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## Trends in the evolution of edaphic specialists with an example of parallel evolution in the *Lasthenia californica* complex

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**Abstract:** Adaptation to unusual soil conditions is a common phenomenon in plants, and closely related taxa are frequently distinguished by their edaphic tolerances. The biology of edaphic endemics further suggests that parallel evolution of edaphic adaptations is not unusual, and that such adaptations may often have effects on gene flow between derivative populations and their progenitors. These observations imply that the study of edaphic divergence is fertile ground for understanding the role of natural selection in adaptation and perhaps in speciation as well. Our work on the *Lasthenia californica* complex provides a case in point. Two races occupy distinct edaphic habitats throughout the range of the complex: race A occupies ionically extreme habitats, while race C occurs in ionically more moderate but drier sites. Phylogenetic data reveal that neither race is monophyletic, and further suggest that race C populations have originated multiple times from race A. Physiological data indicate consistent differences between the races in sodium tolerance, with race A having 20-fold greater sodium uptake rates than race C. Populations of each race also differ in  $\text{Ca}^{2+}/\text{Mg}^{2+}$  uptake, with race A having 2-fold higher uptake rates. Preliminary data further suggest that reproductive isolation is stronger between populations of different races than between populations of the same race. Taken together, these findings point to the development of parallel changes in reproductive compatibility accompanying parallel evolution of edaphic races in the *L. californica* complex. While this example represents one of the better documented cases of parallel adaptation in plants, our characterization of edaphic specialists suggests that these will likely provide further cases that will reveal the importance of edaphic adaptation in plant speciation.

*“Recurrent evolution of taxa not only is possible, but may not be unusual in complexes that have undergone ecological diversification.”* D.A. Levin (2001)

### Introduction

The study of adaptation marks a central focus in evolutionary biology, with one major thrust of these endeavours being to determine the relative importance of adaptive evolution in diversification. When putative adaptive traits have a conspicuous effect on reproductive isolation, e.g., traits that affect pollinator preferences (Schemske and Bradshaw 1999) or key innovations such as nectar spurs (Hodges and Arnold 1995), the link between adaptation and speciation is easiest to draw. However, less conspicuous features of organisms that have effects on fitness and are subject to natural selection may also contribute to speciation and diversification of lineages. For example, a number of plant species are known to occur only under specific, typically extreme soil conditions, being excluded from other substrates (Kruckeberg 2002). The range of extreme soils that harbour edaphic endemics includes hyper-saline soils such as vernal pools and alkaline flats, soils rich in heavy metals, such as mine tailings and serpentine soils, and nitrogen-rich sites such as guano deposits. Where edaphic endemics represent derived expansion and specialization onto these substrates, adaptation to specific soil conditions may have played a key role in diversification (Macnair 1987; Macnair and Gardner 1998). The link between edaphic adaptation and reproductive isolation may be achieved through various means, including direct impacts on reproductive isolation, for example via pleiotropy or linkage, and indirect effects, e.g., reinforcement, accumulation of pre- or post-zygotic isolating barriers in allopatry.

We wish to focus attention on the potential significance of edaphic divergence in plant speciation. We begin with a synopsis of some key aspects of the biology of edaphic specialists that contribute to their value as systems for the study of adaptation and speciation. We focus in particular on parallel adaptive shifts because these cases provide key opportunities to study the selective pressures and genetic mechanisms that contribute to the establishment of adaptation. In addition, cases of parallel adaptation provide the chance to study the relationship between adaptation and reproductive isolation with the benefit of natural replication. We then summarize our recent research characterizing edaphic specialization in the *Lasthenia californica* complex, which we believe is

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among the best documented cases of parallel evolution of edaphic specialization in plants. Although the *Lasthenia* work characterizes parallel adaptation in greater detail than has been done in many other systems, it seems clear that parallel shifts are likely a common outcome of edaphic specialization. Whether parallel adaptation is commonly accompanied by parallel reproductive isolation remains unclear, but further research in this area will likely provide valuable insights into the role of natural selection in diversification.

### The natural history of edaphic specialization

Most plant population biologists are familiar with the classic papers titled “*Evolution in closely adjacent plant populations*” of Bradshaw, McNeilly, Antonovics and co-workers (e.g., McNeilly 1968; McNeilly and Antonovics 1968; Antonovics and Bradshaw 1970) that focused primarily on the evolution of metal tolerance in populations on mine tailings. Much of the work was conducted on two species of grasses, *Anthoxanthum odoratum* and *Agrostis tenuis*, and provides a classic demonstration of the power of strong natural selection in maintaining distinct sub-populations despite the potential for gene flow (McNeilly 1968; McNeilly and Bradshaw 1968). These classic papers, along with subsequent work on mine tailings also illustrate two especially intriguing features of the biology of edaphic specialists that are relevant to speciation. First, edaphic specialists seem to have a tendency to evolve tolerance of specific conditions multiple times, that is there is a tendency towards parallel establishment of edaphic specialization. Whether this represents *de novo* evolution of alleles conferring specialization, or parallel increases in frequency of alleles that have arisen a single time remains unclear (see below). Second, edaphic shifts often have direct or indirect effects on patterns of reproductive isolation.

