

INDIVIDUAL AND COMBINED EFFECTS OF Ca/Mg RATIO AND WATER ON TRAIT EXPRESSION IN *MIMULUS GUTTATUS*

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Abstract. Low Ca/Mg ratios (a defining component of serpentine soils) and low water environmental conditions often co-occur in nature and are thought to exert strong selection pressures on natural populations. However, few studies test the individual and combined effects of these environmental factors. We investigated the effects of low Ca/Mg ratio and low water availability on plant leaf, stem, stolon, and floral traits of *Mimulus guttatus*, a bodenvag species, i.e., a species that occurs in serpentine and non-serpentine areas. We quantified genetic variation and genetic variation for plasticity for these leaf, stem, stolon, and floral traits at three hierarchical levels: field-habitat type, population, and family, and we evaluated the relative importance of local adaptation and plasticity. We chose two populations and 10 families per population from four distinct field “habitat types” in northern California: high Ca/Mg ratio (non-serpentine) and season-long water availability, high Ca/Mg ratio and seasonally drying, low Ca/Mg ratio (serpentine) and season-long water availability, and low Ca/Mg ratio and seasonally drying. Seedlings were planted into greenhouse treatments that mimicked the four field conditions. We only detected genetic variation for stem diameter and length of longest leaf at the field-habitat level, but we detected genetic variation at the family level for nearly all traits.

Soil chemistry and water availability had strong phenotypic effects, alone and in combination. Our hypothesis of an association between responses to low water levels and low Ca/Mg ratio was upheld for length of longest leaf, stem diameter, corolla width, and total number of reproductive units, whereas for other traits, responses to Ca/Mg ratio and low water were clearly independent. Our results suggest that traits may evolve independently from Ca/Mg ratios and water availability and that our focal traits were not simple alternative measures of vigor. We found genetic variation for plasticity both at the field-habitat type and family levels for half of the traits studied. Phenotypic plasticity and genetic variation for plasticity appear to be more important than local adaptation in the success of these *M. guttatus* populations found across a heterogeneous landscape in northern California. Phenotypic plasticity is an important mechanism maintaining the broad ecological breadth of native populations of *M. guttatus*.

Key words: calcium to magnesium ratio; Ca/Mg ratio; drought; genetic variation; habitat heterogeneity; local adaptation; *Mimulus guttatus*; phenotypic plasticity; serpentine.

INTRODUCTION

Across a natural landscape, many aspects of the environment are heterogeneous, and these complex patterns of heterogeneity contribute to observed species distributions and to variation in trait expression. Two factors, which are not mutually exclusive, have been advanced to explain the maintenance of variation in plant performance in heterogeneous environments. First, phenotypic plasticity and the maintenance of genetic variation for plasticity can allow taxa to persist in multiple environments (e.g., Schlichting and Pigliucci 1998, Donohue 2003). Second, populations may become

locally adapted when they occupy distinct ecological habitats that are sufficiently spatially distant from one another to retard gene flow (e.g., Silander and Antonovics 1979, Schemske and Bradshaw 1999, Galloway and Fenster 2000). Local adaptation may also lead to speciation (e.g., Macnair and Gardner 1998, Schluter 1998, Rajakaruna and Whitton 2004).

Two critical components of environmental heterogeneity for plants are soil mineral composition and water availability. In particular, scattered in distribution throughout the world, ultramafic (serpentine) influenced soils are extreme soil–rock systems characterized by high magnesium content in relation to calcium (low Ca/Mg ratio), low nutrients, and often, high heavy metal concentrations (Kruckeberg 1954). The texture of serpentine soils is also characteristically associated with low water availability (e.g., Whittaker 1954, Hull and Wood 1984, Rajakaruna and Bohm 1999, Rajakaruna et al. 2003b). Nevertheless, serpentine influenced habitats

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vary broadly in both mineral composition and water availability (Brooks 1987, Proctor and Nagy 1992, Hughes et al. 2001, Berglund et al. 2004). Extreme water and mineral contents of soils can act to restrict or exclude certain species or subspecies from particular areas, whereas other endemic species may become locally adapted to those environments (e.g., Antonovics et al. 1971, Brooks 1987, Macnair 1987, Sultan and Bazzaz 1993, Harrison 1997, 1999, Rajakaruna et al. 2003a). In contrast, some taxa have distributions that span multiple environmental conditions (Fritas and Mooney 1996). Together, soil chemistry and water conditions may act as strong selection pressures, shaping the distribution, trait expression, and fitness of local plant taxa. As the effects of Ca/Mg ratio and water availability have not been examined simultaneously, it is unclear how these factors independently or in combination are influencing the evolution of plant populations.

Two recent studies have documented phenotypic plasticity and genetic variation for phenotypic plasticity of serpentine taxa to competitive environments (Jurjavcic et al. 2002) and across a watering gradient (Rajakaruna et al. 2003a) for vigor and fitness traits. Examinations of plant responses to serpentine soils (e.g., Kruckeberg 1967, Proctor 1971, Freitas and Mooney 1996, Rajakaruna et al. 2003a, b) have collected data primarily on overall plant size or vigor, and demonstrated reduced performance in serpentine habitats. However, populations persisting in serpentine habitats have characteristics that are typically also associated with water stress situations, such as reduced leaf size (Proctor 1971, Lello 1995, Taylor and Levy 2002), reduced leaf dissection (Gardner and Macnair 2000, Iturralde 2001), reduced biomass (Freitas and Mooney 1996), and early flowering date (e.g., Rajakaruna et al. 2003a). However, the long-standing hypothesis that performance in environments with low water and low Ca/Mg ratio may be related (e.g., Kruckeberg 1967, Proctor 1971, Freitas and Mooney 1996, Hughes et al. 2001, Rajakaruna et al. 2003a, b) has not been empirically investigated.

Genetic differentiation between serpentine and non-serpentine populations is evident from the numerous species or subspecies pairs where one member of the pair occurs on and the other off a serpentine habitat (Kruckeberg 1961, Brooks 1987, Macnair and Gardner 1998). Reciprocal transplants of bodenvag, a species that occurs both in serpentine and non-serpentine areas (sensu Kruckeberg 1967), and other species have demonstrated local adaptation to serpentine habitats (e.g., Kruckeberg 1961, Shaw 1991, Nagy and Proctor 1997, Wilcox Wright et al. 2006), while other studies have not identified evidence for local adaptation (e.g., Jurjavcic et al. 2002) or have found that plasticity may be more important (e.g., Rajakaruna et al. 2003). At this point, the relative importance of local adaptation and the maintenance of genetic variation for phenotypic plasticity for bodenvag species occurring in landscapes heterogeneous in serpentine and water remains unclear.

By taking a whole-plant perspective, our aim is to examine how a variety of morphological and life history traits respond to both high and low water and high and low Mg/Ca ratios, and to test the relative importance of phenotypic plasticity and local adaptation in the maintenance of *Mimulus guttatus* populations. Using this bodenvag species, we reexamined traits that others have examined (including vigor related traits), and added several traits that have not been previously examined in manipulative ecological studies of serpentine populations, although we have observed them to respond to water availability in the field (e.g., stolon production promoting late flowering; Dole 1992; C. J. Murren and M. R. Dudash, *unpublished data*). We used replicate populations of *Mimulus guttatus* growing in four habitat types: (1) low Ca/Mg ratio and low water availability as the growing season progressed; (2) low Ca/Mg ratio and continuous, season-long water availability; (3) high Ca/Mg ratio and low water availability as the growing season progressed; and (4) high Ca/Mg ratio and high, continuous, season-long water availability. Replicate populations within field-habitat types were separated by several kilometers. Within each population, we sampled multiple maternal families. The sampling scheme allowed us to investigate genetic variation at three levels: field-habitat type, population, and family. We examined these four hypotheses:

1) There is genetic variation for traits associated with performance in response to low Ca/Mg ratio and low water availability at all three genetic levels (habitat type, population, and family).

