

# Pollinators cause stronger selection than herbivores on floral traits in *Lobelia cardinalis* (Lobeliaceae)

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Received: 26 September 2011

Accepted: 15 November 2011

New Phytologist (2012) 193: 1039–1048

doi: 10.1111/j.1469-8137.2011.04013.x

**Key words:** floral evolution, herbivore-mediated selection, *Lobelia*, natural selection, plant–animal interactions, pollen limitation, pollinator-mediated selection, selection gradients.

## Introduction

Measures of selection on floral traits in flowering plants are frequently motivated by the assumption that pollinators are the primary agents of selection. Broad associations between floral traits and pollinators – pollination syndromes – support the importance of plant–pollinator interactions, and suites of floral characters have been shown to be associated with particular functional groups of pollinators (Fenster *et al.*, 2004 and references therein). Several studies have also shown pollinators to exert selection on floral traits such as morphology (e.g. Campbell, 1989; Campbell *et al.*, 1991; Alexandersson & Johnson, 2002; Maad & Alexandersson, 2004; Sletvold *et al.*, 2010), phenology (Sandring & Ågren, 2009) and inflorescence architecture (Harder & Barrett, 1995; Nattero *et al.*, 2010; Sletvold *et al.*, 2010). Despite a growing body of selection studies in plants, very few have quantified the contribution of pollinators to total selection on floral traits (Galen & Cuba, 2001; Alexandersson & Johnson, 2002; Fishman & Willis, 2008; Sandring & Ågren, 2009; Parachnowitsch & Kessler, 2010; Sletvold *et al.*, 2010). This is problematic for two reasons. First, traits typically assumed to have evolved for pollination effectiveness (such as floral shape and flower number) may have been shaped by other nonpollinator sources of selection such as herbivores

## Summary

- Measures of selection on floral traits in flowering plants are often motivated by the assumption that pollinators cause selection. Flowering plants experience selection from other sources, including herbivores, which may enhance or oppose selection by pollinators. Surprisingly, few studies have examined selection from multiple sources on the same traits.
- We quantified pollinator-mediated selection on six floral traits of *Lobelia cardinalis* by comparing selection in naturally and supplementally (hand-) pollinated plants. Directional, quadratic and correlational selection gradients as well as total directional and quadratic selection differentials were examined. We used path analysis to examine how three herbivores – slugs, weevils and caterpillars – affected the relationship between floral traits and fitness.
- We detected stronger total selection on four traits and correlational selection ( $\gamma_{ij}$ ) on three trait combinations in the natural pollination treatment, indicating that pollinators caused selection on these traits. Weak but statistically significant selection was caused by weevil larvae on stem diameter and anther–nectary distance, and by slugs on median-flower date.
- In this study, pollinators imposed stronger selection than herbivores on floral traits in *L. cardinalis*. In general, the degree of pollen limitation and rate of herbivory are expected to influence the relative strength of selection caused by pollinators or herbivores.

(reviewed in Strauss & Irwin, 2004 and Strauss & Whittall, 2006). Second, in partially self-fertilizing populations where inbreeding depression affects both fitness and trait values, a correlation between phenotype and fitness is automatically established independent of any plant–animal interaction. In such cases, selection is in fact occurring but the role, if any, of presumed selective agents is wholly unknown (Willis, 1996).

Understanding how selective agents enhance or oppose one another remains a major goal in evolutionary ecology (Herrera, 2000). Flowering plants face the problem of simultaneously attracting pollinators and avoiding herbivores, and thus are likely to experience selection from several sources (reviewed in Strauss & Irwin, 2004 and Strauss & Whittall, 2006). Traits such as floral morphology (Galen & Cuba, 2001), nectar production (Adler & Bronstein, 2004) and plant size and floral display (Sletvold & Grindeland, 2008) have been shown to simultaneously attract pollinators and herbivores. Like pollinators, herbivores can exert selection on floral traits by targeting specific individuals. Herbivores can also affect pollinator-mediated selection either by altering pollinator behavior (e.g. damaged plants are less likely to be visited by pollinators; Irwin, 2006) or by masking pollinator preferences (e.g. if herbivores cue in on the same traits as pollinators, stronger herbivore-mediated selection will overwhelm

pollinator-mediated selection; reviewed in Strauss & Irwin, 2004; Gomez, 2003). The evolutionary effect on traits experiencing multiple sources of selection, however, is not easily predicted (Herrera, 2000). Understanding the forces that shape plant trait evolution thus requires assessing how multiple sources of selection interact.

In this study, we examined the relative importance of three herbivores – caterpillars, *Trichordestra legitima* (Noctuidae); weevils, *Cleopmiarus hispidulus* (Curculionidae); and slugs *Arion subfuscus* (Arionidae) – and a hummingbird pollinator, *Archilochus colubris* (Trochilidae), in causing selection on floral traits through seed production in *Lobelia cardinalis* (Lobeliaceae). We directly quantified the selective role of pollinators by comparing selection in naturally pollinated and supplementally (hand-) pollinated subpopulations. If pollinators are exerting selection on floral traits through seed production, then selection should be stronger in the naturally pollinated treatment. Because pollinators are likely to act as agents of selection via seed production when individuals are pollen-limited (Johnston, 1991a,b; Ashman & Morgan, 2004), we also assessed the degree of pollen limitation within the population. We could not experimentally manipulate the rates of herbivory, and therefore studied selection caused by herbivores using mediation analysis (Preacher & Hayes, 2008) within a path analysis framework (reviewed in Mitchell, 2001).

