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ESTIMATING UNCERTAINTY IN MULTIVARIATE RESPONSES TO SELECTION

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Predicting the responses to natural selection is one of the key goals of evolutionary biology. Two of the challenges in fulfilling this goal have been the realization that many estimates of natural selection might be highly biased by environmentally induced covariances between traits and fitness, and that many estimated responses to selection do not incorporate or report uncertainty in the estimates. Here we describe the application of a framework that blends the merits of the Robertson–Price Identity approach and the multivariate breeder's equation to address these challenges. The approach allows genetic covariance matrices, selection differentials, selection gradients, and responses to selection to be estimated without environmentally induced bias, direct and indirect selection and responses to selection to be distinguished, and if implemented in a Bayesian-MCMC framework, statistically robust estimates of uncertainty on all of these parameters to be made. We illustrate our approach with a worked example of previously published data. More generally, we suggest that applying both the Robertson–Price Identity and the multivariate breeder's equation will facilitate hypothesis testing about natural selection, genetic constraints, and evolutionary responses.

KEY WORDS: **Bayesian, MCMC, multivariate breeder's equation, response to selection, Robertson–Price Identity, secondary theorem of selection, selection differential, selection gradient.**

Observing and quantifying the components of the evolutionary process—genetic variation, natural selection, and evolutionary responses—is one of evolutionary biology's fundamental goals. Toward this end, enormous effort has been devoted to estimating patterns of genetic (co)variation in quantitative traits (Falconer and Mackay 1996; Lynch and Walsh 1998) and comparing those estimates across populations, types of traits, or experimental treatments (Shaw 1991; Phillips and Arnold 1999; Cheverud and Marroig 2007; Calsbeek and Goodnight 2009). Likewise, the Lande–Arnold framework has revolutionized our understanding of natural selection in natural populations (Lande and Arnold 1983; Kingsolver et al. 2001; Kingsolver and Diamond 2011), with extensive work devoted to the regression framework for estimating selection (Mitchell-Olds and Shaw 1987; Phillips and Arnold 1989; Wade and Kalisz 1990; Shaw and Geyer 1997; Blows and Brooks 2003; Hereford et al. 2004; Stinchcombe et al. 2008). To date, however, two challenges have limited our ability to combine estimates of genetic variation and selection to predict evolution. First, many of the relationships observed between traits and fitness in natural populations might be environmentally induced, meaning they fail to contribute to responses to selection (Schluter et al. 1991; Rausher 1992; Kruuk et al. 2001, 2002; Stinchcombe et al. 2002). Second, until recently, statistical tools for evaluating uncertainty in the multivariate response to selection have incompletely captured variation in either the genetic variation underlying traits or the selection acting on them.

Recently, Morrissey et al. (2010, 2011, 2012a) have advocated the secondary theorem of selection (STS; Robertson 1966; Price 1970) as a superior method of meeting these challenges, compared to the traditional multivariate breeder's equation. By estimating the genetic covariance between traits and relative fitness in a bivariate or multivariate model, one can directly obtain predicted responses to selection, and in a way that is unbiased by environmentally induced covariances between traits and fitness. The downside of the STS approach, however, is that it does not allow direct and indirect selection to be distinguished—a key advance of the Lande and Arnold (1983) framework.

Here we outline an approach for estimating multivariate responses to selection that are unbiased by environmentally induced covariances, allow direct and indirect selection to be distinguished, and in a way that allows estimates of uncertainty to be placed on all parameter estimates. We illustrate this approach with an empirical example.

ESTIMATING G, β, s, AND $\Delta\bar{z}$ IN A SINGLE FRAMEWORK

Robertson (1966) and Price (1970) showed that selection on a phenotypic trait can be represented by the covariance between a trait and relative fitness:

$$s = \mathrm{cov}(w,z), \tag{1}$$

a relationship known as the Robertson–Price Identity (Lynch and Walsh 1998). An evolutionary response to selection will only occur if there is a genetic covariance between breeding values for a trait and fitness:

$$R = s_g = \mathrm{cov}_a(w,z). \tag{2}$$

Here R indicates the response to selection, s_g the genetic selection differential, and cov_a is an additive genetic covariance (Robertson 1966; Price 1970; Crow and Nagylaki 1976; Rausher 1992). Equation 2, the STS, shows that evolutionary responses to selection equal the genetic covariance between a trait and fitness. Equation (2) generalizes completely to multivariate form.

