

SHORT COMMUNICATION

Pollen limitation and its influence on natural selection through seed set

M. P. BARTKOWSKA¹ & M. O. JOHNSTON

Department of Biology, Life Sciences Centre, Dalhousie University, Halifax, NS, Canada

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Abstract

Stronger pollen limitation should increase competition among plants, leading to stronger selection on traits important for pollen receipt. The few explicit tests of this hypothesis, however, have provided conflicting support. Using the arithmetic relationship between these two quantities, we show that increased pollen limitation will automatically result in stronger selection (all else equal) although other factors can alter selection independently of pollen limitation. We then tested the hypothesis using two approaches. First, we analysed the published studies containing information on both pollen limitation and selection. Second, we explored how natural selection measured in one Ontario population of *Lobelia cardinalis* over 3 years and two Michigan populations in 1 year relates to pollen limitation. For the Ontario population, we also explored whether pollinator-mediated selection is related to pollen limitation. Consistent with the hypothesis, we found an overall positive relationship between selection strength and pollen limitation both among species and within *L. cardinalis*. Unexpectedly, this relationship was found even for vegetative traits among species, and was not found in *L. cardinalis* for pollinator-mediated selection on nearly all trait types.

Introduction

Although it is well established that natural selection varies through time and space (Thompson, 2005; Siepielski *et al.*, 2009, 2013; Kingsolver & Diamond, 2011), for most populations the reasons for this variation are unknown. Pollen limitation – the observation that fecundity is limited either by pollen quantity or quality rather than abiotic resources – is a pervasive feature of many plant species and populations (Ashman *et al.*, 2004; Knight *et al.*, 2005; Burd *et al.*, 2009) and may be one factor that underlies variation in selection. For instance, in populations where pollinator service is scarce, selection may favour traits that reduce reliance on pollinators, such as autonomous self-pollination

(Morgan & Wilson, 2005) or clonal growth (Eckert, 2002; see also Harder & Aizen, 2010). Because pollen limitation may increase competition among plants for pollinator service, pollen limitation may also influence the intensity of selection (Johnston, 1991a, b). This is expected to be particularly relevant for phenotypes that increase pollinator attraction and efficiency. Thus, pollen limitation is expected to have the greatest influence on the portion of selection caused by pollinators.

Recent studies have quantified pollinator-mediated selection by comparing selection in naturally pollinated plants to those receiving supplemental hand pollination. By experimentally altering pollen limitation, such studies also provide a direct test of the hypothesis that pollen limitation influences selection. The results are mixed. Although some studies detect an increase in selection strength under natural pollination (e.g. Campbell, 1989; Totland *et al.*, 1998; Alexandersson & Johnson, 2002; Sandring & Ågren, 2009; Sletvold & Ågren, 2010, 2014; Sletvold *et al.*, 2010; Bartkowska & Johnston, 2012), others detect weaker (Campbell & Bischoff, 2013) or no change (Fishman & Willis, 2008) in selection, and still

Correspondence: Mark O. Johnston, Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, Nova Scotia, B3H 4R2, Canada. Tel.: (902)-494-8005; fax: (902)-494-3736; e-mail: mark.johnston@dal.ca

¹Current address: Department of Ecology & Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario M5S 3B2, Canada

others find stronger selection under natural pollination despite lack of pollen limitation (Caruso *et al.*, 2010; Parachnowitsch & Kessler, 2010).

To understand why pollen limitation might influence selection, it is helpful to explore the relationship between these two quantities. Pollen limitation describes the proportional decline in seed number of naturally compared to supplementally pollinated plants and is quantified as

$$PL = 1 - \frac{\bar{W}_n}{\bar{W}_s}$$

where \bar{W}_n and \bar{W}_s are the average seed numbers of naturally and supplementally pollinated plants. The directional selection gradient β represents the slope of the regression of relative fitness (here seed number) on phenotype z (standardized to zero mean and unit standard deviation here and throughout; Lande & Arnold, 1983). Letting b represent the slope of the regression of absolute seed number on phenotype, we have

$$\beta_n = \frac{b_n}{\bar{W}_n} \text{ and } \beta_s = \frac{b_s}{\bar{W}_s}. \quad (1a)$$

Thus,

$$\beta_n = \frac{b_n}{\bar{W}_s(1-PL)} \text{ and } \beta_s = \frac{b_s(1-PL)}{\bar{W}_n}. \quad (1b)$$