In the early characterization of mine tailing populations in *Agrostis* and *Anthoxanthum*, parallel evolution was suspected because of the geographical separation of various populations on mine tailings, and the widespread occurrence of non-tolerant populations (Gregory and Bradshaw 1965). Furthermore, copper tolerance was shown to be present at low frequencies in seed samples collected in separate populations of *Agrostis tenuis* growing on non-contaminated soils, pointing to the likely parallel establishment of the trait in isolated localities (McNeilly and Bradshaw 1968). The hypothesis of parallel evolution of metal tolerance in *Agrostis tenuis* has since been further supported by analysis of genotype  $\times$  environment interaction ( $G \times E$ ) in copper tolerant populations from distinct localities, which reveals that copper tolerant populations display significant heterogeneity in their response to levels of copper (Nicholls and McNeilly 1982). While these patterns may reflect distinct origins of copper tolerance, they could also reflect the presence of unique modifiers of a common mechanism of tolerance that in fact had a single origin (Schat et al. 1996).

In much of the literature discussing the origins of metal tolerance, the geographical distribution of metal-tolerant populations again provides the primary indication of parallel origins. While this is a reasonable hypothesis, it is also possible that tolerant populations have been established by long-distance dispersal, perhaps aided by accidental transport by

mine workers (Schat et al. 1996). Further evidence of multiple origins comes from crossing studies that examine patterns of segregation of tolerance that would indicate the action of multiple independent loci. The action of independent loci would be revealed either by a breakdown of metal tolerance in the F2 generation in crosses between homozygous tolerant individuals, but might also be suggested by the presence of transgressive segregation in the F1 generation. To date, such tests tend to support a common genetic basis for tolerance, though the presence of modifiers has been noted in some populations. For example, in *Silene vulgaris*, crosses among copper, zinc and cadmium tolerant and non-tolerant populations from Germany and Ireland reveal that a single locus is responsible for tolerance to each metal, though at least one additional locus contributes to additional copper tolerance in one population (Schat et al. 1993). In this case, the modifier could have arisen subsequent to the establishment of the widespread tolerant genotype at this site. While a common genetic basis for tolerance could be regarded as evidence against parallel evolution of tolerance, studies of the genetic basis of metal tolerance suggest that constraints may limit the number of loci that can successfully mutate to yield tolerant genotypes. Therefore it is possible that crossing studies fail to show a breakdown of tolerance because mutation of the same genetic locus has given rise to tolerant genotypes independently. Finally, it should be noted that even if the same allele is responsible for tolerance in distinct populations, this allele may have been present at low frequencies in non-tolerant populations and thus may have become established in parallel at multiple localities (Schat et al. 1996). In such cases, parallel evolution of metal tolerance could be indicated by phylogenetic data that support multiple origins of tolerant populations. Where phylogenetic studies of edaphically distinct populations have been conducted, it is noteworthy that these uniformly point to parallel evolution.

Under the extreme conditions presented by severely metal-contaminated sites, the role of natural selection in establishing tolerance is understood, given that non-tolerant individuals generally do not survive the mine tailing environment. In less dramatic cases, the pattern of parallel evolution itself is suggestive of the action of natural selection, because parallel establishment of ecologically relevant traits is unlikely to occur as a result of stochastic processes (Levin 2001). For example, in previous work on *Lasthenia californica*, analysis of isozyme variation (Desrochers and Bohm 1998) and phylogenetic data suggested the existence of geographically-based subdivisions within the complex. Analysis of chemical characteristics of soils and plant tissue demonstrated the existence of edaphic races and suggested that divergent natural selection might play a role in diversification of the races (Rajakaruna and Bohm 1999). Combining these data has revealed a pattern of parallel evolution of an ecologically relevant suite of traits (Rajakaruna et al. 2003c), which serves as evidence for the action of natural selection in establishing racial differences. Given that relatively few systems have been explored and that many of these provide some indication of parallel evolution, this tendency would seem to be a common characteristic of edaphic specialists.

A phenomenon that may be related to parallel evolution of tolerance is the evolution of multiple tolerances within spe-

cies. In many cases, lineages that have evolved tolerance to one edaphic extreme have also adapted to other extreme factors. For example, tolerance of multiple populations to four elements (copper, nickel, zinc and lead) was assessed (Gregory and Bradshaw 1965) in *Agrostis tenuis*. Although most populations occur on pasture soils that have trace levels of heavy metals and comprise plants that are not tolerant of heavy metal contamination, mine tailing populations variously display tolerance to zinc, copper and lead, generally matching their tolerance to levels present in soils at the collection locality (Gregory and Bradshaw 1965). The authors also demonstrated that tolerance of each of these metals did not confer tolerance to the others, indicating somewhat distinct mechanisms in each case. Still, that this species has evolved tolerance to normally toxic levels of multiple ions suggest that an underlying trait, perhaps involving tolerance of low pH (Gregory and Bradshaw 1965), drought (Hughes et al. 2001) or hyper saline conditions is widespread in the progenitors of edaphic specialists and contributes to their ability to evolve tolerance to specific ionic extremes. It seems plausible that such underlying traits could facilitate either the evolution of multiple tolerances or the parallel evolution of tolerance of a single heavy metal.

