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ACROSS-ENVIRONMENT GENETIC CORRELATIONS AND THE FREQUENCY OF SELECTIVE ENVIRONMENTS SHAPE THE EVOLUTIONARY DYNAMICS OF GROWTH RATE IN *IMPATIENS CAPENSIS*

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Trade-offs can exist within and across environments, and constrain evolutionary trajectories. To examine the effects of competition and resource availability on trade-offs, we grew individuals of recombinant inbred lines of *Impatiens capensis* in a factorial combination of five densities with two light environments (full light and neutral shade) and used a Bayesian logistic growth analysis to estimate intrinsic growth rates. To estimate across-environment constraints, we developed a variance decomposition approach to principal components analysis, which accounted for sample size, model-fitting, and within-RIL variation prior to eigenanalysis. We detected negative across-environment genetic covariances in intrinsic growth rates, although only under full-light. To evaluate the potential importance of these covariances, we surveyed natural populations of *I. capensis* to measure the frequency of different density environments across space and time. We combined our empirical estimates of across-environment genetic variance–covariance matrices and frequency of selective environments with hypothetical (yet realistic) selection gradients to project evolutionary responses in multiple density environments. Selection in common environments can lead to correlated responses to selection in rare environments that oppose and counteract direct selection in those rare environments. Our results highlight the importance of considering both the frequency of selective environments and the across-environment genetic covariances in traits simultaneously.

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Understanding the evolution of correlated traits is fundamental for the study of evolutionary topics such as life-history theory (Roff 1992; Roff and Fairbairn 2007), costs of adaptation (Futuyma and Moreno 1988; Brodie and Brodie 1999; MacLean et al. 2004), the evolution of plant defense strategies and mating systems (Fineblum and Rausher 1995; Stinchcombe and Rausher 2001; Sargent et al. 2007), sexual selection (e.g., Kilpimaa et al. 2004), and the evolution of aging and senescence (e.g., Bonduriansky and Brassil 2005). In the quantitative genetic framework, trait correlations are typically considered in the context of the genetic variance–covariance matrix (**G**), in which the off-diagonals represent genetic covariances between traits, arising because of either the pleiotropic effects of individual loci on multiple traits or linkage disequilibrium between loci (Falconer and MacKay 1996; Lynch and Walsh 1998; Gardner and Latta 2007). Genetic covariances between traits will lead to a correlated response to selection, which may constrain trajectories of adaptive evolution (Lande 1979; Via and Lande 1985, 1987). Combining estimates of **G** with estimates of natural selection on those traits (β or selection gradients; see Kingsolver et al. 2001a; Stinchcombe et al. 2002; Hereford et al. 2004) allows not only quantitative prediction of the response to selection of the traits in question, but also of whether the genetic covariances affect the rate of adaptation (see e.g., Hansen and Houle 2008; Agrawal and Stinchcombe 2009; Kirkpatrick 2009).

Genetic correlations can exist across environments as well (Falconer 1952). If there is a strong, positive genetic covariance between the same trait in multiple environments (suggesting that the same loci contribute to the trait in each environment) and natural selection is acting in opposite directions on the trait, the across-environment genetic covariance can act as a constraint (Via and Lande 1985, 1987). For this reason, the role of across-environment genetic covariances has been investigated heavily in theoretical and empirical studies of phenotypic plasticity (e.g., Fry 1990, 1992; Scheiner et al. 1991; Gomulkiewicz and Kirkpatrick 1992; Scheiner 1993; Andersson and Shaw 1994; Pigliucci et al. 1995; Via et al. 1995; Pigliucci 1996; Grill et al. 1997; Donohue and Schmitt 1999; Donohue et al. 2000b; Czesak et al. 2006). Likewise, the appropriate analytical methods for estimating across-environment genetic correlations and testing their statistical significance has been intensely investigated (e.g., Rausher 1984; Via 1984; Shaw 1987; Fry 1992; Windig 1997; Astles et al. 2006). Despite the considerable attention paid to across-environment genetic correlations, however, progress in understanding their role as evolutionary constraints has been hindered by three factors: (1) We have comparatively few estimates of across-environment genetic correlations that span multiple environments, rather than just a few, (2) estimates of across-environment genetic correlations are rarely paired with data on the natural frequency of those environments, and (3) the potential consequences of across-environment genetic variances and covariances are rarely evaluated quantitatively, rather than qualitatively.

The need to estimate across-environment genetic correlations across more than two or three environments stems from the fact that most organisms inhabit a range of environments. For example, many plant species inhabit environments that exhibit substantial (and frequently, continuous) variation in intraspecific and interspecific density, light availability, soil moisture and nutrient availability, in addition to other abiotic and biotic variables. Estimating across-environment genetic correlations that encompass this range of environments will provide a more comprehensive estimate of the genetic architecture of species inhabiting multiple environments. These estimates, however, will be most useful when paired with data on the naturally occurring frequency of the environments. Theoretical work has shown that the evolution of phenotypic plasticity can depend critically on the frequency of environments, but usually focusing on the effects of selection within those environments (i.e., the frequency of selective environments: Via and Lande 1985, 1987; Gomulkiewicz and Kirkpatrick 1992); empirically measuring the natural frequency of environments is quite challenging, and examples are rare (but see Weis and Gorman 1990; Kingsolver et al. 2001b; Arnold and Peterson 2002; Huber et al. 2004). The relevance of the frequency of environments to across-environment genetic correlations is clear: if a given environment is rare, across-environment genetic correlations involving it might be less likely to be important in constraining (or facilitating) the evolution of traits expressed in those environments.

A key element to assessing the role of both across-environment genetic correlations and the frequency of selective environments to the evolution of quantitative traits is dispersal. For example, if an environment is common in nature but organisms rarely disperse there, that environment is expected to contribute relatively little to the overall evolutionary trajectory of the traits in question. For organisms that experience coarse-grained environments (sensu Levins 1968), simple predictions from theory suggest that limited dispersal should promote local adaptation or specialization of ecotypes (e.g., Via and Lande 1985), whereas greater dispersal has been implicated in the evolution of plasticity (e.g., Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992; Sultan and Spencer 2002). Empirically, one approach to evaluating the role of dispersal in the context of the frequency of selective environments would be to define environments or patches based on the presence of the organism (implying that they or their ancestors dispersed there) and measure gene flow between extant habitat patches. Molecular and allozyme marker surveys typically reveal extensive gene flow between natural populations (see e.g., Hamrick et al. 1995). Moreover, it is important to note that genetic differentiation and local adaptation, dispersal, and phenotypic plasticity are not mutually exclusive evolutionary forces and

outcomes: populations can show differentiation and local adaptation for traits and their plasticities (Dudley and Schmitt 1995; Donohue et al. 2000a) even in the face of high levels of gene flow (von Wettberg et al. 2008), provided that selection is strong.

The growth response of plants to variable density and light availability represents a promising suite of traits to estimate across-environment genetic correlations and the frequency of environments for several reasons. First, many plant species are commonly found across a range of light environments, ranging from full sun to shady habitats. As photosynthetically active radiation is necessary for carbon fixation and plant growth, changes in the light environment have the potential to affect a suite of growth-related traits simultaneously (e.g., Schmitt et al. 1986; Schmitt 1993; Dorn et al. 2000). Second, intraspecific density frequently varies widely within and across plant populations (Harper 1977; Linhart and Grant 1996; Donohue et al. 2000a,b). The presence of neighboring plants alters the ratio of R to FR light (600–690 nm and 690–800 nm, respectively) due to the absorption of red light by chlorophyll (Holmes and Smith 1977). Changes in the R:FR ratio of light transmitted through foliage or reflected from neighbors initiate a suite of phytochrome-mediated plastic responses in plants known as the "shade-avoidance syndrome," including stem and internode elongation, decreased branch production, and accelerated reproduction (Smith 1982; Ballaré et al. 1990; Schmitt and Wulff 1993; Ballaré 1999; Weinig 2000a; Schmitt et al. 2003). As such, changes in light availability have the capacity to affect biomass growth, whereas changes in density (which alter the R:FR ratio) have the capacity to affect elongation growth through the shade-avoidance pathway. Finally, intraspecific density of plants is a trait that can be readily scored in multiple habitats in the field in a given year and across several years, providing estimates of the frequency of different density environments.

