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QUANTIFYING EVOLUTIONARY GENETIC CONSTRAINTS IN THE IVYLEAF MORNING GLORY, *IPOMOEA HEDERACEA*

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The ability of a population to respond to natural selection will be determined by the patterns of genetic variation and covariation in traits under selection. In the quantitative genetic framework, these patterns of genetic variation and covariation are described by the **G** matrix, which for a given pattern of selection will determine the size and direction of evolutionary responses. Several methods have been developed to evaluate the nature of evolutionary constraints imposed by **G**, although this multitude of methods has never been applied to a common data set to compare their strengths and weaknesses, or the similarity of evolutionary inferences they produce. Here we compare several multivariate methods that calculate genetic constraint using a quantitative genetic field study in the ivyleaf morning glory, *Ipomoea hederacea*. We focus on a tractable number of traits (size at flowering, final size, and flowering time), which allows us to pair multivariate quantitative methods with qualitative interpretations of both **G** and the pattern of natural selection. In methods that rely on either the geometry of **G** or the multivariate orientation of **G** and the pattern of natural selection ($\boldsymbol{\beta}$), we found high levels of inferred constraint. In contrast, when one considers how genetic covariances are likely to affect the rate of adaptation over very short timescales, we inferred relatively low levels of constraint. Two consistent results emerge from our analyses. First, the inferences about evolutionary genetic constraints from all of these metrics are very sensitive to whether traits are unstandardized, standardized by the standard deviation, or standardized by the mean. In general, weaker evolutionary genetic constraints are inferred for metrics utilizing a mean standardization. Second, the discordance between methods that consider the geometric orientation of **G** and $\boldsymbol{\beta}$ and those that evaluate how covariances affect the short-term rate of adaptation suggests that alternative constraint metrics might be informative, depending on whether the goal is to evaluate adaptation in general or the evolution of particular traits.

Keywords: genetic constraints, g_{max}, natural selection, evolvability, rate of adaptation, flowering time.

Introduction

Understanding the evolution of correlated traits remains one of the central goals in evolutionary ecology (Gardner and Latta 2007). For example, correlations between ecologically important traits lie at the heart of theoretical explanations for alternative defense strategies (van der Meijden et al. 1988; Fineblum and Rausher 1995), whether plants should grow or defend (Herms and Mattson 1992), alternative life-history strategies (Lande 1982; Roff and Fairbairn 2007), the evolution of generalists and specialists and phenotypic plasticity (Via and Lande 1985; van Tienderen 1991), male and female fitness in hermaphrodites (e.g., Charnov et al. 1976; Charlesworth and Charlesworth 1981), and the evolution of drought avoidance strategies (Juenger et al. 2005; McKay et al. 2008). Empirically, the dominant approach to detecting correlations among traits has been quantitative genetics, in which correlations between traits are measured as either genetic covariances or correlations (Roff 1996, 2000; Lynch and Walsh 1998).

The role of genetic covariances in the evolution of quantitative traits can easily be seen from considering the equation for the evolution of two traits:

$$\begin{bmatrix} \Delta\bar{z}_1 \\ \Delta\bar{z}_2 \end{bmatrix} = \begin{bmatrix} G_{11}G_{12} \\ G_{21}G_{22} \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix}. \quad (1)$$

In equation (1), the left-hand side represents the evolutionary change in the mean value of two phenotypic traits, 1 and 2. These evolutionary changes can be predicted from the product of the matrix **G**, which contains genetic variances on the diagonal and genetic covariances on the off-diagonals, and the vector $\boldsymbol{\beta}$, which describes the strength of natural selection on traits 1 and 2 (Lande 1979; Lande and Arnold 1983). When genetic covariances between traits are nonzero—for instance, because of the pleiotropic effects of single genes or linkage disequilibrium between genes—the evolution of the traits in question will not be independent. The evolutionary consequences of nonzero covariances in equation (1) have

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been the primary theoretical motivator (either explicitly or implicitly) of the vast majority of empirical quantitative genetic studies of correlated traits.

Abundant data from the literature exist for the component parameters of equation (1). For example, the heritabilities of traits are frequently greater than 0; that is, G_{11} and G_{22} are often nonzero (Mousseau and Roff 1987). In fact, estimates of genetic variation in traits closely related to fitness are higher than for other characters, when measured appropriately (Houle 1992). Likewise, there are numerous estimates of genetic correlations (i.e., $G_{12} > 0$; Roff 1996), and natural selection ($\boldsymbol{\beta}$) appears to be widespread, common, and strong in natural populations (Kingsolver et al. 2001; Hereford et al. 2004). While these findings at face value would appear to suggest that we have all the parameter estimates necessary to solve equation (1), they paradoxically raise two difficult empirical and theoretical challenges. First, the presence of abundant genetic variation and strong natural selection are difficult to reconcile with each other, since selection is expected to reduce genetic variation (Johnson and Barton 2005). Second, there is accumulating evidence that microevolutionary stasis is common, even when natural selection is acting on genetically variable traits (e.g., Merila et al. 2001; Kruuk et al. 2002). What resolutions exist to these challenges?

An emerging body of theoretical and statistical work suggests that progress toward resolving these challenges can be gained by considering the geometry encapsulated by equation (1)—in other words, the relative magnitude of the elements and the axes of variation described by the matrices and vectors, their relative orientation in multivariate space, and the influence of individual elements on evolutionary trajectories (Dickerson 1955; Pease and Bull 1988; Schluter 1996; Blows et al. 2004; Hine and Blows 2006; Blows 2007; Blows and Walsh 2008; Hansen and Houle 2008; Smith and Rausher 2008*b*; Kirkpatrick 2009; for a review, see Walsh and Blows 2009). At their core, these methods seek to answer a handful of questions about equation (1) that are simple to pose yet difficult to answer: (1) Do the eigenvalues of **G** suggest that there are as many independent axes of genetic variation as there are traits, or are there combinations of traits for which there is no genetic variation (i.e., eigenvalues of zero, and **G** is singular)? (2) Is $\boldsymbol{\beta}$ favoring combinations of traits for which there is abundant genetic variation, or is $\boldsymbol{\beta}$ aligned in multivariate space with combinations of phenotypic traits for which there may be phenotypic variance but little to no genetic variance—suggesting that perhaps no response should be expected? (3) How do the genetic covariances in **G** alter the relationship between the trait combinations favored by selection and the resulting evolutionary response? Do genetic covariances accelerate or constrain the rate of adaptation? (4) How much of the evolutionary response is in the direction favored by natural selection? Answers to these questions from diverse systems, traits, and life histories would go a long way to evaluating competing hypotheses for the maintenance of genetic variation and the presence of microevolutionary stasis.

One drawback to previous empirical approaches to these problems is that the various metrics measuring the geometry of equation (1) have rarely been applied to the same set of data (see, e.g., Schluter 1996; Blows et al. 2004; Hansen and Houle 2008; Smith and Rausher 2008*b*; Agrawal and Stinchcombe 2009; Kirkpatrick 2009). Consequently, it has been difficult to compare the strengths and weaknesses of different metrics, the evolutionary inferences they produce, and whether the various methods devised to answer the four questions listed above often lead to the same conclusion. Here we apply the most commonly used metrics of measuring quantitative genetic evolutionary constraints to a single data set, purposefully focusing on a small handful of traits that allow multivariate methods to be paired with qualitative interpretation of both **G** and $\boldsymbol{\beta}$. In particular, we focus on three traits in the annual vine *Ipomoea hederacea* (midseason size, final size, and flowering time), for which we have specific a priori predictions about the nature of **G** and $\boldsymbol{\beta}$. We expect midseason size and final size to be highly correlated because of growth; similarly, relationships between size and flowering time are expected for annuals, since individuals that delay flowering can achieve larger size (Lacey 1986; Bolmgren and Cowan 2008). Likewise, we expect that natural selection should favor larger size in annual plants (e.g., Callahan and Pigliucci 2002; Blair and Wolfe 2004) but that flowering time should be under strong selection, depending on season length (Inouye 2000).

Specifically, we ask the following questions: (1) What are the patterns of **G** and $\boldsymbol{\beta}$ for midseason size, final size, and flowering time for ivyleaf morning glory (*I. hederacea*), and how are these affected by the presence of a closely related competitor (*Ipomoea purpurea*) ? (2) What evidence, if any, exists for evolutionary genetic constraints in this system, and do different constraint metrics lead to the same inferences? (3) Does ecological complexity—namely, the presence or absence of a competitor—alter the degree of evolutionary genetic constraint?

Methods

Study System

Ipomoea hederacea (L.) Jacquin (Convolvulaceae), ivyleaf morning glory, is an annual weedy vine, typically growing in agricultural fields, disturbed habitats, and cultivated gardens. Whether ivyleaf morning glory is native to North America is uncertain, but herbarium specimens indicate that it has resided in North America for at least 150 yr (Bright 1998), and early floras indicate that it has been present since the 1700s (Pursh 1814). Ivyleaf morning glory typically germinates from May to August. Flowering in the field typically begins 4–6 wk later and continues until the kill frost in the fall; growth and leaf production continue after flowering has been initiated. In greenhouse and field conditions, flowering is accelerated by short days (Greulach 1943) and drought (J. R. Stinchcombe, personal observation). Seed capsules (containing 1–6 hard-coated seeds) usually take 4–6 wk to mature and dehisce (Bright and Rausher 2008). Ivyleaf morning glory is largely selfing (average = 63%; from Hull-Sanders et al. 2005). Despite high selfing rates in its current range, Hull-Sanders et al. (2005) found relatively little population differentiation (average $F_{ST} = 0.035$ for 11 populations in Alabama) and hypothesized that agriculturally mediated seed

dispersal was a major source for gene flow. The competitor, *Ipomoea purpurea*, was chosen because it typically occurs in sympatry with *I. hederacea* populations in the southeastern United States (Smith and Rausher 2007) and it has a similar range distribution (USDA 2009).

