Experimental Evolution of Phenotypic Plasticity: How Predictive Are Cross-Environment Genetic Correlations?*

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abstract: Genetic correlations are often predictive of correlated responses of one trait to selection on another trait. There are examples, however, in which genetic correlations are not predictive of correlated responses. We examine how well a cross-environment genetic correlation predicts correlated responses to selection and the evolution of phenotypic plasticity in the seed beetle *Stator limbatus*. This beetle exhibits adaptive plasticity in egg size by laying large eggs on a resistant host and small eggs on a high-quality host. From a half-sib analysis, the cross-environment genetic correlation estimate was large and positive ($r_A = 0.99$). However, an artificial-selection experiment on egg size found that the realized genetic correlations were positive but asymmetrical; that is, they depended on both the host on which selection was imposed and the direction of selection. The half-sib estimate poorly predicted the evolution of egg size plasticity; plasticity evolved when selection was imposed on one host but did not evolve when selection was imposed on the other host. We use a simple two-locus additive genetic model to explore the conditions that can generate the observed realized genetic correlation and the observed pattern of plasticity evolution. Our model and experimental results indicate that the ability of genetic correlations to predict correlated responses to selection depends on the underlying genetic architecture producing the genetic correlation.

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Natural selection on a trait can influence the evolution not only of the trait under selection but also of other traits genetically correlated to the trait under selection (Lande 1979; Roff 1997; Lynch and Walsh 1998). Genetic correlations among traits can arise when traits are affected by the same loci (i.e., loci have pleiotropic effects) or when loci affecting the correlated traits are in linkage disequilibrium. Genetic correlations are often very good predictors of correlated responses to selection (Falconer 1954; Li and Margolies 1993, 1994; Roff and Fairbairn 1999; Czesak and Fox 2003). However, there are a growing number of experimental studies in which genetic correlations have not been predictive of correlated responses (e.g., Falconer 1960; Palmer and Dingle 1986; Wilkinson et al. 1990; Gromko et al. 1991; Bult and Lynch 2000; Worley and Barrett 2000).

Most organisms live in complex environments, and the phenotype of the individual depends not only on the individual's genotype but also on the environment in which it is raised; that is, phenotypes are plastic in response to environmental conditions (Scheiner 1993; Via 1994; Pigliucci 2005). This phenotypic plasticity can be due to environment-specific expression of genes (i.e., genes expressed in only some environments) or environmental sensitivity of alleles (i.e., allelic effects varying with the environment; Schlichting and Pigliucci 1993). The degree to which selection on a trait in one environment affects the evolution of that same trait when expressed in a different environment can be measured as a cross-environment genetic correlation (Falconer 1960). However, the degree to which cross-environment genetic correlations predict (and constrain) the evolution of traits in complex environments and the degree to which they predict how phenotypic plasticity should evolve in response to selection are not well understood (Pigliucci 2005). In general, selection on a trait toward the overall mean of the population (across envi-

ronments) should lead to a reduction in phenotypic plasticity, whereas selection away from the overall mean should lead to an increase in plasticity (the Jinks-Connolly rule; Falconer 1952, 1990; Jinks and Connolly 1973; reviewed in Scheiner 2002), a result commonly observed in selection studies (e.g., Gavrilets and Scheiner 1993*b*; Perez and Garcia 2002), though specific combinations of genetic variances and covariances can lead to exceptions (e.g., when correlated responses are greater than direct responses to selection; Falconer 1990).

In this study we test whether a cross-environment additive genetic correlation estimated from a half-sib quantitative genetic breeding design accurately predicts the correlated evolution of a trait in one environment to selection in a different environment (and vice versa) and whether the evolution of phenotypic plasticity is predictable from this cross-environment genetic correlation. We found that this cross-environment genetic correlation poorly predicts the observed correlated responses; the realized genetic correlations depend on both the direction of selection (increased or decreased trait size) and the environment in which selection is imposed. We also find that the degree to which phenotypic plasticity evolves in response to selection depends on the environment in which selection is imposed. We suggest a simple, biologically meaningful genetic model that can explain the observed pattern of evolution of the cross-environment genetic correlation and the associated evolution of plasticity. In this model, some pleiotropic loci affect the expression of a trait in two environments, while other loci affect the expression of the trait in a single environment. This is analogous to a situation where an additional set of genes are "turned on" in one environment and have phenotypic effects only in that environment. This model explains the observed pattern of experimental evolution and demonstrates the importance of understanding the underlying genetic architecture producing genetic correlations between traits.

The Study System

Stator limbatus is a seed-feeding beetle that exhibits adaptive plasticity in egg size in response to host species that differ in their suitability for larval development. Larval survivorship is poor on seeds of blue paloverde *Parkinsonia florida* (<50%; previously *Cercidium floridum*) but very high on seeds of cat-claw acacia *Acacia greggii* (>95%; Fox and Mousseau 1996). On *P. florida*, larvae hatching from large eggs have much higher survivorship than larvae hatching from small eggs. Thus, there is strong directional selection for large eggs when females oviposit on seeds of *P. florida*. In contrast, there is no fitness benefit for larvae hatching from larger eggs on *A. greggii* seeds because larvae hatching from a range of egg sizes survive equally well on this host. Thus, fecundity selection drives the evolution of small eggs on seeds of *A. greggii* (Fox and Mousseau 1996; Czesak and Fox 2001). Presumably in response to this host-specific difference in selection, *S. limbatus* has evolved egg size plasticity in which females lay larger eggs on *P. florida* seeds and smaller eggs on *A. greggii* seeds (Fox et al. 1997). Laboratory studies have demonstrated that there is genetic variation in this plasticity within populations (Fox et al. 1999).

Material and Methods

This project is the second half of a larger life-history study. Herein we present only the features of the design that are important for the questions addressed in this article. Additional details of the experimental design and maintenance of the selected lines are presented by Czesak and Fox (2003).

The Study Population

The colony of beetles used for these experiments was collected along Mountainview Road in Apache Junction, Pinal County, Arizona, near the base of the Superstition Mountains (in central Arizona; 33°48′N, 111°47′W) in August 1998. At this location, beetles have access to both *Acacia greggii* and *Parkinsonia florida*. The laboratory colony was established with >300 individuals collected from >20 *A. greggii* trees.

