Do extreme environments provide a refuge from pathogens? A phylogenetic test using serpentine flax[1]

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Abiotically extreme environments are often associated with physiologically stressful conditions, small, low-density populations, and depauperate flora and fauna relative to more benign settings. A possible consequence of this may be that organisms that occupy these stressful habitats receive fitness benefits associated with reductions in the frequency and/or intensity of antagonistic species interactions. I investigated a particular form of this effect, formalized as the "pathogen refuge hypothesis," through a study of 13 species of wild flax that grow on stressful serpentine soils and are often infected by a pathogenic fungal rust. The host species vary in the degree of their serpentine association: some specialize on extreme serpentine soils, while others are generalists that occur on soils with a wide range of serpentine influence. Phylogenetically explicit analyses of soil chemistry and field-measured disease levels indicated that rust disease was significantly less frequent and severe in flax populations growing in more stressful, low-calcium serpentine soils. These findings may help to explain the persistence of extremophile species in habitats where stressful physical conditions often impose strong autecological fitness costs on associated organisms. Ancestral state reconstruction of serpentine soil tolerance (approximated using soil calcium concentrations) suggested that the ability to tolerate extreme serpentine soils may have evolved multiple times within the focal genus.

Key words: edaphic specialization; *Hesperolinon*; Linaceae; *Melampsora lini*; pathogen refuge hypothesis; plant–pathogen interactions; serpentine soil; soil calcium; Uredinales.

Organisms that inhabit physically extreme environments experience habitat-associated fitness costs that are absent or reduced in more benign settings (Bijlsma and Loeschcke, 2005). These costs can be a direct result of the harsh conditions to which these organisms are exposed (e.g., resulting in slower growth and delayed maturation at lower temperatures, reduced fecundity when resources are scarce) or an indirect consequence of the adaptations that allow them to cope with such stressors. Through trade-offs or constraints, the morphological, physiological, and behavioral traits that make harsh environments tolerable can render the organisms that possess these traits less vigorous and/or fecund than conspecifics or relatives that lack such characteristics in more benign environments (Sibly and Calow, 1989; Zera and Harshman, 2001; Ricklefs and Wikelski, 2002). The persistence of "extremophile" species over evolutionary time, however, suggests that such costs must be balanced or eclipsed by fitness benefits associated with the occupation of stressful or low quality habitats.

One possible benefit of inhabiting extreme environments could be a reduction in the frequency and/or magnitude of antagonistic species interactions (Hoffmann and Hercus, 2000; Waterman, 2001; Thrall et al., 2007) that effectively renders these settings a refuge from attack by natural enemies (Tobler et al., 2007). Extreme environments are generally characterized by a relatively depauperate flora and fauna (i.e., lower levels of abundance, density, and diversity compared to more benign settings), and the rate of establishment and spread of enemies such as parasites and pathogens should be limited in these numerically and geographically small, low-density host populations. This effect could be further enhanced if the biochemistry or physiology of individuals occupying these stressful niches is altered in a manner that reduces their quality as victims/hosts and the number and/or performance of enemies able to exploit them. Selection acting on organisms in extreme environments may thus represent a balance between two qualitatively different sets of forces: costs imposed by the abiotic environment and benefits associated with the nature and intensity of biotic interactions (or lack thereof) in those environments. While research on extremophile species often focuses on the autecological costs associated with organism–environment interactions, quantifying the relative contribution of the synecological benefits arising from altered species interactions is critical to understanding evolutionary dynamics in extreme environments.

Plants associated with ultramafic (serpentine) soils represent an attractive focus for studies of evolution in extreme environments. Serpentine soils are characterized by physical and chemical properties that are highly stressful to most plant taxa (Proctor, 1999). These include a coarse and rocky texture, low water-holding capacity, elevated and potentially toxic concentrations of metals (e.g., magnesium, iron, cobalt, nickel), and extremely reduced availability of essential plant nutrients (e.g.,

calcium, nitrogen, phosphorous) (Proctor and Woodell, 1975). While most plant species are physiologically unable to tolerate these inhospitable soil conditions, the intense selective pressures associated with this edaphic niche have fostered the evolution of distinct assemblages of plants strongly associated with or endemic to serpentine soils (Brooks, 1987; Brady et al., 2005). This phenomenon is particularly apparent within the California Floristic Province, considered one of the world's biodiversity hotspots (Myers et al., 2000). Although serpentine soil accounts for <2% of the surface area of the state, more than 200 plant species, representing almost 10% of California's endemic flora, grow exclusively on these soils (Kruckeberg, 1984; Safford et al., 2005). The ability of plants to tolerate serpentine soils and the factors that promote their persistence there may thus have important consequences for the generation, maintenance, and distribution of floristic biodiversity.

The evolution of serpentine tolerance has received considerable attention from plant ecologists (see reviews by Kruckeberg, 2002; Rajakaruna, 2004; Brady et al., 2005), but only a handful of studies have evaluated the phenomenon within a phylogenetic framework. Some of these have focused on whether serpentine-associated taxa, which typically have narrow and discontinuous biogeographic distributions, are recently evolved and have yet to expand their range (neoendemics) or are remnants of older lineages with patchy distributions resulting from range contraction and fragmentation (paleoendemics) (Mayer and Soltis, 1994; Baldwin, 2005). In others, mapping of serpentine tolerance onto plant phylogenies has indicated multiple origins of serpentine-tolerant forms within floristic groups (Setoguchi et al., 1998; Pepper and Norwood, 2001; Patterson and Givnish, 2004; Kawase et al., 2007). This result is consistent with the frequent observation of serpentine and nonserpentine sister taxa (Brooks, 1987; Chan et al., 2002; Sudarmono and Okada, 2007) and suggests that serpentine tolerance has evolved rapidly and repeatedly within a number of plant lineages. While these studies have provided important insights into the evolution of serpentine endemism, they each focus almost exclusively on a single, qualitative aspect of the process: the origin of serpentine-tolerant taxa within clades that are comprised predominantly of nonserpentine species. To date, no published study has quantitatively evaluated phylogenetic variation in the degree of serpentine specialization following the origin of tolerance within a lineage. Without such studies, it is difficult to explain why in many groups of serpentine-tolerant plants, some species appear specialized on extreme serpentine soils while close relatives are generalists that occur on soils that vary widely in their serpentine influence (Kruckeberg, 1984; Roberts and Proctor, 1992). Documenting phylogenetic trends in edaphic specialization within serpentine-tolerant lineages could provide clues about the manner in which autecological selective costs of serpentine association are manifested. For example, if the majority of the costs are overcome as part of the initial development of tolerance (i.e., a threshold response), then a pattern of increasing serpentine specialization in more derived forms within a lineage might be expected, especially if synecological benefits are greater in more extreme serpentine environments. Alternatively, if costs increase additively with increasing specialization (e.g., if physiological stress is proportional to the concentration of heavy metals in the soil), then an optimal, intermediate level of specialization might be favored.