## Materials and Methods

### Study species and site

*Lobelia cardinalis* L. (Lobeliaceae) is a short-lived herbaceous perennial distributed throughout eastern North America (Bowden, 1959). Throughout its Canadian range, *L. cardinalis* is pollinated solely by *A. colubris* (Trochilidae), the ruby-throated hummingbird (Bertin, 1982; Johnston, 1991a). Although individuals are self-compatible, pollinators are required for seed production (Johnston, 1991b). Mating system studies in other populations have found intermediate selfing rates and high inbreeding depression (Johnston, 1992). Near the end of the flowering season, plants produce one (occasionally more) overwintering basal rosette.

We studied plants growing along the Petawawa River in Algonquin Provincial Park in Ontario, Canada, from 15 July to 20 October 2009. In this area, *L. cardinalis* was attacked by three herbivores: larvae of *C. hispidulus* (Curculionidae; Anderson, 1973) fed on ovules and developing seeds; caterpillars of *T. legitima* (Noctuidae) ate anther tubes; and slugs, provisionally *A. subfuscus* (Arionidae; R. Forsyth, pers. comm.), consumed either whole or large parts of inflorescences.

### Experimental treatment: pollen supplementation

In July 2009, 854 inflorescences were tagged. Of these, 619 were naturally pollinated and 239 received supplemental pollination. We assumed that inflorescences represented separate individuals, although a small proportion may have been genets arising from multiple basal rosettes formed at the end of previous flowering

seasons. All individuals in the supplemental hand-pollination treatment were fully pollinated by saturating all receptive stigmas every 3 d. Individuals received a mixture of outcross and self-pollen. Because *L. cardinalis* is mixed mating, using a mixture of outcross- and self-pollen more closely emulates natural pollination.

### Traits measured, herbivory and female fitness

On each plant, we measured the total width of the bottom three petals taken as the distance between the tips of the outermost petals; the length of the middle petal; the width of the middle petal; and the distance from the anthers to the bottom of the nectary. Whenever possible, two female-phase and two male-phase flowers were measured. Individual-level estimates of these traits were taken as the average of measured flowers. Flower size was estimated as the geometric mean of total width of the bottom three petals, the length of the middle petal and the width of the middle petal (Mosimann & James, 1979). The total number of flowers produced, the date the median flower was female and the average number of flowers open per d of flowering (a measure of daily floral display) were estimated from surveys of open flowers conducted throughout the flowering season, typically every 3–4 d between 29 July and 14 September. We also measured the stem diameter 5 cm above ground. Stem diameter is a good proxy for plant size; it is strongly correlated with plant height ( $r = 0.75$ ,  $P < 0.0001$ ,  $n = 422$ ). We used stem diameter rather than height in our analysis because we had more complete information for stem diameter. Damage caused by herbivores was treated as a continuous variable and scored as the proportion of flowers or fruit damaged by each herbivore. Individual plants were surveyed for mature fruit every 3–4 d from mid-September to mid-October. All fruit were collected as they matured, including damaged fruit. Female fitness was quantified as the total number of seeds produced per individual.

### Selection analysis

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 (Lande & Arnold, 1983) for univariate and multitrait models. For clarity we refer to directional and nonlinear estimates from univariate models as ‘selection differentials’ and to estimates from multitrait models as ‘gradients’. We estimated the following: total directional selection differential ( $s_j$ ), total quadratic selection ( $\gamma_{ii}$ ), directional selection gradients ( $\beta_j$ ), quadratic selection gradients ( $\gamma_{ii}$ ) and correlational selection ( $\gamma_{ij}$ ). The quadratic selection differentials and gradients presented for each trait are a doubling of the regression coefficients estimated by statistical software (Lande & Arnold, 1983; Stinchcombe *et al.*, 2008). Correlational selection ( $\gamma_{ij}$ ) estimates were obtained from multitrait regression models that included linear and quadratic terms for each trait and all pairwise products among traits.

All coefficients were estimated using Proc GLM in SAS version 9.2 (SAS Institute, 2008). The residuals from all regression models were heteroscedastic and not normally distributed. Thus,

regression coefficient *P*-values and 95% bias-corrected and accelerated (BCa) confidence intervals (Efron & Tibshirani, 1993) were determined using 10 000 bootstrap iterations in programs written in Mathematica (Wolfram Research, Inc., 2010).

We assessed whether pollinators exerted selection on the six plant traits by comparing selection coefficients between the natural and hand-pollination treatments using randomization tests (10 000 iterations, programs written in Mathematica; Wolfram Research, Inc., 2010). These comparisons were conducted for selection differentials (univariate regression) and selection gradients (multitrait regression), and in each case for both directional selection and nonlinear selection.