Etterson and Shaw (2001) provided a rigorous application of (2) with maximum likelihood to predict evolutionary responses to climate change, and tested whether estimates were significantly different from zero, although they did not present estimates of uncertainty in the predicted responses. The secondary theorem approach also eliminates the problems of environmental covariances between traits and fitness and of linear regression with best linear unbiased predictors (BLUPs; Hadfield et al. 2010). Equation (2) includes the net effects of direct and indirect selection on the traits of interest, whether correlated traits were measured or even included in the analysis. The familiar multivariate breeder's equation, in contrast, is limited to measured traits:

$$\Delta\bar{\mathbf{z}} = \boldsymbol{G}\boldsymbol{\beta}, \tag{3}$$

where $\Delta\bar{\mathbf{z}}$ indicates the change in the mean of the phenotypic traits, $\mathbf{G}$ is the genetic covariance matrix, and $\boldsymbol{\beta}$ is a vector of selection gradients (Lande 1979).

An advantage of equation (2) over (3) is that it can be estimated in a single analysis, and hypothesis testing about genetic covariances between traits and fitness can be performed with either likelihood (Shaw 1991; Etterson and Shaw 2001) or Bayesian methods (e.g., Hadfield 2008, 2010; Morrissey et al. 2012a). One disadvantage of equation (2), regardless of implementation method, is that distinguishing direct selection on the trait itself and indirect selection on correlated traits is impossible. Equation (3) has completely converse advantages and disadvantages: selection gradients distinguish indirect and direct selection (Lande and Arnold 1983; Rausher 1992), but the terms on the right-hand side (RHS) of (3) are estimated independently, with no obvious way to perform hypothesis testing about the left-hand side (Simonsen and Stinchcombe 2010). In addition, selection gradients only partition direct and indirect selection accurately when all of the correlated traits under selection have been included in the model (Lande and Arnold 1983).

Morrissey et al. (2010, 2011) have pointed out that most complex, ecologically important traits are likely to involve life history, physiology, and behavior, and that it is unlikely investigators will ever measure all the necessary traits for equation (3) to yield accurate predictions. Accordingly, they have advocated use of the secondary theorem (2) to avoid the problem of correlated traits being omitted from the analysis. However, because the secondary theorem approach (eq. 2) does not allow a distinction between direct and indirect selection, a key advance of (3) is lost by using (2).

We advocate a combined approach that blends the merits of equation (2) and (3), allows evolutionary responses to be estimated directly, indirect and direct selection (and responses) to be distinguished, with uncertainty estimates to be placed on parameters. We start by estimating a genetic covariance matrix, $\mathbf{G_{zw}}$, where one of the traits is relative fitness (Fig. 1); we use $\mathbf{G_{zw}}$ to distinguish this matrix from the genetic covariance matrix for all other traits, $\mathbf{G}$. The diagonal element of $\mathbf{G_{zw}}$ corresponding to relative fitness is the genetic variance in relative fitness, which indicates the upper limit on the rate of evolution (Fisher's fundamental theorem; Fisher 1930). The matrix elements of the column (or row) corresponding to relative fitness, excluding the diagonal element, indicate genetic covariances between traits and relative fitness. Arranged as a vector, these elements are $\mathbf{s_g}$, the genetic selection differentials, and are predicted evolutionary responses for each trait (eq. 2). The remaining rows and columns for traits other than relative fitness represent $\mathbf{G}$ for the measured traits. One can estimate a genotypic selection gradient, $\boldsymbol{\beta}_\mathbf{g}$, using the definition of a selection gradient (Lande and Arnold 1983; Rausher 1992):