While intrinsic, autecological properties of plants play a fundamental role in the evolution of serpentine tolerance and specialization, selective pressures arising from biotic interactions also appear to make important contributions. Historically, most work on this topic has focused on plant–plant competition as an explanation for the fact that serpentine-tolerant species generally grow well in nonserpentine soils in the greenhouse but are rarely found growing off of serpentine soil in the wild. Because serpentine taxa tend to be relatively slow growing and small in stature, they are often thought to be competitively inferior to, and excluded from nonserpentine soils by, intolerant species (Kruckeberg, 1954; Kruckeberg and Rabinowitz, 1985). Serpentine soils may thus act as spatial refugia for tolerant plant species displaced from more fertile soils by competitively superior, intolerant species (e.g., exotic invaders) (Huenneke et al., 1990; Gram et al., 2004). An analogous scenario involving pathogens was proposed by Kruckeberg (1992). According to this "pathogen refuge hypothesis", plant populations or species associated with more extreme serpentine soils should experience lower disease pressure than those growing in less serpentine soils (Springer et al., 2007). A pathogen refuge effect could generate spatial and/or phylogenetic variation in disease-related fitness costs that might reinforce edaphic associations among serpentine plants and promote greater serpentine specialization. The only empirical assessments of this theory to date, however, found the opposite pattern. Studies of the serpentine-associated species *Hesperolinon californicum*, including both a greenhouse soil manipulation experiment (Springer et al., 2007) and epidemiological surveys of natural populations (Springer, 2009), indicated that the frequency and severity of disease caused by the fungal rust *Melampsora lini* were higher among host plants growing in more extreme serpentine soils. While this relationship has yet to be examined in other plant species, these results suggest that plants that are serpentine specialists, in contrast to the predictions of the pathogen refuge hypothesis, may tend to have higher and less variable levels of disease than serpentine generalists.

In this study, I sought to test the pathogen refuge hypothesis using multiple serpentine-tolerant species and a phylogenetically explicit analytical framework. I focused on the genus *Hesperolinon,* which consists of 13 species of wild flax that vary in their degree of serpentine specialization. I quantified soil characteristics and the prevalence and severity of disease symptoms in multiple populations of all *Hesperolinon* species to evaluate whether disease levels were lower in populations associated with more extreme serpentine soils. Additionally, in an attempt to gain insights into the manner in which costs of serpentine specialization are manifested (e.g., additive vs. threshold cost profiles), I mapped soil characteristics onto the host phylogeny and used ancestral state reconstruction to examine phylogenetic trends in the evolution of edaphic specialization within the focal genus.

MATERIALS AND METHODS

Host and pathogen species—The genus *Hesperolinon* (Linaceae) consists of 13 species of wild flax (Table 1); their morphology, biogeography, and ecology are described in detail in a monograph by Sharsmith (1961). All are ephemeral annuals, diminutive in size (mature plants 20–40 cm tall) and possessing thin stems and leaves. With one exception (*H. micranthum*), all are endemic to California (USA), where they occur primarily in the Coast Ranges of the western part of the state. The region associated with Napa and Lake counties appears to be the center of diversity for the genus because all species occur here and are more abundant than in more peripheral areas of their respective distributions (Sharsmith, 1961). Each *Hesperolinon* species grows in association with serpentine soils, and extensive observational studies by Sharsmith suggest that the degree of affinity for serpentine soils varies widely among the taxa. On the basis of visual assessments of the soils on which she observed them growing, she classified eight species as obligate or near-obligate serpentine

TABLE 1.   Names of the 13 *Hesperolinon* species found in California, USA, and identification codes used to refer to them throughout the study. Edaphic categorizations were proposed by Sharsmith (1961) based on observational studies of serpentine associations in natural populations.

Species code	Species name	Edaphic categorization
1	*H. adenophyllum* (A. Gray) Small	Specialist
2	*H. bicarpellatum* (H. Sharsm.) H. Sharsm.	Specialist
3	*H. breweri* (A. Gray) Small	Generalist
4	*H. californicum* (Benth.) Small	Generalist
5	*H. clevelandii* Small	Generalist
6	*H. congestum* (A. Gray) Small	Specialist
7	*H. didymocarpum* H. Sharsm.	Specialist
8	*H. disjunctum* H. Sharsm.	Specialist
9	*H. drymarioides* (Curran) Small	Specialist
10	*H. micranthum* (A. Gray) Small	Generalist
11	*H. sharsmithiae*[*] R. O'Donnell	Specialist
12	*H. spergulinum* (A. Gray) Small	Specialist
13	*H. tehamense* H. Sharsm.	Specialist

[*]*Hesperolinon sharsmithiae*, formerly known as *H. serpentinum* N. McCarten, was discovered following the publication of Sharsmith's work and has been categorized based on observations made by botanists familiar with the genus Hesperolinon.

endemics (hereafter termed specialists) and four as facultative serpentine species (hereafter generalists) (Table 1) because of their occurrence on soils with a wide range of serpentine influence and periodically on soils that are not serpentine derived (e.g., volcanic sediments). *Hesperolinon sharsmithiae* (formerly *H. serpentinum*) was described after the completion of Sharsmith's work (McCarten, 1993; O'Donnell, 2006) but is considered a serpentine specialist (J. Callizo, Wantrup Wildlife Refuge, personal communication). Prior to the present study, this categorization scheme had never been evaluated using quantitative data on soil geochemistry. Greenhouse studies indicate that all 13 species grow vigorously in potting soil (Y. Springer, unpublished data), so these edaphic distinctions between species appear to arise from one or more physiological and/or ecological mechanisms acting in the wild. While not demonstrated conclusively, it is unlikely that any *Hesperolinon* species is a metal hyperaccumulator because hyperaccumulation has never been reported in the Linaceae (A.J.M. Baker, University of Melbourne, personal communication).

Melampsora lini Persoon (Uredinales) is an autoecious, macrocyclic, rust fungus that attacks stem and leaf tissue of plants in the family Linaceae (Flor, 1954; Lawrence et al., 2007). It is an obligate pathogen, causing localized, nonsystemic lesions on plant stems and leaves that reduce host plant vigor via destruction of photosynthetic tissue (Littlefield, 1981). Pronounced reductions in seedling survival and adult fecundity were documented in a concurrent field investigation of *M. lini* infection of *H. californicum* (conducted in the same populations used in the present study). Plants with moderate to severe levels of disease (>30% of photosynthetic tissue covered with rust pustules) had mortality rates approximately six times higher than healthy conspecifics as seedlings and produced roughly one-eighth the number of viable seed capsules as adults (Springer, 2009). These fitness costs resulted in significant decreases in within-population host plant density following years of high disease prevalence (defined here as the percentage of plants on which disease symptoms are apparent). *Melampsora lini* is wind dispersed and has an asexual generation time of ~12–14 d. Aside from rare and relatively mild damage caused by feeding of an herbivorous chrysomelid beetle (Y. Springer, personal observation), no other natural enemies of *Hesperolinon* species are known. Additionally, within the study area, plant species other than *Hesperolinon* spp. that could potentially be infected by *M. lini* (e.g., *Linum bienne*) are extremely uncommon.

Selection of study populations—I determined the biogeographic range of each *Hesperolinon* species using distribution data gathered from the monograph by Sharsmith (1961), herbarium records at the University of California Berkeley Jepson Herbarium (http://ucjeps.berkeley.edu/consortium), the California Department of Fish and Game Natural Diversity Database (http://www.dfg.ca.gov/biogeodata/cnddb/), and interviews with local naturalists and plant ecologists with extensive experience studying California serpentine flora. Five study populations were selected for each species with the exceptions of *H. congestum* (six) and *H. californicum* (16). In total, 77 *Hesperolinon* study populations (Appendix S1, see Supplemental Data with online version of this article) and five populations of the putative outgroup species *Linum neomexicanum*

(online Appendix S2) were selected. The latitude and altitude of each study population were determined using a handheld global positioning system.

