A META-ANALYSIS OF NATURAL SELECTION ON PLANT FUNCTIONAL TRAITS

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A common assumption in plant physiological ecology is that variation in functional traits reflects the adaptation of organisms to their abiotic environment. This assumption can be tested by estimating natural selection as the relationship between a functional trait and a fitness component (i.e., survival or reproduction) within a population. To understand how natural selection operates on plant functional traits, we compiled directional selection gradients ($\beta$), which estimate direct selection, and differentials ($S$), which estimate both direct and indirect selection, from studies conducted in manipulated and unmanipulated environments. We found that, relative to manipulating biotic factors, manipulating abiotic factors had a ~5.7 times larger effect on $\beta$ and ~16 times larger effect on $S$, suggesting that functional traits primarily evolve in response to the abiotic environment. We found that the strength of selection on functional traits ($|\beta|$) did not vary with trait type, performance/fitness component, or measurement context (i.e., common garden vs. natural population). However, the direction of selection did differ between some trait types: $\beta$ was positive for plant size traits but negative for phenology traits. Last, we found that the absolute value of selection differentials ($|S|$) was ~2 times larger than the absolute value of selection gradients ($|\beta|$), indicating that there was indirect selection on functional traits. Overall, our meta-analysis illustrates that natural selection on plant functional traits is common and that estimates of selection on these traits in experimentally manipulated environments can be used effectively to test hypotheses about the causes of adaptation.

Keywords: abiotic environment, agents of selection, leaf morphology, phenology, phenotypic selection, photosynthesis.

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Introduction

The study of plant physiological ecology has long been informed by the assumption that variation in nature reflects the adaptation of organisms to their environment (Warming and Vahl 1909; Raunkiaer 1934). This assumption is the foundation of the comparative method, in which variation in organismal function is correlated with environmental variables (Salisbury 1927; Mooney and Billings 1961; Chapin 1980; Givnish 1988). For example, comparisons of plant function across environmental gradients have demonstrated the potential adaptive significance of functional traits such as photosynthetic capacity (Vogan and Maherali 2014), rooting depth (Schenk and Jackson 2002), stomatal behavior (Oren et al. 1999), and leaf shape (Givnish 1979). These correlations, along with convergence in physiological function among distantly related species that occupy the same environment (Warming and Vahl 1909; Mooney and Dunn 1970; Ackerly 2004), imply that the primary cause of diversification in plant form and function is adaptation to abiotic factors such as moisture, temperature, and soil nutrients.

Although evolutionary thinking has shaped the study of plant functional traits, a common criticism of the comparative method is that inferences about adaptation are not informed by an “evidentiary connection between function and fitness” (Ackerly and Monson 2003, p. S1). One way to demonstrate a connection between functional traits and fitness is by estimating natural selection (i.e., phenotypic selection; reviewed in Conner and Hartl 2004). Selection can be estimated by measuring a functional trait (e.g., photosynthetic rate) and a fitness component (i.e., survival or reproduction) for a sample of individuals within a population. When the trait is standardized to a mean of zero and a variance of one and fitness is relativized by dividing by population mean fitness, then directional selection can be estimated as the slope of the regression between a trait and fitness: a positive slope indicates selection for larger trait values (e.g., higher photosynthetic rate), a negative slope indicates selection for smaller trait values (e.g., lower photosynthetic rate), and a steeper slope indicates stronger selection on a trait. This approach is distinct from explorations of the relationship between functional traits and fitness components among species (e.g., Visser et al. 2016; Ameztegui et al. 2017) and may not lead to
the same conclusions about the relationship (or lack thereof) between function and fitness (Yang et al. 2018). In addition, estimates of selection within populations can be used to make predictions about how functional traits will evolve (reviewed in Conner and Hartl 2004).

Measurements of phenotypic selection can be used to determine which environmental factors have caused the adaptive evolution of plant functional traits. Although plant functional traits are thought to evolve primarily in response to selection by abiotic factors such as moisture, temperature, and soil nutrients (Gutschick and BassiriRad 2003), they can also evolve in response to selection by biotic factors such as competition, herbivory, and mutualism (Grime 2006; McGill et al. 2006). The relative importance of abiotic and biotic factors as causes of selection on functional traits can be tested experimentally: if an environmental factor is experimentally manipulated and selection on a trait differs between treatments, then selection on that trait was caused by the manipulated environmental factor (Wade and Kalisz 1990). For example, increased photosynthetic capacity in arid climates (Vogan and Maherali 2014) may have evolved in response to soil moisture (Sherrard and Maherali 2006) or in response to more intense competition for water (Cohen 1970; Gutschick and BassiriRad 2003). If soil moisture and competition are experimentally manipulated and selection on photosynthetic capacity differs between soil moisture treatments but not between competition treatments, then we can conclude that selection on photosynthetic capacity was caused by the abiotic factor soil moisture. Assuming that the same environmental factors that cause selection in contemporary populations also operated in historical populations (Harder and Johnson 2009), we can infer that variation in photosynthesis evolved in response to an abiotic rather than a biotic factor. Although the effects of abiotic and biotic factors on selection on plant functional traits have been estimated in specific study systems (e.g., Sherrard et al. 2015), meta-analysis has not been used to evaluate their relative importance more broadly (Geber and Griffen 2003).