Experimental Design

Our field experiment utilized inbred lines of *I. hederacea*, which were selfed in the greenhouse for three generations. After three generations of selfing, 93.75% of initially heterozygous loci will be homozygous. The variation and covariation among our inbred lines will approximate the total genetic variation and covariation among traits for highly selfing populations of *I. hederacea*, although it will be less accurate for mixed-mating populations. (We note that the high selfing rates of *I. hederacea* suggest that additive genetic variance and covariance estimates from a typical paternal half-sib design would be of questionable relevance to natural populations.)

The inbred lines we used were originally collected from six subpopulations of the Piedmont region of North Carolina. We included lines from multiple populations in an attempt to include a representative sample of the variation typically found in several subpopulations and to expand the range of phenotypic variation included in our study. Because we used lines derived from several subpopulations, the matrix we estimated is more analogous to $\mathbf{G}_w$, a single, common genetic variance-covariance matrix for all populations (see Zeng 1988; Chenoweth et al. 2010). The geometry of **G** likely differs between subpopulations as a result of several evolutionary forces, including selection, drift, and mutation. Variation among the inbred lines within populations represents total genetic variation (additive and nonadditive genetic components) along with maternal effects. However, in highly selfing species, selection acts on both additive and nonadditive genetic variation (Roughgarden 1979). We attempted to equalize maternal environmental effects among lines by growing parental plants in the greenhouse under common conditions, germinating experimental plants simultaneously, and then growing them in the greenhouse under common conditions for 10 d.

Twenty-four individuals from each of 50 inbred lines of *I. hederacea* ($n = 1200$) and competitor individuals, *I. purpurea* (purchased from American Meadows, Williston, VT), were germinated in the greenhouse on June 12 and transplanted into a recently plowed and disked old field at the Koffler Scientific Reserve (http://ksr.utoronto.ca; 44°03′N, 79°29′W), north of Toronto, Ontario. We purchased competitors from seed suppliers because we hypothesized that a horticultural variety that had potentially been artificially selected for growth and increased flowering could impose stronger competition on focal *I. hederacea*.

Plants were transplanted on June 22 in a randomized, blocked design, with 1.5 m between rows and columns of plants. Each block ($n = 6$) received four randomly distributed replicates of each inbred line, two of which were randomly assigned to the competition treatment. Seedlings of *I. purpurea* were randomly selected and transplanted 15 cm away from *I. hederacea* the next day. To evaluate the possibility that some transplants varied in condition as a result of maternal environmental effects, we tested for variation among our lines in open leaf number at the time of transplant. We failed to detect any variation among our lines (line effect, $\chi^2 = 0$, $P > 0.99$), suggesting that maternal environmental effects were equalized among experimental plants.

Two weeks after transplant, we removed all nonexperimental vegetation within 0.5 m; weeding was done only once. Each experimental plant was provided with a 2-m bamboo stake to twine around. Throughout the summer and fall, we measured four variables for each individual *I. hederacea*: post-flowering size (open leaf number in mid-August), final plant size (open leaf number in mid-September), date of first flower, and viable seed production. We collected seeds until a killing frost (October 28) ended the experiment. On the basis of a viability assay of 30 randomly selected frost-damaged seeds, total viable seed production consisted of the sum of viable seeds plus 16.7% of frost-damaged seeds.

Statistical Analysis

Calculating selection gradients $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$. Selection gradients were calculated from genotypic data rather than phenotypic data to minimize confounding effects due to environmental variation (Rausher 1992); this approach sacrifices statistical power to avoid environmentally induced covariances (Stinchcombe et al. 2002). We estimated inbred line means for both size traits, flowering time, and fitness as least squares means from fixed-effect ANOVAs that included treatment, block, and inbred line (nested within source population). We elected not to use best linear unbiased predictors (BLUPs) because they have several undesirable properties for use in the estimation of selection gradients (see Postma 2006; Hadfield 2008; Hadfield et al. 2010). We estimated relative fitness in each treatment by dividing our fitness estimates by the mean within each treatment (i.e., each treatment $\overline{w} = 1$).

Selection gradients were estimated separately within each competitive treatment. We estimated standard linear ($\boldsymbol{\beta}$, directional) and nonlinear (γ, disruptive, stabilizing, correlational) selection separately from partial regression coefficients in first- and second-order polynomial regressions (Brodie et al. 1995). For linear regressions, all variance inflation factors were <3, suggesting little multicollinearity. For quadratic regressions, we found high variance inflation factors associated with our estimate of quadratic selection on final size in the competition treatment (10.57) as well as for correlational selection on midseason and final size in both the control and the competition treatments (11.75 and 13.61, respectively). However, for both quadratic regressions, the maximum condition index was 8.02, below the number suggested where regression estimates will be affected (10; Belsley et al. 2005). Convex (negative coefficient) or concave (positive coefficient) coefficients are interpreted as stabilizing and disruptive selection, respectively, only if a stationary point is present (Mitchell-Olds and Shaw 1987). We doubled quadratic regression coefficients for individual traits (but not cross products) to match the original γ matrix from Lande's (1979) equations (Stinchcombe et al. 2008). Preliminary analyses indicated nonnormal residuals, so we used bootstrapping to estimate uncertainty in selection gradients. To obtain 95% confidence limits for each selection gradient, we bootstrapped the residuals of each multiple regression model.

In brief, we resampled the residuals of the multiple regression with replacement, added these to predicted values of the multiple regression, and performed the regression again 1000 times (for an overview, see Stine 1989).

Genetic variation in plant traits. We used univariate mixed-model ANOVA (Proc Mixed, SAS Institute) to determine the presence of genetic variation in flowering time and plant size traits. To do this, we analyzed models of the following form:

$$\begin{aligned}\text{trait} = \mu &+ \text{block} + \text{population} + \text{treatment} \\ &+ \text{line(population)} + \text{block} \times \text{line(population)} \\ &+ \text{treatment} \times \text{line(population)} + \varepsilon,\end{aligned}$$

where μ is the grand mean or intercept of the model (fit by default in most statistics packages); terms involving inbred line are random effects; and source population, treatment, and block are fixed effects. We included block as a fixed effect because our spatial blocks were not chosen at random, and our goal was not to generalize about spatial variation based on them. *P* values of random effects were calculated from one-tailed likelihood ratio tests, comparing models with and without the random effects. We interpreted significant inbred line effects as evidence of total genetic variation (additive and nonadditive), indicating a source of heritable variation.

Calculating **G** *matrices.* We estimated genetic covariances between traits by fitting a multivariate model including all traits (flowering time, plant size immediately after flowering, and final plant size), accounting for the fixed effects of block; as before, line was nested within source population. We tested whether genetic covariances differed from 0 with likelihood ratio tests in which the covariances were individually constrained to equal 0. Likelihood ratio tests follow a mixture of χ^2 distributions when a parameter is held at a boundary constraint (e.g., a variance held to 0; Self and Liang 1987), although this does not apply to covariances held to 0. We present estimated standard errors for all **G** matrix elements, although we caution that these are based on large-sample, asymptotic theory.

To ensure that the **G** matrix used for further calculations was positive definite, we used a full-fit factor analytic model with nonstandardized traits (Proc Mixed, type = FA0(3)). The **G** matrix from this model was subsequently used for the Smith and Rausher (2008*b*), Schluter (1996), and Blows et al. (2004) methods. For Hansen and Houle's (2008) method, the same **G** matrix was standardized by the phenotypic means for each trait. A different matrix was constructed for Agrawal and Stinchcombe's method. In their method, **G** is represented by trait heritabilities on the diagonals and genetic correlations on the off-diagonals. The heritabilities (diagonal elements) of **G** were calculated using standardized traits in a factor analytic model (type = FA0(3), Proc Mixed), while genetic correlations were calculated directly from the genetic variances and covariances of the traits.

Evaluating Genetic Constraints

For both the competition and the control treatment, we applied several well-known metrics designed to measure multivariate evolutionary constraints. We do not apply hypothesis testing to any of the metrics to test whether these estimates differ from each other or 0, since methods for doing so are undeveloped for almost all of them. Moreover, our primary interest is to compare the performance of each method when applied to a common data set of tractable size and interpretability, rather than to compare any particular metric to a null distribution. As such, the data from our experiment serve as a common frame of reference to compare the methods themselves.

Angles Approach: Schluter and Blows's Method

The first formal method that incorporated multiple traits (more than two) was developed by Schluter (1996). Schluter (1996) hypothesized that evolution was most likely to proceed along the axis of greatest genetic variation in **G** (referred to as g_{max}, or principal component 1 [PC1] of **G**), or the genetic line of least resistance. Thus, evolutionary constraint can be measured as the angle (θ) between g_{max} and $\boldsymbol{\beta}$: the larger the angle, the larger the genetic constraint. Blows et al.'s (2004) method is similar but also calculates the cosine between $\boldsymbol{\beta}$ and multiple eigenvectors of **G** to explore how multiple dimensions of **G** are oriented relative to selection. Likewise, they describe methods for comparing the orientation of **G** to γ, the matrix of stabilizing and disruptive selection gradients. Because both of the advanced methods of Blows et al. (2004) require using less than one-half of the PCs (of either **G** or γ) and we have only three phenotypic traits, we did not implement them. However, we did estimate the angle between **G** and $\boldsymbol{\beta}$ and the angle between **G** and γ as suggested by Arnold et al. (2001) and implemented by Blows et al. (2004). As with all vector comparisons, angles were calculated using normalized PCs and vectors.

