil from that in serpentine soil – positive values indicate the family flowered later in serpentine and negative values indicate the family flowered earlier in serpentine. T-tests were not conducted on pairs for which less than two nonserpentine individuals survived in serpentine soils (NAHX, CABE_CAST, CLDV_CLHT, NARS_NAHX). Multiple pairs had low nonserpentine survival in serpentine soils, as in reflected in the low degrees of freedom and high p-value, despite a large difference in mean reaction norm slopes between the seed sources.

Pair name	Pair type	Mean reaction norm slope (n)		t	df	p
		Nonserpen- tine taxon	Serpentine taxon			
MGUT	T	46.429 (2)	-1.929 (14)	11.473	5.712	3.69E-05
CABR	T	9.346 (19)	-1.083 (24)	2.260	40.256	0.029
CACO	T	-1.644 (26)	3.875 (24)	-0.440	47.938	0.662
COHT	T	13.123 (5)	2.077 (13)	1.650	9.335	0.132
COSP	T	23.955 (2)	-2.455 (11)	1.997	1.184	0.264
NAHX	T	NA (0)	-14.077 (26)	NA	NA	NA
NAPB	T	-3.054 (24)	12.929 (28)	-0.809	44.920	0.423
PLER	T	24.952 (16)	-0.577 (26)	5.027	17.024	1.03E-04
TWILD	T	19.03 (19)	9.286 (28)	7.260	26.493	9.28E-08
CABE_CAST	E	NA (0)	7.818 (11)	NA	NA	NA
CAGT_CAG A	E	-1.533 (24)	0.95 (20)	-0.435	41.999	0.666
CLDV_CLHT	E	43 (1)	15.588 (17)	NA	NA	NA
COGR_COSP	E	18.667 (9)	-3.667 (18)	5.027	24.236	3.78E-05
LADI_LAGL	E	7.792 (19)	20.944 (18)	1.100	32.970	0.279
MNUD_MG UT	E	14.064 (5)	6.136 (22)	1.194	4.276	0.295
NAJP_NAHN	E	24.9 (2)	8.1 (10)	3.074	5.328	0.025
NARS_NAH X	E	NA (0)	-2.433 (30)	NA	NA	NA

Table 2.5: Taxon-level phenotypic selection analysis on flowering onset in serpentine soils. Red selection coefficients indicate selection on early flowering. Bolded p-values indicate significance following corrections for multiple comparisons. Taxa for which there are no statistics are those with 5 or less individuals surviving in serpentine soil.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.00$

Pair name	Taxon	Sample size	Slope (β)	t	p
MGUT	NS	2	NA	NA	NA
MGUT	S	17	-1.943	-3.915	0.001 ***
CABR	NS	26	-0.124	-0.517	0.610
CABR	S	29	-0.353	-1.953	0.061
CACO	NS	28	-0.542	-0.969	0.341
CACO	S	26	-0.939	-1.990	0.058
COHT	NS	6	0.200	0.251	0.814
COHT	S	19	-2.037	-1.349	0.195
COSP	NS	2	NA	NA	NA
COSP	S	20	1.395	0.534	0.600
NAHX	NS	NA	NA	NA	NA
NAHX	S	30	-11.158	-3.308	0.003 **
NAPB	NS	26	-2.438	-2.412	0.024 *
NAPB	S	30	-1.277	-2.420	0.022 *
PLER	NS	17	-4.128	-1.684	0.113
PLER	S	27	0.229	0.272	0.788
TWILD	NS	21	-4.772	-1.473	0.157
TWILD	S	29	1.071	0.671	0.508
CABE_CAST	NS	NA	NA	NA	NA
CABE_CAST	S	15	-1.792	-1.981	0.069
CAGT_CAGA	NS	27	-0.511	-4.693	8.25E-05 ***
CAGT_CAGA	S	24	-0.967	-4.822	8.11E-05 ***
CLDV_CLHT	NS	1	NA	NA	NA
CLDV_CLHT	S	24	-0.750	-1.431	0.167
COGR_COSP	NS	9	-0.888	-0.286	0.783
COGR_COSP	S	19	-4.281	-3.890	0.001 ***
LADI_LAGL	NS	24	-0.328	-0.737	0.469
LADI_LAGL	S	26	-0.885	-2.103	0.046
MNUD_MGUT	NS	6	-4.885	-4.654	0.010 **
MNUD_MGUT	S	23	-1.205	-0.834	0.414
NAJP_NAHN	NS	3	-10.764	-4.601	0.136
NAJP_NAHN	S	14	-26.103	-6.255	4.22E-05 ***
NARS_NAHX	NS	NA	NA	NA	NA
NARS_NAHX	S	30	-0.840	-2.565	0.016*

Figure 2.1: The extent to which flowering time shifts are genetically-based versus plastic affects the permanence of reproductive isolation due to flowering overlap. A) Serpentine and nonserpentine sister taxa are growing in their home soils, and there is a flowering time shift to earlier flowering in the serpentine taxon, such that B) the flowering time distributions of the serpentine taxon (blue line) and nonserpentine taxon (green line) do not overlap and phenological isolation is strong. C) Given dispersal and survival to flowering of serpentine seed in a common nonserpentine habitat, D) phenological isolation will remain strong (i.e., be “permanent”) if flowering time shifts are genetically-based. E) If flowering time shifts are plastic, serpentine migrant individuals will have similar flowering schedules as the nonserpentine local individuals, and phenological isolation will be lost (i.e., be “ephemeral”).

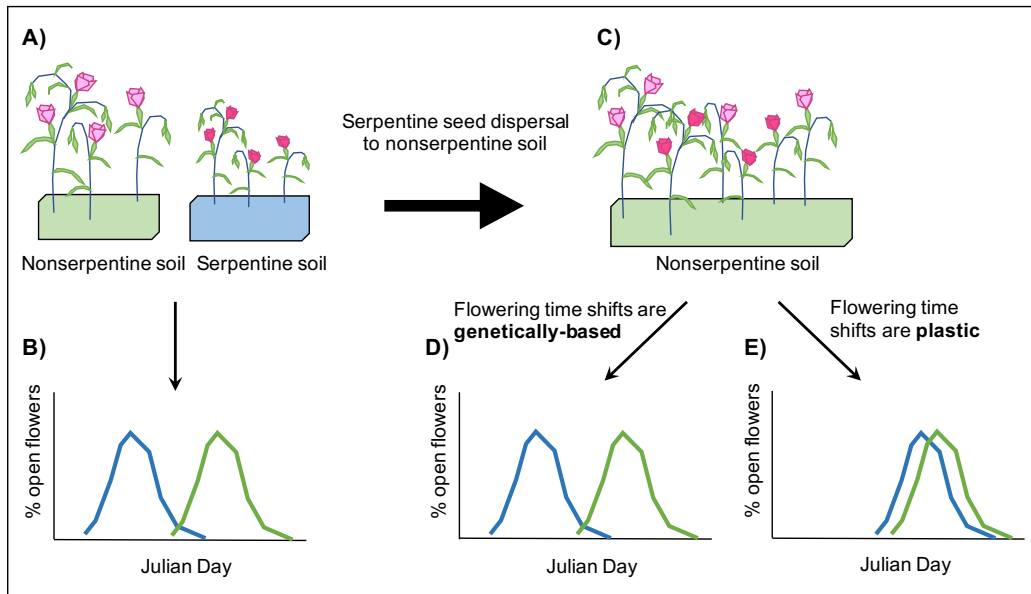


Figure 2.2: The majority of flowering onset shifts in serpentine-adapted taxa are towards later flowering. All pairs but the *Clarkia breweri* pair (CABR) show significant differences in flowering onset between serpentine and nonserpentine taxa (Table 1). Points represent the difference in mean flowering onset of serpentine plants in their home soil and mean flowering onset of nonserpentine plants in their home soil for a given sister taxa pair. Values great than 1 indicate the serpentine taxon flowered later than the nonserpentine taxon, whereas values less than 1 indicate the serpentine taxon flowered earlier than the nonserpentine taxon. Filled circles represent tolerator pairs and open diamonds represent endemic pairs.

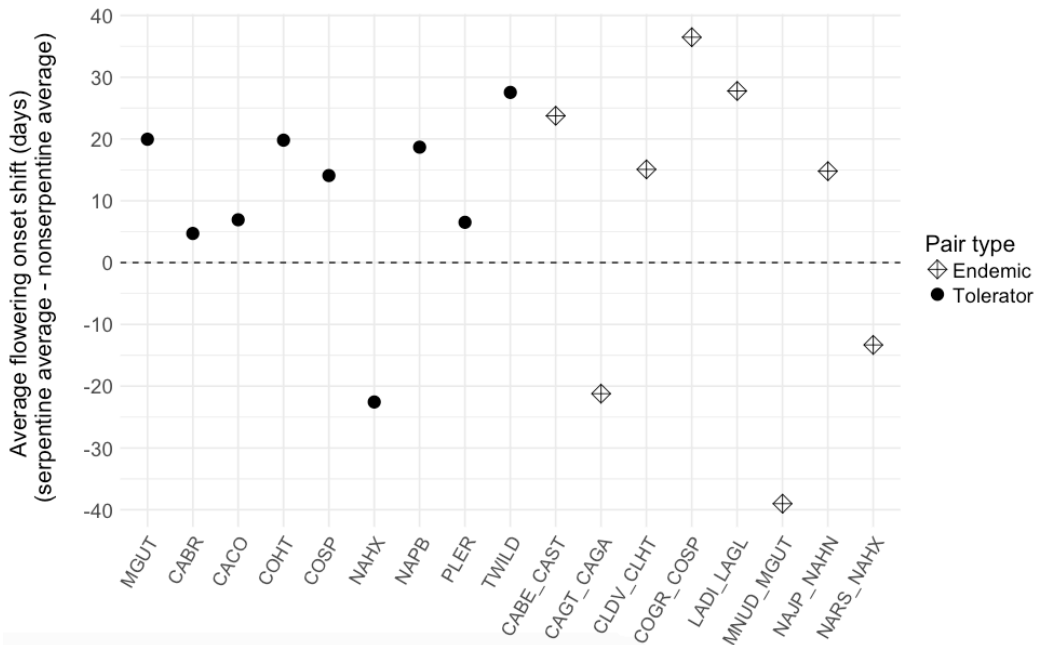


Figure 2.3: Characterization of the contribution of genetic versus plastic differentiation on flowering time shifts of serpentine plants in serpentine soils versus nonserpentine sisters in nonserpentine soils. A) Cartoon of one pair (NAHX) showing treatments we contrasted to determine the genetic and plastic effects. Differences in flowering onset between serpentine and nonserpentine seeds in a common nonserpentine soil environment is due to genetic effects, whereas differences in flowering onset between serpentine seeds in nonserpentine versus serpentine soils is due to plastic effects. B) For each pair (column), variance in flowering onset between serpentine seeds growing in serpentine soil and nonserpentine seeds growing in nonserpentine soil due to the genetic differentiation (seed effect), plasticity (soil effect) and residual variance (within treatment variation).

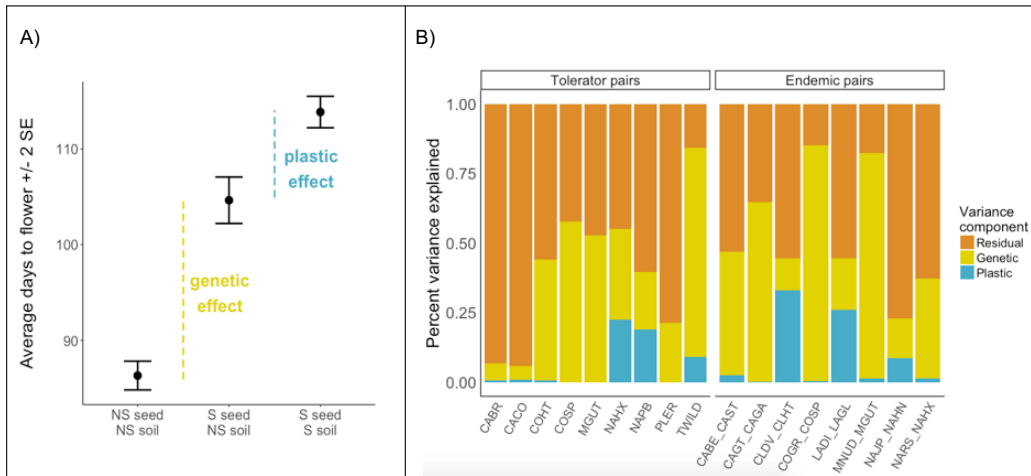


Figure 2.4: Per-pair differences in flowering onset between three seed/soil treatments – nonserpentine (NS) seeds in NS soil, serpentine (S) seeds in NS soil, and S seeds in S soil – that mimic those shown in Figure 3A. The lower and upper edges of the boxplots show the first and third quartiles, respectively, and points indicate data points that are farther than 1.5 times the interquartile range.

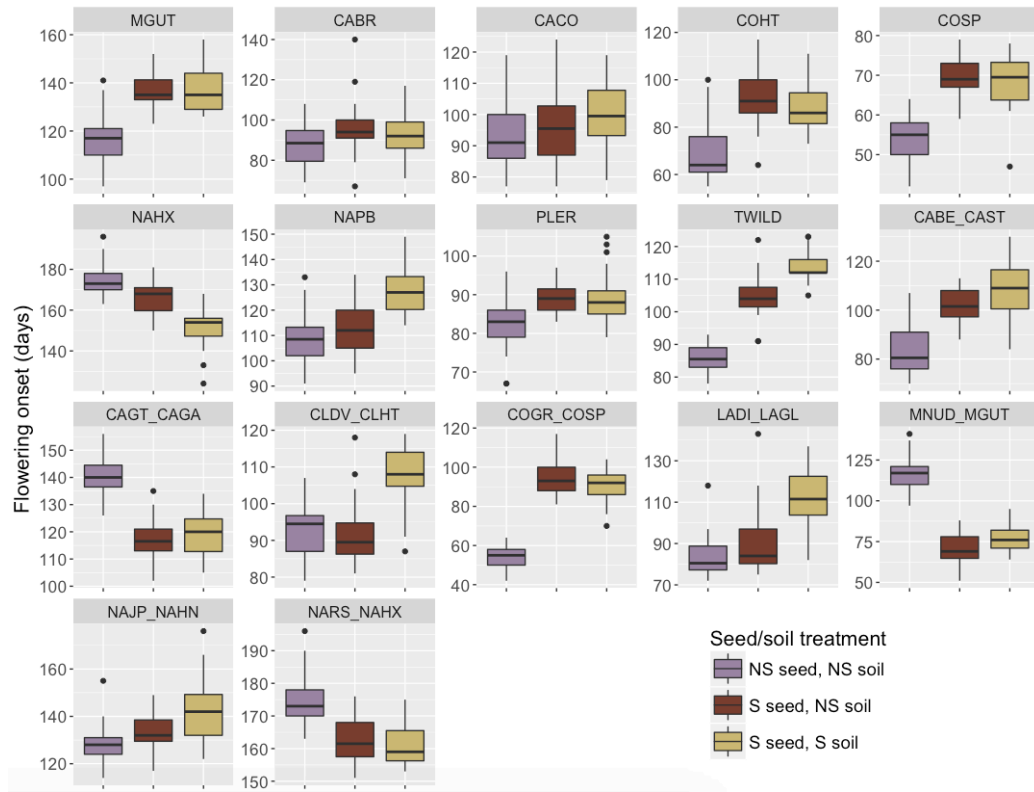


Figure 2.5: Variation in maternal family reaction norms in flowering onset within the nonserpentine and serpentine seeds of each pair. The y axis is the reaction norm slope per family, and is calculated by subtracting the family value when in nonserpentine soil from the family value when in serpentine soil. Points above the dashed zero line are families that flowered later in serpentine soils relative to nonserpentine soils, and points below the dashed zero line are families that flowered earlier in serpentine soils relative to nonserpentine soils. The lower and upper edges of the boxplots show the first and third quartiles, respectively, and points indicate data points that are farther than 1.5 times the interquartile range.

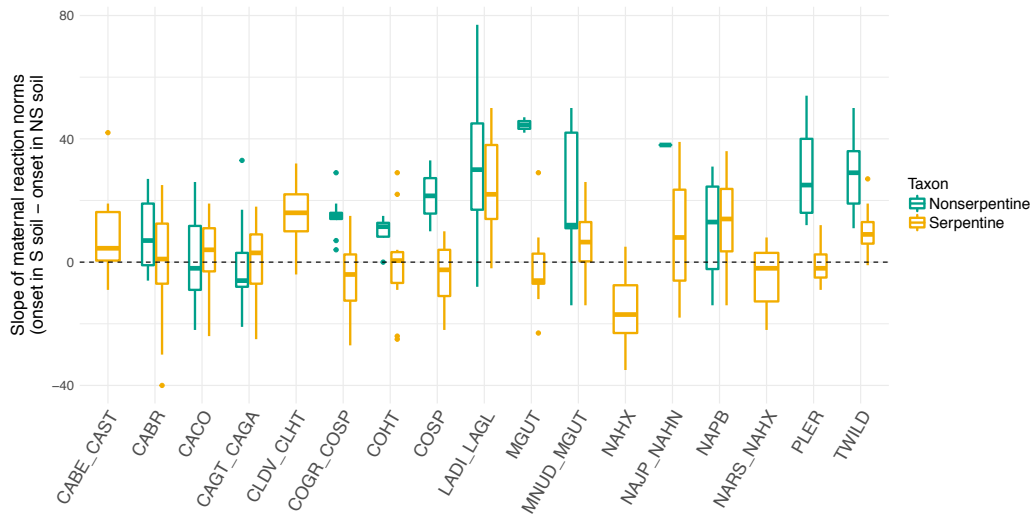


Figure 2.6: Selection on flowering time of both nonserpentine and serpentine taxa in serpentine soil. The solid line represents the linear regression line of best fit and the gray band is the 95% confidence interval.

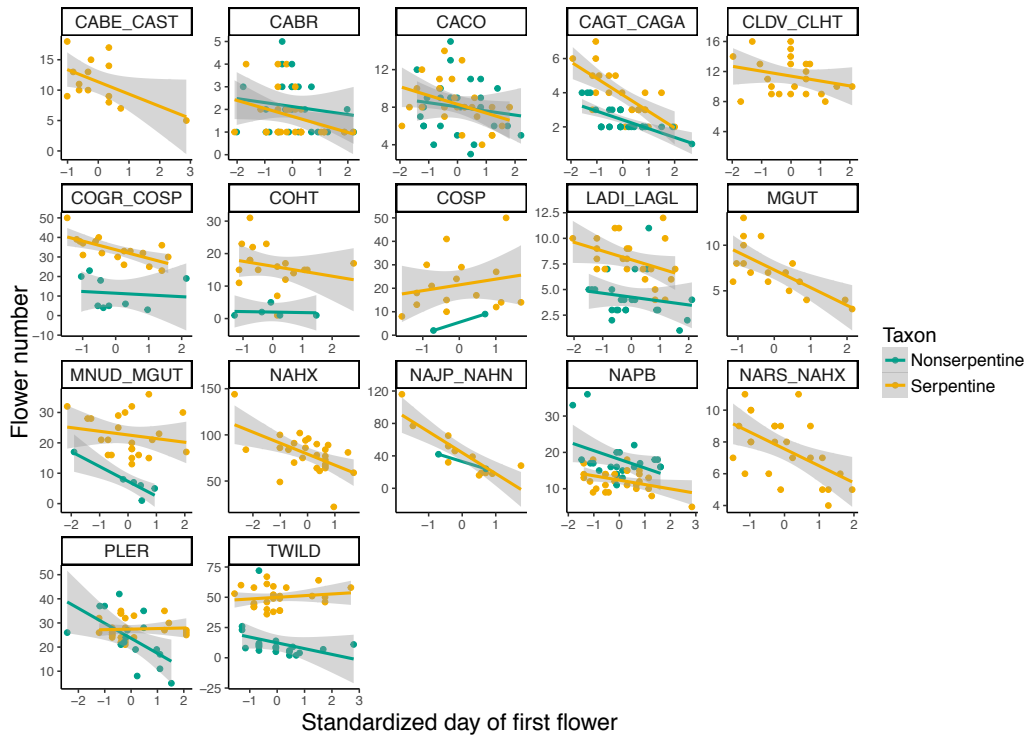


Figure 2.7: Phenological isolation among endemic and tolerator sister taxa pairs does not differ. Red symbols indicate phenological isolation between sister taxa when each is in its home soil. Blue symbols indicate phenological isolation between sister taxa when each is in a common nonserpentine soil. Larger differences between phenological isolation between ecological contexts represents less permanence in phenological isolation.

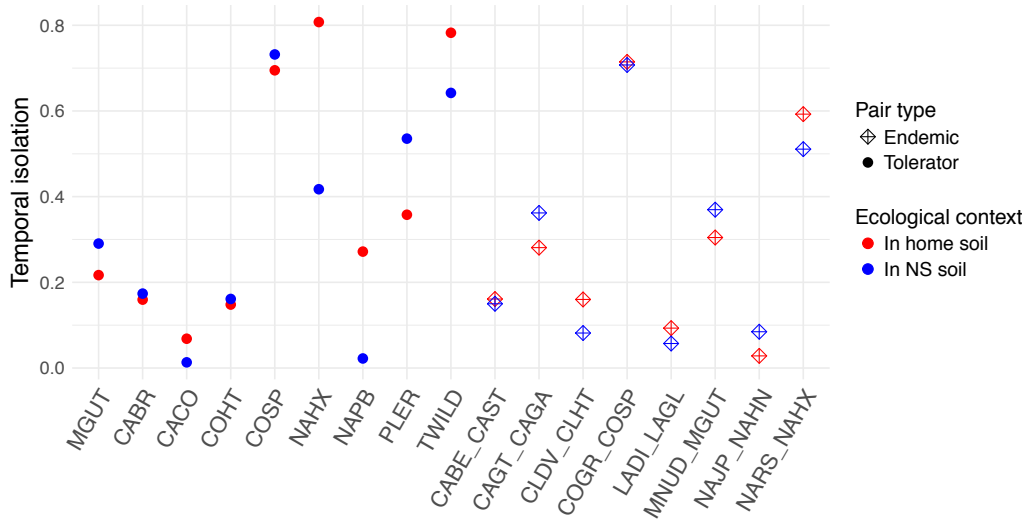


Figure 2.8: Multivariate soil distance between serpentine and nonserpentine sister taxa explains part of the variation in the magnitude of flowering time shifts in sister taxa pairs. Each dot represents one pair. PGLS p-value = 0.051.

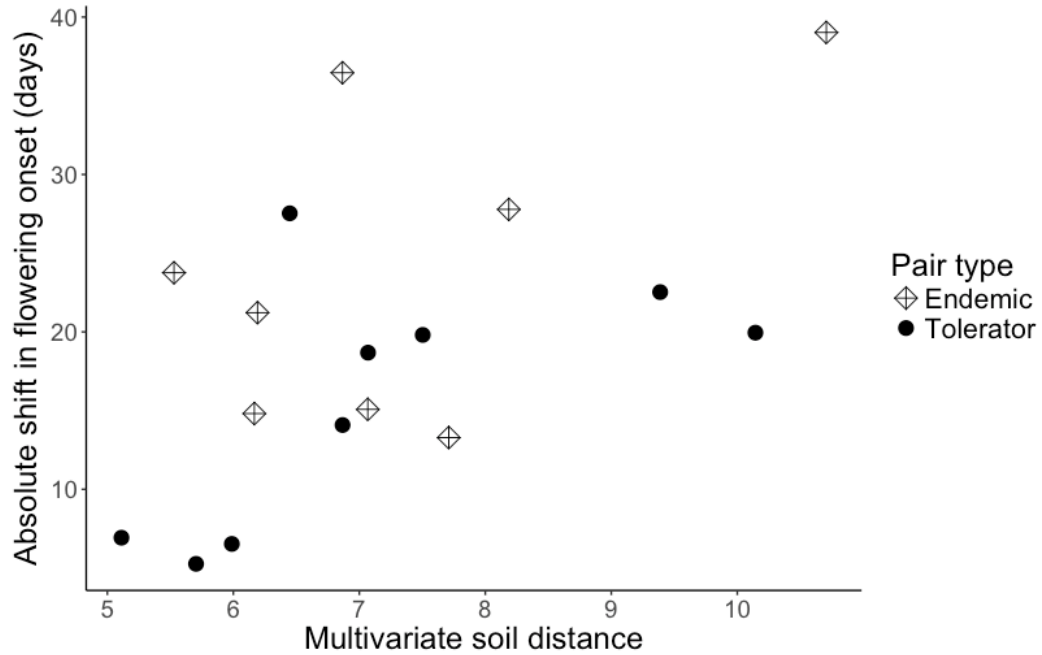


Figure 2.9: Multivariate climatic differences within sister taxa do not explain the magnitude of shifts in flowering time within sister taxa. PGLS, $p = 0.68$.

