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GENETIC VARIANCE AND COVARIANCE FOR PHYSIOLOGICAL TRAITS IN *LOBELIA*: ARE THERE CONSTRAINTS ON ADAPTIVE EVOLUTION?

CHRISTINA M. CARUSO,[1,2,3,4*] HAFIZ MAHERALI,[2,3,5*] ALISON MIKULYUK,[6,7] KJARSTIN CARLSON,[6,8] AND ROBERT B. JACKSON[3]

[1]*Departments of Biology and Mathematics, Grinnell College, Grinnell, Iowa 50112*
[3]*Department of Biology, Duke University, Durham, North Carolina 27708*
[6]*Department of Biology, Grinnell College, Grinnell, Iowa 50112*

Abstract.—Physiological traits that control the uptake of carbon dioxide and loss of water are key determinants of plant growth and reproduction. Variation in these traits is often correlated with environmental gradients of water, light, and nutrients, suggesting that natural selection is the primary evolutionary mechanism responsible for physiological diversification. Responses to selection, however, can be constrained by the amount of standing genetic variation for physiological traits and genetic correlations between these traits. To examine the potential for constraint on adaptive evolution, we estimated the quantitative genetic basis of physiological trait variation in one population of each of two closely related species (*Lobelia siphilitica* and *L. cardinalis*). Restricted maximum likelihood analyses of greenhouse-grown half-sib families were used to estimate genetic variances and covariances for seven traits associated with carbon and water relations. We detected significant genetic variation for all traits in *L. siphilitica*, suggesting that carbon-gain and water-use traits could evolve in response to natural selection in this population. In particular, narrow-sense heritabilities for photosynthetic rate (A), stomatal conductance (g_s), and water-use efficiency (WUE) in our *L. siphilitica* population were high relative to previous studies in other species. Although there was significant narrow-sense heritability for A in *L. cardinalis*, we detected little genetic variation for traits associated with water use (g_s and WUE), suggesting that our population of this species may be unable to adapt to drier environments. Despite being tightly linked functionally, the genetic correlation between A and g_s was not strong and significant in either population. Therefore, our *L. siphilitica* population would not be genetically constrained from evolving high A (and thus fixing more carbon for growth and reproduction) while also decreasing g_s to limit water loss. However, a significant negative genetic correlation existed between WUE and plant size in *L. siphilitica*, suggesting that high WUE may be negatively associated with high fecundity. In contrast, our results suggest that any constraints on the evolution of photosynthetic and stomatal traits of *L. cardinalis* are caused primarily by a lack of genetic variation, rather than by genetic correlations between these functionally related traits.

Key words.—Genetic correlations, heritability, *Lobelia cardinalis*, *Lobelia siphilitica*, photosynthetic gas exchange, quantitative genetics, water-use efficiency.

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Plants exhibit striking diversity in traits that influence resource uptake and utilization, including photosynthetic gas exchange, leaf structure, and biomass allocation. Comparative studies have shown that inter- and intraspecific variation in physiology is correlated with environmental gradients of water, light, and nutrients (Ehleringer and Monson 1993; Ackerly et al. 2000; Maherali et al. 2004). These correlations between phenotype and environment provide indirect evidence that natural selection is the primary evolutionary mechanism driving the diversification of physiological traits. More direct evidence comes from quantitative genetics, which can be used to estimate the rate and direction of evolution in response to natural selection. Under this approach, if there is heritable genetic variation for physiological traits and covariation between fitness and physiological phenotype, then these traits can evolve in response to natural selection (Falconer and Mackay 1996).

Although photosynthetic and stomatal physiology are key traits that control the uptake of carbon and loss of water in plants, genetic variation for these traits has rarely been measured. Significant heritable genetic variation for photosynthetic and stomatal traits has been detected in a few, primarily selfing species (reviewed by Arntz and Delph 2001; Geber and Griffen 2003). Because of the sensitivity of physiology to abiotic conditions, estimates of heritable genetic variation for photosynthetic and stomatal traits may be biased downward (Arntz and Delph 2001). Thus, there is no consensus on the potential for the quantitative genetics of photosynthetic and stomatal traits to constrain or facilitate their adaptive evolution in natural populations, especially in outcrossing species (reviewed by Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003).

The quantitative genetics of physiological traits can constrain their short-term adaptive evolution in two ways. First, evolution in response to phenotypic selection will be more rapid if there is substantial standing genetic variation for the selected trait (Falconer and Mackay 1996). Although insufficient genetic variation is not generally thought to be an important constraint on adaptive evolution (e.g., Rice 1988), some physiological traits may be constrained in this way. For example, heritable variation for photosynthetic rate is nonexistent in some species (*Brassica campestris*, Evans 1991) and very low in others (*Cakile edentula*, Dudley 1996b), suggesting that this trait would evolve slowly in response to any natural selection.

*First authorship is shared.
[2] Present address: Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.
[4] E-mail: carusoc@uoguelph.ca.
[5] E-mail: maherali@uoguelph.ca.
[7] Present address: 1121 E Johnson Street, Madison, Wisconsin 53703.
[8] Present address: School of Biological Sciences, 408 Manter Hall, University of Nebraska-Lincoln, Lincoln, Nebraska 68588.

Second, genetic correlations among physiological traits can constrain adaptive evolution if there are correlated responses to selection (e.g., Lande 1979, 1982; Via and Lande 1985; Caruso 2004). Strong negative genetic correlations could hinder adaptation if only one trait is under selection or if both traits are selected in the same direction. For example, there is a negative genetic correlation between photosynthetic rate and leaf size in *Polygonum arenastrum* (Geber and Dawson 1990), suggesting that simultaneous selection for higher photosynthetic rate and larger leaves (Dudley 1996a) would not result in adaptive evolutionary change. Alternatively, strong positive genetic correlations can slow adaptation if the correlated traits are selected in opposite directions. For example, if photosynthetic rate and stomatal conductance are positively genetically correlated because increased stomatal opening is necessary to increase carbon dioxide diffusion into leaves (Dudley 1996b; Geber and Dawson 1997), then plants may be constrained from simultaneously maximizing carbon fixation and minimizing water loss, as would be favored by selection in a dry environment (Farris and Lechowicz 1990; Dudley 1996a; Arntz et al. 1998, 2000; Heschel et al. 2002, 2004a, 2004b). Given the increasing interspecific evidence for coordination among suites of physiological traits (Reich et al. 1999), genetic correlations among these traits may be common and play an important role in the evolution of plant function (Arntz and Delph 2001).

