

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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PREMISE: 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.

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.

KEY WORDS Bayesian; competition; edaphic divergence; endemic; generalist; habitat bareness; habitat harshness; serpentine; specialist; trade-offs.

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 (E) and tolerator (T) 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, which 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 allow species 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* [Brassicaceae], 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

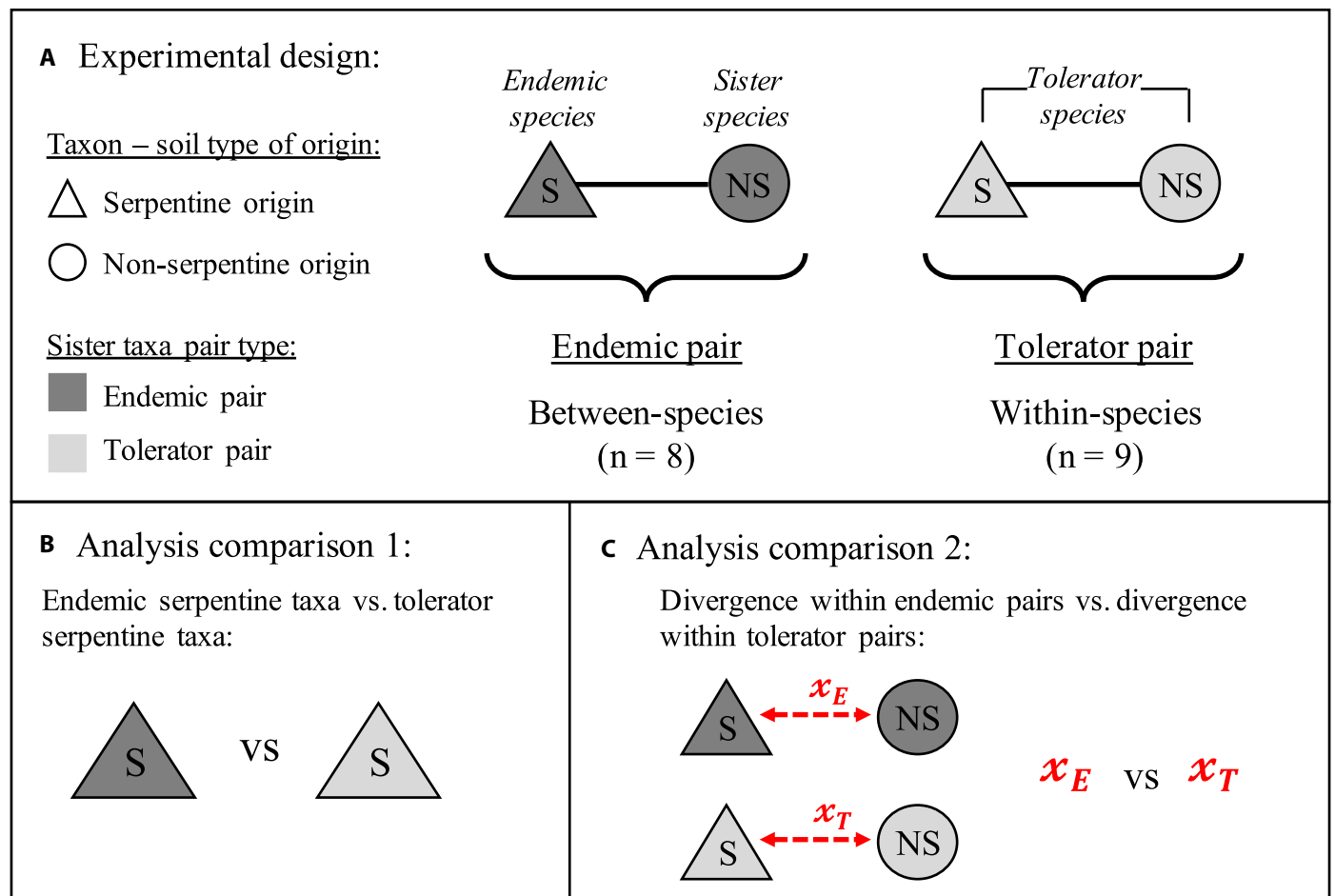


FIGURE 1. Conceptual diagram of the experimental design (A) and two comparisons used throughout the analyses (B, C). Our experimental design (A) consists of sister-taxa pairs. Each pair contains a taxon from serpentine soil and a taxon from nonserpentine soil, but the pairs differ in whether they are 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).

sister taxa pairs that have all undergone edaphic divergence but vary in whether that divergence is associated with serpentine endemism or serpentine tolerances (Fig. 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 Fig. 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 Fig. 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; 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, USA. 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 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 University of California, Davis, 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. The majority of our collections occurred at the McLaughlin Reserve, which spans

TABLE 1. Serpentine and nonserpentine taxa of nine tolerator pairs and eight endemic pairs used in this study. Species codes are provided for subsequent figures. Each species code is followed by whether that species is an endemic (E), tolerator (T), or non-tolerator (NT) and its serpentine affinity scores from Safford et al. (2005)^a if included in the study (otherwise listed as NA). 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.