Quantitative Genetic Analysis

We used a standard paternal half-sib breeding design (Falconer and Mackay 1996) to measure the additive genetic variances in egg size within each host species and the cross-environment additive genetic correlation (r_A) for egg size. We mated each of 127 sires sequentially to three different dams on average (range = two to five), producing 404 full-sib families. Offspring from the first 20 eggs laid by a female were raised to adult at 30°C, 16L : 9D, on seeds of *A. greggii*. We used seeds of *A. greggii* here because larval mortality is very low on this host (larval mortality during this experiment was only 1.2%; larval mortality is quite high on seeds of *P. florida*, which would impose substantial selection during the experiment). Note that beetles do not exhibit plasticity in egg size in response to their rearing environment, only in response to oviposition environment. Daughters emerging from these seeds were mated with a nonsibling male within 12 h of adult emergence and confined with eight seeds of either *A. greggii* or *P. florida* and allowed to lay eggs. We checked seeds for eggs every 12 h and measured egg length and width of two to three randomly chosen eggs per female from their first 12-

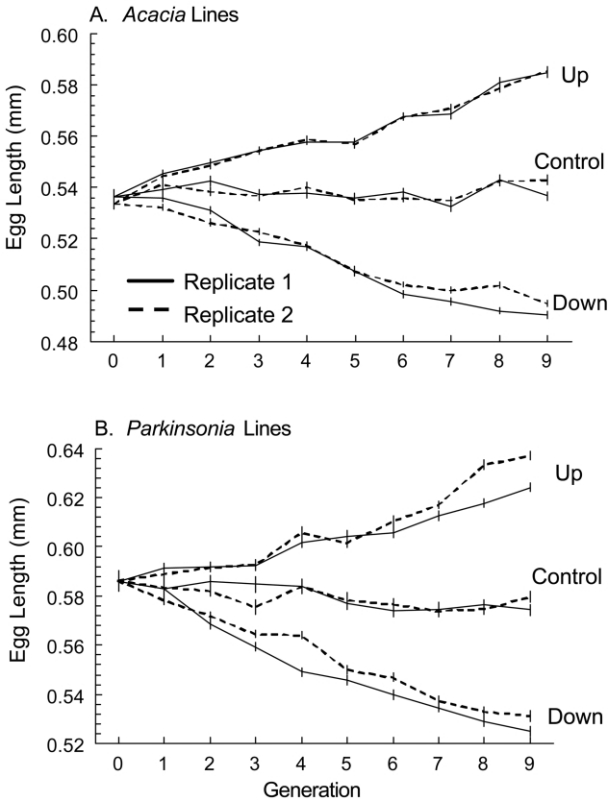

Figure 1: The evolution of mean egg length (±SE) of *Stator limbatus* during an artificial-selection experiment for increased (up) or decreased (down) egg length on *Acacia greggii* (*Acacia* lines; *A*) and *Parkinsonia florida* (*Parkinsonia* lines; *B*) seeds. Data are for two replicates (from Czesak and Fox 2003).

h period of oviposition, using an optical micrometer in a ×55 stereomicroscope (0.005 mm precision; eggs are glued to seeds and cannot be weighed). Eggs from the first 12-h period of oviposition were measured because egg size changes with female age (Savalli and Fox 2002). Egg size was calculated as the average size of three eggs laid during this first 12-h period of oviposition.

The additive genetic covariance between host species was estimated from the sire variance component ($\sigma^2_{\text{sire (both hosts)}}$) from a complete mixed model of ANOVA (Fry 1992; Astles et al. 2006) using SAS (REML estimates). Reduced models, one for each host species, were used to calculate the additive genetic variance in egg size within environments: σ^2_{Ag} for *A. greggii* and σ^2_{Pf} for *P. florida* (Falconer and Mackay 1996). Additive genetic variances were compared between hosts with a Wilcoxon signed-rank test for related samples. Heritabilities were estimated as V_A/V_P, where V_P is the total phenotypic variation (Falconer and Mackay 1996). Cross-environment additive genetic correlations (r_A) were calculated using sire variances and covariances as $r_A = \sigma^2_{\text{sire (both hosts)}}/\sigma_{\text{Ag}}\sigma_{\text{Pf}}$. All parameter estimates and standard errors were estimated by jackknifing the genetic variances and covariances (Roff and Preziosi 1994; Sokal and Rohlf 1995; Windig 1997).

Selection Experiment

We compared the half-sib estimate of the cross-environment additive genetic correlation for egg size to the evolutionary responses observed in an artificial-selection experiment in which we selected on egg length for nine generations. We imposed selection separately on each host species (half of the lines selected on *A. greggii* and half on *P. florida*) and measured the evolutionary response on the other host. Lines selected for egg size on *A. greggii* are referred to as "*Acacia* lines" and lines selected for egg size on *P. florida* as "*Parkinsonia* lines." "Up lines" refers to lines selected for increased egg size (top 20% of the population each generation), and "down lines" refers to lines selected for decreased egg size (bottom 20% of the population), whereas control lines experienced no selection. Each line was replicated twice. All lines were grouped as trios, with each trio containing one up, one down, and one control line created from the same group of beetles in generation 0; we had four trios of lines, for a total of 12 lines (two up lines, two down lines, and two control lines on each host species). The selected lines were maintained at ~400 beetles per generation; this is ~200 females per generation, 40 of which were selected to create each subsequent generation, from which we raised 10 eggs per female. Control lines were maintained at 200 beetles per generation (100 females, two eggs per female). Selection intensities (i) of the selected lines were as follows (averaged over nine generations of selection): *Acacia* up replicate 1 = 1.25, replicate 2 = 1.28; *Acacia* down replicate 1 = −1.23, replicate 2 = −1.22; *Parkinsonia* up replicate 1 = 1.12, replicate 2 = 1.14; *Parkinsonia* down replicate 1 = −1.22, replicate 2 = −1.23. All matings were between one virgin female and one virgin male from the same line. The direct responses to selection are

Table 1: Realized heritabilities (h^2) for *Stator limbatus* egg length on *Acacia greggii* and *Parkinsonia florida*

	Up lines	Down lines
Acacia lines:		
Replicate 1	.41 ± .03	.61 ± .04
Replicate 2	.47 ± .03	.49 ± .04
Parkinsonia lines:		
Replicate 1	.31 ± .02	.47 ± .03
Replicate 2	.45 ± .05	.45 ± .03

Note: Estimates are for females ovipositing on *Acacia greggii* (*Acacia* lines) and *Parkinsonia florida* (*Parkinsonia* lines) seeds following nine generations of artificial selection for increased (up) and decreased (down) egg length for two replicates.

shown in figure 1. Realized heritabilities are listed in table 1, calculated as the slope of the relationship between the direct response and the selection differential. In control lines, variances of mean egg sizes over nine generations of selection were very low (<0.00002 for all control lines).