One advantage to studying genetic correlations involved in shade-avoidance responses is that the fitness benefits and costs of shade avoidance have been well investigated in a variety of plant species, providing rich details on its adaptive value (Dudley and Schmitt 1995, 1996; Donohue and Schmitt 1999; Dorn et al. 2000; Donohue et al. 2000a,b, 2001; Weinig 2000a,b; Huber et al. 2004; von Wettberg and Schmitt 2005). The plastic response to density is adaptive (i.e., elongation is favored over suppression in high density, and vice versa under lower density, Dudley and Schmitt 1996), and may evolve independently several times within a species (von Wettberg et al. 2008). However, the elongation response incurs costs (Maliakal et al. 1999; Weinig and Delph 2001; Huber et al. 2004) and can be subject to heterogeneous selection depending on ecological context (Weinig 2000b; Huber et al. 2004; McGoey and Stinchcombe 2009).

Here, we examine across-environment genetic correlations in shoot elongation rate across a range of density and light environments, and pair that with field surveys of intraspecific density in multiple years in two habitats that differ in light availability. Using a greenhouse experiment, we examined shoot elongation in 49 recombinant inbred lines of *Impatiens capensis* grown in five density environments (spanning more than an order of magnitude of variation in intraspecific density) in both full sun and neutral shade conditions. We could thus examine genetic variation in response to different competitive environments under high and low light resource availability. We used Bayesian curve fitting to model individual intrinsic growth rates. To examine across-environment genetic correlations, we developed a mixed-model principal components analysis (PCA) approach that accounts for within-RIL and model fitting variation to estimate across-environment genetic covariances. In particular, we sought to answer the following questions: (1) What is the genetic correlation in intrinsic growth rates across density environments in each of the light treatments? (2) How frequently do these density environments occur in nature? (3) Do correlations that would likely lead to constraints in the evolution of intrinsic growth rate involve rare or common environments?, and (4) How do across-environment correlations and the frequency of selective environments interact to determine the evolutionary response of intrinsic growth rates?

Materials and Methods

STUDY SPECIES AND QUANTITATIVE GENETIC DESIGN

Jewelweed (*I. capensis* Meerb. (Balsaminaceae)) is a North American herbaceous annual, commonly found in moist forest understories and open wetlands. *Impatiens capensis* has a mixed mating system, producing exclusively self-fertilizing cleistogamous flowers, and chasmogamous flowers that outcross 30–70% of the time (Waller and Knight 1989). Furthermore, the production of cleistogamous and chasmogamous flowers is frequently environmentally and density dependent, with as few as 1–2% of individuals producing chasmogamous flowers in some situations (Schmitt et al. 1987). Natural populations of *I. capensis* experience tremendous variation in the amount of light and intraspecific density, with woodland populations typically occurring in lower light, lower density environments, whereas open populations are characterized by more light and higher densities. In early spring, plants typically germinate and emerge in high light conditions prior to overhead canopy closure (if one exists). In both open and woodland populations, *Impatiens* frequently forms dense monocultures where it is the dominant understory plant (Winsor 1983). Seed dispersal in *Impatiens* is autoballistic, with the majority of observed seeds landing close to the parental plant (Schmitt et al. 1985). However, similar to many other plants (Hamrick et al. 1995), genetic data indicate longer distance dispersal in *I. capensis* (von Wettberg et al. 2008), which is likely due to

secondary transport of seeds by water and flooding (E. von Wettberg, unpubl. data).

Past studies have shown that populations inhabiting "sun" and "woods" sites exhibit genetic differentiation and local adaptation to differing light and density conditions (Schmitt 1993; Dudley and Schmitt 1995; Donohue et al. 2000a,b; von Wettberg and Schmitt 2005; von Wettberg et al. 2008). To study the quantitative genetics of growth curves, we used 49 recombinant inbred lines (RILs, hereafter) that had been developed from a cross between a single inbred line from a woods population and another inbred line from a sun population; these populations have been shown to exhibit quantitative genetic differentiation and local adaptation (Dudley and Schmitt 1996; Donohue et al. 2000a,b). The F_1 of this cross was allowed to self-fertilize, and approximately 100 of the F_2s were advanced by single-seed descent for six generations to produce the RILs (see Heschel et al. 2004; Stinchcombe and Schmitt 2006 for more details).

The genetic structure of these lines is likely similar to many natural populations of *I. capensis* in which rare outcrossing events are followed by several generations of self-fertilization. In particular, von Wettberg et al. (2008) showed that gene flow between sun and woods populations (including the source populations for our parental lines), as estimated from presumably neutral markers, is on the order of one migrant per generation. Outcrossing events, either in the form of pollen movement between populations, or seed dispersal followed by outcrossing, will produce F_1 individuals. Selfing of these F_1s—for example from the exclusively selfing cleistogamous flowers—will produce recombinant F_2 individuals. Any further selfing will likely produce plants with a genetic structure akin to RILs, especially if density and environmental effects inhibit the production of chasmogamous flowers capable of outcrossing (Schmitt et al. 1987). The use of RILs also facilitates growing nearly genetically identical individuals in multiple environments, facilitating the estimation of across-environment genetic covariances.

GREENHOUSE EXPERIMENTAL DESIGN

We grew the 49 RILs in a factorial combination of density and light treatments. Prior to the experiment, seeds were gathered and cold-stratified at 4°C for approximately 4 months in 96-well plates filled with distilled water. After stratification, seeds were planted in late April into cone-tainers (Stuewe and Sons, Inc., Corvallis, OR) that had been filled with Metromix 360 (Scotts-Sierra Horticultural, Marysville, OH). Cone-tainers were randomly distributed on a bench in the Brown University greenhouse, and for the first week were kept consistently moist with top-watering to promote germination and seedling establishment; throughout the experiment, we did not use any supplemental lighting. After approximately one week of growth, replicates of each line were moved into experimental treatments.

The experimental design crossed a light treatment (ambient greenhouse light and neutral shade cloth) with manipulations of intraspecific density. We used five greenhouse benches (blocks), in which half of each bench was covered with neutral shade cloth to reduce the light reaching plants in a split-plot design. Because we wished to manipulate light resource availability independent of the R:FR cue, the shade cloth did not alter the R:FR ratio of light, but reduced photosynthetically active radiance (PAR) by 78% (mean 173 μmol $m^{-2}s^{-1}$ vs. 778 μmol $m^{-2}s^{-1}$ during midday; see Heschel et al. 2004). These light level reductions are within of the natural range of light levels in sun and shaded sites (Heschel and Hausmann 2001), and are thus ecologically relevant. By altering the spacing of cone-tainers in plastic racks, we created five intraspecific density treatments: 64, 144, 289, 625, and 1225 pl/m^2. These densities span the natural range of densities found in sun and woods sites (see below). To prevent lateral, incident light from reaching plants, and plants from different density or light treatments from interacting with each other, each density treatment was surrounded with an aluminum foil; as plants grew, we added to the aluminum foil wrappings as needed. The total starting sample size was 2450 plants (=5 benches $\times$ 2 light treatments/bench $\times$ 5 density/light treatment $\times$ 49 individuals/density treatment). The position of individual replicates within each density array was randomly assigned.

To ensure equal moisture and nutrient levels of plants in different light-density treatment combinations, once plants were in experimental treatments, we bottom-watered them daily using Ebb-Flo Flood irrigation. Plants were fertilized biweekly with Peters 20-20-20 (N-P-K, Scotts-Sierra Horticultural Products, Marysville, OH).

To characterize genetic variation in growth trajectories under the 10 light and density treatment combinations, we measured the height of every plant to the tip of the apical meristem six times over the growing season (18, 26, 33, 39, 47, 57 days). All plants were measured to the nearest millimeter by two investigators (JRS and MSH), and data were entered directly into a handheld PDA (see below). At 57 days, we harvested plants and recorded a final height estimate. The duration of our experiment approximates a short growing season for *I. capensis*, which can occur because of unpredictable early season droughts (Heschel and Riginos 2005). Survivorship over the course the experiment was high (96.8%).