The effect of pollen limitation on selection should apply specifically to the portion of selection caused by pollinators, which can be estimated as $\Delta\beta_{\text{poll}} = \beta_n - \beta_s$ (Totland *et al.*, 1998; Sandring & Ågren, 2009). Pollinator-mediated selection is thus related to pollen limitation as

$$\Delta\beta_{\text{poll}} = \frac{b_n + b_s(PL-1)}{\bar{W}_s(1-PL)} \quad (2a)$$

or equivalently

$$\Delta\beta_{\text{poll}} = \frac{b_n + b_s(PL-1)}{\bar{W}_n}. \quad (2b)$$

The prediction that increased pollen limitation will strengthen pollinator-mediated selection (Johnston, 1991a,b) arises from the idea that there will be increased competition among individuals, causing those with larger or smaller phenotypes to produce more seeds. Equations (1) and (2), however, show that pollen limitation will influence selection even without a change in the relationships between trait and seed number (b_n , b_s). Specifically, a change in only mean natural seed number, \bar{W}_n , as might occur with a change in pollinator abundance, or in only mean supplemental seed number, \bar{W}_s , as might occur when abiotic resources change, will change pollinator-mediated selection despite no change in the relation between trait and seed number.

Here, we used two approaches to evaluate the relationship between population-level pollen limitation and

the strength of selection. First, we examined all published studies that report both pollen limitation and estimates of natural selection in the same population and year, updating the analysis of Ashman & Morgan (2004). We tested whether there was a general correspondence between pollen limitation and natural selection, and whether any such relationship depended on trait class. Second, we studied pollen limitation and selection in one Canadian population of *Lobelia cardinalis* (Campanulaceae) over three years and linked these data to those from a previous study of two USA populations to explore how natural selection scales with pollen limitation within this species. We quantified pollinator-mediated selection as both gradients ($\Delta\beta_{\text{poll}} = \beta_n - \beta_s$) and differentials ($\Delta s_{\text{poll}} = s_n - s_s$). We addressed the following questions in *L. cardinalis*: (1) What traits experienced selection and pollinator-mediated selection? (2) Did differences in selection or pollinator-mediated selection among years correspond with pollen limitation? In the *L. cardinalis* study, we predicted that pollen limitation would show a stronger relationship with pollinator-mediated selection than with net selection. For both studies, we predicted that the relationship between pollen limitation and selection would be stronger for traits most closely related to pollinator attraction, such as plant and flower size, than for vegetative traits such as leaf size.

Materials and methods

Literature survey

Using Web of Science, we found 26 studies representing 21 species and 13 families for which pollen limitation and selection were estimated concurrently in the same population (Table S1). For each study, we categorized traits into the following five classes: flower size (includes petal size, corolla width, corolla length, anther-nectary distance, nectar-spur length, petal area); flower number; plant size (includes plant height, height to first flower, number of inflorescences); and phenology (includes date of first flower opening, date of median-flower opening and date of flower initiation). When a study included several traits that could be classified as the same type (e.g. both petal width and length would be classified as floral size), we selected the trait with the highest selection coefficient to be representative of that trait type for that year. Thus, for each study population and for each of the trait classes evaluated, only one estimate per year from each study population was used in the regression analysis of selection via female fitness and pollen limitation.

Because different fitness measures were used to assess pollen limitation and a single study often reported multiple estimates, we used the 'best pollen limitation estimate available' by prioritizing the estimates from each study as follows: (1) mean number (or

percentage) of seeds, (2) mean number (or percentage) of fruits, (3) mean number (or percentage) of seeds per flower, (4) mean number (or percentage) of seeds per fruit, (5) mean number (or percentage) of fruits per flower and (6) mean number (or percentage) of seeds per ovule. The absolute value of the selection coefficient was regressed on the best estimate of pollen limitation. This analysis was conducted separately for selection differentials and gradients and for the five trait types. Here, we report the results based on selection gradients, but include results based on differentials in the supplemental data. All analyses were conducted using R V. 2.15 base package (R Development Core Team, 2010).