Although selection on egg size was imposed on the size of eggs laid on either *A. greggii* or *P. florida* seeds, larvae were always reared on *A. greggii* seeds (on which larval survival is high) to avoid natural selection on egg size on *P. florida* seeds during the experiment. We allowed all females to oviposit on their test host (*A. greggii* or *P. florida* seeds) until they laid at least three eggs. These eggs were measured. Females were then transferred to seeds of *A. greggii* and allowed to oviposit until they laid >10 eggs. These eggs were raised for the next generation.

After nine generations of selection, we raised all lines for one generation without selection and then, in generation 11, measured egg size on both *A. greggii* (half of the daughters from each full-sib family) and *P. florida* seeds (the other half of the daughters) for all lines ($n \sim 1{,}800$ beetles in each selected line, 900 beetles in each control line). The realized cross-environment additive genetic correlations were estimated as

$$r_A = \frac{CR_Y}{[(h_X h_Y) i \sigma_Y]/2}, \quad (1)$$

where CR_Y is the correlated response of trait Y estimated as the difference between control and selected lines (in generation 11), $h_X h_Y$ is the product of the square roots of the narrow-sense heritabilities of each trait estimated from half-sib analysis, i is the selection intensity, and σ_Y is the standard deviation of the distribution in trait Y estimated from half-sib analysis (Falconer and Mackay 1996). The denominator of this equation is divided by 2 because selection was applied to one sex only (egg length is a trait of females). Standard errors of realized genetic correlations were approximated by $[(1 - r_A^2)/(n - 2)]^{1/2}$, where r_A is the realized genetic correlation coefficient and n is the number of egg size measurements on both hosts (Sokal and Rohlf 1995). Realized genetic correlations were compared using a test of homogeneity (Sokal and Rohlf 1995).

Results

In the half-sib experiment, additive genetic variance (V_A) in egg length was higher when females oviposited on *Parkinsonia florida* seeds than when they oviposited on *Acacia greggii* seeds, but the standard errors for the V_A estimates were large, and thus the difference in V_A between hosts was nonsignificant ($P = .817$; table 2). Despite higher V_A when eggs were laid on *P. florida*, the heritability of egg length on this host was lower, though not significantly, than the heritability on *A. greggii* seeds because of the high environmental variance (V_E) on *P. florida* (table 2). The size of eggs that females laid on *A. greggii* seeds was highly positively genetically correlated with the size of eggs they laid on *P. florida* seeds (cross-environment $r_A \pm SE = 0.99 \pm 0.06$; table 2).

Table 2: Variance components ($\pm$SE) for egg length (mm) of *Stator limbatus* ovipositing on two hosts, *Acacia greggii* and *Parkinsonia florida*

	A. greggii	*P. florida*
V_A ($\times 10^{-4}$)	2.00 ± .43	2.49 ± .64
V_E ($\times 10^{-4}$)	.86 ± .24	2.48 ± .40
h^2	.70 ± .15	.50 ± .13

Note: Cross-environment covariance ($\times 10^{-4}$) = 2.22 ± 0.44. Cross-environment $r_A = 0.99 \pm 0.06$. V_A = additive genetic variance, V_E = environmental variance, h^2 = heritability, r_A = cross-environment additive genetic correlation. Covariance = additive genetic covariance. Estimates are from the base population before selection.

Selection on the size of eggs that females laid on *A. greggii* resulted in the correlated evolution of the size of eggs laid on *P. florida*, and vice versa (fig. 2). The realized cross-environment genetic correlations calculated from the correlated responses were positive for all selected lines, but most were smaller than the estimate from the half-sib experiment, especially for the *Acacia* up lines (table 3). The realized correlations varied substantially among the selection lines within host species (within *Acacia* lines: $\chi^2 = 92.5$, df = 3, $P < .001$; within *Parkinsonia* lines: $\chi^2 = 702.3$, df = 3, $P < .001$) and differed between the two host species, especially for the up lines (table 3). When selection was imposed on the size of eggs laid on *A. greggii*, the realized cross-environment genetic correlation was highly asymmetrical; r_A was ≥ 0.71 when we selected for small eggs but ≤ 0.45 when we selected for large eggs (replicate 1: $\chi^2 = 57.9$, df = 1, $P < .001$; replicate 2: $\chi^2 = 16.4$, df = 1, $P < .001$; table 3). In contrast, when selection was imposed on the size of eggs laid on *P. florida* seeds, the realized cross-environment r_A estimates were less asymmetrical (significantly asymmetrical in replicate 2 [$\chi^2 = 498.2$, df = 1, $P < .001$] but not significantly asymmetrical in replicate 1 [$\chi^2 = 0.375$, df = 1, $P = .540$]; table 3).

When selection was imposed on the size of eggs laid on *P. florida* seeds, selection for small eggs resulted in decreased plasticity, and selection for large eggs resulted in increased plasticity ($P < .008$; nonsignificant replicate effect, $F = 0.07$, df = 3, 447, $P = .975$; fig. 3), consistent with the expected pattern for $r_A < 1.0$. However, when selection was imposed on the size of eggs laid on *A. greggii* seeds, there was no significant change in plasticity regardless of the direction of selection (relative to the control

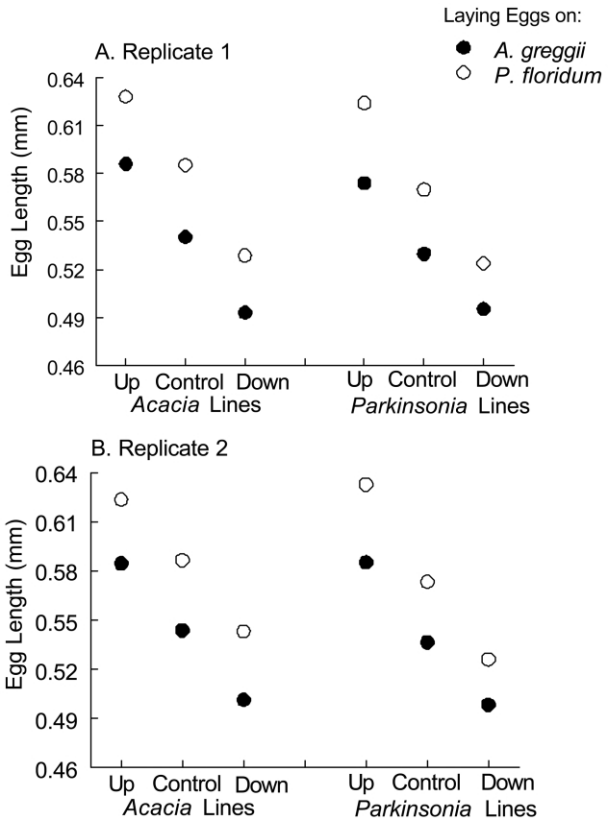

Figure 2: Direct response of egg length and correlated response of egg length on the alternate host species for *Stator limbatus* females ovipositing on *Acacia greggii* and *Parkinsonia florida* seeds following nine generations of artificial selection for increased (up) or decreased (down) egg length for two replicates. Standard error bars are present but are smaller than the points.

lines; $P > .168$; nonsignificant replicate effect, $F = 0.66$, $\mathrm{df} = 3, 443$, $P = .576$; fig. 3).

