

A QUANTITATIVE SYNTHESIS OF POLLEN SUPPLEMENTATION EXPERIMENTS HIGHLIGHTS THE CONTRIBUTION OF RESOURCE REALLOCATION TO ESTIMATES OF POLLEN LIMITATION¹

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Our understanding of pollen limitation depends on a realistic view of its magnitude. Previous reviews of pollen supplementation experiments concluded that a majority of plant species suffers from pollen limitation and that its magnitude is high. Here, we perform a meta-analysis and find evidence that publication bias, experimental design, and the response variable chosen all influence the magnitude of pollen limitation. Fail-safe numbers indicate that publication bias exists for some measures of pollen limitation; significant results are more likely to be published and therefore available for review. Moreover, experiments conducted on only a fraction of a plant's flowers and reproductive episodes report ~8-fold higher effect sizes than those on all flowers produced over the entire lifetime, likely because resource reallocation among flowers and across years contributes to estimates of pollen limitation. Studies measuring percentage fruit set report higher values of pollen limitation than those measuring other response variables, such as seeds per fruit, perhaps because many plant species will not produce fruits unless adequate pollen receipt occurs to fertilize most ovules. We offer suggestions for reducing the bias introduced by methodology in pollen supplementation experiments and discuss our results in the context of optimality theory.

Key words: life history trade-off; meta-analysis; pollen limitation; pollination; plant reproduction.

For many plant species, pollination is necessary for sexual reproduction, and therefore it affects a variety of ecological and evolutionary processes, such as population persistence (Bond, 1994; Kearns et al., 1998; Ashman et al., 2004) and selection on plant mating system and floral attraction (Ashman et al., 2004; Ashman and Morgan, 2004). Sexual selection theory and optimality arguments predict that plant reproduction should not be limited by pollen receipt (Janzen, 1977; Willson and Burley, 1983; Haig and Westoby, 1988; Wilson et al., 1994). Sexual selection theory further predicts that female resources rather than male gametes should limit the number of seeds produced. Based on these arguments, Haig and Westoby (1988) contend that plants should evolve to optimally allocate resources towards attraction and seed maturation, and should not be limited by either.

In contrast, recent reviews indicate that pollen limitation (PL) is widespread in plants (Burd, 1994; Larson and Barrett, 2000; Knight et al., 2005). Ashman et al. (2004) suggest that the incongruence between theoretical predictions and experimental results may indicate that wild populations are generally not in equilibrium or are in an equilibrium that is determined by

a stochastic pollination environment or that the experimental measure of PL is biased. Here we address the third possibility.

Pollen supplementation experiments (PSE) test for PL by comparing plants given supplemental pollen (i.e., those receiving a saturating amount of pollen from conspecific individuals) to control plants (i.e., those receiving ambient pollen loads). If plants (or inflorescences or flowers) produce more fruits or seeds when given supplemental pollen, then it is concluded that fecundity is limited by pollen. Three reviews of PSE report high prevalence (e.g., 62–73% of species or cases; Burd, 1994; Ashman et al., 2004) and high average magnitude of PL (e.g., 67% increase in fruit set [Larson and Barrett, 2000] and 42% increase in seeds/plant [Ashman et al., 2004] following pollen supplementation). However, the widespread occurrence and high magnitude of PL may be at least in part due to biases in publication of PSE, as well as to aspects of the experimental design or the response variables chosen. These possibilities need to be addressed if we are to gain a realistic picture of the extent of PL and if we hope to understand the causes of PL (e.g., bet hedging or non-equilibrium conditions; reviewed in Ashman et al., 2004), or to use measures of PL as indicators of the health of pollination systems (Thomson, 2001), of mutualism-dependent extinction risk or invasion success (e.g., Bond, 1994; Parker and Haubensak, 2002), and of the potential for natural selection through pollination (Ashman and Morgan, 2004).

Publication bias occurs when studies showing nonsignificant results are less likely to be published (and are therefore available for review) than studies demonstrating significant results (Gurevitch et al., 2001). Publication bias is common in studies where only a positive outcome is worthy of publication. However, many PSE are conducted as a part of larger studies aimed at characterizing plant mating system or pollination environment, and thus it is likely that even nonsignificant results will be published. However, to date, no review of PSE has quantified the extent to which publication bias influences our view of PL.

