Ecological Causes and Evolutionary Consequences Underlying Fitness Variation in *Lobelia cardinalis*

by

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ABSTRACT

Understanding the functional relationship between characters and components of fitness is a central goal of evolutionary biology. The studies in this thesis examined the ecological causes and evolutionary consequences underlying differences in fitness among individuals of *Lobelia cardinalis*.

Flowering plants experience selection from many sources, which may enhance or oppose selection by pollinators. In the second chapter of this thesis, the role of pollinators and herbivores in shaping selection on floral characters was investigated. Floral traits experienced pollinator-mediated selection and weak selection by weevil larvae and slugs.

Because pollinators also forage according to local density of flowers, in the fourth chapter I explored how local density of individual plants and flowers influences fitness of individual plants. Plants at dense sites produced more seeds, consistent with pollinator preference for denser patches. Individual female-phase flowers produced more seeds as the density of surrounding male-phase flowers increased and female-phase flowers decreased. This study highlights how plant phenotype and local density influence pollination and subsequent plant fitness.

In *L. cardinalis* rosette formation (a life-history character) partly shapes the distribution of plants, and may influence plant survival and fitness. In the fifth chapter, I explored how variation in allocation to clonal reproduction among plants (ramets) and genets influenced survival and fitness. Plants that produced more and larger rosettes realized higher survival independent of the phenotype of the parental. Plants that produced one rosette in 2009 produced more seeds in 2010 than plants that produced more than one rosette. This pattern was reversed in the following time period; plants that produced more rosettes in 2010 produced more seeds in 2011.

The relative importance of pollinators versus other selective agents in shaping floral traits, as well as the intensity of competition among individual plants and flowers likely depend on the extent to which reproduction is pollen limited. In the third chapter, I explored how pollen limitation affected selection on floral traits via female fitness and found a weak relationship. Although this seems to contradict intuition, several reasons may limit the influence of pollen limitation on selection.

LIST OF ABBREVIATIONS AND SYMBOLS USED

cm	centimeter
s_i	total directional selection differential
γ_{ii}	quadratic selection
γ_{ij}	correlational selection
BCa	bias corrected accelerated
Δs_{poll}	directional pollinator-mediated selection
$\Delta\gamma_{poll}$	quadratic pollinator-mediated selection
DE	direct effect
IE	indirect effect
PL	pollen limitation
W	fitness
CI	confidence interval
NIH	National Institute of Health

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CHAPTER 1 INTRODUCTION

1.1 Introduction

Understanding the functional relationship between characters and components of fitness is a central goal of evolutionary biology. Only by quantifying the relationship between traits and fitness and identifying the mechanism(s) underlying the relationships is it possible to explain phenotypic diversity. Although biologists have questioned the processes driving diversity in floral form for over two centuries (discussed in Galen 1996), the mechanisms underlying the association between fitness and plant traits remains unknown for many systems. Thus, the goal of my dissertation work has been to examine the ecological causes and evolutionary consequences underlying differences in fitness among individuals in angiosperms, using *Lobelia cardinalis* (Campanulaceae) as a model system. This has involved: quantifying selection on plant traits and identifying the agent(s) of selection (Chapter 2), exploring how pollen limitation may change trait-fitness relationships (Chapter 3), examining how local density influences pollination and subsequence reproductive success (Chapter 4), and investigating how investment strategies in a life history trait (clonal reproduction) affects individual fitness (Chapter 5).

Biotic agents of selection cause trait-fitness relationships

Multiple agents of selection may operate concurrently on the same trait(s), thus making it difficult to discern the evolutionary trajectory of particular trait(s) (reviewed Strauss and Irwin 2004, Strauss and Whittall 2006). For instance, floral traits typically assumed to be adaptations promoting pollination effectiveness (such as floral shape and flower number) may have been shaped by other non-pollinator influences, such as herbivores (reviewed Strauss and Irwin 2004, Strauss and Whittall 2006) or abiotic factors (e.g., (Schemske and Bierzychudek 2007). Despite a growing body of selection studies in plants, very few have quantified the selective agents on floral traits (Galen and Cuba 2001, Alexandersson and Johnson 2002, Fishman and Willis 2008, Sandring and Ågren 2009, Sletvold et al. 2010, Parachnowitsch and Kessler 2010), thereby limiting our understanding of the causes of trait-fitness relationships.

For traits that experience pollinator-mediated selection, pollen limitation—constraint of reproductive success by pollen receipt, rather than abiotic resources —may be one factor that shapes the strength of selection on floral traits (Johnston 1991a, 1991b, Ashman and Morgan 2004). When pollinators are incapable of servicing all flowers in a population, competition among individuals for pollinator service likely magnifies differences among individuals in their trait-dependent abilities to export and acquire pollen. In this manner, pollen limitation may alter a trait's influence on fitness by making fitter individuals disproportionately more fit relative to less fit individuals. Under pollen limitation, traits that enhance pollinator visitation or reliability of service such as flower shape and size (e.g. Campbell et al. 1991, Alexandersson and Johnson 2002, Maad and Alexandersson 2004, Sletvold et al. 2010), phenology (e.g. Sletvold et al. 2010) and inflorescence architecture (e.g. (Sletvold et al. 2010, Nattero et al. 2010) are likely to experience stronger selection. On the other hand, when pollen limitation is severe, traits that reduce reliance on pollinators by promoting reproductive assurance through increased autonomous self-pollination (Morgan & Wilson, 2005), or that increase clonal growth (Eckert 2001) may be favored.

Although the hypothesis that pollen limitation influences the strength of selection is often cited in studies of selection on floral traits, it is not well supported. Ashman and Morgan (2004) conducted the first test of this hypothesis using estimates obtained from 12 studies and found that selection increased with the magnitude of pollen limitation. Results from recent studies that have measured selection and pollen limitation concurrently do not support this expectation (e.g., Parachnowitsch and Kessler 2010, Caruso et al. 2010), suggesting that there may not be a strong link between pollen limitation and selection. Thus, a more rigorous test of this hypothesis is needed to clarify the influence of pollen limitation on the evolution of floral traits may be understood.

Local density of individuals and flowers influences trait-fitness relationships

Although trait-fitness relationships may exist for many reasons, in plants environmental factors shaping local plant density may be particularly important. Density may directly influence trait-fitness relationships directly by affecting competition for abiotic resources available to produce leaves and flowers and to mature seeds, or indirectly by influencing pollination and reproductive success. Reproduction of sexually polymorphic species (e.g. dioecious, heterostylous, dichogamous and self-incompatible species) may be particularly sensitive to variation in local density. In these systems, populations are subdivided into individuals or floral morphs that mate primarily or exclusively with contrasting types. Local density of individuals or flowers that are interfertile will determine the likelihood that pollen carried by the pollinator will result in fertilization and, in the case of dichogamous or dioecious species, determine the amount of pollen transferred (Price and Waser 1982, 1982, Aizen 2001, Bianchi and Cunningham

2012). Because the spatial distribution of individuals and flowers influences reproduction, variation in local density may play a role in the ecology and evolution of such species (Stehlik et al. 2008, Weber and Kolb 2013). Very few studies, however, have simultaneously examined the influences of phenotype and local density simultaneously, thus limiting our understanding of the direct effect of these factors on fitness.

The spatial distribution of individuals within perennial plants can arise from environmental heterogeneity and also by variation among individuals in their propensity for clonal reproduction. Most perennial species reproduce to some extent via asexual reproduction (reviewed in Vallejo-Marin et al. 2010). Clonal reproduction provides many potential benefits, including: persistence when pollination opportunities are scarce, ability to spread risk of death among ramets and the potential for integration allowing division of labour between ramets and specialization for certain tasks, such as sexual reproduction, nutrient uptake, or storage (reviewed in Vallejo-Marin et al. 2010). Clonal reproduction can also influence the spatial distribution of individuals (density and genetic identity of flowering stems), and thus can influence pollination and reproductive success. For instance, geitonogamous selfing (self-pollination among flowers of the same genet) may be influenced by the local density of ramets of the same genet (e.g. Eckert 2000). Despite the importance of clonal propagation for populations, little is known about how variation among individuals in allocation to clonal reproduction influences fitness.

1.2 Thesis objectives

The primary objective of my thesis was to better understand the causes and consequences of fitness variation of individuals in the mixed-mating, herbaceous perennial, *Lobelia cardinalis* L.. To do this, I conducted four years of field study of the associations of female reproductive success to phenotypic variation in various traits. In addition, I assessed the influences of local density and pollen limitation on individual fitness. I specifically addressed this objective as described in four data chapters (Chapters 2 – 5) and a concluding discussion (Chapter 6), as outlined below:

Chapter 2: Pollinators cause stronger selection than herbivores on floral traits

Animal-pollinated plants commonly experience selection from a multitude of sources. However, few studies have quantified pollinator-mediated selection or assessed the relative importance of different selective agents in shaping floral traits. Based on field measurements taken during 2009, I quantified pollinator-mediated selection on six floral traits by comparing selection in naturally pollinated plants to that experienced by plants that received supplemental hand-pollination. Because herbivory could not be manipulated, I also used path analysis and structural equation modeling to examine how three herbivores (slugs, weevil larvae, and geometrid caterpillars) affected selection on floral traits. This study has been published in the March 2012 issue of *New Phytologist* (Bartkowska and Johnston 2012). I conducted the data collection, data analysis and writing of the manuscript. M. Johnston assisted with the randomization procedure and provided suggestions for improving the clarity of the manuscript.

Chapter 3: The influence of pollen limitation on the strength of natural selection

Although often cited, the expectation that pollen limitation influences the strength of selection via female fitness has not been rigorously tested. I tested this hypothesis using data from a survey of literature of angiosperm species. I conclude that the role of pollen limitation in mediating selection on plant characters is not straightforward and depends on the relative importance of pollinators versus other selective agents. This study was submitted for publication.

