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EVOLUTIONARY ECOLOGY OF PLANT ADAPTATION TO SERPENTINE SOILS

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■ **Abstract** Plant adaptation to serpentine soil has been a topic of study for many decades, yet investigation of the genetic component of this adaptation has only recently begun. We review the defining properties of serpentine soil and the pioneering work leading to three established physiological and evolutionary mechanisms hypothesized to be responsible for serpentine tolerance: tolerance of a low calcium-to-magnesium ratio, avoidance of Mg toxicity, or a high Mg requirement. In addition, we review recent work in serpentine ecology documenting the high proportion of endemic species present, the adaptive morphologies of serpentine-tolerant plants, and the distinctive structure of serpentine communities. Studies of the physiological mechanisms proposed to confer serpentine tolerance have shown that uptake of particular ions and heavy metals varies between serpentine-tolerant and -intolerant species. Recent studies examining the genetic basis of serpentine adaptation have shown serpentine-adaptive quantitative trait loci (QTL) to have large phenotypic effects, drought tolerance to be as important as metal tolerance, and serpentine adaptation to have evolved independently multiple times within species. Investigations of plant races and species adapted to contrasting soil types have shown disparate flowering times, divergent floral morphologies, and pollen incompatibility to contribute to reproductive isolation. Finally, we propose that future studies involving serpentine systems should merge the fields of ecology, evolution, physiology, and genetics.

1. INTRODUCTION

> "Nothing can be more abrupt than the change often due to diversity of soil, a sharp line dividing a pine- or heather-clad moor from calcareous hills."
>
> —Alfred Russel Wallace (1858)

Wallace (1858) and Darwin (1859) put forth the idea that adaptation is the signature of evolution by the hand of natural selection, and that adaptation to novel environments results in the origin of new species. Though the importance of adaptive evolution in shaping the Earth's diverse biota is undisputed (Schluter 2001), our understanding of the process for organisms living in their natural habitats is remarkably superficial.

Wallace (1858) recognized that plant adaptation to different soil types is evidence of the strong natural selection imposed by ecological discontinuities. Among such examples of edaphic specialization, plant adaptation to serpentine soils is a system ideal for studies in evolutionary ecology and satisfies key requirements for addressing mechanistic questions of adaptive evolution in nature. First, there is extensive literature documenting serpentine flora and systematics, natural history, ecology, and physiology. Second, differential adaptation of closely related plants to serpentine and "normal" soil is phylogenetically and geographically widespread, having evolved independently many times. Third, divergence in adaptive phenotypes is readily demonstrated via reciprocal transplants (see Figure 1). Fourth, agents of natural selection are often apparent—e.g., soil chemical and physical properties—and amenable to manipulative experiments in the field, greenhouse, and laboratory. Finally, physiological mechanisms of serpentine tolerance have been described at the whole-plant level, providing valuable clues for the discovery of adaptations at lower levels of organization (e.g., tissue, cellular, and genetic).

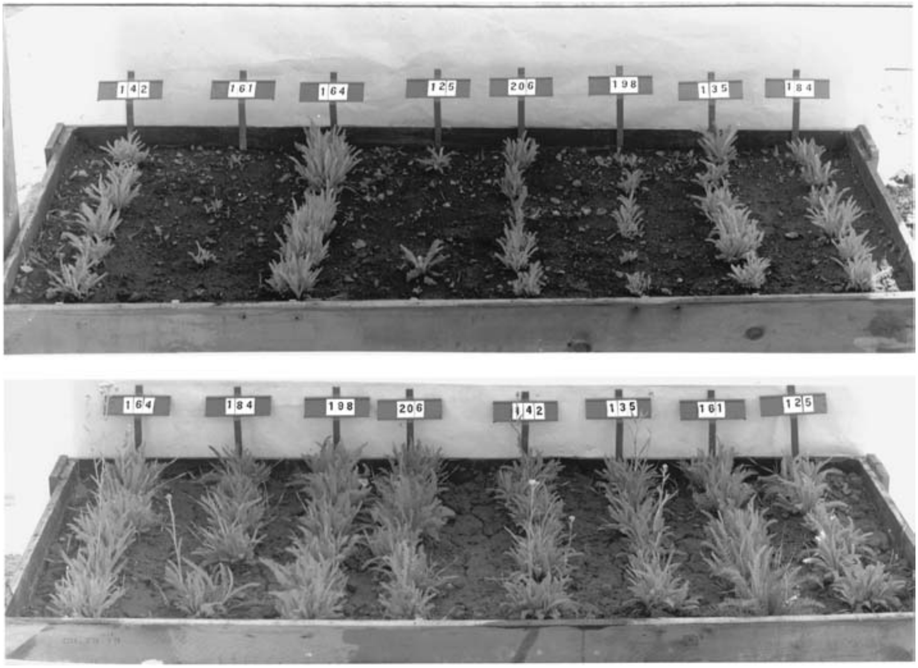

Figure 1 Responses of eight strains of *Achillea borealis* to serpentine soil (*upper panel*) and nonserpentine soil (*lower panel*). The serpentine strains are S-142, S-164, S-135, and S-184. Photo by A.R. Kruckeberg.

In this review, we begin with a brief description of the "serpentine problem," followed by a historical overview of research on serpentine systems, a discussion of the present state of the field, and suggestions for the directions in which future research might proceed. We propose that future studies involving serpentine systems merge the fields of ecology, evolution, physiology, and genetics, with the goal of identifying all of the genes (and mutations within them) that produce the novel phenotypes required for serpentine adaptation.

2. THE SERPENTINE SYNDROME

> "It is the obdurate physical adversity of things such as peridotite bedrock which often drives life to its most surprising transformations."
>
> —David Raines Wallace (1983)
> In *The Klamath Knot*

Serpentine soils are formed by the weathering of ultramafic rocks, those igneous or metamorphic rocks comprised of at least 70% ferromagnesian, or mafic, minerals (Kruckeberg 2002). Although serpentine is present, it is by no means the exclusive mafic mineral in these soils, hence it has been acknowledged that "serpentine soil" is, in fact, a misnomer (Proctor 1999). However, this term is cemented in the field (Brooks 1987).

Serpentine soils are ubiquitous, but patchily distributed. Although some variation occurs between sites, Whittaker (1954) identified three collective traits: (*a*) poor plant productivity, (*b*) high rates of endemism, and (*c*) vegetation types distinct from those of neighboring areas. Based on these features, Whittaker (1954) divided the serpentine problem into three parts: the edaphic, the plant species-level response (autecology), and the plant community-level effect (synecology).