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The design of PSE can differ in numerous ways, including the level and the duration of application of the pollination treatments. In some studies, all flowers on experimental plants receive supplemental pollen (hereafter, plant-level studies), whereas in others only a fraction of the flowers or a portion of the plant is supplemented (hereafter, flower-level and inflorescence-level, respectively). In addition, in some studies, the treatments are implemented for the entire lifetime of the plant (particularly with monocarpic plants), whereas in others, only a portion of the flowers produced during a plant's lifetime are supplemented. Because plants may be able to reallocate resources among flowers or across years (Stephenson, 1981; Zimmerman and Pyke, 1988), studies conducted on less than all flowers and reproductive bouts may overestimate the magnitude of PL by not accounting for the increased resources available to the treated flowers or reproductive episode.

There are three ways that plants can reallocate resources, which have consequences for the magnitude of PL (Table 1A). First, total allocation to flowering may depend on pollen receipt (Stephenson, 1981). For instance, plants may allocate more resources to maturing the fruits and seeds of pollen supplemented flowers and fewer resources to the production of more, larger, or longer-lived flowers. As a result, PSE conducted on a subset of flowers produced by a plant may overestimate the magnitude of PL. If plasticity in allocation during flowering is common, then we expect that the magnitude of PL will be greater in flower-level studies than in plant-level studies.

Second, resources may be unequally allocated among flowers receiving different amounts of pollen (Zimmerman and Pyke, 1988). PSE conducted on fractions of plants (i.e., flower-level studies) may overestimate the magnitude of PL if

seeds in supplemented flowers are preferentially supported at the expense of seeds in unmanipulated flowers. Moreover, because plants exhibit modular growth and resources are allocated locally, resource reallocation may be more likely between nearby flowers than between distant inflorescences. Thus, we expect to find the highest magnitudes of PL when studies are conducted at the flower level, intermediate magnitudes at the inflorescence level, and the lowest magnitudes at the plant level.

Third, resource reallocation may occur across years in polycarpic plants. When extra pollen is applied to flowers in a given year, the plant may allocate more resources to current reproduction, and less to future reproduction, growth, or storage (Primack and Hall, 1990; Calvo, 1993; Ehrlén and Eriksson, 1995; Primack and Stacy, 1998). Therefore, we expect greater PL when treatments are applied during a single season than when treatments are applied over the plant's entire lifetime.

The idea that resource reallocation can inflate estimates of PL has been known for decades (e.g., Stephenson 1981, Zimmerman and Pyke, 1988) and has been tested empirically on a local, species-specific scale (e.g., Zimmerman and Pyke, 1988). However, this has not been accounted for in previous reviews designed to quantify the prevalence and magnitude of PL on a global, across-species scale. In this study, we compare the magnitude of PL among studies utilizing experimental designs that allow for resource reallocation to those that do not.

Researchers also measure a wide variety of response variables in PSE. Common variables include percentage fruit set (fruits/flower), percentage seed set (seeds/ovule), seeds/fruit, seeds/flower, and seeds/plant. Because plants can respond

TABLE 1. Description of the allocation trade-offs involved with increased pollen receipt and experimental evidence for these trade-offs from pollen supplementation experiments. (A) Allocation trade-offs and their potential consequences for the magnitude of pollen limitation (PL). Flower-level studies supplement pollen on only a fraction of the flowers per plant, whereas plant-level studies treat all flowers on a plant. (B) Summary of the effects of pollen supplementation on other maternal plant (current flowering-flower number, size or longevity; future fitness-growth, reproduction, or survival) or progeny (seed mass, germination, or viability) traits (details in Appendix S2, see Supplemental Data with online version of this article). A decrease in the response variable indicates a statistically significant trade-off between the trait and increased seed number following pollen supplementation, as reported in the original study. (C) Summary of sign tests and *P* values for effects of pollen supplementation on maternal plant or progeny traits. Studies showing no response to pollen supplementation with respect to maternal and progeny traits (see part B) were rescored for trends to increase or decrease.