In addition to varying with biotic and abiotic factors, estimates of selection on plant functional traits may also vary as a function of trait type, fitness/performance component, and measurement context (i.e., common garden vs. natural populations). For trait type, selection on physiology may be weaker than selection on other types of functional traits. This is because measurements of physiological traits such as photosynthesis and transpiration are particularly sensitive to the abiotic micro-environment (e.g., light, soil moisture, temperature; Ackerly et al. 2000), making it difficult to accurately measure trait variation among individuals. Such measurement error, by obscuring any relationship between physiological traits and fitness, could cause the strength of selection on physiology to be underestimated. For fitness component, selection on functional traits estimated via performance (sensu Arnold 1983) may differ from selection estimated via fitness. Selection on functional traits has often been estimated via growth rate or biomass allocation, components of performance that may mediate the relationship between traits and fitness (i.e., survival or reproduction; Dijkstra and Lambers 1989; Farris and Lechowicz 1990; Arntz et al. 1998). However, estimates of selection measured via performance and fitness can differ, either because the relationship between performance and fitness is nonlinear or because a portion of the relationship between a trait and fitness is not mediated by performance (Franklin and Morrissey 2017). For measurement context, selection on functional traits may differ between studies conducted in controlled environments and studies conducted in natural populations (Geber and Griffen 2003). The measurement context can change both the distribution of functional traits and the population mean fitness (e.g., Latta and McCain 2009). If traits or fitness differ between measurement contexts, then the strength and/or direction of selection can also differ (Weis et al. 1992; Steele et al. 2011; Caruso et al. 2017a). Although variation in selection among plant functional trait types, fitness/performance components, and measurement contexts has been evaluated using meta-analysis (Geber and Griffen 2003), selection on these traits has not been analyzed using newly developed meta-analytic models that account for sampling error (reviewed in Morrissey 2016).

Finally, estimates of selection gradients ($\beta$) and selection differentials ($S$) on plant functional traits may differ from each other. Gradients estimate the direct effect of a trait on fitness, whereas differentials estimate both direct and indirect effects (reviewed in Conner and Hartl 2004). Consequently, any difference between selection gradients and differentials indicates that there is indirect selection via correlated traits. Plant functional traits may be particularly likely to experience indirect selection because correlations among functional traits are common (Donovan et al. 2011). For example, photosynthetic rate, leaf nitrogen, and specific leaf area are typically positively correlated as part of the leaf economics spectrum (Reich et al. 2003), and thus, selection on any one of these traits may occur indirectly via correlations with the other traits (Donovan et al. 2011).

To understand how natural selection operates on plant functional traits, we compiled databases of estimates of directional selection gradients ($\beta$) and differentials ($S$) from manipulated and unmanipulated environments. We then analyzed these databases using meta-analytic models (Morrissey 2016; Caruso et al. 2017a) to answer three questions. First, does manipulating abiotic factors have a larger effect on selection (both $\beta$ and $S$) than manipulating biotic factors, as expected if abiotic factors are the primary cause of adaptive variation in functional traits? Second, does selection ($\beta$) on functional traits vary as a function of trait type, fitness/performance component, or measurement context? Third, do selection gradients ($\beta$) and differentials ($S$) differ from each other, as expected if functional traits have indirect effects on fitness?

**Material and Methods**

**Database Construction**

We used three strategies to identify published articles for inclusion in our databases of selection estimates. First, we searched the articles that were included in the appendix of Geber and Griffen’s (2003) meta-analysis of selection on plant functional traits. Second, we searched published databases of phenotypic selection estimates (Kingsolver et al. 2001a, 2001b; Caruso et al. 2017a, 2017b, 2019a, 2019b; Siepielski et al. 2017a, 2017b). Third, like many previous meta-analyses of selection (e.g., Siepielski et al. 2009; Fugère and Hendry 2018), we searched the Web of Science (ver. 5.31; Clarivate Analytics, Philadelphia, PA) for articles that cited Lande and Arnold (1983).
This search was restricted to articles that cited Lande and Arnold (1983) and were published between 2002 (when Geber and Griffen’s 2003 literature search ended) and 2018. Our databases have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.sn02v6x0g; Caruso et al. 2020).

An article was included in one or both databases if it met three criteria: variance-standardized directional selection differentials ($S$) and/or gradients ($\beta$) were measured for one or more quantitative functional traits, standard errors for differentials and/or gradients were reported, and selection was measured on phenotypic values rather than on genotypic or breeding values (i.e., genotypic selection; Rausher 1992). Articles that met these three criteria were included in the experimental database if selection was measured in ≥2 experimentally manipulated environments and were included in the observational database if selection was measured in ≥1 unmanipulated environments. Articles that reported selection estimates from populations transplanted into contrasting environments were included in the observational database because the environment was not manipulated. Each article in the experimental database contributed a minimum of two and mean ± SE of 13.30 ± 1.79 records, where a record included a selection differential and/or gradient for a single trait, via a single fitness or performance component, in a single treatment. Each article in the observational database contributed a minimum of one and mean of 11.01 ± 1.72 records, where a record included a selection differential and/or gradient for a single trait, via a single fitness or performance component, in a single population of a single species in a single year. Articles that measured selection in both manipulated and unmanipulated environments contributed records to both databases. A list of the articles that contributed estimates of selection to the experimental and/or observational database is included in the appendix (available online).

Both the experimental and the observational databases contained more estimates of selection gradients ($N = 483$ and 759 for the experimental and observational databases, respectively) than of selection differentials ($N = 250$ and 222 for the experimental and observational databases, respectively). Other meta-analyses (e.g., Geber and Griffen 2003; Siepielski et al. 2017a) have also found that gradients are reported more frequently than differentials. For the same data set, selection differentials are more likely to be statistically significant than selection gradients (Conner 1988). Consequently, it is unlikely that differentials are reported less frequently because they are nonsignificant. Instead, selection gradients may be reported more frequently because, unlike differentials, they estimate only direct selection on a trait and thus can be used to determine whether a trait is a target of selection (reviewed in Conner and Hartl 2004).