Edaphic profiles—Between 2002 and 2003, I collected soil samples from each of the study populations to characterize edaphic conditions experienced by associated plants. Six samples per population were collected from each population except for three relatively large populations of *H. californicum*, from which 10–18 samples were collected. Within populations, soil was collected from haphazardly selected locations in an effort to sample the spatial extent of the population as completely and evenly as possible. Each sample consisted of four bulked equivalently sized scoops (~250 mL) of the upper 10 cm of soil taken from each of the corners of a 0.25-m^2 quadrat. I processed and analyzed soils using methods described by Springer et al. (2007), measuring the concentrations of 17 cations (Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Si, Sr, and Zn) using a Perkin Elmer (Waltham, Massachusetts, USA) Optima 4300 DV internally coupled plasma optical emissions spectrometer. Nonmetric multidimensional scaling (NMDS) and SIMPER analyses (Clarke, 1993), both performed using the program Primer version 5.2.9 (Primer-E Ltd., Lutton, Ivybridge, UK), were used to (1) determine how well populations of the 14 plant species could be distinguished by their edaphic profiles and (2) identify the focal cations that contributed most significantly to this differentiation. Only these focal cations were considered in subsequent analyses involving edaphic characteristics.

Epidemiological profiles—I collected data on the severity of rust disease in all *Hesperolinon* populations in 2002, 2003, and 2004 (a subset of the *H. californicum* populations was also surveyed in 2001) and in all *L. neomexicanum* populations in 2003. Disease surveys were conducted in late May/early June at the onset of flowering. I used a modified James scale (James, 1971) to visually estimate disease severity and assign each plant examined to one of nine severity categories based on the percentage of leaf and stem tissue covered by rust pustules: 0 (no visible pustules), 1%, 5%, 10%, 25%, 50%, 75%, 90%, and 100%. Two survey techniques were employed. Because *H. californicum* was the focus of a separate study requiring a higher degree of spatial resolution, I surveyed populations of this species using a "fixed plot" technique described elsewhere (Springer, 2009). Data for all 12 congeners of *H. californicum* and *L. neomexicanum* were collected using a "random walk" technique. Based on the spatial distribution of plants, I categorized each population as being either linear (e.g., plants occurring along an ecotonal boundary where a serpentine outcropping merged into a nonserpentine meadow) or nonlinear (plants scattered within a serpentine patch with no apparent linear pattern of distribution). In linear populations, I randomly chose a spot to begin surveys and scored disease severity of plants while walking along a transect that ran parallel to the linear axis of the population. To insure that I did not disproportionately sample in areas where plant density was high, I surveyed plants in batches; after the disease status of 25 plants had been recorded, I stopped surveying, walked 5 m along the transect, then resumed scoring disease symptoms until I had examined 25 more plants or I reached the boundary of the population. For nonlinear populations, I scored disease along transects radiating at randomly determined compass headings from a central point within the population. I randomly selected the distance from the transect origin point that I would begin plant scoring, then counted no more than 25 plants per transect heading. I based the frequency for counting plants along transects on rough visual estimates of plant abundance at the time of survey. For populations of fewer than 200 plants, I scored the first 100 plants encountered and every other plant thereafter. For populations between 200 and 500 individuals or larger than 500 individuals, every fifth and eighth plant, respectively, were scored. Depending on the species, location, and year, population sizes varied between fewer than 100 and more than 50 000 individuals.

Phylogenetic reconstruction—I used one sample (tissue from a single plant) from each of the 77 *Hesperolinon* study populations and one of the outgroup species, collected between 2002 and 2003, for sequence-based phylogenetic analyses. For each sample, DNA was extracted from ~1 g of dried tissue using DNeasy plant extraction kits (Qiagen, Valencia, California, USA) and four chloroplast loci (*trnT-trnL* spacer, *trnL-trnF* spacer, *trnK* 3′ intron, and *rpl16* intron) were sequenced using the polymerase chain reaction. Details on primer sequences and PCR reaction mixes and conditions are provided in Appendix S3 (see online Supplemental Data). PCR products were cleaned using Zymoclean gel DNA recovery kits (Zymo Research, Orange, California, USA) or PureLink PCR Purification kits (Invitrogen, Carlsbad, California, USA). Sequencing was performed either by Macrogen (Seoul, Korea) or facilities associated with the Allan Wilson Center for Molecular Ecology and Evolution (Palmerston North, New Zealand). Forward and reverse sequencing reactions were performed for all samples.

Sequences were edited using the program Sequencher (Gene Codes Corp., Ann Arbor, Michigan, USA) and aligned by eye using the program Se-Al version 2.0 a11 (Rambaut, 2003) and based on the similarity criterion (Simmons, 2004). A number of polynucleotide indels and mononucleotide repeats were present in the sequences. I coded indels according to the simple indel coding scheme of Simmons and Ochoterena (2000). Length variation in repeats with a mean number of eight or more bases [type I (a) repeats, Golenberg et al., 1993] was manually standardized among samples because these regions could not be aligned unambiguously and their inclusion would likely have introduced homoplasious characters into the dataset (Small et al., 1998). Because the four loci examined are effectively part of a single coalescent gene (i.e., the chloroplast genome [Hudson, 1990]) that is generally believed to be free of recombination, I concatenated the edited sequences to produce a single master sequence for each sample.

I constructed phylogenetic trees using Markov chain Monte Carlo (MCMC) Bayesian analysis (Yang and Rannala, 1997) in the program MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). The analysis used settings of the best-fit TVM+I+G model, selected by Akaike information criterion (AIC) (Akaike, 1974) in the program Modeltest 3.7 (Posada and Crandall, 1998) with rate variation across sites approximated using a gamma distribution (Yang, 1993) with a proportion of sites being invariant ("rates=invgamma"). I ran the analysis for six million generations with five runs, five chains, and a temperature of 0.2. Based on the cold-chain log-likelihood values of a preliminary analytical run, I selected a highly conservative "burnin" fraction of 0.2. Three runs were performed to ensure that the tree topology and posterior probabilities were stable. In addition to the Bayesian posterior values produced using this approach, I also generated bootstrap values (Felsenstein, 1985a) for the tree using a maximum likelihood analysis in the program PAUP* version 4.0b10 (Swofford, 2002). Tree building (using the same TVM+I+G model settings) was done using a heuristic search with tree-bisection-reconnection (TBR) branch swapping and 100 random replicates of stepwise addition. Bootstrap values were generated using a full heuristic search with 10 replicates.

Data analysis—I tested whether Sharsmith's (1961) edaphic categorization scheme, based on qualitative, observational assessments of soil associations, would be borne out by quantitative soil analyses. Using mixed-models, I compared species classified using Sharsmith's specialist vs. generalist scheme according to their edaphic associations and measured rates of disease. Each model included a fixed effect term for Sharsmith's categorization and random effect terms for species and population nested within species. Response variables of the different models included calcium and magnesium concentrations of individual soil samples collected from generalist and specialist populations, population-level coefficients of variation for both cations (among soil samples), disease prevalence (annual means for host populations), and population-level coefficient of variation for disease prevalence (among survey years). Data were transformed as necessary to meet assumptions of normality. For the prevalence analyses, I only considered data from populations where disease had been observed at least once. Evidence from greenhouse inoculation trials, in which I was able to infect all 13 species with *M. lini* spores, suggests that disease-free populations were more likely uninfected due to a failure of the pathogen to colonize those populations rather than to some mechanism of host resistance (Y. Springer, unpublished data).

I performed phylogenetically independent contrast (PIC) analyses (Garland et al., 1992, 1993), using the PDAP module version 1.11 (Garland et al., 1993; Midford et al., 2008) within the program Mesquite version 2.5 (Maddison and Maddison, 2008), to assess whether levels of rust disease were lower in *Hesperolinon* populations associated with more extreme serpentine soils. This method allows the relationship between edaphic ecology and disease levels to be assessed while controlling for the degree of evolutionary relatedness between host taxa (Felsenstein, 1985b). Soil cation concentrations and disease measures were transformed as necessary to normalize the data. As with the species-level analysis of disease properties, I only considered data from populations where disease symptoms had been documented at least once. For each analysis (focal cation), I examined a plot of the absolute values of the standardized independent contrasts vs. their standard deviations to determine which branch lengths adequately standardize the data (Garland et al., 1992). In every case, branch lengths of the consensus tree were all set equal to one because this provided the best standardization. Branches with Bayesian posterior values <85% (*N* = 5) were pruned from the tree, and while all polytomies were regarded as "hard" (Garland and Díaz-Uriarte, 1999), results were qualitatively identical when they were treated as "soft."