Pair type ^b	Family	Population origin ^c	Species	Latitude	Longitude	Species Code	Distance between taxa (km)
Tolerator ¹	Onagraceae	Serpentine ¹	<i>Clarkia concinna</i>	38.82403	−122.35441	CACO (T; NA)	1.85
		Nonserpentine ¹	<i>Clarkia concinna</i>	38.86488	−122.37175		
Tolerator ²	Onagraceae	Serpentine ²	<i>Clarkia breweri</i>	37.41108	−121.43060	CABR (T; 3.8)	3.03
		Nonserpentine ²	<i>Clarkia breweri</i>	37.42246	−121.34293		
Tolerator ²	Plantaginaceae	Serpentine ¹	<i>Plantago erecta</i>	38.86189	−122.41630	PLER (T; 1.0)	1.75
		Nonserpentine ³	<i>Plantago erecta</i>	38.85107	−122.36597		
Tolerator ³	Phrymaceae	Serpentine ¹	<i>Mimulus guttatus</i>	38.85797	−122.40932	MGUT (T; NA)	6.17
		Nonserpentine ⁴	<i>Mimulus guttatus</i>	38.75227	−122.28473		
Tolerator ²	Plantaginaceae	Serpentine ¹	<i>Collinsia sparsiflora</i>	38.86065	−122.41167	COSP (T; 1.7)	2.48
		Nonserpentine ³	<i>Collinsia sparsiflora</i>	38.83809	−122.34328		
Tolerator ⁴	Plantaginaceae	Serpentine ²	<i>Collinsia heterophylla</i>	37.41087	−121.43031	COHT (T; NA)	2.23
		Nonserpentine ²	<i>Collinsia heterophylla</i>	37.42264	−121.36665		
Tolerator ²	Fabaceae	Serpentine ¹	<i>Trifolium wildenovii</i>	38.85826	−122.40930	TWILD (T; 1.3)	1.57
		Nonserpentine ³	<i>Trifolium wildenovii</i>	38.84920	−122.36364		
Tolerator ²	Polemoniaceae	Serpentine ¹	<i>Navarretia pubescens</i>	38.85821	−122.40356	NAPB (T; 2.0)	1.44
		Nonserpentine ³	<i>Navarretia pubescens</i>	38.84825	−122.36241		
Tolerator ¹	Polemoniaceae	Serpentine ⁵	<i>Navarretia heterodoxa</i>	37.46160	−122.28256	NAHX (T; 2.8)	37.76
		Nonserpentine ⁶	<i>Navarretia heterodoxa</i>	38.33951	−122.22940		
Endemic ^{2,4,5}	Polemoniaceae	Serpentine ¹	<i>Navarretia jepsonii</i>	38.85993	−122.41154	NAJP (E; 5.6)	37.58
		Nonserpentine ⁷	<i>Navarretia heterandra</i>	39.36490	−121.49095	NAHN (NT; NA)	
Endemic ^{2,5,6}	Polemoniaceae	Serpentine ⁸	<i>Navarretia rosulata</i>	37.96745	−122.62773	NARS (E; 6.0)	20.87
		Nonserpentine ⁶	<i>Navarretia heterodoxa</i>	38.33951	−122.22940	NAHX (T; 2.8)	
Endemic ^{2,7}	Onagraceae	Serpentine ¹	<i>Clarkia gracilis</i> ssp. <i>tracyi</i>	38.86001	−122.41724	CAGT (E; 5.0)	52.05
		Nonserpentine ¹⁰	<i>C. gracilis</i> ssp. <i>albicaulis</i>	39.90193	−121.61798	CAGA (T; 2.1)	
Endemic ^{2,8}	Polemoniaceae	Serpentine ¹	<i>Collomia diversifolia</i>	38.86873	−122.38710	CLDV (E; 5.6)	11.06
		Nonserpentine ¹¹	<i>Collomia heterophylla</i>	38.80779	−122.70823	CLHT (NT; NA)	
Endemic ⁹	Asteraceae	Serpentine ¹²	<i>Layia discoidea</i>	36.37538	−120.73183	LADI (E; 6.1)	6.19
		Nonserpentine ¹²	<i>Layia glandulosa</i>	36.27188	−120.61047	LAGL (NT; NA)	
Endemic ^{2,3}	Phrymaceae	Serpentine ¹	<i>Mimulus nudatus</i>	38.85968	−122.41819	MNUD (E; 5.6)	6.4
		Nonserpentine ⁴	<i>Mimulus guttatus</i>	38.75227	−122.28473	MGUT (T; NA)	
Endemic ^{2,10}	Plantaginaceae	Serpentine ³	<i>Collinsia greenii</i>	38.84411	−122.37722	COGR (E; 5.2)	1.17
		Nonserpentine ³	<i>Collinsia sparsiflora</i>	38.83809	−122.34328	COSP (T; 1.7)	
Endemic ^{2,11}	Onagraceae	Serpentine ¹²	<i>Camissonia benitensis</i>	36.35913	−120.76188	CABE (E; 6.1)	4.58
		Nonserpentine ¹²	<i>Camissonia strigulosa</i>	36.32100	−120.63835	CAST (T; NA)	

Notes: ^aSafford et al. (2005) serpentine affinity scores: 5.5–6.1 = “strict endemics”, >95% occurrences on serpentine; 4.5–5.5 = “broad endemics”, 85–94% occurrences on serpentine; 2.5–3.4 = “strong indicators”, 65–74% occurrences on serpentine; 1.5–2.4 = “weak indicators”, 55–64% occurrences on serpentine; 1–1.4 = “indifferent”, 50–54% occurrences on serpentine.

^bCitations 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.

^cCollection locations in California, USA: 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 Creek Mgt. Area, ¹²Napa Co: Knoxville Wildlife Reserve.

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 compared 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 (Fig. 1A). In all cases we selected our non-serpentine sister taxa by using occurrence data from CalFlora (website <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 in our analyses of pairwise divergence (i.e., as in Fig. 1C).

We used three of our nonserpentine sister taxa as the nonserpentine sister taxon in two pairs. For example, *Mimulus nudatus*

