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Plant Speciation

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The world's serpentine regions are known for their striking levels of endemism and the distinctive flora they possess relative to surrounding areas. Although much work has been done to catalog the plant diversity on serpentine, including taxonomic, morphological, and physiological diversity, relatively little has been done to understand the evolutionary origins of serpentine species. Yet serpentine species present an excellent system to study the general processes of plant speciation. We critically evaluate the theory and evidence for the mechanisms of plant speciation on serpentine. We highlight the contributions that studies of serpentine plants have made to the general understanding of speciation processes, suggest directions for future research, and call for efforts to conserve serpentine habitats and the unique opportunities they provide for ecological and evolutionary studies.

Serpentine habitats possess a seemingly insurmountable set of obstacles to successful colonization by plants. Among the many characteristics of serpentine that might limit adaptation are high concentrations of toxic metals, low calcium:magnesium ratios, thin soils prone to rapid desiccation, high gene flow with adjacent nonserpentine habitats, and spatial isolation from source populations. Nevertheless, there is ample evidence that serpentine adaptation has evolved repeatedly and independently in a wide diversity of plants (Kruckeberg and Rabinowitz, 1985; Rajakaruna, 2004; Anacker, 2010). Furthermore, in proportion to

their land area, serpentine habitats harbor more endemic species per area than surrounding habitats (Kruckeberg, 1984).

PATTERNS OF ENDEMISM SUPPORT A ROLE FOR SERPENTINE IN SPECIATION

Serpentine plants show a gradient of tolerance and restriction to serpentine, from widely tolerant to narrowly endemic. In early studies of the evolutionary ecology of serpentine plants, Kruckeberg (1951, 1954) characterized plants found on serpentine as bodenvag, indicator, or endemic species. Bodenvag species appeared indifferent to the soil but often showed differences in tolerance to serpentine at a population scale. Indicators were typically found on serpentine but also occurred occasionally off serpentine, and endemics were restricted wholly to serpentine soil. He reinterpreted Stebbins's (1942) ideas about the origins of endemism through the prism of serpentine soils. Stebbins proposed two routes to endemism. Depleted species, or paleoendemics, were once widespread and genetically diverse but had lost many or most of their biotypes, resulting in endemism on a narrow set of environmental conditions. In contrast, insular species, or neoendemics, developed on a habitat island from a small group of initial founders. Kruckeberg (1986) thought that both routes could be represented among serpentine species, with bodenvag and indicator species possibly showing the process of biotype depletion and narrow endemics representing insular species.

Raven and Axelrod (1978), in a comprehensive treatment of the evolutionary and fossil history of the California flora, largely supported this view. They believed that Californian serpentine exposures were very young, less than 10 Ma (but see Harrison et al., 2004), and that most bodenvag and indicator species predated the exposures. Thus, indicator species that are largely but not wholly restricted to serpentine, such as *Cupressus sargentii* (now classified as *Hesperocyparis sargentii*) and *Quercus durata*, were interpreted as paleoendemics. Although serpentine was not likely involved in the origin of these species, the patchy and isolated distribution of serpentine habitat islands could contribute to further divergence within these species. Strict endemic species, on the other hand, could have diversified substantially since the exposures of California serpentine and were suggested to primarily represent neoendemic or insular taxa.

Raven and Axelrod (1978) further noted patterns in the types of California plants that fall into these categories. Putative neoendemics are comprised chiefly of herbaceous lineages, whereas many paleoendemics are woody. They hypothesized that mesic-adapted woody taxa were gradually outcompeted in nonserpentine areas as the climate became warmer and drier during the summer, whereas herbaceous lineages with Madro-tertiary affinities gave rise to many serpentine neoendemics. Notable exceptions to these categories exist, such as the wide-ranging

herb *Streptanthus glandulosus*, which shows a genetic structure consistent with paleoendemism followed by allopatric divergence among regions (Kruckeberg, 1957; Mayer et al., 1994; Mayer and Soltis, 1994, 1999).

The patterns of endemism found on serpentine are consistent with recent, insular origins of many serpentine endemics. Again, the most comprehensive data on endemism are available for the California Floristic Province. California serpentine outcrops have low species richness compared to nonserpentine areas of similar size, but the turnover in species among serpentine regions, or beta diversity, is extremely high (Harrison and Inouye, 2002). Whereas there are many serpentine endemic species, composing approximately 10% of the plants unique to California (Kruckeberg, 1984), many of these are narrowly distributed. Although serpentine species are rare in absolute terms, owing to the small land area comprised of serpentine habitats, their diversity is far greater than might be expected given the apparent harshness of the habitat and the small land area involved. From a phylogenetic perspective, tolerance to serpentine is often gained and lost, but endemism is less common (Anacker, 2010). The number of endemic serpentine species in a region increases with the proportion of the region exhibiting serpentine soil and the time since exposure (Harrison et al., 2004; Anacker, 2010). These patterns suggest that adaptation to serpentine provides a stimulus for speciation and is not simply a refuge for relictual taxa. Notably, however, the radiation of serpentine clades is uncommon, except where nonserpentine substrates are absent (Spencer and Porter, 1997; Pepper and Norwood, 2001; Patterson and Givnish, 2004; Heads, 2008).

WHAT DOES IT TAKE TO LIVE ON SERPENTINE?

Serpentine soils are characterized by a low Ca:Mg ratio, deficiency in essential macronutrients, elevated concentrations of heavy metals, and low water-holding capacity. They are typically rocky, shallow soils vulnerable to erosion. These inhospitable soils harbor sparse vegetation, further contributing to nutrient limitation and erosion. Serpentine soils vary from bare, rocky outcrops to deeper, more fertile grasslands even within local areas (Alexander et al., 2007). This variation likely creates an evolutionary mosaic that is oversimplified by a dichotomous view of serpentine versus nonserpentine (Brady et al., 2005; Alexander et al., 2007; Springer, 2007; Kazakou et al., 2008).