A) Allocation trade-offs and their potential consequences for the magnitude of PL			
Trade-off predictions	Trade-off with		
	Current flowers	Progeny quality	Future maternal fitness
Description of trade-off	Plants receiving supplemental pollen allocate more resources to seed production and less to current flowering.	Plants receiving supplemental pollen allocate more resources to progeny in supplemented flowers at the expense of those in control flowers.	Plants receiving supplemental pollen allocate more resources to current reproduction at the expense of future maternal fitness.
Consequences for the magnitude of PL	$PL_{flower} > PL_{plant}$	$PL_{flower} > PL_{plant}$	$PL_{season} > PL_{life}$
B) Number of studies measuring a response to pollen supplementation in other allocation traits			
Type of response	Current flowers	Progeny quality	Future maternal fitness
Decrease	7	11	25
Increase	3	15	6
C) Results of sign tests			
Two-sided probabilities	Current flowers	Progeny quality	Future maternal fitness
<i>P</i> value	0.34	0.56	0.0009

Note: Pollen limitation (PL) PL_{flower} = at flower level, PL_{plant} = at plant level, PL_{season} = in a single season, PL_{life} = in an entire lifetime.

to pollen supplementation in any of these components of fitness and trade-offs could occur between them, the response variable measured could influence the magnitude of PL and the conclusions drawn from PSE. For example, if there is a threshold of pollen receipt necessary for a flower to set a fruit (Mitchell, 1997), then inadequate pollen receipt may result in PL when the percentage fruit set is measured, but not when seeds per fruit are measured.

Here, we address whether the aforementioned issues of design, data collection, and publication of PSE influence our estimate of the magnitude of PL and thus our understanding of its prevalence. We answer the following questions: (1) At what frequency are PSE conducted at the flower, inflorescence, and plant level, and what response variable is most often recorded? (2) Does the magnitude and distribution of PL depend on response variable, and is there evidence of publication bias? (3) Does the level at which the treatment is imposed affect the magnitude of PL? (4) Is there evidence of reallocation in response to pollen supplementation in terms of altered allocation to current flowers, progeny quality, or future maternal fitness?

MATERIALS AND METHODS

The data—We searched the literature for PSE published between 1981 and 2003 using ISI's Web of Science and Biological Abstracts and the keywords "pollen limit*," "supplement* poll*," and "hand poll*" (Appendix S1, see Supplemental Data accompanying online version of this article). We also solicited unpublished data, which was generously provided by T.-L. Ashman, C. Ivey, S. Kephart, J. Steets, R. Wesselingh, L. Wolfe, and H. Young. We included only studies that report sample size, mean, and a measure of variance (with the exception of the binomially distributed variable fruit set, for which variance could be estimated from the mean and sample size). Data published in graphical form were digitized using Grab It (version 1, DataTrend Software, 1998). For each study, we recorded whether the treatments were applied at the flower, inflorescence (or ramet, branch), or plant level, whether the treatments were applied over single or multiple years, and which response variable(s) was measured. We only included data from plants that were not manipulated in other ways. We treated each year and population as a separate data record in studies that were conducted in multiple populations and multiple years. However, when studies included within-year or -population variation, we calculated a mean value per year or population. For the few studies in which pollen was supplemented for more than one year, only the first year of the study was included. In all, we had 655 data records from 263 studies, which were conducted on 306 plant species.

At what frequency are PSE conducted at the flower, inflorescence, and plant level, and what response variable is most often recorded?—We determined the frequency of each experimental method and response variable in the 263 studies. In addition, we determined if these differed among plant life forms (tree, shrub or herb) using χ^2 tests.

Does the magnitude and distribution of PL depend on response variable, and is there evidence of publication bias?—For each data record and response variable, we calculated the magnitude of PL (effect size) as the log response ratio ($\ln R$), $\ln R = \ln(\bar{X}^E/\bar{X}^C)$ where \bar{X} is the mean and E and C denote experimental (supplement) and control treatments (Hedges et al., 1999). A value of 0 reflects no difference in fecundity between plants in the supplement and control treatments, a positive value indicates higher fecundity in the supplement treatment, and a negative value indicates the opposite. For all analyses, we used a random effects model and calculated a weighted mean effect size (\bar{E}) for each response variable, $\bar{E} = (\sum_{i=1}^n w_i \ln(R)_i) / \sum_{i=1}^n w_i$ where n is the number of records and $\ln(R)_i$ is the effect size for the i th record (Gurevitch et al., 2001). The weight for the i th record (w_i) is the reciprocal of the sum of its variance (sampling error, v_i) and the pooled study variance (random component of variation, σ_{pooled}^2): $w_i = 1/(v_i + \sigma_{pooled}^2)$. The estimated variance in the effect size is

$$v_i = \frac{(s^E)^2}{N^E(X^E)^2} + \frac{(s^C)^2}{N^C(X^C)^2},$$

where s is the standard deviation and N is the sample size (Hedges et al., 1999). In all cases, significant PL is indicated by bootstrap 95% confidence intervals not overlapping zero.