Curran ex Greene [Phrymaceae] is a serpentine endemic hypothesized to be derived from within *Mimulus guttatus* DC. (Macnair and Gardner, 1998). We chose a *M. guttatus* nonserpentine population to serve as the nonserpentine sister taxa for *M. nudatus*. However, because *M. guttatus* is a serpentine tolerator itself, we also used the same nonserpentine population as the nonserpentine sister taxon for a serpentine taxon of *M. guttatus*. We used this same overlapping design for the *Collinsia greenei* A. Gray - *C. sparsiflora* Fisch. & C.A. Mey. [Plantaginaceae] endemic pair and *C. sparsiflora* tolerator pair, and for the *Navarretia rosulata* Brand - *N. heterodoxa* Greene [Polemoniaceae] 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* L. [Caryophyllaceae], *Alyssum bertolonii* Desv. [Brassicaceae], the *Lasthenia californica* DC. ex Lindl. complex, *M. guttatus*, and *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz [Brassicaceae] (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. glandulosus* Hook. (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 sublocations within the plant population, each collected within the first 10 cm from the surface. We sent soils to the University of Maine Analytical Laboratory and Soil Testing Service, Orono, Maine, USA, for chemical and texture analyses of the following: (1) soil pH, calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K; neutral ammonium acetate extractions); (2) calculated cation exchange capacity; (3) electrical conductivity; (4) nitrate (NO_3^-) and ammonium (NH_4^+) (KCl extraction); (5) phosphorus (P), sulfur (S), boron (B) (modified Morgan extract, pH 4.8); (6) micronutrients (zinc (Zn), manganese (Mn), iron (Fe), aluminum (Al), and copper (Cu)) and heavy metals (nickel (Ni), chromium (Cr), cobalt (Co); (7) diethylenetriaminepentaacetic acid (DTPA) extraction); (8) microbial activity (burst respiration method); and (9) particle size (percent clay, sand, and silt; all 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) by centering a 25 cm \times 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 incorporated 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: (1) the *Navarretia rosulata* (endemic species) serpentine population; and (2) both the serpentine and nonserpentine population of *N. heterodoxa* (tolerator species). Thus, these taxa were 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 as described in Baldwin (1992). PCR reactions consisted of 6.25 μL GoTaq Colorless Master Mix (Promega, Madison, Wisconsin, USA), 0.75 μL each of the ITS5 and ITS4 primers, 1 μL of DNA, and 3.75 μL 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 University of California, 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., 2012), 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 Markov chain Monte Carlo (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 (R Development Core Team, 2008) using the read_annotated command in the R package phylotate version 1.2. We ultrametricized our trees with the Grafen method (Grafen, 1989) using the compute.brlen function in ape version 5.2 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 version 5.2 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 Fig. 1B). We a priori parsed our soil variables down to just 12 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` version 3.1-137. The correlation structure was made with the `corBrownian` function in `ape` version 5.1, 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 Fig. 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_{0j} , is estimated based on taxon identity and the phylogenetic relationship among taxa. β_0 was 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 `vcv()` function from `ape` version 5.1., as in Mitchell et al. (2015). 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 were 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 version 3.5. We implemented the model in JAGS version 4-6, 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 (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 quantified pairwise divergence (i.e., as in Fig. 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 included 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 Euclidean distances separating sister taxa of endemic pairs is greater than the Euclidean distances separating sister taxa of tolerator pairs with a PGLS model that included geographic distance between sister taxa as a covariate.

Divergence in microhabitat bareness between endemic and tolerator sister taxa—Lastly, we asked whether sister taxa of endemic pairs have more divergence in microhabitat bareness than sister taxa of tolerator pairs (i.e., as in Fig. 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 we estimated 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 predicted 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).

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

$$\text{pair.type.mean}_m = \frac{\sum_{k=1}^{K_m} \text{pairwise.divergence}_k}{K_m} \quad (\text{Eq. 6})$$

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 inputted both of these values into a second hierarchical Bayesian model that incorporates our original sampling error (Eq. 9), the error associated with factors not captured in our deterministic model (Eq. 10), and a deterministic model to test for the effects of endemism and tolerance on pairwise divergence (Eq. 11):

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

$$\text{var.PD}_k = \text{variance}(\text{pairwise.divergence}_k) \quad (\text{Eq. 8})$$

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

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

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

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 version 4-6, 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 tested for correlations between the habitat variables used in the above analyses, specifically between microhabitat bareness and the individual soil variables. We subsetted the data to test for correlations among habitat variables from just serpentine taxa, from just nonserpentine taxa, and then from all taxa. We used 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 adjusted *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; $F_{1,14} = 17.45$, $P = 0.002$); Fig. 2). However, there are no other statistically significant differences between endemics and tolerators in any of the other soil harshness variables tested after correcting for multiple comparisons (Table 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 (Fig. 3; Appendix S1 and S2). 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 in the 12 soil harshness variables does not differ between sister taxa of endemic pairs and sister taxa of tolerator pairs (Appendices S3 and S4). The PCA of soil chemistry and texture of all populations used in this study shows clustering of serpentine and non-serpentine taxa, respectively (Fig. 4A). The first five principal components explained over 75% of the variation in