2) As *Mimulus guttatus* is a bodenvag species, i.e., occurring in serpentine and non-serpentine habitats, phenotypic plasticity is prevalent for a variety of traits associated with serpentine (low Ca/Mg ratios) and low water conditions, and genetic variation for plasticity exists at all three genetic levels.

3) It has been repeatedly suggested that phenotypic responses to low water availability and serpentine (low Ca/Mg) conditions are associated. However, we hypothesized that a widespread bodenvag species occurring in a heterogeneous landscape would perform best in the most benign environment (high Ca/Mg ratio, high water availability). Additionally, we predicted that the details of direction and associations of trait responses to water and Ca/Mg treatments would be trait dependent.

4) Although phenotypic plasticity and local adaptation are not mutually exclusive, we hypothesized that phenotypic plasticity and genetic variation for plasticity for a variety of traits are responsible for the widespread distribution of *M. guttatus* on and off serpentine soils in northern California.

METHODS

Study species and site description

Mimulus guttatus (historically in Scrophulariaceae, but recently placed in Phrymaceae [Beardsley and Olmstead 2002]) is native to western North America,

with a range from Alaska to Mexico (Grant 1924, Thompson 1993). Populations in coastal areas act as perennials, yet many inland populations (and all studied here) act as annuals, regenerating between seasons using seeds (Sweigart and Willis 2003); there is substantial phenotypic variation among populations (Vickery 1974, Dole 1992, Carr and Fenster 1994, Fenster and Ritland 1994, Kelly and Willis 2001). Some inland populations may also produce stolons that contribute to late-season flowering. Through field observations, we confirmed that all of our populations senesced at the end of the season and ultimately acted as annuals.

Populations are generally found in wet or seasonally wet creeks or seeps. Water availability, light, and soil types are heterogeneous both within and among populations (Galloway 1995, Lello 1995; C. J. Murren and M. R. Dudash, *unpublished data*). Seeds were collected in June 2002 from eight annual populations in Napa and Lake Counties of California, within a 40-km radius. These eight annual populations were categorized into four field-habitat types (high Ca/Mg ratio, season-long water availability (Field HW) corresponds to MGW and CIRS populations; high Ca/Mg ratio, seasonally drying (Field HD) corresponds to SC and MR populations; low Ca/Mg ratio, season-long water availability (Field LW) corresponds to LC4 and KB populations; and low Ca/Mg ratio, seasonally drying (Field LD) corresponds to M13 and LTR populations). The populations were initially classified based on experience (J. Callizo, *personal communication*) and confirmed by soil testing (A and L Western Agricultural Laboratories, Modesto, California, USA), and water levels observed in April–June 2002. Field plants in seasonally drying sites had access to water during germination, but by time of flowering, water availability was reduced; at fruiting, soil moisture was dramatically decreased. In contrast, sites with season-long water availability were populations in seeps or creeks with running water through the entire flowering and fruiting period (April–June). We collected seeds from 30 maternal plants along transects through each population, and randomly selected 10 families per population for inclusion in our study (80 families total).

Greenhouse design, treatments, and measured variables

In October 2002, at the University of Maryland greenhouse, three replicate 7.5×7.5 cm pots (3-inch pots, Kord, Brampton, Ontario, Canada) were used per family. Seeds were sown into these pots on moist Metro-Mix 510 (Scotts, Marysville, Ohio, USA), and were allowed to germinate under natural light conditions with continuous water availability. Following germination, seedlings were randomly chosen and transplanted, one plant per Kord pot, for use in the experiment. Seedlings were bottom-watered and allowed to acclimate for one week after transplanting before treatments were imposed. We utilized sodium vapor lights to extend the day length to 18 h, which triggers flowering under green-

house conditions (Dudash et al. 1997). Seedlings that died from transplant shock prior to initial treatment were replaced by a randomly chosen individual of the same maternal family from those remaining in the germination pots. There was no genetic component to transplant shock (data not shown).

We utilized a two \times two factorial split-block design (Littell et al. 1996) to examine the effects of simultaneously varying water availability and soil chemistry on plant performance. Each of six blocks was divided into four sub-blocks, which represent each of the four treatments (low Ca/Mg ratio and wet corresponds to LW, low Ca/Mg ratio and dry corresponds to LD, high Ca/Mg ratio and wet corresponds to HW, high Ca/Mg ratio and dry corresponds to HD). Sub-blocks (one tray containing 20 pots per treatment) were used to control for microenvironmental variation within the greenhouse. We planted seedlings from all eight California source populations across the blocks. A member of each of the population and family combination was represented in each of the sub-blocks (8 populations \times 10 families/population = 80 plants total per sub-block). The entire experiment included 8 populations \times 10 families/population \times 4 water and soil chemistry treatments \times 6 replicates per treatment for a total of 1920 individuals.

Ca/Mg ratio greenhouse treatments were designed to mimic the average natural variation of these levels in the soil of our serpentine and non-serpentine field study sites (soil testing A and L Western, Agriculture Laboratories). The average ratio of exchangeable elemental content of $\text{Ca}^{++}/\text{Mg}^{++}$ for the serpentine (L) sites was 0.16 and for the non-serpentine (H) sites was 3.1. To adjust the Ca/Mg ratios in the greenhouse to those observed in the field, we used solutions and methodologies of Hewitt (1966), Jhee et al. (1999), Gardner and Macnair (2000), and A. J. Pollard (*personal communication*), which utilize modified Rorison's solutions. Our solutions included a combination of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), potassium phosphate (K_2HPO_4), and ferric sodium salt to create solutions with molarities of Ca and Mg that reflected the average ratios from the serpentine and non-serpentine field sites. The Metro-Mix soil-less medium contains negligible N, P, or K (100 ppm) and no other micronutrients, and these modest macronutrients would be leached within a week under our growing conditions prior to the beginning of our soil chemistry and water treatments (F. Hulme, *personal communication*). No other additional nutrients were added during the experiment. For the high-water treatments (W), 1 L of the appropriate solution (L or H) was given to each tray two times per week during the experiment. This treatment kept the medium wet. For the low-water treatments (D), 10 mL of appropriate solution (L or H) was given to each individual pot two times per week.

Throughout the experiment, we measured morphological, life history, and reproductive characters. We measured the length of the longest leaf in the rosette

stage for each plant during week four as a measure of early developmental vigor. Date of first flower was calculated from day of transplant. For plants that flowered, we measured the corolla width of the first flower. We harvested the plants after seven weeks from onset of treatments, as most of plants in the dry treatments had senesced. At harvest, we recorded rosette leaf number, node number on the main stem after bolting, stem diameter, and stolon production. We quantified two measures of fitness: number of reproductive units (including flowers, fruits, and buds, referred to as RU) and total aboveground biomass (following drying to 50°C). RU and biomass were highly correlated (data not shown); thus we only report the results for sexual reproduction here. At harvest, we noted whether plants had flowered and senesced or had died before flowering; we used this as our measure of survival. After harvest, we measured leaf dissection on a single leaf per plant. We used Scion Image software (Scion, Frederick, Maryland, USA) to measure specific leaf area, which was quantified as the total leaf perimeter to area ratio and interpreted as a simple measure of leaf dissection.