The second intriguing feature of edaphic endemics is the relationship between the shift in edaphic tolerance and changes in patterns of reproductive isolation. Such changes may arise as a direct consequence of adaptive shifts, either because reproductive compatibility is a by-product of physiological adaptation to new edaphic conditions or because of linkage or pleiotropy of loci affecting ecological shifts and those affecting reproductive isolation. Alternatively, enhanced reproductive isolation between divergent edaphic specialists may reflect the action of reinforcement, i.e., selection for reduced gene flow to avoid maladaptive hybridization following a period of divergence in allopatry. It is important to note that the two alternatives are not mutually exclusive. For instance, in the classic mine tailing studies, heavy metal tolerant and non-tolerant populations of both *Anthoxanthum odoratum* and *Agrostis tenuis* were shown to have genetically-controlled differences in flowering time (McNeilly and Antonovics 1968), with tolerant populations flowering earlier in both cases. Plants closest to the mine boundary showed the greatest difference in flowering times, which McNeilly and Antonovics (1968) interpret as evidence for the action of reinforcement, though they state that a portion of the flowering time shifts also arises as a by-product of adaptation to local conditions. They noted a relationship between flowering time and soil temperatures to support this claim. It is likely that changes in phenology can also evolve as a direct by-product of shifts in edaphic tolerances. For example, flowering time differences are associated with differences in sodium accumulation in wheat (Taeb et al. 1992). Furthermore, even if the initial divergence in flowering time is entirely environmentally-determined, this pattern can contribute to the accumulation of genetically-based flowering time differences. Stam (1983) demonstrated theoretically that spatially structured differences in flowering time can lead to spatially-coincident, genetically-based differences in flowering time. Of course as different edaphic habitats are almost by necessity spatially isolated from one another, individuals that occur in distinct edaphic environ-

ments are likely to experience decreased gene flow relative to individuals that occur in the same habitat (L. H. Rieseberg, pers. comm.). Another tendency that has been repeatedly observed in edaphic endemics is a shift towards increasing self-fertility. Increased selfing rates are thought to have arisen as a mechanism to prevent gene flow between mine and non-mine populations in both *Anthoxanthum odoratum* and *Agrostis tenuis* (Antonovics 1968), though this explanation was not favored to explain increased self-fertility in *Armeria maritima* populations from zinc mines (Lefèbvre 1970). Lefèbvre (1970) instead interprets increased self-fertility as having provided reproductive assurance during long distance colonization of mine sites, as there are no adjacent non-mine populations in this region.

Examples of changes in post-mating reproductive isolation accompanying edaphic shifts are less common, as might be expected given that these are generally more likely to accumulate as a by-product of divergence. However, post-mating reproductive isolation can include mechanisms that act prior to zygote formation, such as pollen-pistil incompatibilities. Such post-mating, pre-zygotic isolation can be subject to reinforcement, and thus may be an indirect outcome of adaptive divergence. The most widely cited example of post-mating reproductive isolation associated with edaphic shifts comes from populations of *Mimulus guttatus* adapted to copper mine tailings. Macnair and co-workers have documented that the genetic locus that confers copper tolerance is closely linked to or has a pleiotropic effect on viability of hybrids between tolerant and non-tolerant individuals (Christie and Macnair 1983). Searcy and Macnair (1990) also demonstrated that copper uptake may contribute to pollen pistil incompatibilities, suggesting that crossing studies involving extremes should perhaps be conducted.

### **The case for parallel evolution of edaphic races in the *Lasthenia californica* complex**

*Lasthenia californica* DC. ex Lindl. *sensu* Ornduff (1966; 1993), the common goldfields of California, provides an ideal system for the study of parallel speciation driven by edaphic forces. Common goldfields, as previously delimited, displays the widest range of edaphic tolerances within the genus, occupying diverse habitats within the Californian Floristic Province (Ornduff 1966; 1993). Despite the range of habitats occupied by populations within the complex, a survey of soil features and elemental composition of plants (Rajakaruna and Bohm 1999) concluded that flavonoid races previously described for the complex (Bohm et al. 1989; Desrochers and Bohm 1993) correspond to edaphic races. Race A plants predominate in ionically-extreme habitats such as coastal bluffs, alkaline flats, vernal pools, and serpentine outcrops; these plants are characterized by the presence of sulfated flavonoids (Bohm et al. 1989). Race C plants are found in ionically-benign but drier sites such as roadside pastures and oak-woodlands; these plants lack the sulfated flavonoids characteristic of race A. Interestingly, the two races grow in parapatry on a serpentine outcrop at Jasper Ridge Biological Preserve (Stanford University, San Mateo County, CA), but at this site, each race occupies microhabitats that correspond to the edaphic conditions that distinguish the races throughout the range of the complex.



Given that we suspect that selection has contributed to the establishment and maintenance of the patterns of distribution of the two races, both at Jasper Ridge and throughout the species range, we initiated a study to examine relationships among populations of the complex. The major findings, outlined below substantiate the role of selection and point to parallel origins of at least one of the edaphic races.