### Mediation analysis

It was not possible to experimentally manipulate herbivory without affecting the natural reproduction of *L. cardinalis*; herbivore exclusion would exclude hummingbirds as well, and heavy use of pesticide is undesirable in a natural area. We employed mediation analysis (Preacher & Hayes, 2008) to assess whether herbivores exerted selection on phenotypic characters. Mediation analysis tests whether a variable exerts its effect on an outcome variable through one or more intervening or mediating variables (reviewed in Preacher & Hayes, 2008). Unlike other approaches, the multiple-mediator model of Preacher & Hayes (2008) can estimate the total indirect effect caused by multiple intervening variables as well as the specific indirect effect caused by each unique mediator. As such, the relative magnitudes of the specific indirect effects associated with each of the herbivores can be readily compared. In addition, the indirect effects and the corresponding confidence intervals are bootstrapped in the multiple-mediator model. Indirect effects are not normally distributed (discussed in Preacher & Hayes, 2008) and thus nonparametric techniques are preferred.

We devised a path model (for the naturally pollinated plants) where each trait and the damage caused by each herbivore had a direct effect on fitness. We applied the path analysis only to the natural pollination treatment group using standardized trait values, relativized seed number, and proportion of flower or fruit damaged. Individuals with incomplete information were removed from the analysis, leaving a sample size of 408. The SPSS mediation analysis macro written by Preacher & Hayes (2008) was used to obtain estimates of total and specific indirect effects and the corresponding confidence intervals. We used 2000 iterations for the bootstrapping procedure and present the BCa bootstrapped confidence intervals (Efron & Tibshirani, 1993).

We present the path model that includes all direct effects of the traits on fitness (so that the path model is comparable to the results obtained for pollinator-mediated selection) and the indirect effects that differed from zero. The indirect effects (paths leading from traits to herbivores to seed number) in our path model indicate whether herbivores were agents of selection on the measured traits. We used AMOS 7 (Arbuckle, 2006) to obtain an estimate of model fit, specifically, the chi-squared goodness of fit (Mitchell, 2001). A nonsignificant chi-squared value indicates that a model is not significantly different from the

**Table 1** Total selection: comparison of total directional selection ( $s_i \pm SE$ ) and total quadratic selection ( $\gamma_{ii}$ ) for six traits in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*

Trait	Naturally pollinated			Hand pollinated			Selection difference			
	$s_i \pm SE$	$P$	$\gamma_{ii} \pm SE$	$s_i \pm SE$	$P$	$\gamma_{ii} \pm SE$	$ \Delta s_{poll} $	$P$	$ \Delta \gamma_{poll} $	$P$
Anther–nectary distance	0.29 ± 0.09	0.0018	0.17 ± 0.11	0.18 ± 0.02	0.020	-0.15 ± 0.11	0.27	0.11	0.23	0.073
Flower size	0.26 ± 0.09	0.0032	-0.03 ± 0.08	0.29 ± 0.08	< 0.0001	-0.14 ± 0.11	0.16	0.03	0.42	0.33
Stem diameter	0.95 ± 0.07	< 0.0001	0.50 ± 0.11	0.63 ± 0.07	< 0.0001	0.09 ± 0.10	0.53	0.32	0.023	0.052
Flower number	1.04 ± 0.07	< 0.0001	-0.06 ± 0.10	0.73 ± 0.06	< 0.0001	-0.02 ± 0.07	0.73	0.31	0.050	0.43
Daily floral display	0.75 ± 0.07	< 0.0001	-0.21 ± 0.10	0.48 ± 0.07	< 0.0001	-0.13 ± 0.12	0.16	0.27	0.037	0.35
Median-flower date	-0.65 ± 0.07	< 0.0001	0.30 ± 0.08	-0.42 ± 0.07	< 0.0001	0.11 ± 0.07	0.12	0.23	0.034	0.084

Coefficients are in units of standard deviation. For each trait, the absolute value of the difference in selection coefficients ( $|\Delta s_{poll}|$  and  $|\Delta \gamma_{poll}|$ ) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

observed correlations in the data and is therefore a good fit (Mitchell, 2001). Because our data do not meet the assumptions of multivariate normality, we used Bollen–Stine (Bollen & Stine, 1993) adjusted  $P$ -values for the chi-squared goodness-of-fit test.

## Results

### Pollen limitation, trait means and trait correlations

Seed and fruit number were pollen limited in this population of *L. cardinalis*, with naturally pollinated plants making 55% fewer seeds per flower and 22% fewer seeds per fruit than plants receiving supplemental pollen. Although plants in the naturally pollinated treatment made more flowers on average than plants in the hand-pollinated treatment, naturally pollinated plants made 42% fewer seeds (naturally pollinated plants made 1836 seeds on average vs hand-pollinated plants which made 1066 seeds on average,  $P < 0.0001$ ; Supporting Information, Table S1). All six traits were significantly correlated with one another in both treatments (Table S2).

### Comparison of selection on traits in two pollination treatments

All traits in both treatments experienced total directional selection (Table 1), which was statistically significantly stronger in the naturally pollinated treatment for stem diameter, flower number, daily floral display and median-flower date ( $|\Delta s_{\text{poll}}|$  in Table 1). In the naturally pollinated treatment, there were also statistically significant positive quadratic selection differentials ( $\gamma_{ii}$ ) for stem diameter and median-flower date (Table 1). This is not indicative, however, of disruptive selection for either trait, because the minima were out of range of the trait values observed in the population (plots not shown; Mitchell-Olds & Shaw, 1987). These nonlinear effects were marginally statistically greater in the naturally pollinated treatment ( $|\Delta \gamma_{\text{poll}}|$  in Table 1).