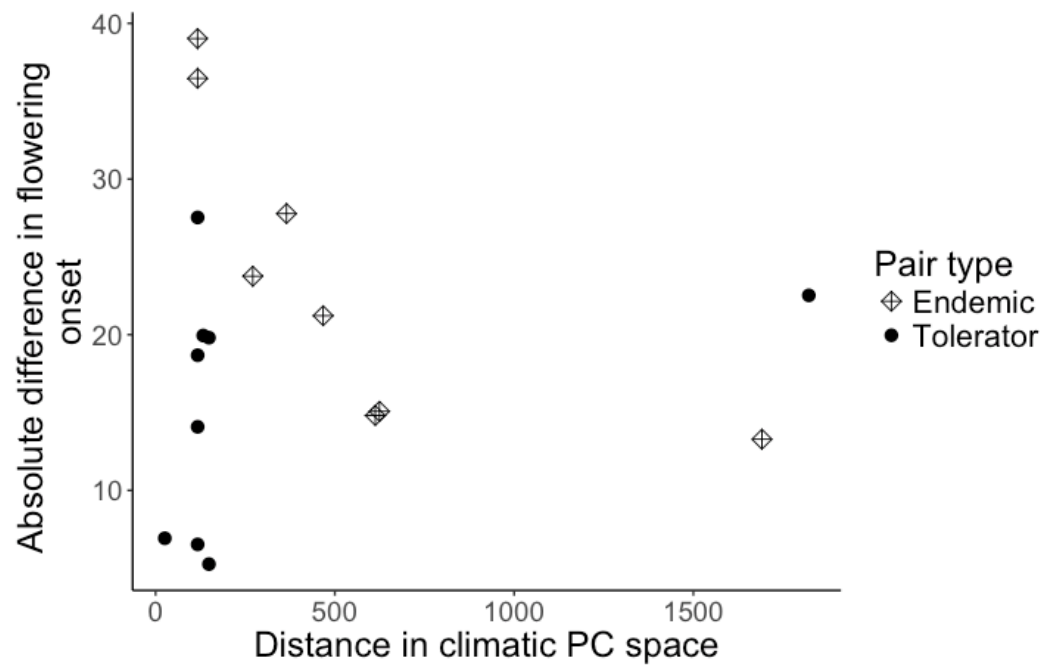
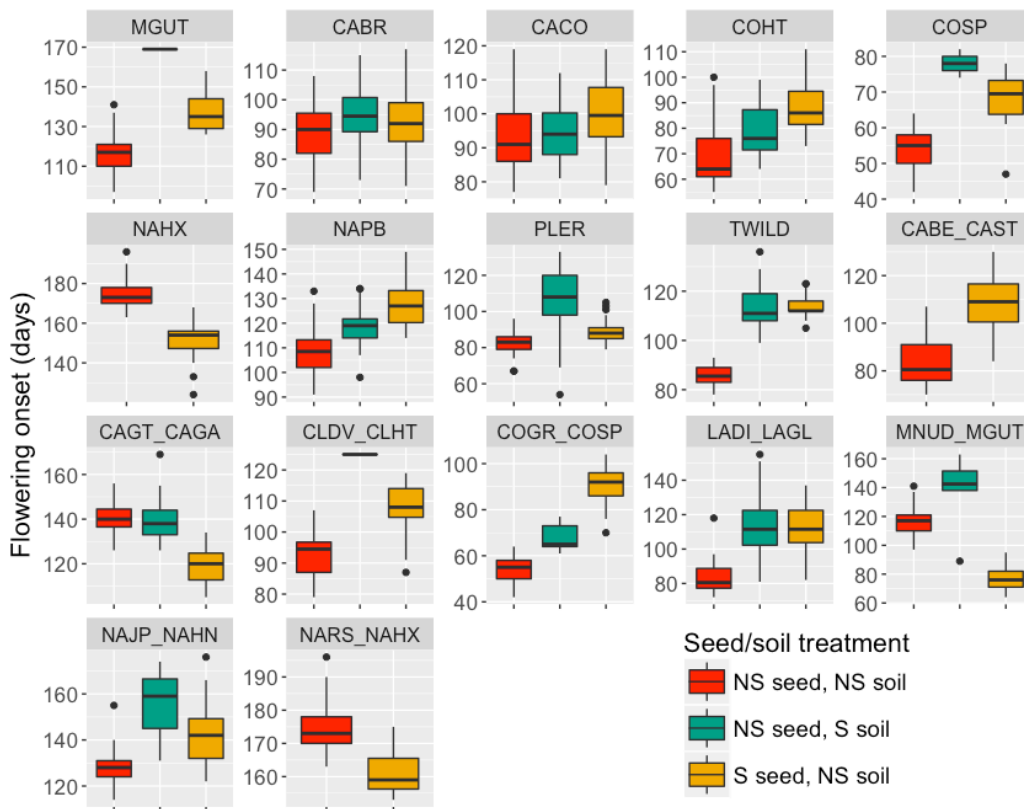


Figure 2.10: Per-pair differences in flowering onset among three treatments that show plastic shifts of nonserpentine taxa in serpentine soil (red versus green boxplots), relative to the flowering onset of serpentine plants in serpentine soils (yellow boxplots). Patterns consistent with genetic assimilation are seen in *Navarretia pubescens*, *Collinsia heterophylla*, and *C. greenei*-*C. sparsiflora*. Patterns consistent with genetic compensation are seen in *Mimulus guttatus*, *C. sparsiflora*, *Plantago erecta*, *Collomia diversifolia*-*C. heterophylla*, *Mimulus nudatus* – *M. guttatus*, and *N. jepsonii*-*N. heterandra*.



Chapter 3

Trade-offs between serpentine adaptation and competitive ability are associated with the evolution of serpentine endemic species but not through habitat isolation

ABSTRACT

Ecological divergence among populations can drive speciation. However, speciation is not the only evolutionary result of populations that undergo ecological divergence, as evidenced by species composed of locally adapted ecotypes. The probability of speciation increases when local adaptation of populations to different habitats is accompanied by fitness trade-offs, in which adaptation to one habitat comes at a cost to fitness in another habitat. Fitness trade-offs may be associated with habitat isolation when they involve selection against migrants within a habitat type. We use replicated instances of adaptation to serpentine soils in the California flora that have led to both population- and species-level divergence to ask whether speciation following ecological divergence is associated with stronger fitness trade-offs and habitat isolation than within-species ecological divergence. Adaptation to chemically and physically harsh serpentine soils is hypothesized to trade-off directly with competitive ability, rendering serpentine-adapted species unfit in more productive nonserpentine habitats. We quantify fitness and competitive abilities of 17 serpentine and nonserpentine sister taxa pairs that vary in their progress towards

speciation in a field-soil based reciprocal transplant greenhouse experiment. We find that adaptation to serpentine soils is associated with a greater loss in competitive ability in between-species pairs versus within-species pairs, which is the first multi-species evidence to support this classic paradigm. However, we don't find evidence of patterns of local adaptation in the between- versus within-species pairs that are consistent with the hypothesis that greater fitness trade-offs lead to stronger habitat isolation and speciation of serpentine endemics.

INTRODUCTION

Ecological divergence among populations can promote speciation (Darwin, 1859; Mayr, 1947; Kirkpatrick and Ravigne, 2002; Coyne and Orr, 2004; Schluter, 2009; Schemske, 2010; Sobel et al., 2010). Local adaptation is a common phenomenon (Leimu and Fischer, 2008; Hereford, 2009), and comparative studies indicate a relationship between the amount of reproductive isolation and ecological differences among taxa (Funk et al., 2006). However, adaptive divergence among populations does not always lead to speciation, as shown by species occupying a broad range of habitats. The paradox of why adaptive divergence only *sometimes* leads to speciation is a critical gap in our understanding of speciation.

One factor championed to promote speciation is divergent natural selection (Sobel et al., 2010; “ecological speciation” sensu Schluter, 2001; Rundle and Nosil,

2005a). Spatially structured divergent natural selection counteracts gene flow to produce local adaptation among populations (Kawecki and Ebert, 2004) and sets the stage for among-habitat fitness trade-offs. Fitness trade-offs are central to theories of ecological specialization and endemism (Futuyma and Moreno, 1988), the maintenance of polymorphism (reviewed by Felsenstein, 1976) and local adaptation (Kawecki and Ebert, 2004). Strong fitness trade-offs between locally adapted populations contribute to habitat isolation, a form of reproductive isolation wherein assortative mating within habitats is promoted through selection against migrant individuals (Dobzhansky, 1937; Coyne and Orr, 2004; immigrant inviability sensu Nosil et al., 2005). Habitat isolation may be one of the first forms of reproductive isolation to evolve through divergent natural selection, and given that it acts early in the life cycle, it is likely to be an important barrier in speciation. Yet, empirical evidence from reciprocal transplant experiments suggests that fitness trade-offs are not ubiquitous (Hereford, 2009).

Comparative studies of taxa pairs that have undergone ecological divergence to similar characteristics but vary in progress to speciation can illuminate how factors that promote speciation evolve. Thus far, most studies using a replicated approach do so in a narrow phylogenetic context, e.g., among populations within a species or species within a genus (McKinnon and Rundle, 2002; Lowry et al., 2008; Nosil and Sandoval, 2008; Thorpe et al., 2008; but see, Rosenblum and Harmon, 2010). However, when this approach is used at a broad scale, across

distant taxon pairs, we gain insights into broader patterns of how speciation proceeds.

Plant species that have adapted to serpentine soils are an ideal system to study factors that promote speciation via adaptive divergence. Serpentine soils are chemically and physically harsh habitats, imposing strong selection on plants (Kruckeberg, 1951; Brady et al., 2005). Because of the island-like distribution of serpentine outcrops, strong divergent selection can occur over small spatial scales (Kay et al., 2011). Adaptation to serpentine has independently led to the evolution of both serpentine endemics (species only occurring on serpentine) and serpentine tolerators (species with populations on and off serpentine) in 39 plant families in California (Anacker, 2011), allowing for the selection of replicated instances of ecological divergence that vary in their progress towards speciation.

Given the strong selection imposed by serpentine, habitat isolation is hypothesized to be an important form of reproductive isolation in this system (Kay et al., 2011). Habitat isolation between nonserpentine and serpentine populations in serpentine habitats is often strong and driven by a lack of tolerance alleles in nonserpentine populations, even among ecotypes within species (Kruckeberg, 1951; Brady et al., 2005; Wright, Stanton, et al., 2006; Kay et al., 2011). However, the role of habitat isolation in blocking gene flow within nonserpentine habitats is less clear. The influential work of Kruckeberg (1951;

1967) demonstrated that serpentine-adapted taxa don't require the peculiar chemistry of serpentine and have equal or higher fitness when planted in nonserpentine soils. He hypothesized that serpentine endemic species were instead restricted to serpentine soils through a direct trade-off between serpentine adaptation and competitive ability. This hypothesis, known as the competition trade-off hypothesis (Rajakaruna, 2017) is the primary paradigm explaining the restriction of serpentine endemics. Serpentine tolerators, in contrast, are predicted to experience little to no fitness trade-offs between serpentine adaptation and competitive ability, allowing gene flow to maintain species cohesion. Kruckeberg (1951) didn't extend the competition trade-off hypothesis to the evolution of reproductive isolation, but it serves as a potential mechanism by which habitat isolation is achieved in nonserpentine soils.

Here, we use 17 replicated instances of edaphic divergence across a wide range of eudicot phylogenetic diversity to experimentally test whether larger fitness trade-offs associated with ecological divergence promote speciation through strong habitat isolation. We use 9 serpentine tolerator species and 8 serpentine endemic species, and pair one serpentine population from each with a nonserpentine sister taxon. We chose a nonserpentine population from within the same tolerator species for the tolerator serpentine taxon, forming "tolerator taxa pairs" that represent adaptive divergence at the population level. We chose a nonserpentine population from the sister species of each serpentine endemic species, forming

“endemic taxa pairs” that representing adaptive divergence that has led to speciation. We perform within-pair reciprocal transplants in a greenhouse using field-collected seed and soil, and a standardized competitor, to quantify 1) fitness trade-offs across environments, 2) habitat isolation within environments and 3) competitive ability (Fig. 3.1) to disentangle the effects that soil chemistry and competition have on fitness trade-offs and habitat isolation.

We first test the effects of soil chemistry on fitness trade-offs and habitat isolation. We predict that nonserpentine taxa will have large fitness trade-offs (Fig. 3.1, contrast 1), leading to strong habitat isolation in serpentine soils (Fig. 3.1, contrast 2). Even though ecotypic differentiation has been documented in some tolerator species (Kruckeberg, 1951, 1967; Sambatti and Rice, 2006; Wright, Stanton, et al., 2006; Kay et al., 2011), other tolerator species may occur on and off serpentine via plasticity. Thus, we predict that on average, endemic pairs will have stronger habitat isolation in serpentine than tolerator pairs, and that the fitness trade-off of nonserpentine sister taxa will be greater in endemic pairs than tolerator pairs. In nonserpentine soils, we predict that, consistent with Kruckeberg’s (1951) findings, there will be weak or no fitness trade-offs in serpentine taxa across all pairs (Fig. 3.1, contrast 3) when competition is not included. Likewise, we predict that habitat isolation will be low in the plain nonserpentine soil across all pairs (Fig. 3.1, contrast 4).

Next, we ask whether fitness trade-offs of serpentine taxa across habitats (Fig. 3.1, contrast 5) and habitat isolation within nonserpentine habitats (Fig. 3.1, contrast 6) is achieved through the effects of competition. We predict that, unlike in the plain nonserpentine soils, in the competitive nonserpentine treatment, endemic pairs will have higher habitat isolation than tolerator pairs. We quantify the fitness trade-offs of serpentine seeds in the competitive nonserpentine treatment to assess the effects of both soil and competition on fitness trade-offs. We predict that the endemic serpentine taxa will have larger fitness trade-offs in the competitive nonserpentine soil than tolerator serpentine taxa.

If competitive exclusion restricts species to serpentine, then we predict endemic serpentine taxa to have lower competitive abilities than tolerators. We quantify the competitive abilities of all serpentine taxa in their sister taxon's nonserpentine soil with and without a standard competitor (Fig. 3.1, contrast 7) to ask whether endemic serpentine taxa are worse competitors than tolerator serpentine taxa. Endemics could be worse competitors than tolerator serpentine populations for one of two reasons: either they evolved from taxa that were also poor competitors, or a loss in competitive ability resulted following adaptation to serpentine. To distinguish between these two hypotheses, we ask whether endemic sister taxa pairs have more divergence in competitive ability than tolerator sister taxa pairs (Fig. 3.1, difference between contrasts 7 and 8).

METHODS

Study system and sister taxa pair selection— We chose 9 tolerator sister taxa pairs and 8 endemic sister taxa pairs that represent independent origins of serpentine adaptation (Table 3.1). Each taxa pair is comprised of one serpentine and one nonserpentine populations. To choose populations and species, we generated a list of annual serpentine tolerators and serpentine endemics that occur in the coast ranges of California. We used a mix of serpentine affinity scores from Safford et al (2005), phylogenetic relationships and serpentine status of 23 genera generated by Anacker et al. (2011), and supplemental phylogenies for genera not included in Anacker et al. (2011) study (see Table 3.1 for species-specific citations) to generate the initial list. We searched for locally abundant populations in serpentine habitats at the UC McLaughlin Reserve, Mt. Tamalpais in Marin Co, serpentine grasslands in the west San Francisco Bay area, in the Mt. Diablo Range, and in the serpentine barrens of New Idria in southern San Benito Co. We chose serpentine taxa that were easy to access and had a nonserpentine sister taxon close to the location of the serpentine taxon. We searched for a spatially proximate non-serpentine sister taxon using CalFlora occurrence data to minimize environmental differences other than the edaphic habitat. However, due to allopatric distributions, our sister taxa pairs vary in geographic distance (Table 3.1). When we found multiple populations per taxon, we chose the population that was the easiest to access and had the largest population size. Our final list of sister taxa pairs spans six plant families and nine genera.

We use three of our nonserpentine taxa as the nonserpentine sister taxon in two pairings each. For example, *Mimulus nudatus* is a serpentine endemic hypothesized to be derived from within *Mimulus guttatus* (Macnair and Gardner, 1998). We chose a *M. guttatus* nonserpentine population to serve as the nonserpentine sister taxon for *M. nudatus*. However, because *M. guttatus* is a serpentine tolerator itself, we also use the same nonserpentine population as the nonserpentine sister taxon for a serpentine population of *M. guttatus*. We use this same overlapping design for the *Collinsia greenei* - *C. sparsiflora* endemic pair and *C. sparsiflora* tolerator pair, and for the *Navarretia rosulata* – *N. heterodoxa* endemic pair and the *N. heterodoxa* tolerator pair. Assuming serpentine adaptation evolved independently for the tolerant population and the endemic species, this is a powerful paired way to compare endemics and tolerators to a common reference taxon. Serpentine adaptation has been shown to evolve independently multiple times within tolerator species, e.g., within *Cerastium alpinum*, *Alyssum bertolonii*, the *Lasthenia californica* complex, *Mimulus guttatus*, and *Arabidopsis lyrata* (Nyberg Berglund et al., 2001, 2004; Mengoni et al., 2003; Rajakaruna and Whitton, 2004; Turner et al., 2010; Selby, 2014; Selby and Willis, 2018). Likewise, independent origins of serpentine adaptation within tolerator species has led to the evolution of endemic species. For example, there are at least 3 local *Streptanthus* endemic species hypothesized to be derived from the tolerator *Streptanthus glandulosus* (Kruckeberg, 1957), and there are two

local/restricted *Mimulus* endemic species hypothesized to be derived from the tolerator *Mimulus guttatus* (Macnair and Gardner, 1998).

Differences in fitness trade-offs, habitat isolation, and competitive ability between endemic and tolerator sister-taxa pairs may be due to the amount of time sister taxa have had to diverge, with the between-species pairs predicted to be more genetically isolated than the within-species pairs. We sequenced ITS for all taxa used in this study (for details, see Sianta and Kay, 2019) and counted the number of nucleotide substitutions between sister taxa as a metric of genetic distance. Genetic distances are reported in Table 3.1.

Seed and soil collections-- At each population, we collected seed from 30-40 maternal plants, i.e., “families”. Maternal plants were haphazardly selected throughout the population, avoiding seed from individuals within 1-2 meters of each other to maximize genetic diversity. Most of the plant taxa we chose have gravity-dispersed seeds, and thus clusters of individuals are likely closely related. Collected fruits were stored in coin envelopes and kept at 4 °C until planting.

We collected approximately 15 liters of soil from within the plant population to use in a greenhouse reciprocal transplant experiment. Soil was collected from within the top 20 cm and stored in open 4-liter plastic bags at room temperature. We discarded rocks larger than 3.5 cm in diameter, but otherwise retained the natural variation in particle size in the soil.

Greenhouse reciprocal transplant experiment – For each sister taxa pair, we set up a greenhouse reciprocal transplant experiment in field-collected soil. Families from each taxon were planted into three treatments: the pair’s serpentine soil, the pair’s nonserpentine soil, and the pair’s nonserpentine soil with a competitor. We used the same species as a competitor for all of the taxa used in the study to provide a standard metric of competitive ability. The competitor was *Bromus carinatus*, which is a California native annual grass that occurs in a wide range of habitats. Seeds of *B. carinatus* were obtained from Lerner Seeds (Bollinas, CA).

Per taxa pair, soil from each population was homogenized and potted into 90 RayLeach Conetainers (3.8 x 21 cm), including all rocks that would fit. On average, 30 pots per treatment (n = 6) were sowed with seed. Seeds from each maternal family were potted into one pot per soil type. In each pot, we planted anywhere between 1-5 seeds to ensure that at least one would germinate. Seeds were sown on the surface and watered down into the soil. In competition pots, we planted 3 *B. carinatus* seeds, ultimately thinning to one individual per competition pot. We put all pots into a stratification treatment (no light, 4° C, daily misting) in a growth chamber (Convicon model E15) to induce germination for a period of 2 weeks or until seeds started germinating, whichever came first. Pots were then moved to a seedling establishment growth chamber (Convicon model E15; 20° C day, 15° C night, 10-hour days, daily top-watering) for an average of 5.4 weeks.

We planted seeds in early November to mimic the cycle of most annual plants in a Mediterranean climate (cool, wet winters and hot, dry summers).

Four of the sister taxa pairs were germinated in petri dishes. The *Layia discoidea*-*L. glandulosa* pair were germinated on filter paper soaked with 150 ppm gibberellic acid because of their germination requirement for light (B. Baldwin, personal communication). The *Camissonia benitensis*-*C. strigulosa*, *Mimulus nudatus*-*M. guttatus*, and the *M. guttatus*-*M. guttatus* pairs were all germinated on filter paper soaked in DI water. The seeds of these species were all too small to see in the pots, and thus it was difficult to distinguish the planted seeds from seeds that recruited naturally. All petri dishes were kept in the stratification growth chamber until germination. Once radicles emerged, seeds were transplanted into pots and put into the seedling establishment growth chamber.

After seedling establishment, all pots were moved to the greenhouse on the same day (within each experimental round, see below), where pots from all sister taxa pairs were randomized. Plants experienced the natural day lengths in the greenhouse, with supplemental lights turned on in between sunrise and sunset if the light intensity fell below 400 Wm². Pots were top-watered with DI water every 2-5 days for 16 weeks, and then reduced to watering pots every 8-10 days for 2.5 weeks, and in the 2018 experiment (see below) at a subsequent 15- and 6-day interval, before ultimately cutting the water off completely. The tapering of

the water regime was designed to mimic the precipitation cycle in California, where winter rains taper off in May.

Because of the large sample size of this experiment, we split the pairs into two experimental rounds. In the first year (2016-2017; “2017”) 5 tolerator pairs and 3 endemic pairs were grown and in the second year (2017-2018; “2018”) 4 tolerator pairs and 5 endemic pairs were grown (Table 3.1). Among the two years, there were some differences in growing conditions. For example, in the first experimental round, pairs spent an average of 23 days in the seedling establishment chamber, and in the second experimental round pairs spent an average of 50 days in the seedling establishment chamber. In the first experimental round, the greenhouse temperature controls were set to maintain day temperatures around 15° C and night temperatures around 18° C, whereas in the second experimental round, the controls got flipped and day temperatures were maintained around 21° C and night temperatures were maintained around 13° C. Because we grew a mix of endemic and tolerator pairs, any differences we see between endemic and tolerator pairs in the traits measured should not be driven by the variation in greenhouse temperatures across years. To statistically correct for the greenhouse variation among years, we use year as a covariate in analyses below.

Fitness measurements—We removed all pots from the experiment that did not germinate. Because we sowed seeds on the surface of the soil, we were

able to quantify germination as the day at which the radicle emerged from the seed, allowing us to capture death at this early life history stage. Death of a plant after the radicle had emerged but before cotyledons emerged was common in some of the nonserpentine taxa growing in serpentine soils. A total of 2,226 individual pots germinated and were included in the analyses.