I visualized phylogenetic patterns of serpentine specialization within the genus *Hesperolinon* using squared-change parsimony analysis, performed in Mesquite, to reconstruct soil associations for ancestral nodes within the consensus tree. For each focal cation, I standardized the concentration predicted for each node to that generated for the most basal node in the tree to produce a relative measure of changes in cation concentration between ancestral nodes. I used these measures to scale the widths of branches in the consensus tree and thereby facilitate visualization of relative changes in soil associations between ancestral forms of *Hesperolinon*. I also performed ancestral state reconstruction using disease prevalence data.

RESULTS

Edaphic profiles—The *Hesperolinon* study populations were distributed along a relatively linear edaphic gradient (Fig. 1), and SIMPER analysis indicated that of the 17 measured cations, magnesium (mean ± SD percentage contribution to solution = 42.25 ± 4.21%) and calcium (39.82 ± 4.47%) had the largest influence on this solution. Potassium (6.29 ± 2.46%) was the only other cation with a mean contribution exceeding 5%. Stress of the two-dimensional NMDS plot was 0.03 or less in all of the 500 restarts. On the basis of these results, calcium and magnesium were selected as the two focal cations for use in subsequent analyses involving edaphic parameters. Their respective concentrations in individual soil samples (*N* = 487) ranged from 102.43 to 6209.79 mg·kg^{-1} and 37.32–4940.82 mg·kg^{-1} (Fig. 2).

Results of the mixed model tests of Sharsmith's (1961) edaphic categorization scheme indicated that serpentine specialists occurred on soils with significantly lower mean calcium concentrations (mg·kg^{-1}, log$_{10}$ transformed) than generalists (among species untransformed mean ± SE: specialists, 704.67 ± 293.88 vs. generalists, 1374.47 ± 117.4, *N* = 487 soil samples, $F_{1,8.8} = 9.21$, *P* = 0.015, Fig. 2), but there was no difference between groups in terms of magnesium concentrations (specialists, 2090.4 ± 166.82 vs. generalists, 1812.38 ± 250.23, *N* = 487 soil samples, $F_{1,11.7} = 0.38$, *P* = 0.55) or coefficient of variation for calcium (specialists, 0.40 ± 0.034 vs. generalists, 0.34 ± 0.051, *N* = 77 populations, $F_{1,4.6} = 0.25$, *P* = 0.64) or magnesium (specialists, 0.23 ± 0.016 vs. generalists, 0.20 ± 0.024, *N* = 77 populations, $F_{1,29} = 3.26$, *P* = 0.17).

Epidemiological profiles—There was considerable variation in the frequency and severity of disease symptoms among *Hesperolinon* species and study populations (Figs. 2, 3). Because regression analysis (using all population and year combinations for which prevalence was nonzero) showed a strong positive correlation between disease prevalence and severity (severity = − 2.91 + (0.36 × prevalence) + (0.005 × (prevalence − 25.96)^2, r^2_{adj} = 0.81, MS = 13382.8, $F_{2,143} = 306.2$, *P* < 0.0001), prevalence was the only measure of disease used in subsequent analyses. Twenty-one of the 77 *Hesperolinon* study populations, including at least one population from seven of the 13 host species, were omitted from disease-related analyses because symptoms of disease were never observed within these populations. Of these 21 excluded populations, nine were associated with serpentine generalists and 11 with specialists; *t* tests indicated that there were no significant differences between excluded and included populations in terms of associated concentrations of soil calcium (*t* = 1.38, df = 75, *P* = 0.17) or magnesium (*t* = 1.57, df = 75, *P* = 0.12). In populations where disease was observed at least once, overall rust prevalence (mean ± SD) averaged 15.9 ± 24.06% across all population/year combinations (*N* = 235) and 25.96 ± 26.17% when symptoms were actually observed in a particular year (*N* = 144).
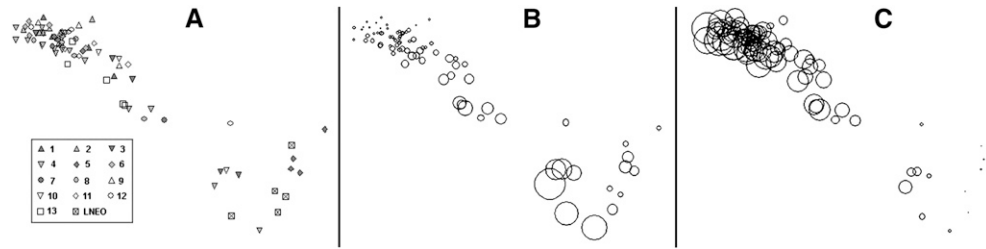
Fig. 1.   Results of (A) NMDS soil analyses from 77 *Hesperolinon* and five *Linum neomexicanum* study populations on serpentine soils in California and (B, C) bubble plots showing the influence of (B) calcium and (C) magnesium on the NMDS solution. Stress of the 2D plot was 0.03 or less in all of the 500 restarts. Circle diameters in plots B and C are proportional to the mean calcium and magnesium concentrations calculated for each study population, respectively.

At the level of host species, disease prevalence (mean ± SE) ranged from 39.42 ± 4.91% in *H. micranthum* to zero in *H. breweri*, the only host species for which disease was never observed. On average, diseased plants lost 26.19% of their photosynthetic tissue to symptom-related damage. Rust disease of *L. neomexicanum* was never observed.