Statistical analyses

We employed mixed-model ANOVA of morphological, life history, and reproductive traits using the mixed procedure in SAS (PROC MIXED; SAS 2003). This procedure allowed for the inclusion of fixed and random effects, as well as accounting for the split-block experimental design in the ANOVA models. The parameters of the model included block, sub-block (nested within block), field water type, field soil type, population (nested within field water type [W or D] and field soil type [H or L]), family (nested within population and field water and field soil type), greenhouse water treatment, greenhouse soil chemistry treatment, and pairwise, three-way, and four-way interactions of the fixed effects.

The model allowed us to assess if habitat types, populations in different ecological habitats, and families nested within populations were genetically differentiated for a range of morphological traits. The field-water-type main effect assessed genetic differences between seeds from seasonally drying versus wet habitats. The field-soil-type main effect assessed genetic differences between plants derived from the low- and high-Ca/Mg ratio habitats. Phenotypic plasticity of traits to greenhouse-manipulated water levels, to Ca/Mg ratios, and to the interaction of Ca/Mg and water are assessed by the treatment and treatment interaction effects. We examined genetic variation of plasticity by examining the interaction between field-habitat types and treatments to assess if populations grouped within the four field-habitat types had varied responses to the greenhouse-imposed treatments. We examined genetic variation for plasticity at the population level by assessing the interaction between populations nested within field soil

and field water types and greenhouse-imposed treatments. We also examined genetic variation for plasticity at the family level by assessing the interaction between families (nested within population and field soil and field water types) and the greenhouse-imposed treatments. Means for field habitats, greenhouse treatments, and their combination are reported as least-squares means \pm SE.

Block, sub-block, population, family, and their interactions with other effects are considered random effects in our models. Block and sub-block(block) were included in the model to account for microenvironmental variation in the greenhouse in our split-block design. The divisors of the fixed effects mean squares were based on combinations of the estimated variance components of the appropriate random effects to compute *F* ratios. The degrees of freedom for these synthesized error terms used the Satterthwaite approximation and are reported to the nearest integer value. Degrees of freedom from the random effects where variance components were zero were pooled with appropriate higher order random effects. Features of the mixed-model procedure were used to partition the residual variance into multiple residuals when variance heterogeneity was detected. When data were non-normal, we used generalized linear mixed models (GLIMMIX macro) and specified the appropriate distribution for each response variable. For example, survival was a binomial response; thus we specified a binomial distribution of responses for the model and the default logit link. As we had high numbers of degrees of freedom for most random effects of interest (Littell et al. 1996), the Wald *Z* statistic test of the random variance components was used (PROC GLIMMIX, SAS 2003). Because the Wald *Z* is a conservative test of significance, we include all *P* values <0.1 in the table and appendices. BLUPs (Best Linear Unbiased Predictors) were determined for populations and families at each level of the fixed effects (following methods in Littell et al. [1996] and methods described in Agrawal et al. [2002] and Johnson and Agrawal [2005]). BLUPs are used for estimates of random effects and are analogous to least-squares means for fixed effects (Robinson 1991, Littell et al. 1996). Our calculated BLUPs are analogous to family means for each treatment, but are less biased by dominance and micro-environmental effects (Johnson and Agrawal 2005). We used BLUPs to evaluate local adaptation and to visualize population- and family-level reaction norms across treatments, which are more accurate than family or population means because they take into account all available model information (Johnson and Agrawal 2005).

RESULTS

Genetic variation for morphological, life history, and fitness traits

We detected genetic variation primarily at the historic field-habitat type and family levels. At the level of the

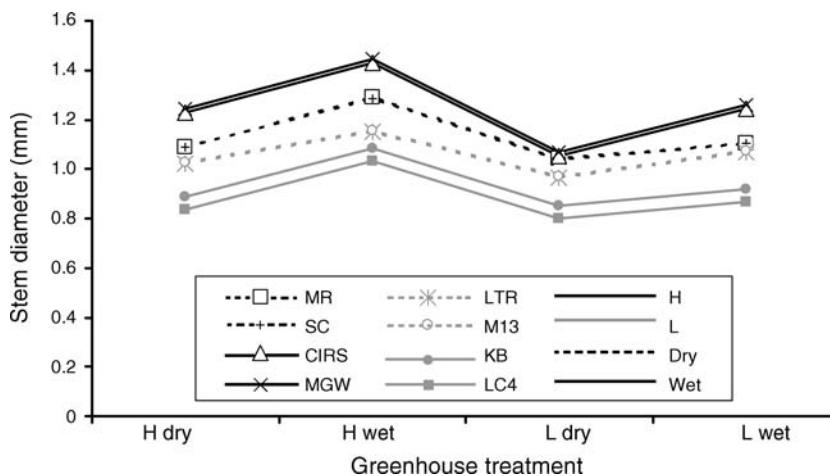


FIG. 1. Reaction norm diagram for stem diameter across the four greenhouse treatments. Values are the best linear unbiased predictors (BLUP, following methods in Agrawal et al. [2002] and Johnson and Agrawal [2005], see *Methods* for details) for each population and treatment combination. Populations from the high Ca/Mg ratio field-habitat type (H, non-serpentine) are depicted with black lines and symbols, and populations from the low Ca/Mg ratio habitat type (L, serpentine) are in gray. Dotted lines indicate populations that are from seasonally drying field-habitat types, and solid lines indicate populations derived from field sites that are continuously wet seeps.

historic field-habitat type, plants derived from high Ca/Mg areas had significantly larger leaf length than plants derived from low Ca/Mg habitats (all values are reported as least-squares means \pm se; Field H = 11.9 ± 0.51 cm; Field L = 9.8 ± 0.51 cm; Table 1; Appendix A). Stem diameter was significantly greater for plants derived from Field HW sites than those from Field HD sites. However, the order was reversed for plants derived from Field L sites, with plants from Field LD sites having greater stem diameter than plants from Field LW sites (Fig. 1, Table 1, Appendix A).

At the population level, we detected no significant differentiation for traits measured among the eight field populations (Table 1, Appendices A, B, and C). While the population effects were not significant, we found significant genetic variation at the family level for length of longest leaf, stem diameter, number of nodes on the main stem, rosette leaf number, days to first flower, corolla width, and stolon number (Table 1, Appendices A, B, and C). For other traits where we detected a significant family main effect, we also detected a significant block \times family interaction. For RU, we found no significant main effect for family alone; however, we did find a significant effect of block \times family interaction, which contained the family level genetic variation as well as the block effects (Table 1, Appendix C).

Evaluation of greenhouse water and soil chemistry treatments

We detected significant effects of water and/or soil chemistry treatments and, thus, significant phenotypic plasticity, on all morphological, life history, and fitness-related traits (Table 1, Appendices A, B, and C). Plants grown in the H (high Ca/Mg) treatment had significantly

greater morphological and life history trait values than when grown in the L (low Ca/Mg) treatment (e.g., both left and right axes; Fig. 2; Table 1). Plants in the L treatments flowered earlier than those in the H treatments (L = 38.9 ± 1.2 days, H = 40.9 ± 1.2 days; Table 1; Appendix B). Corolla width was significantly larger in the H than L treatment (H = 13.7 ± 0.64 mm, L = 12.3 ± 0.57 mm; Table 1; Appendix B). Rosette leaf number was significantly larger in H than L treatments (H = 6.0 ± 0.38 leaves, L = 5.4 ± 0.38 leaves; Table 1; Appendix A). Additionally, total number of reproductive units (Fig. 2b, Table 1, Appendix C) and stolon production (Fig. 3a, Table 1, Appendix C) were significantly greater in the H than the L treatment.