To detect publication bias, we calculated a fail-safe number for each response variable. Fail-safe number indicates the number of unpublished studies with zero effect size that would need to be added to a meta-analysis to reduce an overall statistically significant observed result to nonsignificance. A large fail-safe number ($>5 \times$ number of data records + 10) means that the meta-analysis is robust to publication bias (Rosenthal, 1991). Rosenberg (2005) developed a fail-safe calculation for random effects models and weighted effect size, which we apply to our data set. In addition, for each response variable, we calculated a heterogeneity value (Q_T); significant heterogeneity among data records indicates that further analyses to examine the factors responsible for this variation are warranted (Gurevitch et al., 2001).

Does the level at which the treatment is imposed affect the magnitude of PL?—To determine if PL depends on the level of treatment, we calculated weighted mean effect size and bootstrap 95% confidence intervals for flower-, inflorescence-, and plant-level studies for each response variable. Because all effect sizes were not normally distributed (see Results), we used randomization to test for significant differences in effect size between treatment levels. All statistics were calculated using Metawin 2 (Rosenberg et al., 2000).

Ideally, to determine if PL depends on whether the treatment was conducted on all or a fraction of the plant's reproductive episodes, we would compare effect size for polycarpic plants given supplemental pollen over their entire lifetime to those treated only in a single year. Unfortunately, this is not possible because few studies have applied treatments to polycarpic plants over multiple years. As an alternative, we compared the weighted mean effect sizes of monocarpic and polycarpic plants. Because monocarpic plants have only one reproductive episode, pollen supplementation applied at the plant level in a single year reflects treatment of the entire reproductive lifetime of the plant. However, in polycarpic plants, PSE performed in only one year reflect treatment of only a fraction of the entire reproductive lifetime, and the effect size may be influenced by resource reallocation across years. We determined that breeding system did not differ between monocarpic and polycarpic plants in our data set ($P = 0.60$) and that this factor is not likely to confound our results.

One reason why PL may differ with treatment level and between monocarpic and polycarpic plants is that woody plants are more likely to be studied at the flower level and are almost always polycarpic. To control for plant life form, we compared levels of treatment separately for herbs, shrubs, and trees (sufficient sample size for these analyses was only available for percentage fruit set). We also compare the effect size of monocarpic and polycarpic herbs studied at the flower and plant levels (sufficient sample size for these analyses was only available for percentage fruit set and was not available at the inflorescence level).

Is there evidence of reallocation in response to pollen supplementation in terms of altered allocation to current flowers, progeny quality, or future maternal fitness?—For the studies that reported significant PL and measured responses in additional traits, we tallied whether the increased seed production following supplemental pollination resulted in decreases or increases in (1) current flowers (flower number, size, and longevity), (2) progeny quality (seed mass, germination, and viability), and/or (3) future maternal fitness (growth, survival, and probability of flowering in future years). For each of the three fitness trait classes we performed sign tests to determine if trait reduction (evidence of reallocation) occurred more often than predicted from chance alone.

RESULTS

At what frequency are PSE conducted at the flower, inflorescence and plant level, and what response variable is most often recorded?—Of the 263 data records, over two-thirds treated only a fraction of the plant (Table 2). Most (163) considered only a single response variable, usually fruit set. Only 19 records considered three or more response variables,

TABLE 2. Frequency of pollen supplementation studies conducted at (A) each treatment level (pollination treatment was applied to flowers [i.e., one or more flowers pollinated on a plant], inflorescences [all flowers on an inflorescence or branch of a plant pollinated], or to the entire plant) and (B) each response variable measured. Frequencies are further separated by plant life form.

Experimental design	Life form			All
	Herb	Shrub	Tree	
A) Treatment level				
Flower	74	26	17	117
Inflorescence	25	29	10	64
Plant	76	6	0	82
B) Response variable				
Fruit set	112	58	25	195
Seed set	52	7	4	63
Seeds/flower	26	2	1	29
Seeds/fruit	55	14	3	72
Seeds/plant	26	2	0	28

and many of these were performed with species that only produce a single flower (i.e., seeds/flower is equivalent to seeds/plant). Treatment level and response variable differed among life forms ($\chi^2 = 50.61$; $df = 4$; $P < 0.001$; $\chi^2 = 34.42$; $df = 8$; $P < 0.001$, respectively). Studies on trees never applied treatments at the plant level and typically measured only fruit set, whereas studies of shrubs and herbs applied treatments at any of the levels and measured any of the response variables (Table 2).