$$\boldsymbol{\beta}_g = \boldsymbol{G}^{-1}\boldsymbol{s}_g. \tag{4}$$

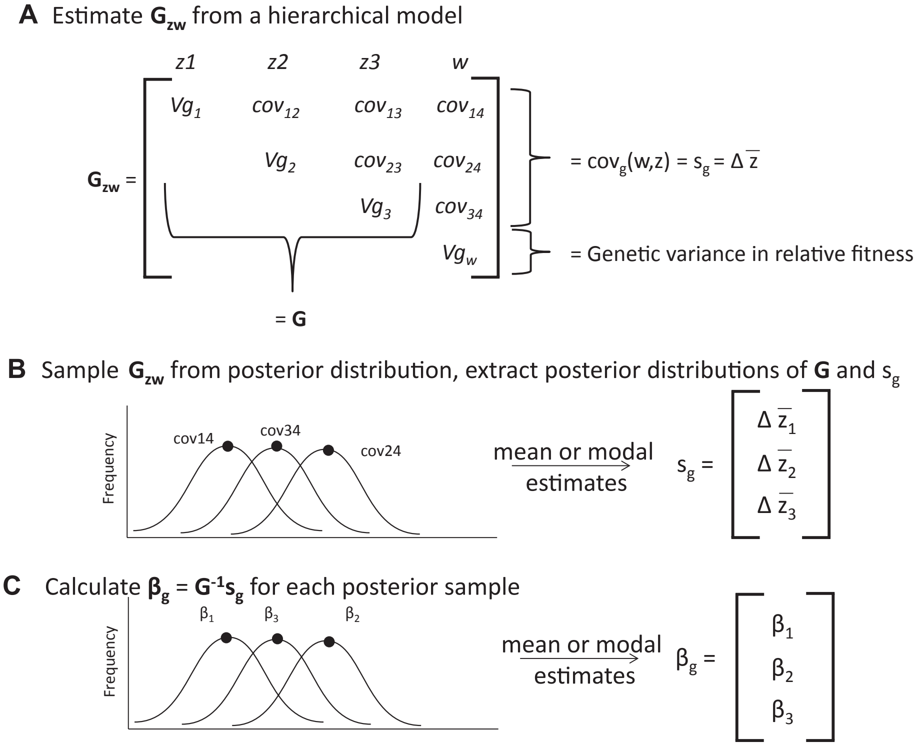

Figure 1. Overview of the analytical approach for estimating multivariate responses to selection and genetic selection gradients, and accompanying estimates of uncertainty, in a fashion unbiased by environmental covariances.

A prerequisite for using (4) is genetic variation in traits and relative fitness (Rausher 1992). Estimating $\boldsymbol{\beta}_\mathbf{g}$ from the component parts of $\mathbf{G}_\mathbf{zw}$ offers some key advantages that do not appear to have been previously recognized. First, it provides estimates of $\mathbf{G}$, $\boldsymbol{\beta}_\mathbf{g}$, $\mathbf{s}_\mathbf{g}$, and $\Delta\bar{\mathbf{z}}$ in a single analysis, with parameters unbiased by environmentally induced covariances. Second, it allows the genetic covariance between traits and fitness to be partitioned between direct and indirect selection, combining the merits of the STS and selection gradient approaches.

The estimate of $\boldsymbol{\beta}_\mathbf{g}$ from (4) will be sensitive to the omission of genetically correlated traits (Rausher 1992), but estimates of $\Delta\bar{\mathbf{z}}$ extracted from $\mathbf{G}_\mathbf{zw}$ will not. To see how, note that substitution of the RHS of (4) into (3) shows clearly that genetic covariances between traits and fitness, $\mathbf{s}_\mathbf{g}$, equal predicted evolutionary responses ($\Delta\bar{\mathbf{z}}$). A substantial predicted evolutionary response ($\mathbf{s}_\mathbf{g} = \Delta\bar{\mathbf{z}} >> 0$) in the presence of near-zero selection gradients ($\boldsymbol{\beta} \approx 0$) indicates that predicted evolutionary responses are due to indirect selection on genetically correlated traits, rather than direct selection. Formally, indirect responses to selection in focal trait i from selection on n correlated traits can be estimated as $\sum_j^n G_{ij}\beta_j$, where G_{ij} are the genetic covariances between trait i and other traits, β_j are selection gradients for other traits, and $j \neq i$.

Estimating $\mathbf{G}_\mathbf{zw}$ with Bayesian methods facilitates estimating uncertainty in evolutionary parameters: posterior distributions of $\mathbf{G}_\mathbf{zw}$ remain valid for any calculations or algebraic operations performed on it (Hadfield 2010; Wilson et al. 2010, 2011). While others have estimated and presented uncertainty in genetic covariances between traits and fitness and subsequently derived evolutionary metrics (Morrissey et al. 2012b), it does not appear to have been applied to partition $\mathbf{s}_\mathbf{g}$ into $\boldsymbol{\beta}_\mathbf{g}$. More generally, the posterior distributions of $\mathbf{G}_\mathbf{zw}$ and derived parameters can be used for evaluating uncertainty in other evolutionary metrics, including the angle between $\Delta\bar{\mathbf{z}}$ and $\boldsymbol{\beta}$ (Schluter 1996), the angle between $\boldsymbol{\beta}$ and $g_{\max}$ (PC1 of $\mathbf{G}$; Blows et al. 2004), the spectral decomposition of the response to selection (Walsh and Blows 2009), the relative magnitude of eigenvalues of $\mathbf{G}$ (Kirkpatrick 2009), and constraint metrics that rely on $\Delta\bar{\mathbf{z}}$ (Hansen and Houle 2008; Agrawal and Stinchcombe 2009; Conner 2012). While we employ a Bayesian approach for estimating uncertainty in $\boldsymbol{\beta}_\mathbf{g}$ and $\Delta\bar{\mathbf{z}}$ (following Morrissey et al. 2012b), other alternatives exist, a point we consider in Discussion.