We estimated the genetic variance-covariance matrices (**G**) for physiological traits of one population of each of two *Lobelia* species. The *L. cardinalis* population that we studied grows in a consistently wet site with nutrient-rich soils, whereas our *L. siphilitica* population grows in a drier and more variable location (Caruso et al. 2003b). These interpopulation habitat differences suggest that physiological traits associated with carbon gain (photosynthesis) and water use (stomatal conductance and water-use efficiency [WUE]) have been subject to differential selection. If this selection is strong enough to alter gene frequencies, then the **G** matrices should also differ between our *L. cardinalis* and *L. siphilitica* populations such that they are not proportional to each other (Lande 1979; reviewed by Roff 2000).

We used the **G** matrices for *L. cardinalis* and *L. siphilitica* to answer three questions: (1) Is there statistically significant genetic variation for physiological traits in natural populations of *Lobelia*? (2) Do patterns of genetic variation for and genetic correlations between physiological traits of *Lobelia* have the potential to constrain or facilitate their adaptive evolution? (3) Does the **G** matrix differ between *L. cardinalis* and *L. siphilitica* populations?

Materials and Methods

Study Species

Lobelia cardinalis and *L. siphilitica* (Lobeliaceae) are closely related (E. B. Knox and A. M. Muasya, pers. comm.) short-lived, herbaceous perennial wildflowers with contrasting floral morphologies (Johnston 1991a and references therein). *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). Although *L. siphilitica* and *L. cardinalis* are self-compatible (Johnston 1992), the complete separation between staminate and pistillate phases of flower development promotes outcrossing by ensuring that any self-fertilization is due to geitonogamy (Johnston 1991b). *Lobelia siphilitica* seeds germinate in the spring and typically flower that fall, whereas *L. cardinalis* rosettes generally overwinter prior to flowering (Johnston 1992).

Crossing Design

As part of a larger experiment (Caruso et al. 2003a; Caruso 2004), we analyzed the genetics of a *L. cardinalis* population (CB) and a *L. siphilitica* population (Krumm) located in central Iowa (for population descriptions see Caruso et al. 2003b). The *L. siphilitica* population used for the current study occurs at a site with both lower and more heterogeneous soil moisture than the *L. cardinalis* population that we used (Caruso et al. 2003b). Open-pollinated fruits were collected from 85 *L. cardinalis* and 67 *L. siphilitica* plants. *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. Seeds from each maternal family were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 4°C for eight weeks (Johnston 1992). Approximately 20 seeds/pot were sown onto moist MetroMix 380 (Scotts Company, Marysville, OH) and placed in standing water in the greenhouse at Grinnell College, Grinnell, Iowa. Two pots were planted for each maternal family. Germination and early survival varied among families (C. M. Caruso, pers. obs.). Consequently, after eight weeks we transplanted 1–9 seedlings/family (38 *L. cardinalis* and 55 *L. siphilitica* families) into 9 × 9-cm plastic pots. A total of 90 *L. cardinalis* and 222 *L. siphilitica* seedlings were transplanted. Plants were watered as necessary, fertilized with Osmocote 14-14-14 (Scotts), and exposed to supplemental light (16-h days).

At flowering, we crossed one randomly chosen plant with male-phase flowers to two randomly chosen plants with female-phase flowers to create a series of full-sib families nested within half-sib families (35 *L. cardinalis* and 75 *L. siphilitica* half-sib families). All individuals used in crosses were sampled without replacement. We pollinated 3 flowers/female plant. The complete separation between staminate and pistillate phases of flower development prevents autogamous self-fertilization (Johnston 1991b). Although female flowers used in the crosses were not bagged, few flowers that were not hand-pollinated set fruit (C. M. Caruso, pers. obs.), indicating that accidental pollination was rare.

Fruits were collected from successful crosses (24 half-sib families from *L. cardinalis* and 49 from *L. siphilitica*) and seeds were stratified and germinated as described above in the greenhouse at Duke University, Durham, North Carolina. Twenty-four pots were planted for each half-sib family, equally divided between the two nested full-sib families. After six weeks, we transplanted 18 seedlings/half-sib family, again equally divided between the two nested full-sib families, into 9 × 9-cm plastic pots. One-third of the offspring

in each full-sib family were randomly assigned to each of three greenhouse benches. Plants were grown in the greenhouse without supplemental lighting, watered to maintain soil at field capacity, and fertilized weekly. Temperature varied diurnally between 20°C and 30°C. We blocked on bench in the genetic analyses to control for potential spatial variation in the greenhouse environment. The overall environmental homogeneity in the greenhouse and the maintenance of plants under well-watered conditions was more similar to the field environment experienced by *L. cardinalis* than by *L. siphilitica*. Nevertheless, the greenhouse environment is much less heterogeneous than almost any field environment (e.g., Conner et al. 2003), suggesting that the greenhouse was a novel environment for both *Lobelia* populations.

Phenotypic Measurements

All traits were measured 12–16 weeks after planting, when plants had formed rosettes but had not yet flowered. Although variation in physiological traits may be greater during the reproductive phase of the life cycle (e.g., *Xanthium strumarium*, Farris and Lechowicz 1990), variation in these traits in prereproductive plants may be biologically important because even small differences in resource uptake early in the life cycle can translate to large differences in the resources available for later reproduction. We assigned one-third of the offspring in each full-sib family to be measured in each of three 10-day blocks. This temporal block effect was stratified with respect to the spatial blocks (e.g., one-third of the plants in each spatial block were included in each temporal block), and was included in the genetic analyses to account for plant growth across the 4-week measurement period. This resulted in 3 spatial blocks $\times$ 3 temporal blocks $\times$ 2 nested full-sib families $\times$ 24 half-sib families = 432 plants for *L. cardinalis* and 3 spatial blocks $\times$ 3 temporal blocks $\times$ 2 nested full-sib families $\times$ 49 half-sib families = 882 plants for *L. siphilitica*.