Does the magnitude and distribution of PL depend on response variable, and is there evidence of publication bias?—Effect sizes for all response variables were leptokurtically distributed with medians slightly above zero, indicating that some plants show very extreme levels of PL (Fig. 1, Table 3). Fail-safe numbers for all response variables are less than the critical value indicating that meta-analysis for these are subject to publication bias (Table 3). Fruit set showed the highest overall PL (0.55): supplemented plants set 75% more fruit per flower than controls. Likewise, differences between supplemented and control were 27% for seed set, 16% for seeds/fruit, 48% for seeds/flower, and 25% for seeds/plant. The magnitude of PL was correlated among these response variables ($r = 0.45–0.99$). There was significant heterogeneity (Q_T) in all response variables (Table 3). Further analyses were conducted to determine whether aspects of experimental design contributed to this heterogeneity.

Does the level at which the treatment is imposed affect the magnitude of PL?—When considering all life forms together, the weighted mean effect size depended on the level that the treatment was imposed (Fig. 2A). Studies at the flower level report significantly higher PL in terms of fruit set and seed set than studies at the whole-plant level. Studies at the inflorescence level reported magnitudes of PL that were intermediate to but not significantly different from the PL at the other treatment levels for percentage fruit set (Fig. 2A). When only data on herbs were analyzed, the general pattern is maintained with the exception that inflorescence-level studies had lower fruit set effect sizes than either flower- or plant-level studies, likely due to the low sample size of inflorescence-level

studies ($N = 22$). In addition, with respect to fruit set, PL did not vary with treatment level for shrubs ($N = 129$, $P = 0.44$), but the magnitude of PL was twice as high for trees pollinated at the flower level than those pollinated at the inflorescence level (effect size of 1.13 vs. 0.51, $N = 48$, $P = 0.05$).

Polycarpic plants were more pollen limited than monocarpic plants with respect to percentage fruit set (Fig. 2B). Within monocarpic herbs, effect size was lower for plant-level data records than for flower-level data records (Fig. 3).

Is there evidence of reallocation in response to pollen supplementation in terms of altered allocation to current flowers, progeny quality, or future maternal fitness?—The strongest evidence for trade-offs between seed production and other traits was seen for future maternal fitness (Table 1B, C; Appendix S2, see Supplemental Data); 25 of the 31 cases showed that supplemental pollination reduced components of future maternal fitness (Table 1B), a significant pattern that demonstrates widespread resource reallocation (Table 1C). Although seven of 10 cases reported trade-offs with current flowers and 11 of 26 cases reported declines in progeny fitness