Hesperolinon species classified by Sharsmith as serpentine specialists tended to be associated with lower levels of disease prevalence (square-root transformed), but this relationship was not significant (among species untransformed mean ± SE: specialists, 15.24 ± 3.67% vs. generalists, 28.12 ± 6.37%, $N = 173$ population/year combinations, $F_{1,8.2} = 2.00$, $P = 0.19$, Fig. 2).
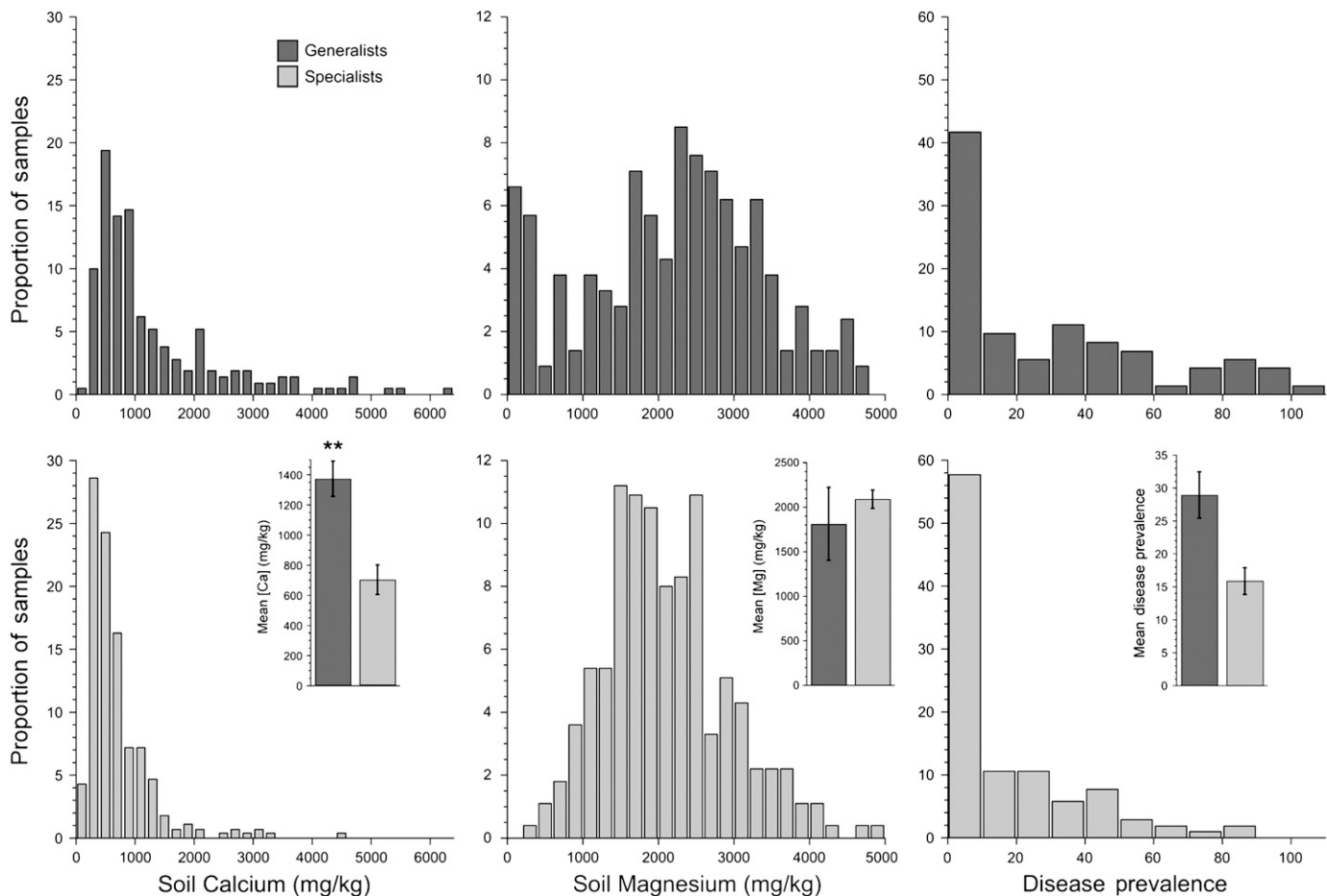

Fig. 2.   Edaphic characteristics and disease prevalence measured in *Hesperolinon* study populations in serpentine soils in California. Samples associated with species classified as edaphic generalists and specialists are distinguished by dark and light gray bars, respectively. Because sample sizes were unequal between the two categories, the proportion of samples in each category, rather than the absolute frequency, are plotted in histograms. For soil calcium and magnesium measurements, data represent individual soil samples ($N = 211$ for generalists, $N = 287$ for specialists). For disease prevalence, data represent all population/year combinations from populations where disease symptoms were observed at least once ($N = 72$ for generalists, $N = 104$ for specialists). Bar graphs show comparisons of the two edaphic categories overall, with grand means calculated by averaging species-level means. Error bars in bar graphs denote one standard error.

The difference in mean CV of prevalence between serpentine generalists and specialists was also not significant (specialists, 0.95 ± 0.082 vs. generalists, 0.82 ± 0.14, $N = 56$ populations with mean prevalence >0, $F_{1,7.8} = 0.35$, $P = 0.57$). Of the four serpentine generalist species, only three were considered in these analyses because rust disease of *H. breweri* was never observed in the field.

Phylogenetic reconstruction—The total length of the master sequence created from the four concatenated DNA loci was 2949 nucleotides. From the original raw sequences, seven regions containing mononucleotide repeats of variable length were truncated, and six indels were coded as single nucleotides.

Among the 77 *Hesperolinon* samples, 2683 sites (91.0%) were invariant and 111 (3.8%) were variable. Of the variable sites, 82 (73.9%) were parsimony informative. There were a total of 46 unique haplotypes identified. Raw nucleotide sequences for each of the individual samples (each locus deposited separately) are available in GenBank (Appendix S4).

The topologies of the trees produced using MrBayes and PAUP* were identical (Fig. 3). The associated data matrix and tree file are available from the database TreeBase (http://treebase.org, accession S2418). The three MCMC analyses produced consensus trees with the same topology, and posterior probabilities of branches were generally identical among trees and never varied by more than 1%. *Hesperolinon drymarioides*
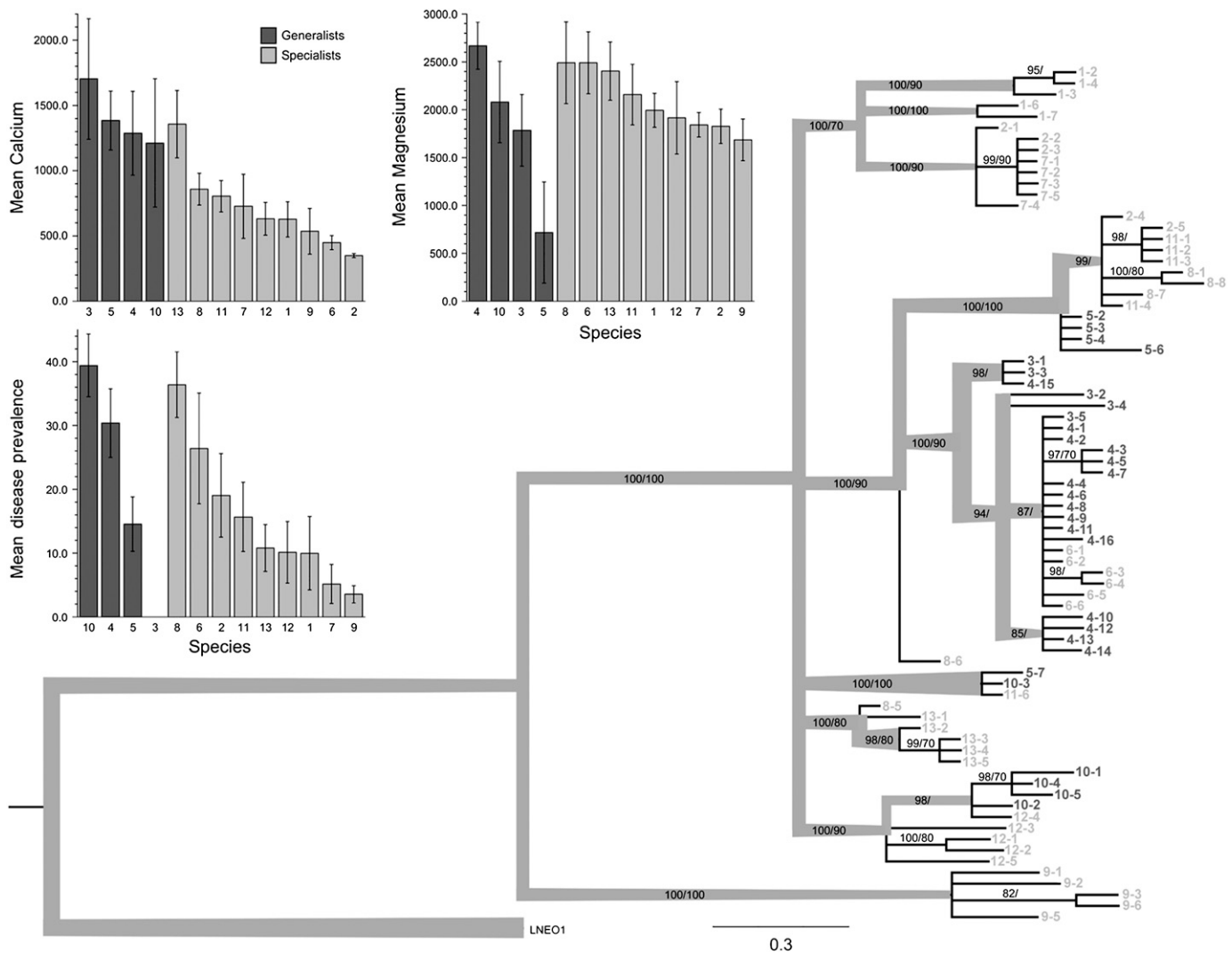

