

PLASTICITY OF PHYSIOLOGY IN *LOBELIA*: TESTING FOR ADAPTATION AND CONSTRAINT

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Abstract.—Phenotypic plasticity is thought to be a major mechanism allowing sessile organisms such as plants to adapt to environmental heterogeneity. However, the adaptive value of many common plastic responses has not been tested by linking these responses to fitness. Even when plasticity is adaptive, costs of plasticity, such as the energy necessary to maintain regulatory pathways for plastic responses, may constrain its evolution. We used a greenhouse experiment to test whether plastic physiological responses to soil water availability (wet vs. dry conditions) were adaptive and/or costly in the congeneric wildflowers *Lobelia cardinalis* and *L. siphilitica*. Eight physiological traits related to carbon and water uptake were measured. Specific leaf area (SLA), photosynthetic rate (A), stomatal conductance (g_s), and photosynthetic capacity (A_{\max}) responded plastically to soil water availability in *L. cardinalis*. Plasticity in A_{\max} was maladaptive, plasticity in A and g_s was adaptive, and plasticity in SLA was adaptively neutral. The nature of adaptive plasticity in *L. cardinalis*, however, differed from previous studies. *Lobelia cardinalis* plants with more conservative water use, characterized by lower g_s , did not have higher fitness under drought conditions. Instead, well-watered *L. cardinalis* that had higher g_s had higher fitness. Only A_{\max} responded plastically to drought in *L. siphilitica*, and this response was adaptively neutral. We detected no costs of plasticity for any physiological trait in either *L. cardinalis* or *L. siphilitica*, suggesting that the evolution of plasticity in these traits would not be constrained by costs. Physiological responses to drought in plants are presumed to be adaptive, but our data suggest that much of this plasticity can be adaptively neutral or maladaptive.

Key words.—Adaptation, cost of plasticity, drought, *Lobelia cardinalis*, *Lobelia siphilitica*, phenotypic plasticity, photosynthetic gas exchange.

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Phenotypic plasticity is thought to be a major mechanism allowing organisms to adapt to environmental heterogeneity. In particular, plastic responses in sessile organisms such as plants are so common that they are often assumed to be adaptive (reviewed by van Kleunen and Fischer 2005). The adaptive significance of some plastic responses, such as stem elongation in response to shading, is well supported (e.g., Schmitt et al. 1995; Dudley and Schmitt 1996). However, other common plastic responses, such as heterophylly, are not adaptive in all species (reviewed in Winn 1999). Consequently, the adaptive value of plasticity needs to be tested by linking specific plastic responses to fitness (Pigliucci 2005; van Kleunen and Fischer 2005).

Even when plasticity is adaptive, costs may constrain its evolution. Costs of plasticity include the energy necessary to acquire information about the environment, maintain regulatory pathways for plastic responses, and produce a phenotype via plastic rather than fixed development (DeWitt et al. 1998). If individuals produce the same phenotype within an environment, but those from more plastic families have lower fitness within that environment than those from less plastic families, then plasticity is inferred to be costly (van Tienderen 1991; DeWitt 1998; Scheiner and Berrigan 1998; van Kleunen et al. 2000; Stinchcombe et al. 2004). In a recent review, van Kleunen and Fischer (2005) found that only eight studies have attempted to test for costs of plasticity in plants, and 13% of the analyses within these studies detected significant costs. Costs may be more apparent within stressful environments (reviewed by van Kleunen and Fischer 2005), as well as for traits for which plasticity is adaptive (DeWitt

et al. 1998; but see Dorn et al. 2000). However, the importance of costs as a constraint on the evolution of plasticity in plants remains unclear.

Physiological traits that control the uptake of carbon dioxide and loss of water are highly plastic (e.g., Sultan and Bazzaz 1993a; Sultan et al. 1998; Heschel et al. 2004) and key determinants of plant growth and reproduction (Ackerly et al. 2000). Plasticity in physiology may be the result of reduced function and growth in response to resource limitation or developmental and regulatory responses to specific environmental cues (Pigliucci 1996). For example, plants may respond plastically to water limitation by closing their stomata, resulting in increased limits to CO₂ diffusion and decreased photosynthesis (Farquhar and Sharkey 1982). Alternatively, stomatal closure and the subsequent decrease in leaf intercellular CO₂ (C_i) can lead to changes in the biochemical regulation of photosynthesis that either enhance or limit carbon fixation (Sultan et al. 1998; Anderson et al. 2001; Farquhar et al. 2002; Lawlor 2002; Flexas et al. 2004).

It has been hypothesized that plasticity occurring through developmental and regulatory responses is more likely to be adaptive than plasticity in response to resource limitation (Pigliucci 1996), because the machinery to produce developmental and regulatory responses would not be maintained unless it was favored by natural selection. However, the adaptive value of plasticity in plant physiological traits occurring through developmental and regulatory responses has not been estimated. Moreover, the available evidence suggests that plastic physiological responses occurring through both mechanisms can be adaptive or maladaptive in plants, depending on the ecological context. For example, stomatal closure under drought can be adaptive because of increased water con-

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servation (Ehleringer 1993; Dudley 1996; Heschel et al. 2002, 2004, 2005) or maladaptive because of associated reductions in photosynthetic rate (Arntz et al. 1998). Similarly, increased photosynthetic capacity through the up-regulation of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) activity could allow plants to maintain relatively high instantaneous photosynthetic rate despite decreased C_i (Anderson et al. 2001). Finally, increased Rubisco activity and synthesis could be associated with increased maintenance respiration and a reduction in the amount of nitrogen allocated to nonphotosynthetic functions such as seed and pollen production (Sage 1994).

It has also been hypothesized that plasticity occurring through developmental and regulatory responses is more likely to be costly than plasticity in response to resource limitation (e.g., DeWitt et al. 1998; Dorn et al. 2000). This is because maintaining the machinery to produce developmental and regulatory responses requires extra energy and materials, whereas plastic responses to resource limitation do not require this machinery. To our knowledge, no studies have estimated costs of plasticity in photosynthetic and stomatal traits, regardless of the mechanism causing the plasticity.

We used a greenhouse experiment to estimate the costs and adaptive value of plasticity in physiological traits of two *Lobelia* species in response to soil water availability. Water availability limits terrestrial plant productivity across ecosystems (Knapp and Smith 2001) and can explain year-to-year variation in productivity within ecosystems (Knapp et al. 2001). This significant effect of water availability on fitness suggests that there would be strong selection for plasticity in physiological traits that regulate water loss (Ackerly et al. 2000).