Angles Approach: Smith and Rausher's Method

We calculated genetic constraint using Smith and Rausher's (2008*b*) angles approach (see method 2 in Smith and Rausher 2008*a*). Smith and Rausher (2008*b*) calculated three measures of genetic constraint with respect to linear selection. The first calculates the angle (θ_1) between $\boldsymbol{\beta}$ and the predicted response, $\Delta\bar{z}$ (see also Blows and Walsh 2008). The predicted response to selection will deviate from $\boldsymbol{\beta}$ because of the effects of genetic covariances between traits and unequal genetic variances in traits; the larger the angle, the larger the difference between the evolutionary response and the combination of traits favored by selection. The second compares the predicted response to selection with and without genetic covariances between traits, measured as the angle (θ_2) between the response with the observed variances and covariances ($\Delta\bar{z}$) and the response that would have occurred in the absence of genetic covariances ($\Delta\bar{z}_{nc}$). The final angle (θ_3) focuses on contribution of unequal genetic variances in the traits by comparing the angle between $\boldsymbol{\beta}$ and the predicted response to selection in the absence of genetic covariances, ($\Delta\bar{z}_{nc}$).

Magnitude Approach: Hansen and Houle

Hansen and Houle (2008) introduce two measures of multivariate evolvability and constraint, respondability (*r*) and

evolvability (e). Qualitatively, respondability measures the predicted magnitude of change of the mean trait values in the next generation relative to the selection gradient. Mathematically, this is simply represented as the norm of the response vector relative to the norm of $\boldsymbol{\beta}$; that is,

$$r(\beta) = \frac{|\Delta\bar{\mathbf{z}}|}{|\beta|}. \quad (2)$$

Evolvability, in contrast, measures how much of the response to selection is in the direction of selection, and it corresponds to the length of the projection of $\Delta\bar{\mathbf{z}}$ onto $\boldsymbol{\beta}$. Following Hansen and Houle (2008), we estimated evolvability as

$$e(\beta) = \frac{\beta'\mathbf{G}\beta}{|\beta|^2}, \quad (3)$$

where the prime indicates transposition. Because these metrics explicitly incorporate the magnitude of the elements of $\boldsymbol{\beta}$ and $\mathbf{G}$, it is essential that all data are scaled appropriately; we followed their recommendation of a mean standardization. Although these metrics differ from past approaches to estimating genetic and evolutionary constraints, they have several useful properties. First, the genetic line of least resistance is the direction in multivariate space in which multivariate evolvability is maximized (Hansen and Houle 2008). Second, these metrics have a natural interpretation because all of the data have been mean standardized: evolvabilities can be interpreted as percentage or proportional changes in traits when the mean-standardized strength of selection is equal to 1 (Hansen and Houle 2008).

Rate of Adaptation Approach: Agrawal and Stinchcombe

While previous approaches directly compare the geometry of either $\Delta\bar{\mathbf{z}}$, $\boldsymbol{\beta}$, or PC1 of $\boldsymbol{\gamma}$ to $\mathbf{G}$, Agrawal and Stinchcombe (2009) utilize the predicted change in fitness of the mean phenotype ($\Delta W(\bar{\mathbf{z}})$) with and without the effects of genetic correlations among traits. Specifically, they estimate the fitness of the mean phenotype as

$$\Delta W(\bar{\mathbf{z}}) = \Delta\bar{\mathbf{z}}^T\boldsymbol{\beta} + \frac{1}{2}\Delta\bar{\mathbf{z}}^T\boldsymbol{\gamma}\Delta\bar{\mathbf{z}} \quad (4)$$

(Agrawal and Stinchcombe 2009). In this formulation, $\Delta\bar{\mathbf{z}}$ is estimated from the equation $\Delta\bar{\mathbf{z}} = \mathbf{G}_{\text{corr}}\beta_\sigma$, where $\mathbf{G}_{\text{corr}}$ is a matrix with heritabilities on the diagonal and genetic correlations on the off-diagonals and β_σ are selection gradients estimated from data with a mean of 0 and a variance of 1 (Agrawal and Stinchcombe 2009). Their index, rate of adaptation R, compares the predicted change in fitness with observed genetic correlations, ΔW_c, to the predicted change in fitness when traits are assumed to be genetically uncorrelated (setting all correlations to 0), ΔW_{nc}, using the ratio

$$R = \frac{\Delta W_c(\bar{\mathbf{z}})}{\Delta W_I(\bar{\mathbf{z}})}. \quad (5)$$

If $R < 1$, covariances are constraining the rate of adaptation, and if $R > 1$, covariances between traits are accelerating the rate of adaptation. This method is similar to Smith and Rausher's (2008*b*) approach but differs in two respects. First, it measures the effects of genetic correlations in terms of how fitness of the mean phenotype is affected, rather than in terms of the angles between $\boldsymbol{\beta}$ and $\Delta\bar{\mathbf{z}}$. Second, it incorporates the effects of nonlinear selection and is also able to detect when covariances accelerate or slow the rate of adaptation.

Dimensions Approach: Kirkpatrick's Dimensionality

Kirkpatrick's (2009) index, as opposed to previous approaches, strictly considers the geometry of $\mathbf{G}$ without regard to the direction of selection and the predicted response. This method determines the effective number of dimensions, n_D, in a $\mathbf{G}$ matrix by measuring whether there is an even distribution of genetic variation explained by all eigenvalues. If most of the genetic variation occurs in the first one or two dimensions, the matrix is ill conditioned and will permit evolution only in few dimensions. Consequently, Kirkpatrick suggests measuring n_D as the sum of the eigenvalues of $\mathbf{G}$ divided by the largest eigenvalue; that is,

$$n_D = \sum_{i=1}^{n} \frac{\lambda_i}{\lambda_1}. \quad (6)$$

If n_D is close to 1, most of the genetic variation in $\mathbf{G}$ is explained by the first and largest eigenvalue, and the matrix has an effective dimension of 1.

Effects of Data Standardization

The effects of data standardization can have important consequences for whether an evolutionary metric is considered to be of small or large magnitude (e.g., Houle 1992; Hereford et al. 2004; Hansen and Houle 2008). To evaluate the sensitivity of the methods developed above to data standardization, we recalculated the angles metrics (Schluter and Blows, Smith and Rausher) using mean-standardized estimates of $\mathbf{G}$ and $\boldsymbol{\beta}$. Because two of our traits are size related (leaf counts), we expected the variance in the traits to scale with the mean and to potentially lead to different evolutionary inferences of constraint when using mean-standardized data. We also explored the sensitivity of the angles comparisons to using standard deviation standardized estimates of $\boldsymbol{\beta}$; estimates of this type are the most frequently used and reported in the literature (e.g., Kingsolver et al. 2001; Siepielski et al. 2009). However, if traits have different variances, converting raw $\boldsymbol{\beta}$ estimates to standard deviation standardized estimates will change the relative magnitudes of the elements of $\boldsymbol{\beta}$ and thus potentially alter any angle-based constraint metric that relies on a normalized estimate (i.e., $|\boldsymbol{\beta}|$).

Results

Competitive Effects and Patterns of Trait Variation and Selection

Phenotypic effects of competition on flowering time, size, and fitness. The presence of an *Ipomoea purpurea* competitor delayed flowering time in *Ipomoea hederacea* by less than half a day but not significantly ($F_{1,1037} = 3.47$, $P = 0.14$; table 1).

Table 1

Effects of an Interspecific Competitor, *Ipomoea purpurea*, on *Ipomoea hederacea* on Inbred Line Means of Flowering Time, Plant Size, and Fitness

	Control treatment			Competition treatment		
	Mean	Phenotypic σ	Line σ	Mean	Phenotypic σ	Line σ
Flowering time (d)	72.68	4.51	3.47	72.99	4.77	3.86
Midseason plant size (leaf no.)	144.83**	40.33	13.91	120.20	31.78	12.45
Final plant size (leaf no.)	454.03**	148.68	61.85	338.12	118.70	56.47
Viable seed	204.56**	138.71	92.79	170.60	114.52	79.22

Note. The standard deviation of both phenotypes and inbred line means is also presented. Asterisks indicate significant differences between competition treatments.

** $P < 0.001$.

The effects of competition substantially reduced midseason size, final size, and seed set in *I. hederacea* ($F_{1,1088} = 154.64$, $P < 0.0001$; $F_{1,1088} = 264.77$, $P < 0.0001$; $F_{1,1088} = 34.52$, $P < 0.0001$, respectively; table 1). There was a weak positive phenotypic correlation between flowering time and final size (control, $r = 0.21$, $P < 0.0001$; competition, $r = 0.18$, $P < 0.0001$) and a weak negative correlation between flowering time and midseason size (control, $r = -0.14$, $P = 0.0004$; competition, $r = 0.06$, $P = 0.13$).