Fig. 3. Phylogeny of the genus *Hesperolinon* based on gene sequences of four chloroplast loci. The topologies of trees generated using Bayesian MCMC and maximum likelihood analyses were identical. Numbers above branches represent Bayesian posterior probabilities (before slash) and bootstrap values generated by 10 replicates of a full heuristic search (after slash). Branches with posterior probabilities <85 have been collapsed, and posterior probabilities <85 and bootstrap values <70 are not shown. Branch tip identifiers include the species code (from Table 1, before hyphen) and population ID number (after hyphen). Widths of gray horizontal branches are proportional to the changes in soil calcium concentrations between nodes as predicted by parsimony-based character history reconstruction in Mesquite 2.5. Branch widths are scaled to the mean value of the most ancestral node of the tree. Inset histograms depict variation in average soil calcium concentrations, magnesium concentrations, and disease prevalence values among the 13 *Hesperolinon* species. Means for edaphic parameters calculated by averaging population-level means for each cation. Mean disease prevalence was calculated using all population/year combinations from populations where disease symptoms were observed at least once. Species categorized by Sharsmith as serpentine generalists and specialists are designated by dark and light gray bars (or text of branch tip identifiers), respectively. Error bars denote one standard error.

was the most deeply divergent species within the genus (sister to all other dwarf flaxes), and the remaining species were distributed among five major clades. Incomplete lineage sorting or chloroplast capture was evidenced by the fact that for five of the 13 *Hesperolinon* species (*H. bicarpellatum*, *H. clevelandii*, *H. disjunctum*, *H. micranthum*, and *H. sharsmithiae*), samples from different study populations were assigned to different clades. Further, in instances where all study populations from more than one species were assigned to a single clade, samples of different species within the same clade often could not be differentiated (e.g., *H. breweri*, *H. californicum*, and *H. congestum*).

Phylogenetic independent contrasts and trait mapping—Host populations occurring on soils with low calcium concentrations (log_{10} transformed) were associated with significantly lower levels of disease prevalence (square-root transformed) (contrasts in sqrt mean prevalence = 3.05 × positivized contrasts in log_{10} soil [Ca], $r^2 = 0.10$, $F_{1,54} = 6.23$, $P = 0.016$, Fig. 4). Of the 55 contrasts involved in this analysis, a significantly higher number were positive ($N = 34$) than negative ($N = 21$, $P = 0.052$). Phylogenetically uncorrected analyses of the data using linear regression produced a qualitatively identical result (sqrt mean prevalence = −3.82 + 2.75 × log_{10} soil [Ca], $r^2_{adj} = 0.07$, $F_{1,55} = 5.44$, $P = 0.023$). There was no significant relationship when the PIC analysis was repeated using soil magnesium concentrations ($r^2 = 0.007$, $F_{1,54} = 0.38$, $P = 0.54$). The relationship between soil calcium and disease prevalence was nonsignificant when all 77 populations (including those where disease was never observed) were included in the PIC analysis ($r^2 = 1.74E-4$, $F_{1,75} = 0.013$, $P = 0.45$).

On the basis of findings of the soil NMDS, *t* tests on edaphic parameters, and results of the PIC analyses, calcium was the only cation considered in analyses of phylogenetic patterns of serpentine specialization. Mapping species-level means of soil calcium concentrations onto the phylogeny was not possible because of the fact that lineage sorting within the genus *Hesperolinon* appears to be incomplete or that chloroplast capture has occurred on a limited scale. In general, however, each of the four species classified as generalists had sister species that were categorized as specialists (Fig. 3): *H. congestum* (specialist, species no. 6, ranked 12th of 13 in descending order of mean soil calcium concentrations) and *H. californicum*/*H. breweri* (generalists, nos. 3 and 4, ranked first and third, respectively), *H. micranthum* (generalist, no. 10, ranked fourth) and *H. spergulinum* (specialist, no. 12, ranked ninth), and *H. clevelandii* (no. 5, ranked second) and *H. disjunctum*/*H. sharsmithiae* (nos. 8 and 11, ranked sixth and seventh) (Fig. 3). Phylogenetic mapping of ancestral states reconstructed using Fitch (1971) squared-change parsimony analysis suggests that the ability to tolerate extreme serpentine soil may have evolved multiple times within the genus, almost exclusively from edaphically general progenitors (Fig. 3, Appendix S5). Examples include *H. drymarioides* (no. 9), *H. spergulinum* (no. 12), *H. congestum* (no. 6), and the clade containing *H. adenophyllum* (no. 1), *H. bicarpellatum* (no. 2) and *H. didymocarpum* (no. 7). These patterns appear consistent with predictions of a threshold model for the evolution of specialization on extreme serpentine soils.

DISCUSSION

Results of the phylogenetically independent contrast analyses of edaphic and disease data support the pathogen refuge hypothesis and suggest that organisms that inhabit abiotically stressful environments may experience reductions in the frequency and severity of disease. For serpentine plants, this effect appears to be related to the concentration of calcium in underlying soils. At the scale of host population, plants growing in more extreme, low-calcium serpentine soils were associated with significantly lower levels of rust prevalence when the pathogen was present. This finding was supported somewhat by qualitatively similar, albeit nonsignificant, results produced by the mixed-model comparison at the level of host species: Sharsmith's (1961) serpentine specialists occurred on soils with significantly lower calcium concentrations compared to congeners classified as edaphic generalists, and specialists tended to be associated with lower levels of rust prevalence. Together, these results suggest that for plants able to grow on extreme, low-calcium serpentine soils, autecological costs of occupying this abiotically stressful niche may be countered to some degree by reduced levels of disease.

While consistent with predictions of the pathogen refuge hypothesis, mechanistic explanations of the observed relationship between soil calcium and disease are complicated by prior findings in two ways. First, the result appears to run counter to the general conclusions of a large body of research on calcium-mediated plant disease development in agricultural systems. Collectively, this work provides evidence of numerous important roles played by calcium in the biochemical and cellular pathways used by plants to recognize and respond to attacking pathogens (Lamb et al., 1989; Levine et al., 1996; Blumwald
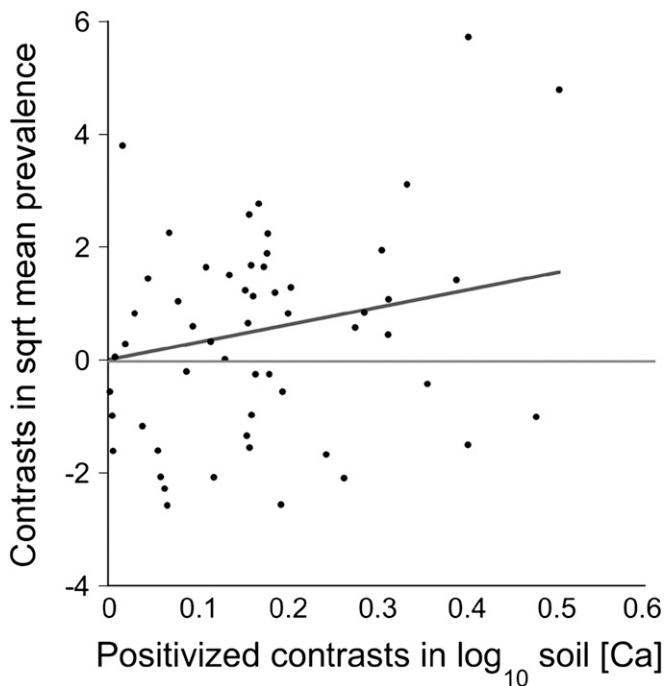
Fig. 4.   Results of independent contrast analysis of mean soil calcium concentration (log_{10} transformed) and mean disease prevalence (square-root transformed [sqrt]) for the 56 *Hesperolinon* study populations in serpentine soils in California where symptoms of rust disease were observed at least once (contrasts in sqrt mean prevalence = 3.08 × positivized contrasts in log_{10} soil [Ca], $r^2 = 0.10$, $F_{1,54} = 7.67$, $P = 0.01$).