Traits and taxa can evolve in parallel by means other than natural selection. For example, multiple independent origins have been documented for numerous plant polyploids and for diploid hybrid taxa. Here, we are concerned with parallel evolution *sensu* Schluter and Nagel (1995) and Levin (2001), implying the action of natural selection. Levin (2001) suggests two criteria that are necessary to demonstrate parallel evolution of a taxon. First, populations of the descendent taxon must be phylogenetically independent. Second, the shared characteristics must be the products of natural selection. Where parallel evolution results in parallel reproductive isolation, parallel speciation is demonstrated. Schluter and Nagel (1995) provide two additional criteria necessary to document parallel speciation. The third criterion states that separate descendant populations that are found in similar environments must be reproductively isolated from the ancestral populations. Finally, the separate descendant populations must not be reproductively isolated from one another. Few cases exist in which all four criteria have been addressed (Schluter and Nagel 1995) and Levin (2001) indicates that there are almost no unassailable examples of parallel speciation in the plant literature. We will present evidence to address each of these four criteria in *Lasthenia californica*. While the evidence is not complete in each case, in our opinion, it clearly demonstrates parallel evolution, and points toward parallel speciation. We will note areas in need of further corroboration and outline our plans for additional studies.

#### Evidence of parallel origins of edaphic races in the *Lasthenia californica* complex

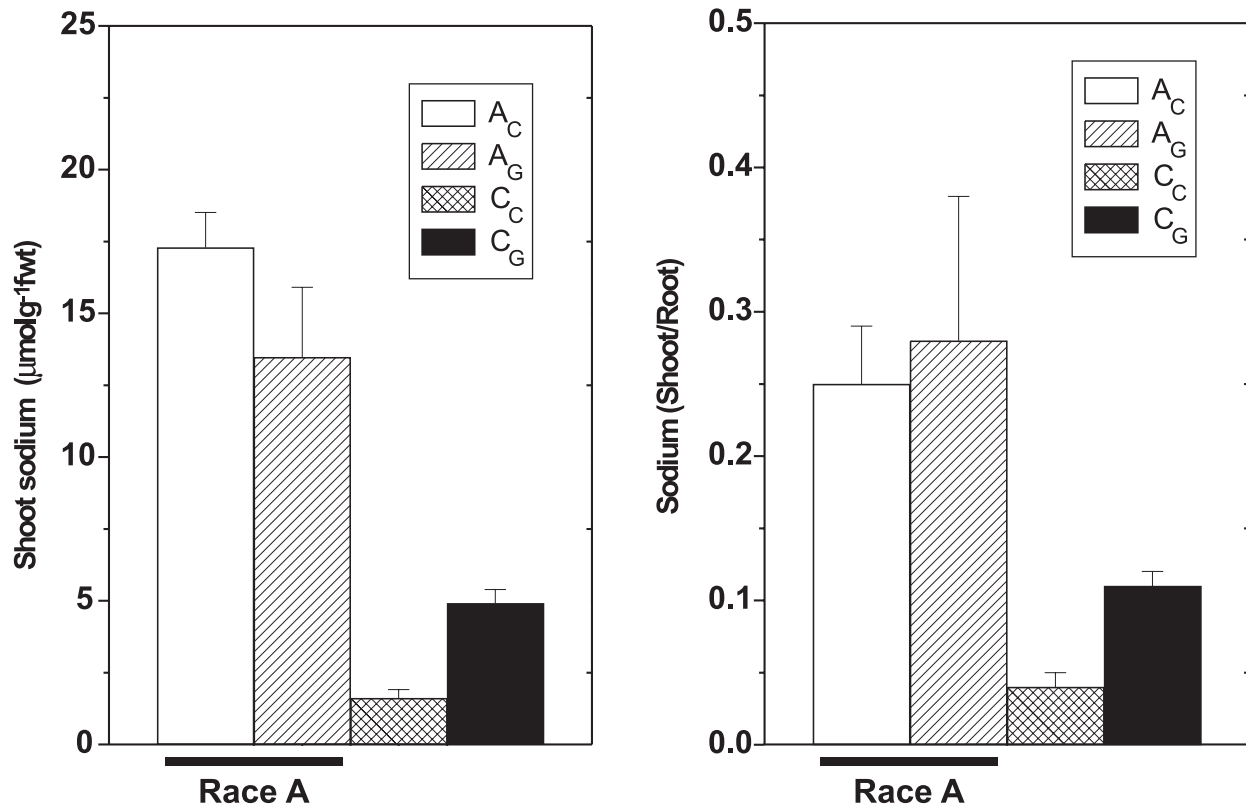
Based on a comprehensive ITS/ETS/cpDNA phylogenetic study, Chan et al. (2001; 2002) found that *L. californica sensu* Ornduff represents two geographically-based, non-sister clades. Chan (2001) and Chan et al. (2002) recognized the clades as two cryptic taxa, *L. californica* subsp. *californica* representing the northern clade and *L. gracilis* DC. (Greene) representing the southern clade. The molecular phylogeny allowed us to examine the relationship of the edaphic races to the newly recognized phylogenetic taxa. If ecological selection has played a role in the origin of edaphic races, then similar edaphic tolerances may have evolved in parallel within one or both of the phylogenetic taxa. Indeed, a previous study of allozyme variation (Desrochers and Bohm 1995) indicated that single populations of race A and C cluster with sets of populations belonging to the alternative race. We determined the edaphic race and phylogenetic affinities of 33 populations from throughout the range of the complex. Flavonoid profiles were used to assign populations of Chan et al. (2001, 2002) to edaphic races and characterization of nuclear ribosomal ITS regions of several representatives from the two edaphic races were used to determine phylogenetic affinities (Rajakaruna et al. 2003c).