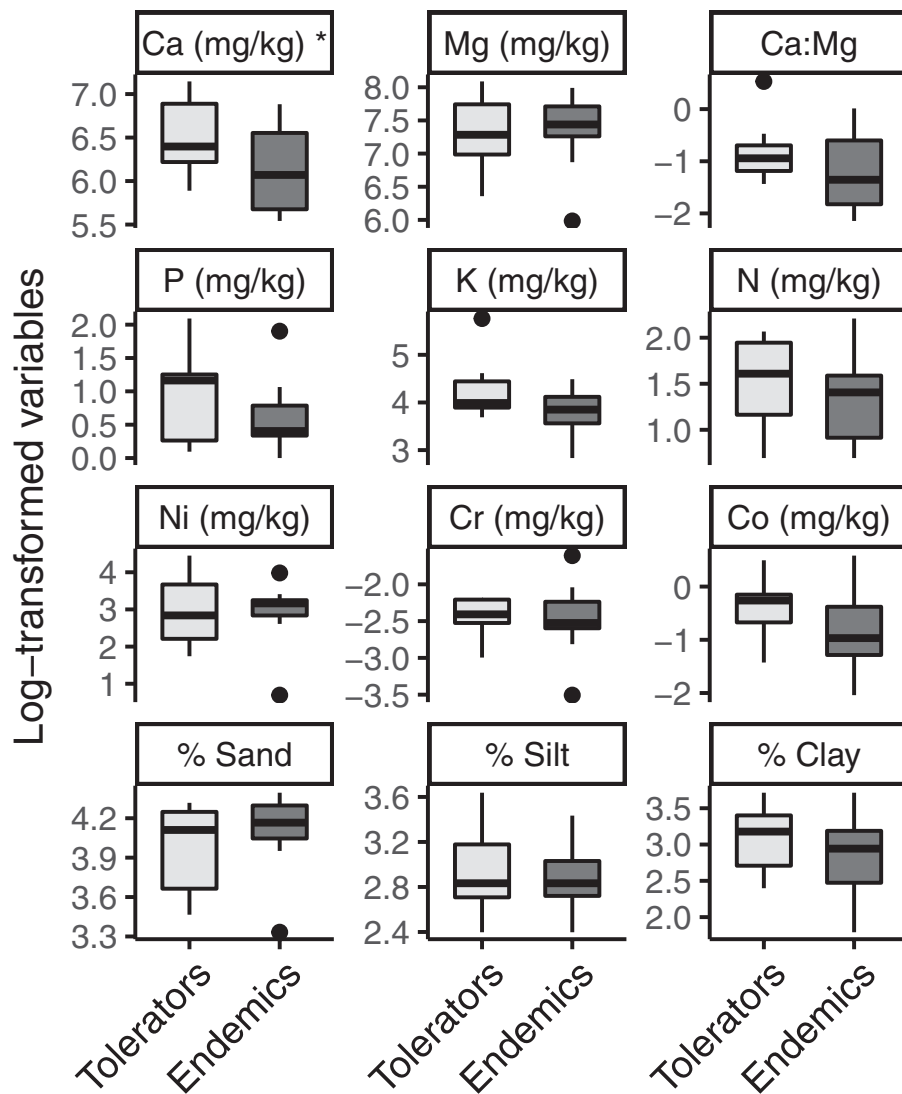


FIGURE 2. Differences in soil harshness variables between endemic and tolerator serpentine taxa. The lower and upper edges of the boxplots show the first and third quartiles, respectively, and points indicate data points that are farther than 1.5 times the interquartile range. Only Ca is statistically different between endemics and tolerators (*).

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 Fig. 4A). The soil distances of the serpentine tolerator pairs does not differ from the soil distances of the serpentine endemic pairs, nor is there an effect of geographic distance between sister taxa pairs on soil distances (Fig. 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 (Fig. 5, Appendices S6 and S7), the endemic pairs have a higher average pairwise divergence than the

tolerator pairs (Fig. 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 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).

TABLE 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
Calcium and magnesium	Ca:Mg	0.720	6.172	0.025
	Mg	−0.222	0.628	0.440
	Ca	0.498	13.346	0.002
Macronutrients	N	0.390	2.482	0.136
	P	0.081	0.253	0.623
	K	0.370	1.444	0.248
Heavy metals	Ni	0.455	1.108	0.309
	Cr	0.260	3.063	0.100
	Co	0.746	6.989	0.018
Texture	% Sand	−0.111	0.660	0.429
	% Silt	0.083	0.279	0.605
	% Clay	0.262	1.461	0.246

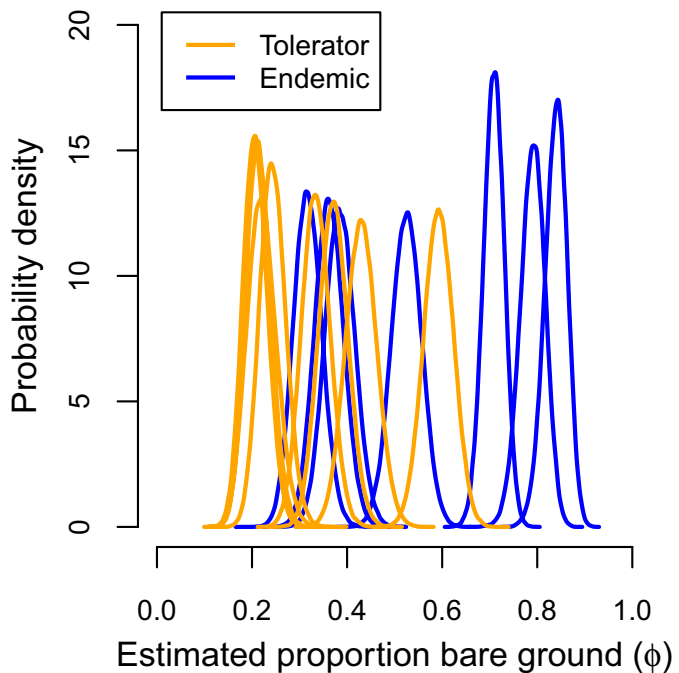


FIGURE 3. Endemic serpentine taxa occur in barer serpentine habitats than tolerator serpentine taxa, in a model that incorporates phylogenetic relatedness. Lines show the individual posterior distributions of the ϕ parameter, i.e., the estimated proportion of bare ground, for the 8 tolerator serpentine taxa and 7 endemic serpentine taxa.

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 benitensis* P.H. Raven [Onagraceae], *Collomia diversifolia* Greene [Polemoniaceae], *Clarkia gracilis* (Piper) A. Nelson & J. F. Macbr. ssp. *tracyi* (Jeps.) Abdel-Hameed & R. Snow [Onagraceae]) and some tolerator serpentine taxa had relatively low soil Ca levels (e.g., *Collinsia sparsiflora* [Plantaginaceae], *Plantago erecta* E. Morris [Plantaginaceae], and *Collinsia heterophylla* Buist ex Graham [Plantaginaceae]). 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 that 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)