Genotypic selection on flowering time and size. Flowering time was highly genetically variable, as was midseason and final plant size (line(population) effect, $\chi^2 > 3.4$, $P < 0.0325$ for each). The presence of genetic variation indicates that a portion of the phenotype is heritable. Our multivariate analysis suggests weaker evidence for genetic variation in midseason size in the control treatment (note the large standard error), even though evidence for genetic variation in this trait is significant in a univariate analysis.

Flowering time and final size were positively genetically correlated in control ($r_g = 0.58$, $P = 0.0001$) and competition ($r_g = 0.31$, $P = 0.073$) treatments. Flowering time was negatively correlated with midseason size in the control treatment ($r_g = -0.23$, $P = 0.58$) and uncorrelated in the competition treatment ($r_g = 0.08$, $P = 0.75$). As expected, mid- and late-season size were positively correlated in each treatment (control, $r_g = 0.44$, $P = 0.48$; competition, $r_g = 0.97$, $P < 0.0001$). Likelihood models constraining all of the genetic covariances (and hence genetic correlations) to equal 0 provided significantly worse fit to the data ($P < 0.0001$ for each treatment).

Estimation of selection gradients for each treatment revealed patterns of strong linear selection favoring earlier flowering time ($\boldsymbol{\beta}_1$; table 2), with only marginally significant selection on one of the size traits (midseason size, $\boldsymbol{\beta}_2$, competition treatment; table 2). Comparison of bootstrapped confidence intervals showed that linear selection on flowering time did not significantly differ between treatments (table 2; fig. 1). There was no significant nonlinear selection in either treatment, perhaps because of the limited experimental power of using 50 inbred line means for analysis (table 2).

Table 2

Linear and Nonlinear Selection Gradients (with Bootstrapped 95% Confidence Intervals [CIs])

Selection type	Control	CI	*P*	Competition	CI	*P*
Flowering time:						
β_1	−.377	(−.462, −.291)	<.0001	−.377	(−.444, −.300)	<.0001
γ_{11}	−.023	(−.179, .138)	.60	.020	(−.050, .075)	.19
Midseason size:						
β_2	.008	(−.078, .094)	.87	.100	(.004, .199)	.06
γ_{22}	−.099	(−.315, .134)	.81	−.023	(−.115, .098)	.79
Final season size:						
β_3	.016	(−.088, .111)	.76	−.078	(−.178, .018)	.15
γ_{33}	−.039	(−.332, .230)	.80	.051	(−.109, .159)	.76
Flowering time × midseason size:						
γ_{12}	.044	(−.097, .180)	.58	−.0720	(−.207, .072)	.36
Flowering time × final size:						
γ_{31}	.030	(−.152, .216)	.77	.116	(−.080, .323)	.32
Midseason size × final size:						
γ_{32}	.110	(−.133, .071)	.35	−.021	(−.201, .157)	.85

Note. All traits have been standardized to $\mu = 1$ and SD = 1, and fitness is relativized to $w = 1$ in each treatment. Linear selection gradients (β) and associated *P* values were estimated in a model containing only linear terms, while nonlinear selection gradients (γ) and associated *P* values were estimated from a model containing linear and all quadratic terms. Quadratic regression coefficients for individual traits (but not the cross-product term) and associated confidence intervals are doubled.

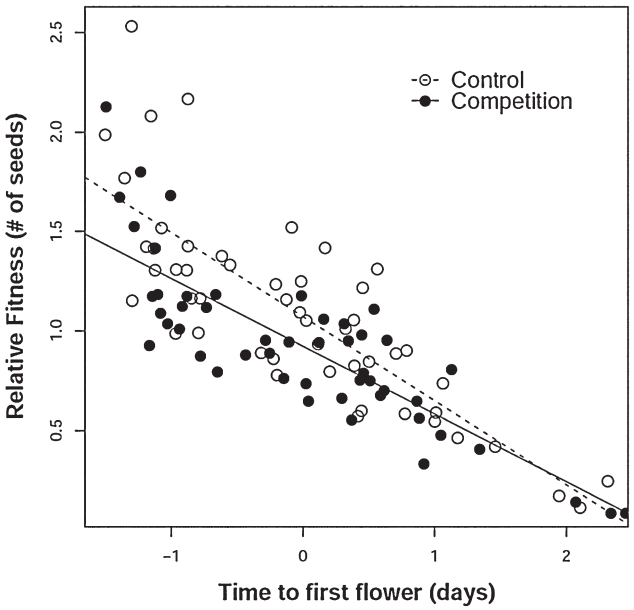

Fig. 1 Selection gradient plots portraying directional selection for earlier flowering time. Data points represent 50 inbred line means for each treatment, with traits standardized to a mean of 0 and a variance of 1.

Multivariate Estimation of Constraints

Angles I: Schluter and Blows. Comparing the major axis of genetic variation in our experimental treatments to the vector of selection gradients revealed a substantial angle between them. For example, in the control treatment, the angle was 87.78°, while in the competition treatment, the angle was 88.64° (appendix). These angles indicate that directional selection favored combinations of traits for which there was not much genetic variation. Inspection of **G** and $\boldsymbol{\beta}$ suggests that this is likely because the majority of selection was on flowering time, the trait for which there was the least genetic variation (on the raw scale). These estimates were relatively unaffected when $\boldsymbol{\beta}$ was estimated from traits with unit variance (89.81° and 80.18°, respectively).

The angle between PC1 of $\boldsymbol{\gamma}$ and PC1 of **G** indicates the correspondence between patterns of genetic variation and nonlinear selection. PC1 of $\boldsymbol{\gamma}$ indicates the combinations of traits for which there is the most nonlinear variation in relative fitness, that is, combinations of traits for which there should be the most selective resistance (Arnold et al. 2001). PC1 of $\boldsymbol{\gamma}$ defines an axis of selective resistance; trait changes along this axis are associated with the greatest curvature in the fitness surface (i.e., the strongest stabilizing/disruptive selection). The major axis of the nonlinear fitness surface for our data was poorly aligned with the major axis of genetic variation in the traits (88.71°, 82.88°; appendix). These data suggest that there is genetic variation in our population that would permit changes in the traits that would not be constrained by strong curvature in the fitness surface. In contrast to the linear case, these estimates changed markedly when $\boldsymbol{\gamma}$ was estimated from traits with unit variance (58.10°, 51.07°).

Angles II: Smith and Rausher. Smith and Rausher's (2008*b*) approach differs from Schluter's (1996) in that it considers the angle between $\boldsymbol{\beta}$ and $\Delta\bar{\mathbf{z}}$. The first angle (θ_1) between $\boldsymbol{\beta}$ and $\Delta\bar{\mathbf{z}}$ reflects how unequal genetic variances and covariances alter the response to selection compared with the combinations of traits favored by selection. In both the control and the competition treatments, we found substantial angles (82.66°, 73.84°; appendix). More than likely, these angles were driven by substantial responses to selection on final size despite weak selection on that trait—responses driven by its large genetic variance and covariances with other traits. The second (θ_2) evaluates the effects of genetic covariances on the response to selection by estimating $\Delta\bar{\mathbf{z}}$ with and without genetic covariances present. In the control treatment, we find a substantial angle (72.61°), while in the competition treatment, this angle is much reduced (15.95°). These data suggest that genetic covariances dramatically alter the response to selection in the control treatment but not in the competition treatment. The likely mechanism behind the latter result is that the patterns of covariance between traits in this treatment are similar to the directions of selection: positively correlated traits are, in general, under the same pattern of selection or very weak selection. The third angle (θ_3) suggested by Smith and Rausher compares the angle between $\boldsymbol{\beta}$ and $\Delta\bar{\mathbf{z}}$ in the absence of genetic covariances, thus revealing the contribution of unequal variance in traits. We found a minor angle in the control treatment (24.92°) and a more substantial one in the competition treatment (63.61°). Taken together, these data suggest that the deflections of the evolutionary response from the combinations of traits favored by selection are driven primarily by genetic covariances in the control treatment and unequal genetic variances in traits in the competition treatment.

Evolvability and respondability. The estimates of mean-standardized respondability and evolvability show a qualitatively different picture. To start, they are based on estimates of **G** using mean-standardized data (table 3), which show a remarkably different picture of trait variation. Respondability in the control treatment equaled 0.00341, while in the competition treatment, it equaled 0.00344. The evolvabilities in these two treatments are lower, reflecting the fact that correlations among the traits deflect some of the evolutionary response away from what was favored by selection: 0.002095 for the control treatment and 0.002793 for the competition treatment.