CASE STUDY: PREDICTED RESPONSES TO SELECTION IN MORNING GLORY

We reanalyze data from the control treatment of an experiment first described by Simonsen and Stinchcombe (2010). Because full field methods, natural history, and experimental details have been published, we only provide a brief overview here. We grew

12 replicates of 50 inbred lines of *Ipomoea hederacea* in the field, and measured three quantitative traits (mid-season size, final size, and flowering time) as well as fitness (seed number). Relative fitness for each individual was calculated by dividing total seed number by mean seed number for all individuals in the control treatment. The data analyzed here are identical to those previously published, with three exceptions: (1) all phenotypic traits, except relative fitness, were centered at zero prior to analysis, (2) we did not apply any standardizations (i.e., division by the mean or standard deviation) to phenotypic traits, (3) an observation for an individual plant with an outlier for one trait was removed.

Data Analysis

TRADITIONAL ANALYSIS

We first estimated $\mathbf{G_{zw}}$ using REML as a point of comparison. We used a multivariate mixed model, with $\mathbf{G_{zw}}$ estimated as the unstructured covariance matrix at the inbred line level (type = UN, Proc Mixed, SAS version 9.3). We report the results of REML fits, and discuss the similarities between REML and Bayesian analyses, in Supporting Information.

To estimate $\boldsymbol{\beta}_\mathbf{g}$, we first performed a regression of relative fitness on the three phenotypic traits, using inbred line means in place of phenotypic estimates (Rausher 1992; Stinchcombe et al. 2002). We elected to use inbred line means because it is the most common implementation of the genotypic selection analysis. As our primary interest is in combining the merits of the STS and the multivariate breeder's equation, we do not formally test for environmentally induced covariances, but instead focus on genetically based estimates that avoid this problem. Methods for testing for environmental covariances are well-established (Stinchcombe et al. 2002; Hadfield 2008; Morrissey et al. 2010); preliminary investigation showed that $\boldsymbol{\beta}_\mathbf{g}$ and $\boldsymbol{\beta}_\mathbf{p}$ were very similar. Phenotypic selection analyses using the Lande and Arnold (1983) approach are presented in the Supporting Information.

BAYESIAN ANALYSIS

We estimated $\mathbf{G_{zw}}$ using a multivariate, random effects model in a Bayesian-MCMC framework (MCMCglmm; Hadfield 2010). While we focus on genetic variances and covariances, $\mathbf{P}$, $\boldsymbol{\beta}_\mathbf{p}$, $\mathbf{s_p}$ can be estimated from this model using the sum of genetic and residual variance components. Briefly, the four traits were entered as response variables, with inbred line designated as a random effect, and block as a fixed effect, specifying that the traits had normal distributions. Traits were approximately normal on the raw scale, which we retain to avoid scale issues commonly associated with transformation (Houle et al. 2011). To evaluate how well our model fit the data, in a preliminary analysis we followed the recommendations of Gelman et al. (2004) by comparing the posterior predictive distribution to the observed data. We generated 1000 predicted datasets and compared the observed means and variances to the distribution of means and variances from 1000 predicted datasets. We did not observe any discrepancies between the means and variances between the 1000 predicted datasets and the observed dataset, indicating that the model fit the data.