In the naturally pollinated treatment, stem diameter, flower number and median-flower date were direct targets of selection; the directional selection gradients for these traits and the quadratic selection gradient for median-flower date were significantly different from zero (Table 2). Although there were no statistically significant differences in selection gradients between treatments, the directional selection gradients were 50 and 32% greater in the naturally pollinated treatment for stem diameter and flower number, respectively. In addition, the quadratic selection gradient for median-flower date was 47% greater in the naturally pollinated treatment. Stronger selection gradients in the natural pollination treatment are suggestive of pollinator-mediated selection.

In the full multitrait model, including all linear, quadratic and pairwise trait combinations, 20 out of 27 regression coefficients were larger in absolute value in the naturally pollinated treatment (one-tailed sign test,  $P = 0.0096$ ), indicating that pollinators exerted selection on floral traits. In the hand-pollinated treatment, there were no estimates of correlational selection that were statistically greater than zero (Table 3). In the naturally

**Table 2** Direct selection: comparison of directional selection gradients ( $\beta_i \pm \text{SE}$ ), and quadratic selection gradients ( $\gamma_{ii}$ ) for six traits in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*

Trait	Naturally pollinated			Hand-pollinated			Selection difference			
	$\beta_i \pm \text{SE}$	$P$	$\gamma_{ii} \pm \text{SE}$	$\beta_{ii} \pm \text{SE}$	$P$	$\gamma_{ii} \pm \text{SE}$	$ \Delta \beta_{\text{poll}} $	$P$	$ \Delta \gamma_{\text{poll}} $	$P$
Anther–nectary distance	0.070 ± 0.08	0.39	0.02 ± 0.10	−0.047 ± 0.07	0.47	−0.09 ± 0.10	0.36	0.12	0.19	0.11
Flower size	−0.018 ± 0.08	0.82	0.07 ± 0.10	0.043 ± 0.07	0.52	−0.05 ± 0.12	0.77	0.06	0.34	0.12
Stem diameter	0.22 ± 0.11	0.059	0.27 ± 0.18	0.11 ± 0.09	0.24	0.01 ± 0.16	0.86	0.11	0.28	0.26
Flower number	0.74 ± 0.18	< 0.0001	0.05 ± 0.19	0.50 ± 0.11	0.00024	0.10 ± 0.11	0.40	0.24	0.17	0.05
Daily floral display	−0.13 ± 0.13	0.33	−0.05 ± 0.14	0.046 ± 0.08	0.57	0.07 ± 0.10	0.37	0.18	0.17	0.12
Median-flower date	−0.43 ± 0.08	< 0.0001	0.32 ± 0.10	−0.40 ± 0.07	< 0.0001	0.17 ± 0.07	0.014	0.03	0.42	0.15

Coefficients are in units of standard deviation. For each trait, the absolute value of the difference in selection coefficients ( $|\Delta \beta_{\text{poll}}|$  and  $|\Delta \gamma_{\text{poll}}|$ ) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

**Table 3** Correlational selection: comparison of correlational selection gradients ( $\gamma_{ij} \pm SE$ ) for 15 pairwise trait combinations in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*

	Naturally pollinated		Hand-pollinated		Selection difference	
	$\gamma_{ij} \pm SE$	<i>P</i>	$\gamma_{ij} \pm SE$	<i>P</i>	$ \Delta\gamma_{poll} $	<i>P</i>
Flower size × anther–nectary distance	−0.061 ± 0.12	0.41	−0.016 ± 0.10	0.94	0.045	0.37
Flower size × stem diameter	0.13 ± 0.13	0.44	0.0046 ± 0.10	0.74	0.13	0.29
Flower size × flower number	0.08 ± 0.20	0.84	−0.23 ± 0.12	0.25	0.31	0.25
Flower size × daily floral display	−0.14 ± 0.13	0.54	0.045 ± 0.08	0.99	0.19	0.18
Flower size × median-flower date	0.078 ± 0.09	0.29	−0.032 ± 0.10	0.78	0.11	0.20
Anther–nectary distance × stem diameter	−0.13 ± 0.14	0.46	−0.073 ± 0.09	0.43	0.057	0.45
Anther–nectary distance × flower number	−0.20 ± 0.25	0.29	0.26 ± 0.12	0.12	0.46	0.061
Anther–nectary distance × daily floral display	0.38 ± 0.17	0.0084	−0.073 ± 0.10	0.49	0.45	0.013
Anther–nectary distance × median-flower date	−0.0086 ± 0.09	0.69	−0.061 ± 0.08	0.29	0.052	0.50
Stem diameter × flower number	0.20 ± 0.26	0.64	−0.20 ± 0.15	0.43	0.40	0.18
Stem diameter × daily floral display	−0.16 ± 0.21	0.51	0.20 ± 0.10	0.11	0.36	0.10
Stem diameter × median-flower date	−0.14 ± 0.14	0.36	−0.12 ± 0.10	0.30	0.02	0.50
Flower number × daily floral display	0.54 ± 0.31	0.053	−0.18 ± 0.10	0.23	0.72	0.012
Flower number × median-flower date	−0.77 ± 0.32	0.025	−0.19 ± 0.10	0.065	0.58	0.044
Daily floral display × median-flower date	0.26 ± 0.25	0.38	0.00058 ± 0.10	0.98	0.26	0.18

For each trait combination, the absolute value of the difference in selection coefficients ( $|\Delta\gamma_{poll}|$ ) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

pollinated treatment, however, plants with greater anther–nectary distance and large daily floral display, plants with many flowers that had large daily floral display, and plants with many flowers and early flowering dates were favored (Table 3). Furthermore, correlational selection for these trait combinations was statistically greater in the naturally pollinated treatment ( $|\Delta\gamma_{poll}|$ , Table 3), consistent with the hypothesis that pollinators are selective agents shaping floral traits in *L. cardinalis*.