To determine carbon-gain and water-use characteristics of each individual, we measured light-saturated photosynthetic rate (A), stomatal conductance (g_s), and WUE. We measured these three gas exchange parameters on 10 randomly selected *L. cardinalis* plants per each of 24 half-sib families, evenly divided between nested full-sib families. We randomly selected 31 *L. siphilitica* half-sib families for gas exchange measurements (A, g_s, and WUE). Within each of these families, we randomly selected eight plants for measurement, evenly divided between nested full-sib families. Plants selected for gas exchange were stratified with respect to the temporal blocks (e.g., one-third of the measured plants were from each block), but were selected at random relative to the spatial blocks. Some plants were too small for gas-exchange measurements, resulting in final $N = 220$ for *L. cardinalis* and 230 for *L. siphilitica*. Steady-state leaf gas exchange was measured at saturating irradiance (1500 μmol m^{-2} s^{-1}) with an open gas-exchange system (LI-6400, Li-Cor, Inc., Lincoln, NE) between 0900 and 1200 in June 2001. During measurements, incident irradiance was provided by red-blue light-emitting diodes and cuvette carbon dioxide concentration was maintained at 400 μmol 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 ($\pm$1 SD) of 0.95 $\pm$ 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 mol m^{-2} 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 carbon dioxide and water vapor within the chamber were $< 0.25\%$) within 5 min. To assess the trade-off between carbon dioxide uptake and water loss, we calculated instantaneous WUE as A/g_s. To ensure that samples experienced similar soil moisture during data collection, all plants were watered to field capacity the evening prior to measurement.

To gain a more complete picture of the physiological capacity for carbon gain, we measured four additional traits that influence photosynthesis. We measured chlorophyll concentration on the three youngest fully expanded leaves/plant using a portable chlorophyll meter (SPAD 502, Minolta, Inc., Ramsey, NJ). To determine the photosynthetic capacity of the light reactions, we assessed the maximum quantum efficiency of photosystem II (PSII) by measuring the dark-adapted ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) with a field portable pulse modulated chlorophyll fluorometer (Model FMS2, Hansatech Instruments Ltd., King's Lynn, Norfolk, U.K.). Measurements were made on 1 leaf/plant, and leaves were dark-adapted with leaf clips for 15 min prior to fluorescence measurements. To minimize temporal variation in F_v/F_m, all measurements were made between 0900 and 1100 h. 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. In addition, for those plants not measured for gas exchange, the youngest fully expanded leaf from each plant was also sampled for SLA. Each leaf was excised and its area measured with a leaf area meter (LI-3000, Li-Cor, Inc.). These leaves were dried to constant mass in a forced convection oven at 65°C for 24 h and weighed. SLA was calculated by dividing leaf size by leaf mass. To determine if physiological variables were correlated with plant size (e.g., Maherali et al. 1997), we measured rosette size of all individuals. Rosette size was estimated as the average of two measurements of rosette diameter, taken at 90° angles to each other. Chlorophyll concentration, quantum efficiency, rosette size, and SLA were measured for 408 *L. cardinalis* plants (mean offspring measured/sire $\pm$ 1 SE = 17.00 $\pm$ 0.170) and 847 *L. siphilitica* plants (17.29 $\pm$ 0.113).

Statistical Analysis

Lobelia siphilitica is gynodioecious (e.g., Dudle et al. 2001; Caruso et al. 2003a) and plants cannot be sexed until they flower. Because F_v/F_m, chlorophyll concentration, A, and g_s differed between female and hermaphrodite *L. siphilitica* (Caruso et al. 2003a), we eliminated the 120 known females from our dataset before analysis. However, only 574 of the 847 *L. siphilitica* plants in our dataset were sexed, suggesting that undetected females were included in the analyses de-

scribed below. Assuming that 21% of Krumm offspring were females (calculated from data in Caruso 2004), we estimate that there are approximately 55 undetected females out of the 847 plants for which physiology was measured. The inclusion of these undetected females could bias our estimate of heritabilities and genetic correlations by increasing the phenotypic or genetic variance for physiological traits. Except for SLA, including detected females did not increase V_P for any of the physiological traits that we measured (*F*-test, data not shown). However, we cannot rule out the possibility that the inclusion of undetected females in the *L. siphilitica* dataset increased V_A for physiological traits.

We used *t*-tests to determine if physiological traits differed between populations. We estimated phenotypic correlations among physiological traits as the Pearson product-moment correlation (*r*). Because photosynthetic rate, stomatal conductance, and WUE were measured on only a subset of the plants, two phenotypic correlation matrices were estimated. We used the full dataset to estimate phenotypic correlations among the four physiological traits that were measured on all plants (F_v/F_m, chlorophyll concentration, rosette size, and SLA). We used the subset of plants for which *A*, g_s, and WUE were measured to estimate all other phenotypic correlations. We present *P*-values for these correlations both before and after applying the Dunn-Sidak correction (Sokal and Rohlf 1995) for multiple tests within each population. To determine if phenotypic correlations were bounded away from −1 or 1, we calculated 95% confidence intervals using SAS (program BOOTPCA, S. Tonsor, Univ. of Pittsburgh).

We used restricted maximum likelihood (REML) to estimate narrow-sense heritabilities (h^2) for and genetic correlations (r_A) among physiological traits of *L. cardinalis* and *L. siphilitica*. Unlike nested ANOVA, the traditional method for analyzing half-sib designs, REML is not sensitive to unbalanced data (Shaw 1987). For this analysis, we used the VCE (ver. 4.2.5) package of Neumaier and Groeneveld (1998). Fixed effects were included in all of our models to control for variation in physiological traits among greenhouse benches (spatial blocks) or measurement periods (temporal blocks). Because *A*, g_s, and WUE were measured on only a subset of the plants, genetic variance-covariance matrices were estimated using the full and partial datasets described above. Simulations indicate that subsampling individuals within families does not significantly bias estimates of genetic correlations, although it does result in higher standard errors (Xie and Mosjidis 1999).

The VCE package provides standard errors for estimates of h^2 and r_A, which were used to determine if these genetic parameters were significantly different from zero (as in Elle 1998). Because heritabilities vary between zero and one, whereas genetic correlations range between −1 and 1, we used one- and two-tailed one-sample *t*-tests, respectively, to determine their significance. The *t*-statistic was calculated by dividing h^2 or r_A by the standard error generated by VCE (Zar 1999). We present *P*-values for these *t*-tests both before and after applying the Dunn-Sidak correction (Sokal and Rohlf 1995) for multiple tests within each combination of measure (h^2 and r_A) and population (*L. cardinalis* and *L. siphilitica*). To determine if genetic correlations were bounded away from −1 or 1, we calculated 95% confidence intervals using the standard errors from VCE.

The genetic variances and covariances that we measured for physiological traits of *Lobelia* were likely influenced by the greenhouse growth environment. Field estimates of heritabilities are generally lower than greenhouse estimates for plant functional traits, including physiology (Geber and Griffen 2003). Although the relationship between greenhouse and field estimates of **G** matrices is unclear, the most sophisticated study to date (Conner et al. 2003) suggests that greenhouse estimates of genetic variances are more inflated than are greenhouse covariances relative to field estimates. Thus, our **G** matrices for greenhouse-grown *Lobelia* may overestimate the potential speed, but not the direction, of evolution in response to selection in natural populations.

