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Journal

Proceedings of the National Academy of Sciences of the United States of America, 111(42)

ISSN

0027-8424

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Publication Date

2014-10-21

DOI

10.1073/pnas.1409242111

Peer reviewed

Occupation of bare habitats, an evolutionary precursor to soil specialization in plants

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Plant soil specialists contribute greatly to global diversity; however, the ecoevolutionary forces responsible for generating this diversity are poorly understood. We integrate molecular phylogenies with descriptive and experimental ecological data, creating a powerful framework with which to elucidate forces driving soil specialization. Hypotheses explaining edaphic specialization have historically focused on costs of adaptation to elements (e.g., nickel, calcium/magnesium) and accompanying tradeoffs in competitive ability in benign soils. We combine in situ microhabitat data for 37 streptanthoid species (Brassicaceae), soil analyses, and competition experiments with their phylogeny to reconstruct selective forces generating serpentine soil endemism, which has four to five independent origins in this group. Coupling ancestral state reconstruction with phylogenetic independent contrasts, we examine the magnitude and timing of changes in soil and habitat attributes relative to inferred shifts to serpentine. We find large changes in soil chemistry at nodes associated with soil shifts, suggesting that elemental changes occurred concomitantly with soil transitions. In contrast, the amount of bare ground surrounding plants in the field ("bareness"), which is greater in serpentine environments, is conserved across soil-type shifts. Thus, occupation of bare environments preceded shifts to serpentine, and may serve as an evolutionary precursor to harsh elemental soils and environments. In greenhouse experiments, taxa from barer environments are poorer competitors, a tradeoff that may contribute to soil endemism. The hypothesis of occupation of bare habitats as a precursor of soil specialization can be tested in other systems with a similar integrative ecophylogenetic approach, thereby providing deeper insights into this rich source of biodiversity.

ecological specialization | edaphic specialist | exaptation | ecological trade-off | *Streptanthus*

Ecological specialization is an important driver of biological diversity often associated with diversification through adaptive radiation, or with extinction due to evolutionary "dead ends." For plants, ecological specialization on geologically distinct parent soils (e.g., gypsum, gabbro, serpentine) contributes disproportionately to regional plant diversity, especially in the biodiversity hotspots of the South African Cape, California, and Cuba (1–3). In California, serpentine soils represent less than 1% of the area, but serpentine endemics comprise about 10% of the flora (1). Overall, two main approaches have been followed in the study of edaphic specialization. From a historical perspective, specialization has been placed in a phylogenetic context with the goal of inferring diversification rates associated with soil endemism either within or across groups (4–6). On the other hand, studies with a contemporary ecological perspective have, with experimental manipulations in either field or controlled conditions, tested hypotheses that address the drivers of edaphic specialization by focusing on plant performance in different microhabitats (7); along environmental gradients (8); when plants are grown on different substrates or with different elemental supplements (9–11); or in the presence of neighbors (12, 13), pathogens (14), or herbivores (15, 16).

The integration of molecular phylogenies with extensive clade-wide ecological data collections and experiments is greatly expanding our ability to test hypotheses and mechanisms generating diversity (17). Futuyma and colleagues (18, 19) and Armbruster and colleagues (20, 21) pioneered these approaches using experiments set in a phylogenetic context to identify exaptations and the adaptive significance of traits involved in radiations and ecological specialization. Common garden (*sensu lato*) experiments in which members of a clade are grown together to understand the ecological adaptive functions of traits have also been used (e.g., refs. 22–25). Experiments with an explicit phylogenetic framework can address aspects of trait evolution, niche conservatism, adaptation, historical contingency, exaptations, phylogenetic lag, and genetic constraints (20, 26–30). Here, we expand on these approaches by also incorporating extensive ecological data collections taken across field sites occupied by members of a whole clade, and integrating them with common garden greenhouse experiments and phylogenetic hypotheses. Using field data from 37 plant species of *Streptanthus* and close relatives (Thelypodieae, Brassicaceae) and greenhouse experiments involving 14 populations (seven species), we test hypotheses and reconstruct selective pressures involved with soil specialization in the context of phylogenetic history (31).