For plants that germinated, we tracked survival to flower and flower production on each individual plant. On a subset of plants per treatment we counted ovule numbers in the second flower produced. Four of the pairs (*Navarretia pubescens*, *Trifolium willdenovii*, *Plantago erecta*, and *Layia discoidea-L. glandulosa*) had fixed ovule numbers across all treatments. Our total metric of fecundity for each individual pot was the number of flowers it produced multiplied by the average ovule number for the treatment it was in. Instead of flower production, we counted inflorescence (head) number in the Asteraceae pair *L. discoidea-L. glandulosa*. The heads varied in size between the two species and among the three treatments. On every individual we measured the head diameter of the first head produced and, on a subset of individuals, counted the number of flowers within that head. There is a strong relationship between head diameter and total flower number per head across the two species ($R^2 = 0.6$, $p = 1.26E-11$). Thus, for each *Layia* individual we calculated the fecundity fitness component by multiplying total head production by the average head diameter of individuals in that treatment.

Data analysis—

General model used for fitness contrasts — All of the fitness trade-off and habitat isolation analyses involve 1) calculating differences of relative fitness in two treatments within each pair and 2) testing for differences among the endemic and tolerator pairs. We achieve this through the same 2-part Bayesian hierarchical model. The advantage of using this hierarchical Bayesian approach is that we can 1) model both viability and fecundity fitness components with appropriate distributions, 2) integrate both fitness components into one comprehensive fitness measure, 3) incorporate the within-treatment variation, and 4) include the phylogenetic structure of the data in our model that tests for differences between endemics and tolerators. In contrast, generalized mixed models (e.g., ASTER; Geyer et al., 2007) can incorporate multiple fitness components and intra-treatment variation, but cannot account for phylogenetic structure. Traditional models that incorporate phylogenetic structure (e.g., phylogenetic generalized least squares) require that each taxon is represented by one data point, which would translate to a summary statistic in our data set (e.g., the difference in mean fitness in treatment A and treatment B).

The first part of the full model (Model 1) models the survival of individual i of treatment j of pair k as a Bernoulli distribution, with θ_{jk} representing the probability of survival in treatment j of pair k (Eq. 1). Fecundity is similarly

modeled for every treatment and pair as a Poisson distribution that is parameterized by λ_{jk} , the average value of treatment j of pair k (Eq. 2). The mean fitness within each treatment and pair combination, \bar{w}_{jk} , is derived from the weighted average of all possible fitness values in each treatment/pair combination (Appendix, Eq. 3). We use the mean fitness of each treatment combination to calculate mean fitness for the two treatments in the pair (Eq. 4) and the relative fitness for each treatment/pair combination (Eq. 5) which enables us to compare fitness across the pairs. We then take the difference in mean relative fitness between the two treatments in the pair as a metric of the fitness trade-off or degree of habitat isolation (Eq. 6). We always take the difference such that positive values reflect a fitness trade-off (home – away) or habitat isolation (local – foreign). We use the degree to which the w_{dif_k} is different from zero to determine the significance and effect of a fitness trade-off or habitat isolation in pair k . We use a vague Beta prior on θ_{jk} (beta (0.001, 0.001)), and a vague gamma prior on λ_{jk} (gamma (0.001, 0.001)).

Model 1

$$\text{survial}_{ijk} \sim \text{bernoulli}(\theta_{jk}), \quad (\text{Eq. 1})$$

$$\text{fecundity}_{ijk} \sim \text{poisson}(\lambda_{jk}), \quad (\text{Eq. 2})$$

$$\bar{w}_{jk} = \frac{\theta_{jk}}{1 - e^{-\lambda_{jk}}} \cdot \lambda_{jk}, \quad (\text{Eq. 3})$$

$$\bar{w}_k = \text{mean}(\bar{w}_{jk}), \quad (\text{Eq. 4})$$

$$\text{relative } \bar{w}_{jk} = \frac{\bar{w}_{jk}}{\bar{w}_k}, \quad (\text{Eq. 5})$$

$$\text{w. dif}_k = \text{relative } \bar{w}_{\text{home/local},k} - \text{relative } \bar{w}_{\text{away/foreign},k} \quad (\text{Eq. 6})$$

The second part of the model uses the mean and variance of the fitness difference posterior distribution (w. dif_k) for each pair in Model 1 as the input to the second model, wherein the effect of endemism and tolerance on the fitness differences are tested. For each pair k , the mean of the w. dif_k distribution is modeled from a normal distribution with a mean z_k and a variance that represents the variance in the actual data (Eq. 7). Equation 8 models z_k as a normal distribution with a mean μ_k , that is calculated from a mixed effects deterministic model (Eq. 9), and a variance that represents the error associated with factors not included in the deterministic model (i.e. process error). The deterministic model tests for the effect of pair type (endemism or tolerance) β_1 , while incorporating the effects of phylogenetic structure β_0 , and year the pair was grown in the greenhouse β_3 . We also include a fixed intercept, β_2 , that represents the average μ_k value for tolerator pairs. The phylogenetic effect in the deterministic model is represented by a random intercept, which is sampled from a multivariate normal distribution with the coancestry matrix, Σ , used for the covariance matrix parameter (Eq. 10; Lynch, 1991; Mitchell et al., 2015; Sianta and Kay, 2019). We are specifically interested in the significance of the β_1 distribution (i.e., how much of the distribution is greater or less than zero) and the median (most probable) value of

the β_1 distribution, which indicates the effect that being an endemic pair has over being a tolerator pair on the fitness differences.

Model 2

$$\text{mean}(\text{w. dif}_k) \sim \text{normal}(z_k, \text{variance}(\text{w. dif}_k)), \quad (\text{Eq. 7})$$

$$z_k \sim \text{normal}(\mu_k, \sigma_{\text{process}}^2), \quad (\text{Eq. 8})$$

$$\mu_k = \beta_0 k + \beta_1 \cdot \text{pair_type}_k + \beta_2 + \beta_3 \cdot \text{year}_k, \quad (\text{Eq. 9})$$

$$\beta_0 \sim \mathcal{N}(0, \Sigma) \quad (\text{Eq. 10})$$

The β_1 , β_2 , and β_3 parameters are all sampled from separate normal distributions with a mean of zero and a variance that is sampled from a uniform distribution with the bounds of (0, 100). We implemented the model in JAGS v4-6, running the models 1 and 2 on three chains over 210,000 MCMC generations, discarding the first 10000 as burn-in. We combined the non-burn-in MCMC generations for each parameter into one vector, yielding posterior samples of 600,000 points. Gelman and Rubin's (1992) convergence diagnostic was equal to or less than 1.03 for all parameters, indicating satisfactory convergence.

Fitness trade-offs and habitat isolation—For all fitness trade-off analyses, we compare the same seed source of each pair in its home environment versus an alternative environment. We determine whether there is a fitness trade-off and its

effect size for each pair, by calculating the percent of the w.diff posterior distribution that is different from zero and the median value of the posterior distribution. For all habitat isolation analyses, we compare the serpentine and nonserpentine seeds within the same soil treatment for each pair by calculating the difference between the local and foreign seed sources. Similarly, we determine whether there is habitat isolation and its effect for each pair by calculating the percent of the w.diff posterior distribution that is greater than zero and the median value of the posterior distribution. In all analyses, we determine the effect of endemism and tolerance by examining the posterior distribution of β_1 .

Competitive ability—We compared the fitness of a seed source growing in nonserpentine soil with and without competition to calculate competitive ability. We used log response ratios to calculate competitive ability. An advantage of log response ratios is that changes in the experimental effect (numerator, Eq. 11) and control effect (denominator, Eq. 11) have equal effects on the overall metric, whereas regular response ratios are more affected by changes in the denominator (Hedges et al., 1999). Following Cacho and Strauss (2014) we calculated log response ratios of each individual i in the competition treatment of pair k as:

$$\text{log Response Ratio (lnRR)}_{ik} = \ln \left(\frac{w_{ik} \text{ with competition}}{\bar{w}_k \text{ without competition}} \right), \quad (\text{Eq. 11})$$

where negative values of lnRR indicate a negative effect of growing with the grass, 0 indicates there was no effect of competition and positive values indicate a positive effect of growing with the grass.

We first ask whether endemic serpentine taxa are poorer competitors than tolerator serpentine taxa. We construct another hierarchical Bayesian model to incorporate the within-treatment variation and the phylogenetic structure of the data. The model is very similar to Model 2 (above) except that we model lnRR of just the serpentine taxa as a normal distribution, with a mean of z_k , and a variance σ_k^2 that reflects sampling error. The variance, σ_k^2 , is sampled from a uniform distribution with bounds (0,100). z_k is subsequently modeled as in Model 2 (Eq. 8-10 from above, repeated below), using the same deterministic model (Eq. 9) to estimate the effect of endemism and tolerance on a serpentine taxon's lnRR. Based on how we coded our pair types (tolerators = 0, endemics = 1), we expect β_1 to be negative. The β_1 , β_2 , and β_3 coefficients were all sampled from normal distributions with means of 0 and variances that were drawn from uniform (0,100) distributions. We ran the model on three chains over 110,000 MCMC generations, discarding the first 10000 as burn-in. We combined the non-burn-in MCMC generations for each parameter into one vector, yielding posterior samples of 300,000 points. Gelman and Rubin's (1992) convergence diagnostic was equal to or less than 1.03 for all parameters, indicating satisfactory convergence.

$$\ln\text{RR}_{ik} \sim \text{normal}(z_k, \sigma_k^2), \quad (\text{Eq. 12})$$

$$z_k \sim \text{normal}(\mu_k, \sigma_{\text{process}}^2), \quad (\text{Eq. 8})$$

$$\mu_k = \beta_0 k + \beta_1 \cdot \text{pair_type}_k + \beta_2 + \beta_3 \cdot \text{year}_k, \quad (\text{Eq. 9})$$

$$\beta_0 \sim \mathcal{N}(0, \Sigma)$$

$$(\text{Eq. 10})$$

Next, to test whether differences between endemics and tolerators in competitive ability are due edaphic divergence or preadaptation, we quantify the difference in competitive ability between the serpentine and nonserpentine taxa within each pair. We use the same general 2-Model hierarchical Bayesian model as above (Eq. 1- 10) to test whether endemic pairs have more divergence in competitive ability than tolerator pairs:

Model 1

$$\ln\text{RR}_{ijk} \sim \text{normal}(\alpha_{jk}, \sigma_{jk}^2), \quad (\text{Eq. 13})$$

$$\ln\text{RR.diff}_k = \alpha_{NS,k} - \alpha_{S,k} \quad (\text{Eq. 14})$$

Model 1 models the $\ln\text{RR}$ values of individual i of seed source j of pair k as a normal distribution, with a mean and variance for each seed source/pair

combination (Eq. 13). The variance, σ_{jk}^2 , was sampled from a uniform distribution with bounds (0,100). The mean lnRR of the serpentine seeds is subtracted from that of the nonserpentine seeds for each pair (Eq. 14). We take the difference in this direction because serpentine taxa are assumed to be poor competitors – thus positive values indicate that there is a loss of competitive ability in serpentine seeds relative to their nonserpentine sister taxa.

Model 2

$$\text{mean}(\text{lnRR. diff}_k) \sim \text{normal}(z_k, \text{variance}(\text{lnRR. diff}_k)), \quad (\text{Eq. 15})$$

$$z_k \sim \text{normal}(\mu_k, \sigma_{\text{process}}^2), \quad (\text{Eq. 8})$$

$$\mu_k = \beta_0 + \beta_1 \cdot \text{pair_type}_k + \beta_2 + \beta_3 \cdot \text{year}_k, \quad (\text{Eq. 9})$$

$$\beta_0 \sim \mathcal{N}(0, \Sigma) \quad (\text{Eq. 10})$$

Model 2 estimates the mean of the lnRR. diff_k posterior distribution as a normal distribution, with a mean of z_k and a variance that is taken from the variance of the lnRR. diff_k posterior distribution. z_k is subsequently modeled as in the previous models. We predict that endemic pairs will have more divergence in competitive ability than tolerator pairs, specifically that endemic serpentine taxa will be worse competitors than the tolerator serpentine taxa, and thus expect that the β_1 distribution will be greater than zero. Models 1 and 2 for the divergence in competitive ability were run on three chains over 110,000 MCMC generations, discarding the first 10000 as burn-in. We combined the non-burn-in MCMC

generations for each parameter into one vector, yielding posterior samples of 300,000 points.

RESULTS

Nonserpentine seed fitness trade-offs and habitat isolation in serpentine soil—

Nonserpentine sister taxa in 13 of 17 of the pairs had a significant reduction in fitness when grown in the pair's serpentine soil (Table 3.2A, Fig. 3.2). Only one of the endemic pairs's (*Clarkia gracilis subsp. tracyi* – *C. gracilis subsp. albicaulis*) nonserpentine taxon had a significant fitness increase in serpentine soil. Two tolerator pairs had no change in nonserpentine fitness in serpentine soil (*C. breweri* and *Plantago erecta*) and one tolerator pair's nonserpentine taxon had a fitness advantage in serpentine soil (*C. concinna*). There was no significant difference between endemic and tolerator nonserpentine taxa in the magnitude of their fitness trade-off in serpentine soils (STATS). Likewise, the inability of most nonserpentine taxa to survive or have high fecundity in serpentine soils resulted in the most prominent habitat isolation among the three soil treatments (Fig. 3.3). All of the endemic pairs, and six of the nine tolerator pairs, showed a signal of habitat isolation, where the local (serpentine) taxon performed better than the foreign (nonserpentine) taxon (Table 3.3A). Fitness trade-offs of the nonserpentine seed did not always lead to habitat isolation in serpentine soils – e.g., the *Navarretia pubescens* nonserpentine taxon had a decrease in relative fitness by 0.57 but still had higher fitness than the serpentine taxon in serpentine

soil. There is a nonsignificant trend that endemic pairs have higher habitat isolation in serpentine soils than tolerator pairs (83% of the β_1 posterior distribution is greater than 0, median of β_1 posterior distribution = 0.64).

Serpentine seed fitness trade-offs and habitat isolation in nonserpentine soil—

We found a mix of responses among the pairs in the change in relative fitness serpentine seeds experience in their home serpentine soil versus in nonserpentine soil. In contrast to Kruckeberg's (1951) results, 5 pairs (3 tolerator pairs and 2 endemic pairs) had decreases in fitness in the nonserpentine soil (Table 3.2B, Fig. 3.4). 10 of the pairs (6 endemic pairs and 4 tolerator pairs) performed better in the nonserpentine soil than the serpentine soil, and 2 of the tolerator pairs had equal fitness in the nonserpentine soil. There was a nonsignificant trend that endemic serpentine taxa had weaker fitness trade-offs (and greater fitness advantages) in nonserpentine soil (75% of the β_1 posterior distribution is less than 0, median of β_1 posterior distribution = -0.36). There was very little habitat isolation in the nonserpentine habitat across all of the pairs (Table 3.3B, Fig. 3.5) – only 4 pairs, 2 endemic pairs and 2 tolerator pairs, had the nonserpentine taxon outperform the serpentine taxon. Conversely, the majority of pairs had the serpentine taxon outperform the nonserpentine taxon (6 endemic pairs, 4 tolerator pairs), or had equal fitness as the nonserpentine taxon (3 tolerator pairs). The endemic and tolerator pairs did not differ in the degree of habitat isolation within nonserpentine

soils (40% of the β_1 posterior distribution is greater than 0, median of β_1 posterior distribution = -0.11).

Serpentine seed fitness trade-offs and habitat isolation in nonserpentine soil

with competition—We predicted that while serpentine seed fitness trade-offs many not be driven by soil factors alone, the effects of soil and competition would drive larger fitness trade-offs in endemic serpentine taxa than tolerator serpentine taxa. The majority of our serpentine taxa (14/17 taxa) show a fitness trade-off in the competitive nonserpentine soil, as expected (Table 3.2C, Fig. 3.6).

Surprisingly, the other three serpentine taxa (2 tolerators and 1 endemic) perform better in the competitive nonserpentine environment than their own. However, there is no difference between endemic and tolerator serpentine taxa in the magnitude of their fitness trade-offs in the competitive nonserpentine soil (64% of the β_1 posterior distribution is greater than zero, median of β_1 posterior distribution = 0.20). Despite fitness trade-offs in the competitive nonserpentine soil being common among serpentine taxa, we do not find strong evidence for habitat isolation within the competitive nonserpentine soil. Nine of the 17 pairs show a pattern consistent with habitat isolation – wherein the nonserpentine taxon has higher fitness than the serpentine taxon (Table 3.3C, Fig. 3.7). Two of the pairs show no difference in fitness between sister taxa, and the remaining 6 pairs have the serpentine taxon outperforming the nonserpentine taxon in the competitive environment. There is a slight and nonsignificant trend that there is a

higher magnitude of habitat isolation in the competitive nonserpentine soil in endemic pairs than in tolerator pairs (76% of the β_1 posterior distribution is greater than zero, median of β_1 posterior distribution = 0.35)

Has adaptation to serpentine substrates resulted in a greater trade-off with competitive ability in serpentine endemics than in serpentine tolerators? —One of the predictions of the competitive trade-off hypothesis is that endemic serpentine taxa are worse competitors than tolerator serpentine taxa. The grass neighbor has a competitive effect on all of the serpentine taxa (Fig. 3.8). We find that, for plants that survived to flower, endemic serpentine taxa have a lower competitive ability (log response ratio) than tolerator serpentine taxa (98% of the β_1 posterior distribution is less than zero). The median value of the β_1 distribution is -0.59, and a change in the log response ratio of -0.59 corresponds to a reduction in the fitness ratio of 45% in endemics compared to tolerators.

Finally, we asked whether endemic serpentine taxa were poorer competitors than tolerator serpentine taxa because they evolved from poor competitors or because adaptation to serpentine in endemic lineages came with a greater loss of competitive ability. We find a significant effect that endemic sister taxa pairs have more divergence in competitive ability than tolerator sister taxa pairs, indicated by 94.6% of the β_1 distribution being greater than zero (Fig. 3.9). Specifically, we find that endemic serpentine taxa tend to have a lowered competitive ability relative to their paired nonserpentine taxa. The median value

of the β_2 distribution is 0.08, which is the average degree of divergence in log response ratios between sister taxa of tolerator pairs. The median value of the β_1 distribution is 0.39, which represents the additional magnitude of divergence between sister taxa in endemic pairs.

DISCUSSION

The evolution of new species is achieved through the evolution of reproductive isolation, and a major goal in speciation research is to understand the ways in which natural selection drives reproductive isolation (Coyne and Orr, 2004; Sobel et al., 2010). Habitat isolation occurs when populations are locally adapted to their own habitats, and local adaptation comes with a cost of reduced fitness in different habitats (Dobzhansky, 1937; Nosil et al., 2005). However, fitness trade-offs may not always accompany local adaptation, which may contribute to why many species are composed of locally adapted populations that never attain species status (Hereford, 2009; Nosil et al., 2009). We used a comparative greenhouse-based reciprocal transplant experiment to determine the effects of edaphic divergence and competition in driving both population-level and species-level divergence. Below, we highlight three of our main findings: that adaptation to serpentine has come with a larger cost in competitive ability for serpentine endemics than for serpentine tolerators, that the magnitude of soil-mediated habitat isolation does not differ between endemic and tolerator pairs, and the apparent discrepancy between these two patterns.

The cost of serpentine adaptation— For decades, a hypothesized trade-off between serpentine adaptation and competitive ability was used to explain the restriction of endemic species to serpentine substrates (Kruckeberg, 1951, 1954; Brady et al., 2005; Anacker, 2014). This competitive trade-off hypothesis posits that serpentine tolerators, with populations on and off serpentine connected by gene flow, should have higher competitive abilities than serpentine endemic species. Despite this hypothesis being the main paradigm describing the restriction of serpentine endemics, there has been no direct test of competitive abilities across a range of serpentine endemic and tolerator species. Here, we found that the serpentine endemics used in this study had overall lower competitive abilities than the tolerator serpentine populations when grown with the same competitor, *Bromus carinatus*. We also found that endemic serpentine taxa have lowered competitive abilities relative to their nonserpentine sister taxa than tolerator serpentine taxa. This latter result suggests that the endemic serpentine taxa are not poorer competitors than tolerator serpentine taxa because they evolved from taxa that were also poor competitors. Instead, evolutionary change between the serpentine and nonserpentine taxa of endemic pairs has resulted in greater divergence in competitive ability than between the sister taxa of tolerator pairs.

Certain aspects of serpentine adaptation may result in a larger cost to competitive ability in some lineages than others. For example, adaptation to serpentine can be achieved through multiple physiological mechanisms, and some may be more costly than others (Kay et al., 2011; O'Dell and Rajakaruna, 2011; Palm and Van Volkenburgh, 2014). Given that serpentine habitats vary in their chemical, physical and biotic properties (Whittaker et al., 1954; Yost et al., 2012; Sianta and Kay, 2019), it may be that adapting to harsher serpentine habitats results in a larger cost to competitive ability. In a prior study, we found that the endemic serpentine taxa used in this study occurred in serpentine habitats with lower soil calcium and higher microhabitat bareness than tolerator serpentine taxa.

Adaptation to certain facets of bare serpentine habitats, such as higher rockiness, disturbance, or herbivore pressure, may drive the evolution of traits that trade-off with competitive ability (Cacho and Strauss, 2014). However, neither habitat microhabitat bareness nor serpentine soil Ca correlate with competitive ability in our dataset (data not shown).

A non-mutually exclusive explanation is that endemic sister taxa pairs show more divergence in competitive ability than tolerator sister taxa because they have had more time to diverge. Our comparison of ITS sequence data between sister taxa indicates that, indeed, there are fewer nucleotide differences in ITS of tolerator pairs than endemic pairs, indicating that endemic sister taxa have been genetically isolated for longer than tolerator sister taxa. However, the number of nucleotide

differences in ITS does not correlate well with competitive ability – e.g., the endemic *Mimulus nudatus* has a much lower competitive ability than the endemic *Collinsia greenei*, even though it has only 2 nucleotide differences relative to its sister taxon while *C. greenei* has 18 nucleotide differences. A loss of competitive ability may evolve over time if taxa do not disperse into competitive environments, with alleles that increase competitive ability being lost to drift or selected against if they are costly.

Soil chemistry-mediated fitness trade-offs do not mediate stronger habitat isolation in endemic lineages—Consistent with many studies that document ecotypic variation in serpentine tolerator species (Kruckeberg, 1951, 1967; Sambatti and Rice, 2006; Wright, Davies, et al., 2006; Kay et al., 2011; Selby and Willis, 2018), we find that across nearly all pairs, nonserpentine sister taxa do not possess serpentine tolerance alleles, as indicated by fitness trade-offs of nonserpentine taxa in serpentine soil. While these trade-offs were common, they were not ubiquitous. Three of the tolerator pairs and one endemic pair did not have a decline in fitness in serpentine soil. Interestingly, three of those pairs were from the genus *Clarkia*, indicating there is likely some level of preadaptation to chemically harsh environments, and/or that serpentine tolerance alleles are not costly in nonserpentine habitats in this genus. Fitness trade-offs of the nonserpentine taxon in serpentine soil did not always lead to habitat isolation (as in the *Layia discoidea*-*L. glandulosa* pair and *Navarretia pubescens* pair) because

the nonserpentine taxon outperformed the serpentine taxon in all environments. A fitness disadvantage of the local serpentine taxon in its home soil for the *Layia discoidea*-*L. glandulosa* pair could be due to small population sizes. A meta-analysis of plant reciprocal transplant studies found that local adaptation (what we refer to as habitat isolation here) was much more common in large plant populations (defined as over 1000 individuals) than in small plant populations (Leimu and Fischer, 2008). Small populations may lack the genetic variation needed to respond to selection and locally adapt. We did not census population size in these populations, but *Layia discoidea* occurs in small ($\sim < 200$ individuals), patchy populations in the field.