Adaptations to serpentine soils have been extensively described and recently reviewed (Brady et al., 2005; Kazakou et al., 2008). Evolution of physiological tolerance to serpentine soils appears to carry a corresponding morphology (Kruckeberg, 1954). Plants having the "serpentine syndrome" are typically adapted to dry soils and are of smaller stature than their nonserpentine relatives. Many have strongly developed root systems, presumably to facilitate uptake of water and

nutrients (Brooks, 1987). Other species down-regulate lateral root growth in high Mg soils, allocating more resources to deep-growing roots important in dry conditions.

The low Ca:Mg ratio presents a physiological challenge not only because of Ca's importance in plant growth and signal transduction but also because high levels of Mg are antagonistic to plant uptake of Ca (Marschner, 2002; Brady et al., 2005). The challenge of low Ca:Mg ratio in serpentine soils has elicited a wide range of adaptive responses based on either ion exclusion at the root/soil interface, selective translocation of Ca from root to shoot, sequestration of Mg in the vacuole, or internal mechanisms of tolerance. Selective uptake or transport of Ca, and sometimes lowered uptake of Mg, have been demonstrated in several serpentine lineages (Walker et al., 1955; O'Dell et al., 2006; Asemaneh et al., 2007). Several species adapted to serpentine environments have higher external and internal Mg requirements than their nonserpentine relatives (Brady et al., 2005). Serpentine forms of *Lasthenia californica* and *L. gracilis* show physiological tolerance to internal ionic stresses, rather than excluding Mg ions (Rajakaruna et al., 2003b). Although these many studies have shown a wide range of gross adaptations to the low Ca:Mg ratio in serpentine soil, we still have much to learn about the precise physiological mechanisms of the toxic action of Mg and plant resistance (Asemaneh et al., 2007). Furthermore, the variety of responses shown suggests that the physiological basis for tolerating low Ca:Mg may involve more than one mechanism in a given species (Brady et al., 2005).

Nutrient limitation is a major stressor in serpentine environments, and the mechanisms of adaptation range widely. The deficiency of macronutrients results from low amounts of organic material and the lack of P and K in parent materials. The primary macronutrient deficit appears to vary globally (reviewed in Kazakou et al., 2008)—California serpentine is typically deficient in N, whereas K is the primary deficiency in Europe.

Serpentine soil often exhibits elevated levels of heavy metals, such as iron, nickel, zinc, cadmium, cobalt, chromium, and manganese, which present another set of challenges for plants. The presence of heavy metals in soils can affect plants through direct toxicity, resulting in stunting and chlorosis, antagonism with other nutrients (which can lead to iron deficiency), and inhibition of root penetration and growth (Antonovics et al., 1971). Soil pH mediates heavy metal levels and can both ameliorate and increase their effect on plants (Wang et al., 2006). Adaptive mechanisms include exclusion of metals (either by restricting them to the roots or through the absence of any uptake mechanism), compartmentalization of metals in various organs, or toxicity tolerance. Some plants concentrate heavy metals in their tissues at levels higher than in the soil. These hyperaccumulators (especially of Ni) have received considerable attention, due in part to their economic potential for the bioextraction of valuable metals. Hyperaccumulation as an adaptation to serpentine soils

is uncommon. For example, the majority of Ni accumulators (~400 species) are serpentine endemics, yet these species represent a mere 2% of all serpentine species worldwide (Proctor, 1999; Kazakou et al., 2008; Reeves and Adiguzel, 2008).

Water deficiency has been suggested as another stressor on serpentine soils (Proctor and Woodell, 1975; MacNair and Gardner, 1998; Proctor, 1999; Gardner and MacNair, 2000; Sambatti and Rice, 2007), but this aspect of serpentine tolerance has received relatively little attention (Brady et al., 2005). Water is sometimes (but not always) less available in serpentine sites than in surrounding habitats (Alexander et al., 2007).

Biotic factors may also contribute to serpentine adaptation. For example, plants on serpentine experience reduced competition from invasive species (Kruckeberg, 1984; Harrison, 1999; Gram et al., 2004; Going et al., 2009). Nickel hyperaccumulation may confer a defense against herbivory (Martens and Boyd, 1994), and **<Q1>** symbioses with serpentine-tolerant ectomycorrhizal communities may facilitate adaptation to edaphic stressors on serpentine (Schechter and Bruns, 2008; Urban et al., 2008; Gonçalves et al., 2009; Moser et al., 2009).

The pathogen refuge hypothesis (Kruckeberg, 1992) suggests that plants may escape pathogen pressure on serpentine, either through reduced horizontal transmission rates in sparse serpentine plant communities (Thrall et al., 2007) or through lowered symptom-associated damage. This has been confirmed for extreme serpentine specialists in the genus *Hesperolinon* (Springer, 2009), perhaps through an ability to selectively uptake Ca, which is required to initiate an effective immune response. Less specialized species, therefore, may be more susceptible to disease on serpentine soil (Springer, 2007, 2009). Rather than receiving refuge from pathogens, plants on serpentine may face increased biotic as well as abiotic stressors.

HOW IS SERPENTINE INVOLVED IN SPECIATION?

Serpentine is an excellent system for examining some of the most fundamental questions about speciation. First, how is adaptation involved in speciation? Most studies of speciation indicate a central role of natural selection, but our understanding of the strength of selection and its mode of action is incomplete (Coyne and Orr, 2004; Sobel et al., 2009). Second, how geographically isolated do populations need to be in order to become new species? The idea that speciation can proceed between geographically proximate populations is one of the most controversial in evolutionary biology.