Chapter 4: The sexual neighborhood through time

Reproductive success of flowering plants is influenced by the morphology and timing of reproductive structures, as well as the density of surrounding conspecifics. In species with separate male and female floral phases, like *L. cardinalis*, successful pollen transfer should vary with the density of surrounding male and female flowers. Increased density of surrounding flowers may increase pollinator visitation rates, but the densities of male and female flowers will determine the availability of pollen and the strength of competition for pollen receipt. In this study, I: (1) quantified the influence of surrounding plant density on fruit and total seed production; (2) quantified the influences of surrounding male- and female-phase flower densities on fruit and seed production per flower; and (3) compared the influence of density on fitness to that of focal plant phenotype, specifically stigma-nectary distance and plant height, in a natural, pollen-limited population. This study was submitted for publication.

Chapter 5: Survival and fitness consequences of rosette size and number measured at the ramet and genet level

How floral display (which encompasses total flower number, number of inflorescences, and timing of flowering) evolves is integral to floral evolution. In many perennial species, floral display may vary with life-history traits, such as survival and between-year allocation of resources. For example, many perennials produce multiple ramets (e.g., rosettes that give rise to inflorescences), which may promote geitonogamous selfing. Multiple rosettes may increase the probability of surviving winter, may help mitigate damage caused by herbivores, or increase total reproductive output directly. I evaluated the fitness consequences of variation in the number and size of rosettes produced by individual ramets and by genets as a whole during four growing seasons.

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CHAPTER 2 POLLINATORS CAUSE STRONGER SELECTION THAN HERBIVORES ON FLORAL TRAITS IN *LOBELIA CARDINALIS*

2.1 Abstract

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. I 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. I used path analysis to examine how three herbivores—slugs, weevils and caterpillars—affected the relationship between floral traits and fitness. I detected stronger total selection on four traits and correlational selection (γ_{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, levels of pollen limitation and herbivory are expected to influence the relative strength of selection caused by pollinators versus herbivores.

2.2 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, 1991, Alexandersson and Johnson 2002, Maad and Alexandersson 2004, Sletvold et al. 2010): phenology (Sandring and Ågren 2009): and inflorescence architecture (Harder and 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 and Cuba 2001, Alexandersson and Johnson 2002, Fishman and Willis 2008, Sandring and Ågren 2009, Parachnowitsch and 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 non-pollinator sources of selection such as herbivores (reviewed Strauss and Irwin 2004, Strauss and 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 and Irwin 2004; Strauss and Whittall 2006). Traits such as floral morphology (Galen and Cuba 2001), nectar production (Adler and Bronstein 2004) and plant size and floral display (Sletvold and 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 and 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, I 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), on phenotypic selection (hereafter selection) on floral traits through seed production in *Lobelia cardinalis* (Lobeliaceae). I quantified the selective role of pollinators directly by comparing selection in naturally pollinated and supplementally (hand-) pollinated subpopulations. If pollinators exert 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 1991*a,b*, Ashman and Morgan 2004), I also assessed the degree of pollen limitation within the population. I could not manipulate levels of herbivory experimentally, and therefore studied selection caused by herbivores using mediation analysis (Preacher and Hayes 2008) within a path-analysis framework (reviewed in Mitchell 2001).

2.3 Materials and Methods

2.3.1 **Study species and site**

Lobelia cardinalis (Lobeliaceae) is a short-lived herbaceous perennial distributed throughout eastern North America (Bowden 1959). Throughout its Canadian range, *L. cardinalis* is pollinated solely by *Archilochus colubris* (Trochilidae), the ruby-throated hummingbird (Bertin 1982, Johnston 1991*a*). Although individuals are self-compatible, pollinators are required for seed production (Johnston 1991*b*). 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.

I studied plants growing along the Petawawa River in Algonquin Provincial Park in Ontario, Canada, from July 15 to October 20, 2009. In this area, *L. cardinalis* was attacked by three herbivores. Larvae of *Cleopmiarus hispidulus* (Curculionidae; Anderson 1973), fed on ovules and developing seeds. Caterpillars of *Trichordestra legitima* (Noctuidae) ate anther tubes. Slugs, provisionally *Arion subfuscus* (Arionidae; Robert Forsyth pers. comm.), consumed either whole or large parts of inflorescences.

2.3.2 **Pollen supplementation**

In July 2009, 854 inflorescences were tagged. Of these, 619 were naturally pollinated and 239 received supplemental pollination. I 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 days. 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, limiting spurious results that could over-estimate pollen limitation (Aizen and Harder, 2007).

2.3.3 **Traits measured, herbivory and female fitness**

On each plant, I 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 and James 1979). The total number of flowers produced, the date the median flower was female and the average number of flowers open per day of flowering (a measure of daily floral display) were estimated from surveys of open flowers conducted throughout the flowering season, typically every three to four days between July 29 and September 14. I 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). I used stem diameter rather than height in our analysis because I 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 days 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.

2.3.4 **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 and Arnold 1983) for univariate and multi-trait models. For clarity I refer to directional and nonlinear estimates from univariate models as “selection differentials” and estimates from multi-trait models as “gradients.” I estimated the following: total directional selection differential (s_i), total quadratic selection (γ_{ii}), directional selection gradients (β), quadratic selection gradients (γ_{ii}) and correlational selection (γ_{ij}). The quadratic selection differentials and gradients presented for each trait are a doubling of the regression coefficients estimated by statistical software (Lande and Arnold 1983, Stinchcombe et al. 2008). Correlational selection (γ_{ij}) estimates were obtained from multi-trait 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, Cary, North Carolina, USA). The residuals from all regression models were heteroscedastic and not normally distributed. Thus**,** regression coefficient *P*-values and 95% BCa confidence intervals (Efron and Tibshirani 1993) were determined using 10,000 bootstrap iterations in programs written in Mathematica (Wolfram Research Inc. 2010).

I 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 (multi-trait regression), and in each case for both directional selection and nonlinear selection.

2.3.5 **Mediation analysis**

It was not possible to experimentally manipulate herbivory without affecting the natural reproduction of *L. cardinalis*; herbivore exclusion would exclude hummingbirds, and heavy use of pesticide is undesirable in a natural area. I employed mediation analysis (Preacher and 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 and Hayes 2008). Unlike other approaches, the multiple-mediator model of Preacher and 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 and Hayes 2008) and thus nonparametric techniques are preferred.

I devised a path model (for the naturally pollinated plants) where each trait and damage caused by each herbivore had a direct effect on fitness. I 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 and Hayes (2008) was used to obtain estimates of total and specific indirect effects and the corresponding confidence intervals. I used 2,000 iterations for the bootsrapping procedure and present the bias corrected and accelerated (BCa) bootsrapped confidence intervals (Efron and Tibshirani 1993).

I 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. I used AMOS 7 (Arbuckle 2006) to obtain an estimate of model fit, specifically, the Chi-square goodness of fit (Mitchell 2001). A non-significant Chi-square value indicates that a model is not significantly different from the observed correlations in the data and is therefore a good fit (Mitchell 2001). Because our data do not meet the assumptions of multivariate normality, I used Bollen-Stine (Bollen and Stine 1993) adjusted p-values for the Chi-square goodness of fit test.

2.4 Results

2.4.1 **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 1066 seeds on average versus hand-pollinated plants that made 1836 seeds on average, $P < 0.0001$; Table 2.1). All six traits were significantly correlated with one another in both treatments (Table 2.2).

2.4.2 **Comparison of selection on traits in two pollination treatments**

All traits in both treatments experienced total directional selection (Table 2.3), which was statistically significantly stronger in the naturally pollinated treatment for stem diameter, flower number, daily floral display and median-flower date ($|\Delta s_{\mathrm{poll}}|$ in Table 2.3). In the naturally pollinated treatment, there were also statistically significant positive quadratic selection differentials (γ_{ii}) for stem diameter and median-flower date

Table 2.1 Means, standard deviations and sample sizes for traits and fitness measured in the naturally pollinated and hand-pollinated treatments of *Lobelia cardinalis*.

	Naturally Pollinated			Hand-Pollinated			Difference in mean between treatments
Measure	Mean	Std	N	Mean	Std	N	*P*
Trait							
Anther-nectary distance (mm)	33.4	2.0	438	33.0	1.9	189	0.02
Flower size (mm)	13.2	1.5	440	13.0	1.4	189	0.33
Stem diameter (mm)	4.3	1.4	551	4.3	1.9	218	0.97
Flower number	8.3	7.1	590	6.5	4.1	229	<0.001
Daily floral display	4.2	2.0	509	4.1	1.6	207	0.75
Median-flower date	August 18 2009	5.7	482	August 17 2009	5.0	201	0.12
Fitness							
Seed number	1066	2115.7	615	1836	2241.1	239	<0.0001
Fruit number	2.2	3.2	615	3.1	3.1	239	<0.0001

Table 2.2 Pearson product-moment correlations among six traits and fitness (seed number) measured in naturally pollinated (N= 426-590) and hand-pollinated treatments (N=176-229) of *Lobelia cardinalis*.

Trait	Seed number	Anther-nectary distance	Flower size	Stem diameter	Flower number	Daily floral display
Naturally Pollinated						
Anther-nectary distance	0.16^{*}					
Flower size	0.14^{*}	0.47^{***}				
Stem Diameter	0.50^{***}	0.23^{***}	0.26^{***}			
Flower number	0.54^{***}	0.20^{***}	0.17^{**}	0.73^{***}		
Daily floral display	0.47^{***}	0.28^{***}	0.18^{**}	0.62^{***}	0.78^{***}	
Median-flower date	-0.42^{***}	-0.14^{*}	-0.20^{***}	-0.42^{***}	-0.19^{***}	-0.31^{***}
Hand Pollinated						
Anther-nectary distance	0.15					
Flower size	0.22^{***}	0.43^{***}				
Stem diameter	0.48^{***}	0.31^{***}	0.32^{***}			
Flower number	0.62^{***}	0.21^{*}	0.22^{*}	0.70^{***}		
Daily floral display	0.47^{***}	0.21^{*}	0.23^{*}	0.40^{***}	0.55^{***}	
Median-flower date	-0.44^{***}	-0.16^{*}	-0.28^{**}	-0.17^{*}	-0.01	-0.16^{*}

Note: Randomization tests were used to assess whether the correlations differed from 0 (*P <0.05; **P< 0.001; *** P<0.0001).