Each record of selection was classified in one of five trait categories: leaf chemistry, leaf morphology, phenology, physiology, and plant size (tables 1, 2). The leaf chemistry category included estimates of selection on the concentration of leaf secondary compounds such as glucosinolates and terpenoids. The leaf morphology category included estimates of selection on physical traits of individual leaves, including specific leaf area, trichome density, and leaf length. The phenology category included estimates of selection on the timing of life-history events, including date of first flower, days to germination, and flowering duration. The physiology category included estimates of selection on leaf-level traits that determine carbon and water use, including photosynthetic rate, water-use efficiency, and leaf nitrogen. The plant size category included estimates of selection on whole-plant traits such as aboveground biomass, rosette diameter, and plant height.

Each record of selection was classified in one of three categories based on the performance or fitness component used to estimate selection: fertility, performance, and survival. The fertility category included estimates of selection via female fitness components such as seed and fruit production. The performance (sensu Arnold 1983) category included records of selection estimated via variables that mediate the relationship between functional traits and fitness such as aboveground biomass, pollen production, and number of inflorescences. Because the distinction between performance components and functional traits can be subjective (Geber and Griffen 2003), measurements such as aboveground biomass were considered functional traits in some studies but performance components in other studies. The survival category included records of selection estimated via survival or a composite fitness measure that includes survival (e.g., $\lambda$).

Each record of selection was classified in one of three measurement context categories: indoor controlled environment, outdoor semicontrolled environment, and natural population. The indoor controlled environment category included records of selection estimated on plants growing ex situ in growth chambers and greenhouses. The outdoor semicontrolled environment category included records of selection estimated on plants growing ex situ in transplant gardens, lath houses, and field arrays of potted plants. The natural population category included records of selection estimated on plants growing in situ in the wild. Because the observational database contained only eight records of selection on plants growing in control treatments in growth chambers and greenhouses, the indoor controlled environment and the outdoor semicontrolled environment categories were combined before analysis.

**Statistical Analyses**

**Question 1: Do abiotic factors have a larger effect on selection than biotic factors?** To test whether manipulating abiotic factors has a larger effect on selection on functional traits than manipulating biotic factors, we estimated the magnitude of the difference in selection gradients ($\beta$) and differentials ($S$) between treatments (i.e., mean $|\beta_i - \beta_j|$ and $|S_i - S_j|$, where $i$ and $j$ represent different treatments). To estimate mean $|\beta_i - \beta_j|$ and $|S_i - S_j|$, we went through the experimental database and identified pairs of records of selection gradients ($N = 435$; table 3) and differentials ($N = 241$; table 3) measured on the same trait and via the same fitness/performance component but in different treatments. These pairs of records of selection are hereafter referred to as “trials” (rather than “studies,” as in Caruso et al. 2017a, 2019a). The trials used to estimate mean $|\beta_i - \beta_j|$ and $|S_i - S_j|$ partially overlapped: 39% of the trials used to estimate mean $|\beta_i - \beta_j|$ also reported selection differentials, whereas 70% of the trials used to estimate mean $|S_i - S_j|$ also reported selection gradients. In experiments with $N = 2$ treatments, each record of selection was included in exactly one trial. In experiments with >2 treatments, some records of selection were included in multiple trials. We then classified trials in categories...
To estimate the mean magnitude of the difference in directional selection gradients between treatments for the abiotic and biotic manipulation categories, we did not calculate effect sizes (i.e., $|\beta_i - \beta_j|$) for each trial and then analyze those effect sizes. Instead, we estimated the variance of the distribution of selection gradients for the abiotic and biotic manipulation categories and then used these variances to estimate the mean absolute value of the difference in selection gradients between treatments for each category (as in Caruso et al. 2017a, 2019a). To estimate the variance of the distribution of selection gradients for the abiotic and biotic manipulation categories, we fit the following mixed model:

$$\hat{\beta}_{i,k} = \mu_j + b_k + m_i + e_i,$$

where $\hat{\beta}_{i,k}$ denotes the estimated selection gradients indexed by $i$, $j$ indexes the environmental manipulation category, and $k$ indexes trials. $b_k$ is included in the model to distinguish between selection gradients from different trials. $m_i$ denotes the sampling errors, and $e_i$ denotes the residuals. $b_k$ are treated as random effects distributed according to $b_k \sim N(0, SE^2_k)$, where the variance of the $b_k$ terms ($\sigma^2_b$) is estimated. $m_i$ is drawn from distributions with known variance according to $m_i \sim N(0, SE^2_i)$, where $SE_i$ denotes the reported standard errors of $\hat{\beta}_{i,k}$. The residuals are assumed to be drawn from separate normal distributions for each category $j$ of environmental manipulation $e_i \sim N(0, \sigma^2(e))$, where each residual variance $\sigma^2(e)$ is estimated separately. The variances estimated from equation (1) were used to estimate mean $|\beta_i - \beta_j|$ as $(2/\pi^{1/2})\sigma^2(\beta_i)^{1/2}$, where $\sigma^2(\beta_i)$ is the estimated variance of selection gradients in a given environmental manipulation category.