The Genetic Model

Model Structure

We consider the simplest genetic system that can explain the patterns of experimental evolution observed. We suggest this as the most parsimonious model that not only explains the observed pattern of correlated responses and realized cross-environment additive genetic correlations but also predicts the observed evolution of plasticity. Our model is analogous to the multilocus model of genotype-by-environment interactions presented by de Jong (1990), but we apply the model to an analysis of the evolution of plasticity and of the cross-environment genetic correlation, neither of which is analyzed by de Jong (1990).

Following the experimental system, we consider a single trait expressed in two environments as two separate traits (traits X and Y) having phenotypic values z_X and z_Y, respectively. We define the trait "plasticity" (P) as the difference between the values of z_X and z_Y for a given genotype (i.e., $z_P = z_Y - z_X$). We assume that there are two unlinked loci (A and B) in linkage equilibrium, with two alleles at each locus. We consider a two-locus model because it is simple but also flexible and produces general results that hold when we add together multiple additive loci. At each locus we have two alleles: A_1, A_2 at the A locus and B_1, B_2 at the B locus. With two traits, X and Y, we have four additive effects: a_{AX}, a_{BX}, a_{AY}, and a_{BY}, where the subscripts denote the locus being considered (A or B) and the trait affected (X or Y); these are the additive effects of locus i on trait j, where one allele has the genotypic value of $+a_{ij}$ and the other allele $-a_{ij}$. The four alleles have the frequencies p_1, p_2, q_1, and q_2 for A_1, A_2, B_1, and B_2, respectively. We assume a large randomly mating diploid sexual population with discrete generations.

The means of trait X, trait Y, and plasticity are defined (assuming that the "2" allele at each locus is the "+" allele) as

$$\bar{z}_X = \mu_X + a_{AX}(p_2 - p_1) + a_{BX}(q_2 - q_1), \tag{2a}$$

$$\bar{z}_Y = \mu_Y + a_{AY}(p_2 - p_1) + a_{BY}(q_2 - q_1), \tag{2b}$$

$$\begin{aligned}\bar{z}_P &= \bar{z}_Y - \bar{z}_X \\ &= (\mu_Y - \mu_X) + (a_{AY} - a_{AX})(p_2 - p_1) \\ &\quad + (a_{BY} - a_{BX})(q_2 - q_1),\end{aligned} \tag{2c}$$

where μ_i is the mean value of all other genetic (i.e., contributions of loci other than A and B) and nongenetic (e.g., environmental) effects on the trait. The additive genetic variances (V_i) for the three traits X, Y, and P have the values (assuming no linkage disequilibrium)

Table 3: Realized genetic correlations (r_A; ±SE) between *Stator limbatus* egg length on *Acacia greggii* and on *Parkinsonia florida*

	Realized r_A up lines	Realized r_A down lines
Acacia lines:		
Replicate 1	.45 ± .08	.91 ± .04
Replicate 2	.39 ± .08	.71 ± .06
Parkinsonia lines:		
Replicate 1	.75 ± .06	.77 ± .06
Replicate 2	.97 ± .02	.71 ± .06

Note: Estimates are of the realized cross-environment genetic correlations calculated after nine generations of artificial selection for increased (up lines) and decreased (down lines) egg length of females ovipositing on seeds of *Acacia greggii* (*Acacia* lines) and *Parkinsonia florida* (*Parkinsonia* lines).

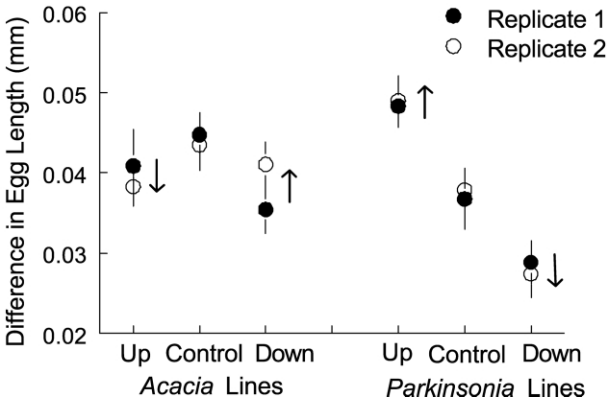

Figure 3: Evolution of egg size plasticity for *Stator limbatus* females ovipositing on *Acacia greggii* and *Parkinsonia florida* seeds following nine generations of artificial selection for increased (up) or decreased (down) egg length. Plotted is the difference in mean egg length (±SE) between full-sib sisters ovipositing on *A. greggii* and *P. florida* seeds for both replicates. Arrows indicate the predicted direction of change in plasticity for $r_A < 1.0$. Note that the pattern observed for the *Parkinsonia* lines is consistent with predictions, but the pattern for the *Acacia* lines is not.

$$V_X = 2a_{AX}^2 p_1 p_2 + 2a_{BX}^2 q_1 q_2, \tag{3a}$$

$$V_Y = 2a_{AY}^2 p_1 p_2 + 2a_{BY}^2 q_1 q_2, \tag{3b}$$

$$V_P = 2(a_{AY} - a_{AX})^2 p_1 p_2 + 2(a_{BY} - a_{BX})^2 q_1 q_2, \tag{3c}$$

and the additive genetic covariances (C_{ij}) between traits have the values

$$C_{XY} = 2a_{AX}a_{AY}p_1 p_2 + 2a_{BX}a_{BY}q_1 q_2, \tag{4a}$$

$$C_{XP} = 2a_{AX}(a_{AY} - a_{AX})p_1 p_2 + 2a_{BX}(a_{BY} - a_{BX})q_1 q_2, \tag{4b}$$

$$C_{YP} = 2a_{AY}(a_{AY} - a_{AX})p_1 p_2 + 2a_{BY}(a_{BY} - a_{BX})q_1 q_2. \tag{4c}$$

From these definitions, it follows that the additive genetic variance for plasticity could be expressed as $V_P = V_X + V_Y - 2C_{XY}$, which is essentially a measure of the independent additive genetic variance in the two environments. It also follows that $C_{XP} = C_{XY} - V_X$ and that $C_{YP} = V_Y - C_{XY}$. Note that the expression for the additive genetic variance in plasticity (V_P) differs from that given in models (such as Scheiner and Lyman 1989) that use the genotype-by-environment (G × E) interaction variance as a measure of the genetic variance for plasticity. We consider the expression of a trait in two environments as two different traits (as in de Jong 1990), and so we have no G × E variance in this model. The two views of a trait in multiple environments give equivalent results, and it can easily be shown that the above expression for V_P is equivalent to the G × E variance of a single trait expressed in two environments.