With respect to water availability, plants were larger and produced more reproductive units in the high-water treatments (W) than the low water treatments (D) (Fig. 2, Table 1, Appendices A and C). In the high-water treatment, corolla width was significantly larger (W = 14.0 ± 0.56 mm, D = 12.0 ± 0.65 mm; Table 1; Appendix B) and flowering date was significantly earlier (W = 40.4 ± 1.2 d, D = 39.3 ± 1.2 d; Table 1; Appendix B) than the low water treatment. Leaf perimeter to area ratio (W = 0.71 ± 0.02 , D = 0.80 ± 0.02 ; Appendix B) was significantly greater in the low water treatment than the high water treatment.

Significant interaction effects between greenhouse soil chemistry and greenhouse water treatments occurred for stem diameter, node number on the main stem, and RU (Table 1, Figs. 1 and 3b, c). For these three traits, plants in the HW treatment were consistently larger than the other treatments and had greater sexual reproductive potential, and LD generally had the lowest values (e.g., Figs. 1, 2, and 3b, c). We also found a significant water and soil chemistry treatment interaction for corolla

TABLE 1. Summary of mixed-model ANOVA *P* levels for all traits examined.

Type of effect and source of variation	Length of longest leaf	Stem diameter	No. nodes	Rosette leaf no.	Days to first flower
Genetic effects					
Random†					
Population‡	NS	NS	NS	0.05–0.1	NS
Family§	<0.05	<0.05	<0.05	<0.05	<0.05
Fixed					
Field soil (FS)	<0.05	NS	NS	NS	NS
Field water (FW)	NS	<0.05	NS	NS	NS
FS × FW	0.05–0.1	<0.05	NS	NS	NS
Environmental greenhouse effects					
Fixed					
Greenhouse soil (GS)	<0.05	<0.05	<0.05	<0.05	<0.05
Greenhouse water (GW)	<0.05	<0.05	<0.05	NS	<0.05
GS × GW	NS	<0.05	<0.05	NS	NS
Genetic × environmental effects					
Fixed					
FS × GW	NS	<0.05	NS	NS	NS
FW × GS × GW	NS	NS	0.05–0.1	NS	NS
FS × FW × GW	NS	NS	NS	0.05–0.1	NS
FS × FW × GS × GW	NS	NS	NS	NS	NS
All other interactions	NS	NS	NS	NS	NS
Random					
Population¶	NS	0.05–0.1	NS	NS	NS
Family#	NS	NS	NS	<0.05	NS

Note: Entries designated NS are not significant ($P > 0.1$); other *P* levels given are significant ($P < 0.05$) and marginally significant ($P = 0.05–0.1$).

† Block and sub-block random effects are described in the Appendices.

‡ Population(field soil and field water).

§ Family(population, field soil, and field water).

|| FS × GS, FW × GW, FW × GS, FS × GS × GW, and FS × FW × GS.

¶ Population(field soil and field water) × greenhouse soil × greenhouse water.

Family(population, FS and FW) × GS × GW.

width such that there was greater corolla width in the wet treatments, yet the corolla width of plants in the LD was greater than in the HD (HW = 15.6 ± 0.68 mm, HD 11.9 ± 0.89 mm, LW = 12.4 ± 0.66 mm, LD = 12.2 ± 0.70 mm; Table 1; Appendix B).

Survival to the end of the experiment, to either reproductive maturity or as a vegetative rosette, varied among treatments. Water treatments alone showed 82% survival in the W treatments and 71% survival in the D treatments. Additionally, when we examined soil chemistry treatments alone, we found 80% survival in the H treatments and 72% survival in the L treatment. For the four soil chemistry and water treatments quantified separately, survival was 67% in the LD treatment, 74% in the HD treatment, 78% in the LW treatment, and 86% in the HW treatment. We did not detect significant variation among populations (Wald $Z = 1.19$, $P = 0.12$) or among families nested within populations (Wald $Z = 0.61$, $P = 0.27$) (data not shown).

*Genetic by environment interaction effects:
genetic variation for phenotypic plasticity*

Crossing reaction norms or changes in slopes of reaction norms were observed across the four greenhouse treatments for several traits when the replicate

populations of the four field-habitat types were contrasted (Figs. 1 and 3c, Table 1, Appendices A and C). A significant interaction exists between field soil and greenhouse water treatment for stem diameter (Table 1, Fig. 1). We found a significant three-way interaction among field water and *both* greenhouse soil chemistry and watering treatments for number of nodes on the main stem (Table 1, Fig. 3b). Finally, we observed a significant four-way interaction among field soil, field water, greenhouse water, and greenhouse soil chemistry treatments for stolon number (Table 1, Fig. 3a). All other interactions with field-habitat type were not significant (Table 1). At the family level, significant genetic variation for plasticity was observed across all four treatments for rosette leaf number (Table 1, Appendix A), stolons (Table 1, Appendix C), and reproductive units (Table 1, Appendix C).

Local adaptation

Two criteria had to be met to detect local adaptation (used in the *Mimulus* system by Lello 1995; but see Silander and Antonovics 1979, Schemske 1984, Schmitt 1993). First, we examined whether there was a significant interaction between field-habitat type and greenhouse soil chemistry and water treatments, such that

TABLE 1. Extended.

Leaf perimeter : area ratio	Corolla width	No. stolons	Sexual reproduction (RU)
NS	NS	NS	NS
NS	<0.05	<0.05	NS
NS	NS	NS	NS
NS	NS	0.05–0.1	NS
NS	NS	NS	NS
NS	<0.05	<0.05	<0.05
<0.05	<0.05	<0.05	<0.05
NS	<0.05	NS	<0.05
NS	NS	NS	NS
NS	NS	NS	NS
NS	NS	NS	NS
NS	NS	<0.05	NS
NS	NS	NS	NS
0.05–0.1	NS	NS	NS
0.05–0.1	NS	<0.05	<0.05

populations would vary in their performance across treatments. Stem diameter met this criterion. Stolon number had a significant four-way interaction among field soil type, field water type, greenhouse soil chemistry treatment, and greenhouse water treatment, also meeting this criterion. Second, we determined if, within a greenhouse treatment, the highest performance of experimental plants occurred for a population from a matching historic field-habitat type. We did not find evidence that plants from a particular field-habitat type perform the best in the analogous greenhouse treatment. The highest ranked population in terms of sexual reproductive units in the HD and LW treatments was M13, a Field LD population (see BLUPs in Fig. 4). The highest ranked population in terms of sexual reproductive units in the HW and LD treatments was MR, a Field HD population. Additionally, in the HW and LD treatments, M13 was the second highest ranked or not significantly different from MR. However, the KB population did exhibit the highest rank in three of the four greenhouse treatments including its home condition, Field LW, for stolon production (Fig. 3a).