FIELD SURVEYS OF INTRASPECIFIC DENSITY

To estimate the frequency of the density environments used in this experiment, we surveyed the density of *Impatiens* seedlings at the clearing and woodland sites described by Dudley and Schmitt (1996), and Donohue et al. (2000a,b, 2001) in locations outside of their experimental plots. We estimated density by counting the number of emerging *Impatiens* seedlings in a series of plots located on transects in each site. Transect lines were separated

by 10 m within sites, and sampling points were located every 5 m on transects. At each sampling point, we counted the number of *Impatiens* in four 0.5 m × 0.5 m quadrants, taking the transect point as the origin, and then summed these counts to estimate *Impatiens*/m^2. We estimated density in the clearing site on four transect lines, for a total of 15 estimates of *Impatiens*/m^2, and in the woodland site on five transect lines, for a total of 25 estimates of *Impatiens*/m^2. These surveys took place yearly between 1996 and 2002, in the early spring after emergence and before self-thinning.

DATA ANALYSIS

Data proofing

Because vertical growth in *I. capensis* is unidirectional (i.e., living plants do not shrink), instances in which successive height measurements show a decline indicate either measurement imprecision or errors in data entry. We identified 57 such cases (≈2.3% of plants, ≈ 0.4% of height measurements); 39 of these were outright entry errors and converted to missing data, whereas 18 were left unaltered because the apparent magnitude of shrinkage was small (mean ± SE = 0.38 ± .07 cm).

Modeling impatiens growth

We modeled *Impatiens* vertical growth curves for individuals using the familiar logistic growth equation, $dh/dt = rh(1 - h/K)$, where dh/dt is the rate of height increase, r is the intrinsic rate of growth, K is the asymptote of height growth, and h is the initial height. We chose the logistic equation for three reasons: (1) In some of our higher densities, we noticed a slowed rate of growth of individual plants, suggesting that they had or were nearing an asymptote, (2) for plants that had not yet leveled off in growth, the logistic equation has the advantage of showing exponential growth in its first phase (i.e., before height reaches half of its eventual asymptote), and (3) the parameters are biologically interpretable—K indicates the asymptotic height of a plant, whereas r describes the intrinsic rate of increase of plants. The logistic equation has been widely used in models of plant growth (e.g., Abrami 1972; Weiner and Thomas 1986; Tsoularis 2001). Using the logistic equation thus allows us to fit a single growth model to all of our data and account for the fact that growth for some plants had started to level off, whereas others had not. However, because fitted asymptote values were frequently beyond the range of observed data (especially in the three lowest density treatments), we focus all of analyses on the fitted values of the maximum rate of height increase (r).

The height of individual i of RIL g at time t, $h_{g(i)}(t)$, was modeled as a logistic curve with measurement error, using the integral form of the logistic equation

$$h_{g(i)}(t) = \frac{K_{g(i)}}{1 + \left(\frac{K_{g(i)}}{H_{g(i)}} - 1\right)\exp(-r_{g(i)}t)} + \varepsilon_{g(i)}, t, \qquad (1)$$

where $H_{g(i)}$ is the initial height, $K_{g(i)}$ is the fitted asymptotic height, and $r_{g(i)}$ is the intrinsic rate of growth of replicate i of RIL g. For simplicity, from here onwards we refer to r as "intrinsic growth rate." The measurement errors $\varepsilon_{g,t}$ are assumed to be independent and normally distributed [$\sim N(0, \sigma_i^2)$]. The three individual parameters ($N_{g(i)}$, $r_{g(i)}$, $K_{g(i)}$) were assumed to be random, to covary, and to have a within RIL variance and between RIL variance. Note that in this model, σ_i^2 represents the measurement error only—within RIL variability is captured by the variability of the parameters $K_{g(i)}$, $N_{g(i)}$, and $r_{g(i)}$. Equation (1) was fit to all individual plants for which we had complete height data at all six time points (N = 1968)—thus our estimate of r encompasses the entire growth trajectory of individuals, with all height measurements made at the same time. We used our initial height estimate (18 days) for $H_{g(i)}$; note that if one used height at time zero (i.e., zero cm tall at zero days), equation (1) would be undefined.

For each individual in each environment, we used a Bayesian approach to fit the model described by equation (1). A Metropolis–Hastings algorithm was run using a flat uniform prior on the three parameters $K_{g(i)}$, $N_{g(i)}$, and $r_{g(i)}$. The measurement error variance, σ^2, was fixed to 1 cm (i.e., that 95% of our measurements were within ±2 cm of the true measurement; as described above, known measurement errors were = 0.38 ± 0.14 cm [mean ± 95% c.l.]). For each individual, we calculated the posterior means of these three parameters (K, N, r) from a posterior sample size of 500, after a burn-in period of 5000 iterations. To evaluate the sensitivity of our results to our assumptions about the error variance, we reran the Bayesian models with the error variance fixed to 2.0 cm. Because the results between model fits were highly similar and data we collected suggest that 1.0 cm is an empirically reasonable estimate of the error variance, we present results from analyses assuming $\sigma^2 = 1$. Model fits were performed in Matlab.

For the logistic growth equation, the rate of height increase per unit time (dh/dt) is maximized at $K/2$. To determine the age at which plants in a particular treatment showed maximum growth, we evaluated equation (1) using the treatment means for r, K, and H, with t ranging from zero to 57 days. We then recorded the time (in days) at which height was the closest to $K/2$. This metric provides an estimate of when during the course of the experiment the rate of height increase (dh/dt) was maximized in each light and density treatment.

Treatment effects on intrinsic growth rate

To estimate the effects of light availability and intraspecific density on intrinsic growth rate, we used a mixed model ANOVA that accounted for the split-plot design of our experiment. For this mixed model, individual values of r were the response variables, whereas light, density, light × density, and greenhouse bench were fixed effects. Random effects included line, line × light, line × density, line × light × density, line × bench, light × bench,

and light × bench × density interactions. We used the Kenward–Roger adjustment for denominator degrees of freedom for F-tests. The random effects terms involving bench were included because of the split-plot nature of the light treatment, which were applied half a bench at a time.

Genetic correlations across environments

To estimate genetic correlations in intrinsic growth rate across density environments, we used a two-step procedure. In the first step, a mixed model was used to fit the genetic variance–covariance matrix for the 10 different environmental conditions after adjusting for within RIL variation and modeling noise. In the second step, PCA was used on the estimated covariance matrix to identify directions of greatest genetic variability. In brief, our goal was to distinguish between three sources of variation in plant intrinsic growth rates: (1) Variation due to model fitting, (2) within-RIL variation, and (3) between RIL variation. For analysis of genetic correlations in intrinsic growth rates across environments, only the third source of variation was of interest. As such, we estimated the genetic variance–covariance matrix of intrinsic growth rates across densities in a manner that reflected between RIL variation only, and not the within RIL or model fitting variation.

Using equation (1) above, we obtained for each individual i of RIL g (i.e., $g(i)$), in a specific density and light environment, dl, a posterior sample of s of the possible values of the parameter. We then assumed that each posterior value of r from model fit s, for replicate i of RIL g in environment dl (i.e., $r_{g(i)}$, s, dl) came from the following mixed effect model:

$$r_{g(i),s,dl} = p_{dl} + bg_{g,dl} + wg_{g(i),dl} + e_{g(i),s,dl}, \qquad (2)$$

where p_{dl} is the fixed population mean for r across all density and light environments, $bg_{g,dl}$ is the effect of RIL g in a given density-light dl environment and $wg_{g(i),dl}$ is the effect of individual $g(i)$ in environment dl on r. Finally, $e_{g(i),s,dl}$ is the individual posterior sampling error in r for the individual in question in a given density light environment. In words, equation (2) is equivalent to: Intrinsic growth rate = Population mean + RIL effect + Within RIL effect + Model fitting effect. In equation (2), the posterior sample size (s) is 500, the density-light index, dl, ranges from 1 to 10 to reflect the 10 experimental conditions, the number of RILs, g, ranges from 1 to 49, and the number of individuals, i, indicates the number of replicates of RIL g in environmental condition dl.