Understanding why some species become restricted to particular soils has challenged biologists for almost a century because most soil endemics are able to grow in more benign substrates (9, 32–36). Costs associated with adaptations to harsh environments are hypothesized to result in reduced competitive ability in zonal (regionally common) soils (9, 37), and have been the main paradigm to explain narrow soil endemism (9, 35, 36). Lately, tradeoffs in competitive ability associated with defense against herbivores and pathogens have also been implicated in the restricted distributions

Significance

Integrating molecular phylogenies with clade-wide ecological data is expanding our ability to address classic ecological questions, such as the origins and maintenance of plant soil specialists, a global source of plant biodiversity. We reconstruct selective forces related to serpentine soil specialization using clade-wide microhabitat characterizations, soil physicochemical data, and phylogenetic hypotheses. Surprisingly, species' occupation of bare environments, not of chemically similar soils, preceded shifts to serpentine soils. Additionally, inhabiting bare environments traded off with competitive ability in multispecies greenhouse experiments, a relationship potentially contributing to soil endemism. We find that combining in situ detailed field ecological data, greenhouse experiments, and phylogenetic hypotheses can reveal underappreciated selective pressures and provide powerful tools to deepen our understanding of evolutionary pathways underlying biodiversity.

of soil specialists (4, 15, 16, 38, 39). For example, tissue replacement may cost more in stressful or poor nutrient environments, selecting for a higher investment in defense that, in turn, compromises growth rates (15, 40).

The bareness of habitats in which plants live may be an underappreciated selective force on soil specialists, and adaptations to bareness may also generate tradeoffs with competitive ability. Our recent work has shown that plants surrounded by bare ground experience greater rates of attack from herbivores owing to greater plant apparency (16). Bareness of habitat may not only make plants more apparent to enemies but may also expose them to greater UV radiation; increased drought stress (41) (Fig. 1); and, by definition, reduced densities of plant neighbors, which can be facilitators in harsh environments (42). Bareness may also be associated with soil texture (41) and rates of disturbance and erosion (43, 44), especially on rocky slopes (41, 43, 45). In studies of Mediterranean plant communities, soil endemics tended to occupy rocky substrates and steep slopes (46). Although the relative contributions of these different aspects of bare habitats are hard to tease apart, we suggest that the amount of bare ground (bareness) surrounding a plant integrates over many of these aspects, and thus we selected it a priori as one metric to capture selective regimes in harsh soil environments.

Here, we use the *Streptanthus* clade (*SI Appendix*, Fig. S1), an example of the "interplay between evolutionary radiation and edaphic endemism" (47), ancestral state reconstruction, and phylogenetic independent contrasts (PICs), to identify possible pathways leading to soil specialization, in particular to serpentine use. Members of the *Streptanthus* clade generally grow on rocky substrates like basalt, gabbro, rhyolite, shale, and granite, as well as in sandy substrates derived from various parent materials. Many species in this clade are soil endemics, with up to one-third restricted to serpentine soils (9), representing four to five independent origins of serpentine endemism (31). The wide range of soil affinities and specialization displayed by species in *Streptanthus* (now, and hereafter, *sensu lato*; *SI Appendix*, Fig. S1) make it an ideal group with which to investigate forces leading to adaptation to harsh soils.

Past investigators have identified challenges to plants specialized on serpentine soils as primarily the high Mg or low Ca/Mg ratio, the high concentrations of heavy metals usually toxic to plants (e.g., Ni, Cr, Co), and the low levels of essential plant nutrients [e.g., N, P, K (11, 41)] that are characteristic of these harsh soils. The vast majority of studies comparing serpentine and nonserpentine populations have focused on differences between serpentine outcrops and soils of adjacent nonserpentine habitats. However, to understand the evolution of soil use of edaphic specialists, which rarely occur on adjacent zonal soils, we believe

the most pertinent comparisons lie between differences in serpentine and nonserpentine soils of clade mates.

To explore whether changes in chemical, textural, or microhabitat aspects of serpentine soils occur concomitantly or in a decoupled fashion from transitions in soil use, we used a combination of ancestral state reconstruction and PICs (48) based on our detailed soil and environment characterizations. Because serpentine and nonserpentine habitats and soils differ significantly in many elements and characteristics (e.g., ref. 9), we predict that there must be large changes in reconstructed soil attributes at some point in the evolutionary history of soil specialists. For example, because a low Ca/Mg ratio is identified as an important selective agent in serpentine soils (9), we might find that use of soils with low Ca concentrations precedes shifts to serpentine, and could facilitate serpentine use.