Examination of flavonoid profiles and ITS sequences revealed that edaphic races are not concordant with the newly circumscribed taxa, *L. californica* subsp. *californica* and *L. gracilis*. Of the 16 populations of *L. gracilis*, 13 were race A and 3 were race C. Of the 17 populations of *L. californica* subsp. *californica*, 11 were race C and 6, race A. Furthermore, a distance-based analysis of RAPD variation (Rajakaruna 2002) rooted using the information from phylogenetic markers indicates that race A is most likely ancestral in the complex, resulting in an inference of parallel origin of race C in each of the two clades. This further implies that the sulfated flavonoid that is diagnostic for race A was lost independently in derived populations of Race C.

#### Parallel physiological adaptation

Schluter and Nagel (1995) and Levin's (2001) second criterion aims at establishing that natural selection is the cause of parallel origins: an adaptive mechanism must be identified and tested. In the original characterization of the races (Rajakaruna and Bohm 1999) two chemical factors that most strongly distinguished both the habitats and the plant tissue elemental composition of races were the levels of sodium and magnesium. Race A plants occur in soils averaging 60.8 ppm sodium and 1147 ppm magnesium, while race C sites average 19.9 ppm and 280.6 ppm of each element, respectively. Further examination of the relationship between tissue and soil sodium levels from the two races at Jasper Ridge suggested that the two races differ in their sodium and magnesium uptake physiologies (Rajakaruna and Bohm 1999). Because these differences are likely to reflect differential adaptation to edaphic conditions, we conducted a hydroponic study of sodium uptake physiology in two populations of each race, including phylogenetically divergent populations of both races. Mean Na<sup>+</sup> uptake rates as well as tissue accumulation patterns for Na<sup>+</sup> were estimated. Results suggest that race A plants from both lineages are better tolerant of sodium: Na<sup>+</sup> uptake rates of race A plants were 20-fold higher than those of race C plants. Further, race A plants translocated ~50% of absorbed Na<sup>+</sup> to the shoot compared with <30% in race C, and thus, when combined with higher uptake rates, race A plants were able to accumulate ~5 fold greater concentrations of Na<sup>+</sup> in the above-ground tissues than race C plants. Race C plants from each of the distinct phylogenetic lineages restricted translocation of Na<sup>+</sup> to shoots, a mechanism often used by plants intolerant of toxic elements (Fitter and Hay, 1987). Figure 1 shows the total shoot accumulation as well as shoot/root ratio of Na<sup>+</sup> in race A and C populations from both taxa. The higher shoot concentrations of Na<sup>+</sup> found in race A plants suggest that a similar mechanism may be involved in conferring tolerance to this potentially toxic cation. In the case of Na<sup>+</sup>, tolerant species are able to maintain higher concentrations in the shoot and sequester the ions in the vacuole (Amtmann and Sanders, 1999) via Na<sup>+</sup>/H<sup>+</sup> antiporters (Apse et al. 1999; Blumwald et al. 2000). We plan to investigate whether the presence/absence or level of expression of the Na<sup>+</sup>/H<sup>+</sup> antiporter gene is responsible for the differences in uptake and accumulation of Na<sup>+</sup> in the two races. Such a study will be essential to address the underlying biochemical/genetic basis of these traits. Germination, root growth, and survivorship

**Fig. 1.** Total shoot sodium (left) and shoot/root sodium (right) for race A and C populations belonging to *L. californica* subsp. *californica* ( $A_C$  and  $C_C$ , respectively) and *L. gracilis* ( $A_G$  and  $C_G$ , respectively). Race A populations of both species allocate significantly more sodium to shoot than race C populations.



estimates also indicated greater tolerance by race A to  $\text{Na}^+$  (Rajakaruna et al. 2003a). Further, significant Genotype  $\times$  Treatment interactions were observed for all tolerance measures, suggesting that these races are genetically differentiated in their tolerance responses.

As described above, results of population genetic studies suggest that race A is ancestral in the complex and therefore both the sulfated flavonoids and  $\text{Na}^+$  tolerance have been lost in race C populations. While the genetic basis of these differences and their correlation with habitats of race C suggest that they contribute to differential adaptation, it is not clear how the loss of these traits is adaptive, unless they result in a relative fitness cost under the conditions in which race C occurs. This hypothesized cost of tolerance seems plausible, and seems to agree with the results of a drought stress experiment that we conducted using seed from race A and race C populations at Jasper Ridge (Rajakaruna et al. 2003b).