An approach to estimating the genetic variance in intrinsic growth rate, r, and its genetic covariances across environments can be seen from rewriting equation (2) in vector form

$$\mathbf{r}_{g(i),s} = \mathbf{p} + \mathbf{bg}_g + \mathbf{wg}_{g(i)} + \mathbf{e}_{g(i),s}, \qquad (3)$$

where $\mathbf{r}_{g(i),s}$ is the vector of observed intrinsic growth rate parameters, r, $\mathbf{p}$ is a fixed effect vector describing the mean intrinsic growth rate of the population, and vectors $\mathbf{bg}_g$, $\mathbf{wg}_{g(i)}$, $\mathbf{e}_{g(i),s}$ are random effects indicating RIL effect, individual effect, and modeling error in intrinsic growth rates, respectively. Note that the between RIL variances and covariances of $\mathbf{bg}_g$ form a matrix, in which the diagonal elements reflect the between RIL variances, and the off-diagonal elements reflect the genetic covariance in the intrinsic growth rate parameter, r, across experimental environments. The genetic covariance across environments is due to the use of the same RILs in each experimental environment. In contrast, the within RIL variances and covariances of $\mathbf{wg}_{g(i),}$ form a diagonal matrix (i.e., with zeroes for covariances) because the measurements for the different RILs in different environmental conditions were made on different individuals, so there is no within-RIL covariance in intrinsic growth rate parameters. Likewise, because model fits were done on different individuals separately, the variances and covariances of the modeling error $\mathbf{e}_{g(i),s}$ also form a diagonal matrix.

Using the algebra outlined in Appendix S1, equation (3) can be solved to obtain an unbiased estimate of the variance of $\mathbf{bg}_g$ that is corrected for unequal sample sizes. The resulting 10 × 10 matrix describes genetic variation and covariation of intrinsic growth rates within and across density environments for both light treatments. To analyze this matrix, we used three PCA models: (1) A PCA of the first five rows and columns, which describes genetic variation and covariation in intrinsic growth rates across density in the shade treatment, (2) a PCA of the rows and columns 6–10, which describes genetic variation and covariation in intrinsic growth rates across density in the sun treatment, and (3) a PCA of the entire 10 × 10 matrix, which describes genetic variation and covariation in intrinsic growth rates across all density and light environments simultaneously.

Matrix comparisons

We used Cheverud's random skewers method (Cheverud 1996; Cheverud and Marroig 2007) to quantitatively compare the across-density genetic variance–covariance matrices for the two light environments. We made comparisons across light environments because (1) light levels are qualitatively different between habitats; (2), the genetic covariance structure of shade-avoidance traits can vary across sites with different light environments (Donohue et al. 2000b); and (3) we assume that the majority of seed dispersal will be within habitats that naturally experience a range of density environments (see below). In the random skewers approach, a pair of variance–covariance matrices are multiplied by a set of random vectors (applied to each matrix), and the mean vector correlation of the response vectors is calculated. Hypothesis testing is performed by comparing the mean vector correlation to the distribution obtained from 10,000 pairs of random vectors (Revell 2007).

Surveys of impatiens density

We used our surveys of naturally occurring *Impatiens* density variation to estimate the frequency of the density environments used in the greenhouse experiment. For the clearing site, we had 105 separate estimates of density, whereas for the woodland site we had 175 separate estimates of density (combining across transect points and years). Temporal consistency of densities in our plots was high—density in a plot in one year was highly correlated with density in that plot in the next year (median Pearson $r = 0.71$ for sun; 0.63 for woods for six successive year to year comparisons; $N = 15$ and 25 plots, respectively). For information on the spatial variation in density within years, see Schmitt et al. (2003).

To estimate the frequency of our experimental treatment densities, we took advantage of the fact that our experimental density treatments are an approximate doubling series (64, 144, 289, 625, 1225 pl/m^2). As such, on a $\log_2$ scale, they are each separated by approximately the same distance (on a $\log_2$ scale, the density treatments were: 6, 7.17, 8.175, 9.288, 10.25). Accordingly, we $\log_2$ transformed our natural density estimates, and created frequency histograms with each "bin" centered on the $\log_2$-transformed values of our experimental density treatments; boundaries between bins were half-way between adjacent values. This approach has two advantages: all of the bins for creating the frequency distribution are of similar size ($\approx$1 unit on a $\log_2$ scale), and it takes advantage of the natural spacing of our experimental units.

Quantitative implications for microevolution

We combined our estimates of the across environment genetic variance–covariance matrix and the frequency of selective environments with hypothetical estimates of natural selection (β) to predict the likely evolutionary response of intrinsic growth rate. We predicted the likely evolutionary response under two scenarios, either equal frequency of selective environments or the estimated frequency of selective environments, using the equation $\Delta\bar{\mathbf{z}} = \mathbf{G}_a\boldsymbol{\beta}_s$. Here, $\Delta\bar{\mathbf{z}}$ is the vector describing the change in the mean intrinsic growth rate in each environment, $\mathbf{G}_a$ is the across-density genetic variance–covariance matrix (i.e., within-density genetic variances on the diagonal, across-density genetic covariances on the off-diagonals), and $\boldsymbol{\beta}_s = \beta f$, where β is the selection gradient for intrinsic growth rate in that environment and f is the frequency of selective environments ranging from zero to 1. We assume that population regulation is local within density environments and leads to a soft-selection model (hence subscript *s* for $\boldsymbol{\beta}$; cf. Gomulkiewicz and Kirkpatrick 1992; Kelley et al. 2005), which may be appropriate for *Impatiens* given past findings of primarily local seed dispersal and microgeographic variation for life history and morphological traits (Schmitt et al. 1985; Argyres and Schmitt 1991). We further assume that any departures from $\Delta\bar{\mathbf{z}} = \mathbf{G}\boldsymbol{\beta}$ caused by *Impatiens*' mixed mating system (as opposed to random mating) are equal across all density environments. We explored scenarios that altered β and f in 2×2 fashion: experimental or empirical estimates of $f \times$ constant or density-dependent β.

For our greenhouse experiment, where each density environment was equally frequent, $f = 0.2$; in this analysis, we explicitly assume that populations will experience all density environments within a habitat (i.e., unlimited and equal dispersal to different density micro-environments or plots within a sun or woods population). To use empirically estimated frequencies of density environments, we pooled all density measurements below 64 pl/m^2 (to the left of the dashed line in Fig. 2) with the lowest density category, ensuring that our frequencies summed to 1. In our use of the natural density frequencies, we assume that populations will experience (and be able to disperse to) the density environments of that habitat in proportion to their actual frequency in nature.

Because of the short duration of our greenhouse experiment, we could not obtain reliable estimates of β, and as such use hypothetical values, following the approach of Steven et al. (2007; also see and Calsbeek and Goodnight (2009)). Although the actual magnitude of the predicted responses to selection will depend on the assumed β, differences between predictions made using the estimated frequency of our density environments and the assumption of equal frequency illustrate the importance of the frequency of selective environments. Similarly, using hypothetical values of β that are constant across all density environments allows an assessment of the relative contributions of differences in the genetic variance–covariance matrices. We considered two scenarios: (1) Constant selection, with mean-standardized β equal to 0.1 for intrinsic growth rate, and (2) a gradual shift of mean-standardized β from -0.1 at the lowest density to $+0.1$ at highest density, with values of -0.05, 0, and 0.05 for the intermediate densities. In the latter scenario, we assumed that selection was acting against rapid intrinsic elongation rates at low densities and in favor of rapid intrinsic elongation rates at high densities, consistent with expectation that overtopping neighbors has a fitness advantage at high density, but that at low density, the costs outweigh the benefits (e.g., Dudley and Schmitt 1996). We used mean-standardized selection gradients because of their ease of interpretation (i.e., a mean-standardized β of 0.1 indicates selection on the trait 10% as strong as selection on fitness; Hereford et al. 2004; Hansen and Houle 2008) and their utility with traits that range from zero to 1, like *r*, which have bounded standard deviations (Stinchcombe 2005).