Selection and pollen limitation in a natural population of *Lobelia cardinalis*

Study species and site

Lobelia cardinalis is a short-lived herbaceous perennial distributed throughout eastern North America with populations also occurring to the west and southward into Central America (Bowden, 1959). It grows in moist habitats near riverbanks, low woods and lake edges. Throughout its eastern range, *L. cardinalis* is pollinated solely by *Archilochus colubris* (Trochilidae), the ruby-throated hummingbird (Bertin, 1982). Although individuals are self-compatible, pollinators are required for seed production (Johnston, 1991b). We studied plants growing along the Petawawa River in Algonquin Provincial Park in Ontario, Canada, from mid-July to mid-October over 3 years (2009–2011). A subset of the 2009 data has been used in an earlier selection analysis (Bartkowska & Johnston, 2012).

Experimental treatment: pollen supplementation

In July of each year, prior to the opening of the first flower, we tagged and assigned inflorescences haphazardly to either a natural or supplemental (hand) pollination treatment. Sample sizes at tagging were 619 (in 2009), 569 (in 2010) and 204 (in 2011) for natural pollination and 239 (in 2009), 234 (in 2010) and 207 (in 2011) for supplemental pollination. Individuals in the supplemental hand-pollination treatment were fully pollinated by saturating all receptive stigmas every 3 days with a mixture of outcross and self-pollen. Partial self-fertilization (approximately 50%) occurs under natural conditions, and seed number does not differ between self- and outcross pollen (Johnston, 1992, 1993).

Traits measured and female fitness

We measured six traits on each plant: anther-nectary distance, flower width, length to last flower (i.e. the length of the stem from ground level to the bottom of the carpel of the uppermost flower), flower number, mean daily floral display and median-flower date (see

Bartkowska and Johnston [2012] for detailed methods). Flower width was measured as the total span of the bottom three petals. Median-flower date was the date the median flower entered the female phase, and mean daily floral display was the average number of flowers open per day over the individual's flowering span. Length was not measured on 110 plants of the naturally pollinated plants in 2010; thus, these individuals were excluded from multitrait selection analysis. Female fitness was quantified as the total number of seeds produced per individual.

Relationship between natural selection, pollinator-mediated selection and pollen limitation

Relative fitness (individual fitness divided by mean fitness) was regressed on standardized trait values (mean 0 and variance 1) to obtain estimates of directional and nonlinear selection for univariate and multitrait models (Lande & Arnold, 1983). For clarity, we refer to directional and nonlinear estimates from univariate models as selection differentials and estimates from multitrait models as gradients, which measure direct selection and control for variation in other traits included in the model (Lande & Arnold, 1983). We estimated the following coefficients: directional selection differential (s_i), quadratic selection differential (γ_{ii}), directional selection gradient (β_i) and quadratic selection gradient (γ_{ii} , doubling the value estimated by statistical software). For simplicity in comparing selection estimates among years and treatments, we do not include estimates of correlational selection (γ_{ij}).

Selection differentials and gradients were estimated separately for naturally pollinated (s_n , β_n) and supplementally pollinated plants (s_s , β_s). For each trait, we subtracted the selection estimate of the hand-pollinated plants from that of the naturally pollinated plants to quantify pollinator-mediated selection (Δs_{poll} , $\Delta \beta_{\text{poll}}$, $\Delta \gamma_{\text{poll}}$). We assessed whether pollinators exerted selection on the six plant traits by examining the confidence intervals of the difference in selection coefficients (subscript 'poll') between the natural and hand-pollination treatments. We also used this procedure to assess: (1) whether selection differentials and gradients (directional selection only) differed among years and (2) whether the difference in selection between naturally and hand-pollinated plants (the strength of pollinator-mediated selection) differed among years.

Using seed number per individual as the fitness measure, we estimated population-level pollen limitation (PL) for each year and assessed whether pollen limitation differed among years. Seed number was assessed after fruit ripening and therefore included any losses due to predispersal seed predation. We coupled selection and PL data from the Algonquin population with estimates reported for two Michigan, USA, populations by Johnston (1991a,b). To assess whether natural

selection was related to population-level PL, we plotted the absolute value of the strength of directional selection differentials and gradients (for the naturally pollinated treatment) obtained from the two studies. We also specifically evaluated how pollinator-mediated selection varied with PL in the Algonquin population by plotting the absolute value of pollinator-mediated selection against PL for 3 years, including the 95% CI for both estimates. All P -values and 95% confidence intervals were determined using bootstrapping (Efron & Tibshirani, 1993; 10 000 iterations) in programs written in R v. 2.15 base package (R Development Core Team, 2010).