At Jasper Ridge (and throughout the range of the complex), race C populations occur in ionically-benign and water stressed environments (Rajakaruna and Bohm 1999), while race A occurs in ionically challenging but wetter sites. We examined the relative fitness of individuals of the two races under varying levels of drought stress. We conducted an experiment in potting soil in the greenhouse that showed that race C plants from the Jasper Ridge site are more drought tolerant than race A plants from this site (Rajakaruna et al. 2003b). Race C plants reach reproductive matu-

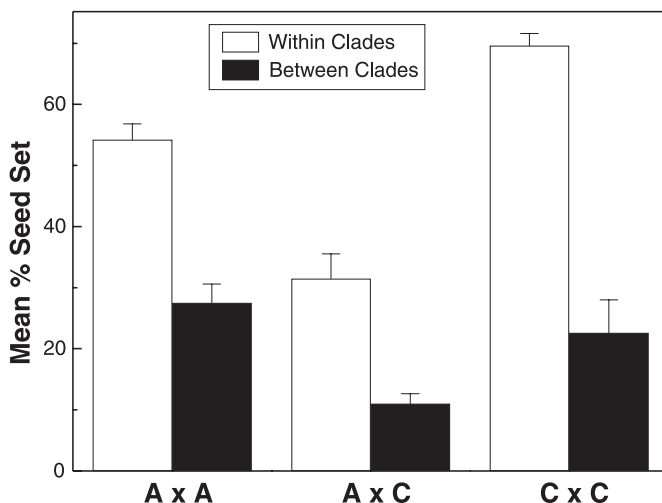
rity faster and have greater reproductive fitness under drought conditions. It is unclear at this stage whether drought tolerance is unique to all race C populations, however it seems likely given the water-stressed nature of their edaphic habitats.

The coincident loss of both sulfated flavonoids and traits responsible for  $\text{Na}^+$  tolerance is of interest in light of the possible link between these traits (Barron et al. 1988). Sulfated flavonoids are often found in plants growing under saline conditions and we recently hypothesized that there may be an ecologically related adaptive mechanism linking their presence to salt tolerance (Rajakaruna et al. 2003c). Hence, it is possible to envision the loss of these potentially linked traits in race C populations not exposed to salinity or other ionic stresses. The loss of these traits combined with a gain in tolerance to water stress is plausible under the known climatic and edaphic shifts that have taken place in California (Howard 1951). Ostensibly, a shift from wet, saline conditions to dry, non-saline habitats could have led to a relaxation of selection on traits conferring salt tolerance while selecting for traits such as early flowering and lower root/shoot ratios that confer drought tolerance. Further, studies have shown that salt tolerance may have a pleiotropic effect on early flowering (Taeb et al. 1992). This study shows a possible link between differential adaptive traits observed for the two races in the *L. californica* complex: early-flowering and  $\text{Na}^+$ -intolerance in race C and later-flowering and  $\text{Na}^+$ -tolerance in race A.

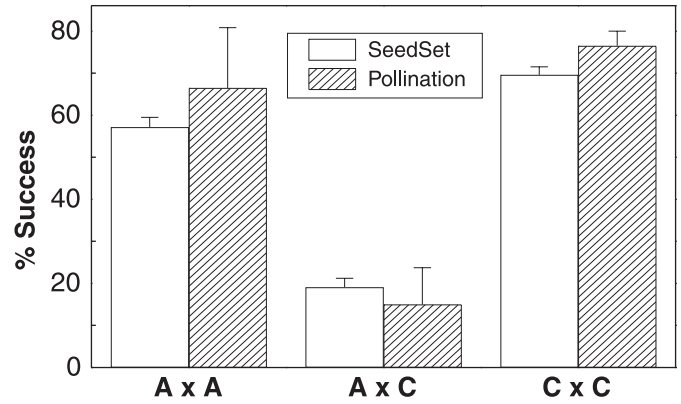
### The case for parallel evolution of reproductive isolation

The third and fourth criteria of Schluter and Nagel (1995) are critical to demonstrate parallel speciation. Although parallel evolution is common (Schluter and Nagel 1995; Levin 2001), parallel evolution of traits conferring reproductive isolation, i.e., parallel speciation, is uncommon in the plant literature (Levin 2001). While examples of parallel evolution provide ideal setting for the study of the action of natural selection in divergence, a causative link between parallel adaptation and parallel speciation need not exist. In fact, it is easy to imagine that a link between parallel adaptation and reproductive isolation could lead to multiple independently derived units each reproductively isolated from one another. However, where cases of parallel evolution are documented, we have the opportunity to examine the link between adaptation and reproductive isolation, as we have attempted to do in the *Lasthenia californica* complex. The parallel occurrence of physiologically distinct races in divergent lineages allowed us to test for reproductive isolation between the races. In order to determine the extent of reproductive isolation between the races, seven populations were used to estimate levels of crossability between the two races within and between the phylogenetic lineages (Rajakaruna 2002). The three *L. californica* subsp. *californica* populations consisted of 2 race A populations and 1 race C population while the four *L. gracilis* populations consisted of 3 race A populations and 1 race C population. Seed set per cross was estimated by counting the number of cypselae that were full and dark (prior germination studies from field-collected cypselae have shown that the two features are good indications of viability) and then averaging the number from two heads used in reciprocal crosses. Figure 2 shows intra- and inter-racial seed set within and between the two phylogenetic lineages. The column representing the race C  $\times$  C within-clade cross includes only intra-population crosses (only 1 race C population from each species was used) and thus should be interpreted with caution. Nevertheless, reproductive isolation

**Fig. 2.** Mean percent seed set within and between race A and C plants belonging to both phylogenetic species (clades). Inter-racial seed set is significantly lower than intra-racial crosses both within and between clades.