Fitting a Bayesian multivariate model requires specifying priors and distributions for the covariance matrix. We explored a variety of priors to ensure that our results were insensitive to prior specifications. For models in which we hypothesized that genetic and residual variances and covariances were sampled from inverse Wishart distributions, we explored priors of either V_p or $0.5 \times V_p$ on the diagonal, and degrees of freedom for the inverse Wishart of 3.003, 3.5, and 4 (with 4 equaling the dimension of the matrices being estimated). We also explored parameter-expanded priors (e.g., Gelman et al. 2004, 2006), specifying that prior means were zero and that the prior covariance matrix was diagonal with variances equal to either 2500 or the REML estimate of V_g. For all models, we used 50,000 burn-in iterations, followed by 500,000 iterations that were thinned every 500 samples, to obtain 1000 estimates of the means, variances, and covariances of the response variables and both the residual and genetic levels. We monitored autocorrelations among posterior samples, and found that they typically were <0.02 between stored samples. For the results presented below, we hypothesized that covariance matrices were sampled from inverse Wishart distributions (with df = 3.003); for priors, we used diagonal matrices of $0.5 \times V_p$. Results from this model had the best combination of a low deviance information criterion and well-behaved posterior distributions. Results of all model fits are in the Supporting Information; differences in priors and distributions had little effect on either quantitative estimates or biological interpretations.

To evaluate uncertainty in predicted evolutionary responses ($\mathbf{s_g}$, $\Delta\bar{\mathbf{z}}$), we evaluated whether the 95% HPD (highest posterior density) interval of the posterior distribution of an estimated parameter overlapped zero. To characterize uncertainty in genotypic selection gradients ($\boldsymbol{\beta}_\mathbf{g}$), we performed the necessary matrix operations on 1000 samples of the posterior distribution of $\mathbf{G_{zw}}$ (Fig. 1) and examined the 95% interval for individual elements of $\boldsymbol{\beta}_\mathbf{g}$. Statistical code in SAS and R for fitting these models and processing the resulting matrices is included in the Supporting Information.

Results

BAYESIAN ESTIMATES OF $\mathbf{G_{zw}}$

We found positive genetic covariances among the phenotypic traits, and substantially larger genetic estimates for mid- and late-season size than flowering time (Table 1). All traits are in

Table 1. Posterior mean MCMC estimates of G_{zw}, for the control and competition treatments, with 95% HPD intervals.

	Flowering time	Mid-season size	Final size	Relative fitness
Flowering time	12.5952 (7.66, 18.43)			
Mid-season size	3.9793 (−16.28, 21.62)	189.67 (94.26, 295.19)		
Final size	88.4242 (10.32, 169.53)	275.7041 (−69.4, 608.17)	3304.48 (1573.65, 5246.04)	
Relative fitness	−1.2484 (−1.90, −0.70)	−0.3290 (−2.56, 2.35)	−8.6367 (−20.93, −0.27)	0.2099 (0.13, 0.31)

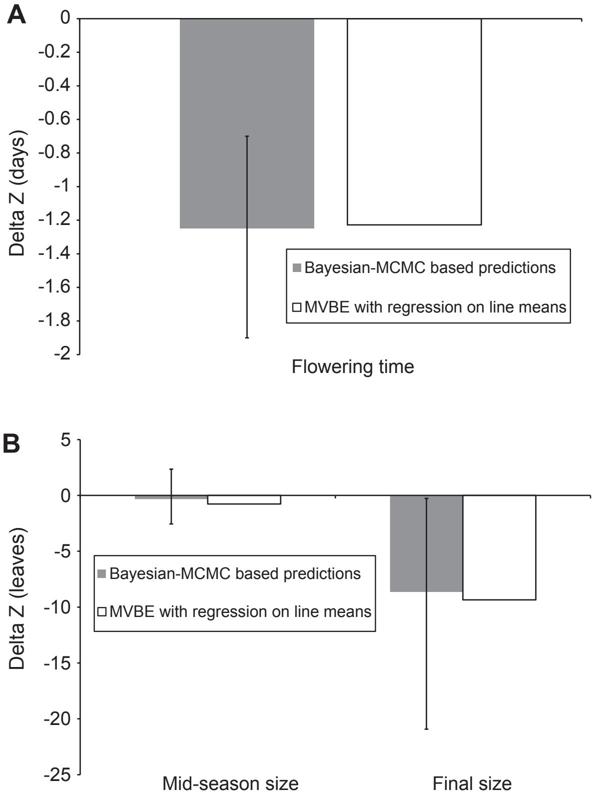

Figure 2. Graphical portrayal of the response to selection for flowering time, mid-season size, and final size. We estimated using genetic selection gradients calculated from Bayesian methods ($\beta_g = G^{-1}s_g$) or regression on line means. Predicted responses to selection using regression were obtained by solving $\Delta\bar{z} = G\beta_g$. Error bars indicate upper and lower 95% HPD intervals for Bayesian-MCMC estimates, while they are not presented for the regression based estimates because of the multistep nature of the estimate. (A) Flowering time, (B) mid-season, and final size.