The edaphic factor of the serpentine problem is multifaceted, involving chemical, physical, and biotic components. Arguably, the most influential factor on plant life is the chemical one (Kruckeberg 1985). Serpentine soils are characterized by low calcium-to-magnesium ratios with Ca at significantly lower concentrations relative to surrounding areas. They also frequently contain elevated levels of heavy metals, such as iron, nickel, chromium, and cobalt, which are toxic to most plants. Serpentine soils are often deficient in essential plant nutrients such as nitrogen, potassium, and phosphorus (Brooks 1987, Gordon & Lipman 1926, Proctor & Woodell 1975, Vlamis & Jenny 1948, Walker 1954).

The physical conditions of serpentine soils also prove inhospitable for many plants. Serpentine outcrops are often steep and comparatively rocky, making them particularly vulnerable to erosion, which results in shallow soils. Silt and clay contents in serpentine soils are generally minimal. Combined, these factors yield an environment with little moisture and depressed nutrient levels (Kruckeberg 2002, Proctor & Woodell 1975, Walker 1954). Therefore, plants inhabiting these sites must often tolerate drought as well as serpentine chemical attributes.

Biologically, serpentine sites frequently host a depauperate flora compared to surrounding regions. Sparse plant cover also encourages erosion and promotes elevated soil temperatures (Kruckeberg 2002). Each of these factors poses an additional stress to plant life. Together, the chemical, physical, and biotic components of the edaphic factor produce what Jenny (1980) coined the "serpentine syndrome," a term which illustrates the fact that it is the cumulative effect of these components to which a plant must adapt.

Plant species adapted to serpentine soils often possess morphologies somewhat distinct from closely related species not adapted to serpentine sites. There are several morphological features characteristic of serpentine-tolerant species and races. First, they typically possess xeromorphic foliage, including reduced leaf size and sclerophylls. Second, the stature of serpentine-tolerant plants is significantly reduced relative to counterparts on nonserpentine soil. Finally, root systems of species growing on and off serpentine sites are often more developed on serpentine soils than on neighboring soils (Krause 1958, Pichi-Sermolli 1948, Ritter-Studnička 1968, Rune 1953).

Serpentine systems are, in part, defined by the presence of a large number of endemic species (Jenny 1980). Cuba and New Caledonia exhibit especially high levels of endemism on serpentine soils. Although serpentine regions comprise only 1% of land area in California (Kruckeberg 1985, 2002), it has been estimated that serpentine-endemic plant species represent up to 10% of the state's total endemic flora (www.biodiversityhotspots.org). Experiments have shown that serpentine-tolerant species and races are limited to serpentine soils because of their inability to compete in nonserpentine environments (Kruckeberg 1950, 1954). This suggests that along the evolutionary trajectory toward serpentine tolerance, genetic trade-offs occur, rendering the serpentine-adapted plant species or race unable to recolonize its historical habitat. Thus, serpentine-tolerant species are often endemic to serpentine regions.

The final aspect of the serpentine problem identified by Whittaker (1954) involves the structure and distribution of serpentine communities. Serpentine landscapes are perhaps most striking from afar because the sharp contrast of vegetation on serpentine and neighboring nonserpentine soils brilliantly delineates the presence of an edaphic discontinuity (see Figure 2). In extreme cases, serpentine sites are barren, leaving no doubt of a steep ecological gradient (see Figure 3).

Each facet of the serpentine problem is important to study in order to understand the ecology and evolution of plant adaptation to serpentine soils. The details of this problem are further discussed in the comprehensive reviews by Krause (1958), Proctor & Woodell (1975), Kinzel (1982), and Brooks (1987).

Figure 3 Extremely barren serpentine slopes above De Roux Forest Camp, upper north fork Teanaway River, Wenatchee Mountains, Washington.

3. SERPENTINE TOLERANCE MECHANISMS: AN OVERVIEW

> "Compared to nonserpentine species, serpentine plants typically have one or more of the following traits: greater tolerance of high Mg and low Ca levels, higher Mg requirement for maximum growth, lower Mg absorption, higher Ca absorption, and Mg exclusion from leaves."
>
> —Tyndall & Hull (1999)
> In *Savannas, Barrens, and Rock Outcrop Plant Communities of North America.*

The mechanism by which a serpentine-tolerant plant copes with elevated levels of Mg and relatively insufficient quantities of Ca in the soil is perhaps its most defining character. There are several physiological and evolutionary mechanisms hypothesized to be responsible for serpentine tolerance.

3.1. The Calcium-to-Magnesium Ratio

Loew & May (1901) first attributed the poor productivity of serpentine sites to the low Ca:Mg ratio present in serpentine substrates. From their experiments, they concluded that the Ca:Mg ratio must be at least unity for optimal growth. Vlamis & Jenny (1948) later proposed that the low concentration of Ca present in serpentine soils is the principal stress of the serpentine syndrome, and high concentrations of Mg further compound the problem by depressing the availability of Ca. This hypothesis is supported by a number of studies (Kruckeberg 1954, Vlamis 1949, Walker 1948, Walker et al. 1955) in which growth of nonserpentine plants in serpentine soil increases significantly with the addition of Ca to the soil. For example, Kruckeberg (1954) found that nonserpentine races of *Phacelia californica* (Hydrophyllaceae) could persist on serpentine soil if the soil was supplemented with Ca; however, plants survived no better on serpentine soil fertilized with N, P, and K than they did on unfertilized serpentine soil.

Walker (1948) tested this hypothesis on tomato (*Lycopersicon esculentum* Mill., var. Marglobe) and a serpentine endemic, *Streptanthus glandulosus* ssp. *pulchellus* (Brassicaceae). In this experiment, Walker grew plants in serpentine soil leached with chloride solutions containing varying concentrations of Ca and Mg and fertilized with N, P, and K. Resulting soils varied in exchangeable Ca from 5% to 80% and Mg from 94% to 19%, respectively. The soil with the highest Ca:Mg ratio resembled productive agricultural soil, whereas the lowest Ca:Mg ratio tested was lower than that found in the original serpentine soil. Growth of tomato was directly correlated with Ca levels, whereas *Streptanthus* growth remained relatively unchanged across different Ca concentrations, suggesting that this species is more tolerant of depleted Ca levels. In soils with low Ca:Mg ratios, tomato took up significantly more Mg than *Streptanthus*. These data led Walker (1948) to conclude that the poor productivity of serpentine soils is a result of the low Ca concentration and concurrent high concentration of Mg, and only those species tolerant of low Ca:Mg levels could survive on serpentine soils.