### Evaluating the effects of herbivory on the relationship between traits and fitness

Herbivory had only minor effects on patterns of selection. The indirect effect caused by weevils for stem diameter and anther–nectary distance and the indirect effect caused by slugs for median-flower date were small, although significantly different from zero (Table 4). Also, the model incorporating herbivore-mediated selection on plant traits appropriately represented the observed data ( $\chi^2 = 19.90$ , *df* = 18, *P* = 0.341, *n* = 408; Fig. 1), suggesting that weevils and slugs were potential agents of selection in this population of *L. cardinalis*. Tall plants and those with slightly greater anther–nectary distance had a slightly higher proportion of weevil damage, indicating that weevils may prefer taller plants or flowers with longer anther–nectary tubes (Fig. 1). Early flowering plants also had a slightly higher proportion of slug damage (Fig. 1). Herbivore-mediated selection was weak, however, as indicated by the very small mediating effects caused by weevils and slugs (column four in Table 5).

### Discussion

Studies of floral trait evolution have often assumed that pollinators are the dominant source of selection. Because plants

commonly interact with many animal species, plant traits may evolve in response to multiple sources of selection (reviewed in Strauss & Irwin, 2004 and Strauss & Whittall, 2006). A thorough understanding of the forces that shape floral evolution thus requires identifying the source(s) of selection, and, when multiple agents of selection are operating, assessing the relative importance of each source of selection. In this study, we evaluated selection on floral characters of *L. cardinalis* and assessed the extent to which selection on these characters was the result of the action of pollinators and herbivores. We found pollinators and not herbivores caused directional as well as correlational selection.

A limitation of our study is the inability to make direct quantitative comparisons of pollinator- vs herbivore-mediated selection. Although the pollination environment in this system could easily be manipulated (by altering the amount of pollen received by each individual), rates of herbivory could not be altered without either damaging a natural environment or affecting the pollination environment. Specifically, manipulating herbivory would require either heavy pesticide application or a method of excluding herbivores, such as bagging plants, which would alter pollinator behavior. Conversely, examining pollinator-mediated selection using a path analysis framework (as was done for herbivore-mediated selection) would require either extensive pollinator observations or another measure, such as stigmatic pollen loads, that could be a proxy for visitation (as was done in Irwin, 2006). Although we could not use an experimental approach to directly compare pollinator- and herbivore-mediated selection, we specifically designed our path model so that the direct effects of traits on fitness would be equivalent to our selection gradient estimates. In this sense the two approaches are comparable; the path analysis with herbivores included shows how selection gradients (direct paths) are altered by herbivores. Despite this limitation, it is clear that pollinators exert stronger selection than herbivores on the traits in this study.

**Table 4** Decomposition of indirect effects of traits on fitness of *Lobelia cardinalis* mediated by caterpillars (*Trichordestra legitima*), weevils (*Cleopmarius hispidulus*) and slugs (*Arion subfuscus*) estimated from path analysis

Trait	Total Indirect Effect		Indirect Effect Via Caterpillar		Indirect Effect Via Weevil		Indirect Effect Via Slug			
	Point estimate ± SE	BC 95% CI	Point estimate ± SE	BC 95% CI	Point estimate ± SE	BC 95% CI	Point estimate ± SE	BC 95% CI		
Anther-nectary distance	0.0005 ± 0.019	-0.035	0.038	0.0046 ± 0.013	-0.0186	0.033	-0.0157 ± 0.010	0.0117 ± 0.010	-0.001	0.044
Flower size	-0.0018 ± 0.018	-0.035	0.036	-0.0047 ± 0.011	-0.0286	0.016	0.0052 ± 0.009	-0.0023 ± 0.007	-0.020	0.011
Stem diameter	-0.0317 ± 0.026	-0.096	0.009	-0.0117 ± 0.014	-0.0608	0.009	<b>-0.0223 ± 0.016</b>	0.0022 ± 0.011	-0.014	0.032
Flower number	0.0154 ± 0.022	-0.022	0.065	0.0137 ± 0.011	-0.0022	0.045	-0.0039 ± 0.014	0.0056 ± 0.009	-0.008	0.030
Daily floral display	0.0043 ± 0.023	-0.045	0.049	-0.0167 ± 0.017	-0.0726	0.004	0.0144 ± 0.012	0.0067 ± 0.008	-0.009	0.025
Median – flower date	0.017 ± 0.016	-0.013	0.049	-0.0013 ± 0.008	-0.018	0.014	0.0026 ± 0.009	<b>0.0153 ± 0.010</b>	<b>0.002</b>	<b>0.042</b>