Consistent with Kruckeberg's (1951) original findings, we find that most serpentine taxa have equal or higher fitness in their sister taxon's nonserpentine soil. We then incorporated competition into our nonserpentine soil to test the hypothesis that fitness trade-offs of serpentine seeds and habitat isolation in nonserpentine habitats were mediated by competition. Not surprisingly, there were larger declines in fitness of serpentine seeds in the competitive nonserpentine soil compared to the regular nonserpentine soil, but competition did not result in endemic serpentine taxa having larger fitness trade-offs than tolerant serpentine taxa. In general, there was more of a signal of habitat isolation in the competitive nonserpentine soil than in the plain nonserpentine soil. The number of pairs that showed a signal of habitat isolation increased from 4 to 9 (out of 17) in

the competitive nonserpentine environment, but again, there were no differences between endemic and tolerator pairs. The increase in habitat isolation that we see when we add in the competitive environment highlights the importance of considering multiple niche axes that drive selection on local and foreign genotypes (Nosil et al., 2009).

Discrepancies between competitive ability and habitat isolation in nonserpentine soils—We found that there was more divergence in competitive ability, with the serpentine taxon being a worse competitor than its paired nonserpentine taxon, in endemic pairs than tolerator pairs. It intuitively follows that we would thus predict nonserpentine taxa to outperform their sister serpentine taxa in the competitive nonserpentine soil for endemic pairs but not for tolerator pairs. However, we did not find a difference between endemic and tolerator pairs in the magnitude of habitat isolation in the competitive nonserpentine soil. Numerically, the discrepancy between the competitive ability and habitat isolation data is due to the serpentine taxon outperforming the nonserpentine taxon in the noncompetitive nonserpentine soil (Table 3.2B) so much that even though the serpentine taxon had a lower competitive ability than the nonserpentine taxon, it still outperformed the nonserpentine taxon in the competitive nonserpentine soil.

Biologically, the discrepancy between the competitive ability and habitat isolation data could be due to the fact that our simulated competitive nonserpentine habitat

(nonserpentine soil plus one *Bromus carinatus* individual) is not reflective of the actual competitive environment in each nonserpentine sister taxon's habitat. We chose to use a standard grass competitor for measures of competitive ability, but there wasn't high grass cover in every one of sister taxon's nonserpentine habitats (personal observation). The community composition and density of competitors likely changes how selection would act on local nonserpentine versus foreign serpentine individuals. Reciprocal transplants in the field would better capture the effect that competition has on selection against serpentine seeds.

Even if the habitat isolation patterns we see in the competitive nonserpentine soil treatment translate to what they would be like in the field, it does not mean that the loss we see in competitive ability has no effect on speciation. Habitat isolation as measured in this study requires that seeds can disperse into each other's habitats. However, the sister taxa that we sampled here vary in their degree of geographic distance, making it quite unlikely for some pairs that a seed from the serpentine population we sampled would disperse into the paired nonserpentine population. A loss of competitive ability in endemic lineages would still block gene flow through selection against serpentine endemics in the nonserpentine matrix that separates sister taxa. Indeed, spatial isolation and a lack of effective dispersal through the nonserpentine matrix have both been implicated in speciation in serpentine systems (Kruckeberg, 1986; Kay et al., 2011).

Conclusion—

We find that fitness trade-offs facilitate strong habitat isolation in one direction of gene flow – from nonserpentine populations into serpentine habitats. In the other direction of gene flow, from serpentine populations into nonserpentine habitats, we find that competition in nonserpentine soils generally increases the effects of habitat isolation. However, these patterns do not differ among endemic and tolerator pairs. This suggests that soil- and competition-mediated fitness trade-offs in sister taxa's habitats do not promote speciation of serpentine endemics over population-level divergence within tolerators. However, we do find that endemic serpentine taxa have undergone a greater loss in competitive ability relative to their paired nonserpentine taxa than tolerator serpentine taxa, rendering endemics worse competitors than tolerators. A trade-off between serpentine adaptation and competitive ability in endemic lineages could promote speciation through the competition exclusion of serpentine populations from the matrix of productive nonserpentine habitats that separates closely related populations.

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Table 3.1: Serpentine (S) and nonserpentine (NS) sister taxa pairs used in this study. The three nonserpentine taxa for which we use as the nonserpentine comparison for two pairs (i.e., *Collinsia sparsiflora*, *Navarretia heterodoxa*, *Mimulus guttatus*) are listed twice in the table – once with the respective tolerator taxa and once with the respective endemic taxa. Pair codes are given to refer to pairs in the subsequent tables and figures. Year refers to the year the pair was grown in the greenhouse reciprocal transplant experiment. The “Nt difference” column refers to the number of nucleotide differences in ITS sequences between the sister taxa. It is used as a metric of genetic divergence and indicates higher levels of genetic isolation between endemics and their sister taxa than between tolerator populations.

Pair type	Population origin	Species	Pair Code	Year	Distance (km)	Number of differences
Tolerator	S	<i>Clarkia concinna</i>	CACO	2017	1.85	0
	NS	<i>Clarkia concinna</i>				
Tolerator	S	<i>Clarkia breweri</i>	CABR	2017	3.03	0
	NS	<i>Clarkia breweri</i>				
Tolerator	S	<i>Plantago erecta</i>	PLER	2018	1.75	0
	NS	<i>Plantago erecta</i>				
Tolerator	S	<i>Mimulus guttatus</i>	MGUT	2018	6.17	0
	NS	<i>Mimulus guttatus</i>				
Tolerator	S	<i>Collinsia sparsiflora</i>	COSP	2017	2.48	1
	NS	<i>Collinsia sparsiflora</i>				
Tolerator	S	<i>Collinsia heterophylla</i>	COHT	2017	2.23	0
	NS	<i>Collinsia heterophylla</i>				
Tolerator	S	<i>Trifolium willdenovii</i>	TWILD	2018	1.57	1
	NS	<i>Trifolium willdenovii</i>				
Tolerator	S	<i>Navarretia pubescens</i>	NAPB	2017	1.44	0
	NS	<i>Navarretia pubescens</i>				
Tolerator	S	<i>Navarretia heterodoxa</i>	NAHX	2018	37.76	4
	NS	<i>Navarretia heterodoxa</i>				
Endemic	S	<i>Navarretia jepsonii</i>	NAJP_	2018	37.58	5
	NS	<i>Navarretia heterandra</i>				
Endemic	S	<i>Navarretia rosulate</i>	NARS_	2018	20.87	5
	NS	<i>Navarretia heterodoxa</i>				
Endemic	S	<i>Clarkia gracilis ssp. tracyi</i>	CAGT_	2018	52.05	2
	NS	<i>C. gracilis ssp. albicaulis</i>				
Endemic	S	<i>Collomia diversifolia</i>	CLDV_	2018	11.06	13
	NS	<i>Collomia heterophylla</i>				
Endemic	S	<i>Layia discoidea</i>	LADI_	2017	6.19	2
	NS	<i>Layia glandulosa</i>				
Endemic	S	<i>Mimulus nudatus</i>	MNUD_	2018	6.4	1
	NS	<i>Mimulus guttatus</i>				
Endemic	S	<i>Collinsia greenei</i>	COGR_	2017	1.17	18
	NS	<i>Collinsia sparsiflora</i>				
Endemic	S	<i>Camissonia benitensis</i>	CABE_	2017	4.58	14
	NS	<i>Camissonia strigulosa</i>				

Table 3.2: Fitness trade-offs summary. For each of the three fitness trade-off contrasts, we report the median value of the w.diff posterior distribution (Eq. 6, main text). Negative median values are in red and represent the seed source doing better in the away versus in its home habitat. We use the percent of the w.diff posterior distribution greater than zero to assess significance. Any pair with at least 95% of the posterior distribution greater than zero has a fitness trade-off (FTO) and is indicated by blue shading. Any pair with less than 5% of the posterior distribution greater than zero has a fitness advantage (FA) in the away habitat, and is indicated by orange shading. Pairs that have marginally significant trends in one direction are shaded with lighter hues. Pairs with no difference (ND) in fitness between habitats are not shaded. A) Fitness trade-offs of nonserpentine seeds in their home nonserpentine soil versus away serpentine soil (S). B) Fitness trade-offs of serpentine seeds in their home serpentine soil versus away nonserpentine soil (NS). C) Fitness trade-offs of serpentine seeds in their home serpentine soil versus away competitive nonserpentine soil (NS+C).

Pair name	Pair type	A) NS seeds --> S soil			B) S seeds --> NS soil			C) S seeds --> NS+C soil		
		Median	% posterior > 0	Fitness effect in S soil	Median	% posterior > 0	Fitness effect in NS soil	Median	% posterior > 0	Fitness effect in NS+C soil
CABR	T	0.03	63.19	ND	-0.19	2.54	FA	0.67	100	FTO
CACO	T	-0.60	0	FA	0.50	100	FTO	1.05	100	FTO
TWILD	T	1.46	100	FTO	-0.58	0	FA	-0.41	0	FA
NAPB	T	0.57	100	FTO	-0.68	0	FA	-0.20	1.70	FA
COHT	T	1.83	100	FTO	0.36	99.36	FTO	1.52	100	FTO
COSP	T	1.87	100	FTO	0.08	83.59	ND	0.35	99.8	FTO
MGUT	T	1.95	100	FTO	-0.17	5.80	ND - FA	0.61	100.0	FTO
PLER	T	0.11	93.53	ND - FTO	-0.15	0.41	FA	0.17	98.1	FTO
NAHX	T	2	100	FTO	0.92	100	FTO	1.48	100	FTO
CABE_CAST	E	2	100	FTO	-0.65	0	FA	0.60	99.98	FTO
CAGT_CAGA	E	-0.56	0	FA	0.67	100	FTO	1.06	100	FTO
CLDV_CLHT	E	2.00	100	FTO	-0.64	0	FA	0.77	100	FTO
NAJP_NAHN	E	1.54	100.0	FTO	-0.56	1.7E-04	FA	0.25	94.88	FTO
LADI_LAGL	E	1.32	100	FTO	-1.37	0	FA	-0.95	0	FA
COGR_COSP	E	1.38	100	FTO	-0.04	4.75	FA	0.84	100	FTO
MNUD_MGUT	E	1.16	100.0	FTO	0.41	99.98	FTO	1.59	100	FTO
NARS_NAHX	E	2	100	FTO	-0.81	0	FA	0.48	100	FTO

Table 3.3: Habitat isolation summary. Habitat isolation is the difference in fitness between the local and foreign seed sources within a habitat. The serpentine seed source is the local seed source in contrast (A). The nonserpentine seed source is the local seed source in contrasts (B) and (C). For each contrast, we report the median value of the w.diff posterior distribution (Eq. 6, main text). Negative median values are in red and represent the foreign seed source outperforming the local seed source. We use the percent of the w.diff posterior distribution greater than zero to assess significance. Any pair with at least 95% of the posterior distribution greater than zero has habitat isolation in a given soil treatment and is indicated by green shading. Any pair with less than 5% of the posterior distribution greater than zero shows the opposite pattern of habitat isolation, wherein foreign seeds outperform local seeds, and is indicated by yellow shading. Pairs with no difference (ND) in fitness between habitats are not shade

Pair name	Pair type	A) Habitat isolation in S soil			B) Habitat isolation in NS soil			C) Habitat isolation in NS+C soil		
		Median	% posterior > 0	Advantage in S soil	Median	% posterior > 0	Advantage in NS soil	Median	% posterior > 0	Advantage in NS+C soil
CABR	T	-0.22	2.15	Foreign	0.06	77.98	ND	0.32	99.40	Local
CACO	T	-0.05	15.44	ND	-0.07	0.04	Foreign	-0.01	31.39	ND
TWILD	T	1.31	100	Local	-0.30	7	Foreign	-0.45	0	Foreign
NAPB	T	-0.38	0	Foreign	0.26	100	Local	0.43	99.99	Local
COHT	T	1.88	100	Local	0.02	67.94	ND	0.75	100	Local
COSP	T	1.88	100	Local	-0.05	22.30	ND	0.11	99.98	Local
MGUT	T	1.88	100	Local	0.61	100	Local	0.35	95.44	Local
PLER	T	0.17	97.46	Local	-0.20	0.0042	Foreign	-0.17	1.78	Foreign
NAHX	T	2	100	Local	-0.91	0	Foreign	-0.35	1.22	Foreign
CABE_CAST	E	2	100	Local	0.65	100	Local	0.24	94.39	Local
CAGT_CAGA	E	0.37	99.99	Local	-0.25	0	Foreign	-0.15	0.13	Foreign
CLDV_CLHT	E	1.98	100	Local	0.80	100	Local	1.31	100	Local
NAJP_NAHN	E	1.63	100	Local	-0.77	0	Foreign	-0.17	0.19	Foreign
LADI_LAGL	E	0.44	99.99	Local	-0.52	0.002	Foreign	-0.16	0.007	Foreign
COGR_COSP	E	1.41	100	Local	-0.11	0.001	Foreign	0.68	100	Local
MNUD_MGUT	E	1.67	100	Local	-0.63	0	Foreign	0.02	54.49	ND
NARS_NAHX	E	2	100	Local	-0.15	2.41	Foreign	0.86	99.80	Local

Figure 3.1: Conceptual diagram of fitness contrasts quantified in this study. We refer to A) fitness trade-offs as the change in fitness of a seed source across our soil treatments. In this diagram, nonserpentine seeds have a large fitness trade-off in serpentine soil. Serpentine seeds have no fitness trade-off in nonserpentine soil but a larger fitness trade-off in the nonserpentine soil with competition. We refer to B) habitat isolation as the difference in fitness between local and foreign seed sources, with greater habitat isolation in cases where the local seed outperforms the foreign seed. In this diagram, there is strong habitat isolation in the serpentine soil and nonserpentine soil with competition, but not in the plain nonserpentine soil. We refer to C) competitive ability as the decrease in fitness of a seed source grown with and without competition. We use log response ratios to calculate competitive ability. In this diagram, the nonserpentine seed source has a higher competitive ability (smaller decrease in fitness with competition) than the serpentine seed source. Numbered contrasts correspond to descriptions in the text.

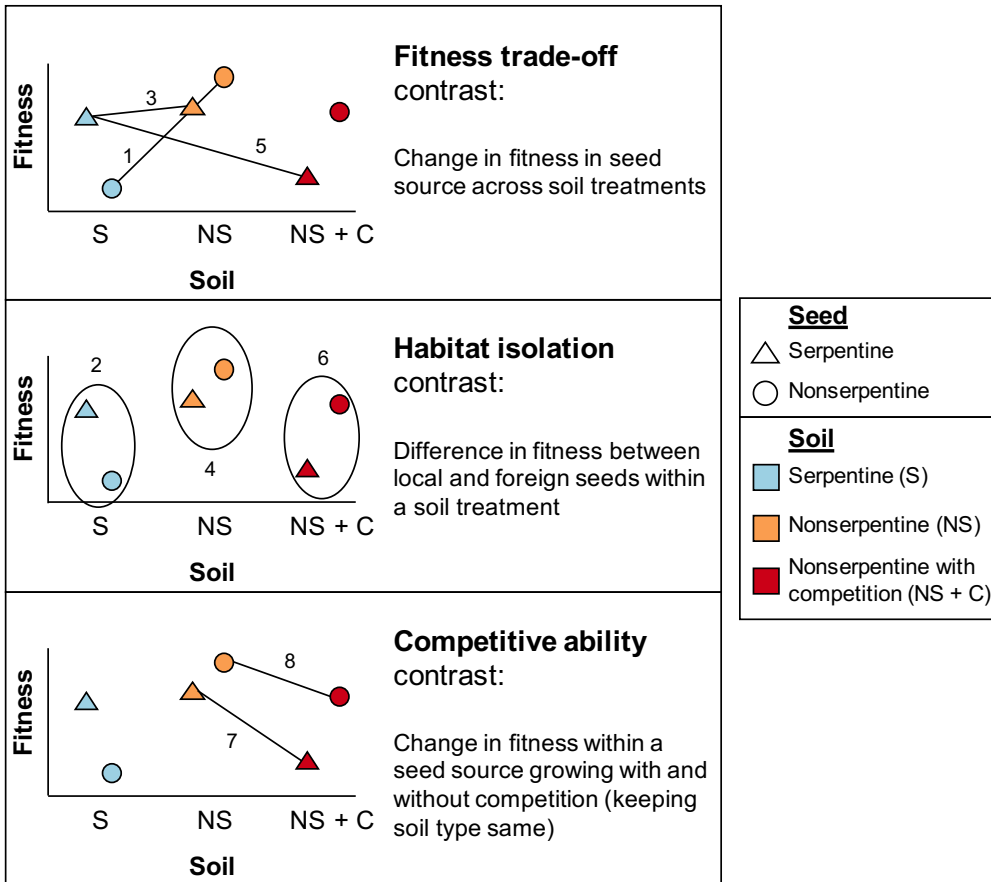


Figure 3.2: Fitness trade-offs of nonserpentine taxa. Nonserpentine taxa in the majority of pairs have large fitness trade-offs in serpentine soil (S; blue boxplots) relative to their home nonserpentine soils (NS; orange boxplots). The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

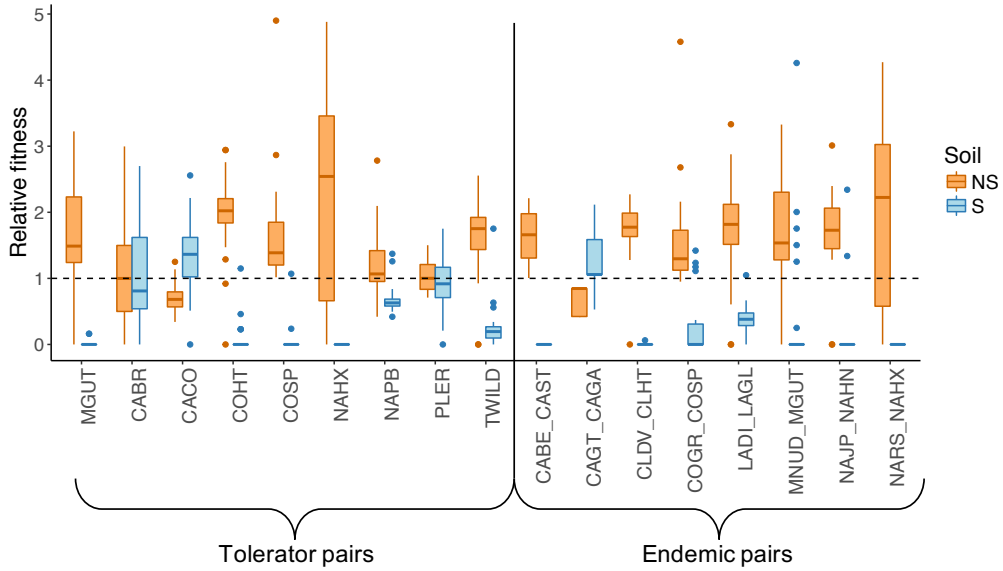


Figure 3.3: Habitat isolation in serpentine soil. There is strong habitat isolation in serpentine soils across the majority of pairs. In 14 out of 17 pairs, the nonserpentine taxon (NS; light blue boxplots) has much lower fitness than the serpentine taxon (S; dark blue boxplots). The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

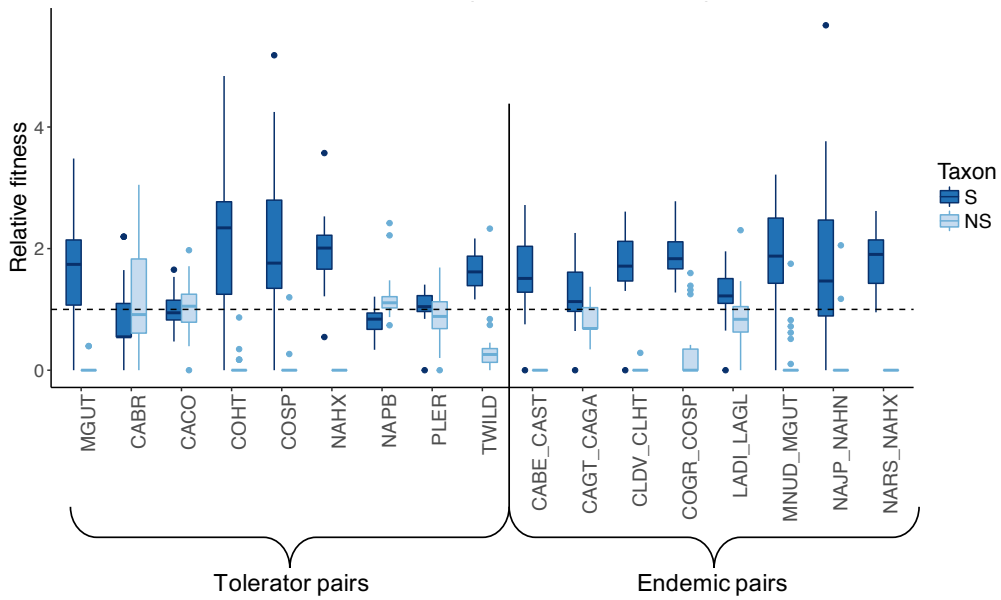


Figure 3.4: Fitness trade-offs of serpentine taxa in nonserpentine soil. Fitness advantages in nonserpentine soil (NS; orange boxplots) relative to their home serpentine soil (S; blue boxplots) are just as common as fitness trade-offs for serpentine taxa. The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

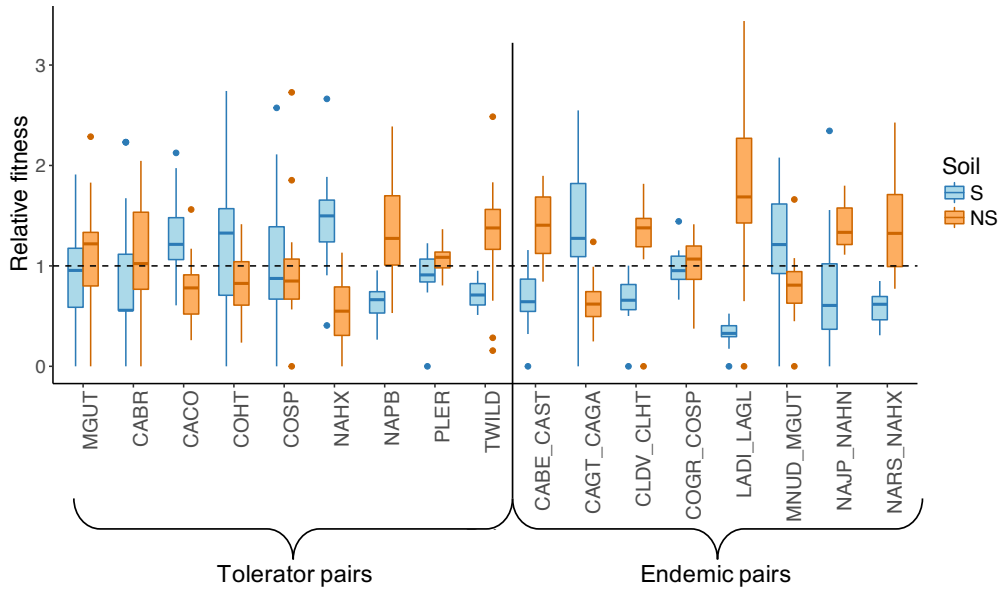


Figure 3.5: Habitat isolation in nonserpentine soil. There is weak evidence for habitat isolation in nonserpentine soils – the serpentine taxon (S; light orange boxplots) in 13/17 pairs have either equal or higher fitness in nonserpentine soils than the local nonserpentine taxon (NS; dark orange boxplots). The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