We used common principal components analysis (CPCA; Flury 1988; Phillips and Arnold 1999; Steppan et al. 2002) to compare genetic (**G**) and phenotypic (**P**) variance-covariance matrices between *L. cardinalis* and *L. siphilitica* populations. CPCA tests hierarchical hypotheses about the relatedness of variance-covariance matrices. The simplest comparison is whether the two matrices are equal. If they are not equal, then CPCA tests whether they differ in size but not shape (proportional). If the matrices are not proportional, then CPCA tests whether they share all or some principal components (PCs) in common. Finally, if the matrices do not share any PCs, then CPCA would allow us to conclude that they are unrelated.

We used the jump-up approach (Phillips and Arnold 1999) to compare variance-covariance matrices. In this approach, each model (equality, proportionality, full CPC, five PCs, four PCs, three PCs, two PCs, and one PC) is tested against the null model that the matrices are unrelated using parametric χ^2-test statistics. The phenotypic variance-covariance matrices for all seven physiological traits were estimated from the subset of plants for which *A*, g_s, and WUE were measured. Rosette size differs from the other six traits that we measured in being a vegetative performance trait that is likely tightly linked to fitness (Geber and Griffin 2003). Because CPCA of matrices with and without rosette size were very similar, we present the results of CPC analysis based on all seven traits. If means and variances are correlated, then differences between variance-covariance matrices can be detected simply because species differ in mean phenotype (Lynch and Walsh 1998). Consequently, we log-transformed all data prior to calculating the **P** and **G** matrices used for the CPC analysis. Because current programs cannot accommodate half-sib designs, the **G** matrices for CPCA were based on variances and covariances of paternal family means. We used P. Phillips's program CPC for all analyses (http://www.uoregon.edu/~pphil/software.html). If two matrices shared any PCs in common, then we presented eigenvalues, component loadings, and corresponding confidence intervals for these PCs. Bootstrapped 95% confidence intervals were calculated using SAS (Program BOOTPCA).

Results

Phenotypic Variation and Covariation

All seven physiological traits differed between *L. cardinalis* and *L. siphilitica* populations. The *L. cardinalis* popu-

TABLE 1. Means (SE) for physiological traits of *Lobelia cardinalis* and *L. siphilitica* populations. Traits were compared between populations using two-sample *t*-tests. F_v/F_m, quantum efficiency of photosystem II; *A*, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE, water-use efficiency.

Trait	*L. cardinalis*	*L. siphilitica*	*L. cardinalis* vs. *L. siphilitica*
F_v/F_m	0.847 (4.54×10^{-4})	0.836 (4.98×10^{-4})	$t = 13.76$ df = 1137 $P < 0.001$
Chlorophyll concentration	44.37 (0.23)	30.55 (0.16)	$t = 49.36$ df = 1137 $P < 0.001$
Rosette size (cm)	19.85 (0.14)	22.99 (0.13)	$t = 15.94$ df = 1137 $P < 0.001$
Specific leaf area ($m^2\ g^{-1}$)	2.85×10^{-2} (2.60×10^{-4})	3.22×10^{-2} (3.80×10^{-4})	$t = 10.88$ df = 1134 $P < 0.001$
A (μmol CO_2 m^{-2} s^{-1})	14.25 (0.16)	12.08 (0.15)	$t = 9.91$ df = 416 $P < 0.001$
g_s (mol H_2O m^{-2} s^{-1})	0.323 (0.01)	0.400 (0.01)	$t = 6.29$ df = 416 $P < 0.001$
WUE	47.81 (0.81)	33.00 (0.71)	$t = 13.60$ df = 416 $P < 0.001$
N	220–408	198–731	

lation had 1.3% higher quantum efficiency (F_v/F_m), 45% higher leaf chlorophyll concentration (Chl), 11% higher light-saturated photosynthetic rate (*A*), and 45% higher WUE than *L. siphilitica*. In contrast, the *L. siphilitica* population had 16% larger rosettes, 13% higher SLA (i.e., thinner leaves), and 24% higher stomatal conductance (g_s) than *L. cardinalis* (Table 1).

Approximately two-thirds of the phenotypic correlations among physiological traits were significant in both the *L. cardinalis* and *L. siphilitica* populations. Among those phenotypic correlations that were significant, approximately 46% were negative in *L. cardinalis* and 40% were negative in *L. siphilitica*. Phenotypic correlations among physiological traits in both species largely reflected functional relationships. For example, Chl and F_v/F_m were both positively correlated with *A*, reflecting coordination between the components of photosynthetic machinery. Similarly, phenotypic correlations between *A* and g_s were strong and positive, suggesting that increased stomatal opening reduces limits on carbon assimilation (Tables 2, 3).

Despite these similarities, the CPC analysis suggested that the pattern of phenotypic correlations among physiological traits differed between *L. cardinalis* and *L. siphilitica* populations (Figs. 1A, B). The jump-up test indicated that the **P** matrices of our *L. siphilitica* and *L. cardinalis* populations were unrelated (all $P < 0.001$). For example, Chl and *A* were negatively correlated with rosette size in the *L. siphilitica* population, whereas correlations between photosynthetic traits and plant size were weak in the *L. cardinalis* population. In addition, both photosynthetic rate and stomatal conductance were correlated with WUE in the *L. cardinalis* population but not in *L. siphilitica* (Tables 2, 3).

Genetic Variation and Covariation

There was significant additive genetic variation for all physiological traits in our *L. siphilitica* population, ranging from a low of 0.103 for F_v/F_m to a high of 0.505 for SLA (mean ± SE = 0.381 ± 0.053). In contrast, narrow-sense heritabilities for physiological traits were generally lower in the *L. cardinalis* population, ranging from 0.047 for g_s to 0.482 for rosette size (mean ± SE = 0.206 ± 0.061). Heritabilities of stomatal conductance and WUE were low (< 0.1) and nonsignificant in the *L. cardinalis* population (Tables 2, 3).