Results and discussion

Two kinds of support for a positive association between pollen limitation and selection

The magnitude and direction of selection frequently change over time and space (Siepielski *et al.*, 2009; Kingsolver & Diamond, 2011), and this variation may reflect differences in the strength of ecological interactions such as herbivory and pollination (Benkman, 2013; Vanhoenacker *et al.*, 2013). We tested the hypothesis that selection is influenced by plant–pollinator interaction strength as represented by pollen limitation using two approaches. Both within *L. cardinalis* and among species, we found support for a general positive association between natural selection and population-level pollen limitation. Within *L. cardinalis*, data from two US populations and one Canadian population over 3 years found that selection gradients, $|\beta_n|$, increased with pollen limitation for all five traits analysed (Fig. 1; pollen limitation, selection coefficients and statistical tests are presented in Tables S3 to S14). Similarly, among species, our literature survey found that gradients were positively related to pollen limitation for all five trait classes and statistically significantly so for flower size ($F_{23} = 4.78$, $P = 0.039$, $R^2 = 0.12$), phenology ($F_{20} = 14.66$, $P = 0.001$, $R^2 = 0.42$) and vegetative traits ($F_8 = 7.44$, $P = 0.026$, $R^2 = 0.48$; Fig. 2, Table S2). The results from our review indicate a weaker general association between selection on floral traits and the degree of pollen limitation, in particular for flower size, than that reported by Ashman & Morgan (2004), who found a strong positive relationship ($r = 0.53$; $P = 0.004$) using data from 12 studies. Although some of this difference probably arises from the increased sample size, a direct comparison is difficult because the earlier study included both differentials and gradients as well as two measures of pollen limitation in a single correlation analysis.

The positive association between selection and pollen limitation is expected to be strongest for traits that are related to pollinator attraction and/or pollination efficiency. Thus, the positive relationship with selection on

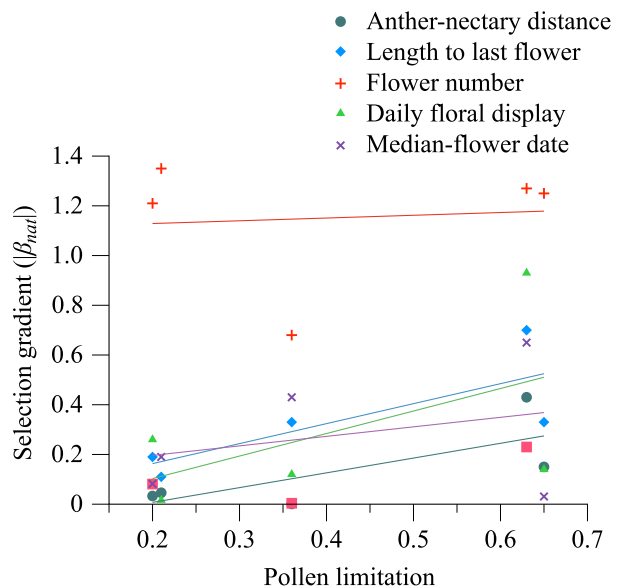


Fig. 1 The influence of population-level pollen limitation on selection gradients (absolute value) measured on five traits in three populations of *Lobelia cardinalis*. The Ontario population was studied in three consecutive years, and the Michigan populations were each studied in a single year. Coefficients are in units of standard deviation.

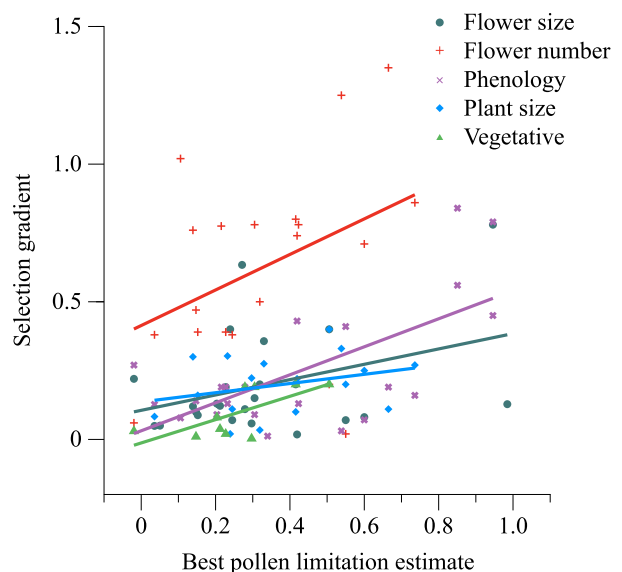


Fig. 2 Strength of direct selection vs. population-level pollen limitation from published studies representing 21 species. Absolute values of selection gradients via female fitness are shown in relation to the best available pollen limitation estimates for five types of traits.

vegetative traits (such as leaf area) was unexpected. Although this association might partly be explained by the increased opportunity for selection under pollen

limitation (Benkman, 2013), it cannot be explained by a simple correlation between vegetative and reproductive traits, because selection gradients account for such correlation. The pattern instead could arise if direct selection is caused by individual differences in levels of inbreeding (Willis, 1996) or by environmental factors driving the covariance between trait and fitness (Rausher, 1992).