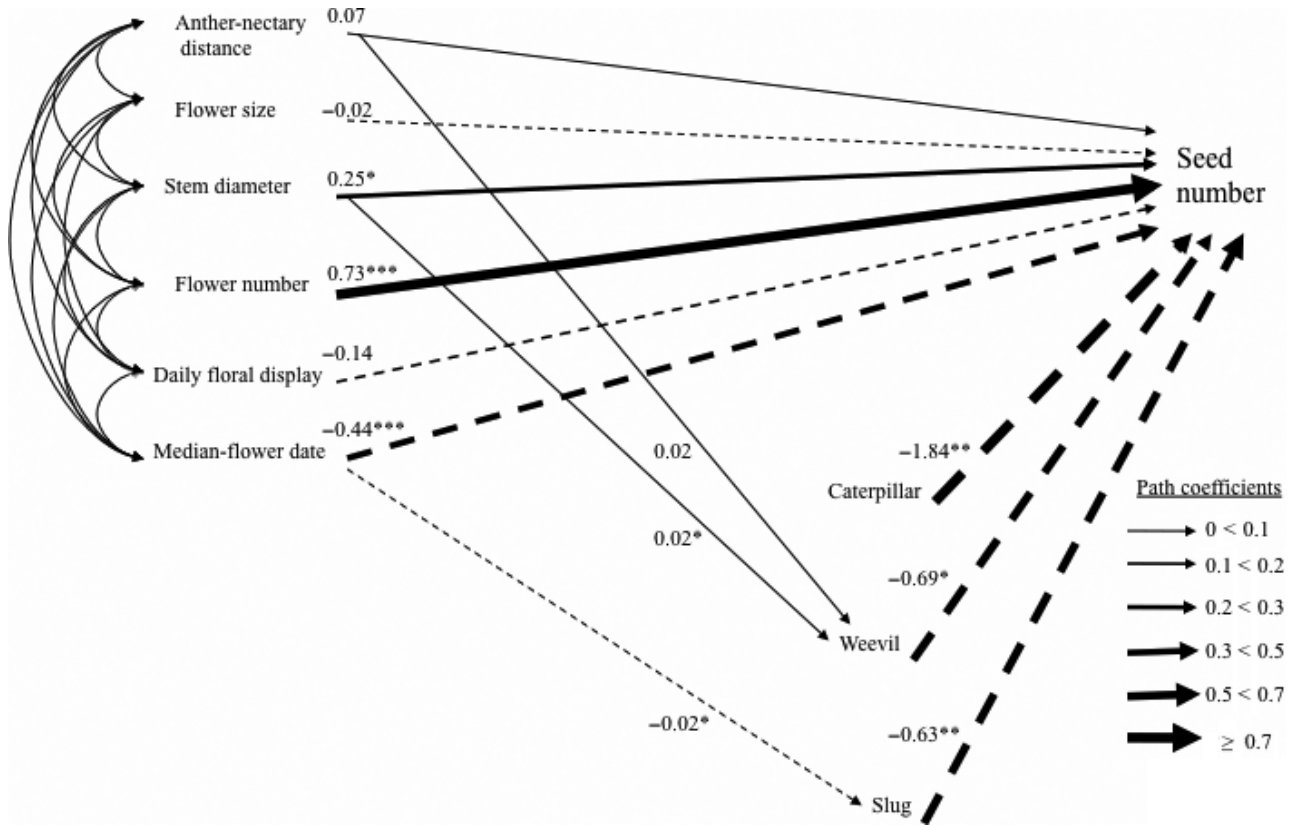
Standard errors and bias corrected bootstrap 95% confidence intervals were estimated using the script written by Hayes and Preacher (2008). Total indirect effects and indirect effects attributed to specific mediators (caterpillars, weevils, and slugs) are presented. Specific indirect effects can be significant even if the total indirect effect is not. Values in bold indicate an indirect effect that is significantly different from zero.

## Pollinators exert selection on floral traits

We evaluated whether selection on floral traits was caused by the action of pollinators by comparing the form and intensity of selection acting on naturally pollinated plants with those receiving supplemental hand-pollination. Estimates of selection differentials and gradients, and of correlational selection were greater for many traits in the naturally pollinated treatment, consistent with the hypothesis that pollinators are selective agents of floral traits in *L. cardinalis*. Total directional selection differentials for stem diameter, flower number, daily floral display and median-flower date were statistically significantly greater in the natural pollination treatment (Table 1). Similarly, for the three traits experiencing significant direct selection (stem diameter, flower number and median-flower date; Table 2), the selection gradients were larger under natural pollination, but the differences were not significant.

Pollinator-mediated selection also favored particular trait combinations, indicated by correlational selection. In the naturally pollinated treatment, plants with greater anther-nectary distance and large daily floral display, plants with many flowers that had large daily floral display, and plants with many flowers and early flowering dates were favored (Table 3).