To examine the role of serpentine in the formation of new species, it is first necessary to define what we mean by species. Like most plant taxa, most serpentine species are initially recognized by their consistent morphological differences from related species (Cronquist, 1978). With the rise of biosystematics, in-depth crossing studies were made of many plant groups to determine the interfertility of

species. These studies at times provided surprising results, with the discovery of strong crossing barriers within some taxonomic species but weak barriers between others. Biosystematic studies of groups including serpentine species were no exception (e.g., Lewis and Lewis, 1955; Kruckeberg, 1957; Ornduff, 1966).

Using the biological species concept, speciation can be considered the process whereby populations evolve genetically based barriers to gene exchange (Dobzhansky, 1940; Mayr, 1942). A major aim of speciation studies has been to identify and explain the traits or genes that cause reproductive isolation. Isolating mechanisms can include ecological factors, such as genetically based differences in habitat affinity or flowering time, in addition to lower success of hybrid crosses and lower fertility of hybrids. Speciation may be most likely to occur when there is divergent selection for traits that confer reproductive isolation as a by-product, such as flowering time differences. Unfortunately, most studies consider only a subset of all possible isolating barriers, and this can severely bias the understanding of speciation mechanisms (Ramsey et al., 2003; Lowry et al., 2008). Moreover, the traits that cause reproductive isolation and their fitness effects are rarely estimated. Identifying and explaining the evolution of isolating barriers is crucial to explaining the origin of species.

Serpentine soils can contribute to speciation in two primary ways. First, adaptation to serpentine soils can contribute indirectly to pre- or postzygotic reproductive barriers that genetically isolate serpentine populations from nonserpentine relatives. Second, the patchy distribution of serpentine can contribute to the geographic isolation of populations. We examine each of these in turn.

We surveyed the literature for empirical studies that have documented mechanisms of reproductive isolation between closely related serpentine and nonserpentine species or between serpentine and nonserpentine populations of the same taxonomic species (Table 4.1). We searched the ISI Web of Science database with the criteria Topic=(serpentine AND ultramafic) and Timespan=All Years through October 2009, and looked for pertinent references cited within these publications. Studies were included if they documented a form of pre- or postzygotic isolation between serpentine and nonserpentine populations or species.

Habitat Isolation

Adaptive trade-offs are a common theme in evolutionary biology—adaptation to one environment is expected to reduce performance in other environments. When accompanied by trade-offs, adaptive differentiation on a local spatial scale can be an effective barrier to gene flow because migrants between habitats have reduced fitness and assortative mating between similarly adapted individuals is increased (Dobzhansky, 1937; Mayr, 1947; Coyne and Orr, 2004). If migrants between serpentine and nonserpentine soils are selected against, most matings will occur between similarly adapted individuals. In this way, adaptation to serpentine can

TABLE 4.1 Forms of Reproductive Isolation Documented between Serpentine (S) and Nonserpentine (NS) Sister Species or Populations

Taxa	Habitat Isolation	Temporal Isolation	Pollinator Isolation	Postmating Isolation	References
Mimulus nudatus (S) and *M. guttatus* (NS)	X	X	X	X	Macnair and Gardner, 1998; Gardner and Macnair, 2000; Hughes et al., 2001
Mimulus pardalis (S) and *M. guttatus* (NS)	X	X	—	—	Macnair and Gardner 1998; Hughes et al., 2001
Mimulus pardalis (S) and *M. marmoratus* (NS)	X	—	—	X	Hughes et al., 2001
Gilia capitata ssp. *capitata* populations	X	—	—	—	Kruckeberg, 1951
Cleome heratensis (S) and *C. foliolosa* (NS)	X	—	—	—	Asemaneh et al., 2007
Calochortus umbellatus (S) and *C. uniflorus* (NS)	—	—	—	X	Ness et al., 1990
Layia glandulosa ssp. *lutea* (NS) and *L. discoidea* (S)	—	—	—	X	Baldwin, 2005
Streptanthus glandulosus complex	X	—	—	X	Kruckeberg, 1951, 1957
Achillea borealis populations	X	—	—	—	Kruckeberg, 1951
Achillea millefolium populations	X	—	—	—	O'Dell and Claassen, 2006
Collinsia sparsiflora populations	X	X	—	—	Wright et al., 2006; Wright and Stanton 2007
Alyssum inflatum populations	X	—	—	—	Ghasemi and Ghaderian, 2009
Cerastium alpinum populations	X	—	—	—	Berglund et al., 2004
Gilia capitata ssp. *capitata* populations	X	—	—	—	Kruckeberg, 1951
Helianthus bolanderi ssp. *exilis* populations	X	X	—	—	Sambatti and Rice, 2007
Mimulus guttatus populations	X	—	—	—	Macnair and Gardner, 1998; Gardner and Macnair, 2000; Hughes et al., 2001
Thlaspi goesingense populations	X	—	—	—	Reeves and Baker, 1984

NOTE: Dashes indicate no known data. Based on an ISI Web search; see text for search criteria.

contribute significantly to prezygotic isolation between the serpentine lineage and a nonserpentine progenitor and protect less abundant serpentine forms from the swamping effects of gene flow. Furthermore, hybrids between differently adapted populations may be relatively unfit, not because of intrinsic genetic incompatibilities but because they are poorly adapted to available habitats (Dobzhansky, 1951). Such habitat isolation, or reproductive isolation based on fitness trade-offs and/or hybrid unfitness among habitats, is particularly relevant for serpentine adaptation, where gene flow between adjacent populations on and off serpentine could otherwise homogenize populations.