(Table 2.3). 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 and Shaw 1987). These nonlinear effects were marginally statistically greater in the naturally pollinated treatment ($|\Delta\gamma_{\mathrm{poll}}|$ in Table 2.3).

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 2.4). Although there were no statistically significant differences in selection gradients between treatments, the directional selection gradient was 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 multi-trait 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 (1-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 2.5). In the naturally 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 2.5).

Table 2.3 Total selection. Comparison of total directional selection (s_i ± SE) and total quadratic selection (γ_{ii}) for six traits in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*. Coefficients are in units of standard deviation. For each trait, the absolute value of the difference in selection coefficients ($|\Delta\ s_{\mathrm{poll}}|$ and $|\Delta\ \gamma_{\mathrm{poll}}|$) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

	Naturally pollinated				Hand pollinated				Selection difference			
Trait	s_i (± SE)	*P*	γ_{ii} (± SE)	*P*	s_i (± SE)	*P*	γ_{ii} (± SE)	*P*	$\|\Delta s_{\mathrm{poll}}\|$	*P*	$\|\Delta \gamma_{\mathrm{poll}}\|$	*P*
Anther-nectary distance	0.29 (±0.09)	0.0018	0.17 (±0.11)	0.19	0.18 (±0.02)	0.020	-0.15 (±0.11)	0.27	0.11	0.23	0.32	0.073
Flower size	0.26 (±0.09)	0.0032	-0.03 (±0.08)	0.84	0.29 (±0.08)	<0.001	-0.14 (±0.11)	0.16	0.03	0.42	0.11	0.33
Stem diameter	0.95 (±0.07)	<0.001	0.50 (±0.11)	<0.001	0.63 (±0.07)	<0.001	0.09 (±0.10)	0.53	0.32	0.023	0.41	0.052
Flower number	1.04 (±0.07)	<0.001	-0.06 (±0.10)	0.66	0.73 (±0.06)	<0.001	-0.02 (±0.07)	0.73	0.31	0.050	0.04	0.43
Daily floral display	0.75 (±0.07)	<0.001	-0.21 (±0.10)	0.16	0.48 (±0.07)	<0.001	-0.13 (±0.12)	0.16	0.27	0.037	0.08	0.35
Median-flower date	-0.65 (±0.07)	<0.001	0.30 (±0.08)	<0.001	-0.42 (±0.07)	<0.001	0.11 (±0.07)	0.12	0.23	0.034	0.19	0.084

Table 2.4 Direct selection. Comparison of directional selection gradients (β_i ± SE), and quadratic selection gradients (γ_{ii}) for six traits in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*. Coefficients are in units of standard deviation. For each trait, the absolute value of the difference in selection coefficients ($|\Delta\,\beta_{\mathrm{poll}}|$ and $|\Delta\,\gamma_{\mathrm{poll}}|$) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

	Naturally pollinated				Hand pollinated				Selection difference			
Trait	β_i (± SE)	*P*	γ_{ii} (± SE)	*P*	β_{ii} (± SE)	*P*	γ_{ii} (± SE)	*P*	$\lvert\Delta\,\beta_{\mathrm{poll}}\rvert$	*P*	$\lvert\Delta\,\gamma_{\mathrm{poll}}\rvert$	*P*
Anther-nectary distance	0.070 (±0.08)	0.39	0.02 (±0.10)	0.82	-0.047 (±0.07)	0.47	-0.09 (±0.10)	0.36	0.12	0.19	0.11	0.29
Flower size	-0.018 (±0.08)	0.82	0.07 (±0.10)	0.33	0.043 (±0.07)	0.52	-0.05 (±0.12)	0.77	0.06	0.34	0.12	0.27
Stem diameter	0.22 (±0.11)	0.059	0.27 (±0.18)	0.10	0.11 (±0.09)	0.24	0.01 (±0.16)	0.86	0.11	0.28	0.26	0.18
Flower number	0.74 (±0.18)	<0.001	0.05 (±0.19)	0.78	0.50 (±0.11)	<0.001	0.10 (±0.11)	0.40	0.24	0.17	0.05	0.41
Daily floral display	-0.13 (±0.13)	0.33	-0.05 (±0.14)	0.73	0.046 (±0.08)	0.57	0.07 (±0.10)	0.37	0.18	0.17	0.12	0.33
Median-flower date	-0.43 (±0.08)	<0.001	0.32 (±0.10)	<0.001	-0.40 (±0.07)	<0.001	0.17 (±0.07)	0.014	0.03	0.42	0.15	0.15

Table 2.5 Correlational selection. Comparison of correlational selection gradients (γ_{ij} ± SE) for the 15 pairwise trait combinations in naturally pollinated and hand-pollinated plants in a population of *Lobelia cardinalis*. For each trait combination, the absolute value of the difference in selection coefficients ($|\Delta\ \gamma_{\mathrm{poll}}|$) between the naturally pollinated and hand-pollinated treatment is presented; statistically significant differences implicate pollinators as agents of selection.

	Naturally pollinated		Hand-pollinated		Selection difference	
	γ_{ij} (± SE)	*P*	γ_{ij} (± SE)	*P*	$|\Delta\ \gamma_{\mathrm{poll}}|$	*P*
Flower size x anther-nectary distance	-0.061 (±0.12)	0.41	-0.016 (±0.10)	0.94	0.045	0.37
Flower size x stem diamter	0.13 (±0.13)	0.44	0.0046 (±0.10)	0.74	0.13	0.29
Flower size x flower number	0.08 (±0.20)	0.84	-0.23 (±0.12)	0.25	0.31	0.25
Flower size x daily floral display	-0.14 (±0.13)	0.54	0.045 (±0.08)	0.99	0.19	0.18
Flower size x median-flower date	0.078 (±0.09)	0.29	-0.032 (±0.10)	0.78	0.11	0.20
Anther-nectary distance x stem diameter	-0.13 (±0.14)	0.46	-0.073 (±0.09)	0.43	0.057	0.45
Anther-nectary distance x flower number	-0.20 (±0.25)	0.29	0.26 (±0.12)	0.12	0.46	0.061
Anther-nectary distance x daily floral display	0.38 (±0.17)	0.008	-0.073 (±0.10)	0.49	0.45	0.013
Anther-nectary distance x median-flower date	-0.0086 (±0.09)	0.69	-0.061 (±0.08)	0.29	0.052	0.50
Stem diameter x flower number	0.20 (±0.26)	0.64	-0.20 (±0.15)	0.43	0.40	0.18
Stem diameter x daily floral display	-0.16 (±0.21)	0.51	0.20 (±0.10)	0.11	0.36	0.10
Stem diameter x median-flower date	-0.14 (±0.14)	0.36	-0.12 (±0.10)	0.30	0.02	0.50
Flower number x daily floral display	0.54 (±0.31)	0.053	-0.18 (±0.10)	0.23	0.72	0.012
Flower number x median-flower date	-0.77 (±0.32)	0.025	-0.19 (±0.10)	0.065	0.58	0.044
Daily floral display x median-flower date	0.26 (±00.25)	0.38	0.00058 (±0.10)	0.98	0.26	0.18

Furthermore, correlational selection for these trait combinations was statistically greater in the naturally pollinated treatment ($|\Delta\gamma_{\text{poll}}|$ Table 2.5), consistent with our hypothesis that pollinators are selective agents shaping floral traits in *L. cardinalis*.

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

Herbivory had only minor effects on patterns of selection; fewer than 20 percent of plants in the population experienced herbivore damage (Table 2.6). 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 2.7). Also, the model incorporating herbivore-mediated selection on plant traits appropriately represented the observed data (χ^2=19.90, *df*=18, *P* =0.341, N=408; Figure 2.1), 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 (Figure 2.1). Early flowering plants also had a slightly higher proportion of slug damage (Figure 2.1). Herbivore-mediated selection was weak, however, as indicated by the very small mediating effects caused by weevils and slugs (column four in Table 2.8).

Table 2.6 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.

	Individuals		Flowers	
Type of damage	Number affected	Proportion affected	Number affected	Proportion affected
Anther tube (caterpillars)	35	0.057	106	0.037
Fruit (weevil larvae)	71	0.115	109	0.038
Grazed inflorescence (Slugs)	92	0.145	321	0.113

Table 2.7 Decomposition of indirect effects of traits on fitness of *Lobelia cardinalis* mediated by caterpillars (*Trichordestra legitima*), weevils (*Cleopmiarus hispidulus*) and slugs (*Arion subfuscus*) estimated from path analysis. 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.

	Total Indirect bootstrapped Effect (± SE)	Total Indirect Effect Lower, and Upper 95% BC CI	Indirect effect via Caterpillar (± SE)	Caterpillar Lower, and Upper 95% BC CI	Indirect effect via Weevil effect (± SE)	Weevil Lower, and Upper 95% BC CI	Indirect effect via Slug (± SE)	Slug Lower, and Upper 95% BC CI
Anther-nectary distance	0.0005 (±0.019)	-0.035, 0.038	0.0046 (±0.013)	-0.0186, 0.033	**-0.0157 (±0.010)**	-0.047, -0.001	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)	-0.009, 0.030	-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	**-0.022 (± 0.016)**	-0.072, -0.001	0.0022 (± 0.011)	-0.014, 0.032
Flower num	0.0154 (± 0.022)	-0.022, 0.065	0.0137 (± 0.011)	-0.0022, 0.045	-0.0039 (± 0.014)	-0.039, 0.012	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)	-0.002, 0.048	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)	-0.014, 0.024	**0.0153 (±0.010)**	0.002, 0.042

Table 2.8 Comparison of direct effects (DE) of floral traits, estimated from path analysis, on fitness with and without herbivore-mediated selection. Size of the mediating effects of weevil (*Cleopmiarus hispidulus*) and slugs (*Arion subfuscus*) on floral traits in *Lobelia cardinalis* is estimated as the percentage change of the direct effect due to the inclusion of an indirect effect (IE) mediated by herbivore damage.

