

REVIEW AND SYNTHESIS

Effects of abiotic factors and species interactions on estimates of male plant function: a meta-analysis

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Abstract

The majority of angiosperms are hermaphroditic with total fitness comprised of both male and female components of reproduction. However, most studies examining the effects of abiotic factors and species interactions on fitness have focussed on female reproduction, potentially biasing our understanding of the consequences of environmental factors on total fitness. Here, we use meta-analysis to test how environmental factors affect male function. We obtained 278 effect sizes from 96 studies that measured male function responses to manipulated environmental factors. We found significant effects of abiotic factors and species interactions on estimates of male function, with responses varying depending on environmental factor identity. Male and female responses were correlated for abiotic factor manipulations, but varied based on the type of species interaction (antagonistic or mutualistic). This suggests that measuring only female function may misrepresent whole-plant reproduction depending on context. Finally, we found differences amongst components of male function in response to environmental factors, suggesting that some male function estimates may fail to capture the effects of environmental factors on male fitness. Our results demonstrate the importance of incorporating male function into ecological and evolutionary studies to provide a more accurate understanding of the effects of environmental factors on total fitness.

Keywords

Effect size, female plant function, literature review, male plant function, meta-analysis, nutrients, pollination, response ratio, species interactions.

Ecology Letters (2013) 16: 399–408

INTRODUCTION

The expression of a plant's phenotype and its associated fitness is subject to the actions of abiotic and biotic agents in its surrounding environment. Examining the strength and direction of the effects of these abiotic factors and species interactions is not only important for understanding how they influence plant fitness and plant population dynamics (Herrera 2000; Maron & Crone 2006), but also how they affect the evolution of ecologically relevant plant traits (Strauss & Irwin 2004; Strauss & Whittall 2006) and mating system expression and evolution (Ashman 2006). Assessing the impact of abiotic factors, such as nutrients or light, and species interactions, such as herbivory or pollination (hereafter collectively referred to as environmental factors), on plant fitness has traditionally been done through measurements of female components of plant fitness (i.e. fruit or seed production). However, the majority of angiosperm species have hermaphroditic or perfect flowers, capable of achieving fitness through both female function and male function (i.e. seeds sired). By assuming that female fitness represents total plant fitness, we ignore the contribution of male gametes and may over- or under-estimate how environmental factors affect the fitness of hermaphroditic plants (Stanton *et al.* 1986; Krupnick & Weis 1999; Lehtilä & Strauss 1999). A comprehensive understanding of how total plant fitness is influenced by interactions between plants and their environment must therefore include the contribution of both male and female fitness components (Charnov 1979; Bell 1985; Stanton *et al.* 1986; Strauss *et al.* 1996, 2001). The goals of this study are to review the effects of environmental factors on estimates of male plant function and to assess whether those effects

reveal similar patterns in comparison to female estimates to determine the importance of incorporating male function when estimating total plant fitness in studies of evolutionary ecology.

Measuring seeds sired is hampered by our ability to trace the fate of pollen (the male gamete) in natural plant populations due to its mobility and small size, as well as the physiological sequence of events that occurs post-pollination to sire seeds (Stanton *et al.* 1992; Snow & Lewis 1993) and the difficulties associated with assigning paternity (reviewed in Ashley 2010). Although advances in methodology have been made, specifically the use of molecular markers for genotyping and likelihood-based techniques for assigning paternity (Snow & Lewis 1993; Ashley 2010), few studies have used these or more basic allozyme approaches in manipulative experiments that test the effects of abiotic and biotic factors on male function (but see Irwin & Brody 2000; Strauss *et al.* 2001). Instead, most studies use correlates or estimates of male function, such as pollen quality, quantity and movement, to estimate the effects of plant–animal or plant–environment interactions on male fitness. For example, environmental factors such as soil nutrients or herbivory can alter pollen production (Lau & Stephenson 1993; Quesada *et al.* 1995) and pollen chemistry (Lau & Stephenson 1994), responses that can affect the number or viability of pollen grains (Delph *et al.* 1997). However, for such quantitative and qualitative differences to translate into effects on male fitness, gametes must be dispersed within natural populations and reach stigmas to facilitate the fertilisation of ovules. Dispersal of male gametes in many plant species is often reliant upon an animal vector (i.e. pollinator) whose foraging and dispersal behaviour can be influenced by plant traits (Schemske & Bradshaw 1999), by plastic responses of these traits to environmen-

tal factors (Strauss & Irwin 2004), or by the environmental factors themselves (Roubik 1982; Ness 2006). Moreover, upon reaching the stigma, gametes face post-pollination processes such as pollen tube competition or female choice that can influence a donor's ability to sire seeds (Snow 1994; Bernasconi *et al.* 2004). Therefore, there are many opportunities, from pollen production to ovule fertilisation, where environmental factors may lead to gains or losses in male fitness. Thus, the estimate of male function used to assess the effects of environmental factors on male plant fitness is critical because not all estimates are good predictors of seeds sired (Stanton *et al.* 1992; Snow & Lewis 1993; Ashman 1998; but see Queller 1997).