Our two study species, *L. cardinalis* and *L. siphilitica*, are short-lived perennials that experience a range of soil water availabilities in the wild but differ in their ecological breadth. The *L. cardinalis* populations that we studied all occurred in soils where standing water was common. Specifically, the volumetric water content (VWC) of the soil was more than 50% during the 2000–2001 growing seasons (Caruso et al. 2003). However, VWC of the soil in our *L. cardinalis* populations can drop substantially below these levels in some years. In late September 2000, for example, VWC in some microsites was as low as 21% (data from Caruso et al. 2003) even though approximately 26 mm of rainfall was received that month (Herzmann 2005). In drier months, such as August 2003, only 8 mm of rainfall was received (Herzmann 2005), and *L. cardinalis* declined at some sites and disappeared from others (C. M. Caruso, pers. obs.). In contrast to *L. cardinalis*, our *L. siphilitica* study populations occupied a variety of habitats, from saturated soils to dry soils that have VWC < 15% in some microsites (September 2000; data from Caruso et al. 2003). Because *L. siphilitica* populations grow in a wider range of soil moisture environments than *L. cardinalis*, *L. siphilitica* may be under selection to maintain physiological performance across environments (e.g., Sultan et al. 1998). If so, then a lack of plasticity in physiological traits that regulate water loss may be adaptive in *L. siphilitica*.

Using these species, we asked three specific questions: Which physiological traits of *Lobelia* respond plastically to variation in soil water availability? Is plasticity of physio-

logical traits in response to soil water availability costly in *Lobelia*? Is plasticity of physiological traits in response to soil water availability adaptive in *Lobelia*?

MATERIALS AND METHODS

Study Species and Seed Sources

Lobelia cardinalis and *L. siphilitica* (Lobeliaceae) are short-lived, herbaceous perennial wildflowers with contrasting floral (Johnston 1991 and references therein) and physiological (Caruso et al. 2005) traits. *Lobelia cardinalis* has 4-cm-long red flowers pollinated by ruby-throated hummingbirds (*Archilochus colubris*) throughout eastern North America (Baker 1975; Bertin 1982). In contrast, *L. siphilitica*'s 3-cm-long blue flowers are pollinated by *Bombus* spp. throughout its range (Beaudoin Yetter 1989). When grown in a common greenhouse environment, *L. cardinalis* had higher photosynthetic rates and lower stomatal conductance than *L. siphilitica* (Caruso et al. 2005). *Lobelia siphilitica* seeds germinate in the spring and typically flower that fall, whereas *L. cardinalis* rosettes generally over winter prior to flowering (Johnston 1992).

We measured plasticity of physiological traits of plants grown from seeds collected from three *L. cardinalis* and three *L. siphilitica* populations. The *L. cardinalis* populations (Airport, Chichaqua Bottoms [CB], and Ditch) were located in the Chichaqua Bottoms Greenbelt, Polk County, Iowa. The *L. siphilitica* populations (Conard Environmental Research Area [CERA], Krumm, and Reichelt) were located in Jasper County, Iowa. See Caruso et al. (2003) for population descriptions.

Experimental Design

We germinated seeds from open-pollinated fruits collected from 10 plants per population. *Lobelia cardinalis* (Devlin 1989) and *L. siphilitica* (Beaudoin Yetter 1989) can produce clonal offshoots, but we attempted to sample fruits from only one ramet per genet. Because controlled crosses were not used to create these fruits, we do not know whether the seeds within fruits are full- or half-sibs, nor do we know the degree of relatedness between our families. Consequently, by using open-pollinated families we may have underestimated genetic variation for plasticity, which would make our tests for adaptive value and costs conservative. Seeds were rinsed with a dilute solution of bleach and ethanol to break dormancy (for details see Dudle et al. 2001). While we cannot rule out the possibility that our germination treatment influenced traits besides germination, to our knowledge there is no published evidence for this kind of an effect. We did not use a hormonal treatment to break dormancy, which can have persistent effects on traits such as leaf size (Fox et al. 1995; Evans et al. 1996). Approximately 20 seeds/pot were sown onto moist Pro-mix PGX (Premier Horticulture, Dorval, Quebec, Canada) and placed in standing water in the greenhouse at the University of Guelph, Guelph, Ontario, Canada. Two pots were planted for each maternal family.

After six weeks, we transplanted six seedlings per family into 9 × 9-cm plastic pots. A total of 180 *L. cardinalis* and 180 *L. siphilitica* seedlings were transplanted. Germination

rates varied among families (C. M. Caruso, pers. obs.). If germination timing was correlated with other performance traits (e.g., Donohue 2002), then variation in germination may have biased the sample of plants included in the experiment. Plants were watered as necessary, fertilized with 4 g of Nutricote Total 13-13-13 (Plant Products, Brampton, Ontario, Canada), and exposed to supplemental light (16-h days).

After approximately eight weeks of growth, we randomly assigned half of the plants in each of 60 families (10 per population) to the wet treatment and half to the dry treatment. We used a split-plot design (as in Wilkinson 1997), with treatment (wet vs. dry) as the between-plot effect and population as the within-plot effect. Consistent with this design, water availability was manipulated on a whole-plot level, with three plots (groups of seven trays) of plants being well-watered and three being droughted. The position of trays within plots was rotated weekly. Plants in the wet treatment were watered as necessary to ensure that they remained in standing water. To decide whether plants in the dry treatment needed to be watered, we measured soil moisture availability of 36 randomly chosen plants (18 per species, evenly divided between the three dry treatment plots) using a Lincoln soil moisture meter (Lincoln Irrigation, Inc., Lincoln, NE) calibrated to a range of soil volumetric water contents (VWC). On days when the mean VWC was <15%, each tray of plants in the dry treatment received approximately 50 ml of water per plant. Mean (± 1 SE) VWC was 40.04% (± 0.02) within the wet treatment and 13.78% ($\pm 3.52 \times 10^{-3}$) within the dry treatment. These treatments were maintained from 14 May 2003 until 7 October 2003. As a result, our watering treatment simulated a sustained drought during the growing season. Although drought conditions in the field may be more temporally variable, historical rainfall records for central Iowa indicate that sustained growing season droughts can occur. For example, in 1985, May–August monthly rainfall at the weather station closest to our *L. cardinalis* population was consistently below the 1951–2005 mean rainfall for these months (37–60% of monthly mean; Herzmann 2005).