	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

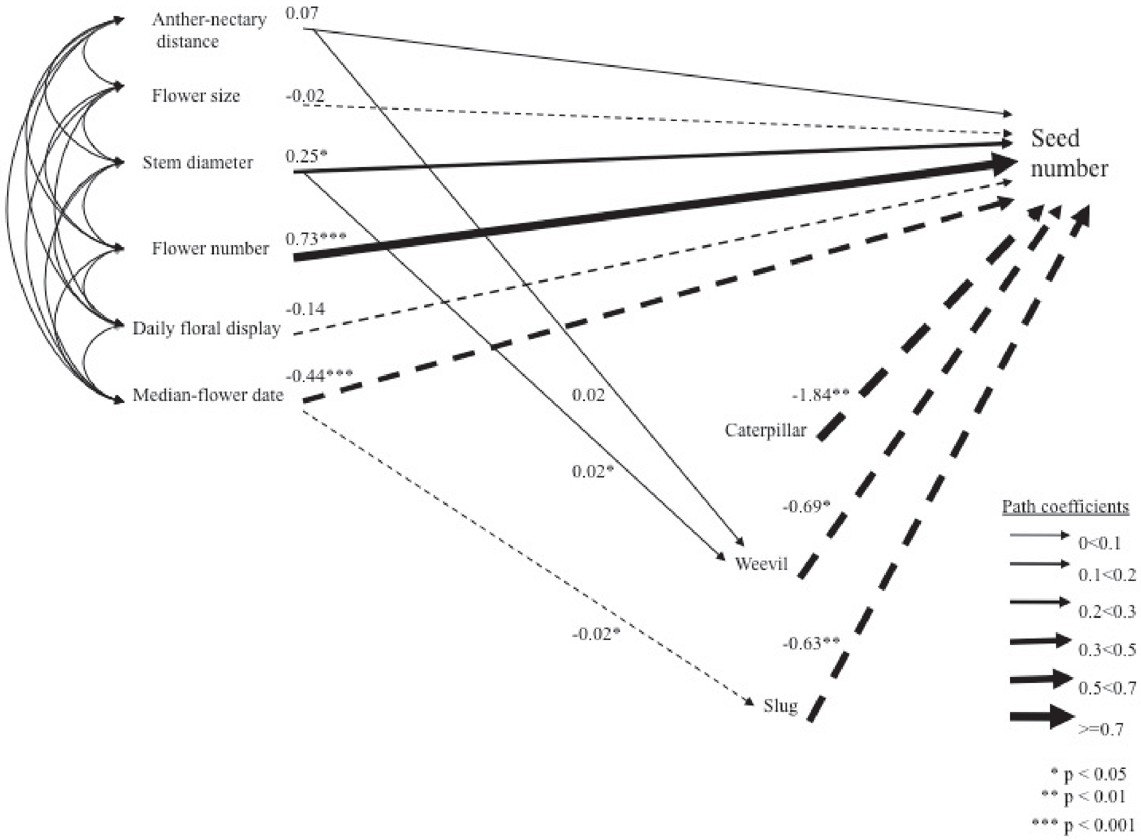

Figure 2.1 Results of path analysis for the effects of herbivore damage on seed set in *Lobelia cardinalis.* Straight arrows reflect causal paths; curved arrows designate correlations. Solid arrows denote positive effects, whereas negative effects are shown by dashed arrows. The strength of the relationship is designated by arrow thickness. Asterisks denote significant paths. 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.

2.5 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 and Irwin 2004 and in Strauss and Whittall 2006). A thorough understanding of the forces that shape floral evolution thus requires: 1) identifying the source(s) of selection, and 2) when multiple agents of selection are operating, assessing the relative importance of each source of selection. In this study, I 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. I 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- versus herbivore-mediated selection. Although the pollination environment in this system could easily be manipulated (by altering the amount of pollen received by each individual), levels 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, like 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 like stigmatic pollen loads that could be a proxy for visitation (as was done in Irwin 2006). Although I could not use an experimental approach to directly compare pollinator- and herbivore-mediated selection, I 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 analaysis 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.

2.5.1 **Pollinators exert selection on floral traits**

I 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 to 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 2.3). Similarly, for the three traits experiencing significant direct selection (stem diameter, flower number and median-flower date; Table 2.4), 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 2.5).

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.; Johnston 1991*b*). 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 I 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 sporadic late in the summer (pers. obs.); thus, plants with many receptive female flowers earlier in the season are more likely to be pollinated.

2.5.2 Evidence for weak herbivore-mediated selection on flowering traits

I 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 (Figure 2.1), thus slug-mediated selection would favor 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 2.5) 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 and 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 I 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 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 and 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 and Caruso 2008). I 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 (Figure 2.1). Anther-nectary distance, although not a direct target of selection (Table 2.2; Figure 2.1), 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 (Figure 2.1; Table 2.4); the inclusion of a path from stem diameter through weevil damage to fitness altered the direct effect of stem diameter to fitness, though the size of this mediating effect was small (Table 2.5). 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 2.4).

2.5.3 **Other factors affecting the relationship between traits and fitness**

Supplementally pollinated plants were not subject to the selective effects of pollinators and yet experienced selection on median-flower date and flower number (Table 2.4). 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 discussion 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 due to 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), I 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 I did not assess the effect of temperature on fruit and seed development it is likely that sub-zero (^{0}C) temperature may inhibit seed production. Frost and sub-zero temperatures were recorded at this site as early as

September 1, 2009 (pers. obs.). This may explain why I found selection favoring early flowering and Johnston (1991*b*) found later flowering dates were favored in more southern populations. Without further information regarding inbreeding depression, and more detailed measures of environmental variables I cannot attribute selection on flowering date to a particular source.

2.5.4 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 and Irwin 2004). The relative importance of pollinators versus 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, *L. siphilitica* (Parachnowitsch and Caruso 2008), in which weevil larvae and not pollinators were attributed with causing selection on floral phenology. The difference in the selective effects of weevil larvae found in this study and the current are likely due to different levels of damage suffered by *L. siphilitca* and *L. cardinalis*; 89% of *L. siphilitica* plants experienced weevil damage compared to 6% of *L. cardinalis* individuals (Table 2.6). 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 versus 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 1991*a*, *b*, Ashman and Morgan 2004, Fishman and Willis 2008). Cariveau et al. (2004), Rey et al. (2006) and Parachnowitsch and Caruso (2008) found that seed herbivores exerted stronger selection on floral traits than pollinators. In contrast, the present study and similar studies of *Dactylorhiza lapponica* (Sletvold et al. 2010) and *Arabidopsis lyrata* (Sandrig and 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. I found hand-pollinated plants produced 42% more seed than naturally pollinated plants. Pollen limitation, however, is not always predictive of pollinator-mediated selection. Parachnowitsch and 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.

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CHAPTER 3 THE INFLUENCE OF POLLEN LIMITATION ON THE STRENGTH OF NATURAL SELECTION

3.1 Abstract

Pollen limitation of seed and fruit production is a pervasive feature of seed plants. Although often cited, the expectation that pollen limitation influences the strength of selection has not been rigorously tested. Here I test this assertion using two approaches. First, based on a literature survey, I regress the strength of selection via female and male fitness on the effect size of pollen limitation for five types of traits (flower number, flower size, plant height, phenology and vegetative). Second, I explore whether the difference in selection between naturally pollinated plants and those with experimentally reduced pollen limitation varies with pollen limitation, as would be expected if it intensifies pollinator-mediated selection. I found weak support for the expectation that pollen limitation results in stronger selection on plant traits: (1) the relationship between pollen limitation and selection, although positive, was weak for male and female fitness and (2) very little of the variance in the strength of selection can be accounted for by the degree of pollen limitation. I discuss why a limited influence of pollen limitation on selection is in fact expected despite contradicting intuition.

3.2 Introduction

Pollen limitation—the observation that supplemental pollen increases seed production compared to naturally pollinated plants—is a pervasive feature of many plant species and populations and is likely to be an important factor shaping the evolution of reproductive traits in angiosperms (Ashman et al 2004, Knight et al. 2005, Burd et al. 2009). Under extreme pollen limitation, traits that reduce reliance on pollinators by promoting reproductive assurance through increased autonomous self-pollination (Morgan and Wilson, 2005), or that increase clonal growth (Eckert 2002) may be favored (see also Harder and Aizen 2010).

When pollen limitation is less extreme, however, competition among individuals for pollinator service may magnify the difference in the ability of individuals to export and acquire pollen. In this case, traits subject to pollinator-mediated selection such as flower shape and size (e.g., Campbell 1989, Campbell et al. 1991, Alexandersson and Johnson 2002, Maad and Alexandersson 2004, Sletvold et al. 2010), phenology (e.g., Sletvold et al. 2010) and inflorescence architecture (e.g., Nattero et al. 2010, Sletvold et al. 2010) may experience stronger selection (Johnston 1991*a,b*, Ashman and Morgan 2004). Although pollen limitation is typically assumed to influence selection via female fitness, pollen limitation may also influence selection via male fitness as traits related to pollen export are also likely to experience stronger selection under pollen limitation. Thus, traits that enhance pollen receipt will experience selection via female fitness, whereas those that influence pollen export will experience selection via male fitness.