Here, we perform a meta-analysis to evaluate the effects of abiotic factors and species interactions on estimates of male plant function. Given the challenges of measuring male plant function, our goals were to quantitatively synthesise how often studies find effects of environmental factors on any estimate of male plant reproduction, determine what estimates of male function were most likely to show responses and assess whether responses were similar to those seen in estimates of female plant reproduction. We focussed our meta-analysis on experimental studies that manipulated an environmental factor or plant trait and measured the response of a male function estimate. We examined the following questions: (1) How do environmental factors affect estimates of male plant function, and do these effects vary depending on the type of abiotic factor or species interaction manipulated? (2) Does the magnitude of the male function effect observed depend on the estimate of male function used? and (3) Are male and female function responses to environmental factors correlated? We hypothesised that male plant function would be affected by interactions with environmental factors based on long-standing theoretical predictions that male function should be particularly sensitive to abiotic factors and species interactions, relative to other morphological and/or reproductive traits (Bateman 1948), but we predicted that the direction of these effects would depend on the specific environmental factor. As male fitness is often more limited by the number of mating opportunities than female fitness (Bateman 1948), we predicted that the magnitude of effect on male function would be greater for environmental factors that affected pollination interactions because the ability of plants to achieve fitness through male function is often dependent on pollination (Baker 1963). Moreover, we hypothesised that effects of environmental factors would vary depending on the estimate of male function measured. We predicted that estimates less proximately linked to male function (e.g. allocation or pollen movement response variables) would exhibit stronger responses to environmental factors, potentially because post-pollination processes can mask the relationship between environmental factors and male fitness (Snow 1994). Finally, we predicted that male and female function responses to environmental factors would be positively correlated, but that estimates of male function would be more sensitive to species interactions than female function as suggested by both theoretical and individual empirical studies (Bateman 1948; Charnov 1979; Stanton *et al.* 1986).

METHODS

Literature search

We searched the literature for experimental studies that evaluated the effects of abiotic factors or species interactions on estimates

of or realised (i.e. seeds sired) male function in plants. We used ISI Web of Science for our literature search spanning the years 1970–2012 (through May 2012). Topics were searched using the following set of keywords: pollen, pollen donation, pollen germination, pollen removal, pollen remaining, pollen tube or siring success. We also included pollen* as a wild card in our search. Results were further refined by category (Plant Sciences, Ecology, Horticulture or Evolutionary Biology) and journal. Using studies that met our literature search criteria (see below), we further examined references to identify additional studies for inclusion in our database.

We included studies in the meta-analysis based on two *a priori* criteria. First, the study had to employ an experimental manipulation of an environmental (abiotic or biotic) factor or plant trait and measure an estimate of or realised male function. Observational studies or studies that verbally discussed implications for male function, but did not provide numerical measures were excluded. Although studies are accumulating that document the importance of paternity in gene flow and spatial population genetic structure using molecular markers (Broyles & Wyatt 1990; Adams *et al.* 1992; Mitchell *et al.* 2005; Ashley 2010), these studies were not included in our meta-analysis because they did not explicitly manipulate an environmental factor. Second, the study had to report means, sample sizes and standard deviations (or some other measure of variance from which a standard deviation could be calculated) to calculate an effect size. We contacted author(s) in cases where some or all of this information was not reported. For data presented in graphical form, we extracted values using DataThief III (Tummers 2006), a generally accepted method of extracting data from figures (Hawkes & Sullivan 2001; Morales & Traveset 2009). In total, we found 96 studies that met our criteria (Appendix S1).

Classifying the data set

Abiotic factors

To test the effects of abiotic factors on male function and how these effects varied depending on the factor manipulated, we classified records into categories for separate analyses. For the first set of analyses, we divided the abiotic manipulations into two subcategories: aboveground and belowground factors. Aboveground records included studies that manipulated light (UV), temperature or carbon dioxide (CO₂), while belowground records encompassed studies that manipulated water or nutrients (including nitrogen, phosphorous and salt) directly. To ensure proper grouping of records, careful consideration was given to designation of 'control' and 'treatment' groups (see *Data Analyses*) because we often encountered studies that manipulated the same factor but some studies augmented it while others reduced it. For example, Galen (2000) examined the effects of drought on male function, while Burkle & Irwin (2009) tested the effects of water supplementation on male function. To group these studies, instead of designating manipulations as 'treatment' and 'control', we designated the manipulations as 'lower water' and 'higher water' to ensure that our meta-analysis was accurately comparing the effects of low vs. high values of environmental factors on male function. This same approach was applied to studies involving other abiotic factors (i.e. low vs. high nutrient availability) and species interactions (i.e. low vs. high levels of herbivory or absence vs. presence of herbivory).

Species Interactions

Biotic manipulations were subdivided into two subcategories: antagonists and mutualists. Antagonists were further categorised by damage type (floral or vegetative) and agent (herbivore, nectar robber or mechanical). Two studies manipulated florivory and one manipulated jasmonic acid; these studies were included in the analysis examining the overall effect of antagonists on male function but not in any of the analyses involving more subdivided categories because of low sample size. We recognise that herbivores and robbers are sometimes not antagonists but can have neutral or beneficial effects on plant reproduction (Morris 1996; Gronemeyer *et al.* 1997); our meta-analysis will allow us to assess whether this categorisation of 'antagonist' is indeed correct via male function. Mutualists were categorised into plant–pollinator and plant–arbuscular mycorrhizal fungi (AMF) interactions. Studies of plant–AMF interactions typically involved direct manipulation of the AMF (e.g. Lau *et al.* 1995); however, studies of plant–pollinator interactions primarily included cases where researchers manipulated floral display, morphological traits or rewards and assessed differences in male function mediated through changes in mutualist behaviour (e.g. Johnson *et al.* 2004). As most of these studies were interested in examining the adaptive function of a floral phenotypic trait, traits were sometimes reduced relative to the ambient or augmented. To ensure we were adequately comparing studies, we designated the manipulations as lower vs. higher trait values. We further categorised plant–pollinator studies into those that manipulated accessory structures (i.e. petals) or rewards (i.e. nectar) to test the degree to which male function was sensitive to changes in morphology vs. rewards.