Stronger directional selection on stem diameter (a proxy for plant height), flower number and median-flower date, as well as correlational selection on particular groups of traits in the natural pollination treatment, are consistent with observations of pollinator behavior. Pollinators visit taller plants first and tend to forage within a stem (pers. obs.; M. O. Johnston, 1991b). Because hummingbirds visit a small number of plants during a feeding bout, tall plants will tend to be pollinated more frequently than shorter plants. Pollinators also are likely to prefer plants with larger floral displays, as has been documented in a number of species (Sletvold *et al.*, 2010 and references therein). Although we also found that pollinator-mediated selection favored plants with many flowers, average daily floral display, when considered independently of other traits, did not influence total seed number. Instead, total positive directional selection on daily floral display results from its correlation with stem diameter (height) and total flower number, which do experience direct selection. Furthermore, tall plants (large stem diameter) with a large floral display did especially well, as shown by the correlational selection gradient. Thus, all else being equal, tall plants and tall plants with large daily floral displays produce more seeds, but for a particular plant height, change in floral display is unimportant. Pollinator migration rather than preference may also account for higher fitness of early flowering plants. Pollinator service likely peaked early in the flowering period before pollinators began their southerly migration (Bertin, 1982); thus early-flowering plants were likely pollinated more frequently than late-flowering individuals. In addition, more frequent pollinator visits earlier in the season, coupled with pollinator preference for plants with many flowers may also explain the correlational selection on flower number and flowering date. Similarly, correlational selection favoring early flowering plants with large daily floral display is consistent with pollinator activity. Pollinator visits to patches of plants were



**Fig. 1** Results of path analysis for the effects of herbivore damage on seed set in *Lobelia cardinalis*. Straight arrows reflect causal paths; curved arrows designate correlations. Solid arrows denote positive effects, and dashed arrows denote negative effects. The strength of the relationship is designated by arrow thickness. All direct effects of traits on seed number (including those not significantly different from zero) are shown. Only the indirect effects mediated by herbivore damage that were statistically different from zero are shown. Asterisks denote significant paths: \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ ; \*\*\*,  $P < 0.0001$ .

**Table 5** Comparison of direct effects (DE) of floral traits, estimated from path analysis, on fitness of *Lobelia cardinalis* with and without herbivore-mediated selection

	DE with no mediation (IE constrained to 0)	DE with herbivore-mediated selection (IE included)	Change in DE caused by IE	Size of mediating effect (percentage change of DE)
Anther-nectary distance (IE via weevil)	0.070	0.069	-0.001	1.4
Stem diameter (IE via weevil)	0.22	0.25	0.03	14
Median-flower date (IE via slug)	-0.43	-0.45	-0.02	4.7

Size of the mediating effects of weevil (*Cleopmarius hispidulus*) and slugs (*Arion subfuscus*) on floral traits in *L. cardinalis* is estimated as the percentage change of the direct effect as the result of the inclusion of an indirect effect (IE) mediated by herbivore damage.

sporadic late in the summer (pers. obs., M. P. Bartkowska); thus, plants with many receptive female flowers earlier in the season are more likely to be pollinated.

### Evidence for weak herbivore-mediated selection on flowering traits

We assessed which traits were possible targets of herbivore-mediated selection by identifying characters that had an indirect effect on fitness mediated by herbivore damage. Slug damage was weakly negatively related to median-flower date (Fig. 1), indicating slug-mediated selection favored later-flowering plants. The mediating effect of slugs, however, was small (inclusion on the indirect effect only changed the direct effect by 4.7%, Table 5)

and likely overwhelmed by other sources of selection favoring early flowering. Herbivores can cause directional selection on flowering time, if, for example, damage is concentrated at some point during the flowering season or herbivores target plants based on traits correlated with flowering time (see review in Strauss & Whittall, 2006). Slugs are unlikely to exert strong selection on flowering date in this system as they were present throughout the entire growing period and we found no evidence that slug damage was related to any other of the studied traits. It is possible, however, that slugs were targeting plants based on an unmeasured character strongly correlated with flowering time. In addition, grazing herbivores like slugs can mask selection caused by other sources (Herrera, 2000; Gomez, 2003) if a sufficient number of individuals are consumed such that the relationship

between a particular trait and fitness is disrupted. If slugs consumed more individuals of *L. cardinalis*, then it is possible that net selection on median-flower date could be altered.

Weevils may be exerting weak selection on plant size. Herbivores that oviposit on flowers of animal-pollinated plants depend on pollinators to provision their larvae, and thus should oviposit on flowers that are attractive to pollinators because they are most likely to set fruit (Strauss & Irwin, 2004). From this perspective, seed predators are likely to exert selection on floral characters and have been shown to exert selection on floral traits in multiple systems (Cariveau *et al.*, 2004; Rey *et al.*, 2006; Parachnowitsch & Caruso, 2008). We found tall plants (stem diameter was used as a proxy for plant height) and those with greater anther–nectary distance to be correlated with weevil damage, indicating that weevils may prefer taller plants and flowers with long anther–nectary tubes (Fig. 1). Anther–nectary distance, although not a direct target of selection (Table 2; Fig. 1), had a weak indirect effect on fitness through its association to weevil damage. Selection acting directly on stem diameter was partially mediated by the effect of weevil larvae (Fig. 1; Table 4); the inclusion of a path from stem diameter through weevil damage to fitness altered the direct effect of stem diameter to fitness, although the size of this mediating effect was small (Table 5). Thus, weevils have the potential to exert selection on floral traits in *L. cardinalis*, as indicated by significant indirect effects attributed to weevil damage; however, weevil-mediated selection was very weak (Table 4).