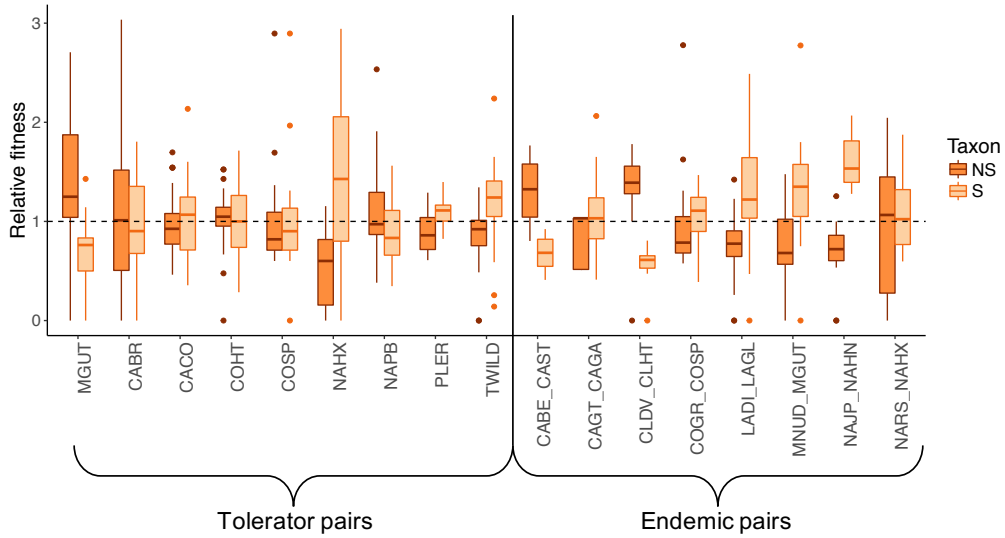


Figure 3.6: Fitness trade-offs of serpentine taxa in nonserpentine soil with competition. Fitness trade-offs of serpentine taxa in the competitive nonserpentine soil (NS+C; red boxplots) relative to their home serpentine soil (S; blue boxplots) are common, but are not greater in endemic pairs than tolerator pairs. The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

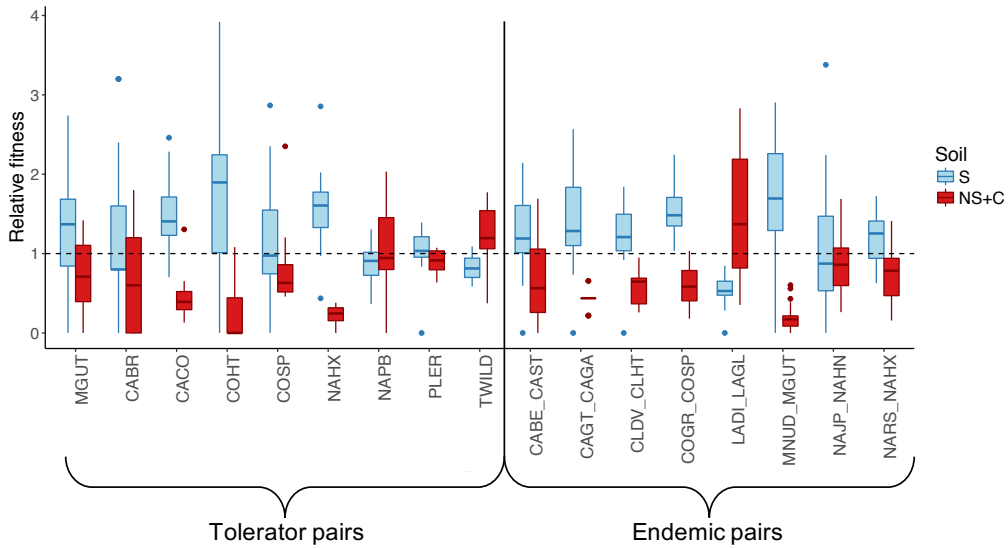


Figure 3.7: Habitat isolation in nonserpentine soil with competition. There is no difference in the potential for habitat isolation in the competitive nonserpentine soil between endemic and tolerator pairs. The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

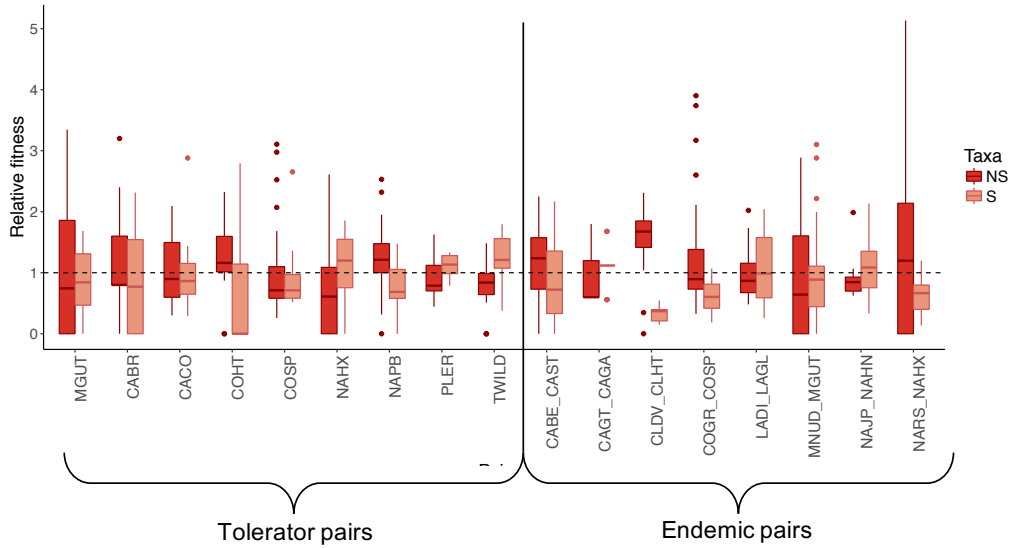


Figure 3.8: Competitive ability among serpentine taxa. Endemic serpentine taxa have lower competitive abilities (log response ratios) than tolerator serpentine taxa. The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.

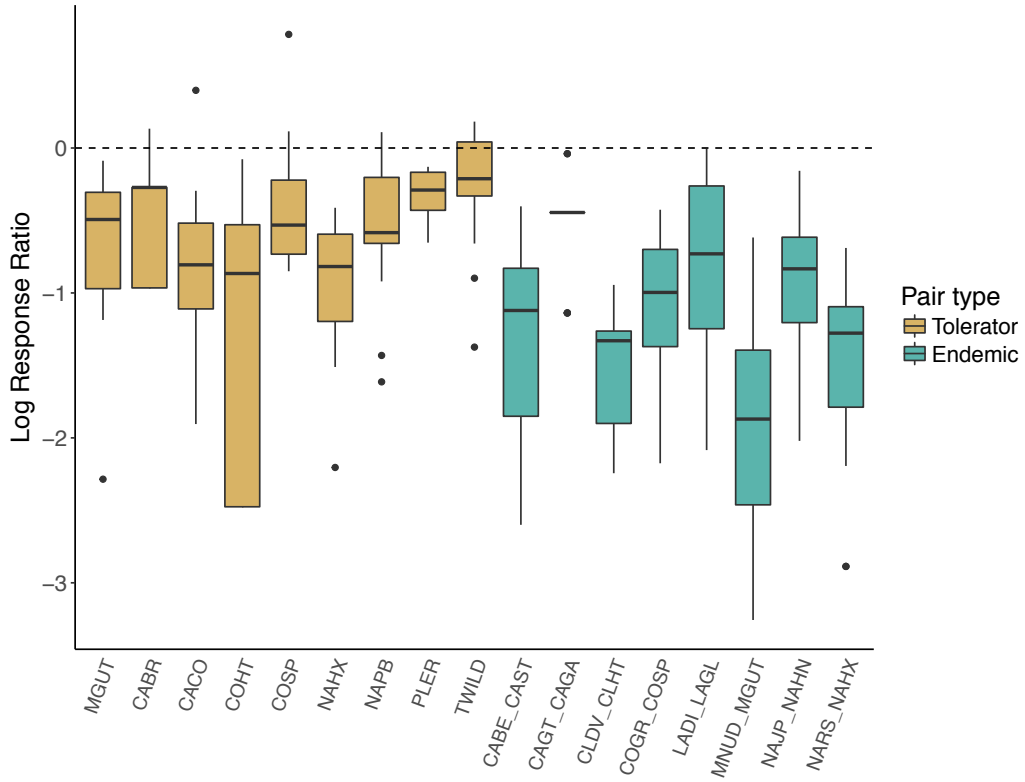
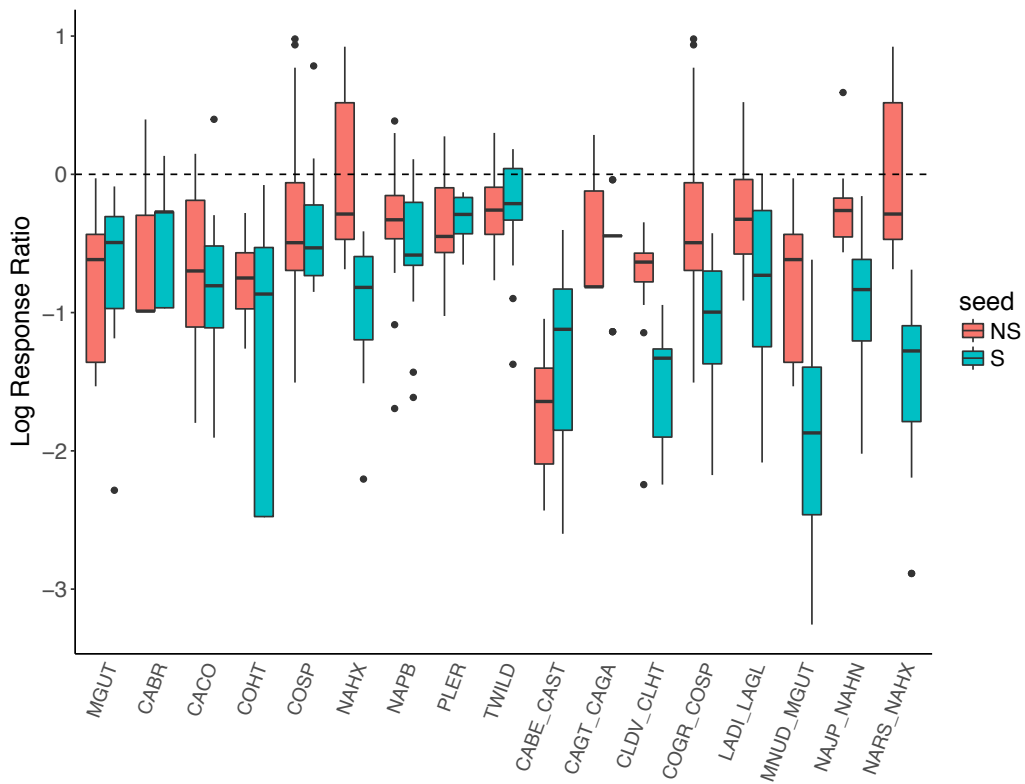


Figure 3.9: Divergence in competitive ability. There is greater divergence in competitive ability in endemic sister taxa pairs than tolerator sister taxa pairs. Each pair is represented by two box plots – one for each sister taxon. The nonserpentine (NS) taxon is on the left and serpentine (S) taxon on the right. The lower and upper edges of the boxplots show the first and third quartiles, respectively, the whiskers extend from the first and third quartiles to 1.5 times the interquartile range, and points indicate data points that are farther than 1.5 times the interquartile range.



APPENDIX

Derivation of mean fitness within each treatment and pair combination, \bar{w}_{jk} .

Mean fitness is derived from the weighted average of all possible fitness values in each treatment/pair combination (Eq. 16)

$$\bar{w}_{jk} = \sum_{w_{ijk}=0}^{\infty} w_{ijk} * P(w_{ijk}) \quad \text{Eq. 16}$$

The probability of any given w_i value is dependent on whether w_{ijk} is equal to or greater than zero (Eq. 17). If $w_{ijk} = 0$, then the probability of getting w_{ijk} is $1 - \theta_{jk}$, where θ_{jk} is the probability of survival inferred from the data (Eq. 1). If $w_{ijk} > 0$, then the probability of w_{ijk} is the probability of survival multiplied by the probability of w_{ijk} drawn from a zero-truncated Poisson distribution with the parameter λ_{jk} , which is inferred from the data (Eq. 2).

$$\Pr(w_{ijk}) = (1 - \theta_{jk})\mathbf{1}_{w=0} + \theta_{jk} * \text{Poisson}(\lambda_{jk}|w > 0) \quad \text{Eq. 17}$$

The indicator function (Eq. 18) is used to calculate the $\Pr(w_{ijk})$ dependent on whether w_{ijk} is greater than or equal to 0.

$$\mathbf{1}_{w=0} = \begin{cases} 1 & \text{if } w = 0 \\ 0 & \text{if } w = 1 \end{cases} \quad \text{Eq. 18}$$

We can sub in Eq. 17 into Eq. 16 (Eq. 19) and simplify (Eqs. 20 – 22). In Eq. 20, we split summation function. The denominator $(1 - e^{-\lambda_{jk}})$ of the last fraction is the correction for the zero-truncated Poisson distribution. In Eq. 21, we pull out the terms within the summation function that do not contain a w_{ijk} . The first half of Eq. 21 reduces to zero because the rules of the indicator function make it such that any value within the summation function will be zero. The summation function in the second half of Eq. 21 boils down to the average of the Poisson distribution, which is λ_{jk} . The final derivation is Eq. 22 (Eq. 3 in the main text), and it describes the average fitness within treatment j of species k as the probability of survival in that treatment/species combination multiplied by the average fecundity value, with an adjustment for the zero-truncated nature of the Poisson distribution.

$$\bar{w}_{jk} = \sum_{w_{ijk}=0}^{\infty} w_{ijk} * \{(1 - \theta_{jk})\mathbf{1}_{w=0} + \theta_{jk} * \text{Poisson}(\lambda_{jk}|w > 0)\} \quad \text{Eq. 19}$$

$$\bar{w}_{jk} = \sum_{w_{ijk}=0}^{\infty} w_{ijk} * (1 - \theta_{jk}) \mathbf{1}_{w=0} + \sum_{w_{ijk}=0}^{\infty} w_{ijk} * \theta_{jk} * \frac{\text{Poisson}(w_{ijk}|\lambda_{jk})}{1 - e^{-\lambda_{jk}}} \quad \text{Eq. 20}$$

$$\bar{w}_{jk} = (1 - \theta_{jk}) \sum_{w_{ijk}=0}^{\infty} w_{ijk} * \mathbf{1}_{w=0} + \frac{\theta_{jk}}{1 - e^{-\lambda_{jk}}} \sum_{w_{ijk}=0}^{\infty} w_{ijk} * \text{Poisson}(w_{ijk}|\lambda_{jk}) \quad \text{Eq. 21}$$

$$\bar{w}_{jk} = \frac{\theta_{jk}}{1 - e^{-\lambda_{jk}}} * \lambda_{jk} \quad \text{Eq. 22}$$

Chapter 4

Phylogenomic analysis resolves a controversial case of putative progenitor-derivative speciation for the serpentine endemic *Clarkia franciscana*

ABSTRACT

There are many models of speciation, but given the time over which most speciation events occur, we often rely on indirect patterns to determine by which mode a species evolved. Budding speciation involves isolation of marginal populations at the periphery of a species range and is typically evidenced by abutting and asymmetric ranges of ecologically-divergent and closely related species. A putative case of budding speciation was studied in the endangered serpentine endemic *Clarkia franciscana* and two closely related widespread congeners by Harlan Lewis, Peter Raven, Leslie Gottlieb and others over a 20-year period, yet its origins remain controversial. Here, we reinvestigate this system with multilocus phylogenetic analysis at the population level to determine whether *C. franciscana* is a recently-evolved derivative serpentine neoendemic phylogenetically nested within one or both of the other two putative progenitor species. In contrast to the hypothesized pattern of relatedness among the three *Clarkia* species, we find no evidence for recent progenitor-derivative relationships. Instead, the data suggest the three species simultaneously and rapidly evolved. We conclude that contemporary range patterns should be cautiously used to infer geographic modes of speciation.

INTRODUCTION

“There are many rich descriptions of [plant] species and how they are reproductively isolated by various mechanisms, but there is little specific evidence about the course of their divergence. Thus, it remains critical to examine particular cases of speciation, and to find out whether the general models of the processes are consistent with the facts.”

-- Leslie Gottlieb, 2004

We have many verbal models of speciation, but it is hard to definitively pinpoint the model by which a particular species evolved, given post-speciation evolutionary changes. Comparative analyses that regress geographic range characteristics of sister taxa (e.g., range overlap and range asymmetry) are often used to identify patterns of speciation. While allopatric speciation via vicariance is supported in five mammal clades (Fitzpatrick and Turelli, 2006), studies across clades spanning the tree of life have found evidence of budding speciation (Barraclough and Vogler, 2000; Malay and Paulay, 2010; Claremont et al., 2012; Anacker and Strauss, 2014; Grossenbacher et al., 2014). Budding speciation occurs when marginal populations become reproductively isolated from the remainder of the species and encompasses multiple named models of speciation, such as peripatric speciation (Mayr, 1954), quantum speciation (Grant, 1981) and catastrophic speciation (Lewis, 1962). In organisms that experience high levels of local adaptation and population structure, such as plants, budding speciation is hypothesized to be a particularly common mode of speciation (Kisel and Barraclough, 2010). Budding speciation results in a progenitor-derivative species pair that should have highly asymmetrical and abutting ranges as well as strong

ecological divergence (Crawford, 2010; Anacker and Strauss, 2014; Grossenbacher et al., 2014). However, patterns of highly asymmetrical and abutting geographic ranges between ecologically divergent taxa can result from other post-speciation processes – for example allopatric speciation via vicariance or dispersal followed by range expansion and/or contraction in one of the sister species (Losos and Glor, 2003) – and may be misleading for identifying cases of budding speciation.

Throughout the 1950s - 1970s, *Evolution* published a suite of influential papers about the prominence of rapid and recent progenitor-derivative budding speciation in the western North American genus *Clarkia* (Onagraceae) by Harlan Lewis, Peter Raven, Leslie Gottlieb, and others (Lewis, 1953, 1962; Lewis and Roberts, 1956; Lewis and Raven, 1958; Bartholomew et al., 1973; Gottlieb, 1973, 1974b), that have been cited 681 times (ISI Web of Science). Lewis (1962) proposed that speciation in *Clarkia* occurred through rapid isolation of peripheral populations through “catastrophic selection” that, unlike Mayr (1954) and Grant’s (1981) peripheral speciation models which invoked a strong role of genetic drift, involved abrupt adaptation to harsher environments accompanied by barriers to gene flow. He predicted the derivative species would be in ecologically marginal and recent habitats, have a smaller and abutting range to the progenitor species, and be morphologically similar but have strong reproductive isolation from the progenitor species.

One of the classic proposed cases of budding speciation was *Clarkia franciscana*, a very restricted serpentine endemic, and two morphologically similar species *C. rubicunda* and *C. amoena* (Lewis and Raven, 1958; Bartholomew et al., 1973; Gottlieb, 1973, 1974a). The three species vary in their range size, but all ranges overlap in the San Francisco Bay area (Figure 4.1). The most widespread and ecological diverse species, *Clarkia amoena*, was hypothesized to be the progenitor species of *C. rubicunda*, through a scenario in which populations at the more arid southern range edge of *C. amoena* gave rise to locally adapted individuals that survived catastrophic selection, resulting in a derivative species with a different chromosomal patterning. *Clarkia rubicunda* then went through a similar process, giving rise to the highly selfing *C. franciscana* that colonized chemically harsh, drought-inducing serpentine soil habitat. Lewis and Raven (1958) proposed that this process happened both rapidly and recently, before the last glacial maximum. Multiple studies documented variation in the chromosomal patterns of the three species and found that the variation in chromosomal patterns among the three species rendered interspecific hybrids sterile (Lewis and Raven, 1958; Snow, 1963, 1964; Bartholomew et al., 1973). To test the hypothesis of budding speciation, Gottlieb (1973) used isozymes to determine whether *C. franciscana* contained a subset of alleles present in *C. rubicunda*, a prediction expected if it recently evolved as a derivative species. Surprisingly, Gottlieb found that *C. franciscana* harbored unique alleles at 6 of the 8 isozyme systems

tested, suggesting that *C. franciscana* was older than hypothesized by Lewis and Raven (1958). However, his results regarding progenitor-derivative speciation were inconclusive because genealogical relationships couldn't be established among isozyme alleles.

Phylogenetic evidence is the most conclusive way to identify progenitor-derivative species pairs (Crawford, 2010), yet the phylogenetic resolution necessary is often elusive. Instead of showing reciprocal monophyly, derivative species are expected to be monophyletic and nested within a paraphyletic progenitor species (Rieseberg and Brouillet, 1994) and the derivative species should be most closely related to peripheral populations from which it evolved. For example, the narrowly-distributed serpentine endemic species *Layia discoidea* (Asteraceae) was found to be phylogenetically nested within the widespread *L. glandulosa* (Baldwin, 2005), and this system is one of the best examples of budding speciation. Lineage sorting and intraspecific gene flow are expected to erase the paraphyly of the progenitor species over time, resulting in two reciprocally monophyletic species (Rieseberg and Brouillet, 1994). Phylogenomic analysis with population-level sampling can remedy some of these issues through the increased signal provided by sampling hundreds of genes and the explicit modeling of gene tree discordance due to incomplete lineage sorting (Degnan and Rosenberg, 2009; García et al., 2017; Carlsen et al., 2018; Morales-Briones et al., 2018). Although most genes sampled may support one topology, gene trees

discordant with the best-supported topology can provide evidence for budding speciation. For example, if budding speciation was not recent, we expect most gene trees to show a topology consistent with reciprocal monophyly (i.e., sister species relationships) because of lineage sorting and intraspecific gene flow. However, we would expect a relatively higher proportion of the discordant gene trees to be consistent with the hypothesized progenitor-derivative pattern of nestedness than other topologies. If speciation did not occur through budding speciation, then we expect no bias in discordant gene trees for one topology over another.

Here, we reevaluate the hypothesized story of budding speciation in the *Clarkia franciscana*-*C. rubicunda*-*C. amoena* triad using phylogenomic analyses. We use population-level samples of *C. franciscana*, *C. rubicunda* and *C. amoena* and targeted sequencing of low-copy genes to infer gene trees and species trees, and to analyze gene tree discordance. We explicitly ask whether there is evidence consistent with phylogenetic nestedness of *C. franciscana* in *C. rubicunda*, and phylogenetic nestedness of both within *C. amoena*.

METHODS

Taxon sampling: We focused our taxon sampling on areas of the ranges of *C. amoena*, *C. rubicunda* and *C. franciscana* at or near the abutting range boundaries in the San Francisco Bay area of California. We sampled a total of 24 individuals

from 14 populations across the 3 species (Figure 4.1; Table 4.1). *Clarkia franciscana* is a California state- and federally-listed endangered species that occurs in only two locations, each on chemically-harsh serpentine soils. Because one of our aims was to understand the ecological transitions associated with progenitor-derivative speciation, we sampled a mix of serpentine and nonserpentine populations of *C. rubicunda* in order to test which ecotype gave rise to the serpentine endemic *C. franciscana*. We used *C. arcuata* as an outgroup because it was the closest diploid relative of the species triad based on a preliminary phylogeny of *Clarkia* built with the same dataset as the one used here. Some of the tissue we used was collected in the field, whereas other tissue was collected from growing field-collected seeds in the greenhouse.

Targeted sequencing: We used a targeted-genome enrichment bait set designed by collaborators at the Chicago Botanic Garden. The bait set was designed using the transcriptomes of two *Oenothera* species, *O. serrulata* and *O. berlanderi* (Onagraceae; Roverson et al., *in preparation*). Transcriptomes were assembled and then mapped to a set of 956 single- or low-copy nuclear loci shared among *Arabidopsis*, *Populus*, *Vitis* and *Oryza* (Duarte et al., 2010). Of the 956 loci, 322 loci were randomly selected for bait design using both *Oenothera serrulata* and *O. berlanderi* as reference sequences. Baits were 120 nucleotides in length and were designed to have a 60 nucleotide overlap (2x tiling), for a total of 19,994

baits. The bait set was manufactured by MYcroarray (now Arbor Biosciences, Ann Arbor, Michigan, USA).