Data Collection

To determine carbon gain and water use characteristics of each individual, we measured light-saturated photosynthetic rate (A) and stomatal conductance (g_s) between 15 and 25 July 2003. During gas-exchange measurements, plants in the wet treatment were watered to maintain soil saturation, whereas dry treatment plants experienced a mean (± 1 SE) VWC of 15.3% (± 0.48). These differences in soil moisture were consistent with the treatment conditions imposed throughout the experiment. We randomly selected plants, evenly divided between species and treatments, for measurement on each day. Some plants were too small for gas-exchange measurements, resulting in final $N = 173$ for *L. cardinalis* and 169 for *L. siphilitica*. Steady state leaf gas exchange was measured at saturating irradiance (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with an open gas-exchange system (LI-6400, Li-Cor, Inc., Lincoln, NE) between 0900 and 1200 h EST. During measurements, incident irradiance was provided by red-blue light-emitting diodes and cuvette CO_2 concentration was

maintained at 400 $\mu\text{mol mol}^{-1}$ to reflect prevailing ambient conditions. A Peltier cooling module maintained leaf temperature at approximately ambient conditions (25–30°C) during each measurement period. We maintained the leaf-to-air vapor pressure deficit (D) at a mean (± 1 SD) of 0.95 ± 0.15 kPa, which permitted the measurement of maximum g_s (Oren et al. 1999). To calculate g_s we used a boundary layer conductance of $1.42 \text{ mol m}^{-2} \text{ s}^{-1}$, which was calculated on the basis of leaf area and fan speed using the energy balance algorithms of the LI-6400. Following enclosure in the leaf cuvette, leaves reached steady-state values (e.g., when the coefficients of variation of CO_2 and H_2O within the chamber were <0.25%) within 5 min. Because leaf thickness can influence photosynthetic rate (Reich et al. 1999), we measured specific leaf area (SLA) on all leaves used for gas-exchange measurements. Each leaf was excised and its area measured with a leaf area meter (AM-200, ADC Bioscientific, Hoddesdon, UK). These leaves were dried to constant mass in a forced convection oven at 65°C for 24 h and weighed. Specific leaf area was calculated by dividing leaf size by leaf mass. We multiplied light-saturated photosynthetic rate and stomatal conductance by SLA to express our area-based estimates of A and g_s on a per unit mass basis.

We characterized two components of leaf photosynthetic capacity for each species. We calculated the maximum velocity of carboxylation of Rubisco (V_{cmax}) and CO_2 saturated photosynthetic rate (A_{max}) based on the response of net photosynthesis (A) to variation in C_i (an $A:C_i$ curve). V_{cmax} is representative of the primary biochemical limitation to carbon fixation capacity when C_i is low, whereas A_{max} is an estimate of limitations on carbon fixation capacity by the regeneration of RuBP when C_i is high (Farquhar et al. 1980). Consequently, V_{cmax} is calculated from the initial slope of the response of A to C_i , whereas A_{max} is calculated at the saturation point of the $A:C_i$ curve (and thus in the absence of CO_2 limitation; Farquhar et al. 1980). Although A_{max} and V_{cmax} limit carbon fixation at differing C_i , they are often correlated (Geber and Dawson 1997).

Because they are time consuming to construct (~ 20 min each), $A:C_i$ curves were measured on a subsample of families ($N = 7$) in each of two *L. siphilitica* (CERA and Krumm) and two *L. cardinalis* (Airport and CB) populations. Curves were fitted with a nonlinear regression model describing an exponential rise to a maximum:

$$A = a[1 - \exp(-bC_i)] + c, \quad (1)$$

where c is the y intercept, $1/b$ is the rate constant, and $a + c$ is CO_2 saturated A (A_{max}). This model provided a good fit to our data ($R^2 > 0.97$) and has been used previously for herbaceous C_3 plants (Jacob et al. 1995; Reid and Fiscus 1998).

V_{cmax} was estimated from the biochemical model of von Caemmerer and Farquhar (1981):

$$V_{\text{cmax}} = CE \left[\Gamma^* + K_C \left(1 + \frac{[\text{O}_2]}{K_O} \right) \right], \quad (2)$$

where the constants were $K_C = 424$ Pa, $K_O = 25.5$ kPa, and Γ^* (CO_2 compensation point in the absence of mitochondrial respiration in the light) = 44.4 Pa at a temperature of 28°C (Harley et al. 1985; Reid and Fiscus 1998) and an $[\text{O}_2]$ of

21 kPa. Carboxylation efficiency (CE), which is the first derivative of equation (1) at the CO_2 compensation point, was calculated as

$$CE = ab \times \exp(-bC_i), \quad (3)$$

where $C_i = (1/b)\ln(a/[a - A + c])$ at $A = 0$ (Reid and Fiscus 1998).

Ninety-five percent of *L. siphilitica*, but less than 2% of *L. cardinalis*, flowered during the course of the experiment. Therefore, we used final aboveground biomass as our estimate of fitness. *Lobelia siphilitica* plants were harvested when they finished flowering or on 7 October 2003, whichever came first. All *L. cardinalis* plants were harvested on 7 October 2003. Plants were dried to constant mass in a forced convection oven at 65°C for 24 h and weighed.

Statistical Analysis

To determine if fitness (plant mass) and six of our physiological traits (leaf size, SLA, photosynthetic rate [area- and mass-based] and stomatal conductance [area- and mass-based]) responded plastically to soil water availability, we used a split-plot ANOVA. All analyses were done separately for each species. Treatment (wet vs. dry) was the between-plot factor and population was the within-plot factor. In this design, treatment was tested over the plot (treatment) term, whereas the population and treatment \times population terms were tested over the population \times plot (treatment) term (Wilkinson 1997). A significant treatment term in the analyses of fitness indicated that our experimental manipulation was successful. A significant treatment term for a physiological trait indicated that the trait responded plastically to soil water availability. Because variation in trays within plots did not have a significant effect on physiological traits or fitness (data not shown), it was not included in any of our analyses. Each response variable was tested for the assumption of normality using Lilliefors's test (Wilkinson 1997). We tested the assumption of homogeneity of residual variance by examining variances for each treatment group.

Because A_{\max} and $V_{c\max}$ were measured for only two populations per species, they were analyzed using a two-way ANOVA, rather than a split-plot. All analyses were done separately for each species. The two factors were treatment (wet vs. dry) and population. All population \times treatment terms were nonsignificant and were dropped from the model. A significant treatment term indicated that the physiological trait responded plastically to soil water availability. Each response variable was tested for the assumption of normality using Lilliefors's test (Wilkinson 1997). We tested the assumption of homogeneity of residual variance by calculating the Spearman rank correlation between the residuals and the dependent variable (Neter et al. 1989).