DISCUSSION

Early work describing the ecological responses of plants to serpentine soils regularly suggested that

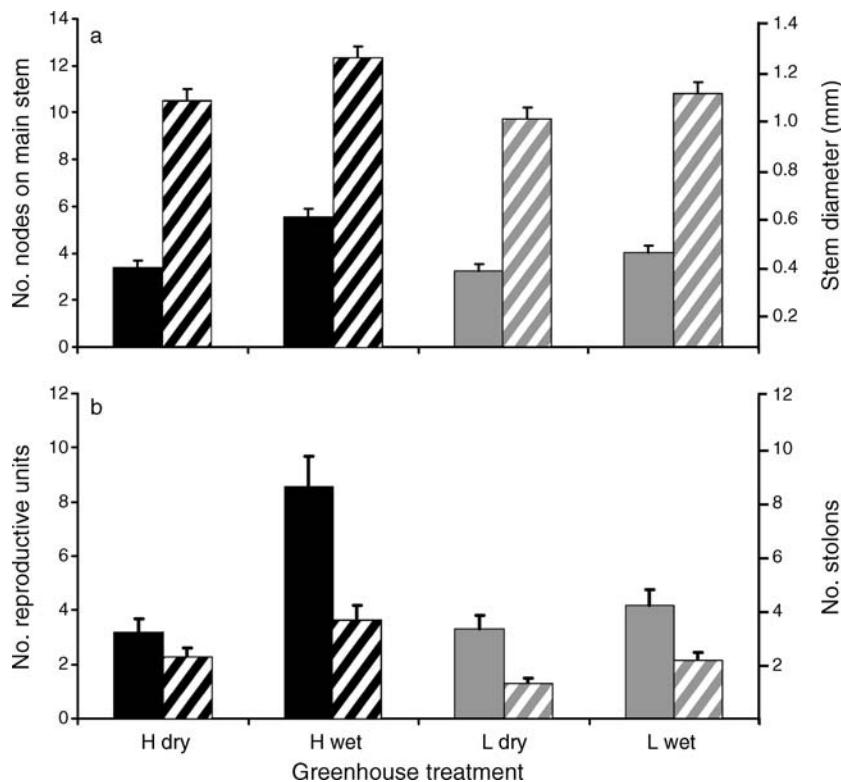


FIG. 2. (a) Least-square means and one standard error for number of nodes on the main stem (solid bars) and stem diameter (mm) (striped bars) across the four greenhouse treatments. (b) Least-square means for the number of reproductive units for buds, flowers, and fruit (solid bars) and total number of stolons (striped bars) across the four greenhouse treatments. Black bars indicate high (H) Ca/Mg ratio treatments; gray bars indicate low (L) Ca/Mg ratio treatments.

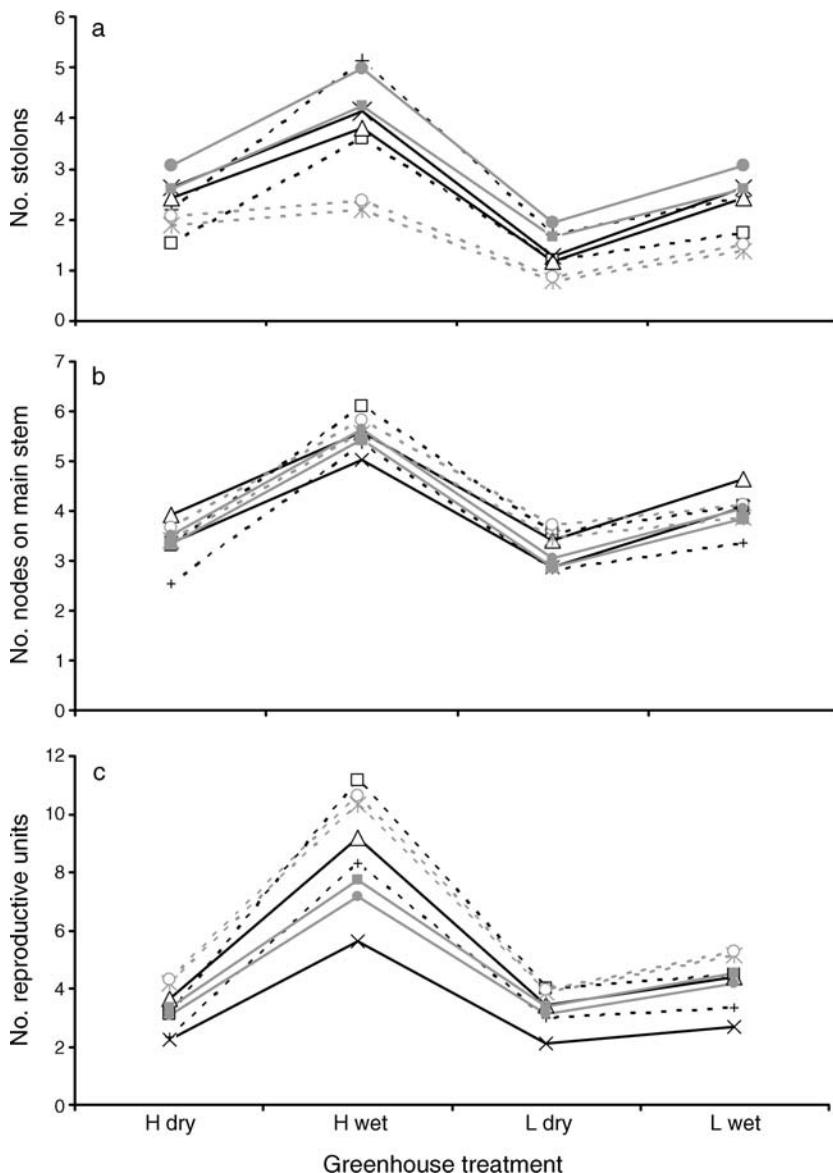


FIG. 3. Reaction norm diagram for (a) number of stolons, (b) number of nodes on the main stem, and (c) number of reproductive units (buds, flowers, and fruit) across four greenhouse treatments. Values plotted are the appropriate best linear unbiased predictor (BLUP). Populations from the H Ca/Mg ratio habitat-type are in black, populations from the L Ca/Mg ratio field habitat-type are gray. The key to symbols is as in Fig. 1.

performance, in relation to soil chemistry and water availability, might be linked because low Ca/Mg ratio (indicative of serpentine soils) and low water availability often co-occur (e.g., Whittaker 1954, Kruckeberg 1961, 1967). Our current study is the first to test the hypothesis that water and soil chemistry jointly *and* alone influence patterns of genetic variation, local adaptation, and the maintenance of phenotypic plasticity in a widespread species. For some traits (e.g., stem diameter), we saw significant responses in the same direction to Ca/Mg ratio and water availability, whereas for other traits (e.g., rosette leaf number and leaf perimeter to area

ratio), responses to Ca/Mg ratio and water availability were independent.

We found little support for local adaptation of these *Mimulus guttatus* populations. Other reciprocal transplant field studies detected significant variation in survivorship across habitats and demonstrated that the greatest performance of populations does not always occur in habitats reflecting home environmental conditions (e.g., Kruckeberg 1967, Jurjavec et al. 2002), which offers additional support for the hypothesis that plasticity is critical for this bodenvag species. Detection of local adaptation varies among species (e.g., Silander and Antonovics 1979, Schemske 1984, Schmitt 1993),

and may reflect differences in methodologies used to detect global vs. local optima (von Wettberg et al. 2005; J. Schmitt, *personal communication*). In a greenhouse study, Gardner and Macnair (2000) demonstrated some evidence for local adaptation in serpentine-derived populations of *M. nudatus* and *M. guttatus*. Our inability to detect local adaptation (consistent with Lello 1995) may, in part, be due to our use of greenhouse soil chemistry treatments that reflected the average of our sampled populations in each habitat type, rather than any individual population's conditions. However, comparing the population with environmental values closest to the treatment values did not offer further support for local adaptation. Finally, variation in rainfall among years (M. R. Dudash and C. J. Murren, *personal observation*) may reduce the differences experienced among sites; thus selection pressures may be spatially and temporally variable.