**Fig. 3.** The relationship between pollination success and seed set in experimental crosses within and between races of *Lasthenia californica*. Most of the isolation comes about by pollen incompatibility reactions, i.e., isolation between races both within and between clades is determined primarily by a prezygotic and postmating mechanism.



appears to be strongest in inter-racial crosses both within and between clades. According to theory, if reproductive isolation is evolving as a direct or indirect consequence of edaphic specialization, we would predict greater reproductive compatibility between independently derived populations of race C than between race C populations and their closely related race A counterparts. Our preliminary observations are consistent with this prediction. Examination of pollen tube growth following experimental crossing (Fig. 3) reveals that the mean pollination success of race C populations with race A populations from the same clade is 5%, while the success rate of crosses between race C populations of distinct origins is 40% (Rajakaruna and Whitton, unpublished). We must caution though, that we consider these findings preliminary, and in particular we note that the patterns of interpopulation crossability are highly variable and will require careful study to tease apart the contributions to reproductive isolation of divergent selection on edaphic tolerances versus other effects. Nonetheless, our observed trends corroborate the final two criteria of Schluter and Nagel (1995), i.e., descendent populations must be reproductively isolated from ancestral populations, and separate descendent populations must not be reproductively isolated.

The role of parallel evolution in the origin of species is at an early stage of discovery and we believe that our studies identify a system that can be used as a model for the study of parallel speciation in plants. Ongoing studies are aimed at providing additional supportive evidence to further our hypothesis of parallel speciation of edaphic races in the *L. californica* complex.

Furthermore, our findings from *Lasthenia californica* must not be taken in isolation, as our review of the literature leads to the hypothesis that parallel evolution may be relatively common outcome of edaphic divergence, and that edaphic divergence can have effects on reproductive isolation that could lead to parallel speciation. Thus, these systems are ideal settings in which to study the link between adaptation and speciation.