Equations (3) and (4) demonstrate the simple result that the additive variances and covariances are the sum of the components contributed by each locus. This holds for an arbitrary number of loci, and therefore the model can easily be expanded to include any number of loci (see de Jong 1990; note, however, that a single locus model does not produce comparable results because the genetic correlation is always ±1 if a locus is pleiotropic and always 0 if a locus is not pleiotropic). For n independent loci, the additive variance of trait X would simply be $V_X = \sum_{i=1}^{n} 2a_{iX}^2 f_{i1} f_{i2}$, where a_{iX} is the additive effect of locus i on trait X and f_{i1} and f_{i2} are the frequencies of the two alleles at locus i (see de Jong 1990). An analogous equation could be written for trait Y. The additive genetic variance of plasticity would be $V_P = \sum_{i=1}^{n} 2(a_{iY} - a_{iX})^2 f_{i1} f_{i2}$. It follows that the cross-environment covariance has the value $C_{XY} = \sum_{i=1}^{n} 2a_{iX}a_{iY}f_{i1}f_{i2}$ and that the covariance between trait X and plasticity is $C_{XP} = \sum_{i=1}^{n} 2a_{iX}(a_{iY} - a_{iX}) f_{i1} f_{i2}$ and the covariance between trait Y and plasticity is $C_{YP} = \sum_{i=1}^{n} 2a_{iY}(a_{iY} - a_{iY}) f_{i1} f_{i2}$. The additive genetic correlations are calculated from these variances and covariances as

$$r_{ij} = \frac{C_{ij}}{\sqrt{V_i V_j}}, \tag{5}$$

making the cross-environment additive genetic correlation (r_{XY}), in which we are primarily interested,

$$r_{XY} = \frac{a_{AX}a_{AY}p_1 p_2 + a_{BX}a_{BY}q_1 q_2}{\sqrt{(a_{AX}^2 p_1 p_2 + a_{BX}^2 q_1 q_2)(a_{AY}^2 p_1 p_2 + a_{BY}^2 q_1 q_2)}}. \tag{6}$$

Using standard evolutionary equations for changes in allele frequencies (see, e.g., Crow and Kimura 1970), we used a deterministic iterative procedure to model positive and negative directional selection on traits X and Y to examine conditions under which the cross-environment genetic correlation would evolve in the manner observed in the selection experiment. Iterations were performed by assigning fitness based on the genotypic values of either trait X or trait Y to match the pattern of selection being examined (i.e., positive or negative selection on each trait) and using these fitness values to calculate changes in allele frequencies at the two loci. Changes in the means of the two traits and corresponding changes in the average level of phenotypic plasticity were calculated using equations (2). The additive genetic variances, covariances, and correlations were calculated for each generation using equations (3)–(6).

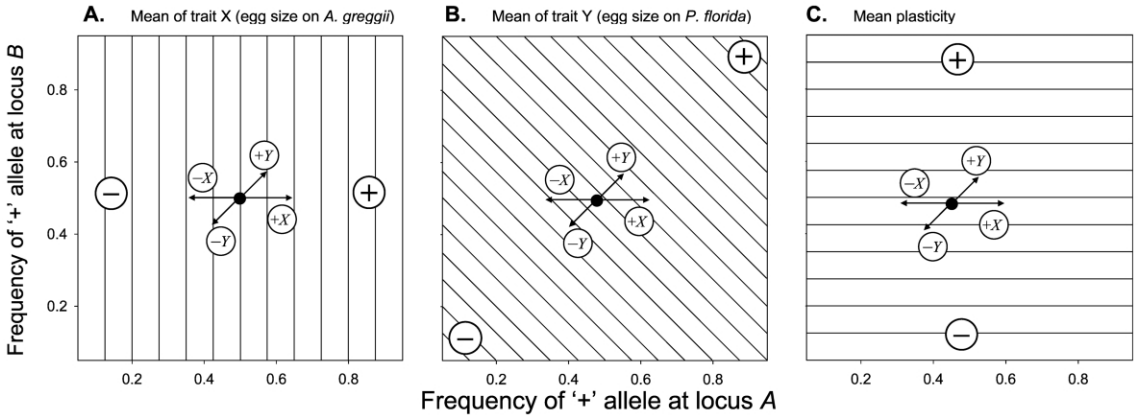

Figure 4: Contour plots showing the means of trait X (egg size on *Acacia greggii*; A), trait Y (egg size on *Parkinsonia florida*; B), and plasticity (C) as a function of the frequencies of alleles with positive effects at a pair of loci (A and B loci). Values were calculated using the genetic model under the asymmetrical genetic architecture (eqq. [2]), assuming that locus A affects both traits X and Y and locus B affects only trait Y (assuming that alleles A_2 and B_2 are the "+" alleles, so that the plots are a function of p_2 and q_2). Contour lines are isoclines of equal value. The relative elevation on the surface is indicated with a plus or minus sign. Overlaid onto each surface is an evolutionary trajectory of a population experiencing directional selection for either larger or smaller values of trait X or Y (the line indicates the evolutionary response of the population, and the arrow indicates the direction of response).

Model Results

Because our goal is to understand our empirical results, we do not provide an exhaustive exploration of the model. Rather, we focus on the conditions under which the model matches the evolutionary patterns observed—specifically, the pattern of evolution of plasticity and the asymmetrical correlated cross-environment response to selection. A more detailed discussion of why other genetic scenarios are unlikely, given our data, can be found in the appendix.