Results

GENERAL PATTERNS OF GROWTH

Curves describing the growth trajectories as a function of time and density for 49 RILs in the 10 experimental conditions are presented in Figure 1. In general, plants elongated earlier and more

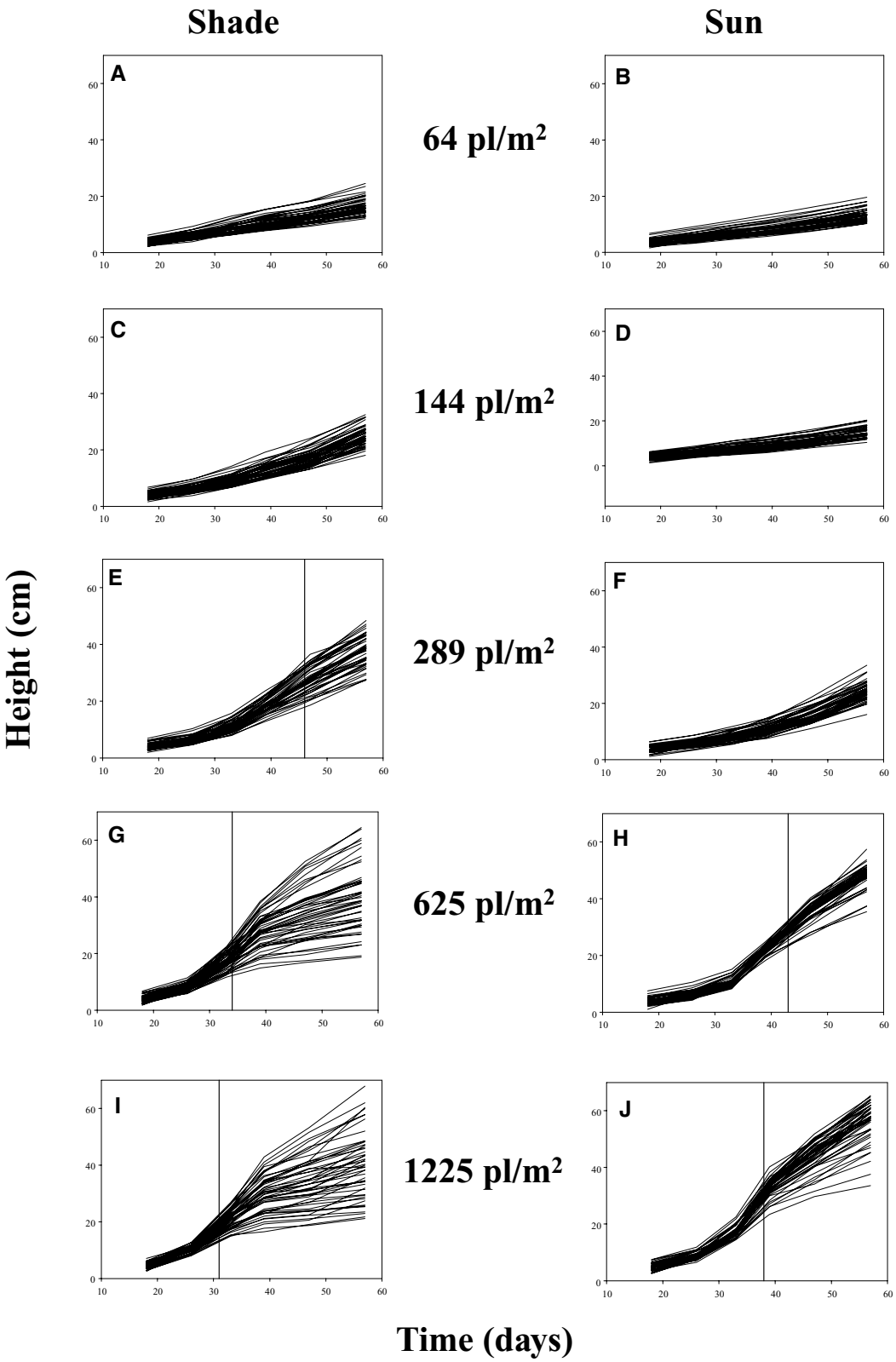

Figure 1. Growth curves for *Impatiens capensis* RILs in the 10 density-light environments. Panels in the left of the column portray the shade treatment (A, C, E, G, I), with density increasing from the top of the page to the bottom. Panels in right column portray the sun treatment (B, D, F, H, J). In several panels, the reaction norms are on top of each other and are not individually distinguishable. The vertical bar shows the time at which the rate of height increase (*dh/dt*) was maximized, if the estimated maximum was within the observed time range of the data.

Table 1. ANOVA for the effects of light and density on maximal intrinsic growth rate (*r*).

Source	df	*F*	*P*
Light treatment	1, 8.11	72.93	<0.0001
Density treatment	4, 38.9	329.88	<0.0001
Light×Density	4, 32.7	5.35	0.0020

rapidly at higher densities, and this effect was greater in the neutral shade treatment, resulting in taller plants at harvest. However, elongation also leveled off earlier at higher densities, especially under neutral shade, suggesting that the shade-avoidance response was resource-limited under those conditions.

Inspection of the growth curves reveals appreciable variation in the timing of maximum growth rate—that is, when during the experiment *dh/dt* was maximized. In general, the highest densities experienced maximum growth rate earliest in the experiment, and this was slightly more pronounced in the shade treatment. For the two lowest densities for the shade treatment and the three lowest densities in the sun, the maximum rate of growth is modeled to have occurred after the end of the experiment. These data indicate that the growth curves in panels A, C, B, D, and F had yet to reach an inflection point, after which height would asymptote and *dh/dt* would decline.

LIGHT AND DENSITY EFFECTS ON *r*

Mixed model ANOVA revealed that both light, density, and the light × density interaction significantly affected the growth rate of the RILs (Table 1). The ANOVA suggests that the treatments were effective in creating different growth trajectories in the RILs. The individual least-square means of *r* (Table 2) suggest that elongation rate increased monotonically with density in both sun and shade, as expected for typical shade-avoidance responses. Elongation rate was always higher in the shade than in the sun treatment for a given density, suggesting that shade-avoidance responses were enhanced under low light conditions.

Table 2. Least square means for *r* (±1 SE), and genetic variance estimates in the 10 light-density treatments.

Density	mean *r*		Genetic variance	
	Shade	Sun	Shade	Sun
64	0.0608 (0.004)	0.0469 (0.004)	0.0261	0.0116
144	0.0659 (0.004)	0.0469 (0.004)	0.0122	0.0065
289	0.1060 (0.004)	0.0623 (0.004)	0.0243	0.0120
625	0.1650 (0.004)	0.1306 (0.004)	0.0374	0.0208
1225	0.1688 (0.004)	0.14442 (0.004)	0.0561	0.0355

Table 3. Across-density genetic correlations for intrinsic growth rate in the two light environments. Above the diagonal: Shade treatment; Below the diagonal: Sun treatment.

Density (pl/m^2)	64	144	289	625	1225
64		0.30	0.26	0.13	0.10
144	0.32		0.08	0.59	0.55
289	0.54	0.35		0.11	0.02
625	0.48	0.36	0.61		0.86
1225	−0.31	−0.24	−0.32	−0.19	

GENETIC VARIATION WITHIN TREATMENTS

Both the spread of the curves in Figure 1 and the genetic variance estimates for *r* (Table 2) suggest significant genetic variation in growth that differs between experimental environments. In general, increasing density increased the expression of genetic variation in intrinsic growth rates in both sun and shade treatments, although the effect was more pronounced in the shade. For a given density, genetic variance estimates were 1.5- to 2-fold higher in the shade than the sun.

MIXED-MODEL PCA OF *r* ACROSS DENSITY ENVIRONMENTS

In the shade environment, intrinsic growth rates were largely positively correlated across all densities (Table 3). Across-environment genetic correlations ranged from weak ($r_{gae} = 0.016$ between densities 289 and 1225) to quite strong ($r_{gae} = 0.86$, between densities 625 and 1225; Table 3). In the shade, most low correlations involved density 289. Although low correlations can be driven by low amounts of genetic variance in one treatment, the genetic variance expressed in this environment is comparable to that expressed in density 64 and 625, and across-density correlations in *r* with these treatments were also weak ($r_{gae} = 0.13$, 0.11, respectively).

In the shade, the largest contribution to PC1 of intrinsic growth rates came from densities 625 and 1225 (Table 4), with less of a contribution from the three lowest density environments (Table 4), suggesting that this PC represented an index of shade-avoidance responses to high density. PC2, in contrast, was loaded on growth at the lowest three densities. Cumulatively, the first three PCs explained 91.7% of the genetic variation in intrinsic growth rates within and across density environments.