More than two-thirds of the genetic correlations among traits were significant in our *L. siphilitica* population, whereas only one-third were significant in *L. cardinalis*. For example, there were significant genetic correlations between WUE and/or g_s and five of the six other measured traits in *L. siphilitica*, whereas WUE and g_s were not correlated with any other trait in *L. cardinalis*. In addition, the magnitude and direction of genetic correlations between rosette size and other physiological traits differed between species. For example, both WUE and g_s were negatively correlated with rosette size in *L. siphilitica*, but neither of these traits were correlated with rosette size in *L. cardinalis*. Only the correlations of *A* and Chl with rosette size were similar in magnitude and direction between populations (Tables 2, 3). Despite these differences for pairs of traits, the CPC jump-up analysis indicated that the *L. cardinalis* and *L. siphilitica* populations shared two PCs in common (CPC3 vs. unrelated; $P = 0.004$; Figs. 1C, D) that together explained 87.0% of the variance in the pooled **G** matrix. WUE loads heavily on PC1 in both species, whereas rosette size (in *L. cardinalis*) and *A* (in *L. siphilitica*) load heavily on PC2 (Table 4).

Comparison of Phenotypic and Genetic Correlations

Of the significant phenotypic correlations among physiological traits in our *L. siphilitica* population, approximately 85% were underlain by a significant genetic correlation of the same sign. Despite this apparent similarity between the **P** and **G** matrices, there were striking examples of discord for correlations of functional importance. In particular, there was no significant genetic correlation between photosynthetic rate and stomatal conductance, despite a strong and significant phenotypic correlation between these traits. There were also traits that were genetically but not phenotypically correlated. For example, rosette size was genetically, but not phenotypically, correlated with stomatal conductance in *L. siphilitica* (Table 3).

TABLE 2. Phenotypic correlations (r; upper off-diagonal), genetic correlations (r_A; lower off-diagonal), and narrow-sense heritabilities (h^2; bold, diagonal) for physiological traits of *Lobelia cardinalis* plants. The 95% confidence intervals for all correlations are in parentheses. One-tailed, one-sample t-tests were used to determine if heritabilities were significantly greater than zero. Two-tailed, one-sample t-tests were used to determine if genetic correlations were significantly different from zero. All significant heritabilities remained significant after Dunn-Sidak correction for multiple tests. F_v/F_m, quantum efficiency of photosystem II; Chl, chlorophyll concentration; SLA, specific leaf area; A, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE, water-use efficiency; $N = 220–408$.

	F_v/F_m	Chl	Rosette size	SLA	A	g_s	WUE
F_v/F_m	**0.118****	0.238***† (0.137, 0.327)	0.104* (0.012, 0.196)	−0.068 (−0.201, 0.065)	0.307***† (0.163, 0.438)	0.214** (0.072, 0.341)	−0.211** (−0.340, −0.080)
Chl	0.368* (0.030, 0.706)	**0.354*****	−0.157** (−0.249, −0.062)	−0.306***† (−0.391, −0.217)	0.250***† (0.118, 0.361)	0.058 (−0.079, 0.176)	0.057 (−0.078, 0.191)
Rosette size	0.424* (0.048, 0.800)	−0.388** (−0.660, −0.116)	**0.482*****	−0.080 (−0.204, 0.038)	−0.088 (−0.208, 0.032)	−0.064 (−0.194, 0.073)	0.050 (−0.087, 0.168)
SLA	−0.287 (−0.687, 0.124)	−0.311 (−0.668, 0.046)	−0.361* (−0.714, −0.008)	**0.165****	0.002 (−0.114, 0.125)	0.237** (0.112, 0.361)	−0.297***† (−0.415, −0.183)
A	0.420 (−0.047, 0.886)	0.623***† (0.301, 0.945)	−0.556** (−0.932, −0.180)	−0.854***† (−1.36, −0.350)	**0.215****	0.784***† (0.733, 0.828)	−0.565***† (−0.650, −0.467)
g_s	0.702 (−0.400, 1.80)	0.079 (−0.742, 0.900)	−0.173 (−0.984, 0.638)	−0.532 (−1.90, 0.834)	0.563 (−0.104, 1.23)	**0.047**	−0.900***† (−0.919, −0.881)
WUE	−0.385 (−1.27, 0.505)	0.528 (−0.192, 1.25)	−0.094 (−0.769, 0.581)	−0.136 (−1.10, 0.832)	0.243 (−0.791, 1.28)	−0.634 (−1.48, 0.210)	**0.058**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Genetic or phenotypic correlation remained significant after Dunn-Sidak correction.

In contrast to *L. siphilitica*, only 30% of the phenotypic correlations among physiological traits in *L. cardinalis* were underlain by a significant genetic correlation of the same sign, reflecting interpopulation differences in the **G** matrices. As in the *L. siphilitica* population, photosynthetic rate and stomatal conductance were not significantly genetically correlated, despite a strong and significant phenotypic correlation between these traits (Table 2).

DISCUSSION

Heritability of Physiological Traits

We detected significant genetic variation for all seven traits in the *L. siphilitica* study population and five of seven traits in the *L. cardinalis* study population (Tables 2, 3). Broad- and narrow-sense heritabilities for photosynthetic rate, stomatal conductance, and WUE in previous studies were typically < 0.1 (Dudley 1996b; Tonsor and Goodnight 1997; Scheiner et al. 1984; but see Geber and Dawson 1997). Relative to these studies, heritabilities of gas exchange traits in the *L. siphilitica* population were high (Table 3). In contrast, heritabilities of these traits in the *L. cardinalis* population were more typical of the previous studies (Table 2). Our results suggest that a lack of genetic variation may constrain the adaptive evolution of physiological traits important for plant function in the *L. cardinalis* population but not in the *L. siphilitica* population. However, estimates of the strength of natural selection on physiological traits of *L. cardinalis* and *L. siphilitica* would be necessary to more definitively test whether a lack of genetic variation constrains the evolution of these traits (e.g., Conner and Via 1992; Caruso 2004).

The presence of significant heritable variation for stomatal conductance and WUE in the *L. siphilitica* population (Table 3), but not in the *L. cardinalis* population (Table 2), does not appear to be caused by lower statistical power or stronger selection on these traits in *L. cardinalis*. We measured gas-exchange traits for 30% more half-sib families of *L. siphilitica* ($N = 31$) than *L. cardinalis* ($N = 24$). However, gas exchange was measured for 44% more offspring per family for *L. cardinalis* ($N \pm \text{SE} = 9.17 \pm 0.62$) than *L. siphilitica* ($N = 6.35 \pm 0.28$). Given that the number of half-sib families and the number of offspring measured per family both influence the power to detect significant genetic variation (Lynch and Walsh 1998), differences in heritabilities of gas exchange traits between populations were unlikely due to differences in power. Another possibility is that genetic variation for g_s and WUE of our *L. cardinalis* population was depleted by strong selection on those traits in previous generations (Fisher 1999). Although we have not measured natural selection on physiological traits of *Lobelia*, studies in other species (*Cakile edentula*, Dudley 1996a; *Impatiens capensis*, Heschel et al. 2002) indicate that neither g_s nor WUE are under significant selection in wet environments such as those occupied by our *L. cardinalis* study population (Caruso et al. 2003b).