Walker et al. (1955) reached similar conclusions from their study on three crop plants and three native California serpentine endemics. Plants were grown in soils prepared using the leaching and fertilizing technique previously described. Growth of crop plants declined considerably as soil Ca levels dropped, whereas the native serpentine species were not significantly affected by changing Ca concentrations. Analysis of plant tissue indicated that, when grown in soils with low Ca:Mg ratios, native species absorbed more Ca and typically less Mg than the crop plants. Thus, Walker et al. (1955) surmised that serpentine-tolerant species survive on soils with depleted levels of Ca because they are still able to absorb sufficient quantities of Ca without taking up excessive quantities of Mg.

Vlamis (1949) grew barley and Romaine lettuce on serpentine soil collected from California and discovered that each crop displayed symptoms of disease and diminished growth. Yields improved somewhat with the addition of N (NH_4NO_3), P, and K to the soil, and significantly increased with the addition of Ca ($CaSO_4$). Disease symptoms also lessened upon addition of Ca. Plants growing in soil with supplemental Ca, N, P, and K produced the greatest yields and appeared perfectly healthy. In contrast, addition of Mg ($MgSO_4$) or K alone drastically reduced growth and exacerbated disease symptoms. Tissue analysis revealed that Ca uptake by plants was considerably reduced when Mg or K was added to the soil. Such results corroborate Vlamis & Jenny's (1948) original suggestion that plant growth on serpentine soil is most affected by the low Ca content, and the excess Mg competitively inhibits Ca uptake by the plant.

3.2. Magnesium Toxicity

Serpentine soils contain potentially toxic concentrations of Mg, leading some researchers to conclude that Mg poisoning is the primary cause of the serpentine syndrome (Brooks 1987; Brooks & Yang 1984; Marrs & Proctor 1976; Proctor 1970, 1971). Proctor (1970) analyzed the effects of elevated levels of Mg on a serpentine-tolerant race of *Agrostis canina* (Poaceae) and a nonserpentine race of *A. stolonifera*, finding the latter to be notably more susceptible to Mg toxicity than the former. Furthermore, Mg toxicity appeared contingent upon sufficiently low Ca concentrations as it subsided upon addition of Ca in both soil and water cultures. Similar conclusions were reached in a second study (Proctor 1971). When grown on serpentine soil, oat plants showed severe symptoms of toxicity, which decreased dramatically upon addition of Ca to soil. Proctor (1971) thus suggested that the serpentine syndrome results not necessarily from limited Ca in the soil, but from the excessive level of Mg, which acts antagonistically to plant uptake of Ca and results in Mg poisoning.

In analyzing tissue of a number of serpentine endemics from Zimbabwe, Brooks & Yang (1984) found the concentration of Mg in plant tissue to be inversely proportional to the concentrations of seven other nutrients: aluminum, Fe, Co, boron, manganese, P, and sodium. Interestingly, a correlation between the concentration of Ca and Mg was not found. However, these data clearly suggest that the uptake of Mg comes at a cost to the plant as the uptake of other elemental nutrients is forfeited. Therefore, Brooks & Yang (1984) proposed that the heightened level of Mg in serpentine soils and its antagonistic behavior toward other elements could be the most important factor in the serpentine syndrome.

Madhok (1965), Madhok & Walker (1969), and Grover (1960) each suggested that competitive inhibition between Ca and Mg in soils with low Ca:Mg ratios is responsible for the differential absorption of the two elements in the serpentine-endemic sunflower *Helianthus bolanderi* ssp. *exilis* (Asteraceae), and the cultivated sunflower *H. annuus*. Grover (1960) showed that the quantity of Ca taken up by *H. bolanderi* ssp. *exilis* roots increased with increasing external Ca concentrations. However, raising the external concentration of Mg inhibited the uptake of Ca. Madhok (1965) and Madhok & Walker (1969) investigated the effects of Ca and Mg levels on these two species. *H. bolanderi* ssp. *exilis* tolerated much higher concentrations of Mg than *H. annuus*, which took up more Mg and less Ca than the serpentine endemic. Likewise, Marrs & Proctor (1976) found serpentine-tolerant strains of *Agrostis stolonifera* displayed greater tolerance to elevated levels of Mg than a nontolerant strain. Furthermore, the *A. stolonifera* strain from the more extreme serpentine site tolerated higher levels of Mg than the strain from the moderate serpentine site.

Main (1970) demonstrated that tolerance to Mg is a heritable trait in *Agropyron spicatum* (Poaceae). The serpentine-tolerant race of *A. spicatum* is significantly more tolerant of high levels of Mg than the nonserpentine race. Crossing these two strains produces an F_1 hybrid that exhibits an intermediate tolerance to Mg. Brooks (1987) suggested that if a specific tolerance to Mg is necessary for plants to survive on serpentine soil and Mg tolerance is heritable, then Mg must certainly be toxic to most plants at the levels present in serpentine regions.

3.3. Magnesium Requirement

Another theory on the physiological aspect of the serpentine syndrome posits a Mg requirement. There is evidence that certain serpentine races require higher concentrations of Mg externally and, in some cases, internally than counterpart nonserpentine populations (Grover 1960; Madhok 1965; Madhok & Walker 1969; Main 1974, 1981; Marrs & Proctor 1976). Main (1974) showed that, when grown on substrates with high levels of Mg, serpentine strains of *Agropyron spicatum* maintained lower concentrations of Mg in their tissue than nonserpentine strains. Thus, Main suggested that higher concentrations of Mg in the soil were necessary in order for the serpentine strain to acquire adequate levels of the element.