We know relatively little about the specific costs of serpentine tolerance. The cost, and thus the magnitude of trade-offs, should vary according to the specific adaptations and peculiarities of the physiology of different plant lineages, as well as with the biotic and abiotic conditions in the surrounding environment (Elmendorf and Moore, 2007). On serpentine, a small, drought-adapted stature and deep roots are advantageous traits but may reduce the growth rate and competitive ability off serpentine. Indeed, Kruckeberg (1954) showed that serpentine endemics are competitively excluded by nonserpentine plants on "normal" soil. More recent ecological studies have also shown that serpentine plants are poor competitors on higher nutrient soils (Rice, 1989; Huenneke et al., 1990; Jurjavcic et al., 2002). A pleiotropic trade-off between early reproduction, which should confer a fitness advantage in drought conditions, and growth has been confirmed in the serpentine-tolerant *Microseris douglasii* (Gailing et al., 2004). There is some suggestion that plants adapted to serpentine have intrinsically lowered growth rates even when grown on more fertile soil (Sambatti and Rice, 2007; Brady unpublished data; Schemske unpublished data), which is expected from plants adapted to stressful environments (Grime, 1977). Adaptation to serpentine may result in an increased demographic susceptibility to herbivory in *Collinsia sparsiflora* (Lau et al., 2008). In contrast, no evidence has been found for a cost to metal tolerance or tolerance of low Ca:Mg ratios in serpentine plants (reviewed in Brady et al., 2005), but this has rarely been directly addressed.

Adaptation to different edaphic habitats likely constitutes an important form of reproductive isolation between serpentine plants and their nonserpentine relatives. Table 4.1 includes examples in which reciprocal soil treatments or ion addition experiments were performed, some fitness component was measured, and fitness trade-offs were quantified between soil types. The apparent strength of the trade-off in performance on serpentine and nonserpentine habitats varied among studies, but some habitat isolation was found in every study (Table 4.1).

Phenological Isolation

Shifts in flowering time are often associated with adaptation to serpentine soils, most likely as result of selection for earlier reproduction in drought conditions or

as a phenotypically plastic response to earlier drying of the soil (Hughes et al., 2001; Brady et al., 2005; Wright et al., 2006). The serpentine endemics *Mimulus pardalis* and *M. nudatus* flower earlier than their progenitor *M. guttatus* (MacNair and Gardner, 1998), as do serpentine populations of *Helianthus bolanderi* ssp. *exilis* (Sambatti and Rice, 2007) and *Collinsia sparsiflora* (Wright et al., 2006; Wright and Stanton, 2007). These shifts in flowering time are sometimes enough to reproductively isolate differentially adapted populations (Rajakaruna and Whitton, 2004). Dry conditions and early flowering may also be correlated with a reduction in flower size, and flower size differences confer mechanical floral isolation between the serpentine endemic *M. nudatus* and its progenitor *M. guttatus* (Gardner and MacNair, 2000). Although in some cases, earlier flowering may be a purely plastic response to the serpentine habitat, it may allow further divergence to proceed in habitat affinity or other isolating factors and can therefore be important in isolating nearby populations (Levin, 2009).

Postzygotic Isolation

If adaptation to serpentine involves catastrophic selection leading to genomic reorganization in a small founder population (Lewis, 1962), then the process of serpentine adaptation could also confer postzygotic reproductive isolation. This was proposed to occur during the formation of the rare serpentine endemic *Clarkia franciscana*, which is isolated from its nearest relatives by intrinsic postzygotic barriers (Lewis and Raven, 1958). However, further genetic work supported an older origin and a more widespread former distribution of *C. franciscana* (Gottlieb and Edwards, 1992). It is unclear how often catastrophic selection contributes to reproductive isolation in serpentine systems. Postzygotic isolation could also evolve over time between geographically isolated serpentine and nonserpentine relatives or different isolated populations of serpentine plants through the accumulation of intrinsic genetic incompatibilities (Kruckeberg, 1957) or from differential adaptation that renders hybrids unfit in the available niches. Few studies have addressed the strength of postmating or postzygotic isolation between serpentine and nonserpentine adapted plants (Table 4.1).

Spatial Isolation among Serpentine Outcrops

The fragmented and patchy distribution of serpentine outcrops also contributes to the genetic isolation and divergence of serpentine species. Paleoendemics that have become restricted to serpentine will lose the intervening nonserpentine populations and experience reduced gene flow. For example, populations of *Streptanthus glandulosus* show substantial genetic isolation and often partial postzygotic isolation among distant serpentine outcrops (Kruckeberg, 1957; Mayer and Soltis, 1994, 1999; Mayer et al., 1994). Neoendemics that arise from the same progenitor species may similarly be isolated from each other and proceed along independent

evolutionary trajectories. Again, we find an example in *Streptanthus*, in which several local endemics have been derived from within *S. glandulosus* and show low interfertility with each other (Kruckeberg, 1957).

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THE BIOGEOGRAPHY OF SPECIATION
ON SERPENTINE

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Traditional models of speciation are classified geographically, and it is a central goal in evolutionary biology to understand their relative importance. We outline these models and showcase empirical examples from serpentine species.

Allopatric Speciation

The well-accepted allopatric model of speciation is that populations become geographically isolated, and through selection or genetic drift, gradually acquire genetic differences that pleiotropically confer reproductive isolation (Muller, 1942; Mayr, 1947, 1963). If incipient species come into secondary sympatry, under this model they will coexist only if the reproductive isolation acquired in allopatry is completely effective in preventing gene flow. The isolating mechanisms can act prezygotically in preventing the species from successfully mating, for example, (Funk, 1998; Rundle et al., 2000) or postzygotically by reducing the fitness of their hybrids, either through intrinsic genetic incompatibilities that cause inviability or infertility (Dobzhansky, 1937) or through poor competitive or mating success in nature (Hatfield and Schluter, 1999). Because of extensive empirical support and a lack of theoretical objections, it is widely acknowledged that allopatric speciation is the most common mechanism of speciation (Coyne and Orr, 2004).