Genetic variation

By replicating populations within a field-habitat type, we were able to assess the hypothesis that field soil chemistry and field water availability contributed to trait differentiation among populations and to assess variation between populations within field-habitat type (rarely explored in the literature, but see Wilcox Wright et al. 2006). Previously, the reduction in leaf size has been attributed to low water availability in low Ca/Mg ratio habitats (e.g., Kruckeberg 1954), yet here we are able to isolate the significant impact of field soil chemistry on this trait differentiation without finding an effect of historic water availability. Our results suggest that if populations in these habitat types were to become isolated, directional selection would contribute to differential character evolution. Furthermore, the historic effects of field water and field soil chemistry are trait dependent and may act either *alone* or *in concert*. Additional evidence for independent trait differentiation among populations comes from two native races of *Lasthenia californica*, where the number of flowering heads varied in relation to distinct water habitats along a gradient of serpentine stress (Rajunkaruna et al. 2003a).

Family-level genetic variation was detected for nearly all traits investigated. This pattern is common for other widespread, roadside, ephemeral habitats that exhibit metapopulation dynamics (e.g., Schmitt et al. 1992, Mazer and Delesalle 1996, Pigliucci et al. 1997, Mutikainen and Delph 1998, Stinchcombe et al. 2004) as well as for prior work on *Mimulus guttatus* (e.g., Galloway 1995, Lello 1995, Dudash et al. 1997, Vickery 1999, Ivey et al. 2004). The maintenance of among-family variation is likely due to environmental heterogeneity of the maternal habitat, the proximity of the four habitat types that allows for potential gene flow among local populations, and variation in maternal outcrossing rates. Additionally, this distribution of genetic variation may explain, in part, our finding of a lack of strong local adaptation.

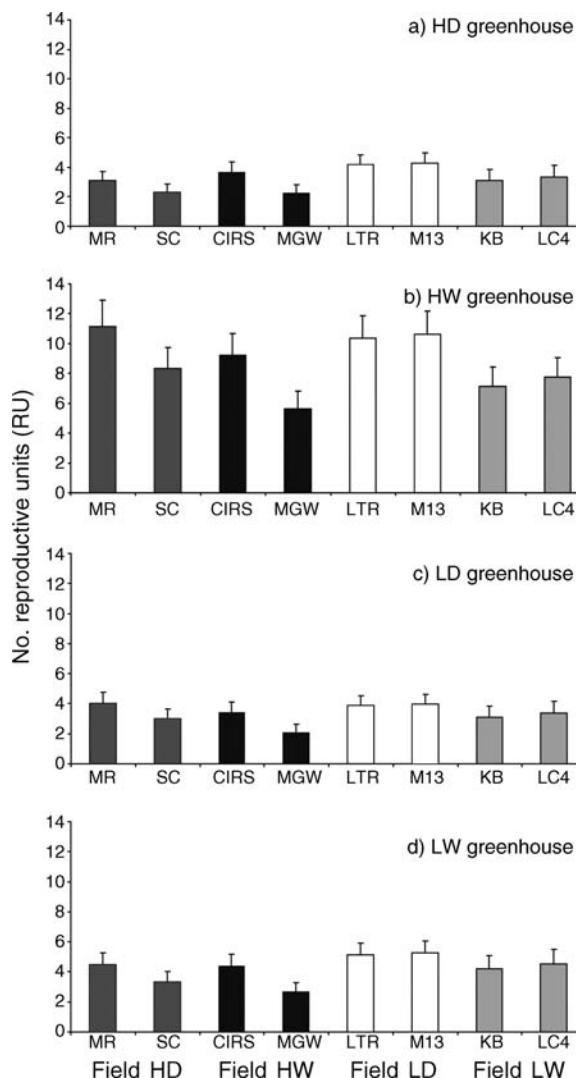


FIG. 4. The best linear unbiased predictors (BLUP) of the random effects (\pm SE for total number of reproductive units (RU) for each population grouped by field soil and field water habitat types used to evaluate local adaptation: (a) HD greenhouse treatment, high Ca/Mg, dry; (b) HW greenhouse treatment, high Ca/Mg, wet; (c) LD greenhouse treatment, low Ca/Mg, dry; (d) LW greenhouse treatment, low Ca/Mg, wet. Field HD populations are indicated by dark gray bars, field HW populations by black bars, field LD populations by white bars, and field LW populations by light gray bars.

Phenotypic plasticity

The patterns of reaction norms of our replicate populations per field-habitat type or among families varied among the observed traits, suggesting that our measured traits were not simply replicate measures of vigor; rather, traits responded independently to water availability and soil chemistry. For example, we observed significant plasticity to water availability, with generally taller plants, larger stem diameters, lower leaf perimeter to area ratios, and greater stolon and sexual

production in our wet treatment. Plants in dry habitats have smaller leaves overall, increasing the perimeter to area ratio, a strategy that reduces water loss in dry habitats (Abrams 1994, Lynn and Waldren 2001, Black-Samuelsson et al. 2003). Several recent studies found plasticity and genetic variation for plasticity of serpentine populations to variable water conditions (e.g., Hughes et al. 2001, Rajakaruna et al. 2003a) for physiological and reproductive traits among taxa or among races. Macnair and Gardner (2000) detected substantial variation for vigor-related traits among replicates of *Mimulus* taxa, and suggested that *M. nudatus*, the serpentine endemic, had greater tolerance to low Ca/Mg ratios. Investigations of genetic variation within species to serpentine are rare (but see Wilcox Wright et al. 2006).

Frietas and Mooney's (1996) is, to our knowledge, the only study that simultaneously examined performance in serpentine and low water environments of root and shoot vigor, as well as physiological traits. They demonstrated that root inhibition contributed to the overall stunted growth of the serpentine treatments. As our plants were all grown in the same soil-less potting medium in the greenhouse, any textural variation was included in the model error term, but may be a real ecological component in the field, warranting further study. Here, we examined aboveground traits, yet we found significant interaction effects of greenhouse soil chemistry and water, resulting in overall smaller individuals with lower RU when grown under the LD treatment. The direction of these observed trait reductions in the LD treatment (smaller leaf size, stem diameter, corolla width, and lower sexual reproduction) reflect similar morphological shifts of these traits from *M. guttatus* to the presumed derived taxon, *M. nudatus*, a serpentine endemic found in seasonally drying microhabitats (Gardner and Macnair 2000). Our data suggest that Ca/Mg ratio and water availability can have strong phenotypic effects both alone and in combination.

Genetic variation for phenotypic plasticity

Habitat heterogeneity is considered a strong selective agent for the maintenance of genetic variation for plasticity (e.g., Schlichting and Pigliucci 1998). Our data support the hypothesis that genetic variation for plasticity plays an important evolutionary role in the maintenance of the ecological breadth of this bodenvag taxon.