In another study, Main (1981) determined that *Poa curtifolia* (Poaceae), a serpentine endemic, requires an abnormally high level of Mg both externally and internally for optimal growth. Growth of this plant is positively correlated with the concentration of Mg in shoots and negatively correlated with Ca levels. Similarly, Marrs & Proctor (1976) showed that serpentine-tolerant strains of *Agrostis stolonifera* require more Mg and contain higher internal levels of Mg than nonserpentine strains. Grover (1960) found that growth of the serpentine-endemic *Helianthus bolanderi* ssp. *exilis* correlated positively with increasing soil Mg concentrations, whereas growth of *H. annuus*, a nonserpentine species, declined precipitously under the same conditions. Madhok (1965) and Madhok & Walker (1969) revealed that *H. bolanderi* ssp. *exilis* also requires a higher internal concentration of Mg relative to *H. annuus*. However, because the serpentine species more readily takes up Ca than Mg, a high external concentration of Mg is necessary for *H. bolanderi* ssp. *exilis* to attain sufficient quantities for optimal growth. Proctor &

Woodell (1975) suggest that studies showing unusually high Mg requirements by some serpentine-tolerant species and races, especially those concentrations typically lethal to plants, could lend insight into serpentine endemism by defining the conditions that restrict serpentine-tolerant plants to serpentine soil.

3.4. Hyperaccumulation

Hyperaccumulation is another mechanism that is hypothesized to allow plants to survive on serpentine soils. Hyperaccumulators are defined as those plants which contain in their tissue more than 1000 μg/g dry weight of Ni, Co, copper, Cr, or lead, or more than 10,000 μg/g dry weight of zinc or Mn (Baker & Brooks 1989). Aside from metal tolerance, hyperaccumulation is thought to benefit the plant by means of allelopathy, defense against herbivores, or general pathogen resistance (Boyd & Jaffré 2001, Boyd & Martens 1998a, Davis et al. 2001). There are at least 400 known Ni hyperaccumulators; however, the majority of serpentine-tolerant species do not fall into this category (Proctor 1999). Therefore, this review does not further explore the phenomenon of hyperaccumulation.

3.5. Preadaptation and Cross-Tolerance

Kruckeberg (1954) described a scenario in which distinct races or species evolve through adaptation to serpentine soil because certain individuals on nonserpentine sites are somewhat preadapted to one or more characteristics of serpentine sites. As these preadapted seeds germinate and reproduce on serpentine soil, their most fit progeny survive to reproduce, and thus a discrete lineage is born through the accumulation of serpentine tolerance alleles.

Macnair (1987) suggested that colonization of areas such as mines is challenging for plants because they must evolve tolerance to toxic levels of heavy metals—e.g., Cu—as well as adapt to a suite of other edaphic restrictions, such as low nutrients or drought conditions. For this reason, it has been suggested that plants that are somewhat preadapted for any of the harsh edaphic conditions of mines successfully colonize these regions more readily (Antonovics et al. 1971, Macnair 1987). Similar inferences might be drawn regarding serpentine sites.

Analogous to the notion of preadaptation is cross-tolerance. Some unfavorable conditions are common between different harsh environments. Thus, plants adapted, for example, to the high levels of Mg present in coastal saline habitats may also be tolerant of the high levels of Mg found in serpentine soil (Proctor 1971). Kruckeberg (1954) noted that a maritime-adapted race of *Achillea borealis californica* (Asteraceae) appeared equally adapted to serpentine soil. Likewise, Proctor (1971) found maritime-adapted *Armeria maritima* (Plumbaginaceae) grew relatively well on serpentine soil, and growth of a maritime-adapted race of *Silene maritima* (Caryophyllaceae) was comparable to the serpentine-tolerant race when grown on serpentine soil.

Boyd & Martens (1998b) offer three theories to explain the "preadaptive" nature of nonserpentine populations to serpentine conditions: (*a*) high rates of gene flow from serpentine to nonserpentine populations bring serpentine tolerance alleles into the latter population, (*b*) a constitutive serpentine adaptive trait presents little or no cost to a plant, or (*c*) a serpentine adaptive trait is adaptive for more than one function.

The first hypothesis aligns with the previously described scenario proposed by Kruckeberg (1954); however, there is little data available to support this idea. Evidence for cross-tolerance lends support to the third suggestion. The second explanation seems the least likely as it would be expected that, if serpentine tolerance presented little or no cost to a plant, then serpentine-adapted plants would grow just as well as nonserpentine plants on "normal" soil. But, it has been shown that serpentine-tolerant plants do not grow as well as nonserpentine plants when both are grown together on nonserpentine soil (Kruckeberg 1950, 1954). Even in the absence of competition with nonserpentine plants, serpentine-tolerant plants seem to possess a slower intrinsic growth potential than nonserpentine plants (K.U. Brady, unpublished data). Nevertheless, Reeves & Baker (1984) suggest that metal tolerance in *Thlaspi goesingense* (Brassicaceae) is a constitutive serpentine adaptive trait and is evident only when plants are grown on soils with high metal concentrations. In their study, seeds from serpentine and calcareous populations of *T. goesingense* were germinated in a greenhouse and grown in various serpentine and artificial limestone soils, some amended with Zn. Plants from each population grown on soils with high concentrations of Ni, Co, Zn, and Mn accumulated high quantities of all metals in shoots. Plants growing on serpentine soils took up much more Mg and considerably less Ca than plants growing on nonserpentine soils, regardless of origin. However, plants from both populations performed better on nonserpentine soil with no heavy metals. The similar metal accumulation and performance of plants from each population on differing soil types suggest that metal tolerance is a constitutive trait within this species.

Boyd & Martens (1998b) reached analogous conclusions in their study on Ni tolerance in *Thlaspi montanum* var. *montanum* (Brassicaceae). Seeds collected from two serpentine and two nonserpentine populations were grown in a greenhouse in soils ranging from "normal" to high-Ni content. Plants from all four populations exhibited hyperaccumulation of Ni in a similar fashion. Thus, Boyd & Martens (1998b) concluded that Ni hyperaccumulation is a constitutive trait of *T. montanum* var. *montanum*.

Similarly, serpentine tolerance appears to be a constitutive trait of *Silene dioica* (Caryophyllaceae), which survives in serpentine soil and tolerates high Ni concentrations regardless of whether plants are from a serpentine or nonserpentine population (Westerbergh 1995, Westerbergh & Saura 1992). The authors suggest that this "preadapted" nature of *S. dioica* has allowed the species to colonize serpentine soils multiple times independently. Taylor & Levy (2002) tested three varieties of *Phacelia dubia* (Hydrophyllaceae) for preadaptation to serpentine soils and found that *P. dubia* var. *georgiana*, an endemic of granite outcrops, displayed a tolerance to lower Ca:Mg ratios than present in its native soil. The authors propose that this type of variation could represent the sort of preadaptive trait that allows a species to inhabit new environments. Likewise, inherent serpentine tolerance in *S. dioica* or metal tolerance in *Thlaspi montanum* var. *montanum* and *T. goesingense* could render each species preadapted to serpentine soils, facilitating colonization of such regions.