Table 2. Two methods for estimating β_g.

	Estimation method	
	G-matrix estimate	Regression (line means)
Flowering time	**−0.09892** **(−0.1316, −0.0729)**	**−0.1075** **(−0.1331, −0.0819)**
Mid-season size	0.0005 (−0.0086, 0.0092)	0.0006 (−0.0063, 0.0077)
Final size	0.000057 (−0.0023, 0.0023)	0.0002 (−0.0015, 0.0020)

For regression, estimates are shown for regression of relative fitness on the three phenotypes using either individual line means. For matrix operations, we estimated β_g as $G^{-1}s_g$. Confidence intervals (95%) for regression coefficients are 1.96 × SE, whereas for matrix operations they represent the upper and lower bound of the 95% HPD. Significant estimates are shown in bold.

original units, and thus variances are in units of flowering time^2, number of leaves^2, and are dimensionless for relative fitness. Genetic covariances between traits and fitness were negative, indicating selection against all traits (Fig. 2). Interpreted at face value, these estimates predict an evolutionary response of flowering time of −1.24 days (95% HPD: −1.9, −0.7), little change in mid-season size (−0.33 leaves,: −2.56, 2.35), and a significant decrease in late-season size of −8.6 leaves (−20.93, −0.27). The potential consequences of evolutionary responses of ~1 day can be judged by comparing it to estimates of **β** (approximately −0.1, see below). An individual or genotype with a flowering time of 1–1.24 days earlier than the mean phenotype of the population (where relative fitness by definition equals 1) would have had a 10–12% (range ~8–16%) advantage in relative fitness.

SELECTION GRADIENTS

Selection gradients estimated from linear regression (using inbred line means) or from matrix operations ($\mathbf{G}^{-1}\mathbf{s}_g$) were highly similar to each other and between phenotypic and genetic data (Table 2; Supporting Information). The correspondence between $\boldsymbol{\beta}_g$ estimated with linear regression and solving $\mathbf{G}^{-1}\mathbf{s}_g$ is notable given that the sample sizes for line means were moderate (maximum of $N = 12$), and as such the line means also reflect within-line components of genetic variance (Arnold 1981; Via 1984) as well as noise. Consequently, inbred-line or family-mean regression coefficients only approximate true genetic covariances and selection gradients (see Rausher 1992, Appendix 4).

As described previously (Simonsen and Stinchcombe 2010), selection significantly favored early flowering. There was no significant selection on mid-season or final size. The absence of a significant selection gradient for final size illustrates the benefits of partitioning the contribution of direct and indirect selection. A significant evolutionary response was predicted (−8.6 leaves, 95% HPD: −0.27 to −20.93 leaves) despite the fact that there was little evidence of a significant selection gradient for this trait (Table 2, Supporting Information). The predicted correlated response to selection was likely driven by strong selection on flowering time and its positive genetic covariance with final size.

We detected little difference in selection gradients depending on whether we used Inverse-Wishart or parameter-expanded priors (Supporting Information). For example, for selection on flowering time, the maximum range in estimates depending on estimation methods was 0.011. By contrast, the standard error of the regression coefficient from a regression using line means was 0.013. For the present data at least, differences in selection gradients introduced by the choice of prior are small in magnitude.

Discussion

Our analysis of predicted responses to selection revealed two major results: (1) application of a combined approach to the secondary theorem and estimation of selection gradients allowed us to distinguish direct and indirect predicted evolutionary responses, in a framework allowing the estimation of uncertainty in parameter estimates, and (2) even after placing uncertainty on both **G** and measures of selection, we still obtained significant predicted evolutionary responses using an experiment with moderate sample sizes achievable in many study systems.

DIRECT AND INDIRECT PREDICTED EVOLUTIONARY RESPONSES

Building on Morrissey et al.'s (2010, 2011, 2012a) approach, we have illustrated how the multivariate approach to estimating genetic covariances between traits and fitness can be partitioned into selection gradients, while using MCMC methods to estimate uncertainty. Application of (4) enables direct selection on individual traits be distinguished from indirect selection on the other measured traits (Rausher 1992). The benefit of distinguishing direct and indirect selection is illustrated by considering final size in the present experiment: much of the response to selection, as predicted by its genetic covariance with fitness, is not due to direct selection on final size per se (because the genotypic and phenotypic selection gradients for it are essentially zero; Table 2, Supporting Information). Rather, the selection differential and predicted response to selection is due to correlated traits: either flowering time or an unmeasured trait with a high genetic correlation with flowering time. In this case, partitioning direct and indirect selection leads to a different interpretation (selection on phenology rather than overall size) from the STS approach, changing the major biological conclusion concerning how selection acts on these traits. Formally, the proportion of the response to selection caused by genetic covariances with other traits can be estimated by calculating $\Delta\bar{\mathbf{z}}$ with and without genetic covariances (Etterson and Shaw 2001; Smith and Rausher 2008; Kirkpatrick 2009).