We extracted DNA with a modified CTAB extraction (Doyle and Doyle, 1987). Libraries for the samples used in this study were prepared in a larger run with 72 other *Clarkia* and *Camissonia* (Onagraceae) samples. We sonicated 200 ng of genomic DNA per sample, targeting 550 bp fragment sizes. We prepared sequencing libraries with the Illumina TruSeq Nano HT DNA Library Preparation Kit (San Diego, California, USA) following the manufacturer's protocol at half reagent volumes following the second addition of AMPure beads (Beckman Coulter, Beverly, Massachusetts, USA). We ligated proprietary Illumina i5 and i7 barcodes. We hybridized libraries to the bait set following the manufacturer's protocol (Mycroarray, Ann Arbor, Michigan, USA). We pooled 12-17 samples in one hybridizing reaction, inputting 100 ng of each library into the hybridization pool. We pooled samples roughly by taxonomic association (e.g., samples within species were pooled together, or closely related species were pooled together). Hybridization was performed at 65°C for 18 hours. We reamplified enriched libraries with 14-18 PCR cycles and performed a final PCR cleanup step with the Qiagen QiaQuick PCR cleanup (Qiagen, Hilden, Germany). We checked molarity and ensured the fragment lengths were appropriate for sequencing using a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). We combined all hybridization pools into one run at equimolar ratios (4 nM) with a 1% molar ratio

of PhiX Control (Illumina) on the Illumina MiSeq (600 cycle,s v3 chemistry). We recovered a total of 7,031,356 paired-end 300-bp reads for our 24 samples (26,974,129 paired-end reads for the whole run of 96 samples) and an average of 292,973 paired-end reads per sample used in this study.

Bioinformatic processing of sequences: We used the bcl2fastq v2.18.0.12 Illumina Conversion Software to demultiplex reads and convert the raw basecall files to fastq files. We used Trimmomatic (Bolger et al., 2014) to remove Illumina adapters and quality filter reads. We removed bases at the leading and trailing ends that were under a phred33 quality score of 10, and trimmed sequences once a sliding window of 4 bases averaged below a quality score of 20. We removed reads that were less than 20 bases and reads that didn't have a mated pair. After quality control, there were a total of 6,276,835 paired-end reads, with an average of 261,534 paired-end reads per sample.

We then used HybPiper (Johnson et al., 2016) to assemble reads into contigs and sort them into gene directories using the sequences of *O. serrulata* and *O. berlanderi* as references for each of the 322 loci. For the samples used in this study, an average of 75% of all trimmed and filtered reads were sorted into a gene directory. HybPiper assembled contigs de novo for each gene separately using SPAdes. The program Exonerate was used to align translated contigs to the translated target sequence for each gene. If multiple contigs overlapped by at least 20 bp, they were merged into a supercontig. If no contigs overlapped, the longest

contig was retained. If there were multiple, long contigs that spanned the target sequence length, HybPiper flagged the gene directory with a paralog warning. An average of 23 of the 322 loci per sample flagged paralog warnings, and these loci were removed. HybPiper mapped exon and intron boundaries and generated an exons-only, introns-only and a supercontig sequence for each gene. We use the supercontigs containing both exon and intron sequences in downstream analyses in an effort to include more variable, non-coding regions (Weitemier et al., 2014). Supercontigs had an average coverage depth of 98 x (with a standard deviation of 24 x). There was an average of 75% of the reads per sample that mapped to a reference gene. The samples had an average of 302 genes mapped with contiguous sequences. An average of 252 genes per sample were at least 50% of the reference sequence length, and an average of 158 genes per sample were at least 75% of the reference sequence length.

We removed one sample, an individual from the *Clarkia rubicunda* Emerald Hills population, that had a low sequencing efficiency (i.e., only 34,844 paired-end reads were recovered) and a low enrichment efficiency (i.e., of 14/322 genes were recovered with a sequence length at least 25% of the reference sequence). We further filtered our set of genes to include only those that were present in at least 22 of the remaining 23 samples and did not flag paralog warnings, resulting in a remaining dataset of 232 loci. Gene sequences were aligned with MAFFT v7.130b (Katoh and Standley, 2013) under the --auto setting. We used TrimAl v1.4.rev.15 (Capella-Gutiérrez et al., 2009) to trim

columns within alignments through an automated algorithm that removes columns with a significant drop in gap score or that are outside of an alignment-wide similarity index range.

Phylogenomic analyses: We first constructed gene trees for each of the 232 genes. We used RAxML-HPC v8.2.0 (Stamatakis, 2014) with *Clarkia arcuata* as an outgroup. We used the GTRCAT model of nucleotide substitution and the rapid bootstrapping method (100 bootstraps per gene).

We inferred species trees in two ways: with a concatenated supermatrix and a coalescent-based summary method (Mirarab et al., 2014). Concatenation has the advantage of adding gene matrices that individually have low phylogenetic signal to increase the power to resolve relationships. However, concatenation assumes that all sites have evolved according to a single evolutionary tree, an assumption that is violated with recombination among genes (Degnan and Rosenberg, 2009). Given that supermatrices implicitly are composed of hundreds of genes, concatenation methods can lead to highly supported but wrong species trees (Edwards et al., 2007). In contrast, coalescent-based methods explicitly model the gene discordance that is expected due to incomplete lineage sorting (ILS; Yu and Kubatko, 2009). The accuracy of concatenation vs. coalescent-based methods is dependent on the level of ILS in the samples, with concatenation being more accurate in low ILS situations and coalescent-based models being more accurate in high ILS situations (Kubatko and Degnan, 2007;

Roch and Warnow, 2015). Given that we sampled three species hypothesized to have evolved recently, ILS should play a large role in discordance among gene trees. However, species tree methods that model discordance due to ILS are sensitive to gene tree estimation error. Given that many of our samples are population-level samples, we expect a relatively high level of gene tree estimation error with the loci we used. Thus we build and contrast two species trees – one inferred under the multispecies coalescent framework and one inferred with concatenation.

We built a species tree from a concatenated supermatrix of all of our genes in RAxML. We concatenated all of our aligned gene sequences into a supermatrix, and created a partition file that characterized the boundary of each gene sequence, which allows different models of evolution to be fit for each of the genes. We used the GTRCAT model and the rapid bootstrap analysis (100 bootstraps).

We then used the program ASTRAL v4.10.2 (Mirarab et al., 2014; Mirarab and Warnow, 2015; Sayyari and Mirarab, 2016) to build a species tree that incorporates ILS. ASTRAL is a summary method ideal for large datasets that takes pre-built gene trees as its input and uses them to infer the species tree. The branch length units are in coalescent units, which are the ratio of the number of generations to the effective population size (Degnan and Rosenberg, 2009). Shorter branch lengths indicate more discordance among the gene trees, and could be reflected by less generations that have passed since divergence or a higher

effective population size. We used the ASTRAL algorithm that computes local posterior probability support values for every branch based on gene tree quartet frequencies (Sayyari and Mirarab, 2016). We inputted RAxML gene trees that had all branches with less than 33% bootstrap support (BS) collapsed, as gene tree estimation error can introduce bias into branch length estimates – specifically underestimating branch lengths when ILS is low (Sayyari and Mirarab, 2016).

We were primarily interested in two aspects of the species tree topology: whether each species was monophyletic, and the relationships among the three species. While both of our species trees had high support for the monophyly of each species (see Results) they differed dramatically in their inferred relationship among the three species. We explored the discordance between these two topologies using the single-site log likelihood (SLL) method developed by Walker et al. (2018). The SLL method calculates per-site log likelihoods for the two species tree topologies at each site in the supermatrix (in RAxML with the “-f g” command). The differences in log likelihoods between the two species trees across all sites is plotted to visually assess outlier loci – i.e., loci that strongly support one topology over the other. We then discarded the outlier loci (see Results) and reran the concatenated RAxML supermatrix analysis to determine whether those outlier loci were the cause of the discordance between the two species trees.

Discordance among gene trees: We explored gene tree discordance in two ways. First, we used the program PhyParts (Smith et al., 2015) to quantify the level of gene tree discordance in topology along each branch of the species tree. We collapsed gene trees at branches with under 33% BS, rooted them with the *C. arcuata* outgroup, and generated a rooted ASTRAL tree to use in the analysis. The output from PhyParts was visualized on the ASTRAL topology with `phypartspiecharts.py` (available at github.com/mossmatters/phyloscripts). The PhyParts analysis outputs the numbers of gene trees that are concordant with the species tree topology at each branch, discordant with the species tree, or are uninformative (i.e., the gene tree has support values lower than 33% at that branch).

To explicitly assess the number of gene trees that show support for hypothesized progenitor-derivative relationships (i.e., a monophyletic derivative species nested within a paraphyletic progenitor species), we quantified the number of gene trees that show support for different patterns of nestedness in R with the code developed by Carlsen et al. (2018; `monophyly.R`; available from <https://github.com/tomas-fer/scripts/>). The script uses the ‘`is.monophyl`’ function from the R package `ape` (Paradis et al., 2004) to assess the monophyly of predetermined groups. In a progenitor-derivative species pair, the derivative should be monophyletic, the derivative + progenitor should be monophyletic, and the progenitor should be paraphyletic. For every gene tree, we assessed whether the following groups were monophyletic: *C. franciscana*, *C. rubicunda*, *C.*

amoena, *C. franciscana* + *C. rubicunda*, *C. franciscana* + *C. amoena*, and *C. rubicunda* + *C. amoena*. If *C. franciscana* was derived from within *C. rubicunda*, we would expect the *C. franciscana* and *C. franciscana* + *C. rubicunda* clades to be monophyletic, but *C. rubicunda* not to be monophyletic. We filtered out gene trees that met these criteria. We used analogous criteria to quantify the number of gene trees that support *C. franciscana* being nested within *C. amoena* and *C. rubicunda* being nested within *C. amoena*.

RESULTS

Gene trees had an average bootstrap support of 65%, with the 10% and 90% quantiles of gene tree average BS ranging being 49% and 80%, respectively.

The original concatenated supermatrix was 523,781 sites in length. The first concatenated (without outlier genes removed) RAxML tree we inferred had 100% BS support for each species being monophyletic (Figure 4.S1). Nearly all of the among-population relationships within each species had 100% bootstrap support, which is likely an artefact of the over-inflated bootstrap support that is commonly seen in supermatrix analyses. The relationships among the three species in the best ML tree show *C. franciscana* and *C. rubicunda* as a clade, with *C. amoena* as its sister. However, there is 0% BS support for the node that comprises *C. franciscana* and *C. rubicunda*.

The ASTRAL species tree also showed strong local posterior probability support for the monophyly of each species (Figure 4.2). The within-species

support values were generally lower than in the RAxML tree. However, individuals from the two populations of *C. franciscana* were still highly supported (local posterior probability (LPP) = 1) as monophyletic groups. Branch lengths, in coalescent units, separating the two *C. franciscana* populations (1.42, 1.32) were comparable in length to those leading to each species. The ASTRAL tree resolved *C. franciscana* as sister to *C. amoena* and that clade sister to *C. rubicunda*, with an LPP of 0.87. The branch length supporting that relationship, however, is very short (0.09) which indicates a high level of discordance along that branch.

We analyzed the discrepancy between our two species trees using the single site log likelihood test (Walker et al., 2018). We compared the summed log likelihoods for each gene and identified four genes showing much greater support for the RAxML species tree (Figure 4.S2). The four outlier genes in our dataset were: AT4G29490, AT5G02250, AT5G03905, and AT5G50930. Outlier loci may be caused by misalignments (Walker et al., 2018), but we did not find evidence of large-scale misalignment within these four genes. The RAxML gene trees for these genes did not show a consistent topology (Figures 4.S3-4.S6).

We created a new supermatrix without the four outlier loci that had 507,590 sites and reran the RAxML analysis. The inferred phylogenetic tree resolved each species as monophyletic (100% BS support for each species, Figure 4.3). The new RAxML phylogeny supported the same relationship among the three species as the ASTRAL phylogeny – *C. franciscana* sister to *C. amoena*,

and *C. rubicunda* sister to the former clade. However, the *C. franciscana*-*C. amoena* clade has low support (25% BS).

Gene tree discordance: Our analysis of gene tree discordance with the ASTRAL species tree shows that, while the branch supporting *C. franciscana* as sister to *C. amoena* has high support (0.89 LPP), there is significant discordance at that node (Figure 4.4). Only 44 of the 232 gene trees support *C. franciscana* as sister to *C. amoena*. 26 of the gene trees support a *C. franciscana* as basal to a clade composed of *C. rubicunda* and *C. amoena*. Only 4 gene trees support *C. franciscana* as sister to *C. rubicunda*.

We then explicitly quantified the number of gene trees that would support hypothesized progenitor-derivative relationships. Specifically, we quantify the number of trees in 3 scenarios: 1) *C. franciscana* is nested within *C. rubicunda*, with *C. amoena* as the basal sister, 2) *C. franciscana* is nested within *C. amoena*, with *C. rubicunda* as the basal sister, and 3) *C. rubicunda* nested within *C. amoena*, with *C. franciscana* as the basal sister. There were 2 gene trees that support *C. franciscana* as being derived from within *C. rubicunda*, 3 gene trees that support *C. franciscana* as being derived from within *C. amoena*, and 4 gene trees that support *C. rubicunda* as being derived from within *C. amoena*.

DISCUSSION

Budding speciation that results in progenitor-derivative species pairs is thought to be a common phenomena, especially in plants (Rieseberg and Brouillet, 1994; Crawford, 2010; Anacker and Strauss, 2014; Grossenbacher et al., 2014).

Unfortunately, it is difficult to positively identify progenitor-derivative species pairs because many of the lines of evidence used (geographic range overlap, mating system transitions, etc.) can change post-speciation. Here, we revisited a hypothesized case of budding speciation among three species – wherein the serpentine endemic *Clarkia franciscana* was putatively derived from *C.*

rubicunda, which in turn was putatively derived from *C. amoena* (Lewis and Raven, 1958). Prior work on this group of species drew evidence from range distributions, habitat affinities, chromosomal rearrangements, morphology, mating system and electrophoretic isozyme similarity, and yet the mode of speciation remained controversial. We took a phylogenomic approach, analyzing the history of hundreds of genes, to test Lewis and Raven's (1958) hypothesis.

Our phylogenomic analyses do not support the hypotheses that *C. franciscana* was recently derived from *C. rubicunda*, and that *C. rubicunda* was recently derived from *C. amoena*. Instead of finding patterns of phylogenetic nestedness consistent with budding speciation, we find that each of the three species is highly supported as a monophyletic clade, even the presumed progenitor species, which are expected to be paraphyletic. Even if budding speciation did occur, monophyly of the progenitor species is expected to evolve over time given lineage sorting and

gene flow among populations. However, if budding speciation happened far in the past, we expect to recover a signal of the progenitor-derivative relationships within the gene trees that are discordant with the species tree topology. We found only two out of 232 gene trees that placed *C. franciscana* as a monophyletic clade nested within *C. rubicunda*, and three out of 232 gene trees that show an alternative pattern of nestedness – with *C. franciscana* nested within *C. amoena*. Five gene trees supported *C. rubicunda* and being nested within *C. amoena*. That these discordant tree topologies are so low in number and are similarly represented across different patterns of nestedness suggests that their discordance with the species tree is the result of ILS instead of a remaining signal of budding speciation.

Our analysis does support the claim by Lewis and Raven (1958) that speciation in this triad happened rapidly. Rapid, almost simultaneous, speciation of the three species is indicated by the short branches in both the ASTRAL and the RAxML trees. Although the branch lengths are in different units – coalescent units in the ASTRAL tree and substitutions per site in the RAxML tree – each type of unit is consistent with rapid speciation. Short branches in coalescent units indicate high levels of gene discordance, which is also supported through the PhyParts analysis. While high levels of gene discordance can be due to ILS over short timescales, it can also be due to other phenomena such as hybridization and introgression (Vargas et al., 2017). However, the species used in this study vary in their

chromosomal arrangements and produce sterile interspecific hybrids. Under Lewis and Raven's (1958) hypothesis that catastrophic selection, which fixes chromosomal deviants, drove speciation in this group, we would not expect introgression to be a factor in speciation.

In addition to short branch lengths in the RAxML tree, the branch that supports the *C. amoena* and *C. franciscana* clade is poorly supported (25% BS). Given the sheer number of sites used in the concatenated matrix and the tendency for concatenation analyses to overestimate bootstrap support (Edwards et al., 2007), it is likely that the concatenation analysis shows a true hard polytomy among the three species. Hard polytomies, indicative of rapid divergence and near simultaneous speciation, have historically been hard to distinguish from soft polytomies, which are due to the lack of phylogenetically informative characters. Phylogenomic studies have the advantage of distinguishing between these two alternatives. Similar gene tree conflicts were found in a recent phylogenomic analysis of the Zingiberales, leading the authors to conclude that this tropical group radiated rapidly, perhaps due to the opening of new pollination niches with the rapid radiation of bird and mammal groups (Carlsen et al., 2018).

Gottlieb (1974) was right to take a critical view of progenitor-derivative speciation in this group, and our results are consistent with his isozyme work but far more conclusive. *Clarkia franciscana* contained a large number of unique

isozyme alleles, and *C. rubicunda* and *C. amoena* were also distinct at a number of loci. This pattern of allelic variation could have resulted from the partitioning of ancestral polymorphism into the three species, or from genic evolution within the isolated species following budding speciation. Our work shows that *C. franciscana* alleles are not generally derivative of *C. rubicunda* alleles, nor are *C. rubicunda* alleles derivative of *C. amoena*. Given the high discordance along the branch supporting the relationship of *C. franciscana* and *C. amoena*, it seems most likely ILS of ancestral polymorphism has led to the distinct number of loci in the three species.

Because of its nested geographic range within *C. rubicunda* and its specialization to stressful serpentine habitats, *Clarkia franciscana* was used as a classic case of serpentine neoendemism (Stebbins and Major, 1965). Neoendemics are recently evolved taxa that are specialized to habitat islands (Stebbins and Major, 1965), are hypothesized to evolve from a small group of initial founders (Kay et al., 2011) and thus represent a form of budding speciation. In contrast are paleoendemics, which were once widespread species that became restricted to a narrow ecological niche (Stebbins and Major, 1965). Our phylogenomic evidence is not consistent with the geographically-based assumption of neoendemism because we do not find evidence of recent budding speciation. Additionally, the two *C. franciscana* populations on either side of the San Francisco Bay form well-supported, divergent clades, characterized by low levels of discordance. The genetic

divergence between the two populations was also shown by Gottlieb and Edwards (1992), who found that individuals within populations were uniform at all isozyme loci but that the Oakland Hills population differed in 5 of the 31 genes tested from the Presidio population. Of our 232 gene trees, 136 (58%) support the two populations as being distinct monophyletic clades. It seems more likely that *C. franciscana* was once more widespread throughout the San Francisco Bay area and underwent a process of biotype depletion, wherein nonserpentine populations went extinct as the climate and competitive environment changed (Raven and Axelrod, 1978; Anacker and Harrison, 2011). The self-fertilization mating system and natural fluctuations in population size (Gottlieb, 1973) could have facilitated rapid sorting of ancestral alleles in these two populations.

Conclusions—

Comparative analyses that use geographic range features of closely related species give us insight into patterns of speciation modes that may be operating across clades in the tree of life. However, our results pertaining to Lewis and Raven's (1958) classic story is another cautionary tale of using current species distributions as evidence of when and how speciation happened (Losos and Glor, 2003). While species distributions and additional lines of circumstantial evidence (such as shifts in mating system, specialization to ecologically-marginal habitats, and unique chromosomal arrangements; Crawford, 2010) have been indicators of

known instances of budding speciation (e.g., Lewis and Roberts, 1956; Gottlieb, 1974b, 2004; Baldwin, 2005), they should not be taken as concrete evidence. The *C. franciscana*, *C. rubicunda*, and *C. amoena* species all showed multiple patterns consistent with budding speciation, and yet our phylogenomic analyses indicate that rapid budding speciation did not happen in this group. The high occurrence of ILS in our study system reinforces the importance of sampling a large number of genes to understand evolutionary relationships, particularly when speciation occurred rapidly. Population-level phylogenomic analyses, although a large undertaking, remain our best method for positively identifying progenitor-derivative species pairs. In this way we can better determine whether, as Leslie Gottlieb (2004) said, the “general models of the processes are consistent with the facts.”

PERMITTING

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Table 4.1: Population-level samples used in this analysis, with the number of individuals (No. ind.) sampled per population.

^aBecause *C. franciscana* is an endangered species, exact coordinates are not given.

^bNA's signify vouchers that will be deposited in the UCSC herbarium

Species	Population location	County	GPS^a	Soil type	No. ind.	Herbarium accession^b
<i>Clarkia franciscana</i>	SF Presidio	San Francisco	NA	Serpentine	3	NA
<i>Clarkia franciscana</i>	Redwood Regional Park	Alameda	NA	Serpentine	3	NA
<i>Clarkia rubicunda</i>	Mount Hamilton	Santa Clara	37.327492, -121.670608	Non-serpentine	1	NA
<i>Clarkia rubicunda</i>	Emerald Hills	San Mateo	37.461958, - 122.277237	Serpentine	4	NA
<i>Clarkia rubicunda</i>	Fort Funston	San Francisco	37.72452, -122.50496	Non-serpentine	2	UCSC 7510
<i>Clarkia rubicunda</i>	Coast Creek	Marin	37.98755, -122.81342	Non-serpentine	1	UCSC 7508
<i>Clarkia rubicunda</i>	Ring Mountain	Marin	37.914628, -122.495725	Serpentine	2	UCSC 7432
<i>Clarkia rubicunda</i>	Tennessee Valley	Marin	37.863907, -122.53327	Non-serpentine	1	UCSC 7429
<i>Clarkia rubicunda</i>	Miwok Trail	Marin	37.842533, -122.523132	Non-serpentine	1	NA
<i>Clarkia rubicunda</i>	Bunker Road	Marin	37.832979, -122.508529	Non-serpentine	1	UCSC 7509
<i>Clarkia amoena</i>	Lucas Valley	Marin	38.047367, -122.622803	Non-serpentine	1	NA
<i>Clarkia amoena</i>	Nicasso Valley	Marin	38.017867, -122.663387	Non-serpentine	1	NA
<i>Clarkia amoena</i>	Marshall Beach Rd.	Marin	38.130067, -122.909019	Non-serpentine	1	NA
<i>Clarkia amoena</i>	McClures Beach	Marin	38.187351, -122.962877	Non-serpentine	2	UCSC 7425

Figure 4.1: Geographic ranges and sampling localities of *Clarkia franciscana*, *C. rubicunda*, and *C. amoena*. Green layers indicate serpentine patches.

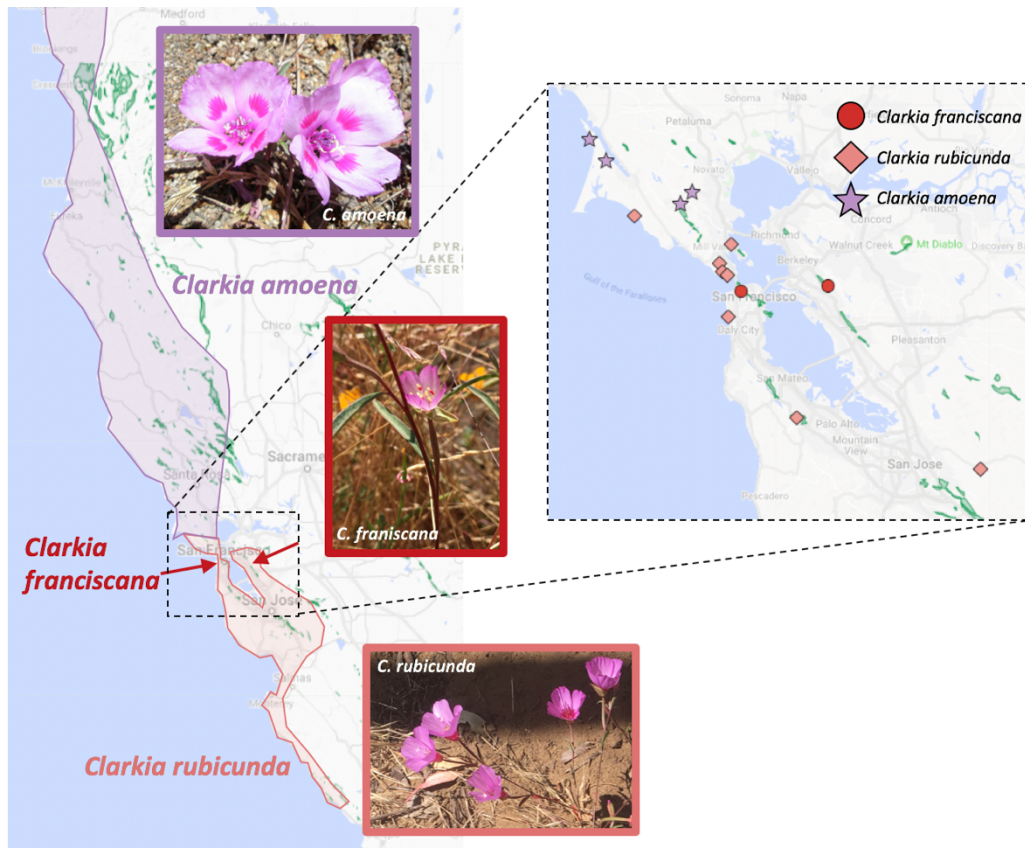


Figure 4.2: ASTRAL species tree, generated with gene trees that were collapsed along branches with < 33% BS. Branch lengths are in coalescent units.