The mean-standardized scale aids in interpretation of the evolvabilities. For instance, these data indicate that if mean-standardized selection on these traits were equal to 1, the expected change in the mean of the three traits in the direction favored by selection would equal 0.2% ($=e(\beta)\times 100$). Over a single generation, this is unlikely to lead to much of an evolutionary response, although change of this magnitude sustained over many generations is likely to be possible. To illustrate, from the definitions of mean-standardized evolvability, the response to selection for our three traits will equal

$$R = 0.002 \times \bar{z}, \tag{7a}$$

with the mean after selection equal to

$$Z^* = \bar{z}(1 + 0.002). \tag{7b}$$

Iterating this for multiple generations (assuming $e(\beta)$ remains constant) leads to

Table 3

Genetic Variance and Covariance Matrices Used for Multivariate Calculations, along with β Estimates

	Unstandardized G, $\boldsymbol{\beta}$, and $\Delta\bar{z}$					Mean standardized $\mathbf{G}_{\mu}$, $\boldsymbol{\beta}_{\mu}$, and $\Delta\bar{z}$					Standard deviation standardized $\mathbf{G}_{\text{corr}}$, $\boldsymbol{\beta}_{\sigma}$, and $\Delta\bar{z}$				
	Flower	Size 1	Size 2	$\boldsymbol{\beta}$	$\Delta\bar{z}$	Flower	Size 1	Size 2	$\boldsymbol{\beta}_{\mu}$	$\Delta\bar{z}$	Flower	Size 1	Size 2	$\boldsymbol{\beta}_{\sigma}$	$\Delta\bar{z}$
Control:															
Flower	11.42440 (2.440)	−5.94441	91.84711	−.10857	−1.21967	.00216	−.0006	.00278	−7.88806	−.01679	.55532	−.2333	.58029	−.37728	−.20197
Size 1	−5.94441 (11.979)	56.82193 (129.69)	155.236	.00060	.72048	−.0006	.00271	.00236	.08681	.00497	−.2333	.00389	.43978	.00834	.09524
Size 2	91.84711 (33.382)	155.236 (222.44)	2192.847 (773.84)	.00026	−9.29964	.00278	.00236	.01064	.11997	−.02048	.58029	.43978	.102584	.01634	−.21359
Competition:															
Flower	14.09702 (3.003)	2.808441	54.41805	−.09780	−1.43117	.00265	.00032	.00221	−7.1335	−.01962	.61043	.086378	.31436	−.37704	−.24597
Size 1	2.808441 (6.8941)	74.98808 (32.0754)	388.434	.00807	−.20563	.00032	.00519	.00956	.96959	−.00171	.086378	.074692	.97291	.10045	−.10085
Size 2	54.41805 (32.056)	388.434 (124.79)	2125.679 (646.47)	−.00138	−5.12139	.00221	.00956	.01859	−.46643	−.01515	.314362	.972909	.15076	−.0779	−.03254

Note. For the unstandardized matrix, traits are in original units, and $\boldsymbol{\beta}$ are in units of trait^{-1}. Asymptotic standard errors are presented in parentheses. For $\mathbf{G}_{\mu}$, variances and covariances have been divided by the square of the mean or the product of the two trait means, respectively. For selection on the mean-standardized scale, $\boldsymbol{\beta}_{\mu}$ indicates how a percentage change in the trait affects relative fitness (i.e., $\beta_{\mu} = -7.8$ indicates that a 1% increase in a trait will decrease relative fitness by 7.8%). For $\mathbf{G}_{\text{corr}}$, the off-diagonals are genetic correlations, while the diagonals are heritabilities. On this scale, $\boldsymbol{\beta}_{\sigma}$ indicates the change in relative fitness for a 1 SD change in the traits. Size 1 and size 2 are midseason and final plant size, respectively. $\Delta\bar{z}$ is the predicted response to selection.

$$Z(t) = \bar{z}(1 + 0.002)^t. \quad (7c)$$

One can use equation (7c) to solve for the number of generations necessary to double or halve the traits (i.e., setting $Z(t)/\bar{z}$ equal to 2 or 0.5); for our data, these calculations suggest that three traits could be doubled or halved in 347 generations. Moreover, these calculations assume $\beta_\mu = 1$, while for our data, selection on flowering time on the mean-standardized scale was much stronger: $\beta_\mu = -7.88$. Put another way, if selection on all three traits were as strong as it currently is on flowering time, the three traits could be doubled or halved in 44 generations.

Rate of adaptation: Agrawal and Stinchcombe. In contrast to previous methods, Agrawal and Stinchcombe's approach evaluates genetic correlations on the basis of their impact on the rate of adaptation. When applied to our data, their method predicts that genetic correlations will slow the rate of adaptation in the control treatment by 11% (i.e., $R = 0.889$) and in the competition treatment by 8.5% ($R = 0.925$). Both of these numbers are similar to an analysis of 45 articles from the literature ($\bar{R} = 0.89$; Agrawal and Stinchcombe 2009). When strongly correlated traits are included and only one or a few of them are under selection, one will infer a large angle between $\boldsymbol{\beta}$ and g_{max}, even though the correlation does not influence adaptation; in these cases, one will infer a large angle and detect a small R (Agrawal and Stinchcombe 2009). In these situations, R will reflect the rate of adaptation measured by changes in fitness, while θ will reflect phenotypic evolution of the traits in question.

Kirkpatrick's dimensionality. The effective number of dimensions of **G** has the potential to constrain the long-term evolutionary dynamics of these traits. Using Kirkpatrick's method for raw data, we estimated $n_{D=1.024}$ for the control treatment and $n_D = 1.007$ for the competition treatment. These estimates were relatively unaffected by scale, with $n_D = 1.30$ and $n_D = 1.11$ for $\mathbf{G}_\mu$ (appendix). These data suggest that there is essentially only one effective trait in our population (which is likely a composite of what we perceive and measure as traits) and that there are evolutionarily forbidden combinations of traits. On the basis of the principle component loadings, this dimension is heavily influenced by final size, with lesser contributions from flowering time and midseason size. Two features suggest that these results are likely to be robust: they hold on the original scale and for mean-standardized data (suggesting that mean-variance relationships do not contribute), and we used a factor-analytic estimation of **G** that required all three eigenvalues to be greater than or equal to 0. Our results are consistent with Kirkpatrick's (2009) findings, in that he found values of n_D less than 2 for five data sets that he reviewed.

Impacts of data standardization. To evaluate the consequences of data standardization, we recalculated the angles metrics estimates of both **G** and $\boldsymbol{\beta}$ that were mean standardized (i.e., we applied principal component analysis to the $\mathbf{G}_\mu$ matrix and normalized a $\boldsymbol{\beta}_\mu$ vector before estimation of θ). For the Schluter and Blows approach, the deviation between PC1 of $\mathbf{G}_\mu$ and $\boldsymbol{\beta}_\mu$ was substantially lower in the control treatment (76.24° for mean standardized vs. 87.78° for unstandardized) but relatively similar in the competition treatment (84.61° for mean standardized vs. 88.64° for unstandardized; appendix). The reduced angle in the control treatment using mean standardized data is likely because PC1 of $\mathbf{G}_\mu$ contains greater trait loadings from flowering time and midseason size (compared with PC1 of **G**) and flowering time is the trait under strongest selection. The substantial angle, however, still exists because final size is the most variable trait (even when correcting for the mean) and is still under relatively weak selection.

For Smith and Rausher's (2008*b*) method, using $\mathbf{G}_\mu$ and $\boldsymbol{\beta}_\mu$ substantially changed the interpretation of the results. For the control treatment, the angles reflecting the effects of the covariances and unequal variances (θ_1) and the covariances alone (θ_2) were both reduced (θ_1, 82.66° → 52.16°; θ_2, 72.61° → 55.45°), as was the angle reflecting only the effects of unequal variances (θ_3, 24.92° → 3.41°; appendix). Thus, in contrast to Schluter and Blows's method, these data indicate weaker inferences of genetic constraints imposed by the effects of genetic covariances on the mean-standardized scale, driven largely by genetic covariances and not simply the effects of unequal variances. For the competition treatment, both θ_1 and θ_3 were substantially affected (73.84° → 35.80°; θ_3, 63.61° → 21.35°; appendix). Collectively, these angles from the competition treatment indicate that unequal genetic variances and genetic covariances deflect the evolutionary response away from trait combinations favored by selection to a much smaller extent on the mean-standardized scale. In other words, in the competition treatment, conclusions about the degree of evolutionary genetic constraint are highly sensitive to scale of measurement and relationships between the mean and variance of traits.

Discussion

Evolution is both ecological and genetic processes, and our experiment allows us to evaluate the ecological and evolutionary effects of competition as well as the nature of multivariate evolutionary genetic constraints on evolution. Two major results emerge from our experiment. First, we find persistent and strong natural selection on flowering time in both treatments, and despite strong ecological effects of competition, estimates of selection in the two treatments appear qualitatively similar. Second, we find a complex pattern of multivariate genetic constraints: metrics based on the geometry of equation (1) show consistent evolutionary constraints (albeit highly sensitive to scale of measurement), while metrics based on the rate of adaptation show weaker evolutionary constraints. These findings suggest that different constraint metrics capture different aspects of evolutionary constraints. Here we discuss both the biology of competition and selection as well as our evaluation of evolutionary constraint metrics.

Competitive Effects and Selection on Size and Phenology

The presence of the interspecific competitor, *Ipomoea purpurea*, dramatically reduced size and fitness of the focal *Ipomoea hederacea*: final size was reduced by 26%, and viable seed set was reduced by 17% (table 1). On the basis of past

studies of *Ipomoea*, it is likely that these competitive effects are driven by a combination of belowground competition for resources and aboveground competition for light (Weiner 1986). Pollinator-mediated competition was unlikely in our experiment since flowering phases did not overlap for both *Ipomoea* species. Our expectation had been that size would be under stronger selection in the competition treatment, because competition in plants is frequently size asymmetric, leading to disproportionate advantages of large size (Weiner 1990). However, our results show that natural selection on size is unaffected by the competition treatment. Thus, the ecological consequences of competition alone are unlikely to affect the evolutionary dynamics of aboveground plant size by altering selection. Our results stand in contrast to Smith and Rausher's (2007, 2008*a*), who found that the presence of *I. purpurea* not only reduced seed set in *I. hederacea* but also significantly altered selection on floral morphology. These data illustrate how ecological interactions between species may not necessarily translate into changes in the evolutionary dynamics of the traits that mediate or are affected by the interactions (Inouye and Stinchcombe 2001).