Although some studies (e.g., Morrissey et al. 2010, 2012a) have indicated differences in evolutionary responses using the secondary theorem approach (eq. 2) and the multivariate breeder's equation (3), these differences likely stem from the use of environmentally biased phenotypic selection gradients ($\boldsymbol{\beta}_\mathbf{p}$) rather than any inherent differences between the equations or approaches, for two reasons. First, with genotypic selection gradients $\Delta\bar{\mathbf{z}}$ is identical, regardless of the expression used to estimate it (substituting the RHS of eq. 4 into 3 recovers eq. 2). Accordingly, estimates of $\Delta\bar{\mathbf{z}}$ (and biological conclusions) will be identical between (2) and (3) when genotypic selection gradients are used for (3). Second, environmental covariances between traits and fitness appear to be frequent (Price et al. 1988; Merila et al. 2001; Kruuk et al. 2002; Scheiner et al. 2002; Stinchcombe et al. 2002; Winn 2004; McGuigan and Blows 2009; Bolund et al. 2011; McGuigan et al. 2011).

Although the omission of genetically correlated traits will not change $\Delta\bar{\mathbf{z}}$ estimates obtained from (2), it will affect the partitioning of direct and indirect selection in $\boldsymbol{\beta}_\mathbf{g}$ using (4). Any interpretation of a difference between the way selection may be acting from a comparison of $\boldsymbol{\beta}_\mathbf{g}$ and $\mathbf{s}_\mathbf{g}$ will be subject to the caveat that the inclusion of an additional trait to the analysis could well influence how selection is partitioned (Lande and Arnold 1983; Rausher 1992). Phenotypic selection gradients can be biased by the omission of environmentally or genetically covarying traits, whereas for genotypic selection gradients the problem is reduced: only the omission of genetically covarying traits from the partitioning will result in altered estimates (Rausher 1992). For traits like size or competitive ability, heritable variation often leads to larger or better territories, or more resources, which in turn enhance the trait and fitness components. Experimental manipulations or common garden experiments that eliminate the covariance between genetic and environmental variance components for these traits are necessary in concert with selection analysis (of whatever variety). Although challenging to implement, prominent examples exist (e.g., Simms and Rausher 1989).

Hadfield (2012) suggests caution in interpreting genetically based estimates of selection and predicted evolutionary responses, from either the STS or the selection gradient approach. His argument is that the STS conflates selection and inheritance, and as such cannot predict responses to selection. The data analyzed here were collected on a single generation, and consequently there was selection but no inheritance. The accuracy of predicted evolutionary responses based on a single generation will depend on temporal changes in aspects of the population such as demography, immigration, environmental conditions, and mutation (Morrissey et al. 2010). A second complication that can arise is that failing to detect nonzero genotypic selection gradients or differentials could be because of either an absence of selection, or a lack of genetic variance in the direction of selection (Blows and Hoffman 2005; Walsh and Blows 2009). The former indicates that the trait does not directly affect fitness. In contrast, the latter indicates that nonsignificant genotypic selection gradients or differentials may reflect evolutionary constraints, rather than simply reduced statistical power. In the current data, mid-season and final size have the largest genetic variances (and mean-standardized variances; Simonsen and Stinchcombe 2010), and there is little evidence of significant selection gradients or differentials for these traits, using either genetic or phenotypic data.