Surprisingly, we find that occupation of bare habitats, rather than any soil element characteristic of serpentine (e.g., Ni, Ca, Mg, K, P), preceded shifts to serpentine. With subsequent common garden experiments, we ask whether taxa from bare environments are, as the competitive exclusion paradigm would predict, poor competitors, a condition that might constrain their distributions to barer soils. A goal of this work was not only to deepen our understanding of pathways of edaphic specialization but also to expand a body of work that gains insights from combined descriptive and experimental ecological data collections within a phylogenetic framework (17).

Results

Characterization of Soils and Environments. Serpentine and non-serpentine soils used by streptanthoids are chemically and texturally different, as revealed by physicochemical analyses of 294 soil samples from 116 populations representing 45 species of *Streptanthus* and close allies (Fig. 2 and *SI Appendix*, Figs. S2 and S3 and Tables S1 and S2). Serpentine soils used by streptanthoids had lower levels of Ca (0.09×), K (0.20×), and P (0.28×), and higher levels of Ni (32×), Mg (5.63×), and Co (6.51×). Differences in other elements were not as pronounced. Texturally, serpentine soils had more coarse particles (>4 mm, 2×) and clay content (< 2 μm, 1.20×) but fewer overall fine particles (<1.7 mm, 0.67×).

Field surveys quantifying the percentage of bare ground surrounding plants across 37 species of *Streptanthus* (with replicate populations per species) revealed that these species typically occur in relatively bare microhabitats, and that those growing on serpentine habitats are surrounded by an even greater proportion of bare ground (1.2×; Fig. 2 and *SI Appendix*, Table S2). Many of the variables characterizing serpentine and nonserpentine soils are, not surprisingly, intercorrelated (*SI Appendix*, Table S3).

Microhabitat Bareness and Soil Texture Are More Strongly Conserved Than Soil Elements. With standard measures of phylogenetic signal, we explore if metrics of contemporary environments (bareness) and soils bear information allowing inferences about past growing regimes. Although there may be pitfalls in extrapolating from current conditions to past ones, the most parsimonious explanation of a strong phylogenetic signal in currently measured ecological characteristics is that current environments reflect past ones; such approaches have been applied in historical biogeography (49) and to test the adaptive significance of traits (17, 20).

Taking phylogenetic uncertainty into account, we find that serpentine affinity in the *Streptanthus* clade has strong phylogenetic signal (median Purvis's $D = -2.193$). The only habitat or soil attributes with K estimates higher than expected under the Brownian model of evolution (95% confidence interval of K estimates not overlapping with $K = 1$) were field microhabitat bareness and soil fine fraction ($K_{Bareness} = 1.292$, $P = 0.01$; $K_{Fine} = 1.443$, $P = 0.01$; Table 1 and *SI Appendix*, Fig. S4 and Table S4). Microhabitat bareness is more phylogenetically conserved (greater mean K) than most soil chemical elements (Table 1 and *SI Appendix*, Table S4), including elements with which it was correlated (*SI Appendix*, Tables S3 and S5), although distributions of K estimates overlap in some instances (*SI Appendix*, Table S4). Soil elements considered

Fig. 1. Microhabitat bareness, the amount of bare ground surrounding plants in the field, is highly variable and integrates many aspects of harsh environments, such as greater apparency to enemies, increased drought and exposure to UV light, and lower density of plant neighbors. (*Left*) *Streptanthus brachiatus* on serpentine soil. (*Right*) *Caulanthus anceps* on nonserpentine fine-texture soil.
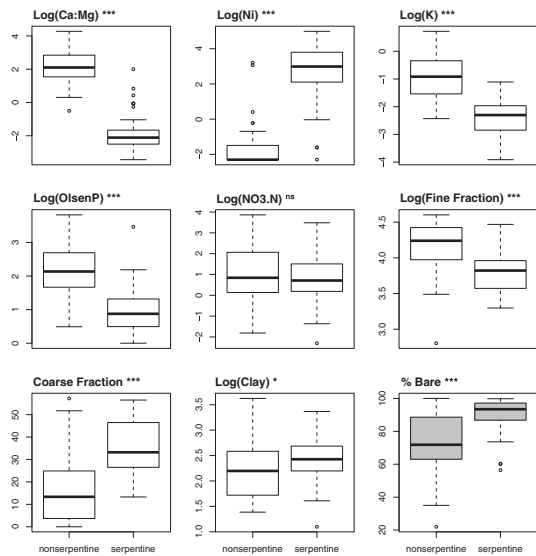
Fig. 2. Serpentine and nonserpentine soils and microhabitats used by streptanthoids are different in soil chemistry, soil texture, and bareness. ***$P \leq$ 0.001; *$P \leq$ 0.05 (*SI Appendix*, Figs. S2 and S3 and Table S2).