The presence of significant heritable variation for stomatal conductance and WUE in *L. siphilitica* (Table 3), but not in *L. cardinalis* (Table 2), may be caused by spatial variation in moisture availability (e.g., Levene 1953; Frank and Slatkin 1990; Ellner and Hairston 1994) or by a recent bottleneck (e.g., Whitlock and Fowler 1999). In general, *L. siphilitica*

TABLE 3. Phenotypic correlations (r; upper off-diagonal), genetic correlations (r_A; lower off-diagonal), and narrow-sense heritabilities (h^2; bold, diagonal) for physiological traits of *Lobelia siphilitica* plants. The 95% confidence intervals for all correlations are in parentheses. One-tailed, one-sample t-tests were used to determine if heritabilities were significantly greater than zero. Two-tailed, one-sample t-tests were used to determine if genetic correlations were significantly different from zero. All heritabilities remained significant after Dunn-Sidak correction for multiple tests. F_v/F_m, quantum efficiency of photosystem II; Chl, chlorophyll concentration; SLA, specific leaf area; A, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE, water-use efficiency; N = 198–731.

	F_v/F_m	Chl	Rosette size	SLA	A	g_s	WUE
F_v/F_m	**0.103*****	0.367***†	−0.187***†	0.168***†	0.312***†	0.152*	−0.018
		(0.301, 0.432)	(−0.251, −0.118)	(0.101, 0.235)	(0.184, 0.445)	(0.013, 0.286)	(−0.169, 0.129)
Chl	0.399**	**0.380*****	−0.402***†	0.034	0.573***†	−0.028	0.378***†
	(0.113, 0.685)		(−0.464, −0.335)	(−0.047, 0.110)	(0.471, 0.664)	(−0.152, 0.095)	(0.226, 0.509)
Rosette size	−0.229	−0.473***†	**0.308*****	−0.071	−0.253***†	0.031	−0.189**
	(−0.506, 0.048)	(−0.661, −0.285)		(−0.144, 0.010)	(−0.376, −0.118)	(−0.097, 0.164)	(−0.351, −0.021)
SLA	0.433**	−0.023	−0.107	**0.505*****	0.173*	0.437***†	−0.405***†
	(0.127, 0.739)	(−0.192, 0.146)	(−0.284, 0.070)		(0.030, 0.308)	(0.319, 0.541)	(−0.507, −0.288)
A	0.345*	0.772***†	−0.177*	0.149	**0.432*****	0.515***†	−0.050
	(0.019, 0.671)	(0.546, 0.998)	(−0.352, −0.002)	(−0.257, 0.555)		(0.394, 0.620)	(−0.196, 0.099)
g_s	0.501**	−0.253	0.708***†	0.490**†	0.277	**0.489*****	−0.814***†
	(0.128, 0.874)	(−0.612, 0.106)	(0.414, 1.00)	(0.166, 0.814)	(−0.076, 0.630)		(−0.844, −0.780)
WUE	−0.399*	0.532***†	−0.833***†	−0.351*	0.177	−0.889***†	**0.453*****
	(−0.772, −0.026)	(0.242, 0.822)	(−1.088, −0.578)	(−0.630, −0.072)	(−0.213, 0.567)	(−0.991, −0.787)	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Genetic or phenotypic correlation remained significant after Dunn-Sidak correction.

populations grow at sites with both lower mean soil moisture and a higher coefficient of variation for moisture than *L. cardinalis* (Caruso et al. 2003b). In a *L. siphilitica* population such as Krumm with high spatial variance in soil moisture (Caruso et al. 2003b), genotypes with gas-exchange traits adapted to high moisture availability may be favored in relatively wet microsites and those adapted to low moisture availability may be favored in relatively dry microsites, maintaining genetic variation for these traits (e.g., Sandquist and Ehleringer 2003a,b). Alternatively, our *L. cardinalis* population may have experienced a recent bottleneck that depleted genetic variation for quantitative traits (e.g., Whitlock and Fowler 1999). This hypothesis is supported by our observation that the CB *L. cardinalis* population declined precipitously between 1999 and 2004, whereas the Krumm *L. siphilitica* population remained relatively unchanged (C. M. Caruso, pers. obs.).

Although evolutionary theory predicts that strong selection on traits that are closely linked to fitness should deplete genetic variation for these traits (Fisher 1999), we detected significant heritable variation for rosette size in both *Lobelia* populations (Tables 2, 3). Rosette size differs from the other six traits that we measured in being a performance trait that is hypothesized to directly affect fitness (Geber and Griffin 2003). Consequently, our finding of heritable variation for rosette size also conflicts with a recent review by Geber and Griffin (2003), where vegetative performance traits were under stronger selection but had lower heritabilities than physiological and morphological traits. There are many possible explanations for the maintenance of genetic variation in traits such as rosette size (reviewed in Barton and Turelli 1989), but one commonly invoked mechanism is antagonistic pleiotropy, where alleles are maintained because they have positive effects on some traits and negative effects on others. For example, we observed a strong negative genetic correlation between rosette size and WUE in our *L. siphilitica* population. If selection favors genotypes with high WUE in microsites or years in which water is scarce (Dudley 1996a), then the strong negative correlation between these traits could plausibly maintain genetic variation for rosette size in our *L. siphilitica* population.

Correlations among Physiological Traits

A strong phenotypic correlation existed between photosynthetic rate and stomatal conductance in both populations, but we failed to detect a significant genetic correlation between these two traits (Tables 2, 3). In our *L. cardinalis* population, the genetic correlation between A and g_s was relatively high but not statistically significant, likely because of the low standing genetic variation for g_s in this population (Table 2). In contrast, there was substantial genetic variation for both A and g_s in the *L. siphilitica* population, but the genetic correlation between these traits was relatively low and not statistically significant (Table 3). Given that increased stomatal opening is a biophysical necessity for increasing the rate of diffusion of carbon dioxide from the air to the inside of plant leaves (Wong et al. 1979; Cowan 1986), this lack of a strong and significant genetic correlation between A and g_s is surprising. One possible explanation is that