We used regression to determine if plasticity of physiological traits was costly (van Tienderen 1991; DeWitt 1998; Scheiner and Berrigan 1998; van Kleunen et al. 2000; Stinchcombe et al. 2004). These analyses were done only for those traits that responded plastically to soil water availability. The dependent variable was family mean fitness within a treatment. The two independent variables were the family mean for each trait within a treatment and a measure of plasticity

across treatments for the same trait. We ran separate regression models for each combination of treatment and trait. We relativized fitness by dividing by mean fitness for all families and standardized each of the independent variables to a mean = 0 and variance = 1. Because A_{\max} of *L. cardinalis* and *L. siphilitica* was higher within the dry treatment, plasticity was estimated by subtracting the family mean for each trait within the wet treatment from the family mean for each trait within the dry treatment. Because area-based A , area- and mass-based g_s , and SLA of *L. cardinalis* were higher within the wet treatment, plasticity for these traits was estimated by subtracting the family mean within the dry treatment from the family mean within the wet treatment. Only a significant negative regression coefficient for the plasticity term indicates that plasticity was costly within that environment. Therefore, we used one-tailed t -tests to determine if this coefficient was significantly less than zero (as in van Kleunen et al. 2000).

We used two complementary regression approaches to determine if plasticity in physiological traits of *L. cardinalis* and *L. siphilitica* was adaptive. First, we regressed each individual plant's relativized fitness on its standardized physiological traits (within-environment phenotypic selection analysis for adaptive plasticity; reviewed by van Kleunen and Fischer 2005). We ran separate regression models for each combination of treatment and trait. If selection on a trait within a treatment was concordant with the direction of significant plastic differences in that trait between treatments, then we inferred that plasticity for that trait was adaptive. Second, we regressed a family's relativized mean fitness across treatments on the standardized family mean for each trait across treatments and a standardized measure of plasticity for each trait (across-environment genotypic selection analysis for adaptive plasticity; van Kleunen and Fischer 2001; Stinchcombe et al. 2004). We ran separate regression models for each trait. We calculated plasticity by subtracting family means for the wet and dry treatments such that the difference between treatments was generally positive. Specifically, because A_{\max} of *L. cardinalis* and *L. siphilitica* was higher within the dry treatment, plasticity was estimated by subtracting the family mean for each trait within the wet treatment from the family mean for each trait within the dry treatment. Because mass-based A , area- and mass-based g_s , and SLA of *L. cardinalis* were higher within the wet treatment, plasticity for these traits was estimated by subtracting the family mean within the dry treatment from the family mean within the wet treatment. When plasticity is calculated in this way, a significant positive regression coefficient for the plasticity term indicates that more plastic families have higher fitness across both environments (i.e., plasticity is adaptive).

We used two complementary regression approaches to determine if a lack of plasticity in physiological traits of *L. cardinalis* and *L. siphilitica* was adaptive, maladaptive, or adaptively neutral. First, we regressed each individual plant's relativized fitness on its standardized physiological traits. We ran separate regression models for each combination of treatment and trait. If a trait did not respond plastically to soil water availability, and there was significant selection on that trait within either treatment, then we inferred that a lack of

plasticity in this trait was maladaptive. If a trait did not respond plastically to soil water availability, and there was no significant selection on that trait in either environment, then we inferred that a lack of plasticity in that trait was adaptively neutral. Second, we regressed a family's relativized mean fitness across treatments on the standardized family mean for each trait across treatments and the standardized plasticity for each trait. We ran separate regression models for each trait. We calculated plasticity as the absolute value of the wet treatment family mean subtracted from the dry treatment family mean (e.g., Scheiner and Berrigan 1998). When plasticity is calculated in this way, a significant negative regression coefficient for the plasticity term indicates that less plastic families, regardless of the direction of plasticity, have higher fitness across both environments (i.e., a lack of plasticity is adaptive). A significant positive regression coefficient for the plasticity term indicates that a lack of plasticity is maladaptive, whereas a nonsignificant coefficient indicates that a lack of plasticity is adaptively neutral.

All analyses of costs and adaptive significance were done separately for each species, but to maximize our sample sizes we pooled populations within species. To account for any variation between populations, we included population as a fixed term in the model. The assumption of normality of residual variance for all regression models presented in this paper was tested using Lilliefors' test (Wilkinson 1997). We tested the assumption of homogeneity of residual variance for all of our regression models by calculating the Spearman rank correlation between the residuals and relative fitness. Variance inflation factors for all analyses of costs of plasticity, as well as the across-environment analyses of adaptive plasticity, were less than 10, indicating there was minimal multicollinearity between family means for a trait and plasticity for that trait (Neter et al. 1989).

We analyzed the adaptive value and costs of plasticity separately for each of our traits for three reasons. First, because physiological measurements are time-consuming, we were able to measure a relatively small number of individuals and families, especially for A_{\max} and V_{\max} , leaving us with limited power for multivariate analyses. Second, because we measured A_{\max} and V_{\max} on a subset of plants in the experiment, we could not include all of our traits as independent variables in a single multiple regression model. Third, two pairs of traits, $A - g_s$ and $A_{\max} - V_{\max}$, were tightly ($r > 0.70$) phenotypically correlated with each other. These strong correlations tend to result in unstable multiple regression coefficients (Mitchell-Olds and Shaw 1987).

RESULTS

Effect of Soil Water Availability on Fitness

The drought treatment decreased fitness of both *L. cardinalis* and *L. siphilitica*, but the effect of soil water availability varied among *L. cardinalis* populations. Although fitness of *L. cardinalis* was 46% higher in the well-watered treatment (Fig. 1I), the treatment main effect was not significant ($F_{1,4} = 3.66$, $P > 0.10$). Instead, there were significant effects of population ($F_{2,8} = 4.56$, $P < 0.05$) and population \times treatment ($F_{2,8} = 11.42$, $P < 0.005$) on the fitness of *L. cardinalis*. Specifically, fitness was higher in the well-watered treatment

in two *L. cardinalis* populations, but did not differ between the drought and well-watered treatments in a third population (Fig. 1I). In contrast, fitness of *L. siphilitica* was 69% higher in the well-watered treatment and the treatment main effect was significant ($F_{1,4} = 211.74$, $P < 0.001$). Neither population ($F_{2,8} = 3.78$, $P < 0.10$) nor population \times treatment ($F_{2,8} = 0.78$, $P > 0.25$) had a significant effect on fitness of *L. siphilitica*.