In the sun environment, PC1 accounted for 52% of the variation, and the first three PCs accounted for 88% of the genetic variation in intrinsic growth rates within and across density environments. However, in contrast to the shade, we detected negative covariances between intrinsic growth rates in different density environments. Specifically, growth at the highest density (1225 pl/m^2) was always negatively correlated with growth in the other four densities ($r_{gae} = -0.19$ to -0.32; Table 3). These

Table 4. Principal components analysis of intrinsic growth rates across density environments. Entries are the elements of the eigenvectors. PCA was performed separately for the shade and sun treatments.

Density	Shade					Sun				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
64	0.1034	0.7359	−0.6337	0.1802	−0.1169	−0.3177	0.2455	0.7884	0.0323	−0.4650
144	0.2378	0.1114	−0.1546	−0.7942	0.5257	−0.1681	0.1161	0.0612	0.9147	0.3433
289	0.0514	0.6472	0.7492	0.0425	0.1242	−0.3545	0.3185	0.2174	−0.4020	0.7510
625	0.6012	−0.0210	0.1149	−0.3329	−0.7216	−0.4301	0.6371	−0.5570	−0.0046	−0.3144
1225	0.7541	−0.1633	−0.0070	0.4803	0.4170	0.7485	0.6472	0.1313	0.0261	0.0547
Eigenvalues	0.0925	0.0316	0.0191	0.0073	0.0057	0.0452	0.0240	0.0073	0.0051	0.0049
% Variance explained	59.22	20.25	12.23	4.68	3.62	52.29	27.73	8.4	5.92	5.66

negative covariances were reflected in the PC analysis, in which the loadings for density 1225 and densities 64–625 had opposing signs for PC1 (Table 4). The concordance between the loadings of PC1 and the individual elements of the variance covariance matrix indicates that this negative covariance contributes appreciably to the multivariate pattern of genetic variation and covariation in intrinsic growth rates.

Analysis of the full 10 × 10 matrix that described genetic variation and covariation in intrinsic growth rates within and across all experimental environments reveals a similar pattern to the separate analyses of each light environment (Table S1). PC1 accounted for 51% of the genetic variation, and had loadings of the same sign for all experimental environments except the highest density in the sun. These results have two implications. First, the contrasting loadings on PC1 for intrinsic growth rate in the highest density in the sun treatment with all other environments (i.e., all densities in the shade and densities 64–625 in the sun; Supplemental Table 1) indicate a pervasive negative across-environment genetic covariance in intrinsic growth rates. In particular, intrinsic growth rates in the highest density environment in the sun are negatively correlated with intrinsic growth rates in other density environments in the shade and sun. Second, the generally positive covariances in intrinsic growth rate across both density and light environments (indicated by loadings of the same sign for all densities but 1225 in the sun) suggest that natural selection on growth rate in one density and light environment will likely lead correlated responses in other light and density environments. We also failed to detect any evidence of a "shade tolerance PC," that would be indicated by a PC with opposite loadings in sun and shade across all densities.

MATRIX COMPARISONS

Analysis of the two across-density genetic variance–covariance matrices by random skewers showed that the response vectors generated by multiplying the sun and shade matrices by random vectors were similar (vector correlation = 0.70), although this correlation coefficient was only marginally significant ($P = 0.06$). The significance of the vector correlation obtained was insensitive to the number of random vectors used—we found $P = 0.06$–0.07 for $N = 1{,}000$; 10,000; and 100,000 skewers.

NATURAL FREQUENCY OF IMPATIENS DENSITY ENVIRONMENTS

Frequency distributions of intraspecific density from natural populations are presented in Figure 2. In general, the woodland population contains more sites in which density is low to moderate (e.g., 16–144 pl/m^2), while the clearing population tends to contain more sites in which density is substantially higher (144–625 pl/m^2). For the experimental density treatments used in our greenhouse study, 64 pl/m^2 occurs less frequently in the clearing population, ≈10%, compared to ≈25% for the woodland population. The second lowest experimental density, 144 pl/m^2 was the modal density for both populations (35% and 31% for clearing and woodland, respectively). In contrast, the three highest experimental densities (289, 625, 1225 pl/m^2) were found much more commonly at the clearing site versus the woodland site (18% vs. ≈7%, 14% vs. 2%, 3% vs. 0%). The absence of the highest density in the woodland site is striking, as appreciable genetic variation for growth in this environment was detected in the experiment. These data suggest that cryptic genetic variation exists that may facilitate adaptation to this novel (and likely rare) environment.

QUANTITATIVE IMPLICATIONS FOR MICROEVOLUTION

The predicted evolutionary responses for the four selective scenarios we calculated are portrayed in Figure 3A–D. Under positive directional selection in each environment, a positive response to selection is observed in all density environments, in both sun and shade (Fig. 3A). The larger evolutionary responses in the shade treatment likely reflect the larger elements of $\mathbf{G}_a$. For the

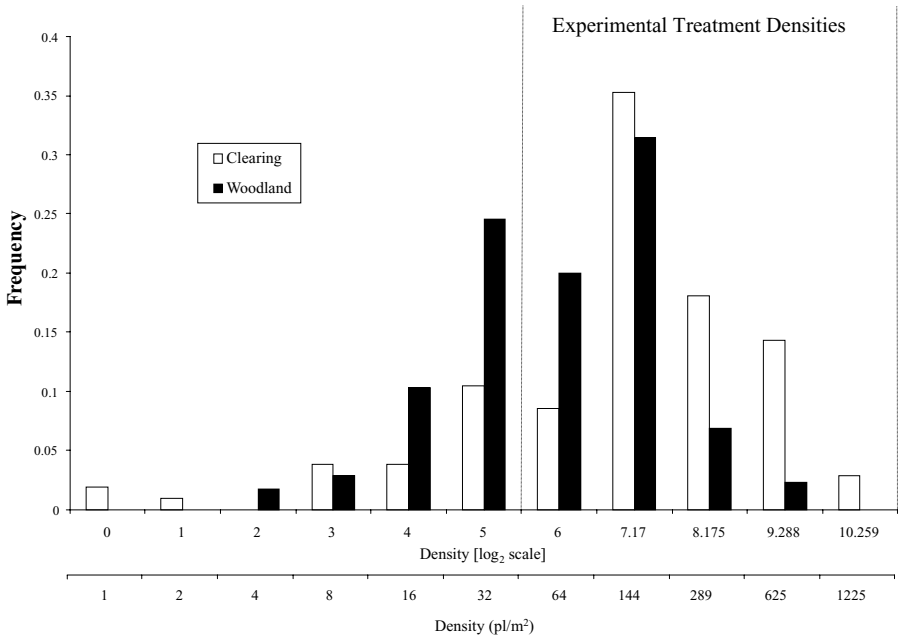

Figure 2. Naturally occurring estimates of the frequency of density environments in *Impatiens* populations. The histogram was created by transforming density to a $\log_2$ scale, to account for the fact that the experimental treatment densities (between the dotted lines) are in an approximate doubling series. Bins are centered on the values shown, with boundaries half-way between adjacent categories.

sun treatment, these findings indicate that the effects of positive selection on intrinsic growth rate in the highest density have a larger contribution to the evolutionary response than selection on negatively correlated traits in other densities. However, when the natural frequency of the selective environments is accounted for, two features emerge: the likely evolutionary response in the shade becomes predominated by low-density environments and the evolutionary response in the sun at high-density becomes negative (Fig. 3B). The former trend is driven by low-density environments being more common in the shade, whereas the latter trend is driven by the relative rarity of high-density environments in the sun. Given a rare occurrence of high-density sun environments, the response to selection at 1225 pl/m^2 becomes dominated by directional selection on negatively correlated traits at other, more frequent densities.

Under a hypothesized selection regime of negative directional selection at low density transitioning to positive directional selection on intrinsic growth rate at high density, the likely evolutionary responses differ markedly, especially when density environments are equally frequent (Fig. 3C). At both the lowest and highest densities, the evolutionary response is in the direction of selection, with larger responses seen in the shade (owing to greater genetic variance in that environment). At the intermediate density (in which there was no direct selection on intrinsic growth rate), the evolutionary response was predicted to be a decrease in mean intrinsic growth rates, because intrinsic growth rate at 289 pl/m^2 showed stronger correlations with intrinsic growth rates at the two lowest, as opposed to highest densities. Similarly, the projected evolutionary response in the shade at 144 pl/m^2 and in the sun at 625 pl/m^2 are in the opposite direction of selection on the traits; again, these trends are driven by across environment genetic covariances (Fig. 3C).