The model described by equation (1) does not control for correlations between environmental manipulation and three other moderator variables (table 3). To control for these correlations, we fit a multivariate version of the model described by equation (1):

$$\hat{\beta}_{i,k} = \mu_j + b_k + m_i + \sum_m e_{i,m}.$$

The notation for equation (2) is the same as for equation (1), except that effects are simultaneously included for each estimate, $\hat{\beta}_{i,k}$, for each associated category $j$ of each moderator variable, indexed $m$. The variance contributed by each category of each moderator variable is independently specified (i.e., such that $e_{l,m} \sim N(0, \sigma^2(e)_{lm})$). This model was used to estimate the mean $|\beta_i - \beta_j|$ and the associated confidence interval for the abiotic and biotic manipulation categories while controlling for correlations with other moderator variables (as in Caruso et al. 2017a, 2019a). However, unlike mean $|\beta_i - \beta_j|$ estimated depending on whether they manipulated abiotic factors (e.g., soil moisture, light) or biotic factors (e.g., competition, herbivory; table A1; tables A1, A2 are available online).

### Table 1

| Moderator variable | No. records ($\beta$) | No. records ($\delta$) |
|--------------------|-----------------------|------------------------|
| Trait type:        |                        |                        |
| Leaf chemistry     | 24                    | …                      |
| Leaf morphology    | 53                    | 33                     |
| Phenology          | 216                   | 108                    |
| Physiology         | 92                    | 92                     |
| Plant size         | 98                    | 17                     |
| Fitness/performance component: | | |
| Fertility          | 311                   | 121                    |
| Performance        | 126                   | 120                    |
| Survival           | 46                    | 9                      |
| Measurement context: |                      |                        |
| Indoor controlled environment | 169 | 92            |
| Outdoor semicontrolled environment | 201 | 110          |
| Natural population | 113                   | 48                     |

**Note.** Estimates of selection were compiled from 43 published studies (15 that manipulated abiotic factors, 23 that manipulated biotic factors, and 5 that manipulated both abiotic and biotic factors; appendix) on 46 species (table A2).
The models used to estimate the mean magnitude of the difference in directional selection gradients between treatments for the abiotic and biotic manipulation categories were also used to estimate the mean magnitude of the difference in experimental directional differentials between treatments (i.e., mean $|S_i - S_j|$). Specifically, the models described by equations (1) and (2) were modified by replacing $\beta$ with $S$. The models described by equations (1), (2) were fit using the MCMCglmm package (Hadfield 2010) in R (R Development Core Team 2013), and all parameters were estimated using data cloning (sensu Caruso et al. 2017a). In data cloning, Bayesian Markov chain Monte Carlo (MCMC) methods are used to calculate maximum likelihood estimates of model parameters and their standard errors (Lele et al. 2007). These estimates, unlike estimates from traditional Bayesian models, are not sensitive to the choice of priors.

To compare mean $|\beta_i - \beta_j|$ and $|S_i - S_j|$ between the abiotic and biotic manipulation categories, we calculated Wald-type confidence intervals (as in Caruso et al. 2017a, 2019a). If manipulating abiotic factors had a larger effect on selection on functional traits than manipulating biotic factors, then mean $|\beta_i - \beta_j|$ or $|S_i - S_j|$ for the abiotic category should be larger than mean $|\beta_i - \beta_j|$ or $|S_i - S_j|$ for the biotic category, and their confidence intervals should not overlap.

**Question 2:** Does selection vary as a function of trait type, fitness/performance component, or measurement context? To test whether the strength and/or direction of selection on plant functional traits varies as a function of trait type, fitness/performance component, or measurement context, we used mixed-effects meta-analysis (as in Kingsolver et al. 2012) to analyze the observational database. This analysis included one of the moderator variables (e.g., trait type) as a fixed effect, as well as species ID and publication ID as random effects to account for any autocorrelation between estimates of selection on traits of the same species measured in the same study. The analysis also included the standard errors for each selection estimate to account for the effects of sampling error (Morrissey and Hadfield 2012). Only selection gradients were analyzed because they were reported more frequently than differentials. All analyses were done using the MCMCglmm package (Hadfield 2010) in R (R Development Core Team 2013). For all analyses, we specified an inverse Wishart prior with $V = 1.000$ and $\nu = 0.002$.

These mixed effect meta-analyses were used to estimate the posterior mode and the 95% credible interval for directional selection gradients $|\beta|$ and the absolute value of directional selection gradients $|\beta|$. Modes and credible intervals were estimated separately for each category of the three moderator variables: trait type, fitness/performance component, and measurement context. We estimated the mode and credible interval, rather than the mean and confidence interval as in question 1 (above), to be consistent with previous meta-analyses (Kingsolver et al. 2012; Morrissey 2016). The posterior modes and 95% credible intervals for $|\beta|$ were corrected by applying the posterior distributions of solutions and variance estimates to a folded normal distribution (as in Kingsolver et al. 2012). This correction accounts for upward bias in estimates of the magnitude of selection caused by taking the absolute value (Herford et al. 2004; Morrissey and Hadfield 2012; Morrissey 2016). For $|\beta|$, if the credible intervals for any two categories of a moderator variable did not overlap, then we concluded that the strength of selection differed between those categories. For $\beta$, if the credible intervals for any two categories of a moderator variable did not overlap, then we concluded that the direction of selection differed between those categories. In addition, if the credible interval surrounding $\beta$ for a category of a moderator variable included zero, then we concluded that that category included both estimates of selection that were negative (i.e., individuals with smaller trait values had higher fitness) and estimates of selection that were positive (i.e., individuals with larger trait values had higher fitness). If the credible interval surrounding $\beta$ for a category of a moderator variable did not include zero, then we concluded that that category included either predominantly negative or predominantly positive estimates of selection.