important indicators of serpentine that exhibit significant phylogenetic signal do not depart from expectations under the Brownian model of evolution ($K = 1$; e.g., $K_{Ca/Mg} = 1.151$, $P = 0.01$; $K_{Ni} = 0.955$, $P = 0.01$; $K_{Co} = 0.829$, $P = 0.01$; Table 1 and *SI Appendix*, Table S4). Interestingly, many chemical elements that differ between serpentine and nonserpentine soils show no or weak ($K < 1$) phylogenetic conservatism (e.g., $K_{Clay} = 0.771$, $P = 0.12$; $K_{Mn} = 0.563$, $P = 0.22$). In contrast, macrotextural soil fractions exhibit a very strong phylogenetic signal (Table 1 and *SI Appendix*, Table S4).

Transitions to Serpentine Are Accompanied by Concomitant Large Changes in Chemical Soil Composition; Changes in Habitat Bareness Preceded Serpentine Shifts. For each of 5,000 randomly selected postburnin trees from a study by Cacho et al. (31), we reconstructed the history of serpentine use and identified nodes associated with soil transitions. We then compared PIC absolute values of soil chemistry and microhabitat attributes at nodes ancestral to soil shifts with values at the rest of the nodes that are not associated with soil transitions (*Methods*; Fig. 3). For a given attribute, similar PIC values between these two kinds of nodes would indicate that its change is not associated with soil shifts. Because we know how the two soil types differ with respect to each of the attributes studied, it is possible to make inferences about the direction of the changes observed. We find that contrasts associated with chemical elements considered diagnostic of serpentine soils (e.g., Ca/Mg, Ni, Co) show large changes between nodes with and without inferred shifts in soil use (e.g., Ca/Mg mean PICs$_{NO-SHIFT}$ = 28.24; Ca/Mg mean PICs$_{SHIFT}$ = 72.09; t test median P value across 5,000 trees = 0.05; Fig. 4 and Table 1).

In contrast to chemical elemental characteristics, changes in bareness (%Bare) at nodes associated with shifts to serpentine soils are significantly smaller than changes at nodes not associated with inferred edaphic shifts (%Bare mean PICs$_{NO-SHIFT}$ = 326.20; %Bare mean PICs$_{SHIFT}$ = 169.4; median P = 0.04; Fig. 4 and Table 1). In other words, nonserpentine habitats ancestral to serpentine shifts were already very bare. These results imply that occupation of bare environments may be a precursor enabling transitions to barer serpentine soils. A similar but weaker pattern is observed in organic matter (OM mean PICs$_{NO-SHIFT}$ = 15.18; OM mean PICs$_{SHIFT}$ = 10.06; median P = 0.15). For the rest of the soil chemical and textural characteristics, there were no significant differences among contrasts at nodes preceding shifts in soil use and the rest of the nodes in 5,000 postburnin trees we

analyzed (Table 1). Even excluding from the analysis the outgroups and the *Streptanthus* clade II, in which there are no serpentine-using species, we still recover these patterns, although they are weaker (PIC $P_{Ca/Mg} = 0.06$; $P_{\%Bare} = 0.11$; *SI Appendix*, Fig. S5 and Table S6).

Could Occupation of Bare Habitats Trade Off with Competitive Ability and Contribute to Soil Specialization? In lath-house assays with raw field soils, we related the average competitive ability of *Streptanthus* species to their average microhabitat bareness surrounding plants in replicate field sites (*Methods*). We found that competitive ability of streptanthoid species is negatively related to species mean field microhabitat bareness ($n = 10$; estimate = −1.33, $P = 0.039$; Fig. 5) and marginally significant when phylogeny is taken into account [phylogenetic generalized least squares (PGLS) estimate = −1.093, $P = 0.087$]. The relationship is stronger when comparing only populations with estimates of both bareness and competitive ability rather than species averages ($n = 14$; estimate = −1.324, $P = 0.004$; PGLS estimate = −0.931, $P = 0.016$; *SI Appendix*, Table S7). The interaction bareness * serpentine was not significant (species-level PGLS $P = 0.25$, population-level PGLS $P = 0.11$).