Plasticity of Physiological Traits

Five of eight physiological traits of *L. cardinalis* responded plastically to soil water availability (Tables 1, 2). SLA was 22% higher in the wet than dry treatment, indicating that plants in the wet environment produced significantly thinner leaves (Table 1; Fig. 1B). Mass-based photosynthetic rate was 34% higher in the wet treatment (Table 1; Fig. 1C). However, *L. cardinalis* exposed to drought conditions had 18% higher A_{\max} (Fig. 1G) than those exposed to well-watered conditions (Table 2). Both mass- and area-based stomatal conductance were significantly higher under well-watered conditions (Table 1), but mass-based g_s increased by 98%, whereas area-based g_s only increased by 65% (Figs. 1E, F). In addition to these significant effects of soil water availability on physiology, SLA (Fig. 1B), mass-based photosynthetic rate (Fig. 1C), and mass-based stomatal conductance (Fig. 1E) all differed significantly among *L. cardinalis* populations (Table 1). However, there were no significant interactions between treatment and population (Table 1).

In *L. siphilitica*, only one of eight physiological traits responded plastically to soil water availability (Tables 1, 2). Plants in the dry treatment had 31% higher A_{\max} (Table 2; Fig. 1G) than those in the wet treatment. In addition to this significant effect of soil water availability on physiology, SLA (Fig. 1B) and mass-based photosynthetic rate (Fig. 1C) differed significantly among *L. siphilitica* populations (Table 1). There were no significant interactions between treatment and population (Table 1).

Costs of Plasticity

We found no evidence that plasticity of physiological traits was costly for *L. cardinalis* or *L. siphilitica*. Families that were more physiologically plastic did not have significantly lower fitness under either well-watered or dry conditions (Table 3).

Is Plasticity Adaptive?

Cross-environmental analyses suggested that plasticity of two of six physiological traits was adaptive in *L. cardinalis* and/or *L. siphilitica*. *Lobelia cardinalis* families with more plastic mass-based photosynthetic rate and stomatal conductance had significantly higher average fitness across well-watered and drought conditions. We did not detect an effect of plasticity in SLA, area-based stomatal conductance, or A_{\max} on fitness of *L. cardinalis* and/or *L. siphilitica* (Table 4).

Within-environment analyses suggested that plasticity of physiological traits in *L. cardinalis* can be adaptive, maladaptive, or adaptive neutral. Plants with higher mass- and

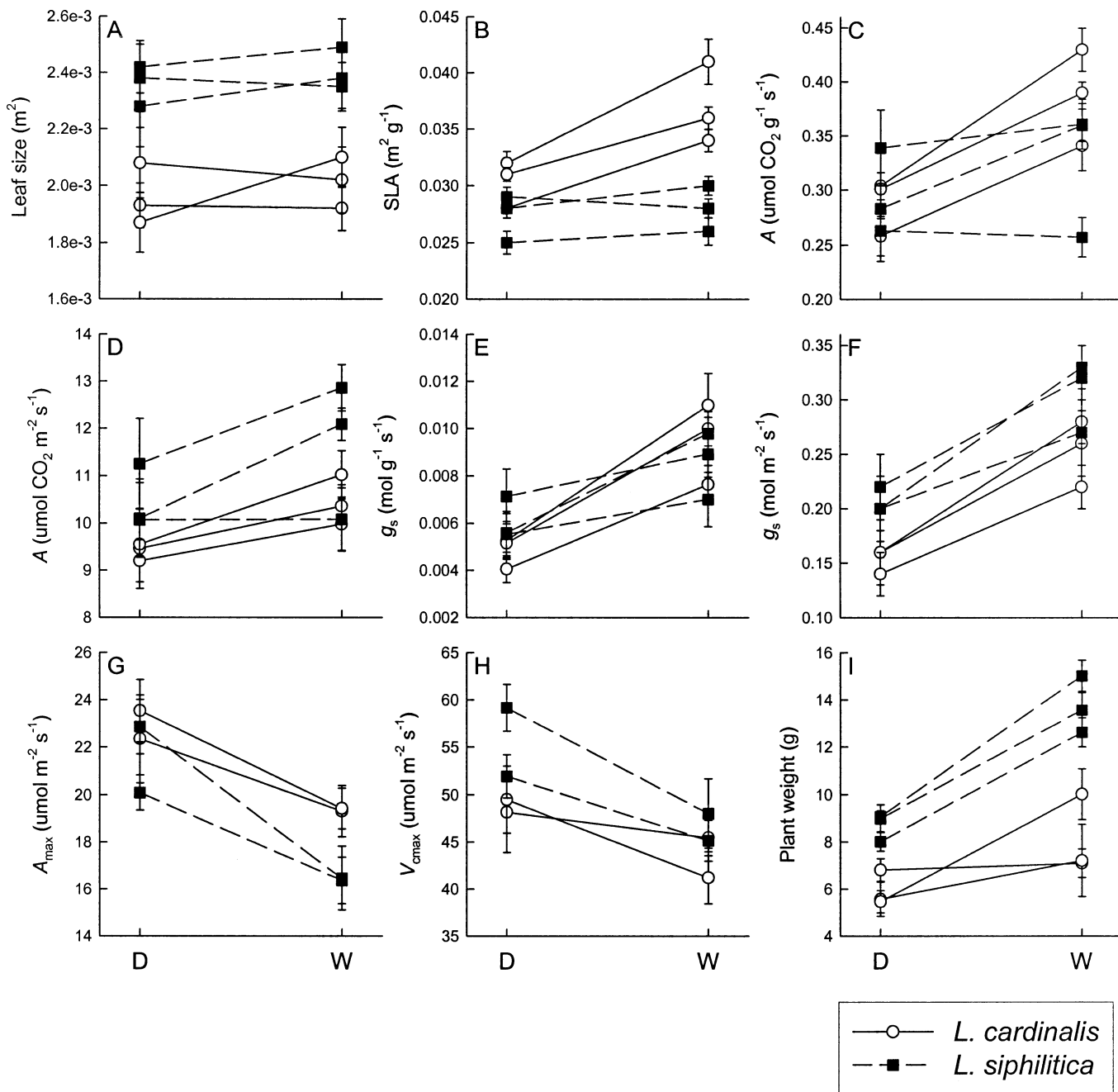


FIG. 1. Mean (± 1 SE) response of eight physiological traits and final plant weight of *Lobelia cardinalis* and *L. siphilitica* to dry (D) and wet (W) treatments. (A) Leaf size, (B) specific leaf area, (C) mass-based photosynthetic rate, (D) area-based photosynthetic rate, (E) mass-based stomatal conductance, (F) area-based stomatal conductance, and (I) plant weight were measured for three *L. cardinalis* and three *L. siphilitica* populations. (G) A_{\max} and (H) V_{\max} were measured for two *L. cardinalis* and two *L. siphilitica* populations.

area-based stomatal conductance had significantly higher fitness under well-watered conditions (Table 5). Given that g_s was higher (Figs. 1E, F) under well-watered conditions, this suggests that plasticity in g_s was adaptive. In contrast, higher A_{\max} (Fig. 1G) under drought conditions was maladaptive. Plants with lower A_{\max} had significantly higher fitness under drought conditions (Table 5), but A_{\max} was higher in the dry treatment (Fig. 1G). Finally, the within-environment analyses

suggest that plasticity in SLA (Fig. 1B) and mass-based photosynthetic rate (Fig. 1C) had no effect on fitness of *L. cardinalis* (Table 5) and were thus adaptively neutral.