Accounting for the natural frequency of these selective environments dramatically changes the picture: predicted responses to selection become negative for all density and light environments, with the exception of 1225 pl/m^2 in the sun. The reason is that the frequency of selective environments is heavily biased toward low-density environments (especially in the shade). The effects of directional selection to reduce intrinsic growth rates in common environments combined with positive across-environment genetic covariances leads to a net reduction in intrinsic growth rates.

Discussion

Understanding how across-environment genetic correlations can shape the evolution of quantitative traits for species that inhabit variable environments requires estimating these important evolutionary parameters across multiple ecological and environmental conditions. By examining many density environments across two light treatments, we found evidence not only for changes in across-density genetic variances and covariances, but also for patterns that we likely would not have detected using fewer experimental environments or treatments. Although the correlations we detected were driven by density environments that occur relatively rarely in nature, according to our field surveys, this does not imply that they are unimportant: it is possible for the

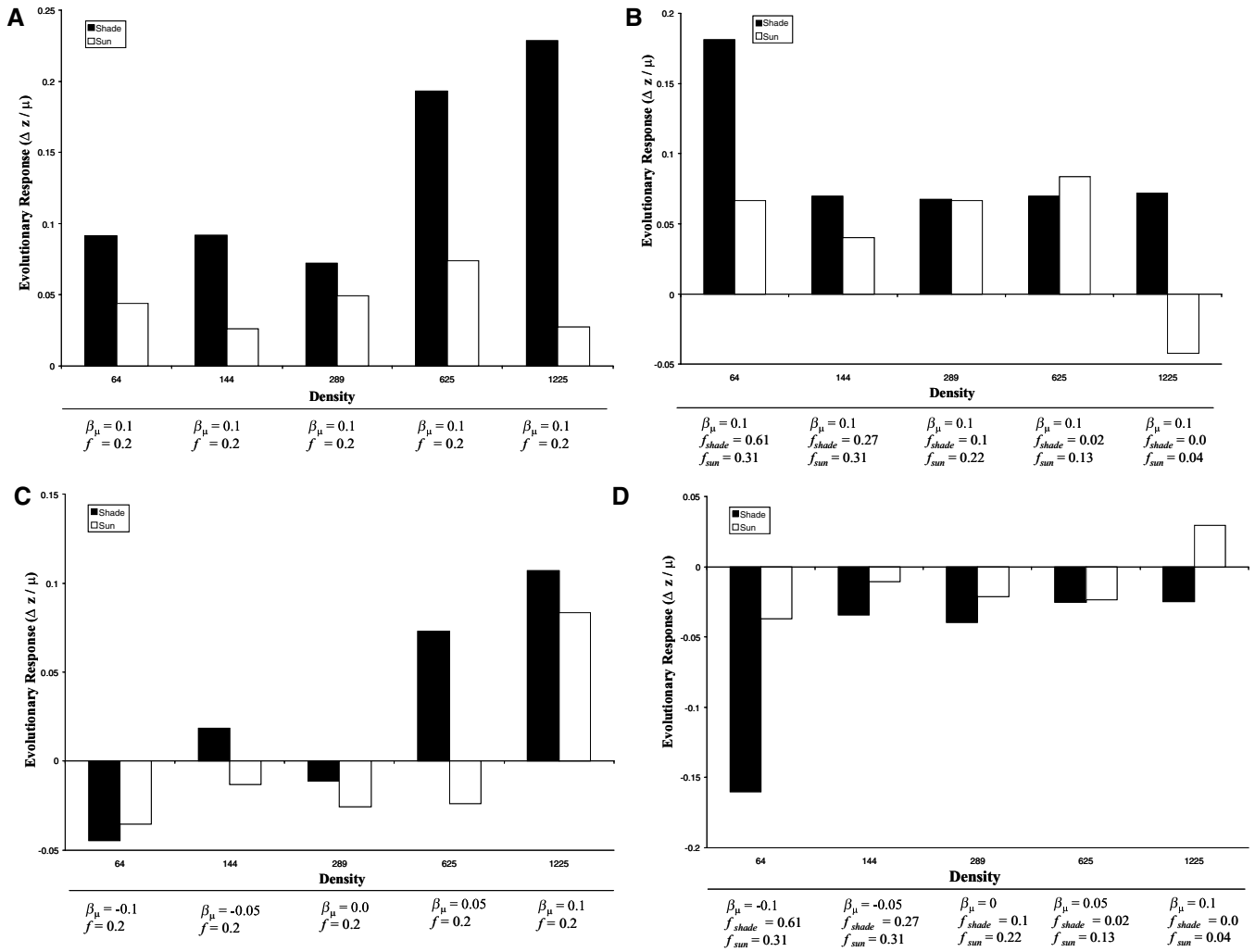

Figure 3. **Predicted responses to selection for *Impatiens* intrinsic growth rates in five density environments, given the estimated across-environment genetic variance–covariance matrices. Responses to selection are given as the evolutionary response ($\Delta\bar{z}$) divided by the preselection mean ($\mu = 0.1$). (A) Constant selection, and equal frequency of density environments (f), (B) Constant selection, natural frequency of density environments, (C) Variable selection, equal frequency of density environments, (D) Variable selection, natural frequency of density environments. Values for f and β_μ used in calculations are given below the bars.**

evolutionary response in rare environments to be reduced, or even reversed, due to effects of selection in common environments and across-environment genetic covariances. Likewise, it is possible for selection in rare environments to alter the evolutionary response in common environments. In the sections below, we first discuss the relationship between our analytical approach and other methods as well as the limitations of our experimental design, before considering the implications of our results for the evolution of these traits within and across heterogeneous environments.

RELATIONSHIP TO ALTERNATIVE MODELING APPROACHES

Several alternative modeling approaches could conceivably be implemented to examine the genetic covariance in growth rate parameters across environments. One alternative is multivariate random regression. In this framework, a regression of size against time is performed, and a deviation from the population mean regression is estimated for each genetic unit (RILs in our case), using a restricted maximum likelihood framework. This approach can be applied using simple (linear, quadratic, or cubic) regression models on the independent variable, regression on orthogonal polynomials of the independent variable (Meyer and Hill 1997; Meyer 1998), or splines (White et al. 1999). Implementing random regression in a multivariate framework allows a simultaneous estimation of the genetic variances and covariances of these slopes within and across environments—that is, do RILs that have positive deviations from the population mean regression slope (i.e., grow faster than average) in environment 1 also have them for environments 2, 3, n? This approach has been implemented successfully in agricultural contexts (with much larger sample sizes;

Veerkamp and Thompson 1999; Veerkamp et al 2001; Karacaoren et al. 2006), but in our attempts to apply it to these data the models failed to converge.

Given the failure of multivariate random regression for our data, several other alternatives are also available: Kirkpatrick and Meyer's method for direct estimation of principal components (Kirkpatrick and Meyer 2004; Meyer and Kirkpatrick 2005), factor analytic modeling of the G matrix (Hine and Blows 2006), and modeling of the G × E interaction matrix with factor analysis (Meyer 2009). Implementation of these methods would take our fitted intrinsic growth rates for individuals as the observed data and then proceed with statistical genetic analysis. We elected not to pursue these approaches as it is not straightforward with these methods to accommodate the model fitting error introduced by the fact that our "trait" is really a parameter value sampled from a posterior distribution. In contrast, the mixed-model PCA approach that we implemented allows us to accommodate and remove the influence of model fitting variation in a straightforward manner.

Past applications of random regression based methods for ecological and evolutionary studies have included investigation of how juvenile weight changes as a function of age (Wilson et al. 2005), growth rate as a function of temperature (Kingsolver et al. 2001b, 2004), and female mating preference functions for male traits (McGuigan et al. 2008). Of these studies, Kingsolver's work on thermal performance curves in caterpillars is the most directly comparable. Using both standard multivariate techniques and random regression on orthogonal polynomials, Kingsolver et al. (2004) detected negative across-temperature genetic covariances in caterpillar growth rates (mg/h) between the two highest temperatures assayed. One similarity to our work is the involvement of extreme, rare environments (high density in our case, high temperature in theirs) with negative genetic covariances that could constrain adaptation to heterogeneous environments; however, the presence of only two studies make conclusions about the generality of this pattern unclear. In Kingsolver's work, caterpillars experienced all temperature environments in succession, simulating the fine-grained fluctuation of temperature experienced by wild animals. In contrast, for our experiment and modeling, individuals experienced only a single density for their lifetime.