Despite a strong theoretical expectation that pollen limitation intensifies pollinator-mediated selection, empirical evidence supporting this hypothesis is limited. Ashman and Morgan (2004) tested the generality of the relationship by regressing selection for floral size on pollen limitation using estimates obtained from 12 studies and found a strong association between selection and pollen limitation ($r = 0.53$; $P = 0.004$). Several recent studies have experimentally manipulated the degree of pollen limitation, either decreasing it through supplemental hand pollination or (potentially) increasing it by pollinator exclusion. These experimental studies are especially powerful because they allow selection to be measured in both the presence and absence of pollen limitation within the same population. The results provide mixed support for the hypothesis. For example, Sletvold et al. (2010) found that in a pollen-limited population of *Dactylorhiza lapponica* naturally pollinated plants experienced stronger selection for taller plants with more flowers and longer spurs, consistent with the hypothesis. In contrast, Fishman and Willis (2008) found that, despite strong pollen limitation in *Mimulus guttatus*, selection on floral traits did not differ between naturally pollinated plants and those with reduced pollen limitation (hand-pollinated plants). Instead, plants with experimentally enhanced pollen limitation (caused by pollinator exclusion) experienced selection for flowers with short and narrow corolla tubes, traits shown to promote autonomous self-fertilization. Thus, in some species, pollen limitation may predominantly influence selection by favoring characters that enhance autonomous self-fertilization. Results from *Lobelia siphilitica* (Caruso et al. 2010) and *Penstemon digitalis* (Parachnowitsch and Kessler 2010) also contradict the expectation that pollen limitation influences selection. In both studies, naturally pollinated plants experienced stronger selection on floral characters, suggesting pollinator-mediated selection was operating on those characters, despite a lack of pollen limitation.

The expectation that pollen limitation influences selection holds only for characters that affect pollen limitation. Any difference in slope between hand- and naturally pollinated plants entirely depends on how pollen limitation scales with trait(s). To illustrate this underappreciated point, I consider how the difference in directional selection on a trait between naturally pollinated and supplementally hand-pollinated plants changes under different pollen limitation conditions (Figure 3.1). In the first two scenarios, naturally pollinated plants produce 40% fewer seeds than supplementally pollinated plants, on average (i.e., pollen limitation = 0.4), but pollen limitation varies differently among plants with trait size. In the first scenario (Figure 3.1 a panels), pollen limitation is constant across trait values. Thus, despite strong pollen limitation, selection in naturally pollinated plants is identical to that of supplementally pollinated plants. In the second scenario (Figure 3.1 b panels), population-level pollen limitation is also 0.40, but pollen limitation is greater in plants with lower trait values (left panel of Figure 3.1 b). In this case, selection differs between naturally pollinated plants and those with experimentally reduced pollen limitation (right panel of Figure 3.1 b). In the last scenario, pollen limitation is much weaker than in the first two scenarios. Nevertheless, compared to the second scenario, neither selection nor the difference in selection between naturally pollinated and supplementally pollinated plants has changed. There are two conclusions from these examples: (1) pollen limitation will only influence selection if it affects fitness disproportionately across phenotype, making fitter individuals disproportionately more fit relative to less fit individuals, and (2) a mean difference in fitness between hand- and naturally pollinated plants will not necessarily predict how the slope of relative fitness on trait(s) changes with pollination treatment.

To assess how pollen limitation influences selection on plant traits (other than favoring traits related to self-fertilization), I reviewed the literature from 1983 to 2013 for studies of angiosperm species for which selection and pollen limitation have been measured. From this search, I created a dataset of population- and species-level estimates of pollen limitation and natural selection. I evaluated the expectation that pollen limitation intensifies selection using two approaches. First, for five types of traits (flower number, flower size, plant height, phenology and vegetative) the strength of selection via female fitness (and separately for male fitness) was regressed on the effect size of pollen limitation. Second, I examined studies that compared selection in naturally pollinated plants to selection in plants receiving supplemental pollination (pollen limitation in this group should be zero or very low). These studies test whether traits experience pollinator-mediated selection and provide an alternative approach to evaluate the relationship between selection and pollen limitation. If pollen limitation intensifies selection on traits, then the difference in the strength of selection between naturally pollinated and hand-pollinated plants should depend on the degree of pollen limitation.

3.3 Methods

3.3.1 **Literature survey**

Using Web of Science, I searched for studies citing Lande and Arnold (1983), the seminal paper outlining the use of a regression of fitness on trait size to measure phenotypic selection. As explained by these authors, the "strength" of selection can be compared among traits and populations when trait values are expressed in units of standard deviations. These standardized selection estimates describe the expected change in relative fitness given a shift in the character by one standard deviation (Lande and Arnold 1983). Based on the publication title, 396 studies were identified to contain selection estimate(s) for a natural population(s). For each of these studies, I searched for the occurrence of the words "pollen", "limitation", or "limited". If any of the words was

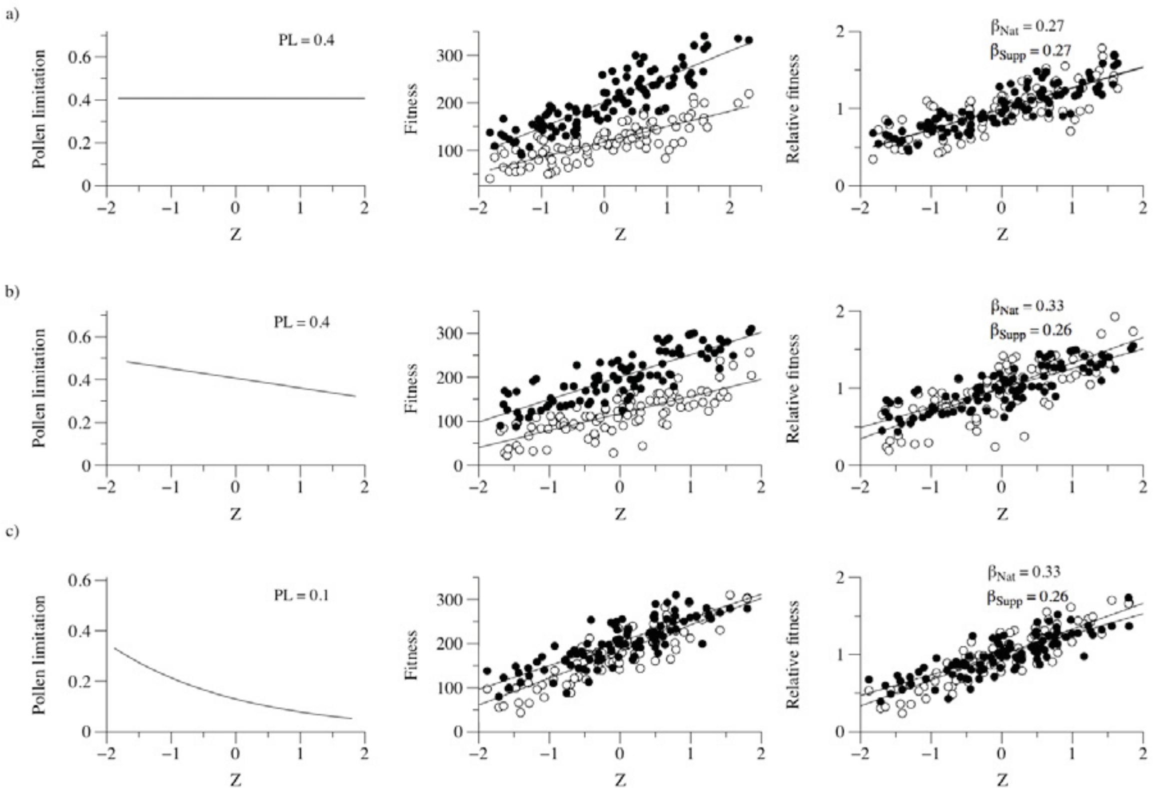

Figure 3.1 Influence of pollen limitation on absolute fitness and natural selection. Leftmost graphs show pollen limitation as a function of trait value, *z*, expressed in standard deviations from a mean of zero. Middle graphs show absolute fitness (for example, seed number) under natural (open circles) and supplemental (closed circles) pollination. Rightmost graphs show relative fitness, where slopes are total standardized selection differentials. These examples were constructed using the following relations. $PL = 1-(W_{\mathrm{natural}} / W_{\mathrm{supplemental}}) = k\alpha^{-(z+m)}$. Absolute fitness values $W_{\mathrm{supplemental}}$ for supplementally pollinated plants were generated as $200+50z+\varepsilon$, where $z \sim N(0, 1)$ and $\varepsilon \sim N(0, 29.12)$. Absolute fitness values W_{natural} for naturally pollinated plants were calculated from the pollen limitation equation plus environmental variance. Values for k, a, m are (a) 0.4, 1, 3; (b) 0.57, 1.09, 4.4; (c) 0.85, 2.02, 3. See text for further explanation.

found, the abstract and/or study methods were read to determine whether an estimate of selection was reported as well as an estimate of pollen limitation. Studies of species for which an estimate of selection and pollen limitation were available were retained for inclusion in the dataset. For most studies that reported an estimate of selection but not pollen limitation was reported, the author(s) referenced a study of pollen limitation for that species.

When dealing with species for which selection and pollen limitation estimates were measured in different populations and/or years, I matched selection and pollen limitation estimates first by location, then by time. In the absence of a clear best estimate of pollen limitation I averaged the pollen limitation estimate from the multiple studies to serve as the best measure of pollen limitation available for a species. In cases that also presented multiple selection estimates, they were averaged. Selection and pollen limitation estimates for multiple populations or years were treated as independent observations in the analysis. I did not correct for possible correlations among estimates from the same species or population, nor did I correct for phylogenetic associations. Because pollen limitation and selection occur at the level of the population and there were so few data, phylogenetic correction was unwarranted. However, I acknowledge that there may be a phylogenetic signal in the association of the strength of selection and pollen limitation (Knight et al. 2005).

3.3.2 **Traits selected for analysis**

For each study, I 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). Among these, flower shape and size (e.g., Galen 1996), flower number (e.g., Parachnowitsch and Kessler 2010), phenology (e.g., Sletvold et al. 2010) and plant size (e.g., Bartkowska and Johnston 2012) have been found to experience pollinator-mediated selection. Only one type of trait from each study population was used in the regression analysis of selection via female fitness and pollen limitation.

3.3.3 **Pollen limitation estimates**

Pollen limitation is typically estimated by comparing some measure of fruit or seed production in hand-pollinated plants to production in naturally pollinated plants. I calculated an effect size for pollen supplementation as the log response ratio,

$$\ln R = \ln(X^{E}/ X^{C})$$

where $\bar{X}$ is the mean of the response variable, and E and C indicate the experimental (supplemental pollination) and control (natural) pollination treatments. The log response ratio is commonly used in meta-analysis as a measure of experimental effect because it quantifies the proportionate change that results from an experimental manipulation (Gurevitch et al. 2001, Hedges et al. 1999). It is particularly useful because: (1) the log linearizes the metric, (2) the log ratio is affected equally by changes in either numerator or denominator, whereas the ratio of treatment to control is more affected by changes in the denominator (Hedges et al. 1999) and (3) the sampling distribution of the log of the effect is approximately normal (Hedges et al. 1999).