Male function response variables

To test whether the effects of environmental factors varied depending on the estimate of male function used, we classified male function response variables into four broad categories: Allocation, Pollination, Fertilisation and Seed (akin to Mutikainen & Delph 1996). Allocation studies were those that measured flower production (either the number of male or perfect flowers) or pollen production (per-flower or per-plant). These measures provided an estimate of how environmental factors influence resources directed by the plant towards male function. Although studies have shown that measurements of allocation to male function are only tenuously linked to seeds sired (Ashman 1998; Karron & Mitchell 2012), we felt it was important to include studies that measured allocation to male function in our analyses for three reasons. First, these measures can provide an upper bound on maximum fitness that can be achieved through male function for an individual plant (Devlin *et al.* 1992). Second, there is precedence for such measures being used as correlates for male reproductive success in the literature (reviewed in Stanton *et al.* 1992). Third, the main analyses assessing the effects of environmental factors on male function hold whether we include vs. exclude male allocation measurements (data not shown), suggesting that the main results of the meta-analysis are not solely driven by their inclusion.

We grouped the following response variables into the Pollination category: pollen remaining, pollen removal and pollen donation (typically measured using powdered fluorescent dyes as pollen analogues). To make results from studies measuring pollen remaining vs. removed comparable in our analyses (see below), we often had to reverse the sign of the effect size for studies that measured pollen remaining. Less pollen remaining would indicate that more

pollen had been removed and dispersed within the natural population; however, this generates a negative effect size, which must be reversed to make a comparison between effect sizes of pollen remaining with those of pollen removal appropriate. Fertilisation studies were those that measured responses important to pollen performance, including pollen germination, pollen tube number and pollen tube growth rate. We only included studies that manipulated the paternal environment and measured subsequent success of pollen donors. Finally, Seed studies measured seeds sired through paternity analysis using phenotypic, protein or molecular markers. These four estimates spanned ecological stages where pre- and post-pollination processes could influence the outcome of a plant's male function. By categorising records in this manner, we aimed to identify the stage(s) where male function may be most sensitive to environmental factors (Fig. 1).

Studies often reported the effects of environmental factors on multiple estimates of male function (i.e. pollen production, removal and seeds sired). In these cases, we used the measurement of male function that was most proximately linked to seeds sired (or we used seeds sired if it was reported). We used a single measurement of male function per study to avoid non-independence (Gurevitch & Hedges 1993).

Male vs. female responses to environmental factors

To determine whether male responses to environmental factors were similar or dissimilar to female responses, we identified those studies from our original list that measured the effects of environmental factors on both male and female function. From this subset of studies, we recorded the female component of plant reproduction that was most closely tied to female reproductive success (i.e. seeds produced) when multiple estimates of female function were provided. Estimates of female function included female flower production, ovule production, pollen receipt, fruit set and seed set.

Data analyses

We used the natural log of the response ratio ($\ln R$) as the effect size metric for this meta-analysis because of its statistical properties (approximate normal distribution) and biological interpretation (Hedges *et al.* 1999; Rosenberg *et al.* 2000). This metric is the natural log of the ratio of the mean outcome in the treatment group divided by the control group. As discussed above, in some cases it was not possible to group manipulations into 'treatment' and 'control' because it would not allow us to combine studies accurately (i.e. combining water manipulation studies that measured the effects of drought vs. water addition on male function). Such differences amongst studies in the direction of predicted effects can present a challenge for researchers in determining how to calculate and group effect sizes in an appropriate manner for conducting meta-analyses. To avoid such a challenge, there may be temptation to calculate and group the absolute values of effect sizes for an analysis to determine the magnitude of effects of a particular factor of interest. This approach however violates key assumptions of traditional meta-analyses (see below), namely a change in the predicted null hypothesis of the test performed (i.e. a difference from zero) and normality, and should be avoided. For the purpose of our analyses, we typically divided the mean outcome of the manipulation with the higher treatment (i.e. higher water) by the lower treatment (i.e. lower water). For comparison, we also conducted the meta-analysis using

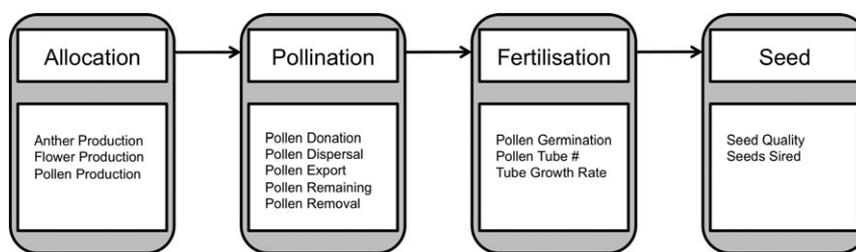


Figure 1 Ecological stages where male function is susceptible to gain or loss as a consequence of abiotic factors and species interactions. In the top box at each stage, we include the category of male function, and in the bottom box we provide the estimates of or realised male function included in the meta-analysis.

Hedges' d (the unbiased standardised mean difference between treatment and control groups measured in standard deviation units, Hedges & Olkin 1985), and found qualitatively similar results (data not shown), suggesting that the results we present are not sensitive to the effect size metric used. Confidence intervals (CIs) were calculated using bias-corrected bootstrap resampling (999 iterations), except for cases where sample sizes were small ($k \leq 10$). In these cases, standard 95% CIs were used because bootstrap procedures would be heavily biased due to resampling from the same limited set of values. An effect size was considered significant if the 95% bias-corrected bootstrap CI did not overlap zero (Rosenberg *et al.* 2000).