IMPLICATIONS OF USING RILs

We argued above that RILs may approximate the natural genetic structure of *I. capensis* populations, and further that RILs present a useful tool for estimating across-environment covariances because replicate individuals that are nearly genetically identical can be grown in multiple environments. Notwithstanding the experimental benefits RILs provide and the plausibility of our arguments about genetic structure, our use of RILs to estimate genetic parameters such as variances and covariances could have several important implications. First, due to transgressive segregation, the RIL population we used may have expressed greater amounts of genetic variation and covariation than typical of natural populations, potentially inflating both our estimates and our statistical power. Second, our use of RILs necessarily only samples whichever alleles were present in the parents originally crossed, rather than representing a random sample of the population. Whether the balance of these effects leads to an overestimation or underestimation of quantitative genetic parameters remains unclear. To some extent this problem is inherent for species with a mixed mating system, as neither selfed inbred lines nor paternal half-sibling crosses will accurately approximate the natural patterns of mating and transmission of alleles and genotypes within populations.

RESOURCE SENSITIVITY OF GENETIC VARIANCES AND ACROSS-DENSITY COVARIANCES

By manipulating intraspecific density (and hence the R:FR cue) and light availability simultaneously, we were able to examine the genetic relationship between a single ecologically important trait—elongation rate—across a range of density environments that are ecologically relevant to *Impatiens* (cf. Arnold and Peterson 2002) and how this relationship depended upon resource availability. There is an increasing appreciation that genetic correlations, much like any other genetic parameter (genetic variance, heritability, etc.), are dependent on the environment in which they are measured (e.g., Donohue and Schmitt 1999; Donohue et al. 2000b; Stinchcombe 2002; see Sgro and Hoffman 2004 for a review).

Our results indicate that across-density genetic correlations are also potentially sensitive to environmental conditions (in this case, resource availability). For example, analysis by random skewers suggests that the two matrices will produce similar, but not identical, responses to selection when multiplied by random vectors. Our primary interest in comparing genetic variance–covariance matrices is in how they affect the response to selection—either for multiple traits in a single environment (Steven et al. 2007; Calsbeek and Goodnight 2009; Stinchcombe et al. 2009) or for a single trait in multiple environments. Because we lacked reliable estimates of $\boldsymbol{\beta}$, we followed the approach of Steven et al. (2007) by applying hypothetical, yet ecologically realistic estimates. For instance, comparisons of between the filled and open bars of Figure 3A,C reflect differences in the underlying matrices, as each matrix has been multiplied by the same $\boldsymbol{\beta}$ vector and the same frequency of selective environments. These results suggest that the different $\mathbf{G}_a$ matrices can lead to greater than twofold (or greater) differences in the predicted response to selection for intrinsic growth rate in a given environment, even if the overall pattern of the response to selection is similar in direction across all environments (e.g., Fig. 3A).

Collectively, our results suggest that generalizations about the across-environment genetic correlations between traits must

be made with caution, as the correlations themselves can be sensitive to which environments are considered and the resource conditions under which they are assessed.

CORRELATIONS ACROSS DENSITY ENVIRONMENTS

The mixed-model PCA that we developed to decompose variation in intrinsic growth rates across density revealed negative across-density covariances, but only in the sun environment. The overall pattern of across-environment genetic correlations in the mixed-model PCA—both from considering light environments separately and from considering them together—suggested that intrinsic growth rate at the highest density (1225 pl/m^2) in the sun was negatively correlated with intrinsic growth rate in all other environments, suggesting a possible trade-off between shade-avoidance responsiveness at high density and intrinsic growth at lower densities. Simply by inspecting the covariances/correlation matrices, it is difficult to discern how much of an evolutionary constraint would be imposed by this relationship—the genetic correlations are moderate in magnitude, and the frequency of the high-density environment is relatively low in the wild. However, interpretation of genetic variance–covariance matrices by inspection of the individual elements is notoriously difficult (Blows et al. 2004; Blows 2007a,b; Houle 2007; Walsh 2007), and the degree to which these genetic covariances and correlations will act as constraints will depend on the magnitude and direction of selection on the associated traits (Lande 1979). For instance, if selection is acting in opposite directions on negatively correlated traits (as may be likely for shade avoidance traits at high vs. low density), the consequences of the correlation will be to accelerate rather than constrain the evolutionary response (Lande 1979; Tiffin and Rausher 1999; Etterson and Shaw 2001; Stinchcombe and Schmitt 2006; Agrawal and Stinchcombe 2009).

Our application of hypothetical selection gradients clarifies the potential influence of the detected across-environment genetic covariances, and the critical importance of the frequency of selective environments (cf. Via and Lande 1985, 1987; Gomulkiewicz and Kirkpatrick 1992; Huber et al. 2004). For the case of constant selection across equally frequent environments ($f = 0.2$ and $\beta = 0.1$ for all environments), the negative across-environment genetic covariances in the sun environment reduces the response to selection that would be predicted based on that environment alone. When accounting for the empirically estimated frequency of selective environments, correlated responses to selection dominate: intrinsic growth rate is predicted to decrease at high density in the sun, despite positive selection on it in that environment (Fig. 3B). The reason is that there is positive selection on intrinsic growth rate in more common environments, combined with the negative across-environment genetic covariances. It is important to note that the assumed strength of β in Figure 3A,B only affects the magnitude of the responses, not the relative differences between Figure 3A,B which are driven by the frequency of selective environments (and similarly for comparisons of 3C and 3D).

Our projection of the likely response to selection in intrinsic growth rates ignores the consequences of selection on other, genetically correlated traits within those environments. The likely consequences of not accounting for these traits and selection on them will depend on the magnitude of the estimated genetic covariances between omitted traits and intrinsic growth rate, and the strength of selection on other traits. If across-environment genetic covariances for the same trait are generally of lower magnitude than within environment genetic covariances between different traits, the response to selection will likely be determined by within-environment forces. However, fully predicting the evolutionary responses of a suite of traits across several environments is likely to be challenging: it also will require an estimate of the genetic covariances between trait 1 in environment 1 with trait 2 in environment 2. For example, considering even two traits in five environments, this would require estimation of 45 unique genetic variances or covariances. Consideration of multiple correlated traits in multiple environments will clearly be a difficult challenge.

Estimating across-environment genetic correlations has produced much discussion in the literature on the most appropriate analytical methods (Rausher 1984; Via 1984; Shaw 1987; Fry 1992; Windig 1997; Astles et al. 2006). Our mixed-model approach was motivated by our desire to account for model fitting variation, unequal sample sizes, and within-RIL variation; these three factors are not explicitly accounted for by an RIL means approach. The ability to account for within-RIL variation is likely to be an important advantage for quantitative genetic designs in which one of the experimental treatments is likely to increase the amount of within-line variation (e.g., if increased density leads to asymmetric competition). Similarly, the variance decomposition approach developed here to account for variation produced by model fitting would seemingly have other application to other function-valued traits (e.g., tolerance, plasticities, and other fitted reaction norms; e.g., Izem and Kingsolver 2005).

CONCLUSIONS

Although much effort has gone into developing methods for comparing **G** matrices as well as estimating across-environment genetic correlations that might act as constraints, comparatively less empirical effort has been devoted to quantifying the frequency of selective environments (but see Weis and Gorman 1990; Kingsolver et al. 2001b; Arnold and Peterson 2002; Huber et al. 2004) despite its well-known theoretical importance (Via and Lande 1985, 1987; Gomulkiewicz and Kirkpatrick 1992). Our results highlight the potential insight of combining estimates of **G** matrices and genetic constraints and the frequency of selective

environments in a single analysis to understand the evolution of complex traits in heterogeneous environments.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Derivation of mixed-model PCA.
Table S1. Mixed-model PCA for *r* across all 10 light-density treatments.

Supporting Information may be found in the online version of this article.