4. THE ECOLOGY OF SERPENTINE SYSTEMS

The ecology of serpentine systems is particularly interesting considering the high proportion of endemic plant species present, the adaptive morphologies of serpentine plants, and the distinctive structure of serpentine communities. Iturralde's (2001) analysis of plants on serpentine soils in Cuba perfectly illustrates the unique ecology of these systems. Plants exhibit various morphological adaptations associated with serpentine tolerance such as sclerophlly, microphylly, and thorny stems, as well as compact statures. The structure of these serpentine communities is characteristically open and short-statured relative to surrounding regions. A high number of endemic species and Ni hyperaccumulators were also recorded for serpentine sites in Cuba (Iturralde 2001). Disproportionate occurrence of endemic species on serpentine soils relative to total species in the surrounding region has also been documented recently in the Piacenza area of North Apennines, Italy (Vercesi 2003). Specht et al. (2001) noted corresponding features in the serpentine region of northern New South Wales, Australia. They found the vegetation on serpentine soils to be distinct from adjoining substrates and the community structure reduced in both height and basal area relative to neighboring areas.

The effects of patch size on local and regional plant diversity on serpentine sites in California have been studied at length (Harrison 1997, 1999; Harrison et al. 2001). In woody species of California serpentine chaparral, it was demonstrated that larger areas of serpentine soil (6 km^2 to 55 km^2) support higher alpha diversity (local species number) than smaller patches (0.5 ha to 3 ha), but that beta diversity (differentiation in species composition among sites) is significantly higher among small patches (Harrison 1997). Similar results were obtained in a study on the diversity of endemic serpentine herbs in the North Coast Ranges of California (Harrison 1999). However, the latter study also showed alpha diversity for all herbaceous species to be higher in small serpentine patches because of a larger number of alien species present. Harrison (1999) found that the number of alien species present in a small serpentine patch directly correlates with the soil Ca level. In contrast, the number of serpentine endemics on large patches is inversely proportional to the concentration of soil Ca. Nevertheless, even when soil conditions do not vary, small serpentine patches still host higher numbers of alien species than large patches (Harrison et al. 2001). Additionally, Gram et al. (2004) found that water availability is strongly correlated with species composition in California serpentine grasslands. Specifically, in a year with high rainfall, the number of exotic species present on rocky serpentine outcrops nearly doubled from 10 exotic species to 19. These analyses suggest that both soil properties and patch size are important factors in determining species composition in serpentine areas of California.

Batianoff & Singh (2001) investigated the differences in plant ecology and frequency of endemism between the upland and lowland landforms in the largest serpentine region in eastern Australia. They discovered that overall species richness correlates negatively with the concentration of Ni in the soil. However, they also found a greater number of endemic species in the uplands, which has higher soil Ni concentrations than the lowlands. Consequently, Batianoff & Singh (2001) concluded that edaphic conditions strongly influence species diversity and levels of endemism in this system.

A study by Cooke (1994) examined dynamics influencing species composition on serpentine and associated nonserpentine areas in the Wenatchee Mountains in the state of Washington. Results indicated substrate to have the greatest effect followed by aspect and disturbance regime. Cooke also identified several traits in serpentine-tolerant races of *Achillea* and *Senecio* (Asteraceae) as pivotal to surviving on serpentine soils. Serpentine species are much more tolerant of depleted Ca levels and elevated concentrations of Mg and Ni in the soil. To minimize water requirements and excessive water loss, serpentine plants are able to reduce water potentials to levels lower than found on nonserpentine soils, as well as keep stomata closed or nearly closed. In addition, serpentine-tolerant plants have a slower growth rate than nonserpentine species and possess morphologies adapted to drought conditions (Cooke 1994).

Several other studies have also revealed the importance of drought tolerance to survival on serpentine soil. Chiarucci (2004) identified drought stress as a more significant challenge to plants on serpentine soil in Tuscany, Italy, than the presence of heavy metals. Freitas & Mooney (1995) showed that populations of *Bromus hordeaceus* (Poaceae) growing on serpentine soil are better adapted to drought stress than *B. hordeaceus* populations growing on sandstone, as represented by both plant growth and root branching patterns. Armstrong & Huenneke (1992) examined the effect of drought on species composition in a California serpentine grassland. Although annual grasses were negatively affected by four consecutive years of drought, native bunchgrasses were little affected, likely because they possess well-developed root systems and are able to access soil moisture at greater depths. Furthermore, exotic annual grasses were more severely affected than native annual grasses. McCarten (1992) found that, in addition to mineral composition, soil depth, slope angle, and aspect are important factors influencing the structure of a California serpentine grassland by virtue of their effects on soil water content. Native perennial bunchgrasses are more commonly found on deeper soils where roots can access deep soil moisture. Native annual grasses with less-developed root systems are more often found on shallower soils. Thus, the combination of soil depth and soil chemical properties creates a patchwork of microhabitats in the serpentine grassland that results in variation in species composition and structure within a small area.

5. THE PHYSIOLOGICAL BASIS OF SERPENTINE TOLERANCE

As previously discussed, serpentine-tolerant plants must endure a variety of adverse chemical conditions. The adaptive mechanism(s) that confer to plants tolerance to soils with depleted quantities of Ca and high concentrations of Mg and heavy metals is still not well understood.

Lee et al. (1997) compared the foliar concentrations of nine elements (N, P, K, Ca, Mg, Ni, Cu, Co, and Cr) in 12 plant species growing on and off serpentine soil in New Zealand. In each case, the foliar concentrations of Mg and Ni were significantly higher in plants growing on serpentine soil than in conspecifics growing on nonserpentine soil. Conversely, the concentration of Ca was significantly less for plants growing on serpentine soil. In several species, the concentration of Cu, Co, and/or Cr was also significantly higher in plants growing on serpentine soil.