**Question 3:** Is there indirect selection on plant functional traits? To test whether there is indirect selection on plant functional traits, we estimated the posterior modes and 95% credible intervals for directional selection gradients ($\beta$, $|\beta|$) and directional selection differentials ($S$, $|S|$) from the observational database. The posterior modes and credible intervals were estimated using the same meta-analytic model described in question 2 (above), except that a moderator variable was not included in the analysis. Because gradients estimate direct selection on a trait, whereas differentials estimate both direct and indirect selection, a difference between gradients and differentials is evidence of indirect selection (reviewed by Conner and Hartl 2004). Assuming that the posterior modes for $S$ and $\beta$ are both positive (as in Kingsolver et al. 2012), we can determine whether...
any indirect selection opposes or reinforces direct selection on plant functional traits by comparing $|S|$ with $|\beta|$. Specifically, if indirect selection opposes direct selection on plant functional traits, then the posterior mode of $|S|$ will be less than the posterior mode of $|\beta|$, and their credible intervals will not overlap. But if indirect selection reinforces direct selection on plant functional traits, then the posterior mode of $|S|$ will be greater than the posterior mode of $|\beta|$, and their credible intervals will not overlap.

**Limitations of the models.** Although there has been considerable progress in developing models for meta-analyses of selection estimates (reviewed in Morrissey 2016), the models that we used to analyze estimates of selection on plant functional traits had two limitations. First, the models that we used to analyze the experimental and observational databases did not include a phylogenetic covariance matrix. This is because, with one exception (Siepielski et al. 2019), none of the models that have been developed for meta-analyses of selection estimates include a phylogenetic covariance matrix. Consequently, we cannot account for the effects of shared evolutionary history on our results. Second, the models that we used to analyze the experimental database (from Caruso et al. 2017a) did not include publication ID as a random effect and thus did not control for any autocorrelation between estimates of selection from the same study. When we modified these models to include publication ID, they generally converged on a solution that was consistent with the results from models that did not include publication ID (results not shown). However, one of the modified models, for the multivariate analysis of selection differentials, did not converge on a solution. Consequently, we continue to analyze the experimental database with models that do not include publication ID (fig. 1).

**Tests for publication bias.** To determine whether our meta-analyses of selection on plant functional traits could be affected by publication bias, we visually examined funnel plots and used Egger’s regression (Egger et al. 1997) as implemented by

![Fig. 1](image-url)  
**Fig. 1** Mean (95% confidence interval [CI]) absolute value of the difference in directional selection between treatments for experiments that manipulated the abiotic environment and for experiments that manipulated the biotic environment. All estimates of selection are on plant functional traits. A, Difference in selection gradients between treatments (i.e., mean $|\beta_i - \beta_j|$, where $i$ and $j$ represent different treatments) estimated from a univariate model that does not control for correlations between environmental manipulation and three other moderator variables (trait type, fitness/performance component, and measurement context). B, Difference in selection gradients between treatments estimated from a multivariate model that does control for correlations between environmental manipulation and three other moderator variables. C, Difference in selection differentials between treatments (i.e., mean $|S_i - S_j|$, where $i$ and $j$ represent different treatments) estimated from a univariate model that does not control for correlations between environmental manipulation and three other moderator variables. D, Difference in selection differentials between treatments estimated from a multivariate model that does control for correlations between environmental manipulation and three other moderator variables. For the multivariate models, mean $|\beta_i - \beta_j|$ and $|S_i - S_j|$ are estimated relative to the reference category of abiotic. Selection gradients estimate direct selection on a trait, whereas selection differentials estimate both direct and indirect selection on a trait.
Nakagawa and Santos (2012) for mixed effect models. To test for an effect of publication bias on analyses of differentials and gradients from the experimental database, we ran a regression on the meta-analytic residuals (sensu Nakagawa and Santos 2012) and estimates of measurement error from the univariate models described in question 1 (above). To test for an effect of publication bias on analyses of differentials and gradients from the observational database, we ran a regression on the meta-analytic residuals and estimates of measurement error from the models used to analyze $S$ and $\beta$ in question 3 (above). All Egger’s regressions were run using the MCMCglmm package (Hadfield 2010) in R (R Development Core Team 2013). If the intercept of an Egger’s regression differed from zero, then we concluded that there was evidence of publication bias.

Results

Description of Experimental and Observational Databases

The experimental database included 483 records of directional selection gradients ($\beta$). There were more records of selection on phenology (45%) than on plant size (20%), physiology (19%), leaf morphology (11%), or leaf chemistry (5%); more records of selection via fertility (64%) than via performance (26%) or survival (10%); and more records of selection in outdoor semiconrolled environments (42%) and indoor controlled environments (33%) than in in situ natural populations (23%; table 1). These 483 records of selection gradients were used to construct 435 trials (i.e., pairs of records of selection estimated in different treatments), with 40% from experiments that manipulated the abiotic environment and 60% from experiments that manipulated the biotic environment (table 3).

The experimental database included 230 records of directional selection differentials ($S$). There were more records of selection on phenology (43%) and physiology (37%) than on leaf morphology (13%) or plant size (7%), more records of selection via fertility (48%) and performance (48%) than via survival (4%), and more records of selection in outdoor semiconrolled environments (44%) and indoor controlled environments (37%) than in in situ natural populations (19%; table 1). These 230 records of selection differentials were used to construct 241 trials (i.e., pairs of records of selection estimated in different treatments), with 33% from experiments that manipulated the abiotic environment and 67% from experiments that manipulated the biotic environment (table 3).