The mean effect size for each group of studies was calculated by determining the weighted mean of the log response ratios from the individual studies. In this case, the weights are the inverse of the sampling variances of the effect sizes in each study. To test for differences in mean effect size amongst groups, we used random-effects models (Gurevitch & Hedges 2001), and examined P -values associated with the between-class heterogeneity statistic Q_b . Q_b represents the variance in effect sizes that can be attributed to differences between categories (Rosenberg *et al.* 2000). Using random-effects models allows for the possibility that the true effect size may vary amongst studies within a group (Gurevitch & Hedges 2001). Categories containing ≤ 5 records were excluded from categorical analyses. Effect sizes and analyses of categorical models were performed with MetaWin 2.0 software (Rosenberg *et al.* 2000). Finally, to determine the degree of association between male and female function responses, we conducted a Spearman Rank correlation using R software, version 2.14.1 (R Development Core Team 2011).

Publication bias

Quantitative reviews of published literature can be subject to the potential problem of publication bias (i.e. the predominance of significant results being published over non-significant ones, Rosenthal 1979). We explored the possibility of publication bias in our data set using both graphical and statistical procedures. Graphically, we used a funnel plot and weighted histogram to explore whether studies with small effect sizes were missing from the distribution of published effect sizes. This would be reflected in a funnel plot if we found skew in the shape and/or distribution of points within the funnel (Palmer 1999). Similarly, bias against publishing non-significant results would be reflected as a depression around zero in a weighted histogram (Greenland 1987). Statistically, we conducted a Spearman Rank correlation to examine the relationship between the standardised effect size and the sample size across studies (Begg

1994). A significant correlation would indicate publication bias if larger effect sizes are more likely to be published than small effect sizes. Lastly, we utilised the fail-safe number calculator to determine the number of non-significant, unpublished or missing studies which would have to be added to our meta-analysis to nullify its overall effect size (Rosenberg 2005). If this number is larger than $5n + 10$ ($n = \#$ of studies), then publication bias can be safely ignored as the results are robust even if publication bias exists (Rosenthal 1979; Rosenberg 2005).

RESULTS

Our final data set consisted of 427 effect sizes ($Male = 278$ measures, $Female = 149$ measures) from 96 studies on 75 plant species (53 hermaphroditic, 7 monoecious and 5 dioecious) from 41 plant families (Table S1). Twenty-six studies manipulated an abiotic factor and measured a subsequent male plant fitness response, while 74 studies manipulated or mimicked a biotic factor. We calculated 78 effect sizes that measured the effects of environmental factors on allocation to male function, 100 that measured male function associated with pollen movement, 77 that measured responses related to pollen performance and 23 that measured seeds sired. For each of the categories examined in our analyses (see below), both standard and bias-corrected bootstrap 95% CIs are provided in an appendix for comparison (Table S2).

Abiotic factors

Above- and belowground abiotic factors differed significantly in their effects on male function ($Q_b = 14.16$, d.f. = 1, $P = 0.005$; Fig. 2a). Manipulation of aboveground abiotic factors had a significant negative effect on male function that was larger in magnitude than that of belowground factors. When we examined individual abiotic manipulations and their effects on male function, we found significant differences amongst types of manipulations in their effects ($Q_b = 28.84$, d.f. = 4, $P = 0.002$; Fig. 2b). UV light exerted the largest negative effect on male function, suggesting that increased UV-B reduced male function on average. Temperature was the only other abiotic factor to exert a negative effect on male function, with rising temperatures reducing estimates of male function. Nutrients, water and CO_2 all exerted positive, non-significant effects on estimates of male function.

Species interactions

As predicted, we detected a significant difference between the effects exerted on male function by antagonists vs. mutualists

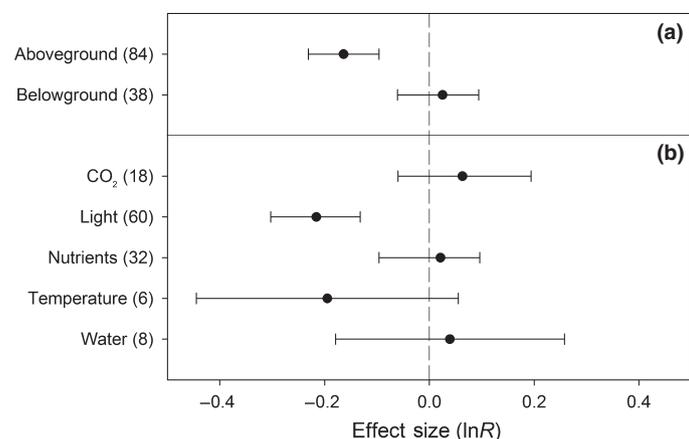


Figure 2 Weighted-mean effect sizes ($\ln R$) and 95% bias-corrected confidence intervals (CIs) for effects of abiotic factors on estimates of male plant function. Comparisons between (a) above- vs. belowground abiotic factors, and (b) individual abiotic factors (CO_2 , light, nutrients, temperature and water) are presented. Effects are considered significant when the CI does not overlap 0 (dashed line). Sample sizes are given in parentheses. Categories with ≤ 10 records have regular 95% CIs.

($Q_b = 10.54$, d.f. = 1, $P = 0.003$; Fig. 3a). Both antagonists and mutualists exerted significant effects, with the magnitude of effect on male function being slightly larger for mutualists. As expected, mutualists had an overall positive effect on male function, whereas antagonists had an overall negative effect on male function. Sensitivity of male function to these interactions was tissue dependent, as male function estimates differed in response to changes in floral traits vs. vegetative traits ($Q_b = 2.76$, d.f. = 1, $P = 0.047$). For mutualist interactions, no significant difference was detected between floral reward and floral morphological trait manipulations on male function ($Q_b = 3.46$, d.f. = 1, $P = 0.177$); both exerted significant positive effects, with the magnitude of effect for reward manipulations ($\ln R = 0.236$ (0.075 to 0.423), $k = 27$) being twice that of morphological traits ($\ln R = 0.113$ (0.045 to 0.210), $k = 39$).