To gain insight into how Mg and Ca are stored in plants growing on serpentine soil, Tibbetts & Smith (1992) used *Sedum anglicum* (Crassulaceae) to analyze accumulation of Ca and Mg in the vacuole. Although *S. anglicum* is not associated with serpentine soils, the authors chose this species because it has large parenchymatous mesophyll cells, and the vacuole comprises more than 95% of cell volume. Therefore sufficient quantities of cell "sap," which essentially reflect the contents of the vacuole, can be easily obtained. In any case, *S. anglicum* can be found on soils relatively rich in Mg, and experiments revealed that the species is tolerant of a wide range of Ca:Mg ratios. However, *S. anglicum* preferentially takes up Ca over Mg. Specifically, the authors found that at a soil Ca:Mg of unity, the cell sap concentration of Ca was about twice that of Mg. Equal cellular concentrations of Ca and Mg were reached when the external Ca:Mg ratio was approximately 1:6. Interestingly, less than half of the total Mg and Ca in the leaf cell sap was in the form of free cations. Instead, chelation of Mg and Ca by soluble carboxylates such as malate, citrate, and isocitrate located in the vacuole result in the formation of metal-ligand complexes. Tibbetts & Smith (1992) thus suggest that chelation may be important for vacuolar sequestration of excessive numbers of ions that might otherwise be toxic to the cell.

Adaptive physiological differences in two races of *Lasthenia californica* (Asteraceae) found on serpentine soil have been studied extensively (Rajakaruna 2003, Rajakaruna & Bohm 1999, Rajakaruna et al. 2003c). Races A and C of *L. californica* coexist on serpentine soil at Jasper Ridge Biological Preserve, California, but inhabit soils of differing physical and chemical properties. Rajakaruna & Bohm (1999) describe race A soil as having a higher water content, percent clay, cation exchange capacity, and Na and Mg concentration than the soil race C inhabits, which has a higher Ca:Mg ratio and higher concentrations of Ca, K, and Ni. Results of these studies revealed that race A is more tolerant of ionic stresses, including high levels of Na and Mg (Rajakaruna et al. 2003c), whereas race C is more tolerant of drought stress (Rajakaruna et al. 2003b). Interestingly, uptake of both Ca and Mg was shown to be twice as high in race A plants as in race C plants. Furthermore, shoot concentrations of Ca and Mg were reported to be 127- and 28-times higher, respectively, in race A than in race C, indicating a greater tolerance of ion accumulation in the shoot in race A (Rajakaruna et al. 2003c). Conversely, ion concentrations in the roots remained approximately equal in both races, suggesting that elevated levels of ions in race A shoots are a result of both an increased rate of uptake and translocation of ions in race A. It is worth noting that these studies in conjunction with earlier research (e.g., Madhok 1965, Madhok & Walker 1969, Walker et al. 1955) suggest that the physiological basis for serpentine tolerance may involve one or more different mechanisms (Rajakaruna et al. 2003c), including ion uptake discrimination at the root level, ion translocation properties, and/or chelation.

6. THE GENETICS OF ADAPTATION TO SERPENTINE SOILS

Little is known about the process by which a serpentine-tolerant population evolves. Studies concerning genetic and adaptive differentiation in serpentine-tolerant and -intolerant races and species are necessary in order to reveal key innovations in the path to tolerance. Nyberg Berglund et al. (2001) examined the genetic differentiation of multiple serpentine populations of *Cerastium alpinum* (Caryophyllaceae) in Fennoscandia and revealed that serpentine tolerance in this species likely evolved two or more times independently. Further investigation (Nyberg Berglund et al. 2004) showed that serpentine populations of *C. alpinum* are more tolerant of elevated levels of Ni and Mg than nonserpentine populations, and that the degree of tolerance is directly correlated with the degree of saturation of Ni or Mg in the soil of the population's origin. These data suggest that serpentine tolerance in this species is locally evolved, supporting the earlier conclusion (Nyberg Berglund et al. 2001) that tolerance in *C. alpinum* has arisen more than once in this region.

Patterson & Givnish (2004) sequenced three segments of chloroplast DNA in *Calochortus* (Liliaceae) and deduced (by phylogenetic analysis using maximum parsimony) that serpentine tolerance arose seven times in this genus. Similarly, Mengoni et al. (2003) examined chloroplast genetic diversity between nine populations of the serpentine-endemic *Alyssum bertolonii* (Brassicaceae) from four serpentine regions in northern Italy. High levels of genetic differentiation were detected between populations within a region as well as between populations of different regions. Mengoni et al. suggest the high percentage of population-specific chloroplast haplotypes is evidence that serpentine tolerance within this species has arisen independently in each population.

As previously discussed, the physical state of serpentine soils produces drought conditions. Accordingly, serpentine-tolerant plants are often drought tolerant. Hughes et al. (2001) analyzed the role of drought tolerance in serpentine tolerance in the *Mimulus guttatus* (Phrymaceae) complex. Physiological responses to drought were analyzed in two serpentine-endemic species (*M. nudatus* and *M. pardalis*), two nonserpentine species (*M. marmaratus* and *M. nasutus*), and *M. guttatus*, which occurs on and off serpentine soil. Although all five species proved to be susceptible to drought, pressure-volume curves imply that the two serpentine-endemic species are more drought tolerant than the other three species, because the former possess higher hydrated osmotic pressures.

Hughes et al. (2001) also developed a population segregating for serpentine tolerance from a cross between *Mimulus nudatus*, a serpentine-endemic species, and *M. marmoratus*, a nonserpentine species. They found that serpentine tolerance in the segregating population was significantly positively correlated with drought tolerance, but not with Ni tolerance. This suggests that drought tolerance may be a more important factor than Ni tolerance in influencing adaptation to serpentine conditions in the *M. guttatus* complex. Interestingly, there seemed to be an important correlation between drought tolerance and plant size, as the most tolerant plants were also the smallest. This suggests that more compact forms may be favored in drought and serpentine conditions, seemingly explaining the characteristically low stature of serpentine communities. However, a more detailed genetic analysis is needed to determine whether the observed correlation between plant size and drought or serpentine tolerance in this system is due to linkage or pleiotropy.

A QTL mapping project by Gailing et al. (2004) identified one large QTL (or two or more tightly linked QTLs) controlling contrasting adaptive traits in serpentine-tolerant *Microseris douglasii* (Asteraceae) and serpentine-intolerant *M. bigelovii*. Adaptive traits are often genetically complex, involving multiple genes with varying effects. QTL maps provide information on the genetic architecture of a quantitative trait by identifying the number of loci involved, their map location, magnitude of effect, and mode of action. The success of QTL maps in analyzing the genetic component of adaptive differences between plant species and races is well documented (Mauricio 2001). Serpentine plants ordinarily flower and set seed earlier than conspecifics or sister taxa on nonserpentine soil in order to avoid drought conditions typical of serpentine systems. Accordingly, serpentine-tolerant *Microseris douglasii* flowers and sets seed earlier than *M. bigelovii*, which grows off serpentine soil. But flowering early comes at a cost to accumulating leaf biomass. Thus, alleles at the major QTL controlling leaf and floral bud production identified by Gailing et al. (2004) have opposite effects in *M. douglasii* and *M. bigelovii*.