Our study indicates that flowering time is an important trait in determining plant fitness: there was strong directional selection to flower early in both competition treatments, which is consistent with numerous other studies that have measured selection on flowering time (e.g., Schemske 1977; Zimmerman and Gross 1984; Stewart and Schoen 1987; Campbell 1991; O'Neil 1997). The likely mechanism behind this selection for early flowering is that we grew plants collected from North Carolina north of their range limit, where season-ending frosts are more common early in the season and at longer day lengths. Support for this interpretation comes from two lines of evidence. First, common garden experiments for populations of *I. hederacea* collected along a latitudinal gradient have found that northern populations generally flower earlier then southern ones (Klingaman and Oliver 1996; B. Campitelli and J. R. Stinchcombe, unpublished data). Second, data from other species suggest that flowering time is strongly influenced by several abiotic cues, such as temperature, light quality, and photoperiod (e.g., from *Arabidopsis thaliana*; Blazquez et al. 2003; Searle and Coupland 2004; Lempe et al. 2005), and other studies have identified a strong genetic response in traits associated with photoperiod cues (Samis et al. 2008; Jackson 2009; Song et al. 2009). Both *I. hederacea* and *I. purpurea* are short-day plants (Greulach 1943), and as such, it is likely that photoperiodic cues are a strong contributor to flowering time.

One caveat to our selection analyses is the potential influence of partial inbreeding. Willis (1996) noted that estimates of selection can be biased by partial inbreeding; in essence, if inbreeding lowers the values of both traits and fitness for only some samples, a biased selection gradient will be obtained. While all of our lines had been selfed for three generations in a common environment, we have no data on whether they suffered from different levels of inbreeding when originally collected. Two lines of argument, however, suggest that partial inbreeding is unlikely to affect our results. First, theoretical work predicts that random mutational effects should contribute to inbreeding depression to a far greater extent than variation in individual inbreeding histories and that the consequences of variation in inbreeding history are likely too small to detect empirically (Schultz and Willis 1995). Second, the reports of inbreeding depression in *I. hederacea* from comparisons of selfed and outcrossed progeny are themselves context dependent: while Hull-Sanders et al. (2005) found inbreeding depression for germination timing, biomass, and flower number, the presence and significance of these effects varied between populations and disappeared for field-grown plants.

Evaluation of Multivariate Genetic Constraints

Our evaluation of multivariate genetic constraints revealed several major findings and challenges, which we discuss in turn. First, our ecological treatment that manipulated competition lead to differential genetic constraints, even though an element-wise comparison of $\mathbf{G}$ and $\boldsymbol{\beta}$ between the two treatments would suggest few differences. Second, all of the constraint metrics that we calculated showed appreciable sensitivity to the underlying measurement scale, that is, whether traits were left in the raw units, standardized by the mean, or standardized by the standard deviation. Third, we detected a consistent difference between metrics based on the geometry of $\mathbf{G}$ and $\boldsymbol{\beta}$ and those based on the rate of adaptation, suggesting that long- and short-term constraints may differ.

Ecological effects on genetic constraints. Initial inspection of $\mathbf{G}$ and $\boldsymbol{\beta}$ suggests few differences between treatments, and comparison of the bootstrapped confidence limits on $\boldsymbol{\beta}$ suggests few significant differences in selection between treatments. Similarly, although we did not perform formal $\mathbf{G}$ matrix comparisons between treatments, the overall structure of the matrices appears largely similar. Yet comparisons of how genetic covariances and unequal variances in quantitative traits affect the direction of evolution reveal substantial differences between treatments. For instance, according to Smith and Rausher's (2008*b*) metrics, θ_2, which measures how genetic covariances affect the response to selection compared with when these covariances are absent, showed strong differences between treatments (control vs. competition: 72.61° vs. 15.95° on the raw scale; 55.45° vs. 21.80° on the mean-standardized scale), suggesting that the response to selection in the control treatment is strongly affected by genetic covariances. Likewise, comparisons of $\boldsymbol{\beta}$ and the response in the absence of covariances ($\Delta\bar{\mathbf{z}}_{\mathrm{nc}}$), which reflect the contribution of unequal variances in traits, revealed substantial differences between treatments on the original data scale (control vs. competition: $\theta_3 = 24.92°$ vs. 63.61° on the raw scale, 3.41° vs. 21.35° on the mean-standardized scale). These data suggest that unequal genetic variances have a greater effect on the evolutionary response in the competition treatment, at least on the scale of original trait units. These data illustrate, especially for θ_2, how small differences in the geometry of $\boldsymbol{\beta}$, $\mathbf{G}$, and $\Delta\bar{\mathbf{z}}$—that is, the alignment between patterns of genetic variation, covariation, and selection—can have substantial effects on evolutionary responses.

Sensitivity to measurement scales. Our data also reveal that inferences about evolutionary genetic constraints are highly sensitive to the scale of measurement and that it is

unlikely that a given scale of measurement will be a panacea. For example, our analysis using Smith and Rausher's (2008*b*) approach, especially in the competition treatment, was highly sensitive to whether we used data in raw units versus in mean standardized units. In this case, the best approach is unclear. For Smith and Rausher's (2008*b*) original study, they investigated a series of size traits measured in the same units (mm), such that the variances and covariances of their traits of interest were on the same scale (mm^2). Accordingly, for their data, it would be straightforward to apply Hansen and Houle's metrics on both the raw and mean-standardized scales, since both the respondability and the evolvability would be measuring evolution of a set of traits of the same units. In contrast, for our data, using the original units would have lead to evolvability and respondability measures that were mixtures of leaf numbers and days to flowering. Interpreting evolutionary responses based on vector norms that are mixtures of trait units is extremely challenging (Hansen and Houle 2008; Stinchcombe et al. 2009).

While the difficulties of considering the evolution of multiple traits in different units is a major challenge, the use of a mean standardization also poses challenges, as both Hereford et al. (2004) and Hansen and Houle (2008) emphasize. Hereford et al. (2004) suggest that a $\boldsymbol{\beta}_\mu$ equal to 1 is a natural benchmark for the strength of selection, since a regression of relative fitness on relative fitness will equal 1. However, mean-standardized gradients can be greater than 1 (e.g., table 2; Stinchcombe 2005). The notion of traits under selection more strongly than fitness itself poses an intuitive challenge to many, especially if one has the world view that fitness is under selection and that other ecologically important traits that are under selection show varying degrees of correlation with fitness (e.g., Orr 2009). Second, the mean-standardized scale is not widely applicable to all traits that biologists may wish to measure (Hansen and Houle 2008). While it may apply quite well to size-related traits that are on either a ratio or log interval scale—implying that both ratios and differences are meaningful (ratio scale) or that ratios but not differences are meaningful (log interval; for a fuller description, see Hansen and Houle 2008)—its applicability for traits such as flowering time is difficult. Phenological traits are often on an interval scale, which permits linear transformations (addition and multiplication). While Hansen and Houle (2008) note that mean standardization is allowed because inferences about differences are unaltered, the actual values for $\boldsymbol{\beta}_\mu$ that one will calculate will be affected by the assumed origin of the traits. For example, if we had measured flowering time as days elapsed since the last snowfall or since the summer equinox (rather than germination), we would have estimated different $\boldsymbol{\beta}_\mu$. These considerations suggest that mean-standardized estimates of selection and constraint for data involving interval traits must be interpreted relative to their original context (i.e., days since germination, snowfall, or equinox).

Estimating uncertainty in genetic constraint metrics. In addition to choice of measurement scale, estimating appropriate uncertainties for **G**, $\boldsymbol{\beta}$, γ, and all subsequent genetic constraint metrics remains challenging. All of these elements have been historically developed and calculated in separate analyses: selection gradients through multiple regression, **G** through multivariate and factor analytic models, and constraint metrics through matrix multiplication. Estimating uncertainty in constraint metrics is especially challenging, since they are calculated from estimates of multiple quantities, each estimated with some error, and it is unclear whether these errors are independent (or not) and how to propagate error and uncertainty through the calculations (also see O'Hara et al. 2008).

Two of the initially most apparent approaches—bootstrapping and BLUPs—are potentially problematic. For bootstrapping, it is unclear what should be sampled with replacement (individuals or quantitative genetic units, or both), and evidence suggests that bootstrapping will tend to lead to an overestimation of the number of significant eigenvalues of **G** (for details, see Hine and Blows 2006), which would lead to biased estimates for metrics, such as Kirkpatrick's dimensionality. Similarly, although BLUPs have several useful properties, recent work by Postma (2006) and Hadfield et al. (2010) suggest that they are poorly suited for use in estimating **G** or $\boldsymbol{\beta}$, because they will frequently be biased and anticonservative. Because the variance in BLUPs is downward biased (Postma 2006; Hadfield et al. 2010), bootstrapping based on BLUPs also underestimates the variance associated with random effects in mixed models (Morris 2002), that is, quantitative genetic variation in the evolutionary context.