Pollen limitation and pollinator-mediated selection

The hypothesized link between pollen limitation and selection should apply specifically to that portion of selection caused by pollinators. For *L. cardinalis*, direct pollinator-mediated selection could be measured only in the Algonquin population, where selection strength, $|\Delta\beta_{poll}|$, was greatest for all traits in 2010 (Fig. 3), when

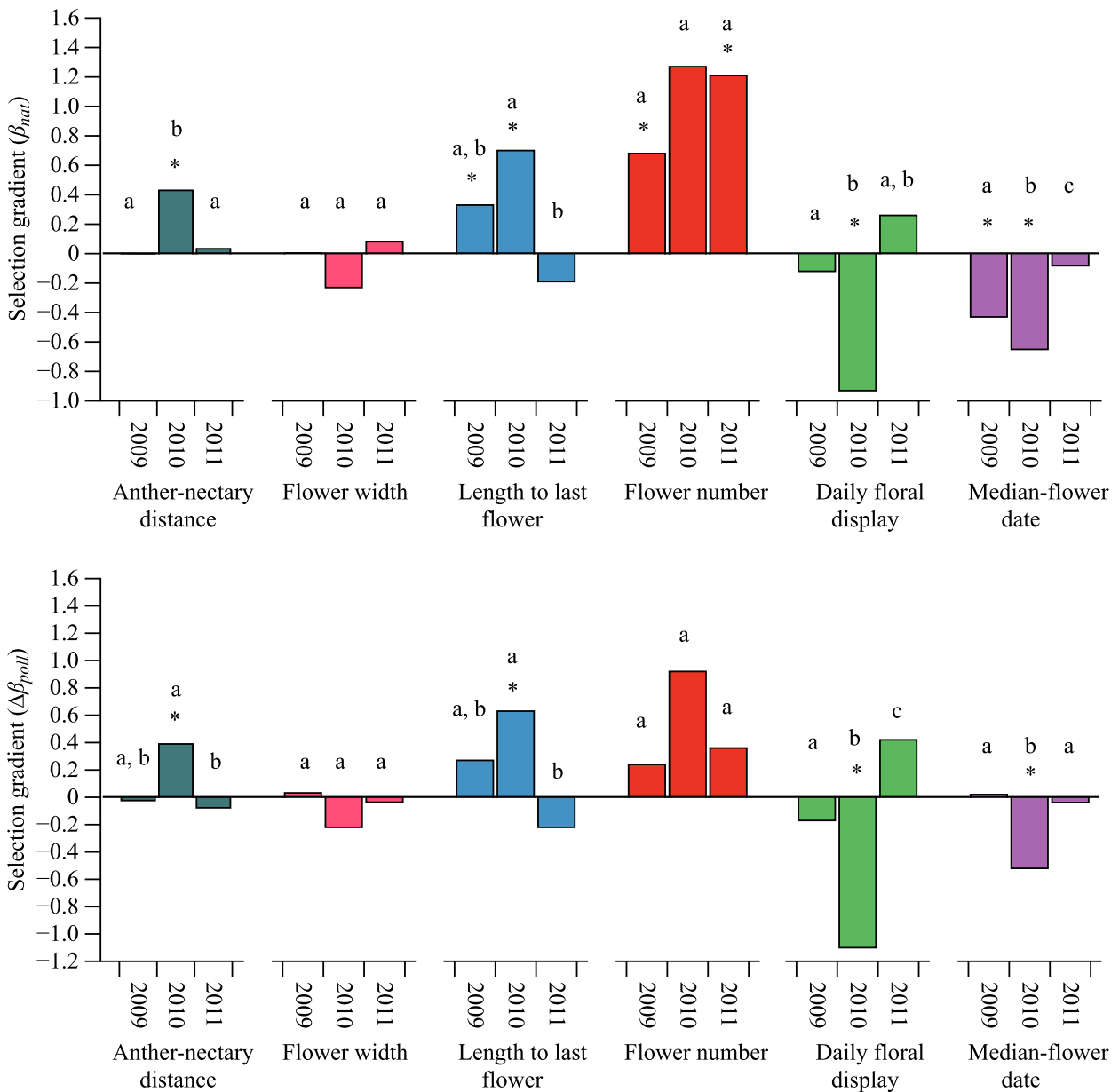


Fig. 3 Direct selection in 3 years for six traits in a naturally pollinated population of *Lobelia cardinalis* in Ontario. Top panel: selection gradients, β_{nat} ; bottom panel: pollinator-mediated selection gradients, $\Delta\beta_{poll}$. Coefficients are in units of standard deviation. For each trait and year, “*” indicates that the estimate of selection was statistically significantly different from 0 ($P < 0.05$). Bars not sharing a letter differed significantly among years for each trait ($P < 0.05$).

pollen limitation was most severe ($PL = 0.63$; Fig. 4). Nevertheless, despite a nearly two-fold difference in pollen limitation between 2011 ($PL = 0.2$) and 2009 (0.36), there was no associated difference in the strength of pollinator-mediated selection, other than for daily floral display (Fig. 4, Table S14). Thus, the expected positive relationship was found only when comparing pollinator-mediated selection in the year (2010) with the most severe pollen limitation to selection in the other 2 years. Sletvold & Ågren (2014) recently studied two populations in each of two orchid species over multiple years. Similar to the results from *L. cardinalis*, they found that pollinator-mediated selection differed among years for some traits but that mean pollen limitation of the population could not consistently predict the strength of pollinator-mediated selection.

Pollen limitation, variance in relative fitness and selection

The maximum intensity of selection is established by the variance in relative fitness, I , also known as the

opportunity for selection (Arnold & Wade, 1984), which is expected to increase in an accelerating fashion with pollen limitation (Benkman, 2013). In the populations studied here, there is no indication that the opportunity for selection increases with pollen limitation (Fig. 5). Specifically, two Michigan populations have very similar values of I (~ 2.5) despite a greater-than-threefold difference in pollen limitation (0.21 vs. 0.65). Furthermore, within the Algonquin population, the opportunity for selection was largest in 2011, when pollen limitation was weakest. Although it is premature to make conclusions regarding the shape of the relation between I and pollen limitation in *L. cardinalis* with only five population-year combinations, it is clear that the opportunity for selection is not well predicted by pollen limitation. This weak relationship suggests that seed production depends on factors other than pollen limitation. Although random factors are certainly involved, another prominent possibility is herbivory and ovule or seed predation. In 2009, Algonquin population slugs and weevils were found to cause a large increase in fitness variance as well as selection on plant