Because different fitness measures are used to assess pollen limitation and a single study often reports multiple estimates, I use 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.

Two studies found that naturally pollinated plants produced slightly more seeds per ovule (Ashman and Diefenderfer 2001) and seeds per flower (Totland 2001) than supplemented plants. Because this is more likely caused by differences in quality or phenotype between supplementally and naturally pollinated plants, I estimated pollen limitation as zero in such cases.

3.3.4 **Selection estimates**

The relationship between selection and pollen limitation may differ for selection differentials and selection gradients. Selection differentials (s_i) may vary more strongly with pollen limitation because they measure both direct selection on a trait and indirect selection caused by phenotypic correlations with other traits. Conversely, selection gradients (β) measure selection acting directly on a trait and thus may be a better estimate to use to explore how the relationship between pollen limitation and selection differs between types of traits. Therefore, both types of estimates of selection are used. All analyses were conducted using JMP software version 10.0 (SAS Institute, 2012, Cary, NC).

3.3.5 Evidence for a relationship between pollen limitation and the strength of selection

I addressed whether pollen limitation intensifies the strength of selection on plant traits with two approaches. First, the absolute value of the selection coefficient was regressed on the effect size of the best estimate of pollen limitation. This analysis was conducted separately for selection differentials and gradients, for female and male fitness, and for the five trait types. Because there were fewer estimates of selection via male fitness, I did not separate the male analyses by trait type. Second, I examined studies for which selection measured in naturally pollinated plants was compared to selection on plants receiving supplemental pollination (pollen limitation in this group should be eliminated or very low). If pollen limitation intensifies selection, then the difference in the strength of selection between naturally pollinated and hand-pollinated plants should depend on the degree of pollen limitation. Because all selection coefficients for phenological characters used in this study were negative, I used the absolute value of the difference. Positive difference values indicated that the magnitude of selection was greater in the natural pollination treatment.

I used simple linear regression to test whether the degree of pollen limitation was associated with the difference in selection (between naturally and hand-pollinated plants). I used all available data, so studies reporting selection on multiple trait types and in multiple populations or years are represented more than once. I repeated this analysis using a single trait for each population that is most likely to experience pollinator-mediated selection. Because not all studies specifically tested for pollinator-mediated selection, I chose a single trait with the greatest selection coefficient under natural conditions as the trait most likely to experience pollinator-mediated selection.

3.4 Results

The dataset includes 30 species and 18 families. Many studies reported both selection differentials and gradients for the same trait. Most estimates for standardized linear selection differentials (*s*) and gradients (β) occurred between -1 and 1 (Figure 3.2a for differentials and Figure 3.2b for gradients).

3.4.1 **Selection estimates for naturally pollinated plants only**

For selection via female fitness, four out of five differentials and four out of five gradients tended to vary positively with the best estimate of pollen limitation, suggesting an overall positive relationship between the strength of selection and pollen limitation (Figure 3.2). Phenology, however, was the only trait for which the relationship differed significantly from zero (differential: R^2=0.25, *df*=16, *F*=4.96, *P*=0.04; gradient: R^2=0.53, *df*=20, *F*=21.19, *P*=0.0002). Selection on vegetative traits varies similarly to that on reproductive traits (Figure 3.2).

A similar pattern was observed for selection via male fitness, but no relation differed significantly from zero (Figure 3.3; differential: R^2=0.23, *df*=8, *F*=2.06, *P*=0.19; gradient: R^2=0.17, *df*=10, *F*=1.84, *P*=0.21).

3.4.2 **Studies reporting paired selection estimates for naturally and hand-pollinated plants**

Ten studies that compared selection gradients between naturally pollinated to hand-pollinated plants. When all traits were included in the regression, there was no evidence that pollen-limited populations consistently experienced stronger selection under natural pollination compared to hand pollination (Figure 3.4 left panel; R^2<0.001, *df*=39, *F*=0.0003, *P*=0.99). After limiting the representation of each study population to a single trait most likely to experience pollinator-mediated selection, I found a positive relationship between the degree of pollen limitation and the difference in selection gradients (Figure 3.4 right panel). This relationship, however, was not statistically significantly greater than zero (R^2=0.01, *df*=13, *F*=0.14, *P*=0.72).

3.5 Discussion

3.5.1 **Evidence for pollen limitation influencing selection**

I found weak evidence supporting the hypothesis that pollen limitation intensifies selection. From the first approach, where I searched for a general pattern across all studies by each trait type, I found the relationship between pollen limitation and the strength of selection via female fitness (differentials and gradients) was positive for eight of the ten tests I conducted. The relationship, however, was statistically significantly different from zero only for phenological traits. Furthermore, the slope of the relationship for vegetative traits–which are not expected to experience selection from pollinators–was similar to that of the other trait types. Selection via male was also positively related to pollen limitation (Figure 3.3), although not statistically significantly

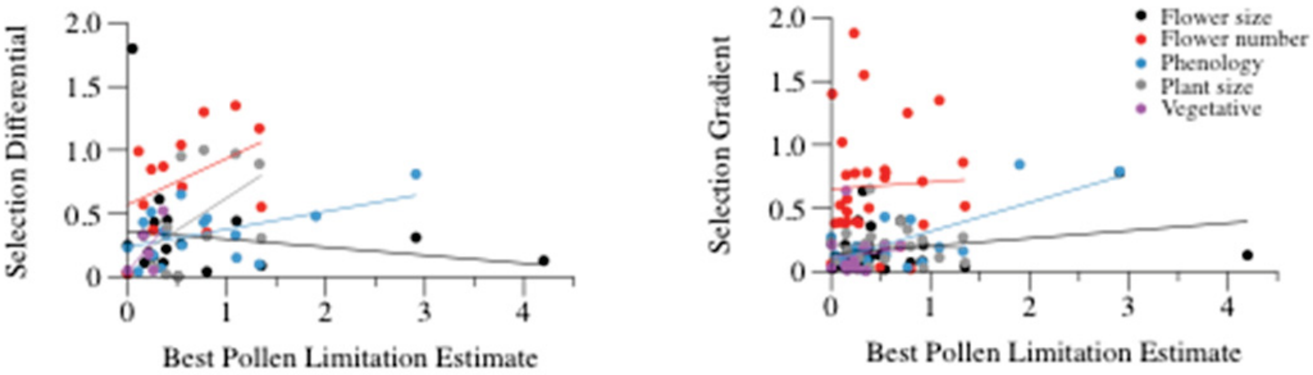

Figure 3.2 Selection differentials (left panel) and gradients (right panel) via female fitness related to the best available pollen limitation estimates for five types of traits.

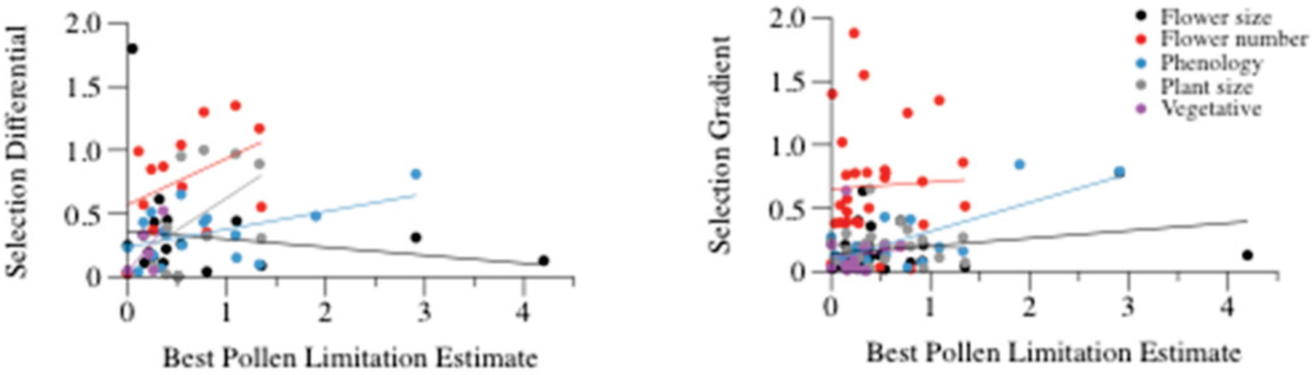

Figure 3.3 Selection differentials (left panel) and gradients (right panel) via male fitness related to the best available pollen limitation estimates.

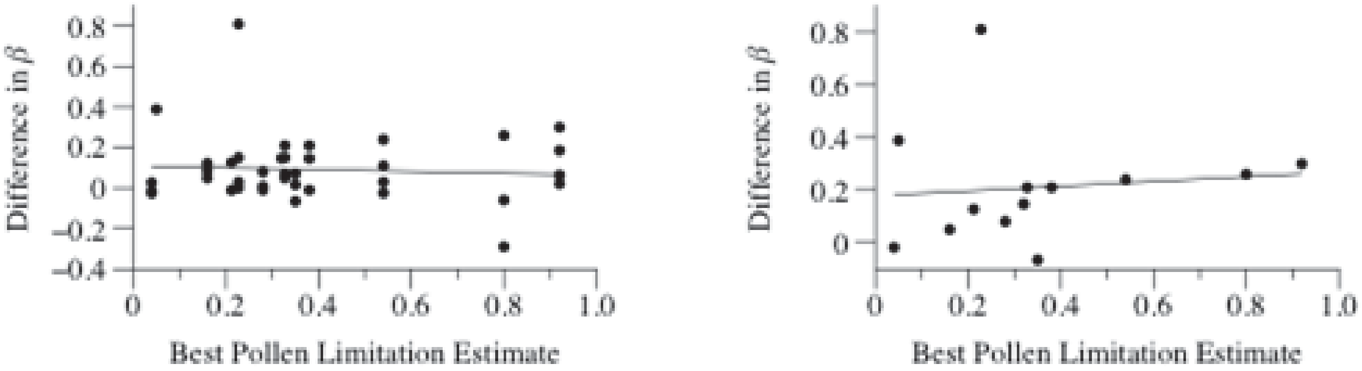

Figure 3.4 Difference in selection gradient between in naturally and hand-pollinated plants related to the best available estimate of pollen limitation. The left panel includes all available estimates for all trait types. The right panel includes only those traits judged most likely to be subject to pollinator-mediated selection

different from zero. For traits likely to experience pollinator-mediated selection, the difference in selection between naturally pollinated and hand-pollinated plants was positively related to pollen limitation, but the relationship was again not statistically significantly different from zero. Overall, our results show a positive but weak relationship between pollen limitation and the strength of selection; furthermore, the variance in selection accounted for by pollen limitation is low, except for phenology. My analyses detected a much weaker general association between selection on floral traits and the degree of pollen limitation than reported by Ashman and Morgan (2004), who found a strong positive relationship ($r = 0.53$; $P = 0.004$) using data from 12 studies. The earlier study was limited to flower-size traits and included both differentials and gradients in one analysis as well as two measures of pollen limitation.