Discussion

Plant soil specialists contribute greatly to global plant diversity, especially in arid and Mediterranean regions (2, 44, 50, 51). By integrating extensive contemporary microhabitat data collections and common garden experiments with phylogenetic history, we

Table 1. Phylogenetic signal and means of absolute values of PICs (absPICs) at nodes associated and not associated with inferred soil shifts, over 5,000 postburnin randomly sampled trees

Variable	Phylogenetic signal Median K	Median P	abs(PICs) NO-SHIFT node	SHIFT node	Median P
%Bare	**1.292**	**0.009**	326.20	169.40	**0.044**
Log (Ca/Mg)	**1.151**	**0.009**	28.24	72.09	**0.050**
Log (Co)	**0.829**	**0.009**	16.25	44.91	**0.074**
Log (Ni)	**0.955**	**0.009**	40.81	93.48	**0.085**
Log (OM)	0.578	0.287	15.18	10.06	0.150
Log (Na)	**0.694**	**0.050**	17.93	11.70	0.160
Log (Mg)	**0.910**	**0.009**	21.78	42.06	0.193
Log (P)	**0.881**	**0.009**	16.20	27.85	0.209
Log (K)	**0.949**	**0.009**	15.96	24.15	0.297
Log (Ca)	**0.960**	**0.009**	22.65	32.46	0.310
Log (B)	**0.650**	**0.050**	18.26	12.39	0.316
Log (CEC)	**0.677**	**0.030**	17.72	13.81	0.320
Log (NO₃N)	0.632	0.149	25.80	36.18	0.370
Log (Mn)	0.563	0.218	18.17	21.66	0.403
Log (Cu)	0.487	0.564	19.04	15.03	0.414
Log (SP)	0.571	0.119	4.85	4.25	0.440
Log (C)	0.583	0.158	14.29	12.17	0.481
Log (Fe)	**0.748**	**0.009**	18.49	17.57	0.583
Log (N)	0.512	0.485	13.18	12.08	0.598
Log (Cr)	0.570	0.327	3.12	2.21	0.606
Log (pH)	**0.630**	**0.030**	2.49	2.44	0.666
Log (Zn)	0.582	0.139	17.93	18.42	0.671
Log (clay)	0.771	0.119	12.06	16.12	0.370
Log (silt)	0.581	0.188	11.72	8.52	0.385
Coarse fraction	**1.117**	**0.009**	286.50	271.10	0.463
Fine fraction	**1.443**	**0.009**	5.39	5.19	0.562
Sand	0.572	0.347	291.80	281.10	0.618

Variables are ordered by significance of difference in PICs within category (bareness, chemistry, and texture). The 95% confidence intervals for K are provided in *SI Appendix*, Table S4. CEC, cation exchange capacity; OM, organic matter; SP, saturated paste. Boldface denotes $P < 0.10$.
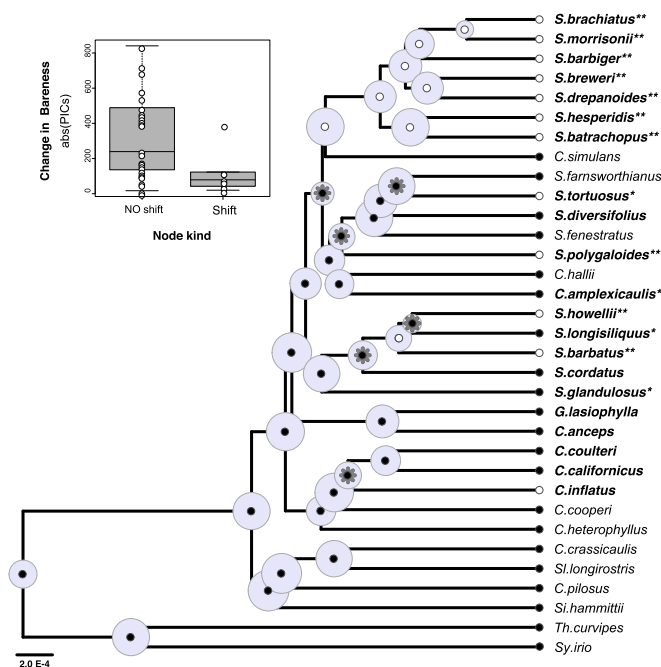
Fig. 3. Single realization [1 of 5,000 randomly sampled postburnin trees (31)] of serpentine and bareness evolution in the *Streptanthus* clade. Small circles represent serpentine states, assigned with a probability equal to the proportion of records on serpentine (PRS) for a given species (white indicates serpentine). Stars represent selected nodes, because they precede soil shifts. Gray circles show size proportional to the absolute value of PICs [abs(PICs)]. Species with PRS > 0 are shown in boldface, serpentine endemics (PRS > 87%) are indicated by two asterisks, and bodenvags (defined as PRS > 11%) are indicated by one asterisk. (*Inset*) Bareness PICs (absolute value) for this particular realization.