Recent work suggests that Bayesian approaches might allow some insight (Hadfield 2008; O'Hara et al. 2008; Hadfield et al. 2010). In particular, a single multivariate model could be fit including all of the traits and fitness, allowing an estimation of the genetic covariances between traits and between traits and fitness (e.g., Etterson and Shaw 2001). Implementing these analyses in a Bayesian framework would allow sampling from the joint posterior distribution of these parameters and could be used to estimate confidence limits on elements of **G** (Hadfield 2008). Similarly, to estimate a genotypic $\boldsymbol{\beta}$, the vector of genetic covariances between traits and fitness (s) could be premultiplied by $\mathbf{G}^{-1}$ to obtain estimates in the form of selection gradients rather than differentials, again using the posterior distribution to estimate confidence limits. By sampling from the posterior distribution of a multivariate model including traits and fitness a large number of times, it might be possible to estimate **G**, $\boldsymbol{\beta}$, and associated angle-based constraint metrics along with uncertainty in the calculated metrics. One current limitation of this proposed approach is that it is unclear how to estimate γ in this framework.

Interpreting constraint metrics. Two features emerged from a comparison of constraint metrics. First, we observed consistent differences in constraint metrics based on the rate of adaptation, which revealed low levels of constraint and constraint metrics based on the geometry of **G** and $\boldsymbol{\beta}$, which revealed high levels of constraint. These discrepancies can occur when highly correlated traits exist in **G** that are only under weak selection (Agrawal and Stinchcombe 2009), as occurred in the current data. For this reason, Agrawal and Stinchcombe (2009) advocated that measures based on θ and the rate of adaptation, R, both be reported. We suggest that angle-based methods are more

useful for determining the evolution of specific traits, while the rate of adaptation approach offers a valuable complement if the interest in **G** and $\boldsymbol{\beta}$ is to predict whether genetic covariances or correlations will inhibit evolution in response to changes in the environment (e.g., Etterson and Shaw 2001). Attempts to link observed **G** matrices to macroevolutionary patterns of traits (e.g., Arnold et al. 2001; Hansen and Houle 2008; Hohenlohe and Arnold 2008) are probably more profitably pursued with angle-based approaches, since these metrics will provide a more explicit consideration of specific phenotypes. Many of these approaches, of course, require assumptions about the stability and evolution of **G**, an important unresolved issue (Steppan et al. 2002; also see Chenoweth et al. 2010).

The second feature to emerge from our analysis of constraint metrics concerns the maintenance of genetic variation and the likely evolutionary response. For example, consideration of the dimensionality metric (n_D) suggests that there is effectively one dimension of multivariate space that could lead to an evolutionary response, appreciably lower than the number of "traits" we measured. Moreover, this dimension of genetic variation is driven largely by final plant size, with much weaker contributions from flowering time and midseason size. Natural selection, in contrast, is acting much more strongly on flowering time, a trait that makes a smaller contribution to the effective number of dimensions. Consideration of the angle-based metrics also supports this view: selection is favoring combinations of traits for which there is relatively little genetic variation, and genetic covariances substantially deflect the evolutionary response away from trait combinations favored by selection. Given these data, the paradox suggested by equation (1) is less challenging: weaker evolutionary responses would be predicted because of genetic covariances and because selection is favoring trait combinations for which there is relatively less genetic variation. Similarly, the challenge of explaining why selection is not eroding genetic variation is lessened by recognizing that selection is acting on trait combinations already exhibiting lesser amounts of genetic variation.

Conclusions

Despite numerous individual estimates of genetic variances, genetic correlations, heritabilities, and selection gradients (e.g., Mousseau and Roff 1987; Roff 1996; Kingsolver et al. 2001), we have relatively few quantitative evaluations of whether those estimates suggest strong multivariate genetic constraints, why genetic variation is maintained (or not), and how genetic correlations affect adaptation. As Walsh and Blows (2009) emphasize, many of these constraints become apparent only in a multivariate framework. Further empirical work from a diversity of study systems will be necessary to evaluate the generality of our findings of low dimensionality of **G**, a misalignment of g_{max} and $\boldsymbol{\beta}$, and a moderate constraint on the rate of adaptation due to genetic covariances.

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Appendix

Supplementary Table

Table A1

Genetic Constraint Indices across Methods and Levels of Standardization

Method, implementation, and index	**G**	$\boldsymbol{\beta}$	Control	Constraint?	Competition	Constraint?	Control/competition
Schluter and Blows:							
Standard:							
$\theta\ (\beta, g_{max})$	**G**	$\boldsymbol{\beta}$	87.785	High	88.643	High	.990
$\theta\ (\gamma, g_{max})$	**G**	γ	88.708	High	82.868	High	1.070
Variant:							
$\theta\ (\beta, g_{max})$	**G**	$\boldsymbol{\beta}_\sigma$	89.813	High	80.181	High	1.120
$\theta\ (\mathrm{PC1}(\gamma), g_{max})$	**G**	γ_σ	58.099	Midrange	51.074	Midrange	1.138
$\theta\ (\beta_\mu, g_{\mu,\max})$	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	76.247	High	84.606	High	.901
Smith and Rausher:							
Standard:							
$\theta_1\ (\beta, \Delta\bar{z})$	**G**	$\boldsymbol{\beta}$	82.665	High	73.838	High	1.120
$\theta_2\ (\Delta\bar{z}_c, \Delta\bar{z}_{nc})$	**G**	$\boldsymbol{\beta}$	72.606	High	15.945	Low	4.554
$\theta_3\ (\beta, \Delta\bar{z}_{nc})$	**G**	$\boldsymbol{\beta}$	24.921	Low	63.615	High	.392

Table A1

(*Continued*)

Method, implementation, and index	G	$\boldsymbol{\beta}$	Control	Constraint?	Competition	Constraint?	Control/competition
Variant:							
θ_1 ($\beta_\mu, \Delta\bar{z}_\mu$)	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	52.166	Midrange	35.802	Midrange	1.457
θ_2 ($\Delta\bar{z}_{\mu,c}, \Delta\bar{z}_{\mu,nc}$)	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	55.452	Midrange	21.794	Low	2.544
θ_3 ($\beta_\mu, \Delta\bar{z}_{\mu,nc}$)	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	3.407	Low	21.354	Low	.160
Hansen and Houle:							
Standard:							
e	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	.002	High	.003	High	.613
r	$\mathbf{G}_\mu$	$\boldsymbol{\beta}_\mu$	.003	High	.003	High	.811
Agrawal and Stinchcombe:							
Standard:							
R	$\mathbf{G}_{corr}$	$\boldsymbol{\beta}_\sigma$ and γ_σ	.890	Low	.925	Low	.961
Kirkpatrick:							
Standard:							
n_D	**G**	na	1.024	High	1.008	High	1.016
Variant:							
n_D	$\mathbf{G}_\mu$	na	1.297	High	1.112	High	1.167

Note. Control/competition indicates the ratio of metrics across treatments. na, not applicable.

Literature Cited

Agrawal AF, JR Stinchcombe 2009 How much do genetic covariances alter the rate of adaptation? Proc R Soc B 276:1183–1191.

Arnold SJ, ME Pfrender, AG Jones 2001 The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112/113:9–32.

Belsley DA, E Kuh, RE Welsch 2005 Regression diagnostics: identifying influential data and sources of collinearity. Wiley, New York.

Blair AC, LM Wolfe 2004 The evolution of an invasive plant: an experimental study with *Silene latifolia*. Ecology 85:3035–3042.

Blazquez MA, JH Ahn, D Weigel 2003 A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nat Genet 33: 168–171.

Blows MW 2007 A tale of two matrices: multivariate approaches in evolutionary biology. J Evol Biol 20:1–8.

Blows MW, SF Chenoweth, E Hine 2004 Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am Nat 163:329–340.

Blows MW, B Walsh 2008 Spherical cows grazing in flatland: constraints to selection and adaptation. Pages 82–102 *in* J van der Werf, HU Graser, R Frankham, C Gondro, eds. Adaptation and fitness in animal populations: evolutionary and breeding perspectives on genetic resource management. Springer, New York.

Bolmgren K, PD Cowan 2008 Time-size tradeoffs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. Oikos 117:424–429.

Bright K 1998 Geographic variation and natural selection on a leaf shape polymorphism in the ivyleaf morning glory (*Ipomoea hederacea*). PhD diss. Duke University, Durham, NC.

Bright KL, M Rausher 2008 Natural selection on a leaf-shape polymorphism in the ivyleaf morning glory. Evolution 62:1978–1990.

Brodie ED, AJ Moore, FJ Janzen 1995 Visualizing and quantifying natural selection. Trends Ecol Evol 10:313–318.

Callahan HS, M Pigliucci 2002 Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis thaliana*. Ecology 83:1965–1980.

Campbell DR 1991 Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. Am Nat 137:713–737.

Charlesworth D, B Charlesworth 1981 Allocation of resources to male and female functions in hermaphrodites. Biol J Linn Soc 15: 57–74.

Charnov EL, JJ Bull, J Maynard Smith 1976 Why be an hermaphrodite? Nature 263:125–126.

Chenoweth SF, HD Rundle, MW Blows 2010 The contribution of selection and genetic constraints to phenotypic divergence. Am Nat 175:186–196.

Dickerson GE 1955 Genetic slippage in response to selection for multiple objectives. Cold Spring Harbor Symp Quant Biol 20:213–224.

Etterson JR, RG Shaw 2001 Constraint to adaptive evolution in response to global warming. Science 294:151–154.

Fineblum WL, MD Rausher 1995 Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517–520.

Gardner KM, RG Latta 2007 Shared quantitative trait loci underlying the genetic correlation between continuous traits. Mol Ecol 16:4195–4209.