et al., 1998; Scheel, 1998; Grant and Mansfield, 1999; Romeis et al., 2001) and of the suppressive effect that calcium addition to soils has on a range of phytopathogens (reviewed in Engelhard, 1989). In his study of agricultural plants grown in serpentine soils, Vlamis (1949) found that disease symptoms were negatively related to soil calcium and that symptom severity was reduced by the addition of calcium to soils. Together, these findings suggest that plants growing in high calcium soils should be more resistant to attack by pathogens. In contrast, a smaller body of research suggests that the observed result in the current study could be caused by a reduction in pathogen virulence under low calcium conditions. Calcium is known to play a critical role in the activation of virulence factors in a number of fungal and bacterial pathogens of mammals (Sibley et al., 1986; Yang et al., 1992; Sebghati et al., 2000; Brandhorst et al., 2005; Marquart et al., 2005; Sarkisova et al., 2005), and studies of fungal plant pathogens have demonstrated that calcium binding is necessary for spore germination and appressorium formation (Magalhaes et al., 1991; Warwar and Dickman, 1996; Lee and Lee, 1998; Shaw and Hoch, 2000; Uhm et al., 2003) and promotes mycelial growth (Simpfendorfer and Harden, 2000). Given that a positive correlation between soil and tissue calcium concentrations has been shown for *H. californicum* (Springer et al., 2007), lower rates of disease among plants growing on more extreme serpentine soils could reflect a reduction in the ability of *M. lini* to infect plants with low concentrations of calcium in leaf and stem tissue. The effects of calcium on disease could also be an indirect result of soil effects on plant morphology or demography. In general, plant species associated with extreme, low calcium serpentine soils tend to be smaller (e.g., height, size, and number of leaves), and present at lower levels of abundance and density than those on less stressful soils (Kruckeberg, 1984; Brooks, 1987). These characteristics would favor lower infection and disease levels by reducing the probability of primary infection and lowering rates of intrapopulation transmission of pathogen propagules following establishment (Burdon et al., 1989). Alternatively, given that overall soil fertility should decline with increasing serpentine influence, plants growing in more extreme, less productive serpentine soils might invest more in defenses against natural enemies (e.g., herbivores, pathogens) because the cost of replacing tissue lost to grazing or disease is relative high (Janzen, 1974; Coley et al., 1985; Bryant et al., 1989; but see Miller and Woodrow, 2008). Via this mechanism, the lower rates of disease observed among plants in stressful environments would be due to intrinsic defense mechanisms rather than environmentally associated protection. Common garden inoculation experiments involving orthogonal manipulations of plant source (populations associated with soils spanning a range of serpentine influence) and soil fertility treatments could be used to distinguish these mechanistic alternatives.

Further complicating interpretation of the soil/disease result is the fact that the only other tests of the pathogen refuge hypothesis involving serpentine plants, both of which focused on interactions between *H. californicum* and *M. lini*, found evidence of the opposite relationship between calcium and disease. In a greenhouse inoculation experiment involving manipulation of calcium concentrations of serpentine soils (Springer et al., 2007), and a 4-year epidemiological field study of disease dynamics in natural populations (Springer, 2009), *H. californicum* growing in lower calcium soils had higher levels of disease caused by *M. lini*. This apparent contradiction could be explained in one of two ways. First, the nature of the calci-

um–disease interaction might vary among host species. The negative relationship shown with *H. californicum* could be obscured if the positive interaction evidenced in the current study was associated with most or all of its congeners. While plausible, this possibility seems unlikely given the close taxonomic relatedness of the host species, the fact that the same pathogen was considered in all three studies, and the disproportionate evidence from the phytopathology literature supporting a disease-reducing effect of calcium. Alternatively, variation in the ability of *Hesperolinon* species to absorb calcium from soils could reconcile the seemingly disparate results. Greater absorption efficiency among species found on more extreme serpentine soils could result in higher absolute tissue calcium concentrations compared to species such as *H. californicum* that occur on soils representing a wider range of serpentine influence. This asymmetry could account for the low rust prevalence among species that predominate in low calcium soils. Because of their lower absorption capacity, species growing in higher calcium soils would still have lower absolute tissue calcium concentrations than those on low calcium soils, increasing prevalence at the upper end of the soil calcium continuum and producing a positive calcium–disease relationship. Such variation in calcium absorption was demonstrated by Rajakaruna and colleagues in studies of two races of the serpentine-adapted *Lasthenia californica*. Although race A grows on soils with lower calcium concentrations it has approximately twice the rate of calcium uptake, and 127 times higher shoot calcium concentrations, than race C (Rajakaruna and Bohm, 1999; Rajakaruna et al., 2003). Manipulative experimental studies (e.g., reciprocal transplants, common garden experiments) would be an ideal way to reconcile these contrasting findings and hypotheses and more definitively assess the relative contribution of the various posited mechanisms, if any, to patterns of disease. That the results of multiple studies consistently show an effect of soil calcium on rust disease in *Hesperolinon* species provides strong motivation for such research.

Findings of the soil analyses reinforce conclusions of previous studies that have identified calcium and magnesium as the principle edaphic factors underlying the "serpentine syndrome" (reviewed in Brady et al., 2005). Of the 17 soil cations examined here, these two elements each accounted for approximately 40% of the edaphic variation among *Hesperolinon* study populations. Only calcium, however, had a significant influence on interactions with pathogens. Results from transplant and soil manipulations have also implicated low calcium availability as a primary factor underlying floristic partitioning in areas where serpentine soils occur (Vlamis, 1949; Kruckeberg, 1954; Whittaker, 1954; Walker et al., 1955; Kruckeberg, 1967). The ability of plants to grow and reproduce in soils with extremely low calcium concentrations thus appears to be central to the evolution of serpentine tolerance (Vlamis and Jenny, 1948).

This study presents the first attempt to quantitatively describe the evolution of serpentine specialization, using soil calcium concentrations as a metric, within a group of edaphically variable, serpentine-tolerant plants. Ancentral state reconstructions of soil calcium concentrations produced a pattern that seems consistent with the evolution of serpentine specialization via a threshold process. The presence of at least one edaphic specialist within five of the seven major clades in the *Hesperolinon* phylogeny and the multiple pairings of closely related specialist and generalist sister species withing clades suggest that the ability to inhabit extreme serpentine conditions has evolved rapidly and repeatedly within the genus. This pattern would be

consistent with similar evolutionary dynamics associated with the appearance of serpentine tolerant taxa in lineages of plants composed primarily of nonserpentine species (Setoguchi et al., 1998; Pepper and Norwood, 2001; Patterson and Givnish, 2004; Kawase et al., 2007). Alternatively, and perhaps more parsimoniously, the fact that the most deeply divergent taxon within the tree (*H. drymarioides*) occurs on extreme serpentine may indicate that this tolerance threshold was passed early on in the evolutionary history of the genus, that serpentine specialization is the rule rather than the exception, and that a reversion to an edaphically generalist lifestyle has occurred three times: once in the clade containing *H. micranthum*, once in the clade containing *H. clevelandii*, and once in the clad containing *H. breweri* and *H. californicum*. In either case, the observed pattern may represent evidence that changes in a small number of physiological traits might be all that is required for colonization of extreme serpentine soils and that the autecological costs of serpentine specialization are associated primarily with the initial development of tolerance. The apparent absence of common evolutionary trajectories among clades and the close relationships between taxa with widely divergent edaphic associations suggest that either the costs of serpentine specialization are not additive or that if they are, their magnitude or the degree to which they are offset by one or more fitness benefits varies among the species. These results are clearly preliminary because they are based on what are essentially observational measures of soil associations in the wild. For these conjectures to be conclusively evaluated, soil transplant experiments are needed to quantify the absolute calcium ranges over which different *Hesperolinon* species are physiologically able to grow. Nevertheless, the findings do suggest some intriguing hypotheses about the evolutionary patterns and mechanisms involved in the colonization of extreme soil environments and indicate that the genus *Hesperolinon* may be an attractive model system in which to study them.