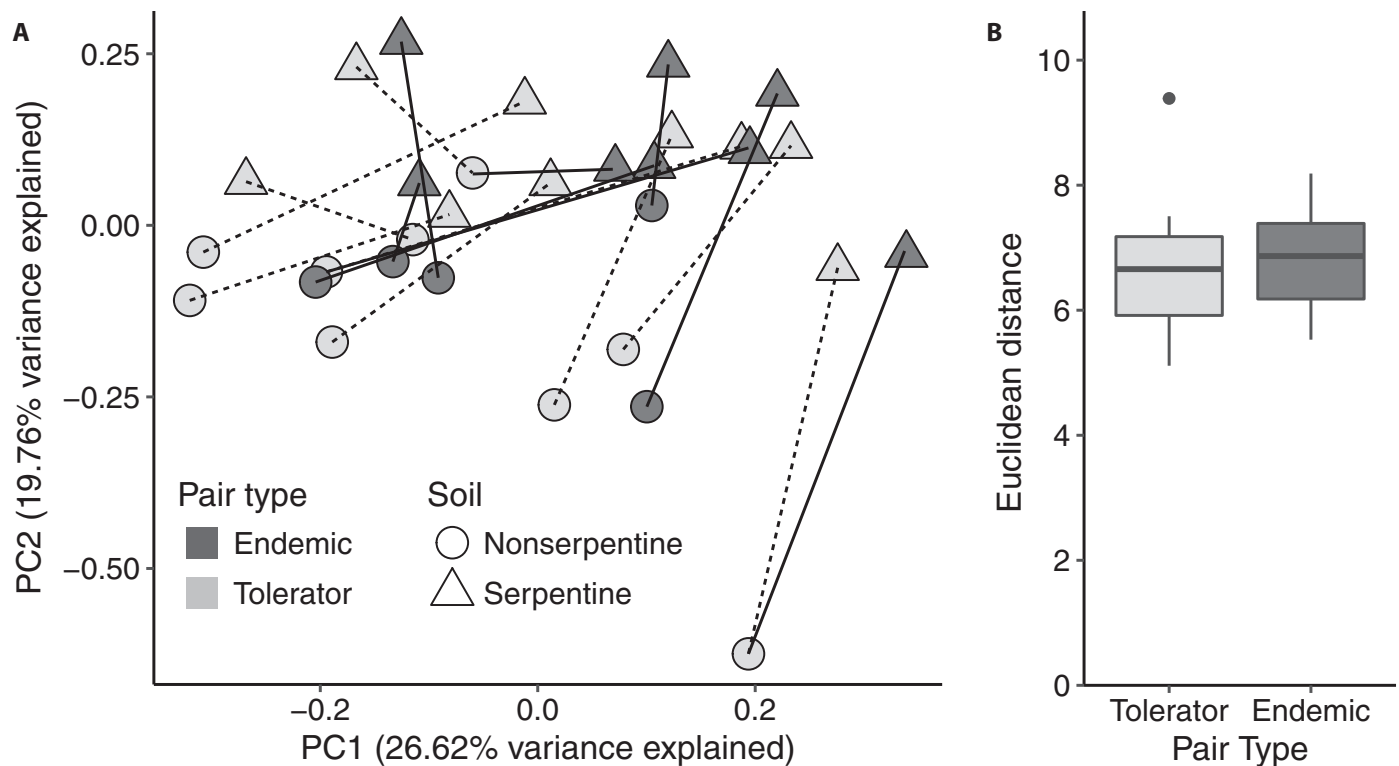


FIGURE 4. Divergence in the multivariate soil environment. (A) The principal component analysis that includes all soil variables. Each point is one taxon. Shapes differentiate the soil that the taxon is from and colors indicate whether the taxon is part of an endemic or tolerator pair. Solid lines connect 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.

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* (A. Gray) Greene [Onagraceae] 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 of South Africa (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 quantitative trait locus (QTL) mapping study between *Microseris douglasii* (DC.) Sch.Bip. [Asteraceae] (serpentine tolerator) and *M. bigelovii* (A. Gray) Sch. Bip. (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

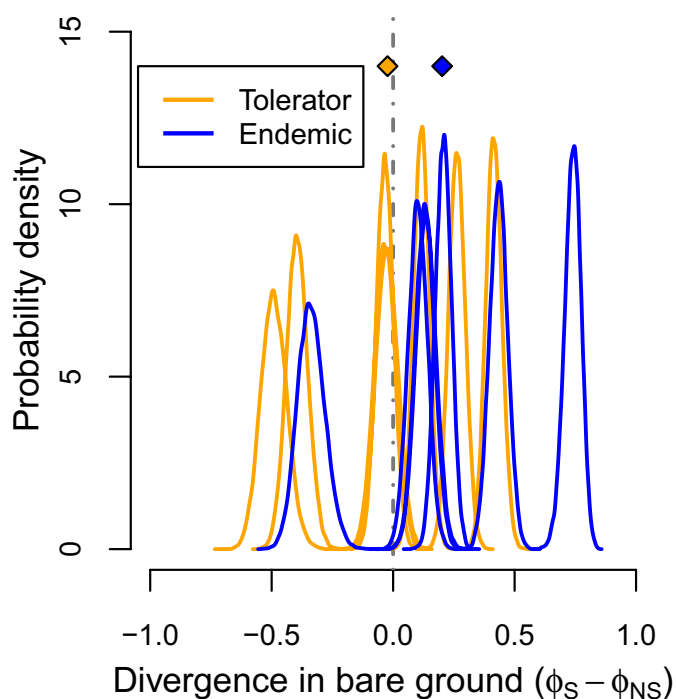


FIGURE 5. Endemic sister-taxa pairs tend to have more pairwise divergence in bare ground (i.e., $\phi_S - \phi_{NS}$) than tolerator sister-taxa pairs. A model that incorporates relatedness among pairs indicates there is a probability of 0.83 that endemic sister-taxa pairs have more divergence in bare 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).

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-Goñi 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* (Fisch. & C.A. Mey.) Greene, *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* [Asteraceae] and *Arenaria* [Caryophyllaceae] 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* Hook. [Brassicaceae] 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* Keck [Asteraceae] (endemic) – *L. glandulosa* (Hook.) Hook. & Arn. [Asteraceae] (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 Fig. 1B) or pairwise divergence (i.e., as in Fig. 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 *Collinsia sparsiflora* and *Mimulus 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 tolerator 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, as well as 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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AUTHOR CONTRIBUTIONS

S.A.S. and K.M.K. designed the study. S.A.S. collected and analyzed the data, and wrote the first draft of the manuscript. S.A.S. and K.M.K. both contributed substantially to revisions.