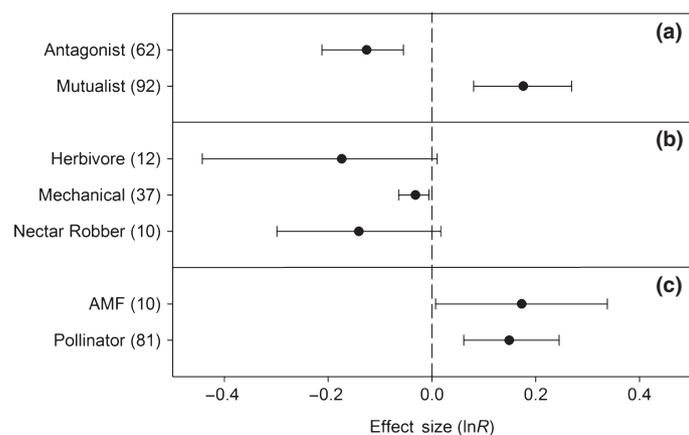


Figure 3 Weighted-mean effect sizes ($\ln R$) and 95% bias-corrected confidence intervals (CIs) for effects of species interactions on estimates of male plant function. Comparisons between (a) species interactions, (b) type of antagonist and (c) type of mutualist are presented. Effects are considered significant when the CI does not overlap 0 (dashed line). Sample sizes are given in parentheses. Categories with ≤ 10 records have regular 95% CIs.

Further analyses revealed significant differences amongst antagonists in their effects on male function ($Q_b = 13.41$, d.f. = 2, $P = 0.019$; Fig. 3b). Herbivores exerted the largest negative effect followed by nectar-robbers, though both were not statistically significant individually. Effect sizes for both herbivores and robbers ranged from negative to positive, suggesting that both can in some cases benefit male function. Although having the smallest effect on male function, mechanical damage that simulated herbivory had a significant negative effect on male function. We did not detect a significant difference between our categories of mutualist manipulations (AMF vs. pollinator) on male function ($Q_b = 0.09$, d.f. = 1, $P = 0.86$; Fig. 3c); both AMF and pollinators exerted significant positive effects.

Male function response variables

Of the records in our data set where an abiotic factor was manipulated and a component of male function was measured, we categorised 43 effect sizes as Allocation responses, 69 as Fertilisation responses, four as Pollination responses and six as Seed responses. In comparison, for records where a species interaction was manipulated or mimicked and a component of male function was measured, we categorised 33 effect sizes as Allocation, 8 as Fertilisation, 96 as Pollination and 17 as Seed.

Analyses revealed no significant difference amongst categories of male response variables in response to abiotic factors ($Q_b = 4.4$, d.f. = 2, $P = 0.23$). We continued to explore the effects of abiotic factors on pollen performance (Fertilisation category) given that these were the only response variables that responded significantly to manipulations of abiotic factors (Fertilisation: $\ln R = -0.140$ (-0.235 to -0.066), $k = 69$). A *post-hoc* analysis revealed that the negative effect size of pollen performance was predominantly driven by UV light ($\ln R = -0.262$ (-0.377 to -0.154), $k = 40$), followed by temperature ($\ln R = -0.195$ (-0.496 to 0.106), $k = 6$). Nutrients ($\ln R = 0.132$ (-0.179 to 0.443), $k = 6$) and CO_2 ($\ln R = 0.110$ (0.052 to 0.158), $k = 13$) generated positive effects on pollen performance; however, effects were only statistically significant for CO_2 .

Our categories of male function response variables varied significantly in their response to species interactions ($Q_b = 29.98$, d.f. = 3, $P = 0.023$). Overall, Pollination variables responded positively to species interactions ($\ln R = 0.137$ (0.070 to 0.216), $k = 96$), whereas Allocation ($\ln R = -0.081$ (-0.172 to -0.001), $k = 33$) and Seed ($\ln R = -0.258$ (-0.515 to -0.084), $k = 17$) variables responded negatively. Further analyses revealed significant differences between types of species interaction on estimates of male function related to pollen movement ($Q_b = 31.24$, d.f. = 1, $P = 0.016$), as antagonists exerted negative effects and mutualists exerted positive effects.

Male vs. female function response variables

Across all environmental factors, we detected a significant difference in outcome between male and female response variables ($Q_b = 5.59$, d.f. = 1, $P = 0.011$). Overall, environmental factors had a significant positive effect on female function ($\ln R = 0.112$ (0.027 to 0.189), $k = 149$) and a non-significant negative effect on male function ($\ln R = -0.018$ (-0.065 to 0.029), $k = 278$). Our test of whether responses were correlated across all studies revealed a significant moderate, positive association between male

and female function responses ($r = 0.43$, $P < 0.001$; Fig. 4). This overall positive association was driven by the degree of association between male and female responses to abiotic factors, as this association was significantly greater than that of the association between male and female responses to species interactions ($Z = 2.23$, $P = 0.033$). Though we detected a significant, positive association between male and female responses to species interactions ($r = 0.39$, $P < 0.001$), further analyses of the interaction type revealed key differences. A positive, though weak, association was found between male and female responses to mutualist interactions ($r = 0.26$, $P = 0.035$); however, no association was detected between fitness estimates to antagonist interactions ($r = 0.11$, $P = 0.458$).