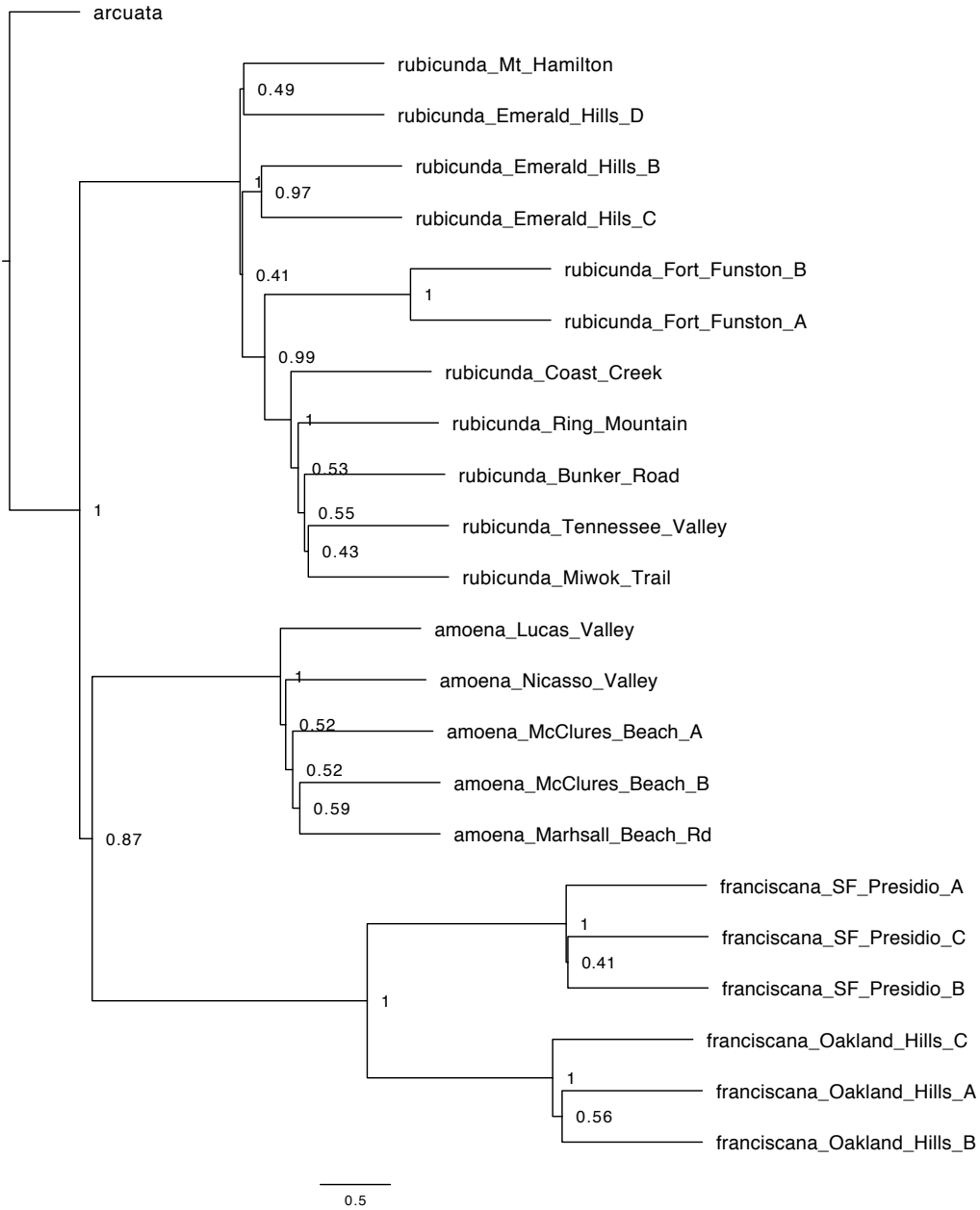


Figure 4.3: The maximum likelihood species tree inferred from RAxML with the supermatrix that omits the four outlier genes. Node values are bootstrap support (%).

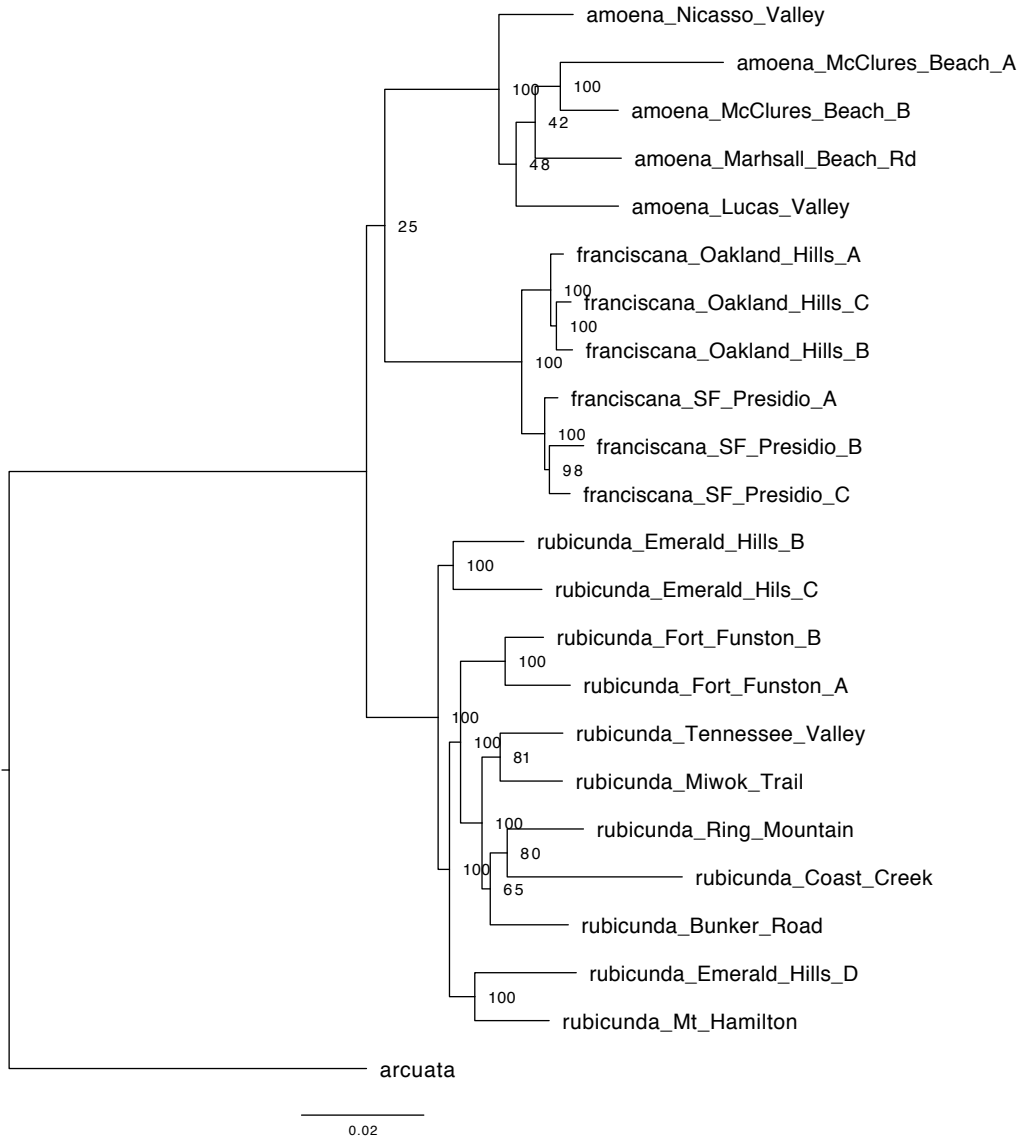


Figure 4.4: Gene tree discordance visualized on the ASTRAL species tree. The pie charts at every node represent the number of gene trees that fall into one of four categories. The blue slice represents gene trees that are concordant with the species tree topology at that node. The green slice represents the number of gene trees that represent the next most common topology at that node. The red slice represents all other gene trees that show a different topology at that node. The grey slice represents uninformative gene trees at that node (i.e., gene trees with less than 33% BS at that node). The number on top of each branch is the number of gene trees concordant with the species tree topology at that node (blue slice). The number on the bottom of each branch is the number of informative alternative topologies at that node (green + red slices).

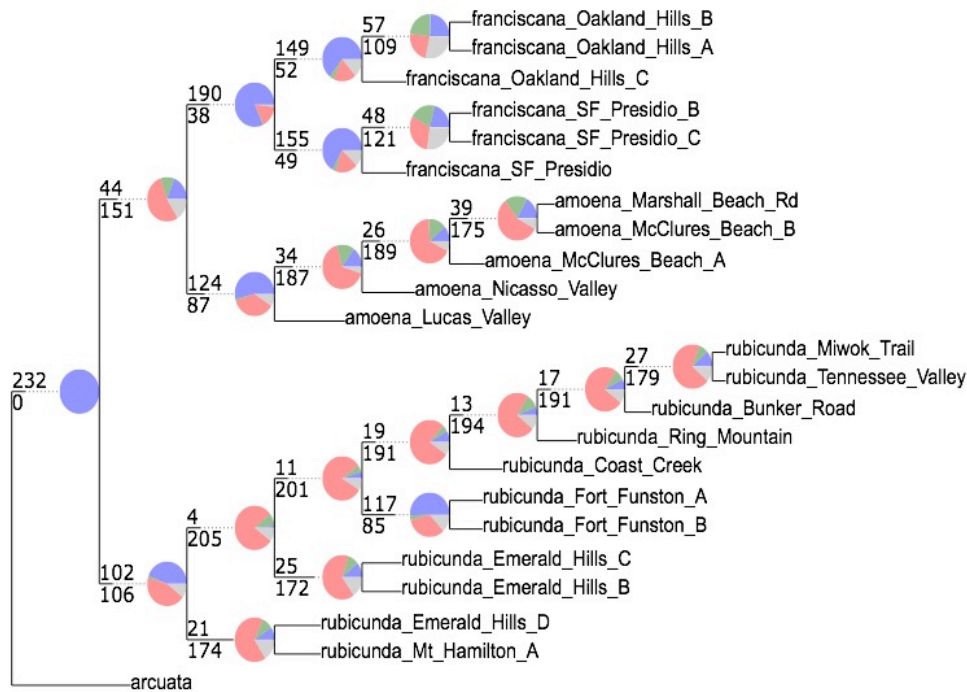


Figure 4.S1: RAxML concatenation tree with the highest maximum likelihood inferred from full supermatrix. Node values indicate bootstrap support (%). *Clarkia arcuata* is the outgroup taxon. Branch lengths are in substitutions/site.

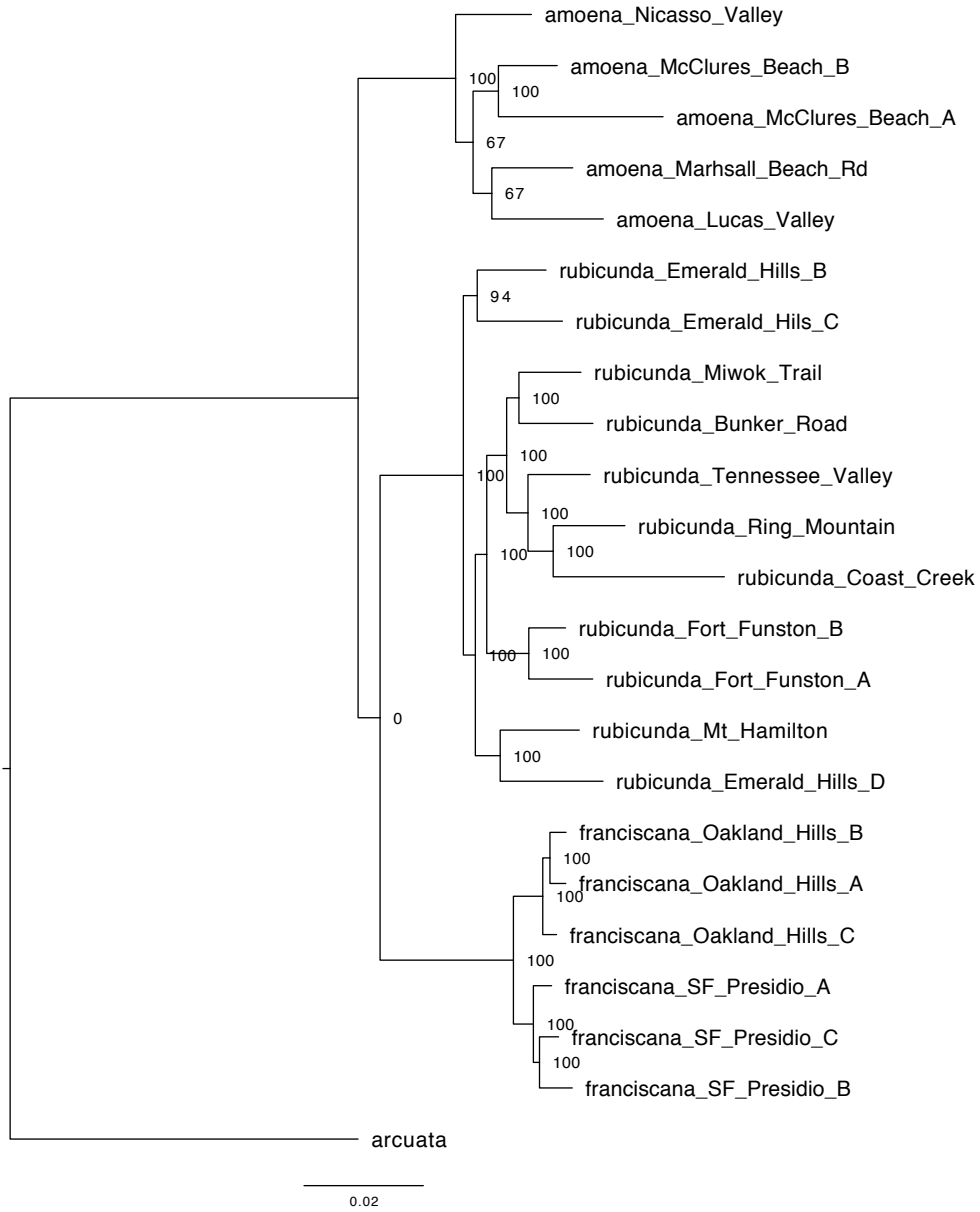


Figure 4.S2: Single Site Log Likelihood test results indicate that there are four genes (highlighted in red box) that show very strong support for the RAxML tree built with the full supermatrix, where *C. franciscana* + *C. rubicunda* clade has 0% BS, over the ASTRAL tree, which places *C. franciscana* as sister to *C. amoena*. These four genes were deemed outlier loci and were removed from the supermatrix for further analysis.

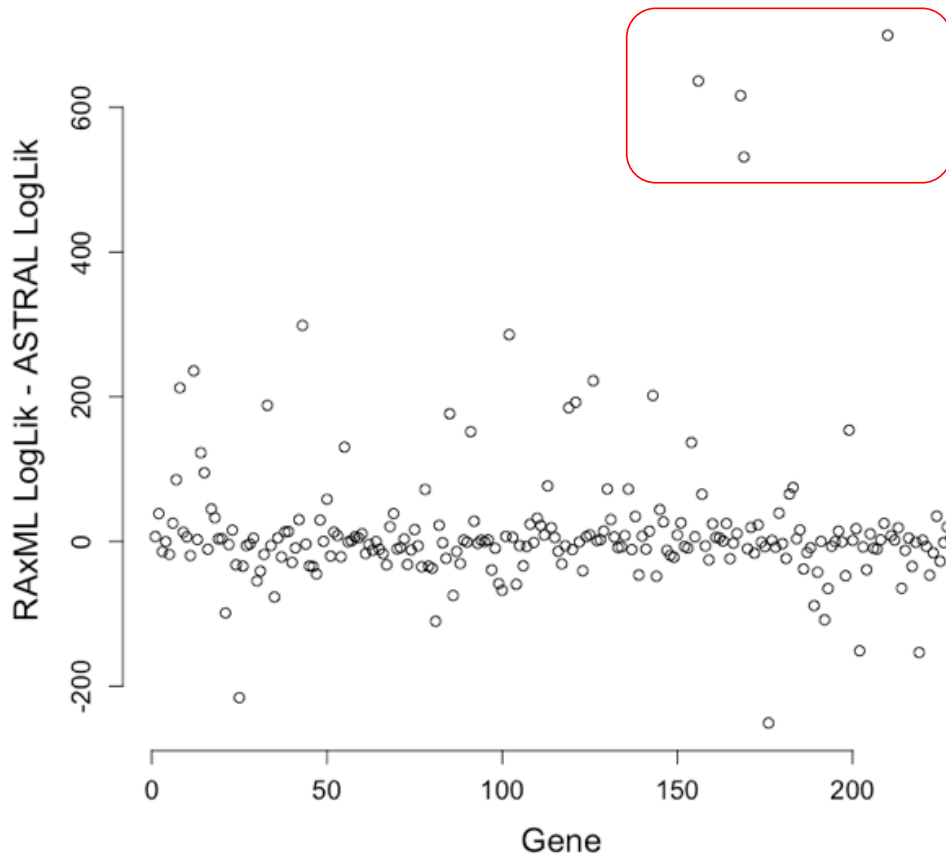


Figure 4.S3: Maximum likelihood gene tree of outlier gene AT4G29490 inferred with RAxML. Node values are bootstrap support (%).

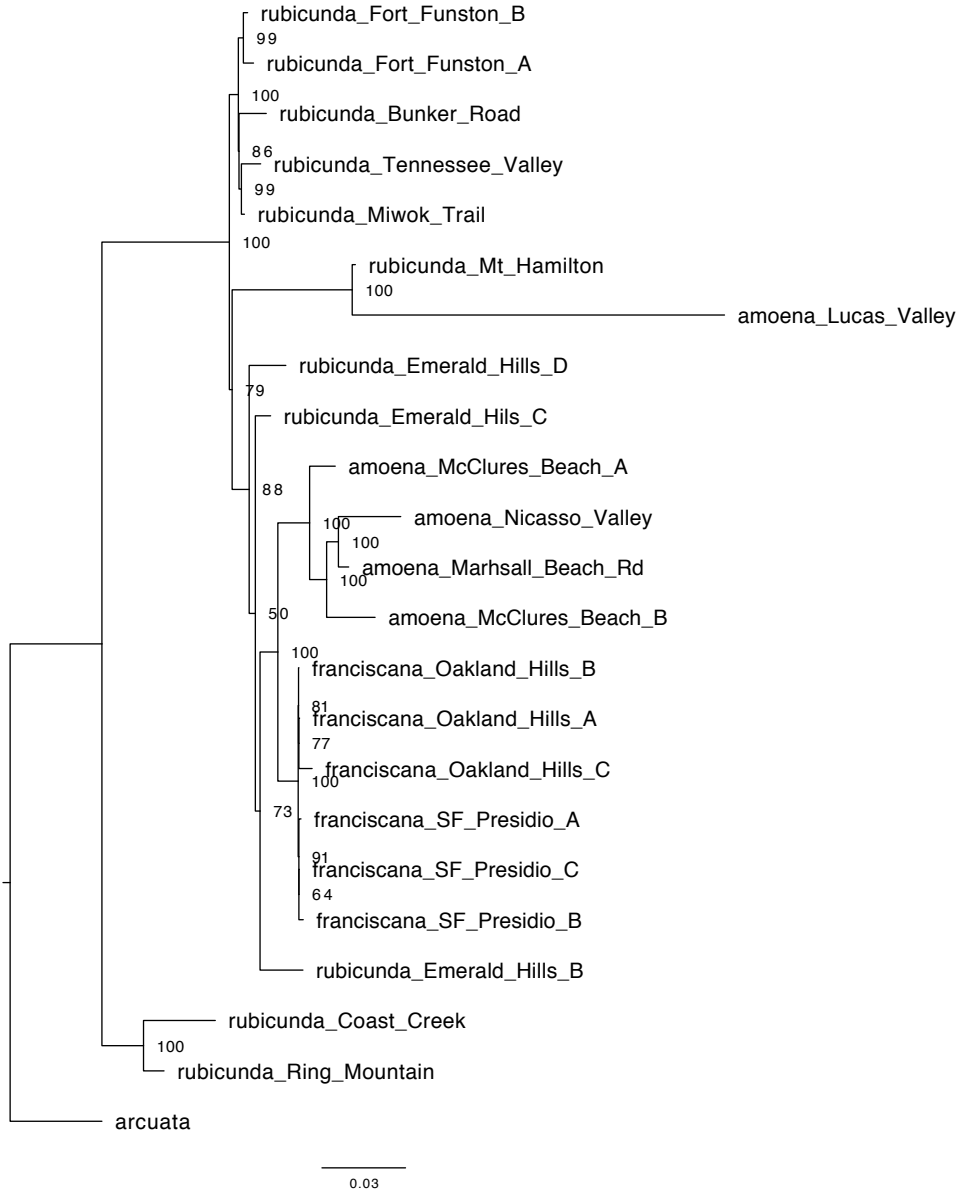


Figure 4.S4: Maximum likelihood gene tree of outlier gene AT5G02250 inferred with RAxML. Node values are bootstrap support (%).