#### Other factors affecting the relationship between traits and fitness

In the hand-pollinated treatment, where we eliminated the selective effects of pollinators, we found selection for median-flower date and flower number (Table 4). Thus, some factor other than pollinator-mediated selection is causing an association between these traits and fitness; it is also unlikely that herbivores are the cause (see above). A positive relationship between flower number and fitness is expected when seed number is used as a proxy for fitness, because the total number of flowers sets an upper limit on potential seed production. Any study in which seed number is used as a measure of fitness will find a positive correlation between fitness and flower number. Direct selection on median-flower date could not be attributed to the action of pollinators. Thus, some other factor is favoring early-flowering plants. The quality of maternal plants may cause a correlation between fitness and flowering date. Some individuals can acquire resources faster, and therefore flower sooner, either because they are located in salubrious environments or because they have genes enhancing resource acquisition. Mating system and inbreeding depression can also affect estimates of selection. If individuals vary in their history of inbreeding, then differences between individuals in traits and fitness, as would be the case in a mixed mating system such as *L. cardinalis* (Johnston, 1990), could be the result of differences in inbreeding depression. Inbreeding depression for quantitative traits can result in a linear relationship between relative fitness and a trait(s) even if there is no causal relationship between the trait(s) and fitness (Willis, 1996). Although

*L. cardinalis* has been shown to suffer inbreeding depression in fitness (Johnston, 1992), we did not assess inbreeding depression in this study and thus cannot evaluate whether inbreeding depression is a contributing factor to the relationship between traits and fitness. Alternatively, the short growing season may select for the early flowering time. Although we did not assess the effect of temperature on fruit and seed development it is likely that sub-zero (°C) temperature may inhibit seed production. Frost and sub-zero temperatures were recorded at this site as early as September 1, 2009 (pers. obs., M. P. Bartkowska). This may explain why we found selection favoring early flowering and Johnston (1991b) found later-flowering dates were favored in more southern populations. Without further information regarding inbreeding depression, and more detailed measures of environmental variables, we cannot attribute selection on flowering date to a particular source.

#### Factors influencing the relative importance of pollinator- and herbivore-mediated selection

There is growing evidence that both herbivory and pollination play important roles in plant fitness (reviewed in Strauss & Irwin, 2004). The relative importance of pollinators vs herbivores in shaping selection on floral characters varies greatly between systems and is likely to depend on the strength of herbivore-mediated selection and the degree of pollen limitation. For instance, our conclusions about the selective effects of weevil larvae contrast with those of a similar study conducted in a sister species, *Lobelia siphilitica* (Parachnowitsch & Caruso, 2008), in which weevil larvae and not pollinators were attributed with causing selection on floral phenology. The difference between the selective effects of weevil larvae found in that study and the current one are likely the result of different amounts of damage suffered by *L. siphilitica* and *L. cardinalis*; 89% of *L. siphilitica* plants experienced weevil damage compared with 6% of *L. cardinalis* individuals (Table S3). Because the intensity of herbivory (the number of individual plants attacked and the damage sustained by attacked individuals) likely depends on factors influencing herbivore abundance and movement, herbivore-mediated selection is likely to vary geographically and over time. Further study is required to understand how herbivore-mediated selection that varies across space and time influences plant trait evolution (see Gomez & Zamora, 2000).

The relative importance of herbivores vs pollinators in shaping floral characters may also depend on the degree of pollen limitation. Pollen limitation is expected to intensify selection on characters that increase attractiveness to pollinators (Johnston, 1991a,b; Ashman & Morgan, 2004; Fishman & Willis, 2008). Cariveau *et al.* (2004), Rey *et al.* (2006) and Parachnowitsch & Caruso (2008) found that seed herbivores exerted stronger selection on floral traits than pollinators. By contrast, the present study and similar studies of *Dactylorhiza lapponica* (Sletvold *et al.*, 2010) and *Arabidopsis lyrata* (Sandring & Ågren, 2009) found pollinators rather than herbivores to be agents of selection on floral characters. In studies where herbivores had greater selective effects than pollinators, plant populations were not



pollen-limited. We found that hand-pollinated plants produced 42% more seed than naturally pollinated plants. Pollen limitation, however, is not always predictive of pollinator-mediated selection. Parachnowitsch & Kessler (2010) found pollinator-mediated selection on floral size and display in *Penstemon digitalis*, but did not find that seed set was pollen-limited. Future studies evaluating the relative importance of different selective agents will benefit from quantifying pollen limitation.

## Acknowledgements

We thank C.G. Eckert and R.G. Latta for helpful discussion; R.J. Mitchell for helpful comments on the manuscript; C.M. Caruso for sharing locations of *L. cardinalis* populations; M. Bartkowski, E. Busing, E. Lapalme, and J. Pollack for assistance in the field; L. Fraughton and G. Cummings for assistance with seed counts; R.G. Forsyth for slug identification; and John Winters, park superintendent, and staff of Algonquin Provincial Park for permits and support in the field. This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (Canada) to M.O.J.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Table S1** Means, standard deviations and sample sizes for traits and fitness measured in the naturally pollinated and hand-pollinated treatments of *Lobelia cardinalis*

**Table S2** Pearson product–moment correlations among six traits and fitness (seed number) measured in naturally pollinated and hand-pollinated treatments of *Lobelia cardinalis*

**Table S3** Number and proportion of individual plants and flowers of *Lobelia cardinalis* damaged by slugs (*Arion subfuscus*), caterpillars (*Trichordestra legitima*) and weevil larvae (*Cleopmiarus hispidulus*) for the naturally pollinated treatment

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