Publication bias

Graphical exploration for publication bias in the studies included in this review revealed bias in reporting of results. Inspection of the weighted histogram showed a bimodal distribution with modes centred on zero and -0.9 (Fig. S1). Moreover, inspection of the funnel plot of effect size vs. variance revealed a slight skewness (Fig. S2). These graphical findings were further supported by our statistical analyses. The correlation between standardised effect size and sample size was weak but statistically significant ($r = 0.188$, $k = 276$, $P = 0.0001$). Though publication bias was detected, we found that the calculated weighted fail-safe number (92709) was dramatically higher than expected (485) without publication bias, suggesting that our results are robust. Similar analyses for publication bias conducted using the effect size Hedge's d yielded the same findings (data not shown).

DISCUSSION

We found that on average, manipulations of some abiotic factors and species interactions can affect estimates of male function. How-

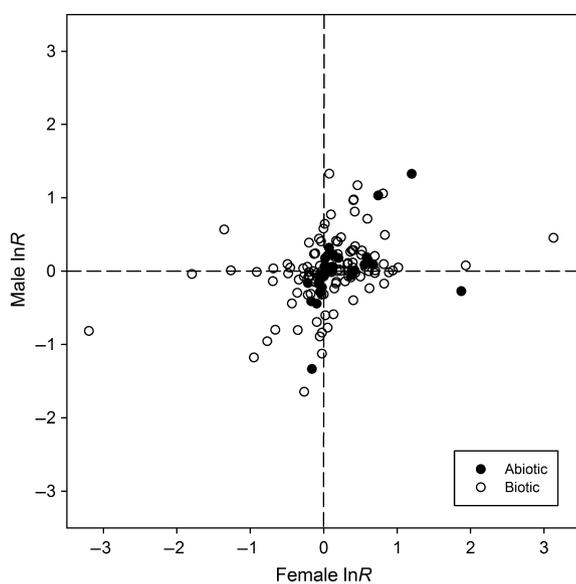


Figure 4 Association between male and female function responses to abiotic (filled circles) and biotic (empty circles) factors. $\ln R$ is the effect size metric of the effect of environmental factors on male and female function estimates.

ever, interpretation of the effects of environmental factors on male function may be largely dependent on the estimate of male function used, in addition to the environmental factor manipulated. Moreover, we found that measuring female fitness components alone may not always paint a complete picture when assessing the effects of environmental factors on total plant fitness. As the first quantitative synthesis of male function responses to environmental factors in plants, these results highlight the importance of accounting for male function when examining the effects of key abiotic factors and species interactions on total plant fitness, in addition to raising caution with respect to interpretation of particular male estimates used. Below, we discuss these patterns, in addition to identifying areas where future research is critically needed to further our understanding of the male contribution to total plant fitness.

Male vs. female plant function

Overall, we found a positive, moderate correlation between male and female variables in response to environmental factors. This finding suggests that although sexual conflict may be common in some systems (Barrett 2002), in general traits or environmental factors that make plants better males will also make plants better females (Grone-meyer *et al.* 1997). However, further examination of the relationship between male and female responses demonstrated that while there was a positive association between male and female responses in studies that manipulated abiotic factors, the association between the two for studies that manipulated species interactions was much weaker. Although theory predicts that female plant reproduction should be more limited by factors that affect plant access to abiotic resources, whereas male reproduction should be more limited by plant access to mating opportunities (Bateman 1948), our findings suggest that components of both female and male function show correlated responses to nutrients and other abiotic factors. The weak or lack of significant positive associations between female and male responses to mutualist and antagonist species interactions could reflect a differential ability to take advantage of mutualistic interactions between the two sexual functions or differential tolerance to antagonistic interactions (Krupnick & Weis 1999; Strauss & Agrawal 1999). It should also be noted that most of the studies that we could find to compare male and female responses to abiotic factors used male allocation responses, whereas the majority to biotic factors used male pollination or seed responses. Thus, we cannot rule out the possibility that the differences in correlations between male and female responses to abiotic factors vs. species interactions is due to the different estimates of male function available for the analyses. Ultimately, more work is needed across a diversity of abiotic factors and species interactions, as well as different female and male components of reproduction, to understand the mechanistic pathways by which female and male reproduction experience gains or losses in fitness and why.

Estimates of male plant function

Due to the challenges of measuring realised paternity in natural populations (reviewed in Stanton *et al.* 1992), studies that manipulate environmental factors often use estimates of male function and then compare those estimates amongst treatments (i.e. Quesada *et al.* 1995; Lehtilä & Strauss 1999). By doing so, these studies assume that measuring estimates of male reproduction provide an understanding of how environmental factors will affect seeds sired.

Our results examining the effects of environmental factors on correlates of male function indicate that caution is warranted when extrapolating to effects on seeds sired. The effects of environmental factors on seeds sired were significantly greater than effects on estimates related to allocation, pollen movement and performance. Although some studies have found positive correlations between estimates of male reproduction and seeds sired (Schoen & Stewart 1986; Broyles & Wyatt 1990; Galen 1992; Stanton *et al.* 1992), these positive correlations are not universal due to pollen loss, pollen inviability and other post-pollination processes which introduce high variance in the success of pollen grains after removal from flowers (Thomson & Thomson 1992; Snow 1994; Wilson *et al.* 1994; Bernasconi *et al.* 2004). Such high variance has been used as an argument for employment of pollen removal measures rather than paternity analyses in studies examining natural selection on plant traits through male function (Queller 1997). Though we detected high variance amongst studies that performed a paternity analysis, which may lend further support to Queller's (1997) argument, our findings suggest that using alternative measures as proxies for seeds sired may underestimate true effects.