Because our experiment was performed on field-collected seed, some of the effects that we observed may be due, in part, to maternal effects (e.g., Roach and Wulff 1987, Galloway 1995, Mousseau and Fox 1998), and may have reduced our ability to detect genetic variation for plasticity. Galloway (1995) demonstrated that maternal effects in *M. guttatus* occurred in vegetative traits and were reduced for adult flower production. Maternal effects may be a component of the mechanism by which *M. guttatus* is able to persist along a range of stressful serpentine and seasonally drying

conditions to moist non-serpentine regions. Additionally, we chose to terminate the experiment when all of the plants in the dry treatments had senesced. If we had allowed the plants in the wet treatments to continue growing, the patterns that we observed would likely have been more dramatic.

We detected evidence for genetic variation for phenotypic plasticity at three hierarchical levels: at the levels of field-habitat type, population (although marginally significant), and families within population. We also found significant variation among traits in the detection of genetic variation for plasticity at these hierarchical levels such that morphological traits have the potential of evolving independently. Populations from continuously wet, non-serpentine habitats had the greatest overall mean for stem diameter across treatments, as well as the greatest plasticity, thus producing the most architecturally stable plants, which are also likely to contribute to efficient conductance of water to their leaves. Late in the season, stolons are produced and can contribute to additional floral production. Stolon production was greater in wet treatments, consistent with wet sites in the field where plants have a longer flowering season. Populations from habitats with historical seasonal drying rapidly allocated biomass toward primary stem sexual reproduction, as a possible mechanism to avoid water stress later in the season. Our results, in part, also corroborate our hypothesis that plasticity to water availability may correspond to plasticity to Ca/Mg ratio at the family level. Yet, we also demonstrate that the magnitudes of the responses to the two sets of environmental conditions can be trait specific (Table 1). Trait-specific responses have been found for other widespread annual species in other environmental treatments (e.g., Pigliucci et al. 1995, Kolodynska and Pigliucci 2003), and for *M. guttatus* in other environments (Vickery 1974, Galloway 1995). The maintenance of genetic variation for plasticity is particularly important for annual species that have high seed and pollen dispersal capabilities (e.g., metapopulation dynamics; Vickery 1999) and habitats that are regionally heterogeneous (Alpert and Simms 2002). For this widespread bodenvag species, plasticity and genetic variation for plasticity enhance the chance of population establishment and persistence in a landscape where soil chemistry and water availability are highly heterogeneous.

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LITERATURE CITED

- Abrams, M. D. 1994. Genotypic and phenotypic variation as stress adaptations in temperate tree species—a review of several case-studies. *Tree Physiology* **14**:833–842.
- Agrawal, A., J. K. Conner, M. T. J. Johnson, and R. Wallsgrave. 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution* **56**:2206–2213.
- Alpert, P., and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology* **16**:285–297.
- Antonovics, J., A. D. Bradshaw, and R. G. Turner. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* **7**:1–85.
- Beardsley, P. M., and R. G. Olmstead. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimulaceae, and *Phryma*. *American Journal of Botany* **89**:1093–1102.
- Berglund, A. B. N., S. Dahlgren, and A. Westerbergh. 2004. Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during colonization of Scandinavia. *New Phytologist* **161**:199–209.
- Black-Samuelsson, S., R. E. Whitley, and G. Junzhan. 2003. Growth and leaf morphology response to drought stress in the riparian broadleaf tree *Ulmus laevis* (Pall.). *Silvae Genetica* **52**:292–299.
- Brooks, R. R. 1987. *Serpentine and its vegetation: a multidisciplinary approach*. Dioscorides Press, Portland, Oregon, USA.
- Carr, D. E., and C. B. Fenster. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity* **72**:606–618.
- Dole, J. A. 1992. Reproductive assurance mechanisms in three taxa of *Mimulus guttatus* complex (Scrophulariaceae). *American Journal of Botany* **79**:650–659.
- Donohue, K. 2003. Setting the stage: phenotypic plasticity as habitat selection. *International Journal of Plant Sciences* **164**:S79–S92.
- Dudash, M. R., D. E. Carr, and C. B. Fenster. 1997. Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. *Evolution* **51**:54–65.
- Fenster, C. B., and K. Ritland. 1994. Evidence for natural selection on mating system in *Mimulus* (Scrophulariaceae). *International Journal of Plant Sciences* **155**:588–596.
- Freitas, H., and H. Mooney. 1996. Effects of water stress and soil texture on the performance of two *Bromus horreaceus* ecotypes from sandstone and serpentine soils. *Acta Oecologia* **17**:307–317.
- Galloway, L. 1995. Responses to natural environmental heterogeneity: maternal effects and selection on life history characters and plasticities in *Mimulus guttatus*. *Evolution* **49**:1095–1107.
- Galloway, L. F., and C. B. Fenster. 2000. Population differentiation in an annual legume: local adaptation. *Evolution* **54**:1173–1181.
- Gardner, M., and M. Macnair. 2000. Factors affecting the coexistence of the serpentine endemic *Mimulus nudatus* Curran and its presumed progenitor, *Mimulus guttatus* Fisher ex DC. *Biological Journal of the Linnean Society* **69**:443–459.
- Grant, A. L. 1924. A monograph of the genus *Mimulus*. *Annals of the Missouri Botanical Garden* **11**:99–389.
- Harrison, S. 1997. How natural habitat patchiness affects the distribution of diversity in Californian serpentine chaparral. *Ecology* **78**:1898–1906.
- Harrison, S. 1999. Local and regional diversity in a patchy landscape: native, alien, and endemic herbs on serpentine soils. *Ecology* **80**:70–80.
- Hewitt, E. J. 1966. *Sand and water culture methods used in the study of plant nutrition*. Second edition. Farnham Royal, Commonwealth Agricultural Bureau, Bucks, UK.
- Hughes, R., K. Bachmann, N. Smirnov, and M. R. Macnair. 2001. The role of drought tolerance in serpentine tolerance in the *Mimulus guttatus* Fisher ex DC. Complex. *South African Journal of Science* **97**:581–586.
- Hull, J. C., and S. G. Wood. 1984. Water relations of oak species on and adjacent to a Maryland serpentine soil. *American Midland Naturalist* **112**:224–234.
- Iturralde, R. B. 2001. The influence of ultramafic soils on plants in Cuba. *South African Journal of Science* **97**:510–512.
- Ivey, C. T., D. E. Carr, and M. D. Eubanks. 2004. Effects of inbreeding in *Mimulus guttatus* on tolerance to herbivory in natural environments. *Ecology* **85**:567–574.
- Jhee, E. M., K. L. Dandridge, A. M. Christy, Jr., and A. J. Pollard. 1999. Selective herbivory on low-zinc phenotypes of the hyperaccumulator *Thlaspi caerulescens* (Brassicaceae). *Chemoecology* **9**:93–95.
- Johnson, M. T. J., and A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86**:874–885.
- Jurjavcic, N. L., S. Harrison, and A. T. Wolf. 2002. Abiotic stress, competition, and the distribution of the native annual grass *Vulpia microstachys* in a mosaic environment. *Oecologia* **130**:555–562.
- Kelly, J. K., and J. H. Willis. 2001. Deleterious mutations and genetic variation for flower size in *Mimulus guttatus*. *Evolution* **55**:937–942.
- Kolodynska, A., and M. Pigliucci. 2003. Multivariate responses to flooding in *Arabidopsis*: an experimental evolutionary investigation. *Functional Ecology* **17**:131–140.
- Kruckeberg, A. R. 1954. The ecology of serpentine soils III. Plant species in relation to serpentine soils. *Ecology* **35**:267–274.
- Kruckeberg, A. R. 1961. Intraspecific variability in the response of certain native plant species to serpentine soil. *American Journal of Botany* **38**:408–419.
- Kruckeberg, A. R. 1967. Ecotypic responses to ultramafic soils by some plant species of northwestern United States. *Brittonia* **19**:133–151.
- Lello, D. 1995. *Evolutionary dynamics of the mating system in a population of Mimulus guttatus*. Dissertation. University of Washington, Seattle, Washington, USA.
- Littell, R. C., G. A. Milliken, W. W. Stoop, and R. D. Wolfinger. 1996. *SAS system for mixed models*. SAS Institute, Cary, North Carolina, USA.
- Lynn, D. E., and S. Waldren. 2001. Morphological variation in populations of *Ranunculus repens* from the temporary limestone lakes (turloughs) in the West of Ireland. *Annals of Botany* **87**:9–17.
- Macnair, M. R. 1987. Heavy metal tolerance in plants: a model evolutionary system. *Trends in Ecology and Evolution* **2**:354–359.
- Macnair, M. R., and M. Gardner. 1998. Evolution of edaphic endemics. Pages 157–171 in D. J. Howard and S. H. Berlocher, editors. *Endless forms: species and speciation*. Oxford University Press, New York, New York, USA.
- Mazer, S. J., and V. A. Delesalle. 1996. Temporal instability of genetic components of floral trait variation trait: maternal family and population effects in *Spergularia marina* (Caryophyllaceae). *Evolution* **50**:2509–2515.
- Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. *Trends in Ecology and Evolution* **13**:403–407.
- Mutikainen, P., and L. F. Delph. 1998. Inbreeding depression in gynodioecious *Lobelia siphilitica*: among-family differences override between-morph differences. *Evolution* **52**:1572–1582.
- Nagy, L., and J. Proctor. 1997. Plant growth and reproduction on a toxic Alpine ultramafic soil: adaptation to nutrient limitation. *New Phytologist* **137**:267–274.