DATA ACCESSIBILITY

The following are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.t12ch7h> (Sianta and Kay, 2019):

1. Soil chemistry and texture data by taxon in .csv format.
2. Bare ground data by quadrat and taxon in .csv format.
3. R script, including sunken JAGs models, for all habitat analyses.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

LITERATURE CITED

- Anacker, B. L. 2011. Phylogenetic patterns of endemism and diversity. In S. P. Harrison, and N. Rajakaruna [eds.], *Serpentine: The evolution and ecology of a model system*, 49–70. University of California Press, Berkeley, California, USA.
- Anacker, B. L., and S. Y. Strauss. 2014. The geography and ecology of plant speciation: Range overlap and niche divergence in sister species. *Proceedings of the Royal Society, B, Biological Sciences* 280: 20132980.
- Anacker, B. L., J. B. Whittall, E. E. Goldberg, and S. P. Harrison. 2011. Origins and consequences of serpentine endemism in the California flora. *Evolution* 65: 365–376.
- Antonovics, J. 2006. Evolution in closely adjacent plant populations X: Long-term persistence of prereproductive isolation at a mine boundary. *Heredity* 97: 33–37.
- Baldwin, B. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.
- Baldwin, B. G. 2005. Origin of the serpentine-endemic herb *Layia discoidea* from the widespread *L. glandulosa* (Compositae). *Evolution* 59: 2473–2479.
- Baldwin, B. G. 2014. Origins of plant diversity in the California Floristic Province. *Annual Review of Ecology, Evolution, and Systematics* 45: 347–369.
- Baldwin, B. G., S. Kalisz, and W. S. Armbruster. 2011. Phylogenetic perspectives on diversification, biogeography, and floral evolution of *Collinsia* and *Tonella* (Plantaginaceae). *American Journal of Botany* 98: 731–753.
- Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken [eds.], 2012. *The Jepson manual: Vascular plants of California*. University of California Press, Berkeley, California, USA.
- Baskin, J. M., and C. C. Baskin. 1988. Endemism in rock outcrop plant communities of unglaciated eastern United States: An evaluation of the roles of edaphic, genetic and light factors. *Journal of Biogeography* 15: 829–840.

- Baythavong, B. S. 2011. Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: Selection favors adaptive plasticity in fine-grained environments. *American Naturalist* 178: 75–87.
- Baythavong, B. S., and M. L. Stanton. 2010. Characterizing selection on phenotypic plasticity in response to natural environmental heterogeneity. *Evolution* 64: 2904–2920.
- Brady, K. U., A. R. Kruckeberg, and H. D. Bradshaw Jr. 2005. Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* 36: 243–266.
- Branco, S. 2009. Are oaks locally adapted to serpentine soils? *Northeastern Naturalist* 16: 329–340.
- Cacho, N. I., and S. Y. Strauss. 2014. Occupation of bare habitats, an evolutionary precursor to soil specialization in plants. *Proceedings of the National Academy of Sciences, USA* 111: 15132–15137.
- Caisse, M., and J. Antonovics. 1978. Evolution in closely adjacent plant populations IX. Evolution of reproductive isolation in clinal populations. *Heredity* 40: 371–384.
- Coley, P. D., J. Lokvam, K. Rudolph, K. Bromberg, E. Tara, L. Wright, T. Brenes-Arguedas, et al. 2005. Divergent defensive strategies of young leaves in two species of *Inga*. *Ecology* 86: 2633–2643.
- Cowling, R. M., E. T. F. Witkowski, A. V. Milewski, and K. R. Newbey. 1994. Taxonomic, edaphic and biological aspects of narrow plant endemism on matched sites in Mediterranean South Africa and Australia. *Journal of Biogeography* 21: 651.
- Crawford, D. J. 2010. Progenitor-derivative species pairs and plant speciation. *Taxon* 59: 1413–1423.
- Dick, C. A., J. A. Herman, R. E. O'Dell, A. Lopez-Villalobos, C. Eckert, and J. B. Whittall. 2014. Cryptic genetic subdivision in the San Benito evening primrose (*Camissonia benitensis*). *Conservation Genetics* 15: 165–175.
- Dittmar, E. L., and D. W. Schemske. 2017. The edaphic environment mediates flowering-time differentiation between adjacent populations of *Leptosiphon parviflorus*. *Journal of Heredity* 109: 90–99.
- Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Ellis, A. G., and A. E. Weis. 2006. Coexistence and differentiation of “flowering stones”: The role of local adaptation to soil microenvironment. *Journal of Ecology* 94: 322–335.
- Ellis, A. G., A. E. Weis, and B. S. Gaut. 2006. Evolutionary radiation of “stone plants” in the genus *Argyrodendron* (Aizoaceae): Unraveling the effects of landscape, habitat, and flowering time. *Evolution* 60: 39.
- Endara, M. J., and P. D. Coley. 2011. The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology* 25: 389–398.
- Escudero, A., S. Palacio, F. T. Maestre, and A. L. Luzuriaga. 2015. Plant life on gypsum: A review of its multiple facets. *Biological Reviews* 90: 1–18.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.
- Fernandez-Goñi, B., and S. Harrison. 2013. Effects of experimental water addition depend on grassland community characteristics. *Plant Ecology* 214: 777–786.