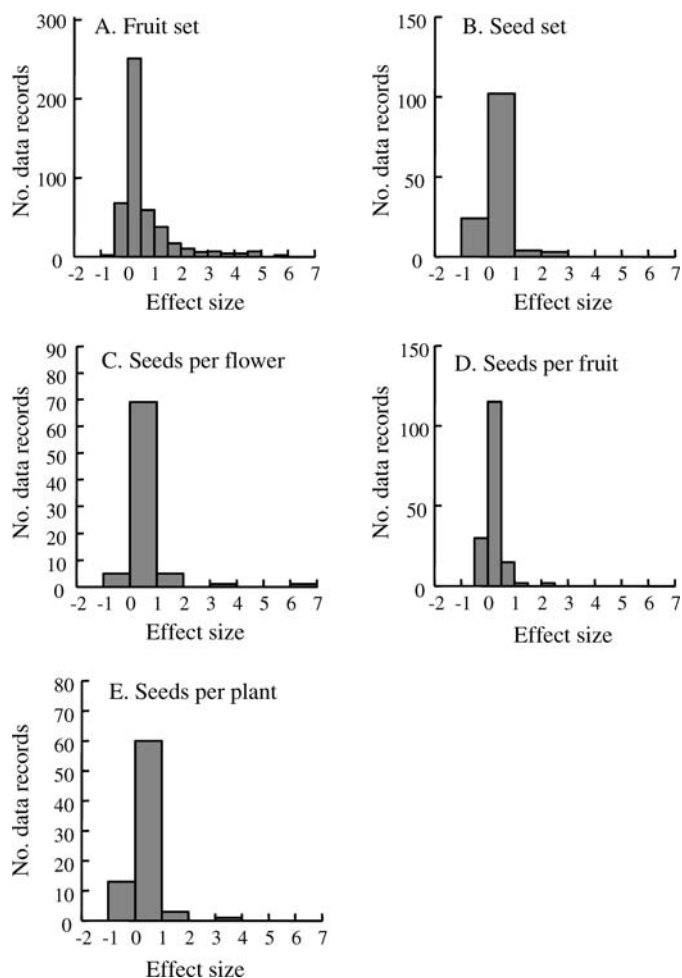


Fig. 1. Frequency histograms of pollen limitation effect size in terms of each response variable: (A) percentage fruit set, (B) percentage seed set, (C) seeds per fruit, (D) seeds per flower, (E) seeds per plant. The effect size measures the magnitude of pollen limitation (see Materials and Methods for details of estimation).

TABLE 3. Number of studies, mean weighted effect size, 95% bootstrap confidence intervals (95% CI), heterogeneity values (Q_T), fail-safe number (N_1), and critical value ($5 \times$ number of data records + 10) for each pollen limitation response variable. Mean weighted effect sizes are estimated using the log response ratio under a random-effects model. An asterisk indicates significant heterogeneity ($P < 0.01$). When $N_1 >$ critical value, then the meta-analysis is robust to publication bias.

Response variable	No. of data records	Effect size	95% CI	Q_T	N_1	Critical value
Fruit set	482	0.5457	0.4573–0.6300	717.3*	18.84	2420
Seed set	133	0.2435	0.1900–0.3025	225.8*	486.0	675
Seeds/fruit	164	0.1479	0.1146–0.1824	248.7*	725.9	830
Seeds/flower	81	0.3921	0.2923–0.5339	121.8*	83.21	451
Seeds/plant	77	0.2194	0.1437–0.3057	135.7*	196.0	395

with supplemental pollination (Table 1B), these patterns were not statistically significant (Table 1C).

DISCUSSION

The results of our meta-analysis corroborate other reviews (Burd, 1994; Larson and Barrett, 2000; Ashman et al., 2004; Knight et al., 2005) in finding that pollen receipt is inadequate and appears to limit female fecundity (Fig. 1, Table 3). However, we also find multiple lines of evidence indicating that the magnitude of PL may be overestimated. Specifically, publication bias (Table 3) and fractional treatment (flowers or

reproductive episodes) of plants led to elevated estimates of PL (Figs. 2, 3). Thus, it is clear that both publication bias and experimental methodology color our understanding of the magnitude, and ultimately the prevalence, of PL.

Publication bias indicates that our measures of PL effect size likely overestimate the true magnitude of PL. Interestingly, studies measuring percentage fruit set had the highest mean effect size, but this response variable was also the least robust to publication bias. We suggest that data on fruit set effect size is best used to compare different categories of plants (e.g., those studied at the plant or flower level), rather than to discuss the average overall effect size and its ecological and evolutionary consequences. As with other quantitative reviews in ecology, our understanding of the true effect size would be greatly improved if studies showing nonsignificant results were not disproportionately underrepresented from the literature.

The magnitude of PL depended on the response variable chosen: the largest PL effect was seen for percentage fruit set, and the lowest effect was seen for seeds/flower and seeds/fruit (Table 2). While plant life form may contribute to these differences (i.e., seeds/fruit was almost exclusively measured on herbs, whereas fruit set was most often measured on trees [Table 2]), this is not the only contributing factor. For example, when we analyzed only the studies measuring both variables, PL remains higher when measured in terms of fruit set compared to seeds/fruit ($P < 0.01$), suggesting that there is a biological reason for differences among these response variables. For instance, this pattern could reflect a threshold amount of pollen required before a fruit is formed (Mitchell, 1997). Thus, a complete understanding of the magnitude of PL requires measurement of multiple response variables.

The magnitude of PL was correlated among these response variables, which may indicate that PL occurs simultaneously at several different stages of the reproductive process, but with varying intensity. However, these correlations are likely inflated by plants that produce few flowers. In such species, multiple response variables are more likely to be measured and may be more likely to give similar magnitudes of PL. For example, information on multiple response variables is almost always available for single-flowering plants species because when these plants set fruit, seeds/flower, seeds/fruit, and seeds/plant are equivalent.