Among serpentine species, a couple of well-studied systems support allopatry as the root cause of divergence. First, there is evidence for incipient allopatric divergence in the widespread species *S. glandulosus*, as both genetic distance and hybrid infertility increase with geographic distance, and geographically isolated populations show morphological divergence as well (Kruckeberg, 1957; Mayer and Soltis, 1994, 1999; Mayer et al., 1994). In addition, phylogenetic work on *Lasthenia* revealed the presence of two cryptic species on serpentine outcrops in the California Coast Ranges, each comprising populations of different edaphic races (Chan et al., 2002; Rajakaruna et al., 2003a). The phylogenetic structuring of these two species across a latitudinal range suggests that although they are now partially sympatric, allopatry was likely during early stages of divergence.

Speciation with Gene Flow: The Theory of Sympatric and Parapatric Divergence

In contrast to allopatry, sympatric and parapatric speciation involves divergence in the face of gene flow between geographically overlapping or adjacent

populations. Theoretically, however, divergence in the face of gene flow is problematic. As Felsenstein (1981) pointed out, recombination between genes for mating preferences and other population-specific traits should lead to a breakdown in species divergence. Imagine a simple situation with a two-allele locus for mating preference and another two-allele locus for a species-specific trait like habitat affinity. With gene flow and recombination, some individuals adapted to the habitat of species A will prefer to mate with species B, and vice versa, reducing the likelihood of divergence.

The most controversial mode of divergence with gene flow is sympatric speciation, in which selection initiates assortative mating without any geographic isolation. Even with strong disruptive selection, sympatric speciation is theoretically implausible except via polyploidization, since linkage disequilibrium must build up de novo (Mayr, 1947; Coyne and Orr, 2004). It is also difficult to exclude past allopatry and range shifts as explanations of presently sympatric species (Mayr, 1947; Templeton, 1981; Coyne and Price, 2000). The few well-documented empirical examples suggest that it may occur under unusual ecological or genetic situations (e.g., Bush, 1969; Ramsey and Schemske, 2002; Seehausen et al., 2008). Because serpentine occurs in discrete patches, sympatric speciation has not been proposed for serpentine plants; however, serpentine endemics often occur within plausible dispersal distance of nonserpentine relatives, and parapatric speciation is often posited.

It is possible that strong selection might overcome the homogenizing effects of gene flow between parapatric populations that are not isolated by geographic barriers. The pioneering work of Clausen, Keck, and Hiesey (1958) demonstrated that local adaptation is common in plant populations and species distributed across altitudinal and climatic gradients. Parapatric populations can be distributed along gradual or steep environmental gradients (as in the boundary between serpentine and nonserpentine soils) or in distinct habitat patches distributed across the landscape in a stepping stone–like pattern (as are serpentine outcrops throughout many tectonic contact zones). Serpentine systems are ideally suited to test the theory of parapatric speciation because populations adapted to serpentine soils are typically distributed as a mosaic of distinct patches surrounded by adjacent populations growing on nonserpentine soils. Indeed, Coyne and Orr (2004) proposed that edaphic plant specialists provide an excellent opportunity to assess the likelihood of parapatric speciation.

The conditions for parapatric speciation are very restrictive, requiring some combination of strong selection and assortative mating (Coyne and Orr, 2004). Population genetic models of speciation investigate the conditions under which barriers to gene flow can arise between populations that are initially exchanging genes (Endler, 1977; Kirkpatrick and Ravigne, 2002; Gavrilets, 2003; Bolnick and Fitzpatrick, 2007). Two main types of models can be described, distinguished by

the spatial distribution of populations. In clinal models, the underlying environmental factors are assumed to vary along a geographic gradient, whereas in stepping-stone models, the habitats are discrete patches distributed in a mosaic across the landscape. In both models, it is assumed that there is geographic variation in selection on a local spatial scale and that gene flow between populations experiencing different selective regimes is the major obstacle to both adaptive differentiation and speciation. The major difference is that the magnitude of gene flow between locally adapting populations is greater for clinal than stepping-stone models, the latter being nearly equivalent to allopatric divergence but without a geographic barrier (sometimes referred to as peripatric divergence).

In clinal models, the steepness of the environmental gradient and the magnitude of selection and gene flow are key factors that determine the opportunity of adaptive divergence and speciation. Steep gradients may restrict adaptive gene substitutions to mutations of large phenotypic effect because the spatial extent of intermediate habitat is limited. Although it is theoretically possible that strong local selection can eliminate all foreign genes that migrate from neighboring populations, achieving complete reproductive isolation between parapatric populations probably requires the evolution of additional isolating barriers. For example, Caisse and Antonovics (1978) modeled assortative mating in plants as a flowering time difference genetically linked to local adaptation. Complete isolation only evolved between populations at the poles of the cline. Although this model had restrictive conditions, the parameter values may be realized in some serpentine habitats.

Divergence and speciation of serpentine plants via a pure stepping-stone model is somewhat analogous to the evolution of species that colonize island archipelagos in marine systems. In both cases, there is a low probability of migration between favorable "islands" of habitat. Successful colonization may provide an opportunity for rapid divergence due to both low gene flow between ancestral and derived populations and novel environmental conditions in the new habitat. Environmental heterogeneity and the geographic area, both of which are probably limiting factors in the case of serpentine adaptation, will determine the opportunity for further diversification.