In contrast to *L. cardinalis*, the within-environment analyses suggested that plasticity of physiological traits in *L. siphilitica* was adaptively neutral. Variation in A_{\max} had no effect on fitness under either well-watered or drought conditions (Table 5).

TABLE 1. Plasticity in six physiological traits of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability. Traits were analyzed using a split-plot ANOVA with treatment (wet vs. dry) as the between-plot effect and population as the within-plot effect. df_{num} , df_{denom} for treatment = 1, 4. df_{num} , df_{denom} for population and treatment \times population = 2, 8.

	<i>L. cardinalis</i>			<i>L. siphilitica</i>		
	MS	F	P	MS	F	P
	Leaf size (m ²)					
Treatment	4.87 \times 10 ⁴	0.23	>0.25	1.29 \times 10 ⁴	0.25	>0.25
Population	2.26 \times 10 ⁴	3.70	>0.05	8.74 \times 10 ³	0.13	>0.25
Treat \times pop	2.96 \times 10 ⁴	4.28	>0.05	2.19 \times 10 ⁴	0.33	>0.25
	Specific leaf area (m ² g ⁻¹)					
Treatment	2.48 \times 10 ⁻⁴	34.36	<0.005	6.90 \times 10 ⁻⁷	0.24	>0.25
Population	4.72 \times 10 ⁻⁵	34.96	<0.0005	2.12 \times 10 ⁻⁵	13.14	<0.005
Treat \times pop	3.82 \times 10 ⁻⁶	2.83	>0.10	1.90 \times 10 ⁻⁶	1.18	>0.25
	Photosynthetic rate (μ mol CO ₂ g ⁻¹ s ⁻¹)					
Treatment	5.04 \times 10 ⁻²	19.48	<0.025	5.77 \times 10 ⁻³	0.83	>0.25
Population	8.11 \times 10 ⁻³	10.27	<0.01	1.45 \times 10 ⁻²	5.68	<0.05
Treat \times pop	2.78 \times 10 ⁻⁴	0.35	>0.25	2.60 \times 10 ⁻³	1.02	>0.25
	Photosynthetic rate (μ mol CO ₂ m ⁻² s ⁻¹)					
Treatment	5.38	1.70	>0.25	6.64	1.31	>0.25
Population	0.83	1.52	>0.25	6.05	3.12	>0.05
Treat \times pop	0.10	0.19	>0.25	1.15	0.59	>0.25
	Stomatal conductance (mol g ⁻¹ s ⁻¹)					
Treatment	1.01 \times 10 ⁻⁴	67.37	<0.0025	4.29 \times 10 ⁻⁵	2.33	>0.10
Population	9.86 \times 10 ⁻⁶	5.54	<0.05	1.05 \times 10 ⁻⁵	1.13	>0.25
Treat \times pop	9.89 \times 10 ⁻⁷	0.55	>0.50	3.04 \times 10 ⁻⁶	0.33	>0.25
	Stomatal conductance (mol m ⁻² s ⁻¹)					
Treatment	0.043	24.66	<0.01	3.87 \times 10 ⁻²	2.24	>0.10
Population	2.65 \times 10 ⁻³	2.14	>0.10	1.85 \times 10 ⁻³	0.42	>0.25
Treat \times pop	2.02 \times 10 ⁻⁵	1.63 \times 10 ⁻²	>0.25	1.82 \times 10 ⁻³	0.42	>0.25

Is a Lack of Plasticity Adaptive?

Cross-environmental analyses suggested that a lack of plasticity in physiological traits of *L. cardinalis* and *L. siphilitica* was adaptively neutral. Families that were less physiologically plastic did not have significantly higher or lower fitness across treatments (Table 6).

Within-environment analyses suggested that a lack of plasticity in physiological traits of *L. cardinalis* can be maladaptive or adaptively neutral. *Lobelia cardinalis* plants that produced larger leaves had higher fitness within the wet treatment (Table 5), but this trait was not plastic (Table 1; Fig. 1A), suggesting that this lack of plasticity was maladaptive. Area-based photosynthetic rate and V_{cmax} had no effect on fitness of *L. cardinalis* in either treatment, suggesting that a lack of plasticity in these traits was adaptively neutral (Table 5).

Within-environment analyses suggested that a lack of plasticity in physiological traits of *L. siphilitica* can also be maladaptive or adaptively neutral. *Lobelia siphilitica* plants that produced larger leaves and a lower SLA had higher fitness within the dry treatment (Table 5), suggesting that a lack of plasticity in these traits (Table 1; Figs. 1A, B) was maladaptive. Area- and mass-based photosynthetic rate, area- and mass-based stomatal conductance, and V_{cmax} had no effect on fitness of *L. siphilitica* in either treatment, suggesting that a lack of plasticity in these traits was adaptively neutral (Table 5).

DISCUSSION

We detected significant plasticity in response to soil moisture availability for five of eight physiological traits in *L. cardinalis* and one of eight traits in *L. siphilitica* (Tables 1, 2). The direction of these significant responses was generally consistent with previous studies of plasticity in response to moisture availability (Sultan and Bazzaz 1993b; Sultan et al. 1998; Heschel et al. 2004; Heschel and Riginos 2005). However, our results do not support the hypothesis that plasticity occurring through developmental and regulatory responses is likely to be adaptive. In addition, our results do not support the hypothesis that plasticity in response to resource limitation is likely to be maladaptive or adaptively neutral (Pigliucci 1996). The two traits for which plasticity was adaptive (mass-based A and g_s ; Table 4) were both responses to water limitation, whereas plasticity in the biochemical regulation of photosynthesis (A_{max}) was maladaptive or adaptively neutral (Tables 4, 5).