The relationship of selection to pollen limitation and selection via male fitness may be similar to that for selection via female fitness. In the first instance, pollen limitation of seed production arises because of limited pollen export. Therefore, if pollen limitation causes females to compete for pollen receipt, they must also compete to export pollen and, following pollination, compete for access to ovules. Thus, pollen limitation may intensify selection on traits related to pollinator attraction via male fitness if pollen export is important, or reduce selection via male fitness on traits such as flower size if access to ovules is more important. Given the paucity of studies on male fitness in plants, it is difficult to generalize how pollen limitation may influence selection via male fitness; pollen limitation, however, has been proposed to be a factor determining the relative importance of selection through male and female function (Ashman and Diefenderfer 2001, Hodgins and Barrett 2008).

3.5.2 Why pollen limitation may have little influence on selection

Testing the expectation that pollen limitation influences selection on traits related to pollinator proficiency, attraction, fit, or necessity for seed production requires knowledge of which traits are related to these functions. Unfortunately, for most systems this is unknown. Partitioning traits into different types that are likely to differ in how they relate to pollinator attraction, proficiency of pollen transfer and reliance on pollinators for reproduction may help discern a pattern. This is nonetheless problematic, as reproductive success may depend on a different trait or trait combination for each species. For example, Chapter 2 demonstrates that pollinators exerted selection on phenology in *Lobelia cardinalis* (Bartkowska and Johnston 2012), whereas in *L. siphilitica* herbivores, but not pollinators, governed selection on this trait (Parachnowitsch and Caruso 2008). Furthermore, there is growing evidence that traits assumed to have evolved for pollinator attraction and service are often subject to selection by factors other than pollinators (reviewed in Strauss and Irwin 2004, see also Strauss and Whitall 2006). When multiple sources of selection (potentially acting in opposition to one another) are acting concurrently on a particular trait, the relationship between selection and pollen limitation will be limited to the component of net selection attributable to pollinators.

Without detailed studies identifying the selective agents acting on each character, it is difficult to correctly choose for each species which trait(s) are important for pollinator attraction or pollen transfer. Including traits not related to pollinator service will obscure the relationship between selection and pollen limitation. Similarly, comparing the relationship between selection and pollen limitation for traits likely to be subject to pollinator-mediated selection to those less likely can be misleading, as demonstrated by the similar results for vegetative and floral traits. Although this could suggest that the influence of pollen limitation is weak for all characters, it could also reflect an association between pollen limitation and selection on vegetative characters. If the relationship between fitness and vegetative trait(s) is caused by individual differences in levels of inbreeding (Willis 1996) and pollen limitation magnifies the difference in the ability of individuals to mature seed, then selection on vegetative traits may be correlated with pollen limitation.

Because diverse adaptive options available to plants to reduce pollen limitation (e.g., increased attractiveness or increased reliance on self-pollination; see discussion in Harder and Aizen [2010] and Knight et al. [2005]), clear influences of pollen limitation on the evolution of plant traits may be elusive. Pollen limitation has likely played a role in the frequent evolution of self-fertilizing lineages (reviewed by Morgan and Wilson 2005, Knight et al. 2005). Recent studies in *Mimulus luteus* (Fishman and Willis 2008) and *M. guttatus* (Carvallo and Medel 2010) found that severe pollen limitation experimentally induced by pollinator exclusion favors traits that enhance self-fertilization, specifically anther-stigma separation. The role of pollen limitation in mediating selection on characters related to pollinator attraction is not straightforward, and likely depends on the relative importance of pollinators versus the number of different agents exerting selection on those traits and whether self-fertilization provides reproductive assurance. Thus, despite a strong theoretical framework, the nature and outcome of natural selection in pollen-limited populations may be difficult to predict.

3.5.3 **Pollen limitation must differ among phenotypes**

Pollen limitation will influence selection only if it changes with phenotype, augmenting the performance of individuals with favored traits. As shown in Figure 3.1, however, the relationship between pollen limitation and phenotype is not sufficient to determine selection. Pollen limitation estimates are based on differences in mean fitness with no information regarding how pollen limitation scales with traits, whereas selection measures the slope of the relationship between relative fitness and a trait. Predicting how pollen limitation may influence selection is complicated by a lack of knowledge of how pollen limitation relates to trait values.

3.6 Conclusions

Although intuition suggests that pollen limitation should intensify selection on reproductive traits, no simple general relationship is evident. Most importantly, the relationship holds only for those traits experiencing pollinator-mediated selection where pollen limitation differs with phenotype. A general relationship will further be weakened whenever different agents—such as pollinators and herbivores—cause opposing selection on the same trait in different populations. Populations will also differ in the opportunity for selection (variance in relative fitness), which sets a limit on the maximum intensity of selection (Arnold and Wade 1984). Furthermore, some species may respond to pollen limitation by increasing levels of self-fertilization, causing selection on traits related to autonomous selfing rather than pollinator attraction. Consistent with these observations, our analyses find a positive but weak association between selection intensity and pollen limitation.

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CHAPTER 4 THE SEXUAL NEIGHBORHOOD THROUGH TIME: COMPETITION AND FACILITATION FOR POLLINATION

4.1 Abstract

Reproductive success in flowering plants is influenced by the morphology and timing of reproductive structures as well as the density of surrounding conspecifics. In species with separate male and female flower phases, successful pollen transfer is also expected to vary with the density and ratio of surrounding male and female flowers. Increased density of surrounding flowers may increase pollinator visitation rates, but the densities of male and female flowers will determine the availability of pollen and the strength of competition for pollen receipt. Here I (1) quantify the influence of surrounding plant density on total seasonal fruit and seed production, (2) quantify the influence of sexual neighborhood (surrounding sex ratio and male- and female-flower sex phases) on fruit and seed production for individual flowers presented within the season and (3) compare the influence of density on fitness to that of focal plant phenotype, specifically stigma-nectary distance and plant height in a natural population of the pollen-limited, hummingbird-pollinated hermaphrodite *Lobelia cardinalis*. These relationships were examined at four spatial scales (10, 20, 50 and 100 cm). This combination of temporal and spatial scales demonstrated that: (1) total seed production per plant decreased with density at the smallest scale but increased with density at all larger scales; (2) at any given time, a female-phase flower benefited from a higher surrounding density of male-phase flowers and a lower density of surrounding female-phase flowers; (3) when sex ratio was explicitly analyzed a female-phase flower benefited from a lower proportion of surrounding female flowers as well as a lower total flower density; and (4)

at the whole-plant level taller plants were more likely to produce fruit (even when accounting for total flowers produced), consistent with pollinator preference for taller floral displays. Our results suggest that the local density of male and female flowers (and surrounding sex ratio) influences successful pollen transfer and by influencing successful pollen transfer, the local floral environment may shape how attraction traits like plant height are related to fitness.

4.2 Introduction

The spatial distribution of individuals within and among populations can greatly influence pollination and reproductive success. Small sparse populations often experience reduced pollinator service and subsequently suffer reduced reproductive success (Ghazoul 2005, Leimu et al. 2006). Within populations, the proximity of neighboring plants will affect whether individuals compete for or facilitate the acquisition of abiotic resources and pollinator service (Ghazoul 2005 and references therein). Pollen or abiotic resources may limit seed production, and local density may determine the relative importance of each factor. For instance, resource competition among plants in dense aggregations may limit an individual's capacity to produce seeds despite receiving sufficient pollen. In contrast, plants with sufficient resources to mature seeds may be limited by the quantity and/or quality of pollen received (de Jong et al. 2005, Zorn-Arnold and Howe 2007, Jakobsson et al. 2009). Isolated plants often receive fewer visits (Klinkhamer and Jong 1990) and may experience higher rates of geitonogamous self-fertilization (e.g., Karron et al. 1995). Even in dense patches that benefit from more frequent pollinator visits, competition for pollinator service among plants and flowers may limit fruit and seed production of individuals, resulting in a fitness plateau at high densities (Rathcke 1983, see also Elliot and Irwin 2009). This also holds for stands that are mixtures of heterospecifics (Feldman et al. 2004, Feldman 2006).

Plants within sexually polymorphic populations (e.g., dioecious, heterostylous, dichogamous and self-incompatible) experience limited mating opportunities and therefore may be particularly sensitive to local density (e.g., Aizen 1997, Stehlik et al. 2006, Stehlik et al. 2008, Brys and Jacquemyn 2010). At the patch level total floral density may influence the rate of pollinator visitation (Feldman 2004). Within a patch, on the other hand, the local density of flowers that are interfertile will determine whether pollination results in fertilization and the amount of pollen transferred (Price and Waser 1982, Aizen 2001, Bianchi and Cunningham 2012). In completely dichogamous species a higher surrounding density of male flowers may increase the amount of pollen deposited on a female flower, whereas a female-biased density may result in female-female competition for pollen. Few studies have examined how local density in sexually polymorphic species influences pollination and reproduction, and only one of these (Aizen 1997) focused on dichogamy. In completely dichogamous flowers optimal allocation to male and female phases is affected by surrounding density and sex ratio, both of which change over space and time. Thus, selection on floral traits including morphology, phenology and investment in sexual functions will be shaped by the sexual neighborhood.