7. THE EVOLUTION OF REPRODUCTIVE ISOLATION

In theory, plant populations growing on contrasting soil types could experience such strong divergent selection that subsequent genetic differentiation of the populations renders them reproductively isolated and, in extreme cases, results in ecological speciation (Schluter 2001). Kruckeberg (1986) suggests diverse edaphic substrates set the stage for ecological speciation because such geologic effects are patchily distributed and thus promote geographic isolation. Adaptation to edaphic conditions may also beget reproductive isolation indirectly via linkage or pleiotropy, or directly via the advent of pre- and postzygotic isolating mechanisms such as reinforcement to prevent hybridization (Rajakaruna & Whitton 2004, Schluter 2001).

Prezygotic isolating mechanisms between plant species or races include shifts in flowering time, a switch to primarily self-fertilization from out-crossing, and alterations in flower morphology that affect pollinator attraction and/or visitation (Macnair 1989). For example, in *Collinsia sparsiflora* (Scrophulariaceae *s.l.*), peak flowering time in serpentine and nonserpentine populations differs significantly (Wright et al. 2005). The serpentine-endemic *Mimulus nudatus* flowers earlier than serpentine-adapted populations of *M. guttatus*, the presumed progenitor of *M. nudatus* (Gardner & Macnair 2000). Furthermore, the primary pollinators of *M. guttatus* and *M. nudatus* are different owing to divergent floral morphologies. In *Lasthenia californica* disparate flowering times and pollen incompatibility reproductively isolate the two edaphic races previously described (Rajakaruna & Whitton 2004). In fact, recent phylogenetic work by Rajakaruna et al. (2003a) suggests that at Jasper Ridge Biological Preserve these races are two distinct species.

Although disparate flowering times have been confirmed for plant races and species adapted to contrasting soil types in several systems, whether shifts in phenology are strictly a result of adaptation to edaphic conditions or a method of reinforcement is not always clear (McNeilly & Antonovics 1968). Nevertheless, both processes function to reproductively isolate differentially adapted plant races or species.

8. FUTURE DIRECTIONS FOR RESEARCH ON PLANT ADAPTATION TO SERPENTINE SOILS

> "When this research was in its embryonic stages, a complete solution of the problem of serpentine endemism seemed manifestly an attainable goal, wholly within the scope of this single study! Like the proverbial mirage, that goal seems elusive as, one by one, the many facets and ramifications of the problem are explored. It would be well at this time then, to take stock of just what is known about serpentine floras and what is yet to be learned."
>
> —A.R. Kruckeberg (1950)
> In *An experimental inquiry into the nature of endemism on serpentine soils*.
> PhD thesis.

In contrast to our detailed knowledge of neutral evolutionary processes, where precision at the DNA sequence level is the norm, there is an almost complete lack of detailed information regarding the genetic basis of adaptive evolution for organisms living in their natural habitats. We attribute this disparity to the fact that remarkably little is known about either the ecology of highly developed model organisms in genomics (e.g., *Drosophila*, *Caenorhabditis*, *Arabidopsis*, *Saccharomyces*), or the genetics of any of the myriad organisms that ecologists have studied in depth. It is not a coincidence that the organisms of interest to ecologists and geneticists are different—ecologists prefer larger, longer-lived species that dominate and structure ecosystems, whereas geneticists prefer smaller, shorter-lived species that prosper in the laboratory. Yet studying adaptive evolution in nature requires the analysis of both the ecology and genetics of the organism. These two disciplines are encompassed in the field of ecological genetics, which seeks to analyze natural selection and genetic variation in concert as a means to studying adaptive evolution (Via 2002).

There are two obvious approaches to advancing the study of ecological genetics. The first is to develop a good working knowledge of the ecology of one or more of the genetic model organisms. The second is to develop powerful genetic tools for organisms and experimental systems whose ecology is already well understood. Although both approaches are worthwhile and are being pursued vigorously by many investigators worldwide, we favor the latter approach. Developing genetic and genomic resources for interesting organisms has become a major scientific enterprise; whole eukaryotic genomes can be completely sequenced in a matter of a few weeks. Ecological research in natural settings cannot match that pace; hence, we feel that it is easier to bring genetics to ecology, rather than vice versa.

Plant adaptation to serpentine soils has the potential to become a general model for ecological genetics in natural populations (Gailing et al. 2004, Pepper & Norwood 2001). Plants—because of their prolific seed production, macroscopic size, sessile growth habit, and ease of estimating darwinian fitness—are nearly uniquely suited to the large reciprocal transplant experiments that will be required for a thorough examination of adaptive genetics in nature. Reciprocal transplant experiments between serpentine populations (or species) and their spatially adjacent populations (or sister species) growing on normal soils demonstrate unequivocally that adaptation to serpentine soils has a genetic basis (Nyberg Berglund et al. 2004; Kruckeberg 1950, 1954; Rajakaruna et al. 2003b,c), but detailed genetic analyses of this adaptation have only recently been initiated (see Section 6 above).

The patchy distribution of serpentine soils in many landscapes means that adaptation often occurs on small geographic scales, with serpentine-adapted populations and their presumed nonserpentine ancestors growing adjacent to each other, but on juxtaposed soil types. The results of reciprocal transplant experiments suggest that natural selection for serpentine tolerance is strong. Strong selection favors adaptation by mutations with large phenotypic effects (Orr 1998), which improves the odds of identifying these adaptive mutations by genetic analysis. Further, as detailed above, the evolution of serpentine tolerance has occurred many times independently, even within a single species, facilitating investigation of the "repeatability" of evolutionary trajectories (Nyberg Berglund et al. 2004). Finally, the physiological basis of serpentine tolerance is likely to involve such mechanisms as ion transport, osmotic control, and temperature stress, topics that are well studied in plant genomic model systems such as *Arabidopsis thaliana* or crop species. This knowledge can be used to develop and test hypotheses about the physiology of adaptations in serpentine-tolerant plants. It is a short step from physiology to biochemistry and from there to genetics and genomics. Our current understanding of the serpentine-tolerant phenotype ("syndrome") is a somewhat vague composite of morphology, physiology, and ecology. Genetic analysis will benefit greatly from a precise description of each component of the tolerance phenotype (e.g., Ca homeostasis, Ni tolerance, drought tolerance), so that each component may be dissected genetically, then reassembled, like a mosaic, into a complete portrait of adaptation.