One surprising result from our analysis was the small effect size observed for allocation to male function in response to manipulation of environmental factors. We expected the magnitude of effect to be greater than or on par with effects observed across other ecological stages, such as pollination or fertilisation. Similarly, we expected to see greater variation in allocation responses considering the plastic nature of plant responses to environmental factors (reviewed in Strauss & Irwin 2004). At least two non-mutually exclusive hypotheses could explain this finding. First, pollination and stamen-pistil interactions may simply respond more strongly to environmental manipulations than traits associated with plant allocation to fitness, especially given that plants may experience constraints or trade-offs in their ability to reallocate resources following changes in their environment (reviewed in Lovett Doust 1989). Second, the weaker effect of environmental factors on allocation to male function components may be due to the scale at which the measurements were made (i.e. per-flower or per-plant). For example, Herlihy and Delph (2009) found no significant impact of water and nutrient stress on pollen production in *Silene latifolia* at the individual flower level, but because nutrient stress reduced flower number, there was a significant reduction in pollen production at the whole-plant level. Due to the modular nature of plants, per-plant measurements may be able to respond more strongly to environmental factors whereas per-flower measurements may be more conserved. *Post-hoc* examination of our data lends support to this speculation as a significant difference was detected between per-plant ($\ln R = 0.569$ (-0.026 to 1.16), $k = 6$) and per-flower ($\ln R = -0.032$ (-0.078 to 0.009), $k = 45$) measures of pollen production in response to environmental factors ($Q_b = 6.68$, d.f. = 1, $P = 0.035$). Thus, variation in the responses of per-flower vs. per-plant estimates make the effects of environmental factors on allocation traits challenging to interpret, and weaker effects of environmental factors on per-flower vs. per-plant estimates of male reproduction may have dampened the effect size for allocation traits.

Abiotic factors

Aboveground abiotic factors were important in driving the effects of environmental factors on male function, with positive effects of CO₂ enrichment and negative effects of UV on male reproduction. The

positive effects of elevated CO₂ on male reproduction, especially pollen performance, reinforce studies that demonstrate the benefits of increased CO₂ for plants. Elevated CO₂ has positive effects on photosynthesis, vegetative growth and seed set, primarily due to increased assimilation rates (Ainsworth *et al.* 2002; Long *et al.* 2004). Pollen quality also improves with exposure to higher CO₂ (Lavigne *et al.* 1999), but pollen quantity, measured as per-anther or per-flower pollen production, does not increase with elevated CO₂ (Lake & Hughes 1999; Koti *et al.* 2005). In contrast, elevated UV-B levels resulted in a significant negative effect on pollen production and performance overall, although this effect was species specific. For example, Wang *et al.* (2006) found that increased UV-B reduced pollen germination and pollen tube growth in *Vicia angustifolia*, improved pollen performance in *Poa annua*, and had no effect on three other plant species. UV-B irradiation can have deleterious consequences for cell membranes, DNA, pollen morphology and, ultimately, pollen viability (Feng *et al.* 2000; Koti *et al.* 2005); however, hypotheses to explain the positive effects that UV-B has on components of male reproduction of some species are lacking (Feng *et al.* 2000).

Unlike aboveground factors, manipulation of belowground factors had no significant effect on estimates of male function. However, it is important to note that across all studies that manipulated nutrients and/or water availability, there was a trend towards a positive correlation between increased availability of these specific resources and measurements of male reproduction (i.e. Lau *et al.* 1995; Pendleton 2000; Poulton *et al.* 2001a, 2002), and while some studies found no effect (i.e. Havens *et al.* 1995; Caruso *et al.* 2005), only one study documented a negative relationship between resource addition and pollen quality (Burkle & Irwin 2009). Trade-offs may exist between male function traits, as is the case in *Abutilon theophrasti*, where high nutrient levels led to more stamens, but low nutrient levels resulted in larger anthers and pollen, leading to an overall neutral effect of increased belowground resources on male plant fitness (Havens *et al.* 1995).

Three caveats are important in interpreting the effects of abiotic factors on male reproduction. First, the costs and benefits of particular abiotic factors for male function may be context-dependent, as 'low' or 'high' levels of a factor may not always be costly or beneficial, respectively, depending on the ecological context of the study. For example, we observed both increases and decreases in male function in response to water addition (Galen 2000; Burkle & Irwin 2009). Second, for belowground factors, timing is a key to determining the degree to which nutrient manipulations will affect male function. As some perennial plants must preform their reproductive structures months or years in advance of flowering (Aydelotte & Diggle 1997; Worley & Harder 1999), both nutrient addition and stress may be most beneficial or costly for male function when implemented prior to flower or pollen formation. And finally, studies in evolutionary ecology are often most meaningful when factors vary within natural populations; yet, for factors such as CO₂, temperature and UV-B, variation in these factors is more relevant amongst populations. Thus, while some abiotic factors may provide for the potential for soft selection to occur via male function, others may simply provide potential forecasting of the effects of environmental change on male function.

Species interactions

Male function estimates were significantly affected by species interactions, with responses differing in response to both antagonistic

and mutualistic interactions. Our results matched initial predictions, as antagonists exerted significant negative effects, while mutualists exerted positive effects. Qualitative examinations of differences in effect size revealed that the effect of mutualistic interactions was greater than that of antagonistic interactions. This finding suggests the potential for plants to exhibit tolerance to negative effects exerted by antagonistic interactions through male function (Strauss & Agrawal 1999), potentially mitigated through the presence of mutualistic interactions (Morris *et al.* 2007). Moreover, costs of tolerance through male function may be low (Agrawal *et al.* 1999).