suggest a new hypothesis: the importance of habitat bareness as a driver of soil specialization. We show that shifts onto serpentine soils likely took place not from chemically similar soils (e.g., with a low Ca/Mg ratio or high Ni content), as has been previously hypothesized (9), but from ancestrally bare microsites. Adaptations to bare habitats may, per se, enable adaptation to harsh soils like serpentine. Batten et al. (52) had similar ideas on forces maintaining rare plant species found in different soils (greenstone, limestone, shale, and others) along the Yukon River in Alaska: "...factors other than the chemical nature of the substrate are responsible for the persistence of these supposed relic species.... [T]hese slopes provide a habitat for species adapted to dry unstable conditions, but intolerant of competition" (also ref. 53).

We point to three lines of evidence suggesting that elemental composition may be an easier evolutionary hurdle than bareness in soil specialization by streptanthoids. First, members of the *Streptanthus* clade use a huge number of parent soils with highly variable elemental composition (*SI Appendix*, Table S2), including three southwestern limestone specialists that, by definition, tolerate high soil Ca (in contrast to low-Ca serpentine). Second, microhabitat bareness tends to be more conserved across the clade than elemental features of soils previously identified as important selective agents in serpentine soils [e.g., Mg, Ni, Ca/Mg ratio (35, 41, 43, 54)]. Third, our ongoing experiments in this clade and work of many others demonstrate that harsh soil-adapted species can grow on zonal soils (32, 33, 35, 43, 53), suggesting more lability in the fundamental niche of soil use than in the realized niche. The main paradigm to reconcile edaphic endemism with the ability of many soil endemics to grow on alternative soils relies on hypothesized tradeoffs between the ability to tolerate peculiar substrate chemistry and the ability to withstand competition in zonal soils [competitive ability tradeoff hypothesis (36, 39–41, 44, 52)]. We show, in addition, that ability to live in bare habitats might contribute to this tradeoff.

Multiple, nonmutually exclusive sources of selection arising from bare environments might result in tradeoffs in competitive ability. Abiotically, life in bare microhabitats might require adaptations to increased disturbance (45), rockiness, drought (55), and exposure to high levels of UV light (36). Adaptations like the production of heat-shock proteins and UV-absorbing phenols and flavonoids may have costs that reduce competitive ability (56, 57). Likewise, adaptations to disturbance, which are associated with a lack of vegetation cover in serpentine and other bare soils (45, 52) and which may perpetuate a lack of vegetation (58), may also have costs. Bare environments were rockier, and macrotextural aspects of soils (coarse and fine fractions, which are not included in "off-the-shelf" analyses) were highly conserved across this clade.

Bare habitats also differ in their biotic selective regimes; by definition, they have a lower biomass of plant neighbors. Other studies have provided evidence for positive, facilitative effects of plant neighbors in a variety of harsh abiotic environments (42, 59), suggesting that a lack of neighbors (bareness) could impose abiotic challenges for plants. However, using both experimental and descriptive approaches, we found the net effect of neighbors measured over the lifetime of two serpentine *Streptanthus* species in the field was negative, not facilitative (16). Low levels of plant competition in bare environments might select for different suites of plant traits than those traits favored in more vegetated and competitive environments (60), as outlined in Grime's plant strategies (61) and in other studies (62).