Figure 4.1 illustrates some of the many possible routes to plant speciation on serpentine. In stage I, plants from the ancestral nonserpentine population colonize the adjacent serpentine habitat and either evolve into a new species in situ (B_s; clinal parapatric speciation) or become locally adapted to serpentine soil without speciation (A'_s; clinal parapatric divergence). In stage II, seeds from the serpentine habitat of the ancestral range colonize nearby serpentine. If the founding population is the new species (B_s), it may persist without further divergence. If the founding population had evolved serpentine tolerance in the ancestral habitat (A'_s), it may speciate in the new site ($A'_s \rightarrow B_s$; stepping-stone parapatric speciation). In
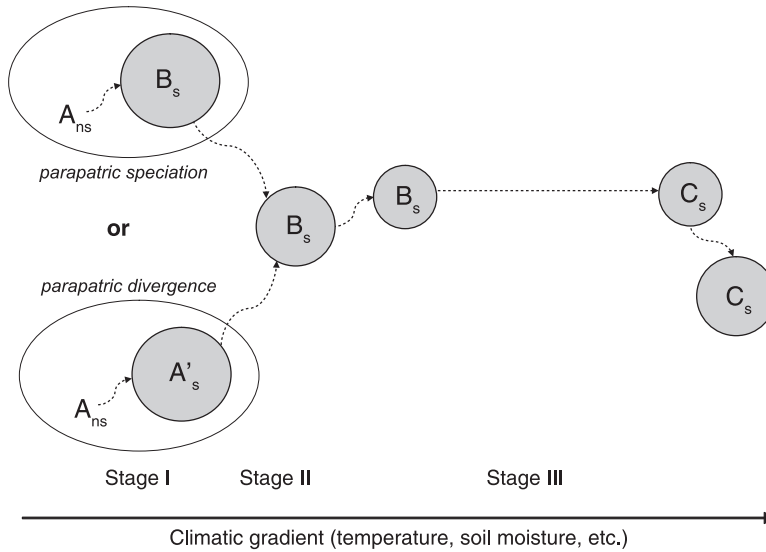
FIGURE 4.1. Hypothetical scenarios for parapatric divergence and speciation on serpentine soils. Shaded circles denote distinct serpentine habitats, and the ellipse represents the geographic distribution of an ancestral species adapted to nonserpentine soils (A_{ns}). Dashed lines indicate migration/colonization. The geographic region depicted spans a climatic gradient. In stage I, plants from the ancestral nonserpentine population colonize the adjacent serpentine habitat and either evolve into a new species (B_s; clinal parapatric speciation) or become locally adapted to serpentine soil (A'_s; clinal parapatric divergence). In stage II, seeds from the serpentine habitat of the ancestral range colonize a serpentine habitat within a similar climatic region, and the founding population either persists without further divergence if migration is from the new species ($B_s \rightarrow B_s$), or speciates in the case of migration from the population adapted to serpentine ($A'_s \rightarrow B_s$; stepping-stone speciation). In stage III, seeds from the new serpentine-adapted species colonize a serpentine habitat in a different climatic region and evolve into a new serpentine-adapted species ($B_s \rightarrow C_s$; stepping-stone speciation).

stage III, seeds from the new serpentine-adapted species (B_s) colonize a distinct serpentine habitat in a different climatic region and evolve into a new serpentine-adapted species ($B_s \rightarrow C_s$; stepping-stone parapatric speciation).

What is the relative importance of clinal and stepping-stone mechanisms of plant speciation on serpentine? If clinal speciation predominates, then we expect repeated and independent colonization of serpentine habitats followed by adaptation and the evolution of isolating barriers in situ. If stepping-stone speciation predominates, we expect that the initial adaptation to serpentine habitats is followed by subsequent diversification as serpentine-adapted species colonize other sites. The opportunities for speciation on serpentine via a pure stepping-stone

model may be somewhat limited because it requires both dispersal to a new island and serpentine adaptation simultaneously. Rather, stepping-stone speciation may be more likely after a population has acquired some serpentine adaptation in parapatry. Phylogenetic data may provide the best opportunity for evaluating the relative importance of clinal and stepping-stone models (Anacker, 2010).

A major challenge to demonstrating a parapatric origin for species in nature is excluding the possibility that speciation (or at least the initial evolution of traits involved in assortative mating) occurred during an earlier period of allopatry. For example, young species that evolve in allopatry and whose ranges subsequently come into contact may appear identical to parapatric species in being young, ecologically divergent, and reproductively isolated along their contact zone. Genetic data may be able to distinguish these situations, and substantial allopatry should lead to divergence at both neutral and selected loci, whereas neutral loci should be fairly homogenized during parapatric divergence. Extensive hybridization and introgression on secondary contact, however, would obscure these differences and make a coalescent-based approach that incorporates variation in the timing of gene tree divergence among loci more appropriate (Hey, 2006; Becquet and Przeworski, 2009).

A Case Study of the Early Stages of Parapatric Divergence: Leptosiphon parviflorus

Leptosiphon parviflorus (Polemoniaceae) provides a clear example of differentiation among parapatric populations growing on and off adjacent serpentine soils. *L. parviflorus* is a small, spring-flowering annual herb abundant in open and wooded habitats in the California Floristic Province (Hickman, 1993). Populations exhibit a single gene polymorphism for flower color, which ranges from white to deep pink, with pink dominant to white (Schemske, unpublished data). At Jasper Ridge Biological Preserve in San Mateo County, serpentine and nonserpentine (sandstone) soils are found in close proximity (<10 m), and *L. parviflorus* grows on both soil types. Populations on serpentine are typically pink-flowered and flower earlier than the adjacent white-flowered populations on nonserpentine soil. In addition, *L. parviflorus* exhibits striking differentiation in tolerance to serpentine soils. In reciprocal transplant experiments conducted in the greenhouse using field-collected soil, plants performed best on their native soil type. "Sandstone" plants grown on serpentine soil have very low survival, and the few survivors are small and produce few flowers. In comparison, "serpentine" plants grown on sandstone soil have high survival, equivalent to that on serpentine soil, but reach a smaller size than "sandstone" plants (Schemske, unpublished data).