## Acknowledgements

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## References

- Allan, W.R., and Sheppard, P.M. 1971. Copper tolerance in some California populations of the monkey flower, *Mimulus guttatus*. Proc. R. Soc. Lond. B Biol. Sci. **177**: 177–196.
- Amtmann, A., and Sanders, D. 1999. Mechanisms of Na<sup>+</sup> uptake by plant cells. Adv. Bot. Res. **29**: 76–112.
- Antonovics, J. 1968. Evolution in closely adjacent plant populations. V. Evolution of self-fertility. Heredity **23**: 219–238.
- Antonovics, J., and Bradshaw, A.D. 1970. Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. Heredity **25**: 349–362.
- Apse, M.P., Aharon, G.S., Snedden, W.A., and Blumwald, E. 1999. Salt tolerance conferred by overexpression of a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter in *Arabidopsis*. Science (Washington, D.C.) **285**: 1256–1258.
- Barron, D., Varin, L., Ibrahim, R.K., Harborne, J.B., and Williams, C.A. 1988. Sulfated flavonoids — an update. Phytochemistry **27**: 2375–2395.
- Blumwald, E., Aharon, G.S., and Apse, M.P. 2000. Sodium transport in plant cells. Biochim. Biophys. Acta **1465**: 140–151.
- Bohm, B. A., Herring, A., Nicholls, K.W., Bohm, L.R., and Ornduff, R. 1989. A six-year study of flavonoid distribution in a population of *Lasthenia californica* (Asteraceae). Am. J. Bot. **76**: 157–162.
- Chan, R., Baldwin, B.G., and Ornduff, R. 2001. Goldfields revisited: A molecular phylogenetic perspective on the evolution of *Lasthenia* (Compositae: Heliantheae *sensu lato*). Int. J. Plant Sci. **162**: 1347–1360.
- Chan, R., Baldwin, B.G., and Ornduff, R. 2002. Cryptic goldfields: A molecular phylogenetic re-investigation of *Lasthenia californica sensu lato* and close relatives (Compositae: Heliantheae *sensu lato*). Am. J. Bot. **89**: 1103–1112.
- Christie, P., and Macnair, M.R. 1983. Complimentary lethal factors in two North American populations of the yellow monkey-flower. J. Hered. **75**: 510–511.
- Desrochers, A.M., and Bohm, B.A. 1993. Flavonoid variation in *Lasthenia californica*. Biochem. Syst. Ecol. **21**: 449–453.
- Desrochers, A.M., and Bohm, B.A. 1995. Biosystematic study of *Lasthenia californica* (Asteraceae). Syst. Bot. **20**: 65–84.
- Fitter, A.H., and Hay, R.K.M. 1987. Environmental Physiology of Plants. Second Edition. Academic Press, London.
- Gregory, R.P.G., and Bradshaw, A.D. 1965. Heavy metal tolerance in populations of *Agrostis tenuis* Sibth. and other grasses. New Phytol. **64**: 131–143.
- Hodges, S.A., and Arnold, M.L. 1995. Spurring plant diversification: are floral nectar spurs a key innovation? Proc. R. Soc. Lond. B Biol. Sci. **262**: 343–348.
- Howard, A.D. 1951 The development of the landscape of the San Francisco Bay counties. California Divisions Mines Bull. **154**: 95–106.
- Hughes, R., Bachmann, K., Smirnoff, N., and Macnair, M.R. 2001. The role of drought tolerance in serpentine tolerance in the *Mimulus guttatus* Fischer ex DC. complex. S. Afr. J. Sci. **97**: 581–586.
- Kruckeberg, A.R. 2002. Geology and plant life: the effects of landforms and rock types on plants. University of Washington Press, Seattle, WA.
- Lefèbvre, C. 1970. Self-fertility in maritime and zinc mine populations of *Armeria maritima* (Mill.) Willd. Evolution **24**: 571–577.
- Levin, D.A. 2001. The recurrent origin of plant races and species. Syst. Bot. **26**: 197–204.
- Macnair, M.R. 1987. Heavy metal tolerance in plants: a model evolutionary system. Trends Ecol. Evol. **2**: 354–359.
- Macnair, M.R., and Gardner, M. 1998. The evolution of edaphic endemics. In Endless forms: species and speciation. Edited by D.J. Howard and S.H. Berlocher. Oxford University Press, New York. pp. 157–171.
- McNeilly, T. 1968. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. Heredity **23**: 99–108.
- McNeilly, T., and Antonovics, J. 1968. Evolution of closely adjacent plant populations. IV. Barriers to gene flow. Heredity **23**: 205–218.
- McNeilly, T., and Bradshaw, A.D. 1968. Evolutionary processes in populations of copper tolerant *Agrostis tenuis*. Evolution **22**: 108–118.
- Nichols M.K., and McNeilly, T. 1982. The possible polyphyletic origin of copper tolerance in *Agrostis tenuis* (Gramineae). Plant Syst. Evol. **140**: 109–117.
- Ornduff, R. 1966. A biosystematic survey of the Goldfield genus *Lasthenia* (Compositae: Helenieae). Univ. California Publ. Bot. **40**: 1–92.
- Ornduff, R. 1993. *Lasthenia*. In The Jepson Manual: Higher Plants of California. Edited by J.C. Hickman. University of California Press, Berkeley, CA. pp. 298–300.
- Rajakaruna, N. 2002. Evolution in the *Lasthenia californica* complex (Asteraceae). Ph.D. thesis, Botany, University of British Columbia, Vancouver, BC.
- Rajakaruna, N., and Bohm, B.A. 1999. The edaphic factor and patterns of variation in *Lasthenia californica* (Asteraceae). Am. J. Bot. **86**: 1576–1596.
- Rajakaruna, N., Siddiqi, M.Y., Whitton, J., Bohm, B.A., and Glass, A.D.M. 2003a. Differential responses to Na<sup>+</sup>/K<sup>+</sup> and Ca<sup>2+</sup>/Mg<sup>2+</sup> in two edaphic races in the *Lasthenia californica* complex (Asteraceae). New Phytol. **157**: 93–103.
- Rajakaruna, N., Bradfield, G.E., Bohm, B.A., and Whitton, J. 2003b. Adaptive differentiation in response to water stress by edaphic races of *Lasthenia californica* (Asteraceae). Int. J. Plant Sci. **164**: 371–376.
- Rajakaruna, N., Baldwin, B.G., Chan, R., Desrochers, A.M., Bohm, B.A., and Whitton, J. 2003c. Edaphic races and phylogenetic taxa in the *Lasthenia californica* complex (Asteraceae: Heliantheae): an hypothesis of parallel evolution. Mol. Ecol. **12**: 1675–1679.
- Schat, H., Kuiper, E., Ten Bookum, W.M., and Vooijs, R. 1993. A general model for the genetic control of copper tolerance in *Silene vulgaris*: evidence from crosses between plants from different tolerant populations. Heredity **70**: 142–147.
- Schat, H., Vooijs, R., and Kuiper, E. 1996. Identical major gene loci for heavy metal tolerances that have independently evolved in different local populations and subspecies of *Silene vulgaris*. Evolution **50**: 1888–1895.
- Schemske, D.W., and Bradshaw, H.D., Jr. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. U.S.A. **96**: 11910–11915.
- Schluter, D., and Nagel, L.M. 1995. Parallel speciation by natural selection. Am. Nat. **146**: 292–301.
- Searcy, K.B., and Macnair, M.R. 1990. Differential seed production in *Mimulus guttatus* in response to increasing concentrations of copper in the pistil by pollen from copper tolerant and sensitive sources. Evolution **44**: 1424–1435.



Stam, P. 1983. The evolution of reproductive isolation in closely adjacent plant populations through differential flowering time. *Heredity* **50**: 105–118.

Taeb, M., Koebner, R.M.D., Forster, B.P., and Law, C.N. 1992. Association between genes controlling flowering time and shoot sodium accumulation in the Triticeae. *Plant Soil* **146**: 117–121.