Some response variables are more suited to addressing specific questions associated with PL. For instance, seeds/plant is the appropriate response variable for questions concerning population persistence or evolutionary dynamics, whereas seed set or seeds/fruit may be more appropriate for questions concerning variation in sexual selection, mate acquisition, or bet hedging. The latter assumes that these response variables accurately reflect the pollination environment, i.e., pollen

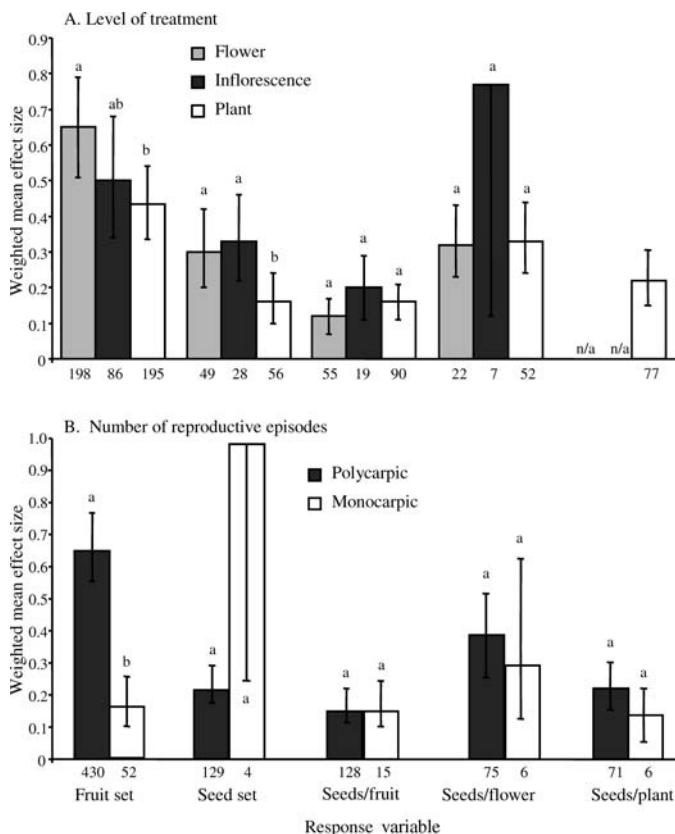


Fig. 2. Weighted mean pollen limitation effect size and bootstrap 95% confidence intervals for studies performed (A) at three treatment levels (flower, inflorescence, and plant) and (B) on monocarpic and polycarpic plants. Within response variables, statistically significant ($P < 0.05$) differences among treatment levels as determined by randomization tests are denoted by different letters. The upper confidence intervals for seeds set of monocarpic plants is 1.88.

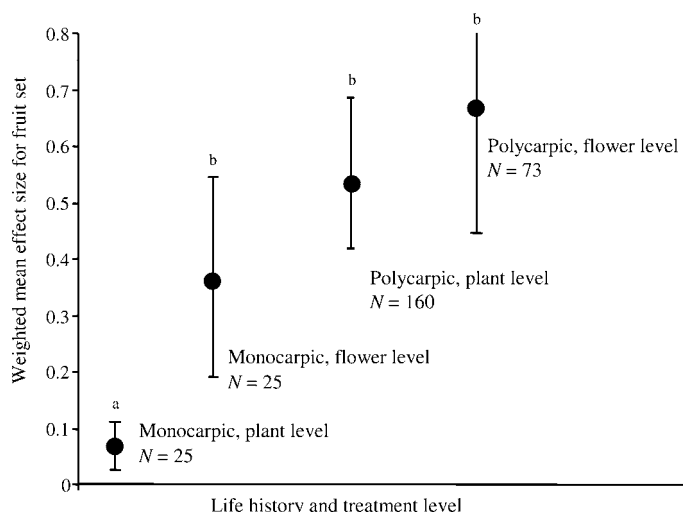


Fig. 3. The weighted mean pollen limitation effect size for percentage fruit set and bootstrap 95% confidence intervals for studies on monocarpic and polycarpic herbs treated at two levels (flower and plant). Statistically significant ($P < 0.05$) differences as determined by randomization tests are denoted by different letters. Sample sizes (N) are presented below the x-axis. Results are not shown for inflorescence-level studies because of limited sample size. The upper confidence intervals for polycarpic herbs studied at the flower level is 0.91.

receipt. To test this assumption, measures of natural variation in pollen receipt should accompany flower-level estimates of PL (see also Ashman et al., 2004).