We can examine the relationship between the model and the experimental results by defining trait X as egg size on *Acacia greggii* and trait Y as egg size on *Parkinsonia florida*. First, consider the empirical finding that plasticity evolved in response to selection on *A. greggii* but not on *P. florida*. This implies that there is no covariance between egg size on *A. greggii* and plasticity (i.e., $C_{XP} = 0$), while the covariance between egg size on *P. florida* and plasticity is positive (i.e., $C_{YP} > 0$). The conditions that lead to this covariance pattern must also make the cross-environment correlation (C_{XY}) positive to be consistent with the empirical estimate. The model strongly suggests a genetic architecture where one locus affects egg size only on *P. florida* while the other locus has approximately equal effects on egg size on both hosts (i.e., either $a_{AY} = a_{AX}$ and $a_{BX} = 0$, $a_{BY} \neq 0$ or $a_{BY} = a_{BX}$ and $a_{AX} = 0$, $a_{AY} \neq 0$; see appendix). This pattern of allelic effects, where the effect of a locus is sensitive to the environment whereas the effect of another locus or loci are not, was proposed by Jinks and colleagues (e.g., Brumpton et al. 1977; Jinks et al. 1977 and references therein) and has been called an "epistasis model" by Scheiner and colleagues (e.g., Scheiner and Lyman 1991; Scheiner 1998; Berrigan and Scheiner 2004) because the final phenotype depends on environmental interactions across locus types. However, both our model and that of Scheiner and colleagues include only additive allelic effects. We thus refer to this form of genetic architecture as "asymmetrical genetic architecture" (AGA) to avoid confusion between the use of the term "epistasis" to refer to within-genome interactions between genotypes at different loci and the use of "epistasis" to refer to interaction effects in the broader sense of Scheiner and Lyman (1991; see also Scheiner 1998 and review in Berrigan and Scheiner 2004).

The effect of AGA on the evolutionary dynamics of allele frequencies and the means of traits X and Y and plasticity P are illustrated in figure 4 under the assumption that locus A affects both traits (X and Y) while locus B affects only trait Y (selection moves a population up or down the surface of mean phenotype in the direction of maximum gradient). The evolutionary trajectories overlaid onto the surfaces illustrate that selection on trait X (fig. 4*A*) will change allele frequencies at locus A but not at locus B, while selection on trait Y (fig. 4*B*) will affect allele frequencies at both loci. It is clear from the trajectories in figure 4*C* why plasticity evolves only when selection acts on trait Y—when selection is on trait X, the population slides along an isocline on the surface of

mean plasticity, but when selection is on trait *Y*, the population moves between isoclines on the surface of mean plasticity.

AGA also allows for the observed asymmetrical cross-environment correlated response to selection (table 3). Figure 5 shows the cross-environment genetic correlation as a function of allele frequencies at the two loci under an AGA. The genetic correlation changes much more rapidly as a function of allele frequencies at locus *A* compared with locus *B* and generally becomes smaller as one moves away from intermediate allele frequencies at locus *A*. This occurs because locus *A* contributes to both the numerator and the denominator of the correlation, while the *B* locus contributes only to the denominator and thus contributes a component of variance that does not change as the covariance changes. This implies that selection on *X* can result in rapid evolution of the genetic correlation by affecting allele frequencies at locus *A*. The trajectories overlaid onto figure 5 also illustrate the limited conditions under which only selection on trait *X* will reduce the cross-environment genetic correlation (r_{XY})—that is, when allele frequencies at locus *B* are intermediate while allele frequencies at locus *A* are midway between 0.5 and fixation for the "+" allele. This implies that pleiotropic alleles that make eggs larger on both *A. greggii* and *P. florida* are at a higher frequency than are alleles that make eggs smaller, while alleles at loci that affect egg size only on *P. florida* are at intermediate frequency.

While the model under the AGA assumption produces the basic patterns observed in the experiment, it is very unlikely that the real genetic architecture of these traits is so simple—that is, only two loci each with two alleles and additive effects. Rather, the genetic architecture is probably considerably more complex. However, the same basic results seen in the simple model hold for an arbitrary (n) number of loci (as long as $n > 1$), and the required conditions of an AGA are met whenever one set of loci has similar effects on egg size on both hosts while a second set of loci affects egg size only on *P. florida*. The model strongly suggests that because the genetic correlation is very large in the control (unselected) population, it is likely either that there are more pleiotropic than nonpleiotropic

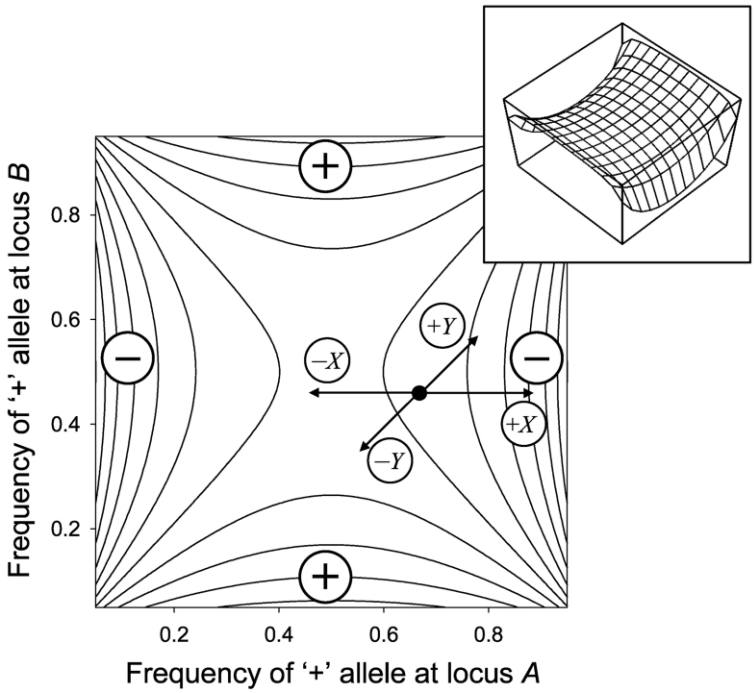

Figure 5: Contour plot of the additive genetic cross-environment correlation (r_{XY}) as a function of allele frequencies at a pair of loci (*A* and *B* loci). Contour lines are correlation isoclines. All values are positive, and relative elevation on the surface is indicated with a plus or minus sign. Values were calculated using equation (6), assuming that locus *A* has the same effect on both traits while locus *B* affects only trait *Y*. Overlaid onto the surface is an evolutionary trajectory of a population experiencing directional selection for either larger or smaller values of trait *X* or *Y* (lines indicates the evolutionary response of the population, and arrows indicate the direction of response), assuming that the "+" allele at locus *A* (A_2) starts at a frequency (p_2) of 0.65, meaning that the "+" allele is more common than the "−" allele at this locus and that the two alleles are at about equal frequency at locus *B*.

loci or that the pleiotropic effects are larger, on average, than the nonpleiotropic effects. Finally, the model predicts that, on average, the alleles with "positive" effects on egg size on both *A. greggii* and *P. florida* are at higher frequency than those with "negative" effects.