Our previous work, and that of others, has shown that bareness or low neighbor density can increase plant apparency to enemies (16, 63). Bareness, both naturally occurring and manipulated, was associated with increased levels of damage from herbivores in the field, and a concomitant reduction in plant fitness, in *Streptanthus breweri* and *Streptanthus hesperidis*. Apparency in bare or open environments might increase the need for enemy defense (64, 65), an investment that could also trade off with competitive ability (15, 16; but also ref. 39). Species of *Streptanthus* exhibit various forms of antiherbivore defense; notably, several species have brown or gray leaves that match the color of their soil outcrop (16). This potentially costly crypsis defense is found only on bare habitats, both serpentine and nonserpentine. Ni hyperaccumulation (66) and mimicry of herbivore pierid butterfly eggs that reduces oviposition by butterflies (67) provide additional evidence that herbivory and bare environments may represent historical selective forces in this
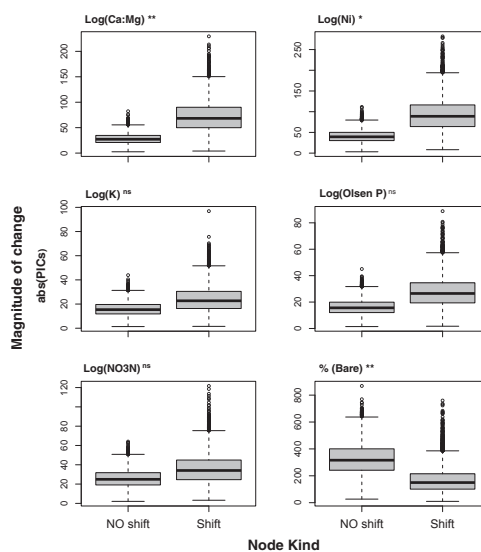
Fig. 4. absPICs at nodes with and without inferred shifts to serpentine soils. Points are mean PICs for each node category (Fig. 3) for each of 5,000 postburnin trees (31). **$P \leq 0.05$; *$P < 0.1$. Estimates and P values are provided in Table 1. ns, not significant.
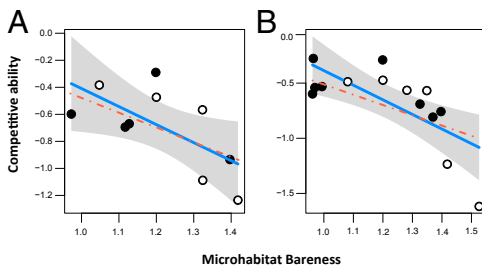
Fig. 5. Field microhabitat bareness [percentage of a 0.25-m² quadrat not occupied by plants; arcsinsqrt(%Bare)] is negatively correlated with competitive ability (log response ratio) against a native grass, as measured in a greenhouse experiment. Serpentine populations are denoted ○, and nonserpentine populations are denoted ●. Fits for linear (solid lines) and PGLS models (dashed lines) are depicted. (*A*) Species-level analysis; serpentine and nonserpentine populations of bodenvag species are treated separately (PGLS lnRR = −1.093 × + 0.614; *P* = 0.08, adjusted *R*² = 0.24). (*B*) Population-level analysis (PGLS lnRR = −0.932 × + 0.419; *P* = 0.02, adjusted *R*² = 0.34). Details are provided in *SI Appendix*, Table S7.

clade. Thus, tradeoffs between competitive ability and bareness might arise from adaptations to a variety of nonexclusive selective forces.

Combined historical and contemporary approaches can point to potentially underappreciated forces shaping biodiversity, but reconstructing past selective forces, sequences of events, and trait changes is challenging. Alternative hypotheses might explain our results. For example, bareness may be a surrogate for a correlated, as yet unmeasured, factor. Disentangling the relative importance of bareness, nutrients, elements, enemies, and competition in pathways leading to soil specialization will require the expansion of experimental and descriptive approaches, for example, a design incorporating specific nutrient and elemental manipulations with varying competition intensity in a phylogenetic context. Additionally, measurement and phylogenetic reconstruction of plant traits and abilities associated with elemental uptake, competition, and drought tolerance may shed light on the sequence of selective forces and trait evolution contributing to soil specialization.

Soil endemism contributes disproportionately to regional floras and to overall global plant diversity (2, 44, 50, 51). Our integrative ecophylogenetic approach can be extended to test the generality of adaptation to bare habitats in other plant groups; in other regions with high levels of soil endemism; and in other bare soil substrate types like gypsum (5, 68), limestone (33, 69), ironstone (70), and dolomite (71). More broadly, ecophylogenetic approaches applied to a variety of systems and questions in evolutionary ecology continue to be an important means through which we can explain the origins and maintenance of biodiversity.