Although the response of photosynthetic capacity to transient drought has been well-studied (reviewed in Lawlor 2002; Flexas et al. 2004), ours is the first test of whether these responses are adaptive. Increased biochemical photosynthetic capacity has been shown to increase fitness (Arntz et al. 1998; Arntz and Delph 2001), but we found that increased A_{max} in response to drought was maladaptive in *L. cardinalis* and adaptively neutral in *L. siphilitica* (Tables 4, 5). Because a substantial portion of leaf nitrogen is typically

TABLE 2. Plasticity in two physiological traits associated with photosynthetic capacity of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability. Traits were analyzed using a two-way ANOVA with treatment (wet vs. dry) and population (two per species) as main effects. None of the interaction terms were significant and were dropped from the analyses. df_{num} , df_{denom} for treatment and population = 1, 9.

	<i>L. cardinalis</i>			<i>L. siphilitica</i>		
	MS	F	P	MS	F	P
			A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
Treatment	57.90	4.90	0.05	80.61	14.17	0.004
Population	0.20	0.02	0.90	2.33	0.41	0.54
			V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
Treatment	1.09×10^2	3.87	0.08	1.83×10^2	2.04	0.19
Population	0.02	5.95×10^{-4}	0.98	1.16×10^2	1.29	0.29

allocated to Rubisco, plasticity in A_{max} of *L. cardinalis* was likely caused by increased allocation to leaf nitrogen under drought (Wright et al. 2001; Huberty and Denno 2004). Consistent with this prediction, we found that chlorophyll concentration, an indicator of nitrogen content (Chapman and Barreto 1997), was higher in the dry treatment in both *L. cardinalis* and *L. siphilitica* (data not shown). Nevertheless, because plasticity in A_{max} was maladaptive or adaptively neutral (Tables 4, 5), the benefit of increased nitrogen for photosynthesis was probably outweighed by carbon costs associated with increased dark respiration (e.g., Wright et al. 2001). In addition, higher A_{max} may have been maladaptive or adaptively neutral (Tables 4, 5) because increased RuBP regeneration capacity is metabolically wasteful, especially when plants are operating at low C_i because of stomatal closure (Sage 1994).

In contrast to previous work (Dudley 1996; Heschel et al. 2002, 2004, 2005; Heschel and Riginos 2005), we found that lower g_s , and therefore increased water conservation (Ehleringer 1993) was not adaptive in the dry environment for *L. cardinalis*. Instead, plasticity of g_s was adaptive in *L. cardinalis* because plants with higher g_s had higher fitness within the wet treatment (Table 5). The contrast between our results and those in the literature may be because of differences in the degree to which stomatal opening limits A of *L. cardinalis* in the wet versus dry environment. Specifically, *L. cardinalis* in the wet environment had higher intercellular CO_2 concentration (C_i) than those in the dry environment (separate-variance t -test; $t = 7.81$, $df = 156.7$, $P < 0.001$). Therefore,

increased g_s under wet conditions may be adaptive because of decreased diffusional limits on CO_2 supply to photosynthesis (Farquhar and Sharkey 1982).

Although thicker leaves (lower SLA) are thought to be a common adaptation to drought (e.g., Niinemets 2001; Wright et al. 2001; Ackerly 2004), the plastic decrease in SLA of *L. cardinalis* under drought was adaptively neutral (Tables 4, 5). However, plasticity in mass-based A in response to drought (Table 1; Fig. 1C) was adaptive in this species (Table 4), whereas area-based A was not plastic (Table 1; Fig. 1D). This suggests that the adaptive plasticity of mass-based A in *L. cardinalis* was driven primarily by plasticity in SLA. Although we could not determine if increased A was adaptive in the wet environment or if decreased A was adaptive in the dry environment (Fig. 1C; Table 5), decreased SLA under drought may represent a structural mechanism to reduce photosynthesis independently of biochemical regulation in *L. cardinalis*. For example, low SLA could reduce photosynthesis (Peterson 1999; Reich et al. 1999) potentially because of leaf structural changes that cause greater internal shading (Terashima and Hikosaka 1995) or greater resistance to CO_2 diffusion (Parkhurst 1994). As a result, decreased SLA could potentially mitigate the effects of the maladaptive increase in A_{max} on photosynthetic rate in the dry environment (Table 5). Nevertheless we cannot rule out that a change in the variance structure of the data caused by multiplying area-based A by SLA may have allowed us to detect adaptive plasticity in mass-based A and not SLA itself (Peterson 1999).

Fewer physiological traits responded plastically to soil

TABLE 3. Analysis of costs of plasticity in physiological traits of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability. Costs were estimated by regressing the family mean fitness within a treatment (wet vs. dry) on the family mean of a trait and a measure of plasticity of that trait. A significant negative regression coefficient (β) for plasticity indicates that it is costly. $N = 29$ for specific leaf area, A , and g_s . $N = 11$ – 12 for A_{max} .

	Wet treatment			Dry treatment		
	β	SE	P	β	SE	P
<i>L. cardinalis</i>						
Specific leaf area ($\text{m}^2 \text{g}^{-1}$)	0.104	0.100	>0.10	0.080	0.043	<0.05
Photosynthetic rate ($\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$)	0.045	0.073	>0.25	0.048	0.064	>0.10
Stomatal conductance ($\text{mol g}^{-1} \text{s}^{-1}$)	0.034	0.081	>0.25	0.039	0.047	>0.25
Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	-0.009	0.065	>0.25	0.003	0.050	>0.25
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.185	0.125	<0.10	0.125	0.076	<0.10
<i>L. siphilitica</i>						
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.058	0.086	>0.10	0.008	0.062	>0.25

TABLE 4. Cross-environment analysis of adaptive plasticity in physiological traits of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability. The family mean fitness across treatments was regressed on the family mean of a trait across treatments and a measure of plasticity of that trait. A significant positive regression coefficient (β) for plasticity indicates that it is adaptive. Significant ($P < 0.05$) coefficients are in bold. $N = 29$ for specific leaf area, A , and g_s . $N = 11-12$ for A_{\max} .