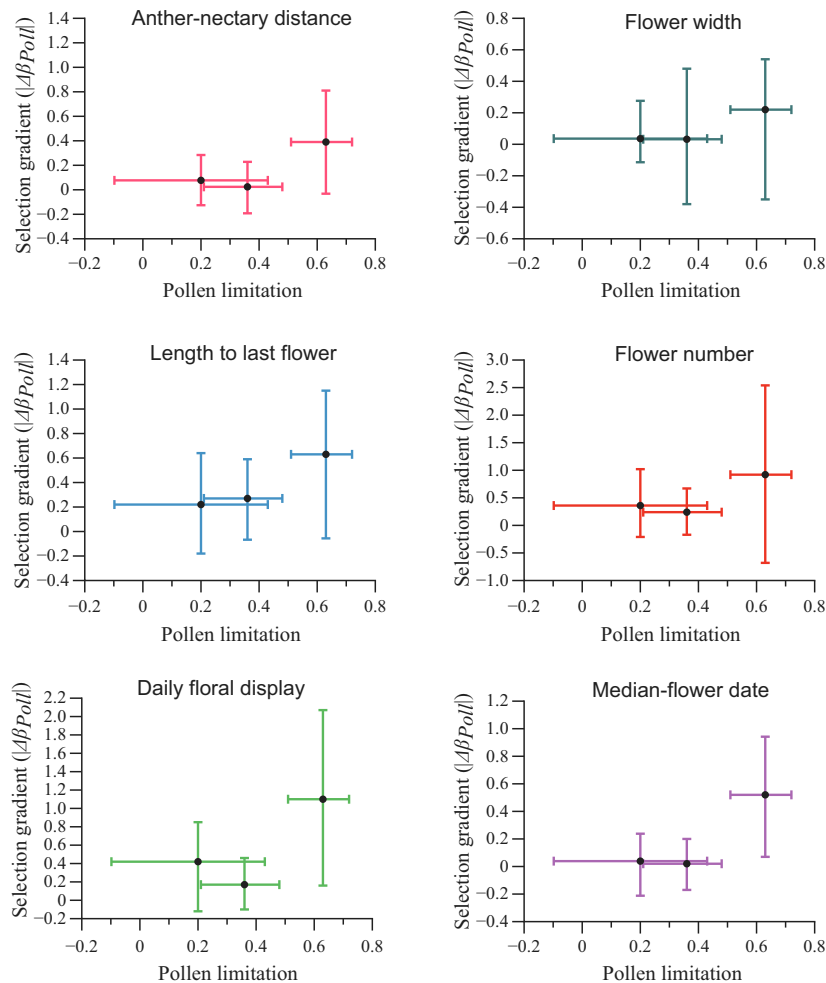


Fig. 4 Strength of direct pollinator-mediated selection ($\Delta\beta_{poll}$) vs. population-level pollen limitation in an Ontario population of *Lobelia cardinalis* over 3 years. Bootstrapped 95% CI are shown for both variables.

size and flowering time (Bartkowska & Johnston, 2012). Interactions such as seed predation and herbivory will increase fitness variance but will obscure its relationship with pollen limitation. Selection gradients in these populations also failed to show evidence of increasing with the opportunity for selection, and only

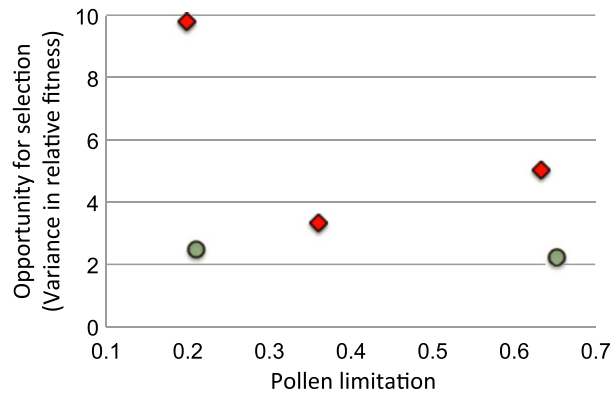


Fig. 5 Levels of pollen limitation and opportunity for selection (variance in relative fitness, I) in five population-year combinations of *Lobelia cardinalis*. Diamonds represent 3 years of the Algonquin (Ontario) population; circles represent two Michigan populations.

the gradients for flower number exceeded one-half the maximum possible (square root of I , Fig. 6). This contrasts with results from two nonrewarding orchid species (Sletvold & Ågren, 2014), where pollinator-mediated selection increased with I .

Conclusions

The results presented here for different populations and years within *L. cardinalis* and for published studies representing 21 species support the hypothesis of increased selection strength with increased pollen limitation (Johnston, 1991, 1992). Unexpectedly, both kinds of support represent net direct selection rather than pollinator-mediated direct selection, which should be most closely related to pollen limitation. The results from *L. cardinalis* and those from two orchid species by Sletvold & Ågren (2014) represent the two largest within-species studies of pollen limitation and pollinator-mediated selection. It is therefore surprising that within all three species pollen limitation fails to predict pollinator-mediated selection.