The observational database included 759 records of directional selection gradients (table 2). There were more records of selection on phenology (45%) than on plant size (22%), leaf morphology (17%), physiology (12%), or leaf chemistry (4%); more records of selection via fertility (70%) than via performance (22%) or survival (8%); and more records of selection in in situ natural populations (67%) than in indoor or outdoor controlled environments (33%).

Question 1: Do abiotic factors have a larger effect on selection than biotic factors? Manipulating abiotic factors had a larger effect on both directional selection gradients and differentials than manipulating biotic factors (fig. 1A). The effect of manipulating abiotic factors on selection gradients (mean $|S| - |\beta|$) was $\sim$5.7 times larger than the effect of manipulating biotic factors (fig. 1A), and the effect of manipulating abiotic factors on selection differentials (mean $|S| - |\beta|$) was $\sim$16 times larger than the effect of manipulating biotic factors (fig. 1C). These differences in the effect of manipulating abiotic versus biotic factors on selection on functional traits were also found in multivariate models (fig. 1B, 1D) that controlled for correlations with trait type, fitness/performance component, and measurement context.

Question 2: Does selection vary as a function of trait type, fitness/performance component, or measurement context? The direction of selection ($\beta$) differed between two of five trait types: plant size and phenology (fig. 2A). Specifically, the modal value of $\beta$ was positive for size traits but negative for phenology traits, and their credible intervals did not overlap with each other. In contrast to size and phenology traits, the direction of selection on the remaining three trait types was similar: the modal values of $\beta$ for leaf chemistry, leaf morphology, and physiology traits were all positive, and their credible intervals overlapped with each other. The credible intervals surrounding the modal values of $\beta$ for leaf chemistry, leaf morphology, and physiology traits also included zero, indicating that selection for both larger and smaller values of these traits was common.

In contrast to the direction of selection ($\beta$), the strength of selection ($\beta$) did not vary among the five types of functional traits that we analyzed (fig. 2D). The modal values of $|\beta|$ for leaf chemistry, leaf morphology, phenology, and physiology traits were similar and had credible intervals that overlapped with each other. The modal value of $|\beta|$ for plant size traits was $\sim$33% larger than the modal value of $|\beta|$ for all other trait types, but its credible interval overlapped with the credible intervals for all other trait types.

The direction of selection ($\beta$) did not vary as a function of fitness/performance component or measurement context. Modal values of $\beta$ for fertility, performance, and survival were similar to each other, and their credible intervals overlapped with each other (fig. 2B). Modal values of $\beta$ measured in controlled environments and in situ natural populations were also similar to each other, and their credible intervals overlapped with each other (fig. 2C). In addition, the credible intervals surrounding the modal values of $\beta$ for all categories of fitness/performance component (fig. 2B) and measurement context (fig. 2C) included zero, indicating that selection for both larger and smaller functional trait values was common.

The strength of selection ($|\beta|$) did not vary as a function of fitness/performance component or measurement context. Modal values of $|\beta|$ for fertility, performance, and survival were similar to each other, and their credible intervals overlapped with each other (fig. 2E). Modal values of $|\beta|$ measured in controlled environments and in situ natural populations were also similar to each other, and their credible intervals overlapped with each other (fig. 2F).

Question 3: Is there indirect selection on plant functional traits? Selection gradients (which estimate direct selection) and differentials (which estimate both direct and indirect selection) did not differ in direction: the modal values of $S$ and $\beta$ were both positive, and their credible intervals overlapped (fig. 3A). But gradients and differentials did differ in magnitude: the modal value of $|S|$ was $\sim$2 times larger than the modal value of $|\beta|$, and their credible intervals did not overlap (fig. 3B).
Tests for Publication Bias

Egger’s regression indicated that most of our meta-analyses of selection estimates were not affected by publication bias. As expected if there is no publication bias, the intercept from the Egger’s regression \( b_0 \) was not significantly different from zero for selection gradients from the experimental database \( (b_0 = 0.166; P = 0.06) \), selection differentials from the experimental database \( (b_0 = 0.165; P = 0.11) \), or selection gradients from the observational database \( (b_0 = 0.158; P = 0.37) \). The only evidence for publication bias was found for selection differentials from the observational database, for which the intercept from the Egger’s regression was significantly different from zero \( (b_0 = 0.940; P = 0.04) \).

Fig. 2  Directional selection gradients \( (\beta; 95\% \text{ credible interval [CI]}) \) and the absolute value of directional selection gradients \( (|\beta|; 95\% \text{ CI}) \) measured on plant functional traits in unmanipulated environments. Results are shown for categories of three moderator variables. A, D, Trait type: leaf chemistry, leaf morphology, phenology, physiology, and plant size. B, E, Fitness/performance component: fertility, performance, and survival. C, F, Measurement context: controlled environment and natural population.