While several authors have warned that resource reallocation may influence estimates of PL (see Stephenson, 1981; Zimmerman and Pyke, 1988), and some researchers have made adjustments in design to account for (or detect) reallocation, our survey reveals that the majority of studies are still conducted on fractions of plants or lifetimes, leaving estimates of PL vulnerable to inflation as a result of resource reallocation. We find that treatment at the flower level results in approximately two-fold higher estimates of PL for fruit and seed set (Figs. 2A, 3) than treatment at the plant level. The comparison between plant-level treatment of monocarpic and polycarpic herbs provides another striking example of the severity of the bias introduced by reallocation. Reallocation among years can occur in the polycarps but not the monocarps, and PL of fruit set is more than three-fold greater in the former (Fig. 3). In addition, we find evidence of a significant negative effect of supplemental pollination on components of future maternal fitness and a trend for reduced current flowering; however, the latter data set is rather small (Table 1B, C). And, although no significant pattern of reallocation associated with progeny quality was found, it is worth noting that reallocation may be dampened for this trait because supplemental pollination may involve higher quality pollen than natural pollination (Ashman et al., 2004). These results indicate that the issue of resource reallocation contributing to the magnitude of PL should be taken seriously, and steps to reduce the potential for resource reallocation or to quantify the magnitude by which resource reallocation affects the results of PSE are needed. Interpretation of PSE within individual studies and syntheses across species should consider the potential for resource reallocation to contribute to differences in PL (Knight et al., 2005).

Experimenters who wish to use PSE to estimate the magnitude of PL should supplement pollen at the whole-plant level and in more than one consecutive year in polycarpic plants to lessen biases introduced by reallocation. However, experimenters may be constrained to apply pollination treatments on a fraction of the flowers per plant for plants with large floral displays (i.e., flowering trees). In these cases, PL estimates will be less influenced by resource reallocation when researchers apply treatments at intermediate levels (e.g., the inflorescence level). We find that the average effect was reduced by 50% when trees were pollinated at an intermediate level compared to those pollinated at the flower level. When it is not possible to utilize methodology that minimizes the contribution of resource reallocation to response, researchers can estimate the degree to which resource reallocation contributes to response by measuring traits potentially involved in trade-offs with increased seed production in supplemented plants (Table 1). For example, studies should attempt to quantify the extent of resource reallocation across years by measuring fitness of the maternal plant in the year following the pollination treatments.

Further, evidence of PL from PSE can be corroborated with other observations, such as pollinator observations. For example, in the PSE by Bosch and Waser (1999), they observed pollinators and counted pollen loads to determine whether plant density influences pollination success. Further, PL may be indicated by style persistence (Wagenius, 2004) or by observing untripped flowers with trip mechanisms (Parker, 1997) in some plant species. These observations not only corroborate results of PSE, but also provide more mechanistic information about the factors causing PL.

In a quantitative review of PSE that did not account for experimental methodology, Larson and Barrett (2000) found that woody plants were more pollen limited than herbs (in their TIPS analysis, which is analogous to our analyses here that do not control for phylogenetic relationships among plant species) and suggested that this may be due to, among other things, stronger selection on traits that reduce pollen limitation in herbs which may be shorter-lived than woody plants. However, we show that treatments are more often applied to fractions of the flowers on woody plants (Table 2). In our data set, we find higher PL in woody plants relative to herbs when we do not consider experimental methodology (effect size of 0.63 vs. 0.43, $N = 482$, $P = 0.001$), but no difference between these groups when we consider only polycarpic plants studied at the flower level ($N = 173$, $P = 0.81$). This illustrates how important it is to consider experimental methodology and the possibility for resource reallocation when interpreting results of PSE and extrapolating results across studies.

The results of prior reviews that suggest a high prevalence and magnitude of PL are in discord with predictions from an optimality argument made by Haig and Westoby (1988) that plants should not respond to additional pollen receipt. Such a result fuels concerns that the widespread PL across angiosperms may be due to recent ecological perturbations (i.e., habitat fragmentation) that disrupt interactions between plants and pollinators (Ashman et al., 2004) or may be due to a global pollinator crisis (e.g., Buchmann and Nabhan, 1996). However, we find that studies conducted on whole plants over their entire lifetime (i.e., monocarpic herbs), in which resource reallocation is not possible, found low levels of PL (Fig. 3). Our results indicate a more optimistic scenario: the extreme

magnitudes of PL found in many PSE represent both limited amounts of pollen and reallocation of resources.

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