The exclusion of uninfected populations had a significant effect on the outcome of disease-related analyses. This was especially true for mixed-model tests of Sharsmith's categorization scheme because the removal of all *H. breweri* populations from the data set left only three species of edaphic generalists. The decision to exclude these data was based on results of greenhouse inoculation trials (Y. Springer, unpublished data) that suggest that uninfected populations were disease-free due to the absence of the pathogen rather than some form of host resistance. All host species are vulnerable to infection, and when challenged with rust spores, some degree of disease invariably results. This is especially true of *H. breweri*, which has particularly low levels of genetic resistance to the fungus. Nevertheless, in repeated surveys of uninfected field populations, many of which are numerically and geographically small and can thus be sampled thoroughly, no signs of the pathogen were discovered despite the frequent observation of disease in nearby host populations. Although possible, it seems unlikely that the pathogen is present and that one or more properties of the soil or environment is rendering these hosts completely resistant to infection. A more plausible explanation for the disease status of uninfected populations is a failure of the pathogen to recruit to these locations. Given that the goal of this study was to examine the effect of soil chemistry on disease when the pathogen is present, the exclusion of these populations from the analyses was deemed justifiable. Comparison of infected and uninfected populations indicated that overall, the exclusion of the latter did not introduce significant edaphic biased into the analyses.

The interpretation of results was also limited by phylogenetic attributes of the host genus. Because only four *Hesperolinon* species were classified as serpentine generalists and rust disease was only documented on three of them, the power of the species-level analyses of edaphic and disease levels was reduced. A seemingly more fundamental weakness of these species-level comparisons was the fact that conspecific populations of several species are interspersed throughout different parts of the phylogenetic tree rather than grouped together, raising questions about possible errors in correct identification of study populations or the status of "species" being assigned to these taxa. The fact that each species possess one or more distinctive morphological traits that have been shown to be heritable in the greenhouse mean that the taxa can be readily distinguished and likely represent fairly distinct evolutionary units rather than environmentally associated phenotypes. The distribution of species within the tree is by and large consistent with patterns of relatedness that would be postulated based on the presumptive age of the genus and the morphological and biogeographic attributes of the constitutive taxa. While these complications were to a great extent circumvented by the population-level analysis, the large number of polytomies in the phylogeny reduced the number of independent contrasts that could be made. As discussed earlier, this feature of the topology is likely an accurate reflection of recent divergence within the genus rather than the result of labeling error, low resolution of the sequencing, or the specific genetic markers used to build the tree. In their monograph of California floristic diversity, Raven and Axelrod (1978) speculated that most serpentine-associated herbs such as those in the genus *Hesperolinon* had originated recently, many from progenitors in the American Southwest, following the exposure of serpentine formations and the onset of warmer, drier mediterranean climate conditions in California. Estimates of the timing of this paleoclimatic transition (reviewed in Harrison et al., 2004) and diversification rates in another lineage of serpentine associated annuals in the state's Coast Range Mountains (Mayer and Soltis, 1994; Baldwin, 2005) suggest that the radiation within *Hesperolinon* probably began sometime within the last 5 million years (Myr) and may have taken less than 1 or 2 Myr. Additional evidence for this recent divergence, indicative of neoendemic origins of *Hesperolinon* species, includes a lack of strong concordance between the tree topology and the biogeographic distribution of constituent species, incomplete lineage sorting or chloroplast capture between some taxa, and observations of morphologically intermediate hybrids for some species pairs (Y. Springer, personal observation). The number and variable edaphic ecologies of the different *Hesperolinon* species may thus reflect recent and rapid divergence within the lineage. Work in other systems suggests that there may even be a causal connection between the two, as edaphic specialization can be associated with changes in plant reproductive morphology and/or phenology that promote reproductive isolation of populations (reviewed by Rajakaruna, 2004). Investigations of such effects using the genus *Hesperolinon* could be particularly fruitful.

Some readers will no doubt take issue with the classification of certain edaphic conditions as "stressful" given that no empirical measures explicitly indicative of stress experienced by the host species were made. It seems reasonable to assert that plants endemic to serpentine soils are presumably adapted to them and therefore do not experience considerable stress as a result of growing on them. While studies that explicitly quantify one or more physiological, morphological, or developmental

indices of stress in *Hesperolinon* spp. growing in soils with varying serpentine influence would be necessary to demonstrate the stressful nature of these environments unambiguously, a qualitative observation is worth nothing. Serpentine-adapted plants have by definition evolved to tolerate soil conditions that are highly stressful to most nonserpentine species. Yet among soil environments that vary in their degree of serpentine influence the observation of extreme serpentine "barrens" from which even serpentine-adapted species are largely or completely excluded indicates that in many instances the abiotic conditions associated with these soils must approach or exceed physiological thresholds beyond which essentially all plants, irrespective of their evolutionary history, can no longer grow. This observation suggests that a continuum of conditions along one or more edaphic axes does represent a stress gradient for serpentine-associated plants. The literature on serpentine geochemistry and serpentine plant ecology points to calcium and magnesium as two of the most likely axes; their scarcity or excess, respectively, are known to have profound effects on plant growth and reproduction. The concentrations of these cations can vary substantially among serpentine outcrops and deviate from levels associated with nonserpentine soils to an especially great degree (very low calcium, very high magnesium) in serpentine "barrens." This evidence provides reasonably strong support for the assumption that variation in calcium and magnesium concentrations associated with serpentine soils represents a continuum of edaphic stress even for serpentine-adapted plants. The findings of this study suggest that this stress may have important consequences for species interactions in these systems and perhaps in other environments where conditions approach the physiological limits of associated organisms.

As evidenced by the results of this study, the ability of organisms to inhabit abiotically stressful environments may represent a selective balance of autecological costs and synecological benefits. The genus *Hesperolinon* appears to be a promising focal system for studies examining this interplay. With the species' short generation time, amenability to greenhouse germination and growth, and easily measured reproductive output, the physiological costs of growth on soils with varying serpentine influence should be readily quantifiable. Similarly, empirical evidence from the *H. californicum–M. lini* interaction indicates that disease can have strong fitness consequences for host plants (Springer, 2009), and three studies have now documented significant impacts of soil calcium on the frequency and severity of disease. Quantifying the contribution of these two forces to the evolution of tolerance and edaphic specialization among serpentine plants could make important contributions to our understanding of patterns of floristic biodiversity associated with this unique environment. More generally, results presented here suggest that the evolution of these traits could be quite rapid in these systems. Serpentine plant communities may thus provide valuable insights into the patterns and mechanisms associated with the evolution of ecological specialization in extreme environments.

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