Fig. 3  Estimates of natural selection measured on plant functional traits in unmanipulated environments. A, Directional selection gradients \( (\beta; 95\% \text{ credible interval [CI]}) \) and differentials \( (S; 95\% \text{ CI}) \). B, Absolute values of directional selection gradients \( (|\beta|; 95\% \text{ CI}) \) and differentials \( (|S|; 95\% \text{ CI}) \). All estimates are posterior modes from a mixed effect meta-analysis. \( N = 759 \) for selection gradients. \( N = 222 \) for selection differentials.
Discussion

Question 1: Do abiotic factors have a larger effect on selection than biotic factors? We found evidence to support the expectation that variation in plant functional traits primarily evolved in response to abiotic environmental factors. Manipulating abiotic factors had a larger effect on selection on plant functional traits than manipulating biotic factors (fig. 1A, 1C), even when controlling for the moderator variables trait type, fitness/performance component, and measurement context (e.g., greenhouse vs. natural population; fig. 1B, 1D). This difference between the effect of abiotic and biotic factors on selection was consistent across analyses of gradients (fig. 1A, 1B) and differentials (fig. 1C, 1D), even though the type of abiotic and biotic factors that were manipulated differed (table A1); for example, moisture was the most common type of abiotic manipulation in studies that measured selection differentials (∼70% of trials; table A1) but not in studies that measured selection gradients (∼27% of trials; table A1). Our inference that variation in plant functional traits primarily evolved in response to abiotic environmental factors is consistent with the plant physiological ecology literature, which has historically placed greater weight on abiotic than biotic environmental factors as causes of the adaptive evolution of functional traits (Warming and Vahl 1909; Raunkiær 1934; Mooney and Billings 1961; Chapin 1980; Givnish 1988).

Our finding that manipulating abiotic factors had a larger effect on selection than manipulating biotic factors has implications for how plant functional traits will evolve in the future in response to anthropogenic activities such as climate change and nutrient deposition. Although anthropogenic activities are expected to cause changes in both abiotic (e.g., temperature, rainfall; Pachauri et al. 2014; soil eutrophication; Penuelas et al. 2013) and biotic (e.g., competition; Grant and Grant 2006; herbivory; Rosenblatt and Schmitz 2016; mutualism; Kiers et al. 2010) factors, our results suggest that changes in abiotic factors will have a larger effect on natural selection. Consequently, predictions of how organismal function will evolve in response to anthropogenic activities should be more accurate if abiotic factors are given greater weight.

Although we found that abiotic factors had a larger effect than biotic factors on selection on functional traits of plants, previous meta-analyses suggest that this result is not generalizable to other trait types or taxa. Previous meta-analyses found that manipulating abiotic and biotic factors had similar effects on selection, both when all classes of traits in plants and animals were analyzed (Caruso et al. 2017a) and when only floral traits of plants were analyzed (Caruso et al. 2019a). This difference between the results of our meta-analysis and previous meta-analyses indicates that there is something distinctive about the biology of plant functional traits that causes abiotic factors to exert particularly strong selection. One possibility is that abiotic factors exert particularly strong selection because they induce greater plasticity in plant functional traits such as physiology (e.g., Sherrard and Maherali 2006), leaf morphology (e.g., Steinger et al. 2003), and phenology (e.g., Franks 2011) than in other trait classes (e.g., floral traits; Caruso 2006). Such plasticity, by shifting the distribution of functional traits within a population, can change the strength of selection even if the underlying fitness surface is static (Weis et al. 1992; Steele et al. 2011). This explanation could be tested by looking at the effect of manipulating environmental factors on both the distribution of and selection on functional traits and then determining whether trait plasticity and $|β| - |β|$ are associated.

Question 2: Does selection vary as a function of trait type, fitness/performance component, or measurement context? We found evidence that the direction of selection varied among some types of functional traits: selection ($β$) on plant size was positive, whereas selection on phenology was negative (fig. 2A). This difference in the direction of selection on size and phenology traits is consistent with previous meta-analyses (Geber and Griffen 2003; Kingsolver and Pfennig 2004; Kingsolver et al. 2012), suggesting that selection for larger size and earlier development is common in both plants and animals. However, evolution in response to selection for larger size and earlier development may be limited for two reasons. First, evolution may be limited because size and phenology traits are positively genetically correlated (e.g., Geber 1990; Sherrard et al. 2009). For example, if larger size is genetically correlated with later development, then the indirect response to selection for larger size would be the evolution of later, rather than earlier, flowering. Second, evolution may be limited because the expression of size and/or phenology traits is condition dependent (Austen et al. 2017). For example, if plants that are in better condition are larger, flower earlier, and have higher fitness, then any response to selection on phenology and size could be limited by a lack of genetic covariance between traits and fitness. As expected if there are limits on evolution in response to selection for larger size and earlier development, contemporary populations that have evolved earlier development have not evolved larger size (e.g., Hamann et al. 2018).

In contrast to the direction of selection, the strength of selection ($|β|$) did not vary among functional trait types: estimates of $|β|$ for plant size, leaf chemistry, leaf morphology, phenology, and physiology had overlapping credible intervals (fig. 2D). This result is not consistent with previous meta-analyses (Kingsolver et al. 2001a; Geber and Griffen 2003), which found that $|β|$ varies among trait types of both plants and animals. But regardless of why estimates of $|β|$ vary among trait types in some meta-analyses but not in others, observing that $|β|$ was similar for different types of functional traits does not support the expectation that selection on physiological traits is relatively weak because measurement error obscures any relationship between physiology and fitness (Ackery et al. 2000; Arntz and Delph 2001). Although physiology is sensitive to micro-environmental conditions, new techniques have been developed that precisely control microenvironmental variation during measurement (Evans and Santiago 2014). These techniques, by more accurately estimating variation in physiology among individuals, may have reduced the likelihood of underestimating selection on physiological traits. While the concern that selection on physiology was relatively weak led to physiological traits being left out of selection studies in the 1980s and 1990s (reviewed in Arntz and Delph 2001), our results suggest that physiological traits should be more frequently included in studies of natural selection.