We used the *L. parviflorus* system at Jasper Ridge to investigate the dynamics of parapatric divergence across a local contact zone. Although the transplant experiments demonstrate that populations are locally adapted to their native soil, we

wanted to know whether the sharp boundary between the pink- and white-flowered plants was maintained by strong selection or simply a lack of migration. Moreover, we wished to understand how the strength of selection varied on different putatively adaptive plant traits. To accomplish these objectives, we estimated differentiation in putatively neutral molecular markers (F_{ST}) across the contact zone and compared this differentiation to estimates of quantitative genetic differentiation (Q_{ST}) for a variety of morphological and life history traits and components of fitness on serpentine. High F_{ST} for neutral markers indicates low levels of gene flow, whereas low F_{ST} indicates extensive migration and gene flow. If estimates of Q_{ST} are higher than the estimates of F_{ST}, then this is strong evidence that quantitative trait differences are maintained by selection in the face of gene flow. The magnitude of the difference between Q_{ST} and F_{ST} can further be used as evidence for the strength of selection on different traits (McKay and Latta, 2002).

Details of this work can be found in Ward (2000) and Figure 4.2. The parental generation was collected in the field as seed and raised in the greenhouse before crossing to eliminate maternal effects. One hundred individuals were sampled from each of two populations: one on serpentine soil and one on sandstone soil, 109 m apart. Morphological and life history traits were assayed on standard greenhouse soil mix, and several measures of fitness on serpentine were assayed in progeny sown directly on field-collected serpentine soil in the greenhouse. An estimate of molecular genetic differentiation (F_{ST}) was derived from analysis of 101 amplified fragment length polymorphism (AFLP) markers in the same progeny (Ward and Schemske, unpublished data).

<Q3>

The results show that differentiation in several plant traits is maintained by selection in the face of substantial migration and gene flow. First, we found that differentiation in the putatively neutral AFLP molecular markers was very low (F_{ST} = 0.120), which is evidence for high gene flow. Second, we found that measures of population differentiation in flower color, flowering phenology, plant size, and fitness on serpentine were significantly higher than the neutral expectation provided by F_{ST} (Figure 4.2), suggesting that these phenotypic traits are subject to strong disruptive selection on the two soil types. Thus, these populations maintain marked adaptive differentiation over a microspatial scale despite considerable gene flow.

L. parviflorus is an interesting model system for the study of serpentine adaptation and speciation. Populations at Jasper Ridge meet the conditions required for parapatric speciation in that strong divergent selection leads to adaptive genetic differentiation despite considerable gene flow. These populations experience strong selection on traits associated with use of the serpentine habitat, selection against migrants from sandstone to serpentine soils, and at least an association of phenotypes affecting fitness (serpentine tolerance) and mate choice (as yielded by differing flowering time). Hence, the system presents an ideal opportunity to examine
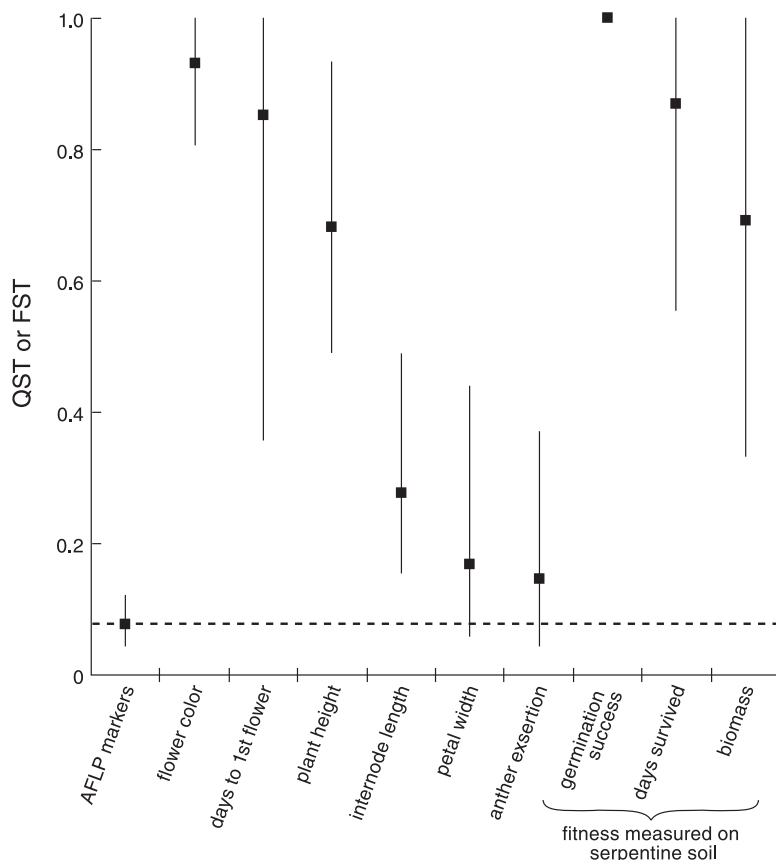
FIGURE 4.2. Estimates of *Leptosiphon parviflorus* population differentiation for putatively neutral AFLP markers (F_{ST}) and quantitative traits (Q_{ST}) measured on progeny grown in the greenhouse on regular nursery mix or field-collected serpentine soil. The dashed line tracks the value of F_{ST} for easy comparison. Controlled crosses for Q_{ST} measures followed the North Carolina II breeding design. Variance components were calculated using restricted maximum likelihood with SAS Proc MIXED METHOD=REML, with the model trait = pop dam(pop) sire(pop) dam*sire(pop). Additive genetic variance was calculated as V_a = 4*sire(pop), and Q_{ST} was calculated from variance components as Q_{ST} = pop/(2* V_a + pop). F_{ST} was calculated from the software program Arlequin (Schneider et al., 2005). Values are the mean of 1000 bootstrap replicates, with 95% bootstrap confidence intervals.