Methods

Species and Phylogenetic History. To integrate over phylogenetic uncertainty, we performed analyses over a random sample of 5,000 postburnin trees of a 50-million–generation Bayesian analysis (31). Due to lack of monophyly in the well-supported *Streptanthus glandulosus* complex (31), which also uses a variety of soil types, we collapsed it to a single lineage in all our evolutionary analyses, sampling tips (with their soil affinities) at random across our 5,000 iterations (a full explanation of methods used is provided in *SI Appendix, Methods in Full*).

Soils and Environments. Soil samples (one to three samples per population, 116 populations, 45 species) were collected from the rhizosphere (top 30 cm of soil) immediately below randomly selected focal plants within each population, dried, and stored. Soils were sifted into three fractions (fine, <1.7 mm;

medium, 1.7–4 mm; and coarse, >4 mm) using standard soil sieves. Physicochemical analyses were done at the University of California, Davis Analytical Laboratory (http://anlab.ucdavis.edu). Microhabitat bareness was quantified at the time of flowering in 2011 and 2013 for 71 populations of streptanthoids (37 species; *SI Appendix*, Table S1) as the percentage of bare ground in a 0.25-m² quadrat centered on focal plants naturally occurring in the field. Plants are patchily distributed in expansive habitats, so we identified focal plants with a combination of targeted and random sampling, first identifying areas where plants occurred, then selecting some areas at random, and then randomly selecting focal plants within selected patches. When possible, we measured replicate populations per species and 15 focal plants per population (a minimum of five plants per site in low-density populations). We investigated the potential effect of year in our estimates using sites measured in both years. We found that bareness estimates between years were variable but correlated (*r* = 0.6, *P* = 0.02 after removing one outlier; *n* = 14) and not significantly different (*P* = 0.29; *n* = 30), justifying our use of data collected across years.

Statistical Analyses. We compared serpentine and nonserpentine soils with Welch's *t* tests to account for unequal variances in R version 3.1; when necessary, soil elemental data were log-transformed to meet normality assumptions. Using only the populations for which we had both field bareness and soil chemical data (*SI Appendix*, Tables S1 and S5), we also analyzed the relationship between bareness and individual soil elements using univariate linear models and correcting for multiple comparisons (Bonferroni).

Phylogenetic Signal and PIC Analyses. We incorporated phylogenetic uncertainty by analyzing 5,000 randomly selected postburning trees (31). We evaluated phylogenetic signal using Blomberg's *K* (72) or Purvis's *D* (73). All analyses were done in R (details and functions used are provided in *SI Appendix, Methods in Full*). For our PIC analyses, we first inferred soil use history to identify soil transitions in each tree. Then, for a given habitat attribute, we compared the PIC absolute values at nodes ancestral to transitions to serpentine, which reflect the magnitude of change associated with soil shifts ("SHIFT" nodes), with the rest of the nodes ("NO-SHIFT" nodes) using a Welch's *t* test that accounts for unequal variances (Fig. 3).

Tradeoffs Between Competitive Ability and Bareness. We explored the relationship between competitive ability [log response ratio (lnRR)] measured in lath-house experiments and field microhabitat bareness using linear models (JMP Pro version 10, SAS Institute Inc.) and PGLS (74) based on the maximum credibility tree of a 50-million–generation Bayesian analysis (31). Competitive ability was estimated as the response ratio of the performance (biomass) of *Streptanthus* plants grown with (B_W) and without (B_{WO}) a grass neighbor (*Bromus laevipes*), calculated as lnRR = ln(B_W/B_{WO}) (75). *B. laevipes* is native to California and occurs at many *Streptanthus* sites. Competition assays were performed under natural light and temperature conditions in natural raw soils also from *Streptanthus* sites. Focal plants and their grass neighbors were collected for dry biomass analyses at the onset of first flower. Full details on germination and growing conditions are provided in *SI Appendix, Methods in Full*. For a species-level analysis, we used species averages of both lnRR and bareness. Three of the seven species measured can be found on and off serpentine (*Streptanthus glandulosus, S. tortuosus, Caulanthus amplexicaulis*), and because serpentine and nonserpentine sites differ in bareness, serpentine and nonserpentine populations were analyzed as separate data points. For an analysis at the population level, we included 14 populations for which we had estimates of both field bareness and lnRR.

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