- Fernandez-Goñi, B., B. Anacker, and S. Harrison. 2012. Temporal variability in California grasslands: Soil type and species functional traits mediate response to precipitation. *Ecology* 93: 2104–2114.
- Ferris, K. G., and J. H. Willis. 2018. Differential adaptation to a harsh granite outcrop habitat between sympatric *Mimulus* species. *Evolution* 72: 1225–1241.
- Fine, P. V., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Sääksjärvi, et al. 2006. The growth-defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.
- Funk, D. J., P. Nosil, and W. J. Etges. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences, USA* 103: 3209–3213.
- Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematics* 19: 207–233.
- Gailing, O., M. R. Macnair, and K. Bachmann. 2004. QTL mapping for a trade-off between leaf and bud production in a recombinant inbred population of *Microseris douglasii* and *M. bigelovii* (Asteraceae, Lactuceae): A potential preadaptation for the colonization of serpentine soils. *Plant Biology* 6: 440–446.
- Gelman, A., and D. Rubin. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–511.
- Gottlieb, L. D., and N. F. Weeden. 1979. Gene duplication and phylogeny in *Clarkia*. *Evolution* 33: 1024–1039.
- Grafen, A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 326: 119–157.
- Green, E. S. 2010. Infrageneric relationships within *Collomia* (Polemoniaceae). M.S. thesis. Brigham Young University, Provo, Utah, USA.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169–1194.
- Harrison, S., and N. Rajakaruna [eds.]. 2011. Serpentine: The evolution and ecology of a model system. University of California Press, Berkeley, California, USA.
- Imbert, E., S. Youssef, D. Carbonell, and A. Baumel. 2012. Do endemic species always have a low competitive ability? A test for two Mediterranean plant species under controlled conditions. *Journal of Plant Ecology* 5: 305–312.
- Kay, K. M., K. L. Ward, L. R. Watt, and D. W. Schemske. 2011. Plant Speciation. In S. P. Harrison, and N. Rajakaruna [eds.], Serpentine: The evolution and ecology of a model system, 71–97. University of California Press, Berkeley, California, USA.
- Kay, K. M., S. Woolhouse, B. A. Smith, N. S. Pope, and N. Rajakaruna. 2018. Sympatric serpentine endemic *Monardella* (Lamiaceae) species maintain habitat differences despite hybridization. *Molecular Ecology* 27: 2302–2316.
- Kazakou, E., P. G. Dimitrakopoulos, A. J. M. Baker, R. D. Reeves, and A. Y. Troumbis. 2008. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: From species to ecosystem level. *Biological Reviews* 83: 495–508.
- Kruckeberg, A. R. 1951. Intraspecific variability in the response of certain native plant species to serpentine soil. *American Journal of Botany* 38: 408–419.
- Kruckeberg, A. R. 1954. The ecology of serpentine soils. III. Plant species in relation to serpentine soils. *Ecology* 35: 267–274.
- Kruckeberg, A. R. 1957. Variation in fertility of hybrids between isolated populations of the serpentine species, *Streptanthus glandulosus* Hook. *Evolution* 11: 185–211.
- Kruckeberg, A. R. 1967. Ecotypic response to ultramafic soils by some plant species of northwestern United States. *Brittonia* 19: 133–151.
- Kruckeberg, A. R. 1986. An essay: The stimulus of unusual geologies for plant speciation. *Systematic Botany* 11: 455–463.
- Lavergne, S., J. D. Thompson, E. Garnier, and M. Debussche. 2004. The biology and ecology of narrow endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. *Oikos* 107: 505–518.
- Loew, O., and D. W. May. 1901. The relation of lime and magnesia to plant growth. *U.S. Department of Agriculture Bureau of Plant Industry Bulletin* 1: 1–53.
- Lynch, M. 1991. Methods for the analysis of comparative data in evolutionary biology. *Evolution* 45: 1065–1080.
- Macnair, M., and M. Gardner. 1998. The evolution of edaphic endemics. In D. Howard, and S. Berlocher [eds.], Endless forms - species and speciation, 157–171. Oxford University Press, New York, New York, USA.
- Maddison, W. P., and D. R. Maddison. 2018. Mesquite: A modular system for evolutionary analysis, version 3.51. Website <http://www.mesquiteproject.org>.
- Mayer, M. S., and P. S. Soltis. 1999. Intraspecific phylogeny analysis using ITS sequences: Insights from studies of the *Streptanthus glandulosus* complex (Cruciferae). *Systematic Biology* 24: 47–61.
- Mayer, M. S., P. S. Soltis, and D. E. Soltis. 1994. The evolution of the *Streptanthus glandulosus* complex (Cruciferae): Genetic divergence and gene flow in serpentine endemics. *American Journal of Botany* 81: 1288–1299.
- McNeilly, T. 1968. Evolution in closely adjacent plant populations III. *Agrostis tenuis* on a small copper mine. *Heredity* 23: 99–108.
- Mengoni, A., C. Gonnelli, E. Brocchini, F. Galardi, S. Pucci, R. Gabbriellini, and M. Bazzicalupo. 2003. Chloroplast genetic diversity and biogeography in the serpentine endemic Ni-hyperaccumulator *Alyssum bertolonii*. *New Phytologist* 157: 349–356.