<Q4>

the adaptation that parapatric populations undergo in what must be the first steps of speciation. Additional studies are needed to determine whether the adaptive differentiation among populations on and off serpentine at Jasper Ridge may ultimately result in parapatric speciation or whether additional external factors are required to further isolate the populations.

A Case Study of Putative Completion of Parapatric Speciation:
Layia discoidea

Layia discoidea, a rare annual herb known only from a small area of serpentine in the inner South Coast Ranges of California, presents perhaps the most well-documented example of an insular neoendemic arising from within the geographic range of its progenitor. Although morphologically distinctive enough to be initially assigned to a new genus, crossing studies and phylogenetic analysis of *L. discoidea* show that it was derived from within the widespread *L. glandulosa* and is most closely related to populations of *L. glandulosa* growing on sandy nonserpentine soils a few kilometers away (Clausen et al., 1947; Gottlieb et al., 1985; Ford and Gottlieb, 1989, 1990; Baldwin, 2005). Moreover, molecular clock dating and the young age of the isolated South Coast Range serpentine exposures support a Quaternary origin of *L. discoidea* (Baldwin, 2005). It will be important to better understand the mechanisms of reproductive isolation between *L. discoidea* and nearby *L. glandulosa*. The two species are highly interfertile in artificial crosses but are presumably strongly isolated by ecological differences because there is no evidence of hybridization and gene flow (Baldwin, 2005). For these species, a prior period of large-scale allopatry is highly unlikely, but it is unclear how much spatial isolation was involved in their initial divergence. Whether they diverged, like *Leptosiphon*, across a contact zone that is no longer present or followed a stepping-stone or peripatric model may never be deciphered. Studies of the strength of different isolating mechanisms, like habitat and floral isolation, would better indicate the likelihood of clinal parapatric divergence.

Assigning speciation events an allopatric or parapatric label may impose an artificial dichotomy on what is essentially a continuum. The amount of spatial separation necessary to disrupt gene flow for divergence to proceed depends on the strength of disruptive selection and whether any selected traits confer reproductive isolation. With limited spatial isolation and strong disruptive selection on traits that pleiotropically confer reproductive isolation, speciation may occur rapidly. Serpentine plants have played and will continue to play a prominent role in biologists' attempts to understand the interplay between gene flow and selection in speciation.

FUTURE DIRECTIONS

Although the plants growing on serpentine have fascinated botanists for many decades, the work of connecting the patterns of endemism and adaptation to speciation processes has just begun. Much of what we understand comes from the well-studied flora of California; we know relatively little about the origin of serpentine taxa in other geographic regions, even though serpentine is a widespread phenomenon. Regions vary in the age and extent of serpentine exposures and in

the amount of endemism. For example, the relatively small island of New Caledonia has 1755 species of serpentine plants, whereas the relatively large island of Japan has only 50 endemics (reviewed in Anacker, 2010). California is on the endemic-rich side of the spectrum, and it is unclear how much of what we know from California applies across the broad range of serpentine regions. It will be important to conduct studies in other regions to understand the aspects of serpentine that promote and constrain neoendemism.

We also need to understand better the physiological and genetic basis of adaptation to serpentine to elucidate the trade-offs involved and the effects on reproductive isolation. How many and what types of mutations are involved, and what pleiotropic effects do they have on other plant traits? What are the exact physiological mechanisms that allow serpentine tolerance, and how do these affect the ability to live off serpentine? How do changes in these traits affect mating patterns? Prior studies of the trade-offs of serpentine tolerance are few, are limited taxonomically and geographically, and often involve transplants or ion addition treatments that do not address the effects of biotic interactions, such as competitors, herbivores, and pathogens, which are likely to be very important in the wild.

Some questions may best be answered in a comparative context. Does the strength of trade-offs to living on versus off of serpentine differ between tolerator and endemic species? This could be addressed in a comparative transplant study with multiple pairs of serpentine and nonserpentine sister taxa, with the expectation that endemics would show a greater cost to living on serpentine than tolerators. We could also ask whether the magnitude of trade-offs vary between types of serpentine habitats, that is, serpentine grasslands versus serpentine barrens or across a moisture gradient. Comparative studies could also better circumscribe the physiological or ecological traits that are coincident with serpentine tolerance or endemism, such as intrinsic growth rates, flowering time, or root depth, and whether these traits are typically plastic responses to the soil environment or genetically based.

Serpentine endemics may provide the best opportunity to address parapatric speciation models with real data. The many evolutionarily independent examples of serpentine endemism should make it possible to find all the different stages of the process of parapatric divergence and speciation. It may be most productive to further develop some model serpentine systems that are experimentally tractable and have extensive genetic tools. *Mimulus*, *Layia*, *Helianthus*, *Thlaspi*, *Lasthenia*, and *Leptosiphon* are all good candidates. All have genetic tools in development (or close relatives with genetic tools), their natural history is well documented, they are experimentally tractable, and they represent a range of stages of divergence and degrees of endemism. In these systems, it should be possible to determine the traits targeted by selection on serpentine soil, the effects of those traits on reproductive isolation, and the genetic and/or physiological basis of those traits.

The work on serpentine plant origins to date confirms the hypothesis that serpentine is a driver of speciation and that neoendemics make up an important portion of serpentine plant diversity. It will be important to protect as many serpentine patches as possible to conserve the evolutionary potential of serpentine plants, as each patch may be unique. We also know that it will be important to conserve these areas as natural laboratories for understanding general processes of adaptation and speciation.

Acknowledgments

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