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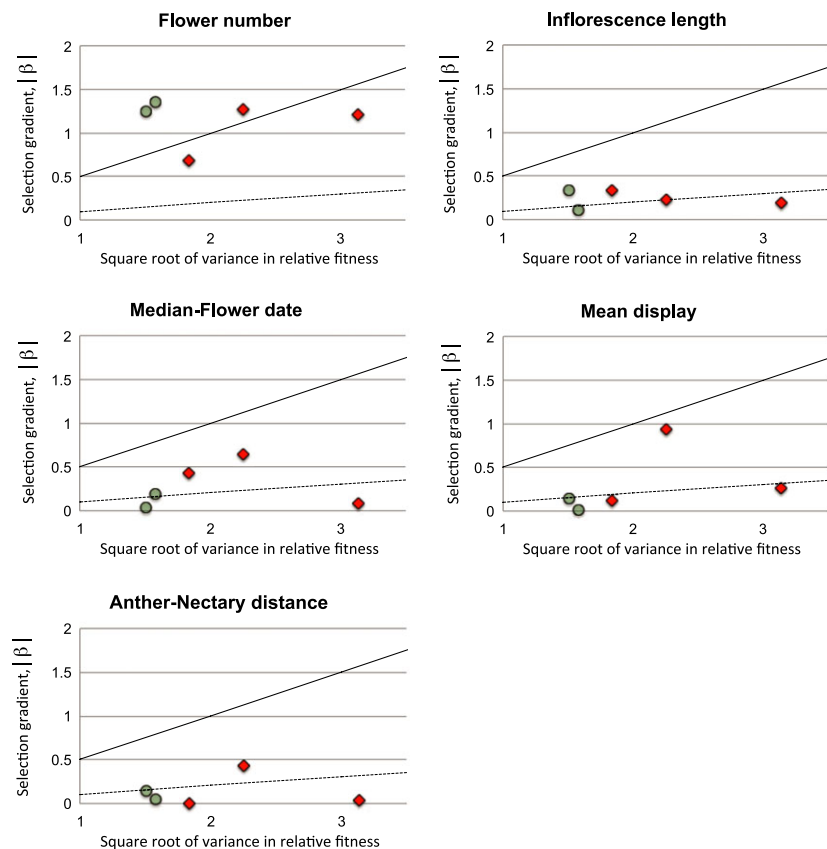


Fig. 6 Relationship between directional selection gradients and the maximum possible (square root of variance in relative fitness) in 3 years of the Algonquin population (diamonds) and two Michigan populations (circles). Lines indicate 50% (solid line) and 10% (dotted line) of maximum possible selection.

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Supporting information

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

Table S1. Dataset created from a literature survey of selection and pollen limitation estimates for species for which both types of estimates are available.

Table S2. Summary of the relationship between selection strength and pollen limitation for five trait types estimated from a survey of all studies reporting selection and pollen limitation published prior to January 2014.

Table S3. Means, standard deviations and sample sizes for traits and fitness measured in the naturally pollinated and hand-pollinated treatments of *L. cardinalis* 2009.

Table S4. Means, standard deviations and sample sizes for traits and fitness measured in the naturally pollinated and hand-pollinated treatments of *L. cardinalis* 2010.

Table S5. Means, standard deviations and sample sizes for traits and fitness measured in the naturally pollinated and hand-pollinated treatments of *L. cardinalis* 2011.

Table S6. Comparison of pollen limitation across three years in a population of *L. cardinalis*.

Table S7. Total selection in 2009: Comparison of total directional selection, (s_i) and total quadratic selection (γ_{ii}) for six traits in naturally pollinated and hand-pollinated plants in a population of *L. cardinalis*.

Table S8. Total selection in 2010: Comparison of total directional selection (s_i) and total quadratic selection (γ_{ii}) for six traits in naturally pollinated and hand-pollinated

plants in a population of *L. cardinalis*.

Table S9. Total selection in 2011: Comparison of total directional selection (s_i) and total quadratic selection (γ_{ii}) for six traits in naturally pollinated and hand-pollinated plants in a population of *L. cardinalis*.

Table S10. Direct selection in 2009: Comparison of directional selection gradients (β_i), and quadratic selection gradients (γ_{ii}) for six traits in naturally pollinated ($N = 367$) and hand-pollinated ($N = 154$) plants in a population of *L. cardinalis*.

Table S11. Direct selection in 2010: Comparison of directional selection gradients (β_i), and quadratic selection gradients (γ_{ii}) for six traits in naturally pollinated ($N = 181$) and hand-pollinated ($N = 199$) plants in a population of *L. cardinalis*.

Table S12. Direct selection in 2011: Comparison of directional selection gradients (β_i), and quadratic selection gradients (γ_{ii}) for six traits in naturally pollinated ($N = 125$) and hand-pollinated plants ($N = 136$) in a population of *L. cardinalis*.

Table S13. Total Selection Among Year: Differences among years in estimates of directional selection differentials (s_i), for six traits in naturally pollinated (s_{nat}) and hand-pollinated (s_{hp}) plants in a population of *L. cardinalis*.

Table S14. Direct Selection Among Years: Differences among years in estimates of directional selection gradients (β_i), for six traits in naturally pollinated (β_{nat}) and hand-pollinated (β_{hp}) plants in a population of *L. cardinalis*.

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