- Mitchell, N., T. E. Moore, H. K. Mollmann, J. E. Carlson, K. Mocko, H. Martinez-Cabrera, C. Adams, et al. 2015. Functional traits in parallel evolutionary radiations and trait-environment associations in the Cape Floristic Region of South Africa. *American Naturalist* 185: 525–537.
- Molina-Venegas, R., A. Aparicio, F. J. Pina, B. Valdés, and J. Arroyo. 2013. Disentangling environmental correlates of vascular plant biodiversity in a Mediterranean hotspot. *Ecology and Evolution* 3: 3879–3894.
- Moore, M. J., J. F. Mota, N. A. Douglas, H. F. Olvera, and H. Ochoterena. 2014. The ecology, assembly and evolution of Gypsophile floras. In N. Rajakaruna, R. S. Boyd, and T. B. Harris [eds.], *Plant ecology and evolution in harsh environments*, 97–128. Nova Science Publishers Inc., New York, New York, USA.
- Nyberg Berglund, A. B., A. Saura, and A. Westerbergh. 2001. Genetic differentiation of a polyploid plant on ultramafic soils in Fennoscandia. *South African Journal of Science* 97: 533–535.
- Nyberg Berglund, A. B., S. Dahlgren, and A. Westerbergh. 2004. Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during the colonization of Scandinavia. *New Phytologist* 161: 199–209.
- O'Dell, R. E., and V. P. Claassen. 2006. Serpentine and nonserpentine *Achillea millefolium* accessions differ in serpentine substrate tolerance and response to organic and inorganic amendments. *Plant and Soil* 279: 253–269.
- O'Dell, R. E., and N. Rajakaruna. 2011. Intraspecific variation, adaptation, and evolution. In S. P. Harrison, and N. Rajakaruna [eds.], *Serpentine: The evolution and ecology of a model system*, 97–138. University of California Press, Berkeley, California, USA.
- O'Dell, R. E., J. J. James, and J. H. Richards. 2006. Congeneric serpentine and nonserpentine shrubs differ more in leaf Ca: Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* 280: 49–64.
- Palm, E. R., and E. Van Volkenburgh. 2014. Physiological adaptations of plants to serpentine soil. In N. Rajakaruna, R. S. Boyd, and T. B. Harris [eds.], *Plant ecology and evolution in harsh environments*, 129–147. Nova Science Publishers Inc., New York, New York, USA.
- Powell, K. I., and T. M. Knight. 2009. Effects of nutrient addition and competition on biomass of five *Cirsium* species (Asteraceae), including a serpentine endemic. *International Journal of Plant Sciences* 170: 918–925.
- Proctor, J. 1971. The plant ecology of serpentine. II. Plant response to serpentine soils. *Journal of Ecology* 59: 827–842.
- Proctor, J., and S. Woodell. 1971. The plant ecology of serpentine: I. Serpentine vegetation of England and Scotland. *Journal of Ecology* 59: 827–842.
- R Development Core Team. 2008. R: A language and environment for statistical computing, Vienna, Austria. Website: <http://www.R-project.org>.
- Rajakaruna, N. 2004. The edaphic factor in the origin of plant species. *International Geology Review* 46: 471–478.
- Rajakaruna, N. 2017. Lessons on evolution from the study of edaphic specialization. *Botanical Review* 84: 39–78.
- Rajakaruna, N., and J. Whitton. 2004. Trends in the evolution of edaphic specialists with an example of parallel evolution in the *Lasthenia californica* complex. In Q. C. B. Cronk, J. Whitton, R. H. Ree, and I. E. P. Taylor [eds.], *Plant adaptation: Molecular genetics and ecology*, 103–110. NRC Research Press, Ottawa, Ontario, Canada.
- Rajakaruna, N., G. E. Bradfield, B. A. Bohm, and J. Whitton. 2003. Adaptive differentiation in response to water stress by edaphic races of *Lasthenia californica* (Asteraceae). *International Journal of Plant Sciences* 164: 371–376.
- Rogers, R., and S. Schumm. 1991. The effect of sparse vegetative cover on erosion and sediment yield. *Journal of Hydrology* 123: 19–24.
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Rune, O. 1953. Plant life on serpentines and related rocks in the north of Sweden. *Acta Phytogeographica Suecica* 31: 1–139.
- Safford, H. D., J. H. Viers, and S. P. Harrison. 2005. Serpentine endemism in the California flora: A database of serpentine affinity. *Madroño* 52: 222–257.
- Sambatti, J. B. M., and K. J. Rice. 2007. Functional ecology of ecotypic differentiation in the Californian serpentine sunflower (*Helianthus exilis*). *New Phytologist* 175: 107–119.
- Schnitzler, J., T. G. Barraclough, J. S. Boatwright, P. Goldblatt, J. C. Manning, M. P. Powell, T. Rebelo, and V. Savolainen. 2011. Causes of plant diversification in the Cape biodiversity hotspot of South Africa. *Systematic Biology* 60: 343–357.
- Selby, J. P. 2014. The genetic basis of local adaptation to serpentine soils in *Mimulus guttatus*. Ph.D. dissertation. Duke University, Durham, North Carolina, USA.
- Selby, J. P., and J. H. Willis. 2018. Major QTL controls adaptation to serpentine soils in *Mimulus guttatus*. *Molecular Ecology* 27: 5073–5087.
- Sexton, J. P., J. Montiel, J. E. Shay, M. R. Stephens, and R. A. Slatyer. 2017. Evolution of ecological niche breadth. *Annual Review of Ecology, Evolution, and Systematics* 48: 183–206.
- Sianta, S. A., and K. M. Kay 2019. Data from: 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. Dryad Digital Repository. <https://doi.org/10.5061/dryad.t12ch7h>.
- Spencer, S. C., and J. M. Porter. 1997. Evolutionary diversification and adaptation to novel environments in *Navarretia* (Polemoniaceae). *Systematic Botany* 22: 649–668.
- Stebbins, G. L., and J. Major. 1965. Endemism and speciation in the California flora. *Ecological Monographs* 35: 1–35.
- Stevens, P. F. 2017. Angiosperm Phylogeny Website, version 14. Missouri Botanical Garden, Saint Louis, Missouri, USA. Website <http://www.mobot.org/MOBOT/research/APweb/>.
- Strauss, S. Y., and N. I. Cacho. 2013. Nowhere to run, nowhere to hide: The importance of enemies and apparency in adaptation to harsh soil environments. *American Naturalist* 182: E1–E14.
- Turner, T. L., E. C. Bourne, E. J. Von Wettberg, T. T. Hu, and S. V. Nuzhdin. 2010. Population resequencing reveals local adaptation of *Arabidopsis lyrata* to serpentine soils. *Nature Genetics* 42: 260–263.
- Verboom, G. A., W. D. Stock, and M. D. Cramer. 2017. Specialization to extremely low-nutrient soils limits the nutritional adaptability of plant lineages. *American Naturalist* 189: 684–699.
- Vlams, J. 1949. Growth of lettuce and barley as influenced by degree of calcium saturation of soil. *Soil Science* 67: 453–466.
- Vlams, J., and H. Jenny. 1948. Calcium deficiency in serpentine soils as revealed by absorbent technique. *Science* 107: 549–551.
- Walker, R. B. 1948. A study of serpentine soil infertility with special reference to edaphic endemism. Ph.D. dissertation. University of California, Berkeley, Berkeley, California, USA.
- Walker, R. B., H. M. Walker, and P. R. Ashworth. 1955. Calcium-magnesium nutrition with special reference to serpentine soils. *Plant Physiology* 30: 214–221.
- Whittaker, R. H., R. B. Walker, and A. R. Kruckeberg. 1954. The ecology of serpentine soils. *Ecology* 35: 258–288.
- Wright, J. W., K. F. Davies, J. A. Lau, A. C. McCall, and J. K. McKay. 2006. Experimental verification of ecological niche modeling in a heterogeneous environment. *Ecology* 87: 2433–2439.
- Wright, J. W., M. L. Stanton, and R. Scherson. 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* 8: 1–21.
- Yost, J. M., T. Barry, K. M. Kay, and N. Rajakaruna. 2012. Edaphic adaptation maintains the coexistence of two cryptic species on serpentine soils. *American Journal of Botany* 99: 890–897.