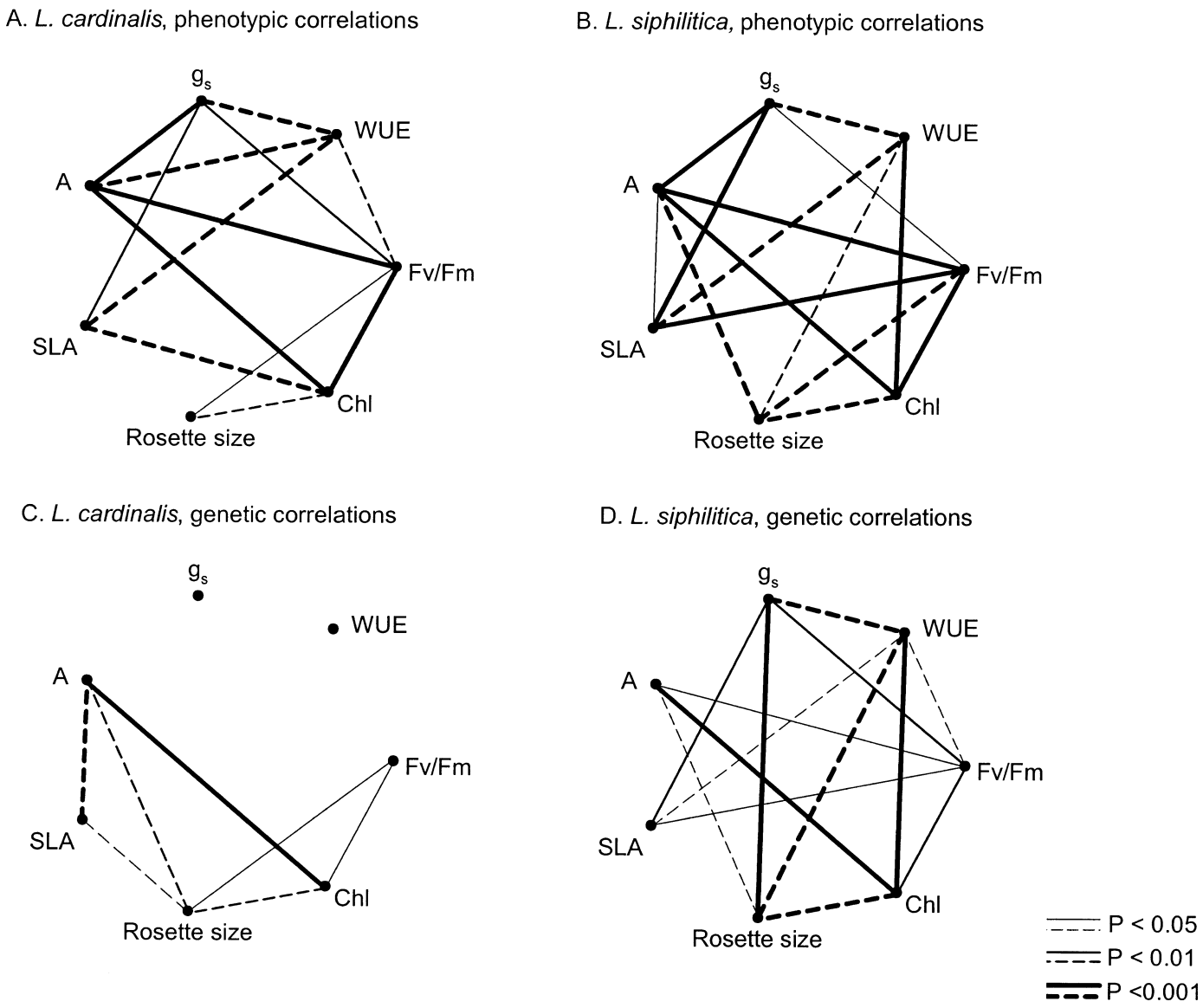

FIG. 1. Phenotypic (A, B) and genetic (C, D) correlations among physiological traits of one *Lobelia siphilitica* and one *L. cardinalis* population. Only statistically significant correlations are shown. Solid lines represent positive correlations, and dashed lines represent negative correlations. Thicker lines represent statistically stronger correlations. Both the genetic and phenotypic variance-covariance matrices differed between populations by a common principal components analysis (see text for details). F_v/F_m, quantum efficiency of photosystem II; Chl, chlorophyll concentration; SLA, specific leaf area; *A*, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE, water-use efficiency. *N* for genetic correlations = 24 half-sib families for *L. cardinalis* and 31–49 half-sib families for *L. siphilitica*. *N* for phenotypic correlations = 220–408 for *L. cardinalis* and 198–731 for *L. siphilitica*.

stomatal limitation to photosynthesis may be secondary to other limiting factors, such as the resistances controlling carbon dioxide diffusion from the substomatal cavity to the site of carboxylation (Nobel 1991). However, significant mesophyll limitations are not expected to occur in fast-growing species with relatively high photosynthetic rates such as *Lobelia* (DeLucia et al. 2003). Regardless of the mechanism responsible for it, the lack of a genetic correlation between *A* and g_s, combined with significant genetic variation for these traits (Table 3), suggests that WUE of our *L. siphilitica* study population could evolve in response to drought. For example, selection to increase *A* alone would not result in a correlated response to selection in g_s, allowing increased WUE to evolve. Artificial selection on gas-exchange traits could be used to test our prediction that the evolution of WUE is unconstrained in this *L. siphilitica* population.

Interspecific (Reich et al. 1997) and intraspecific (Peterson 1999) surveys have found broad support for a positive relationship between photosynthetic rate and SLA, suggesting correlated evolution between these leaf physiological traits (Reich et al. 1999). However, we detected a strong negative genetic correlation between *A* and SLA in *L. cardinalis* (Table 2), suggesting that correlated evolution of both high *A* and high SLA may not occur in all species. In addition, *A* and SLA were not significantly phenotypically correlated in *L. cardinalis* (Table 2). Previous studies have noted that the

TABLE 4. Principal components analysis of the **G** matrices used in the common principal component analysis. Eigenvalues and component loadings are reported for the first two principal components (PCs). Bootstrapped 95% confidence intervals for eigenvalues and component loadings are in parentheses. F_v/F_m, quantum efficiency of photosystem II; Chl, chlorophyll concentration; SLA, specific leaf area; *A*, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE, water-use efficiency; $N = 24$ families for *Lobelia cardinalis* and 31 families for *L. siphilitica*.