- Pigliucci, M., P. DiIorio, and C. D. Schlichting. 1997. Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. *Journal of Ecology* **85**:265–276.
- Pigliucci, M., J. Whitton, and C. D. Schlichting. 1995. Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. *Journal of Evolutionary Biology* **8**:421–438.
- Proctor, J. 1971. The plant ecology of serpentine II. Plant response to serpentine soils. *Journal of Ecology* **59**:397–410.
- Proctor, J., and L. Nagy. 1992. Ultramafic rocks and their vegetation: an overview. Pages 469–494 in A. J. M. Baker, J. Proctor, and R. D. Reeves, editors. *The vegetation of ultramafic (serpentine) soils*. Intercept, Andover Hants, UK.
- Rajakaruna, N., and B. A. Bohm. 1999. The edaphic factor and patterns of variation in *Lasthenia californica* (Asteraceae). *American Journal of Botany* **86**:1576–1596.
- Rajakaruna, N., G. E. Bradfield, B. A. Bohm, and J. Whitton. 2003a. Adaptive differentiation in response to water stress by edaphic races of *Lasthenia californica* (Asteraceae). *International Journal of Plant Sciences* **164**:371–376.
- Rajakaruna, N., M. Y. Siddiqi, J. Whitton, B. A. Bohm, and A. D. M. Glass. 2003b. Differential responses to Na^+/K^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ in two edaphic races of the *Lasthenia californica* (Asteraceae) complex: a case for parallel evolution of physiological traits. *New Phytologist* **157**:93–103.
- Rajakaruna, N., and J. Whitton. 2004. Trends in the evolution of edaphic specialists with an example of parallel evolution in the *Lasthenia californica* complex. Pages 103–110 in Q. C. B. Conk, J. Whitton, R. H. Ree, and I. E. P. Taylor, editors. *Plant adaptation: molecular genetics and ecology*. Proceedings of an International Workshop (December 2002, Vancouver, British Columbia, Canada). NRC Research Press, Ottawa, Canada.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* **18**:209–235.
- Robinson, G. K. 1991. That BLUP is a good thing: The estimation of random effects. *Statistical Science* **6**:15–51.
- Schemske, D. W. 1984. Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. *Evolution* **38**:817–832.
- Schemske, D. W., and H. D. Bradshaw, Jr. 1999. Pollinator preference and the evolution of floral traits in monkey flowers (*Mimulus*). *Proceedings of the National Academy of Sciences (USA)* **96**:11910–11915.
- Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Massachusetts, USA.
- Schluter, D. 1998. Ecological causes of speciation. Pages 114–129 in D. J. Howard and S. H. Berlocher, editors. *Endless forms: species and speciation*. Oxford University Press, New York, New York, USA.
- Schmitt, J. 1993. Reaction norms of morphological and life history traits to light availability in *Impatiens capensis*. *Evolution* **47**:1654–1668.
- Schmitt, J., J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *American Naturalist* **139**:451–466.
- Shaw, A. J. 1991. Ecological genetics of serpentine tolerance in the moss *Funaria flavicans*: variation within and among haploid sib families. *American Journal of Botany* **78**:1487–1493.
- Silander, J. A., and J. Antonovics. 1979. The genetic basis of the ecological amplitude of *Spartina patens*. I. Morphometric and physiological traits. *Evolution* **33**:1114–1127.
- Stinchcombe, J. R., L. Dorn, and J. Schmitt. 2004. Flowering time plasticity in *Arabidopsis thaliana*: a reanalysis of Westerman and Lawrence (1970). *Journal of Evolutionary Biology* **17**:197–207.
- Sultan, S. E., and F. A. Bazzaz. 1993. Phenotypic plasticity in *Polygonum persicaria*. II. Norms of reaction to soil moisture and the maintenance of genetic diversity. *Evolution* **47**:1032–1049.
- Sweigart, A. L., and J. H. Willis. 2003. Patterns of nucleotide diversity are affected by mating system and asymmetric introgression in two species of *Mimulus*. *Evolution* **57**:2490–2506.
- Taylor, S. I., and F. Levy. 2002. Responses to soils and a test for preadaptation to serpentine in *Phacelia dubia* (Hydrophyllaceae). *New Phytologist* **155**:437–447.
- Thompson, D. M. 1993. *Mimulus*. Pages 1037–1051 in J. C. Hickman, editor. *The Jepson manual*. University of California Press, Berkeley, California, USA.
- Vickery, R. K., Jr. 1974. Growth in artificial climates—an indication of *Mimulus*' ability to invade new habitats. *Ecology* **55**:796–807.
- Vickery, R. K., Jr. 1999. The remarkable waxing, waning, and wandering of populations of *Mimulus guttatus*: an unexpected example of global warming. *Great Basin Naturalist* **59**:112–126.
- von Wettberg, E. J., H. Huber, and J. Schmitt. 2005. Interacting effects of microsite quality, plasticity and dispersal distance from the parental site on fitness in a natural population of *Impatiens capensis*. *Evolutionary Ecology Research* **7**:531–548.
- Whittacker, R. H. 1954. The ecology of serpentine soils IV. The vegetational response to serpentine soils. *Ecology* **35**:275–288.
- Wilcox Wright, J., M. L. Stanton, and R. Scherson. 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* **8**:1–21.

APPENDIX A

Restricted maximum-likelihood estimates for morphological traits (*Ecological Archives* E087-156-A1).

APPENDIX B

Restricted maximum-likelihood estimates for life history and for floral and leaf morphological traits (*Ecological Archives* E087-156-A2).

APPENDIX C

Restricted maximum-likelihood estimates for a sexual reproduction/fitness-related trait and an overall architectural trait that contributes to late-season flower production (*Ecological Archives* E087-156-A3).