Discussion

Our experimental and theoretical studies demonstrate two intriguing results. First, the realized genetic correlations varied depending on the environment in which selection was imposed and the direction of selection (table 3). In other words, the realized genetic correlation between two traits, *X* and *Y*, depended both on the direction of selection on trait *X* (but not on trait *Y*) and on which trait (*X* or *Y*) was under selection. Second, the estimated cross-environment genetic correlation was not predictive for how phenotypic plasticity evolved (fig. 3).

Asymmetric Genetic Correlations

Our results go against the common notion in quantitative genetics (based largely on the assumptions of the Gaussian infinitesimal model, hereafter referred to as the GIM) that a genetic correlation can be used to predict the evolutionary response to selection over multiple generations regardless of the direction of selection and the trait on which selection acts (see Arnold 1994 and Roff 1997 for summaries of theoretical and experimental quantitative genetics based on the GIM; see also Turelli 1988; Turelli and Barton 1994; Pigliucci and Schlichting 1997; Pigliucci 2006 for reviews of criticism of analyses based on the GIM). A few studies have demonstrated that observed correlated responses to selection do not agree with predicted correlated responses based on a genetic correlation estimate (e.g., Palmer and Dingle 1986; Gromko 1995; Worley and Barrett 2000), and some studies have observed variation in the correlated responses of traits among replicate selection lines (e.g., Gromko et al. 1991) and between divergent selection lines (e.g., Wilkinson et al. 1990; Worley and Barrett 2000).

Gromko et al. (1991) show that random variation in which loci contribute to the response to selection could create considerable variation among replicate populations in direct and correlated responses to selection. Alternatively, variation among lines in correlated responses could result from sampling error (i.e., experimental error, rather than just variation in which loci contribute to the response). These sorts of stochastic effects (i.e., stochastic variation in the loci contributing to selection response or sampling error) probably explain the asymmetries in genetic correlations between up and down lines observed by Hillesheim and Stearns (1991), for which the direction of asymmetry in estimated cross-environment genetic correlations was inconsistent between the sexes and among generations. However, neither experimental error nor a stochastic selection model as used by Gromko et al. (1991) is adequate to explain the sort of repeatable asymmetrical correlated responses to selection seen in our experiment. The asymmetry and host effects on realized genetic correlations were consistent between replicates, and our study is large enough that experimental error is unlikely to account for the observed patterns.

The more likely explanation for the patterns we observed in *Stator limbatus* is that variation in pleiotropic effects among loci as well as evolving genetic variances and covariances (due to evolving allele frequencies) generate the observed variation in realized genetic correlations among lines. An asymmetrical correlated response to selection is a common outcome of genetic models (see Roff 1997 for a review). In fact, in population genetic models, there is only a limited set of conditions under which an asymmetrical correlated response to selection is not predicted, at least to some degree. For example, when there is only a single locus with additive effects on a pair of traits, the genetic correlation cannot evolve. At the other extreme, there is the GIM, which assumes an infinite number of loci and infinite population size (e.g., Lande 1980; Bulmer 1985), under which genetic variances and covariances remain approximately constant. For all cases that fall between the extremes of the single locus additive model and the GIM, nearly all parameter space predicts some degree of asymmetrical correlated response to selection (see Bohren et al. 1966). Few quantitative traits are affected by only one locus, so the single-locus case has limited applicability. In contrast, the GIM is widely adopted as a suitable representation of quantitative trait evolution, but the constancy predicted by the GIM architecture of quantitative genetic variation depends critically on the assumptions of the model, and related models using different assumptions do not predict such constancy (see Slatkin and Frank 1990; Turelli and Barton 1994; Reeve 2000).

Our finding in *S. limbatus* that correlated responses to selection depend on both the direction of selection and the trait under selection indicates that the extremes of the single-locus additive model and the GIM do not fit the system being studied. We have developed a simple genetic model that not only explains the asymmetrical correlated response to selection but also predicts the observed pattern of the evolution of phenotypic plasticity. We do not suggest and do not believe that the trait genetics are as simple as those suggested by the model, but we do suggest that the genetic architecture of the traits is likely to follow the basic genetic architecture suggested by the model (with this pattern holding for a larger number of loci than the two being examined in the model).

The Evolution of Phenotypic Plasticity

The evolution of phenotypic plasticity in egg size in *S. limbatus* depended on the environment in which selection was imposed: selection on the size of eggs laid by females on *Parkinsonia florida* seeds changed phenotypic plasticity in the predicted direction, whereas selection on the size of eggs laid on *Acacia greggii* did not (fig. 3). Other studies have likewise found that the evolution of plasticity is complex and often dependent on the environment in which selection is imposed (e.g., Scheiner and Lyman 1991; Matsumura 1996; Noach et al. 1997, 1998). For example, Scheiner and Lyman (1991) selected for small and large thorax size of *Drosophila melanogaster* in two environments (19° and 25°C; flies are larger when raised at 19° vs. 25°C). When selection was imposed at 19°C, plasticity evolved in a manner consistent with the Jinks-Connolly rule, whereas the evolution of plasticity at 25°C was inconsistent among lines. Such complex patterns appear to be the norm rather than the exception in selection experiments examining the evolution of phenotypic plasticity. Our model suggests that complex and asymmetric evolutionary responses of plasticity should be a common outcome of such selection experiments and that the specific responses observed are dependent on the genetic architecture underlying the phenotypic expression of the traits in the studied environments.