Greulach VA 1943 Photoperiodic aftereffects in *Ipomoea*. Ohio J Sci 43:65–73.

Hadfield JD 2008 Estimating evolutionary parameters when viability selection is operating. Proc R Soc B 275:723–734.

Hadfield JD, AJ Wilson, D Garant, BC Sheldon, LEB Kruuk 2010 The misuse of BLUP in ecology and evolution. Am Nat 175:116–125.

Hansen TF, D Houle 2008 Measuring and comparing evolvability and constraint in multivariate characters. J Evol Biol 21:1201–1219.

Hereford J, TF Hansen, D Houle 2004 Comparing strengths of directional selection: how strong is strong? Evolution 58:2133–2143.

Herms DA, WJ Mattson 1992 The dilemma of plants: to grow or defend? Q Rev Biol 67:283–335.

Hine E, MW Blows 2006 Determining the effective dimensionality of the genetic variance-covariance matrix. Genetics 173:1135–1144.

Hohenlohe P, S Arnold 2008 MIPoD: a hypothesis-testing frame-

work for microevolutionary inference from patterns of divergence. Am Nat 171:366–385.
Houle D 1992 Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.
Hull-Sanders HM, MD Eubanks, DE Carr 2005 Inbreeding depression and selfing rate of *Ipomoea hederacea* var. *integriuscula* (Convolvulaceae). Am J Bot 92:1871–1877.
Inouye B, JR Stinchcombe 2001 Relationships between ecological interaction modifications and diffuse coevolution: similarities, differences, and causal links. Oikos 95:353–360.
Inouye DW 2000 The ecological and evolutionary significance of frost in the context of climate change. Ecol Lett 3:457–463.
Jackson SD 2009 Plant responses to photoperiod. New Phytol 181: 517–531.
Johnson T, N Barton 2005 Theoretical models of selection and mutation on quantitative traits. Philos Trans R Soc B 360:1411–1425.
Juenger TE, JK McKay, N Hausmann, JJB Keurentjes, S Sen, KA Stowe, TE Dawson, EL Simms, JH Richards 2005 Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: $\delta^{13}C$, stomatal conductance and transpiration efficiency. Plant Cell Environ 28:697–708.
Kingsolver JG, HE Hoekstra, JM Hoekstra, D Berrigan, SN Vignieri, CE Hill, A Hoang, P Gibert, P Beerli 2001 The strength of phenotypic selection in natural populations. Am Nat 157:245–261.
Kirkpatrick M 2009 Patterns of quantitative genetic variation in multiple dimensions. Genetica 136:271–284.
Klingaman TE, LR Oliver 1996 Existence of ecotypes among populations of entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula*). Weed Sci 44:540–544.
Kruuk LEB, J Slate, JM Pemberton, S Brotherstone, F Guinness, T Clutton-Brock 2002 Antler size in red deer: heritability and selection but no evolution. Evolution 56:1683–1695.
Lacey EP 1986 Onset of reproduction in plants: size- versus age-dependency. Trends Ecol Evol 1:72–75.
Lande R 1979 Quantitative genetic analysis of multivariate evolution, applied to brain : body size allometry. Evolution 33:402–416.
——— 1982 A quantitative genetic theory of life history evolution. Ecology 63:607–615.
Lande R, SJ Arnold 1983 The measurement of selection on correlated characters. Evolution 37:1210–1226.
Lempe J, S Balasubramanian, S Sureshkumar, A Singh, M Schmid, D Weigel, J Doebley 2005 Diversity of flowering responses in wild *Arabidopsis thaliana* strains. PLoS Genet 1:e6.
Lynch M, B Walsh 1998 Genetics and the analysis of quantitative traits. Sinauer, Sunderland, MA.
McKay JKR, H James, KS Nemali, S Sen, T Mitchell-Olds, S Boles, EA Stahl, T Wayne, TE Juenger 2008 Genetics of drought adaptation in *Arabidopsis thaliana*. II. QTL analysis of a new mapping population, Kas-1 × Tsu-1. Evolution 62:3014–3026.
Merila J, BC Sheldon, LEB Kruuk 2001 Explaining stasis: microevolutionary studies in natural populations. Genetica 112:199–222.
Mitchell-Olds T, RG Shaw 1987 Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.
Morris JS 2002 The BLUPs are not "best" when it comes to bootstrapping. Stat Probability Lett 56:425–430.
Mousseau TA, DA Roff 1987 Natural selection and the heritability of fitness components. Heredity 59:181–197.
O'Hara RB, JM Cano, O Ovaskainen, C Teplitsky, JS Alho 2008 Bayesian approaches in evolutionary quantitative genetics. J Evol Biol 21:949–957.
O'Neil P 1997 Natural selection on genetically correlated phenological characters in *Lythrum salicaria* L. (Lythraceae). Evolution 51: 267–274.
Orr HA 2009 Fitness and its role in evolutionary genetics. Nat Rev Genet 10:531–539.
Pease CM, JJ Bull 1988 A critique of methods for measuring life history trade-offs. J Evol Biol 1:293–303.
Postma E 2006 Implications of the difference between true and predicted breeding values for the study of natural selection and micro-evolution. J Evol Biol 19:309–320.
Pursh F 1814 Flora Americae Septentrionalis. Vol 1. Cochrane, London.
Rausher MD 1992 The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. Evolution 46:616–626.
Roff DA 1996 The evolution of genetic correlations: an analysis of patterns. Evolution 50:1392–1403.
——— 2000 Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. J Evol Biol 13:434–445.
Roff DA, DJ Fairbairn 2007 The evolution of trade-offs: where are we? J Evol Biol 20:433–447.
Roughgarden J 1979 Theory of population genetics and evolutionary ecology: an introduction. MacMillan, New York.
Samis KE, KD Heath, JR Stinchcombe 2008 Discordant longitudinal clines in flowering time and phytochrome C in *Arabidopsis thaliana*. Evolution 62:2971–2983.
Schemske DW 1977 Flowering phenology and seed set in *Claytonia virginica* (Portulacaceae). Bull Torrey Bot Club 104:254–263.
Schluter D 1996 Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.
Schultz ST, JH Willis 1995 Individual variation in inbreeding depression: the roles of inbreeding history and mutation. Genetics 141:1209–1223.
Searle I, G Coupland 2004 Induction of flowering by seasonal changes in photoperiod. EMBO J 23:1217–1222.
Self SG, K-Y Liang 1987 Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. J Am Stat Assoc 82:605–610.
Siepielski AM, JD DiBattista, SM Carlson 2009 It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol Lett 12: 1261–1276.
Smith RA, MD Rausher 2007 Close clustering of anthers and stigma in *Ipomoea hederacea* enhances prezygotic isolation from *Ipomoea purpurea*. New Phytol 173:641–647.
——— 2008*a* Experimental evidence that selection favors character displacement in the ivyleaf morning glory. Am Nat 171:1–9.
——— 2008*b* Selection for character displacement is constrained by the genetic architecture of floral traits in the ivyleaf morning glory. Evolution 62:2829–2841.
Song X, DN Kristie, EG Reekie 2009 Why does elevated CO_2 affect time of flowering? an exploratory study using the photoperiodic flowering mutants of *Arabidopsis thaliana*. New Phytol 181:339–346.
Steppan SJ, PC Phillips, D Houle 2002 Comparative quantitative genetics: evolution of the **G** matrix. Trends Ecol Evol 17:320–327.
Stewart SC, DJ Schoen 1987 Pattern of phenotypic viability and fecundity selection in a natural population of *Impatiens pallida*. Evolution 41:1290–1301.
Stinchcombe JR 2005 Measuring natural selection on proportional traits: comparisons of three types of selection estimates for resistance and susceptibility to herbivore damage. Evol Ecol 19: 363–373.
Stinchcombe JR, AF Agrawal, PA Hohenlohe, SJ Arnold, MW Blows 2008 Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62: 2435–2440.
Stinchcombe JR, MT Rutter, DS Burdick, P Tiffin, MD Rausher, R

Mauricio 2002 Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. Am Nat 160:511–523.

Stinchcombe JR, C Weinig, KD Heath, MT Brock, J Schmitt 2009 Polymorphic genes of major effect: consequences for variation, selection and evolution in *Arabidopsis thaliana*. Genetics 182:911–922.

Stine R 1989 An introduction to bootstrap methods. Sociol Methods Res 18:243–291.

USDA 2010 The PLANTS database. National Plant Data Center, Baton Rouge, LA. http://plants.usda.gov.

van der Meijden E, M Wijn, HJ Verkaar 1988 Defense and regrowth, alternative plant strategies in the struggle against herbivores. Oikos 51:355–363.

van Tienderen PH 1991 Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45:1317–1331.

Via S, R Lande 1985 Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522.

Walsh B, MW Blows 2009 Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. Annu Rev Ecol Evol Syst 40:41–59.

Weiner J 1986 How competition for light and nutrients affects size variability in *Ipomoea tricolor* populations. Ecology 67:1425–1427.

——— 1990 Asymmetric competition in plant populations. Trends Ecol Evol 5:360–364.

Willis JJ 1996 Measures of phenotypic selection are biased by partial inbreeding. Evolution 50:1501–1511.

Zeng Z-B 1988 Long-term correlated response, interpopulation covariation, and interspecific allometry. Evolution 42:363–374.

Zimmerman M, RS Gross 1984 The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray. Am Midl Nat 111:185–191.