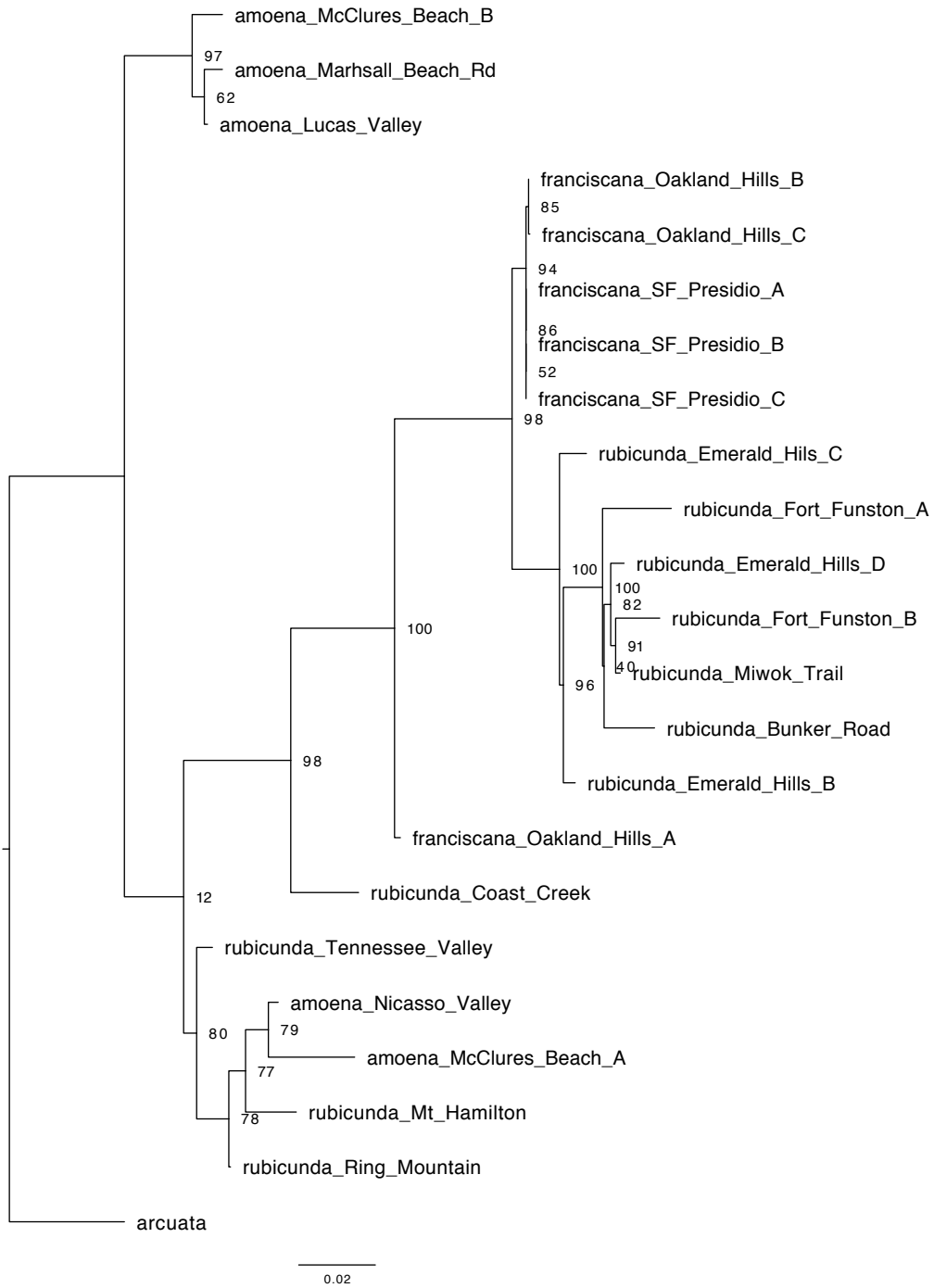


Figure 4.S5: Maximum likelihood gene tree of outlier gene AT5G03905 inferred with RAxML. Node values are bootstrap support (%).

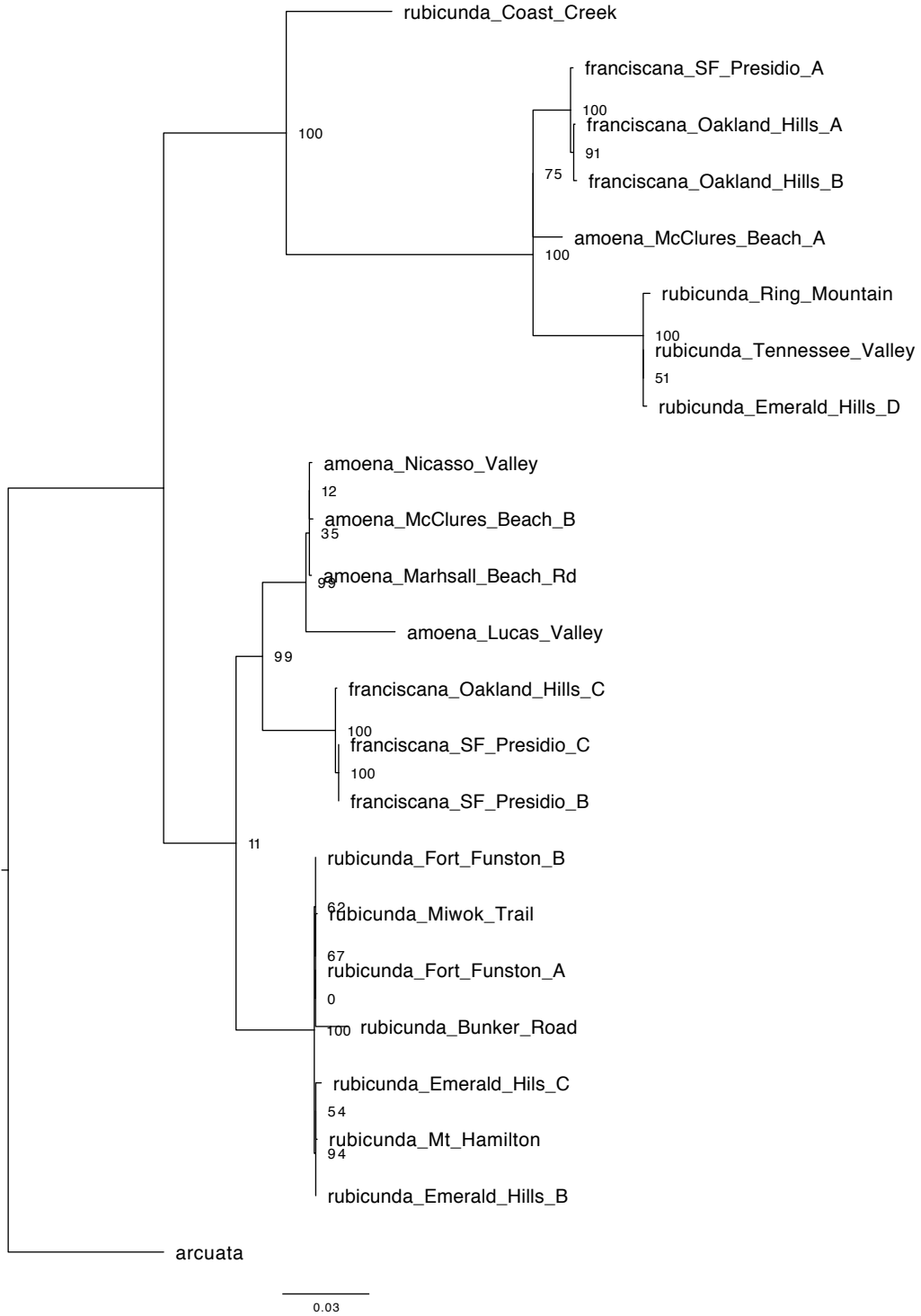
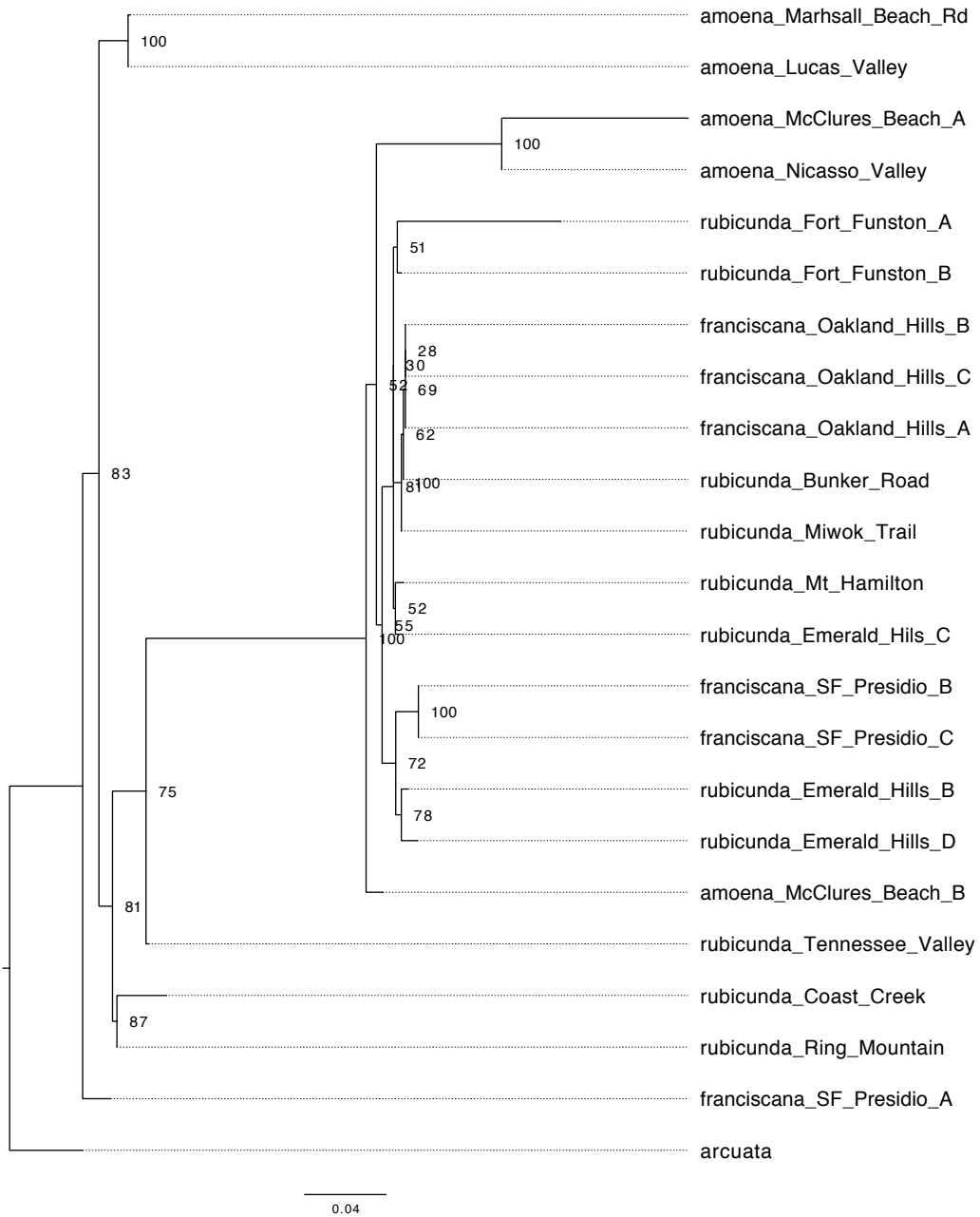


Figure 4.S6: Maximum likelihood gene tree of outlier gene AT5G50930 inferred with RAxML. Node values are bootstrap support (%).



SYNTHESIS

Darwin and Wallace emphasized the role of adaptive divergence among populations in initiating speciation, and studying how natural selection causes reproductive isolation is an active field of research (Darwin, 1859; Kirkpatrick and Ravigne, 2002; Coyne and Orr, 2004; Schemske, 2010). Indeed, local adaptation is known to be a common phenomenon (Leimu and Fischer, 2008; Hereford, 2009), and comparative studies indicate a relationship between the amount of reproductive isolation and ecological differences among taxa (Funk et al., 2006). However, adaptive divergence among populations does not always lead to speciation, as shown by species occupying a broad range of habitats. The overall goal of this dissertation research was to improve our understanding of how speciation occurs following adaptive divergence and why it sometimes does not.

I focused my dissertation research on exploring factors promoting speciation in the California serpentine flora. This was an ideal system because of the strong selection imposed on plants by serpentine soil chemistry (Brady et al., 2005), the independent adaptation to serpentine and evolution of both serpentine generalists (tolerators) and specialists (endemics) across 39 plant families (Anacker, 2011), and the decades of work done in this system that set a strong foundation of hypotheses to test regarding adaptive divergence and speciation. I studied speciation through two lenses. The first lens used pairs of extant sister taxa that all underwent ecological divergence to the same type of selective factor (serpentine vs nonserpentine soils) but varied in their taxonomic status (either

within species or between species pairs), representing different stages in the speciation process. I tested the central hypotheses that speciation was more likely to have occurred when adaptation to extreme or divergent novel habitats comes with large fitness costs in the progenitor/source habitat and when adaptation to a novel habitat has indirect, genetically-based effects on assortative mating. My design is novel in both the number ($n = 17$) and phylogenetic breadth (spanning 9 plant families) of the taxa pairs that I used, achieving a level of generality that many speciation-spectrum studies within species complexes or genera cannot attain. The second lens looked back in time, to understand patterns of evolutionary divergence of a putative serpentine neoendemic, *Clarkia franciscana*, from two potential progenitor species. I took a novel population-level phylogenomic approach to disprove a classic hypothesis regarding the speciation history of this rare serpentine endemic.

In Chapters 2 and 3 I experimentally tested whether a suite of factors promote speciation of serpentine endemic species, and found these factors to be important at different stages of speciation. While I found that adaptive divergence across serpentine and nonserpentine substrates frequently led to genetically-based shifts in flowering times and moderate phenological isolation, flowering time shifts were not stronger in endemic species than in tolerator species. Because between-species pairs have likely been diverging for longer time periods than within-species pairs, my results suggest that (partial) phenological isolation does not drive speciation of serpentine endemics but is instead important at the early

stages of population divergence (as in Levin, 2009), as is seen in another comparative speciation spectrum study (Christie and Strauss, 2018) and in case studies of reproductive isolation between ecotypes (Peterson, 1995; Nagy, 1997; Antonovics, 2006; Lowry et al., 2008; Briscoe Runquist et al., 2014). The level of ecological divergence between diverging populations - in this case, divergence in soil chemistry - was positively correlated with flowering time shifts, a finding that corroborates a comparative study that found positive associations between ecological divergence and reproductive isolation across a variety of organisms (Funk et al., 2006).

Similarly, I did not find a difference in the magnitude of fitness trade-offs or habitat isolation between endemic and tolerator pairs in the field-soil reciprocal transplant experiment, suggesting that habitat isolation due to soil-mediated selection against foreign genotypes also does not drive speciation of serpentine endemics. I did find strong evidence that serpentine populations of endemics are poorer competitors than serpentine populations of tolerators, and that there is more divergence in competitive ability in endemic pairs than tolerator pairs. Based on this result, I would have predicted that endemic pairs have higher degrees of habitat isolation in the competitive nonserpentine treatment than tolerator pairs. However, I did not find that introducing competition resulted in higher degrees of habitat isolation in endemic pairs. It should be noted that competition, and what constitutes a population's competitive environment, is a nuanced concept. The density and community composition of potential

competitors varies across all of the nonserpentine habitats from which we sampled sister taxa. Similarly, the limiting resources – e.g., water, nutrients or light – also likely vary among all of the habitats we sampled, and the different populations used in this study may vary in the types of competition-related traits that are selected in their habitats. Field experiments that test the roles of naturally-relevant competitive environments would complement the standardized competitive ability results we see here. For example, growing serpentine taxa in their nonserpentine sister’s field habitats in a neighbor-removal design (e.g., Sambatti and Rice, 2006) would give us a better idea of how competition affects habitat isolation in the field and the importance of habitat isolation in driving speciation.

The idea that serpentine endemics are restricted to serpentine soils because of competition was proposed by Kruckeberg in the 1950s and has since been one of the primary explanations for why lineages evolve to become endemics versus tolerators (Kruckeberg, 1951; Anacker, 2014). Over the years, studies have accumulated partial or indirect evidence for this hypothesis. For example, some studies comparing endemic and tolerator competitive abilities have had inconclusive sample sizes (Powell and Knight, 2009), have found inconsistent differences between endemics and tolerators in the effects neighbor removal had on fitness (Fernandez-Going and Harrison, 2013), or have quantified functional traits in serpentine and nonserpentine habitats as a proxy for competitive ability (Fernandez-Going et al., 2012). This dissertation work is the first study that

explicitly quantifies competitive abilities of multiple serpentine endemics and serpentine tolerators. Here, I used the same grass species (*Bromus carinatus*) as the competitor for all the species used in the study to get a standardized measure of competitive ability. With this standardized measure of competitive ability, I came to the conclusion that serpentine endemics are worse competitors than serpentine tolerators because of greater trade-offs between serpentine adaptation and competitive ability in endemic lineages. This study is the first empirical support for a primary prediction of Kruckeberg's competitive trade-off hypothesis: that larger trade-offs between serpentine adaptation and competitive ability in endemic lineages cause endemics to be worse competitors than serpentine tolerators.

In conjunction with habitat data collected from serpentine sites of all study taxa in Chapter 1, I was able to identify possible selective agents that may play a role in the evolution of a trade-off between serpentine adaptation and competitive ability. Specifically, I found that serpentine endemics occur in more barren serpentine habitats with lower soil Ca levels than serpentine tolerators. Adaptation to aspects of bare habitats, such higher rockiness or higher herbivore pressure (Strauss and Cacho, 2013), may involve the evolution of traits that trade-off with competitive ability. This idea is supported by a comparative study in the serpentine-endemic rich genus *Streptanthus* found that habitat bareness was negatively correlated with competitive ability (Cacho and Strauss, 2014).

Hypothesized trade-offs between stress-related traits and competition-related traits have a long history in ecology (Grime, 1977). A largely unanswered question in the serpentine system that warrants future work is what the specific traits are, and their genetic architecture, that underlie trade-offs between adaptation to stressful habitats and competitive habitats. For example, do physiological traits related to nutrient uptake have a more negative pleiotropic effect on competitive ability than the physiological traits related to water use efficiency? A fruitful avenue could be to compare a QTL mapping population generated from a serpentine endemic species to one generated from a closely related serpentine tolerator species. Controlled lab experiments that manipulate nutrient availability, water and standardized competition could be crossed with field estimates of fitness in the field to determine what physiological traits have genetic trade-offs with competition in the lab and with fitness in natural, productive nonserpentine habitats. By comparing the traits influencing nutrient and water uptake, and their effects on competitive ability, between a serpentine endemic and closely related tolerator, we could begin to understand the mechanisms producing the trade-off patterns seen in this dissertation.

A common theme in the first three chapters of this dissertation was the high variation among all of the tolerator and endemic sister taxa pairs in a variety of the response variables we measured. This variance could in part be explained by the phylogenetic breadth I had in this study and the idiosyncratic adaptation of different lineages to serpentine soils due to phyletic constraints. Another source of

this variation could be due to the treatment of all sister taxa pairs as categorical – pairs were either endemic or tolerator pairs. Yet, there are different ways and extents to which species can be serpentine tolerators – e.g., the degree of ecotypic differentiation among serpentine and nonserpentine populations or the degree to which a tolerator species is restricted to serpentine may explain some of the variation within this system. A study by Safford et al. (2005) created a quasi-continuous scale to score serpentine-affiliated species based on their degree of restriction to serpentine. However, not all serpentine-affiliated species are on this list, and their metric of serpentine restriction does not follow any statistical distribution. A future direction for comparative studies in the serpentine system would be to use a continuous measure of serpentine affinity, such as the proportion of populations on serpentine, to better understand the causes and consequences of edaphic divergence.

What, then, in addition to a loss in competitive ability promotes speciation of serpentine endemics? One factor I did not quantify is the degree of spatial isolation among sister taxa pairs. Spatial isolation has long been recognized as one of the primary factors driving speciation (Dobzhansky, 1937; Mayr, 1959; Coyne and Orr, 2004), allowing populations to evolve reproductive barriers in the absence of homogenizing gene flow. Spatial isolation is likely an important factor in serpentine systems (Kay et al., 2011). Serpentine outcrops often appear in discrete and island-like patches, and the relative number, size and spacing between outcrops varies across the landscape (Harrison et al., 2000). In regions

where the serpentine and nonserpentine matrix is more finely grained (i.e., more spatial heterogeneity relative to dispersal distance), adaptive plasticity is hypothesized to be favored (Baythavong, 2011), facilitating relatively high levels of gene flow among populations and preventing the evolution of reproductive isolation. Conversely, the evolution of reproductive isolation may be more likely in regions where the edaphic matrix is coarse-grained, and long-distance dispersal to isolated serpentine patches confers enough temporary reproductive isolation for young endemic species to diverge from their progenitor species. Surprisingly, herbaceous endemic serpentine richness is negatively correlated with the patchiness of serpentine in a region (Harrison et al., 2000), suggesting that while spatial isolation may promote the evolution of new species, more continuous areas of suitable serpentine habitat are necessary to maintain endemic diversity through decreasing extinction risk.

Serpentine endemics have historically been thought to evolve through one of two pathways: neoendemism or paleoendemism. The concepts were initially described by Stebbins and Major (1965) to describe ecological endemism in general, and were adapted by Kruckeberg (1954) to explain serpentine endemism. Paleoendemics were once widespread species that underwent a processes of biotype depletion, wherein populations in all but one type of habitat go extinct, resulting in a species that occurs in only one habitat. Neoendemics evolve through the process of budding speciation – where colonization into a new habitat is followed by adaptation and the evolution of reproductive isolation with their

progenitor populations. Stebbins and Major (1965) described paleoendemism in the context of ancient lineages that were once widespread, such as the redwoods *Sequoia sempervirens*, whereas herbaceous annual or perennial endemics were thought to be recently evolved neoendemisms. In Chapter 4, I inferred the evolutionary history of a putative neoendemic species *Clarkia franciscana*. This species shows many of the classic characteristics of a neoendemic – it has a small and abutting geographic range to its presumed progenitor species, it is an herbaceous annual, and it has a putatively derived self-fertilizing mating system. Despite these characteristics, my evidence shows that *Clarkia franciscana* is not a neoendemic in the sense that it was recently derived from a progenitor species. Given the levels of genetic diversity between disjunct populations, it is more likely that the species was once widespread and then underwent a process of population extinction in nonserpentine substrates. One of the primary conclusions from this chapter supports those made by Losos and Glor (2003) that evolutionary biologists should be cautious of using present-day geographic patterns and plant traits to make conclusions about the geographic mode of speciation. This limitation will make it difficult to study the role that spatial isolation plays in the evolution of endemism versus tolerance.

My dissertation work highlights general patterns that emerge from edaphic divergence across serpentine and nonserpentine substrates, such as the early evolution of flowering time shifts with ecological divergence, and the occupation of harsher habitats and lower competitive abilities in serpentine endemics.

Edaphic endemics are an important component of the flora in regions with a complexity of edaphic substrates, and they are typically associated with harsh edaphic substrates (Schnitzler et al., 2011; Molina-Venegas et al., 2013; Baldwin, 2014; Moore et al., 2014). Although the particular chemical and physical challenges of non-serpentine substrates occupied by edaphic endemics are different than serpentine substrates, similar patterns that we find here are likely occurring in those systems. For example, flowering time differences are seen in edaphic ecotypes between populations on “normal” soils and populations on granite (Ferris and Willis, 2018), quartz gravel (Ellis et al., 2006), mine tailings (Macnair et al., 1989; Antonovics, 2006), gypsum (Escudero et al., 2015), or volcanic soils (Hipperson et al., 2016). The commonality of flowering time shifts in other systems of edaphic divergence highlights a tight link between plant nutrient and water relationships on phenology, and suggests flowering time shifts might be a common mechanism facilitating the evolution of ecotypes. Serpentine systems are often treated as binary habitats (i.e., serpentine vs nonserpentine) for convenience, and my work adds to other studies (e.g., Yost et al., 2012) that highlight the variation within serpentine habitats in their harshness and selective factors. Future research can determine how much fine scale variation there is within other harsh edaphic substrates and whether that variation explains the evolution of edaphic generalists or specialists.

Lastly, the concept of trade-offs is central to the ideas of ecological specialization (Futuyma and Moreno, 1988; Sexton et al., 2017), range dynamics

(Holt, 2003), and speciation via adaptive divergence (Schluter, 2001; Sobel et al., 2010). However, fitness trade-offs are not always detected (Bennett and Lenski, 2007; Hereford, 2009; Forister et al., 2012). My research suggests that one reason studies may not detect trade-offs is because trade-offs occur along different niche axes. I found that adaptation to serpentine soil generally doesn't come with a fitness costs in nonserpentine soil, but it does have a cost in the presence of competition. Similarly, salt adaptation in *Daphnia* does not come with a fitness cost in low-salinity environments but instead in predator response (Latta et al., 2012). I also found that fitness trade-offs were more prominent when fitness measures included fecundity, as opposed to just viability, components of fitness. Inclusive measures of fitness across multiple environmental axes will aide in uncovering the presence of fitness trade-offs and understanding their role in specialization and speciation.

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