Most models of the evolution of phenotypic plasticity have been based on the GIM and have focused on the patterns of plasticity expected to evolve under different types of selection in a heterogeneous environment (e.g., Via and Lande 1985; Gavrilets and Scheiner 1993*a*, 1993*b*). A notable exception is the work of Scheiner and colleagues examining how different types of loci ("plastic" vs. "nonplastic" loci) affect the evolution of plasticity (e.g., Scheiner 1998; review in Berrigan and Scheiner 2004). Our model extends this work of Scheiner and colleagues by explicitly defining a model for the genetic architecture (i.e., the effects of plastic loci with environment-dependent effects and nonplastic loci with environment-independent effects) underlying phenotypically plastic traits to model the correlated responses to selection and how well the cross-environment genetic correlation predicts the evolution of phenotypic plasticity. Our model differs from other models of the evolution of plasticity in that we include both loci that are sensitive to the environment and those that are not (in contrast to Castillo-Chavez et al. 1988; Scheiner 1998), we do not assume an infinite number of loci, we do not include plasticity as a parameter (in our model, plasticity is an emergent property of the underlying genetics of the trait, in contrast to Scheiner 1998, which includes the slope of the norm of reaction as a parameter in the model; see also de Jong 1999), and we explicitly examine the genetics and evolution of plasticity and the evolution of the cross-environment genetic correlation (in contrast to de Jong 1990, which is otherwise an analogous model). The models in the literature most analogous to our model proposed here are those that examine how genetic architecture affects the evolution of genetic variances and covariances (and thus genetic correlations), but those models do not take the next step of examining the evolution of plasticity (e.g., Bohren et al. 1966; Reeve 2000). Our simple genetic model indicates that even with only two loci, the details of the allelic effects influence how phenotypic plasticity will evolve in response to selection and how well the cross-environment genetic correlation predicts that evolution. We have focused only on the conditions that produce patterns of plasticity evolution like those observed in the experimental study, but it is clear that a more thorough analysis of the model will find that a wide variety of complex patterns of plasticity evolution are possible with small changes in the starting allele frequencies, allelic effects, and the addition of more loci with epistatic interactions or linkage.

Our simple model also indicates that complex patterns of plasticity evolution can be generated with an entirely additive model, consistent with the results of Scheiner (1998). Thus, while epistatic interactions among loci influence the evolution of genetic variances and covariances (Schlichting and Pigliucci 1993) and can contribute to the evolution of phenotypic plasticity (Pigliucci 2005), they are not necessary to generate complex patterns of plasticity evolution or correlated responses to selection (e.g., Bohren et al. 1966; Scheiner and Lyman 1991), such as the patterns observed here. This is not meant to suggest that dominance and epistasis do not play any role; clearly, the addition of other forms of genetic effects could allow for nearly any pattern of experimental evolution. However, regardless of the presence of other forms of genetic effects, we expect additive effects (even those that arise from epistasis) to dominate the patterns of short-term responses to selection seen in studies of experimental evolution, and we suggest that they are likely to explain the pattern of response to selection seen here.

Our model suggests that the pattern of correlated responses and the pattern of evolution of plasticity observed for *S. limbatus* are a consequence of loci that have environment-specific expression; specifically, one or more loci affect the size of eggs laid on both *Acacia* and *Parkinsonia*, but at least one locus affects only the size of eggs laid on *Parkinsonia*. Recent quantitative trait locus (QTL) studies of phenotypically plastic traits suggest that this is a reasonable genetic model; those studies demonstrate that while some QTLs influence traits expressed in multiple environments (i.e., have pleiotropic effects across multiple environments), other loci affect the phenotype in only

some environments (e.g., *Drosophila* life span [review in Mackay 2002], methyl jasmonate production in *Arabidopsis thaliana* [Kliebenstein et al. 2002], and reproductive timing in *A. thaliana* [Weinig et al. 2002]). Previous experiments with *S. limbatus* have shown that in the absence of host experience, females lay small *Acacia*-sized eggs (Fox et al. 1997). We suspect that females thus default to laying "small" eggs except in the presence of specific stimuli and that at least one gene mediates the "response" (change in egg size) when encountering this stimulus (possibly a regulatory control gene; Schlichting and Pigliucci 1993; Schlichting and Smith 2002). However, the details of the genetic architecture underlying egg size plasticity in *S. limbatus*, such as the number of QTLs and their individual effects, are currently unknown.

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APPENDIX

Support for the Asymmetrical Genetic Architecture

In the main text, we discuss the case of an asymmetrical genetic architecture (AGA). Here, we provide a detailed discussion of why other genetic scenarios are unlikely and further details on the fit between the model and the observed genetic parameters to show why we believe that the experimental results are most compatible with this genetic architecture and are not consistent with other possible genetic architectures.

First, consider the conditions under which $C_{XP} = 0$ while $C_{YP} > 0$ (i.e., conditions under which plasticity would evolve only when selection was imposed on the size of eggs laid on *Parkinsonia florida*) and $C_{XY} > 0$ (since we find a large and positive cross-environment correlation). Consider the contribution of locus A to the covariance between trait X and plasticity (C_{AXP}); locus A contributes a zero covariance component when $a_{AX} = 0$ and/or $a_{AY} = a_{AX}$ (see eq. [4b]). Total covariance of trait X and plasticity (sum for loci A and B) would be 0 when one of the following conditions is true: $a_{AY} = a_{AX}$ and $a_{BY} = a_{BX}$; $a_{AX} = 0$ and $a_{BX} = 0$; or either $a_{AY} = a_{AX}$ and $a_{BX} = 0$ or $a_{BY} = a_{BX}$ and $a_{AX} = 0$. In the first condition, each locus has the exact same influence on traits X and Y (i.e., egg size on *Acacia greggii* and *P. florida*), which also makes $C_{YP} = 0$ (see eq. [4c]) and $r_{XY} = 1$ for all allele frequencies (this is because $C_{YP} = V_Y - C_{XY}$, and thus $V_Y = C_{XY}$, since $V_X = V_Y$, $r_{XY} = 1$; see eqq. [3a], [3b], [5]). We observed realized $r_{XY} < 1$ (table 3), so the first condition can be ruled out. In the second condition, neither locus affects trait X (i.e., egg size on *A. greggii*), which makes $C_{XY} = 0$ (see eq. [4a]) and $V_X = 0$ (see eq. [3a]). We observed $C_{XY} > 0$ (i.e., a positive cross-environment genetic covariance) and $V_X > 0$ (because egg size evolved on *A. greggii*; fig. 2), so the second condition can be ruled out. The third condition occurs when one locus has the same effect in both environments while the other locus has an effect in only one of the two environments. In this case, one locus affects egg size only on *P. florida*, while the other locus affects egg size on both hosts (and has roughly equal effects on both hosts). Thus, $C_{XP} = 0$, and $C_{YP} > 0$ (see eq. [4c]); egg size will evolve in response to selection on the size of eggs laid on *P. florida* (because $C_{YP} > 0$) but not on the size of eggs laid on *A. greggii* (because $C_{XP} = 0$). Thus, the third condition is consistent with our empirical results and is our assumed genetic architecture. The third condition is the AGA.

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