	L. cardinalis		*L. siphilitica*	
	PC1	PC2	PC1	PC2
Eigenvalue	1.362×10^{-3} (1.073×10^{-3}, 1.953×10^{-3})	1.048×10^{-3} (5.815×10^{-4}, 1.255×10^{-3})	0.010 (4.169×10^{-3}, 0.019)	2.547×10^{-3} (1.265×10^{-3}, 3.711×10^{-3})
F_v/F_m	-8.222×10^{-3} (-0.013, 0.011)	2.052×10^{-3} (-0.012, 0.015)	-6.791×10^{-4} (-0.010, 9.536×10^{-3})	0.012 (-5.748×10^{-3}, 0.027)
Chl	-0.099 (-0.382, 0.553)	0.443 (-0.425, 0.701)	0.331 (0.144, 0.485)	0.408 (2.458×10^{-3}, 0.609)
Rosette size	0.287 (-0.717, 0.778)	-0.728 (-0.807, 0.928)	-0.244 (-0.423, -0.077)	-0.025 (-0.489, 0.569)
SLA	-3.719×10^{-3} (-0.014, 8.138×10^{-3})	-6.980×10^{-3} (-0.014, 0.010)	-3.943×10^{-3} (-9.384×10^{-3}, 3.532×10^{-3})	5.071×10^{-3} (-0.011, 0.016)
A	-0.410 (-0.528, 0.637)	0.287 (-0.499, 0.686)	0.257 (-0.118, 0.469)	0.784 (0.402, 0.865)
g_s	-0.286 (-0.299, 0.300)	-0.068 (-0.282, 0.277)	-0.161 (-0.285, -0.072)	0.323 (0.121, 0.365)
WUE	0.811 (-0.808, 0.934)	0.432 (-0.683, 0.901)	0.860 (0.669, 0.916)	-0.339 (-0.611, 0.144)

relationship between *A* and SLA can be modified by phenology and environmental variation in light and nutrients (Reich et al. 1991; Ellsworth and Reich 1992). Therefore, our results suggest that the positive correlation between *A* and SLA found in inter- and intraspecific surveys could be driven by environmental variation rather than an underlying genetic correlation.

We observed significant genetic correlations between traits associated with SAS exchange and rosette diameter, suggesting that leaf function does not evolve independently of overall plant size. In particular, small plant size was linked with low stomatal conductance and high WUE in *L. siphilitica* (Table 3). This trait combination of slow growth and water conservation may be commonly associated with drought adaptation. For example, artificial selection to increase WUE in agricultural species has often resulted in slower growth and reduced seed yield (Condon et al. 2004). Similarly, studies in *Polygonum arenastrum* (Geber and Dawson 1990) and *Arabidopsis thaliana* (McKay et al. 2003) suggest that high WUE is associated with later flowering time and therefore slower development. Genetic correlations between growth rate and water use may also depend on the environment. For instance, in a population of recombinant inbred lines of *Triticum aestivum*, the line mean correlation between WUE and total biomass, as well as grain yield, was negative under well-watered conditions but positive under dry conditions (Condon and Hall 1997). Given that *L. siphilitica* can grow under a variety of soil moisture conditions (Caruso et al. 2003b), the strength and direction of genetic correlations between rosette size, g_s, and WUE may vary among populations and years.

We also observed negative genetic correlations between rosette diameter and *A* in both *L. siphilitica* and *L. cardinalis*, suggesting that small plants have higher photosynthetic capacity. This result is consistent with studies in other species, which have documented that photosynthesis is rarely positively correlated with biomass and growth (Lechowicz 1984; Farris and Lechowicz 1990; Arntz et al. 1998). One possible explanation for this pattern is that, assuming equal soil nitrogen availability across pots, leaf nitrogen concentration will be less diluted in small relative to large plants. That increased nitrogen concentration led to increased photosynthesis is supported by the observation that chlorophyll concentration, an index of leaf nitrogen concentration (Chapman and Barreto 1997), was also negatively genetically correlated with rosette diameter in both *Lobelia* populations (Tables 2, 3). Increased leaf nitrogen concentration and higher photosynthetic capacity in small plants may also compensate for limitations on total plant carbon fixation associated with low leaf area (e.g., Lechowicz 1984).

In contrast to *A* and chlorophyll concentration, F_v/F_m was positively genetically correlated with rosette size in *L. cardinalis* and uncorrelated with size in *L. siphilitica* (Table 2). Dark-adapted F_v/F_m estimates the quantum efficiency of photosystem II and thus represents the efficiency of photon capture under light limitation (Kao and Forseth 1992). Our results suggest that large *L. cardinalis* plants, despite having lower *A* and chlorophyll concentration, use light more efficiently than smaller plants. Self-shading in larger *L. cardinalis*, which forms a dense leaf canopy during the rosette stage, may have caused increased light use at low light at

the expense of light-saturated photosynthesis (e.g., shade acclimation; Givnish 1988). In contrast, large *L. siphilitica* plants, which form rosettes with a relatively open leaf canopy, may not have acclimated to shade when compared to smaller plants.

*Interpopulation Variation in **G** and **P** Matrices*

As in other studies (reviewed by Steppan et al. 2002), we detected greater differences in **P** than in **G** between our *L. cardinalis* and *L. siphilitica* populations. The **P** matrices were completely unrelated, whereas the **G** matrices shared two PCs that together explain 87% of the variation among traits. We may have detected more differentiation in **P** than **G** because of higher statistical power to detect differences in **P** (reviewed in Steppan et al. 2002). Alternatively, our *L. cardinalis* and *L. siphilitica* populations may have undergone more phenotypic than genetic divergence (as in Podolsky et al. 1997; Roff and Mousseau 1999). More generally, our finding that the **G** matrices for *L. cardinalis* and *L. siphilitica* are not proportional to each other suggests that natural selection has driven the evolution of genetic variances and covariances in these populations (Lande 1979; Roff 2000). However, given the methodological difficulties with all current methods of comparing genetic variance-covariance matrices (including CPC analysis; Houle et al. 2002; Steppan et al. 2002), the importance of selection versus drift in **G**-matrix evolution remains an open question.

Conclusions

In summary, the quantitative genetics of photosynthetic and stomatal traits is more likely to constrain adaptive evolution in our *L. cardinalis* population than in our *L. siphilitica* population. Correlations among physiological traits are thought to play an important role in the evolution of plant function (e.g., Arntz and Delph 2001). However, most of the genetic constraints on the evolution of photosynthetic and stomatal traits in our *L. cardinalis* population were associated with a lack of genetic variation rather than genetic correlations. In contrast, there was significant genetic variation for all measured physiological traits in *L. siphilitica*, and photosynthetic rate was not genetically correlated with stomatal conductance. Our results highlight the importance of testing the common assumption that functionally related traits such as photosynthetic rate and stomatal conductance are correlated in a way that constrains their adaptive evolution.

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