The physiology of ion transport is an especially promising area for more detailed study, because serpentine tolerance will universally involve adaptations to deal with the reduced availability of Ca (Hirschi 2004), high levels of Mg, and high levels of one or more heavy metals. Elemental analysis of plant tissues can now be done on tiny samples (10 μm to 50 μm in diameter) using laser ablation and mass spectrometry (Narewski et al. 2000), in principle making it feasible to measure the internal concentration of all mineral ions anywhere in the plant and compare them to the ion concentration in the soil solution (or artificial nutrient solution) in which the plant is growing. Such experiments can be carried out in a high-throughput fashion (Hirschi 2003, Salt 2004). By experimental manipulation of ion concentrations and ratios in artificial nutrient solutions, it should be possible to infer mechanistic details about ion exclusion, uptake, competition, and sequestration in various plant tissues. Indeed, many of the current hypotheses about physiological mechanisms of serpentine tolerance were developed as a result of experiments of this type, albeit in decades past when analytical methods were much less sensitive and sophisticated. Whole-plant investigations can now be followed up with electrophysiological ion flux measurements at the level of single cells (Shabala et al. 1997) down to individual ion channel proteins (Tester 1997).

Much of the selectivity in ion transport is likely to occur in the roots of serpentine-tolerant plants, yet most physiological experiments have been focused on the above-ground organs. Root growth is very sensitive to the ionic composition of the surrounding solution and has been used successfully to assay tolerance to serpentine soils (Nyberg Berglund et al. 2004) and heavy metals (Macnair 1983). Because the essential ionic features of serpentine soil can be recreated in hydroponic culture (Walker et al. 1955) and the flux of ions across roots can be measured at spatial scales comparable to the dimensions of single cells (Newman 2001, Shabala et al. 1997), root physiologists are poised to make substantial progress on the mechanisms of serpentine tolerance.

A reasonably comprehensive understanding of serpentine-tolerance physiology leads immediately to a "candidate gene" approach for identifying individual genes that contribute to serpentine tolerance in natural plant populations. The essence of the candidate gene approach is to narrow the scope of the search for adaptive genes from the whole plant genome (>25,000 genes) to a manageable handful of genes (and their alleles) whose phenotypic effects can be measured directly through labor-intensive breeding or transgenic experiments. Genes that are known or suspected to participate in the relevant physiological processes or other adaptive traits in well-studied plants—crops and genomic models such as *Arabidopsis*—become candidate genes. For example, the genes encoding many ion transport proteins in *Arabidopsis* have been identified (Hirschi 2003, Salt 2004). The orthologs of these genes can be cloned from serpentine-tolerant plants and their sister taxa, then tested for both DNA sequence variation and functional differences. Clearly, this comparative method will depend upon high-resolution phylogenies capable of resolving the sister taxon relationships of serpentine-tolerant plant populations and species. Species- and population-level phylogenies have been less commonly available in plants than in animals, but that is changing as plant molecular systematists turn to more rapidly evolving nuclear DNA sequences (Sang 2002, Small et al. 2004).

Candidate genes may also be identified in mutant screens for serpentine tolerant phenotypes in model plant systems. Intensive efforts to discover *Arabidopsis* mutants that accumulate or exclude specific ions (Hirschi 2004, Lahner et al. 2003, Salt 2004) could have obvious application to wild serpentine plant species with the same phenotype. Likewise, a mutant screen of *Arabidopsis* has shown that null alleles of *CAX1*, a Ca-proton antiporter in the tonoplast (Hirschi et al. 1996), can produce many of the growth and ion uptake phenotypes associated with serpentine-tolerant plants (Bradshaw 2005). Whether mutations in *CAX1* play a role in serpentine tolerance in natural plant populations remains to be seen.

In the absence of a well-developed physiological model for serpentine tolerance, and therefore in the absence of candidate genes, it may be wise to pursue a purely genetic approach to identifying genes that contribute to serpentine tolerance. The most general method is to genetically map and positionally clone the quantitative trait loci that are responsible for the phenotypic differences between serpentine-tolerant and nonserpentine sister taxa. Such an approach has the advantage of being unbiased; i.e., it can point to biochemical pathways or physiological mechanisms that are as yet undescribed. The QTL mapping and cloning approach (reviewed in Mauricio 2001) is initiated by making crosses between individuals from populations (or closely related species), one of which is serpentine-adapted and the other of which is not. From this F_1 hybrid generation, a segregating F_2 population is produced. For each F_2 plant, the serpentine tolerance phenotypes are measured (e.g., biomass on serpentine soil, tissue Mg concentration), and variation in these phenotypes is correlated with genome-wide molecular marker genotypes (e.g., microsatellites, single nucleotide polymorphisms) that distinguish the alleles of the serpentine-tolerant parent from those of the nonserpentine parent. The power of this method is illustrated by the results of the QTL mapping experiment in *Microseris* described previously (Gailing et al. 2004). QTL mapping gives an estimate of the number of loci involved in the fully serpentine-adapted phenotype, as well their relative magnitude of effect on each component of the tolerance phenotype. Positional cloning of QTLs is notoriously difficult (e.g., Frary et al. 2000, Fridman et al. 2000), but if selection for serpentine tolerance is strong, there should be QTLs with large phenotypic effects that make cloning possible. The first such mapping experiment confirms that these large adaptive QTLs exist (Gailing et al. 2004). Once a QTL has been cloned from a single plant species, it will be straightforward to test for its significance in other plant taxa that have evolved serpentine tolerance independently.

Although it has been more than a century since Loew & May (1901) offered a hypothesis for plant tolerance of serpentine soils, for the first time we have methods adequate for determining the phenotypes and genotypes of serpentine-tolerant plants at the level of precision needed to identify individual genes responsible for this striking adaptation. Plant adaptation to serpentine soil represents a valuable and experimentally tractable system for evolutionary ecologists and geneticists. We look forward to seeing its secrets unraveled.

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Figure 2 Yellow goldfields (*Lasthenia californica*) on serpentine (*left*) and grassland on sandstone (*right*) at Jasper Ridge, Stanford University, California. Photo by Bruce Bohm.

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