An important point with any study characterizing selection is the potential influence of invisible fractions (Grafen 1988; Bennington and McGraw 1995; Hadfield 2008; Sinervo and McAdam 2008; Mojoica and Kelly 2010). In brief, if a fraction of the population dies before expressing the traits of interest, the resulting estimates of selection will be biased by the omission of an episode of selection on the trait, before it was even expressed. Accordingly, while estimates of $\Delta\bar{\mathbf{z}}$ from equation (2) are unbiased by the omission of correlated traits (Robertson 1966; Price 1970; Rausher 1992; Morrissey 2012a; cf. Etterson and Shaw 2001), both estimates of $\Delta\bar{\mathbf{z}}$ and $\boldsymbol{\beta}_\mathbf{g}$ will likely be affected by invisible fraction problems. Hadfield (2008) suggests analytical methods for evaluating this problem, taking advantage of relatedness between surviving and nonsurviving individuals (similar to estimating across-sex genetic correlations). Mojica and Kelly (2010), in contrast, used artificial selection experiments to detect strong viability selection on flower size before it was expressed. Future work on the extent of the invisible fraction problem, and assessments of its quantitative importance, are clearly warranted.

UNCERTAINTY IN MICROEVOLUTIONARY PARAMETERS

Placing uncertainty estimates on predicted responses to selection has been an especially stubborn challenge. Although analytical approximations exist (McCulloch et al. 1996), most attempts at placing confidence limits on predicted evolutionary responses have used bootstrapping and been limited to univariate traits (e.g., Galen 1996; Grant and Grant 2006; Franks et al. 2007), or have considered uncertainty in only one set of parameters while holding another constant (Smith and Rausher 2008; Stinchcombe et al. 2009).

An encouraging outcome from applying Bayesian-MCMC methods to the case study data was that even after accounting for uncertainty in both **G** and $\boldsymbol{\beta}$, some estimates of $\Delta\bar{\mathbf{z}}$ could be distinguished from 0, based on the uncertainty reflected in the posterior distribution. Our results suggest some cause for optimism that microevolutionary analyses can be successfully conducted within a robust statistical framework with sample sizes that are achievable in many empirical systems. We note, however, that our use of inbred lines may be a contributing factor here. Inbreeding decreases genetic variance within lines and increases the genetic variance exhibited among lines, and the hierarchical statistical model for analysis has fewer levels than many experimental breeding designs (e.g., half-sibs), all of which are likely to decrease the uncertainty associated with estimates of **G** and $\boldsymbol{\beta}$.

Although Bayesian approaches are quite flexible for estimating uncertainty in evolutionary parameters (e.g., O'Hara 2008; Ovaskainen et al. 2008; Hadfield 2010; Wilson et al. 2010, 2011; Morrissey et al. 2012a,b; Aguirre et al. 2013) and their general applicability to diverse experimental designs (e.g., inbred lines, half-sibs, pedigrees, experimental evolution), other alternatives exist. A common intuition is that bootstrapping would be useful, although it is difficult to implement in pedigree-based or more complex experimental designs, where much effort has been devoted to detecting selection and evolutionary responses (e.g., Kruuk 2004). Another alternative would be to sample **G** or $\mathbf{G}_\mathbf{zw}$ repeatedly from the asymptotic covariance matrix of parameter estimates from standard REML-based mixed model estimates (Shaw et al. 1995; Shaw and Geyer 1997; Lau et al. 2014).

Conclusions

In summary, we have shown how to combine the merits of the STS with the selection gradient approach, in a way that can be implemented to provide confidence on predicted evolutionary responses and genotypic selection gradients. Although only detailed for linear selection here, nonlinear selection on the genetic variance can be directly assessed through the application of the nonlinear version of the Robertson–Price Identity by a genetic analysis of squared trait deviations (Delcourt et al. 2012). Collectively, these approaches allow direct and indirect selection on individual traits to be distinguished in the way originally envisaged by the multivariate breeder's equation (Lande and Arnold 1983), and facilitate hypothesis testing about the nature of selection, genetic constraints, and evolutionary responses.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.384nf.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. REML estimates of $\mathbf{G}_{\mathbf{zw}}$, for the control treatment, with approximate (asymptotic) standard errors.

Table S2. Phenotypic selection gradients, as estimated following the Lande and Arnold (1983) approach.

Tables S3–S10. Model outputs for $\mathbf{G}_{\mathbf{zw}}$ from different prior specifications.

Figure S1. Estimation of the response to selection, with three methods: (1) MCMC-based and 95% HPD intervals, (2) using the multivariate breeder's equation, estimating **G** and $\boldsymbol{\beta}\mathbf{g}$ separately, with no means of incorporating uncertainty in either quantity, and (3) using the multivariate breeder's equation, but substituting **P** for **G**, and using $\boldsymbol{\beta}\mathbf{p}$ instead of $\boldsymbol{\beta}\mathbf{g}$, also without a means of incorporating uncertainty in either quantity.