Although the effect of mutualists on male reproduction was significantly positive, there is a caveat to this result. In contrast to studies of antagonist interactions where an antagonist (or damage) was either present or absent, the majority of mutualism studies involved plant–pollinator interactions and had pollinators present in all treatments and manipulated the visitation rates or behaviours of pollinators by altering floral display, morphological traits or rewards. For our comparison with the results of the antagonist manipulations, it would have been ideal to have studies where pollinators were either present or absent (as in the studies that manipulated AMF). Such data may exist in studies characterising the mating systems of plant species; however, these were beyond the scope of our literature search. Moreover, many plant species, particularly those that are self-incompatible, rely on access to the mutualists for male success; thus, studies that manipulate the behaviours or visitation rates of pollinators may provide more biological insight than those that remove the pollinator completely. Nonetheless, our analyses likely severely underestimate the beneficial effects of pollinators on male function.

One surprise is that our records from 1970 to 2012 were void of interactions between plants and pathogens and their potential direct and indirect effects on male reproduction. Moreover, just as plants can incur costs to male function through induced resistance and tolerance to herbivores as mediated through the jasmonic acid pathway (Agrawal *et al.* 1999), they may similarly be affected by interactions with pathogens and costs associated with induced resistance mediated through the salicylic acid pathway. To our knowledge, no study to date has examined this, nor has any work addressed how cross-talk between defence pathways in response to simultaneous attack from herbivores and pathogens affects male function. Similarly, our database lacked interactions between plants and most other microbial organisms. We included five studies that manipulated mycorrhizal colonisation (Lau *et al.* 1995; Pendleton 2000; Poulton *et al.* 2001a,b, 2002); however, plants interact with a myriad of microbial organisms both above- and belowground, including endophytic fungi (Clay 1988), rhizobia (Reynolds *et al.* 2003) and yeasts (Herrera *et al.* 2008). Further studies are needed that measure the effect of beneficial and deleterious microbial interactions on male plant reproduction to provide insight into these common ecological relationships.

Caveats and conclusions

Three caveats are important in the interpretation of our findings. First, the results of any meta-analysis are only as robust as the studies that are included (Morris *et al.* 2007), in addition to how data from those studies are used. Surprisingly, few studies have measured the effects of environmental factors on seeds sired relative to other estimates of male reproduction. Thus, we cannot rule out the possi-

bility that the observed effects of environmental factors on seeds sired compared to other pollination and fertilisation responses may be influenced by small sample size. Moreover, studies that manipulated abiotic vs. biotic factors frequently measured different estimates of male reproduction. These differences likely reflect, in part, predictions related to what types of environmental factors will affect which estimates of male reproduction, as well as preferential differences in the response variables measured by ecosystem vs. community and population ecologists (see Burkle & Irwin 2010). However, to gain a clearer understanding of the effects of environmental factors on male reproduction, more studies are needed that manipulate environmental factors, measure seeds sired and dissect the mechanisms by which seeds sired may experience gains or losses.

Second, our conclusions on the effects of species interactions on estimates of male plant fitness are limited to those that manipulated an antagonist or mutualist, and we ignored plant–plant interactions. We recognise that plants may interact with other plants directly and indirectly through intra- and interspecific competition (Waser 1978; Bazzaz *et al.* 1987; Weiner 1988), which may have consequences for estimates of and realised male plant fitness. We did not include these references because the effects of plant–plant interactions via pollination have recently been reviewed qualitatively (Mitchell *et al.* 2009; Morales & Traveset 2009), and the male and female responses in many of these studies are tightly intertwined, making the determination of effect sizes via male function difficult to calculate and interpret.

Third, plants experience a suite of abiotic factors and species interactions simultaneously and these factors can lead to non-additive effects. For example, Koti *et al.* (2005) found significant interactions between three abiotic factors and that the negative effects of increased UV-B and temperature on pollen production and germination could not be balanced by the positive effect of elevated CO₂ (Koti *et al.* 2005). Although the importance of the interactive effects of environmental factors on plant growth and seed production are widely recognised (i.e. Hawkes & Sullivan 2001; Morris *et al.* 2007), studies examining the effects of multiple interactions on male plant reproduction are still largely lacking. Although the goal of this analysis was to identify the role of individual factors in shaping male reproduction, factorial experiments that measure male reproduction will increase our understanding of how multiple environmental factors shape male plant fitness in natural systems.

In conclusion, our meta-analysis suggests that measuring female function alone may misrepresent the effects of environmental factors on whole-plant reproduction and environmental factors, especially species interactions, may not affect all estimates of male function equally. Caution is warranted when extrapolating effects of environmental factors on male components to seeds sired, and of primary need are more studies that examine the effects of environmental factors on realised male function (but see Queller 1997). Though studies on paternity and the extent of gene dispersal in plant populations are accumulating due to advances in molecular tools, these studies have primarily focussed on the importance of distance in determining siring success, with a large percentage of variation left unexplained (Ashley 2010). Environmental factors may underlie the considerable variation observed. We encourage researchers to take advantage of systems where molecular tools are available to begin exploring the link in varied male reproductive success and the environmental factors that plants experience. Estimates of male reproduction have proven critical for evaluating

theory on sex allocation, mating system evolution and more recently the contribution of male function to the evolution of floral traits. These estimates, combined with rigorous testing of interactions with environmental factors, may shed light on the mechanisms driving and maintaining the diversity of floral forms around us.

ACKNOWLEDGEMENTS

We thank N. Barber, L. Burkle, J. Conner, J. Cresswell and A. Smithson for kindly providing data for our analyses. We also thank J. Chase, J. Karron, L. Richardson, M. Vallejo-Marín and three anonymous referees for helpful comments that improved the quality of the manuscript. JSM was supported by NSF grant DEB-0841862 to REI.

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Editor, Jessica Gurevitch

Manuscript received 17 September 2012

First decision made 16 October 2012

Manuscript accepted 3 November 2012