Unlike trait type, neither the strength nor the direction of selection on functional traits varied among fitness/performance component, or measurement context.
findings that indirect selection reinforces direct selection on plant functional traits. This is because unless $S$ and $\beta$ differ in sign, reinforcing natural selection will cause the magnitude of total (direct plus indirect) selection ($|S|$) to be larger than the magnitude of direct selection ($|\beta|$; Geber and Griffen 2003). Past meta-analyses have found evidence for indirect selection on functional traits of plants (Geber and Griffen 2003) but not on nonsize traits of plants and animals (Kingsolver and Diamond 2011; Kingsolver et al. 2012). There may be stronger indirect selection on plant functional traits than on other trait types because indirect selection occurs via correlated traits, and correlations among leaf, stem, and root traits are ubiquitous in the angiosperms (i.e., trait spectra; Wright et al. 2004; Reich 2014; Diaz et al. 2016). Alternatively, stronger indirect selection on plant functional traits than on other trait types could be a consequence of how researchers choose traits to include in selection studies; if researchers omit some traits to avoid multicollinearity, then any indirect selection will be underestimated (Kingsolver et al. 2012). But more generally, the finding that indirect selection reinforces direct selection has implications for the adaptive evolution of plant functional traits: if functional traits are positively genetically correlated, then reinforcing indirect selection will accelerate adaptive evolution (reviewed in Wade 2000).

**Limitations of the Database**

Although we compiled >1000 estimates of directional selection on plant functional traits, our databases had two limitations. First, the observational database included only 166 records that reported both selection differentials and selection gradients, and there was evidence for publication bias in the analyses of differentials from the observational database. Consequently, we cannot rule out the possibility that the difference between $|S|$ and $|\beta|$ that we detected (fig. 3B) reflects in part differences between studies that report only selection differentials and studies that report only selection gradients. Second, studies included in the experimental database often did not report the environmental conditions in different treatment groups (e.g., Caruso et al. 2005). We were thus unable to estimate the magnitude of the environmental manipulations imposed by researchers and cannot rule out the possibility that the magnitude of biotic and abiotic environmental manipulations differed.

**Conclusions**

The meta-analysis of natural selection on plant functional traits presented here demonstrates that measurements of selection are valuable because they establish an “evidentiary connection between function and fitness” (Ackerly and Monson 2003, p. S1). This evidentiary connection has historically been missing from studies of the evolution of functional traits (Ackerly et al. 2000; Ackerly and Monson 2003), but it is evident that significant progress on linking function with fitness has been made in the previous two decades (i.e., since the publication of Geber and Griffen 2003). In particular, the present analysis clearly establishes that the connection between plant functional traits and plant fitness is strong, since the magnitude of selection on functional traits (i.e., $|S|$ and $|\beta|$; fig. 3B) is four to eight times greater than the magnitude of selection on all trait classes of both plants and animals (Kingsolver et al. 2012). Such strong selection indicates that measurements of natural selection are valuable for testing hypotheses about functional trait evolution.

The meta-analysis presented here also makes an important contribution to our understanding of functional trait evolution by testing the long-standing (Warming and Vahl 1909; Salisbury 1927; Mooney and Dunn 1970) hypothesis that the diversification of functional traits primarily reflects adaptation to the abiotic environment. The present analysis shows that experimentally manipulating abiotic factors can have more than an order of magnitude larger effect on natural selection on functional traits than manipulating biotic factors, even when controlling for other variables that can also affect selection on these

**Question 3:** Is there indirect selection on plant functional traits? We found evidence of indirect selection on plant functional traits. Specifically, the absolute value of selection differentials ($|S|$), which estimate both direct and indirect selection on a trait, was larger than the absolute value of selection gradients ($|\beta|$), which estimate only direct selection (fig. 3B). Furthermore, the finding that $|S|$ was larger than $|\beta|$ indicates that indirect selection reinforces, rather than opposes, direct selection on plant functional traits. This is because unless $S$ and $\beta$ differ in sign, reinforcing natural selection will cause the magnitude of total (direct plus indirect) selection ($|S|$) to be larger than the magnitude of direct selection ($|\beta|$; Geber and Griffen 2003). Past meta-analyses have found evidence for indirect selection on functional traits of plants (Geber and Griffen 2003) but not on nonsize traits of plants and animals (Kingsolver and Diamond 2011; Kingsolver et al. 2012). There may be stronger indirect selection on plant functional traits than on other trait types because indirect selection occurs via correlated traits, and correlations among leaf, stem, and root traits are ubiquitous in the angiosperms (i.e., trait spectra; Wright et al. 2004; Reich 2014; Diaz et al. 2016). Alternatively, stronger indirect selection on plant functional traits than on other trait types could be a consequence of how researchers choose traits to include in selection studies; if researchers omit some traits to avoid multicollinearity, then any indirect selection will be underestimated (Kingsolver et al. 2012). But more generally, the finding that indirect selection reinforces direct selection has implications for the adaptive evolution of plant functional traits: if functional traits are positively genetically correlated, then reinforcing indirect selection will accelerate adaptive evolution (reviewed in Wade 2000).
traits (fig. 1). However, despite the accumulation of studies that experimentally manipulate the environment and measure selection on functional traits (appendix), there are still not enough studies to test predictions about the effect of specific abiotic and biotic factors on selection. For example, precipitation explains more of the variation in selection in natural populations than temperature does (Siepielski et al. 2017), suggesting that water availability is a more important agent of selection than temperature. Consequently, measuring natural selection on functional traits in experimentally manipulated environments remains a necessity to understand the evolution of plant form and function.

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