	β	SE	P
<i>L. cardinalis</i>			
Specific leaf area ($\text{m}^2 \text{g}^{-1}$)	0.040	0.043	0.371
Photosynthetic rate ($\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$)	0.074	0.035	0.042
Stomatal conductance ($\text{mol g}^{-1} \text{s}^{-1}$)	0.070	0.033	0.044
Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	0.033	0.039	0.397
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.081	0.071	0.294
<i>L. siphilitica</i>			
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.009	0.043	0.833

moisture availability in *L. siphilitica* than in *L. cardinalis* (Tables 1, 2). One explanation for this result is that species with greater ecological breadth, such as *L. siphilitica* (Caruso et al. 2003), have evolved the capacity to maintain constant physiological function in a variety of soil moisture environments. In this case, we would expect that physiological traits such as A and g_s should exhibit very little plasticity and that the absence of this plasticity is adaptive. In contrast, traits that allow this constancy across environments, such as photosynthetic capacity (V_{cmax} and A_{\max}), should exhibit greater plasticity and this plasticity should be adaptive (Sultan et al. 1998). However, we found that the lack of plasticity in A and g_s , as well as plasticity in A_{\max} , was adaptively neutral in *L. siphilitica* (Tables 4–6). Although our results are con-

TABLE 6. Cross-environment analysis of whether a lack of plasticity in physiological traits of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability is adaptive. Family mean fitness across treatments was regressed on the family mean of a trait across treatments and a measure of plasticity of that trait. A significant negative regression coefficient (β) for plasticity indicates that a lack of plasticity is adaptive. $N = 29-30$ for all traits except A_{\max} and V_{cmax} , for which $N = 11-12$.

	β	SE	P
<i>L. cardinalis</i>			
Leaf size (m^2)	-0.006	0.035	0.857
Photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	0.052	0.031	0.107
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.002	0.080	0.978
<i>L. siphilitica</i>			
Leaf size (m^2)	0.033	0.024	0.180
Specific leaf area ($\text{m}^2 \text{g}^{-1}$)	0.047	0.030	0.123
Photosynthetic rate ($\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$)	0.001	0.029	0.968
Photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	-0.001	0.029	0.973
Stomatal conductance ($\text{mol g}^{-1} \text{s}^{-1}$)	0.011	0.028	0.689
Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	0.005	0.027	0.858
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.015	0.042	0.730

sistent with the expectation of physiological constancy in species with greater ecological breadth, they do not support the hypothesis that this constancy is adaptive.

None of our analyses for costs of plasticity were significant (Table 3), regardless of whether plasticity was the result of reduced function and growth in response to resource limitation or developmental and regulatory responses to specific environmental cues. This supports a recent review (van Kleunen and Fisher 2005) that found little evidence for costs of plasticity in plants. However, it is difficult to reconcile these empirical results with theory, which indicates that costs are

TABLE 5. Within-environment analysis of whether plasticity or a lack of plasticity in physiological traits of *Lobelia cardinalis* and *L. siphilitica* in response to soil water availability is adaptive. Fitness within a treatment (wet vs. dry) was regressed on each plastic trait. Significant ($P < 0.05$) coefficients are in bold. $N = 75-77$ for leaf size, specific leaf area, A , and g_s of *L. cardinalis*. $N = 31-32$ for A_{\max} and V_{cmax} of *L. cardinalis*. $N = 86-89$ for leaf size, specific leaf area, A , and g_s of *L. siphilitica*. $N = 34-36$ for A_{\max} and V_{cmax} of *L. siphilitica*.

	Wet treatment			Dry treatment		
	β	SE	P	β	SE	P
<i>L. cardinalis</i> , plastic traits						
Specific leaf area ($\text{m}^2 \text{g}^{-1}$)	-0.114	0.059	0.056	-0.008	0.043	0.850
Photosynthetic rate ($\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$)	-0.009	0.058	0.877	0.032	0.041	0.445
Stomatal conductance ($\text{mol g}^{-1} \text{s}^{-1}$)	0.131	0.055	0.019	0.020	0.041	0.634
Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	0.137	0.054	0.013	0.029	0.040	0.481
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.081	0.107	0.456	-0.148	0.062	0.023
<i>L. cardinalis</i> , nonplastic traits						
Leaf size (m^2)	0.170	0.044	0.000	0.057	0.041	0.165
Photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	0.025	0.048	0.598	0.041	0.040	0.301
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.015	0.042	0.726	0.014	0.054	0.794
<i>L. siphilitica</i> , plastic traits						
A_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	-0.044	0.044	0.321	-0.082	0.055	0.145
<i>L. siphilitica</i> , nonplastic traits						
Leaf size (m^2)	0.021	0.027	0.427	0.072	0.032	0.027
Specific leaf area ($\text{m}^2 \text{g}^{-1}$)	-0.029	0.028	0.294	-0.100	0.033	0.003
Photosynthetic rate ($\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$)	-0.051	0.029	0.077	-0.041	0.034	0.230
Photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	-0.033	0.028	0.246	-0.006	0.033	0.869
Stomatal conductance ($\text{mol g}^{-1} \text{s}^{-1}$)	-0.052	0.027	0.055	-0.041	0.033	0.224
Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	-0.043	0.026	0.110	-0.022	0.033	0.513
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.077	0.079	0.337	-0.113	0.059	0.063

a significant limit on the evolution of plasticity (reviewed in van Kleunen and Fischer 2005). One possibility is that plasticity in physiological traits of *Lobelia* is costly in the field, but not in the greenhouse environment. For example, costs could be more apparent within more stressful resource limited environments (reviewed in van Kleunen and Fischer 2005). Our drought treatment greatly reduced fitness of both *L. cardinalis* and *L. siphilitica* (Fig. 1I), suggesting that the plants were stressed despite growing in the greenhouse. However, plant growth may be more limited by increased variation in the timing of precipitation rather than total rainfall (e.g., Fay et al. 2003). Consequently, we cannot rule out that costs of plasticity in physiological traits of *Lobelia* may be more apparent under variable field rather than more constant greenhouse conditions.

In summary, we found significant phenotypic plasticity for physiological traits of *Lobelia*, but in contrast to previous studies, much of this plasticity was adaptively neutral or maladaptive. Mass-based A and g_s of *L. cardinalis* were the only traits for which we found clear evidence of adaptive plasticity, and the reason why plasticity in g_s was adaptive contradicted other published studies. Specifically, *L. cardinalis* plants with higher g_s had higher fitness under wet conditions, but lower g_s was not favored under dry conditions (Table 5). More generally, our results highlight the importance of testing assumptions about the adaptive value of plastic responses, especially for plant functional traits such as photosynthetic physiology (e.g., Dudley 2004). For example, plasticity in the biochemical regulation of photosynthesis (e.g., A_{max} ; Fig. 1G), which is a regulatory and developmental response to drought, was maladaptive or adaptively neutral (Tables 4, 5). Although there are difficulties with all methods of testing for adaptive plasticity (summarized in Sultan 1995; Pigliucci 2001), recent reviews (e.g., Pigliucci 2005) suggest that such tests are central to our understanding of phenotypic evolution.

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