Understanding how the spatial distribution of individuals affects fitness is difficult because multiple processes underlying an association between density (or distance to nearest neighbor) and fitness can be operating simultaneously and at different spatial scales. For example, a relationship between density of individuals or flowers and fitness at small scales (e.g., less than one meter in *Sabatia angularis*) may reflect partitioning of abiotic resources, but at larger scales (e.g., greater than one meter in *S. angularis*) may capture the effects of density on pollinator visitation (Spigler and Chang 2008). Pollinators also discriminate among plants by phenotype, thereby acting as selective agents (e.g., Sandring and Ågren 2009, Sletvold et al. 2010). Because plant phenotypes may themselves be shaped by density, associations between density and fitness or phenotype and fitness may be confounded. Therefore, to understand the factors underlying fitness variation in natural populations, the role of local density and focal plant phenotype on reproductive success should be studied in concert and at multiple scales. Few studies, however, have integrated local density and plant traits in studies of pollination and/or reproductive success (although see Ohashi and Yahara 2002, Grindeland et al. 2005, Weber and Koln 2013).

In this study, I examine how natural variation in the density of plants (whole-plant level) as well as both the density of male- and female-phase flowers and the sex ratio (flower level), together with two morphological traits (plant height and stigma-nectary distance), influence the likelihood of fruit production and seed number in the dichogamous species *Lobelia cardinalis* (Campanulaceae). I include plant height and stigma-nectary distance in the analyses in order to evaluate the relative importance of density and two phenotypic characters that differ in their function for pollination. Plant height is strongly correlated with stem diameter, which was identified as a direct target of pollinator-mediated selection in the study population (Bartkowska and Johnston 2012). Stigma-nectary distance was chosen because it was not a direct target of pollinator-mediated selection, and was thus unlikely to be the target of pollinator preference, but was expected to influence the efficiency of pollen transfer. Because density effects are known to be scale dependent (Spigler and Chang 2008, Gunton and Kunin 2009), I explored all relationships at four scales (10 cm, 20 cm, 50 cm, 100 cm radii). The roots of *L. cardinalis* are shallow and tend to be shorter than 30 cm (*personal observations*), and the maximum length of leaves on an inflorescence is approximately 20 cm. Thus, competition for abiotic resources is likely to be limited to areas within the circumference of roots and inflorescence leaves. Although hummingbirds can move long distances, foraging bouts tended to be short in duration (most last less than four minutes) and, while visiting a patch, pollinators tended to move among plants that were within one meter of one another (*personal observations*). Thus, the smallest scales are most likely to capture abiotic resource partitioning, while the larger scales should capture competition for or facilitation of pollinator service.

4.3 Methods

4.3.1 **Study species and site**

Lobelia cardinalis is an herbaceous short-lived perennial that is widely distributed throughout North America. It grows in moist habitats and is usually found near the edges of rivers, streams and lakes. The red flowers open acropetally (from the bottom upward) along a single inflorescence and are protandrous with no overlap of sexual phases within a flower. Flowers spend three to ten days in the staminate sexual phase and two to four days in the pistillate sexual phase (Devlin and Stephenson 1984). Mature fruits can produce up to 1763 seeds (data not shown). Throughout its Canadian range, *L. cardinalis* is pollinated solely by *Archilochus colubris* (Trochilidae), the ruby-throated hummingbird (Bertin 1982). Although self-compatible, *L. cardinalis* produces self-fertilized seeds only through geitonogamous transfer of pollen from male to female flowers. Toward the end of flowering, each inflorescence produces one (occasionally two or more) basal rosette for overwintering. In the following season, each rosette may produce a single physiologically independent inflorescence. Thus, density at the smallest scales may be determined by environmental factors and by the propensity for clonal reproduction among individuals. The roots of the inflorescence are shallow and tend to be shorter than 30 cm (*personal observations*). Thus, competition for abiotic resources is likely to be limited to areas within the circumference of roots. I treat all independent flowering stems as individuals, but acknowledge that at the smallest scale (10 cm) surrounding plants may in some cases be ramets of the same genet.

I studied plants growing along the northern side of Lake Travers in the northeastern section of Algonquin Provincial Park in Ontario, Canada, from 15 July to 14 October 2009. Flowering occurred from late July to mid-September. Plants produced mature fruit from August to October.

4.3.2 **Mapping plants**

In July 2009 I tagged and mapped all plants of *L. cardinalis* growing along an 80m length of shoreline. There were very few plants of other species growing within the study site and there were no other flowering conspecifics at least 500m west and 100m east of this site. I extended a rope along the shoreline beginning at the most western individual in the population and placed markers at set intervals. For each plant the distance to the two nearest markers was recorded and its Cartesian coordinates were calculated using trigonometry. A total of 594 plants were tagged; however, only 430 plants were used as focal plants in the study. Plants that produced no flowers, and those where the majority of the inflorescence was eaten by herbivores were excluded as focal plants but were included in estimates of surrounding density.

4.3.3 Reproductive and phenotypic traits measured

On each plant I measured stigma-nectary distance as the distance from the stigma to the bottom of the nectary. Whenever possible two female flowers were measured on each plant, and the average was used in analysis. Plant height was measured as the distance from the ground to the base of the calyx of the uppermost flower on the inflorescence. Because plants continued to grow throughout the season, height on all plants was measured between 8 and 10 September 2009. The relative height of plants was consistent throughout the season.

For each individual I recorded the position along the inflorescence and sex phase of every flower open on 13 dates between 29 July and 14 September, typically every three to four days. I trimmed the dataset to nine dates from 6 August to 7 September because there were few plants with open flowers outside these dates. All fruits from each individual were collected as they became mature. The position of each fruit on the inflorescence was recorded so that for each flower the resulting fruit and seeds could be matched. Seeds were counted by hand.

4.3.4 **Estimating surrounding individual and floral density**

I used the map information to estimate the number of conspecific neighbours surrounding focal individuals at radii of 10 cm, 20 cm, 50 cm and 100 cm radii. For each census date I also estimated the surrounding density of conspecific male and female flowers at the four scales. Density was calculated as the number of plants or flowers per m^2. Plants that suffered extensive damage (specifically plants whose flowers were consumed by slugs or those that were crushed by humans or wildlife) were not used as focal plants, but were included in calculations of surrounding plant and floral density.

4.3.5 **Statistical analysis**

Characterizing surrounding plant densities.—I characterized the distribution of surrounding plant densities using a method similar to Morisita's index of dispersion, a statistical measure of clustering (Morisita 1962). Using a chi-square goodness of fit test for each scale (10 cm, 20 cm, 50 cm, 100 cm), I compared the observed distribution of surrounding plants to a Poisson distribution. For the smallest scale I binned plants in increments of two, and for all other scales I used a bin size of five. Bin sizes were adjusted as necessary, so that the expected number of samples within a particular bin was not less than five (Sokal and Rohlf 1995). For each scale (other than 10 cm), the bin size for the highest density plants was adjusted and the first bin for the 100 cm scale was also increased.

Effects of surrounding individual density on total seed production and focal plant phenotype.—Because density may influence focal plant phenotype and fitness (e.g., plant size and/or seed production may be limited by resource competition), I evaluated whether the surrounding density of plants influenced total seed production, stigma-nectary distance and plant height of focal plants. For all four scales, standard univariate regression was used to examine these relationships. Regression models were fit using the GLM procedure in SAS software, Version 9.2 of the SAS system for Windows (SAS Institute 2008). Although fitness is modeled using a two-part mixture analysis for all subsequent analyses, the univariate regression of total seed production on surrounding plant density enables comparison of our results to those of other studies of density.

Two-part mixture analysis to model fruit and seed production at the whole-plant and flower level.—I used a two-part mixture analysis approach to evaluate the effect of plant and floral density on fitness (Cameron and Trivedi 1998). First, I modeled the likelihood of producing fruit given surrounding density and focal plant phenotype. I treat fruit production as the binary part of the mixture analysis. Second, for plants that produced at least one fruit I tested how density and phenotype influenced the number of seeds produced; this is the count part of the mixture analysis. Factors contributing to fruit production may differ from those shaping the number of seeds produced. For instance, a single visit from a hummingbird may be sufficient for a plant to set fruit (provided that a male flower was visited prior to the female flower). The number of seeds produced, however, may be determined by the duration of the visit and the amount of pollen carried by the hummingbird, which will likely depend on the sequence of visits to male and female flowers within a patch. In addition, many plants and flowers failed to produce fruits, resulting in a zero-inflated distribution for fruit and seed number. No single statistical model can readily account for the longitudinal nature of the data for male and female floral density (i.e. modeling the variance-covariance matrix is required to account for correlations among observations of the same individual taken repeatedly over time) and generate parameter estimates using complex likelihood structures employed in models used for zero-inflated data. Therefore, I modeled reproduction in two parts: 1) factors influencing the likelihood of producing fruit, and 2) factors influencing the number of seeds provided a fruit was produced.

Two-part analysis of whole-plant fruit and seed production.—For all four scales, the total reproductive success of focal plants was characterized by logistic regression to model the likelihood of producing fruit and by standard multivariate regression to model seed production. In both types of models, surrounding density of plants, plant height and stigma-nectary distance were included as explanatory variables. Logistic models were fit using the LOGISTIC procedure in SAS software, Version 9.2 (SAS Institute 2008), assuming a binomial distribution. Regression models were fit using the GLM procedure in SAS software, Version 9.2 of the SAS system for Windows (SAS Institute 2008).

Two-part analysis of flower level fruit and seed production.—Models of flower level fitness accounted for non-independence of multiple observations taken of the same individual over time. The correlation among observations within a plant was modeled using linear mixed-effects models (predicting the average number of seeds produced per day) and a generalized linear mixed model (predicting the likelihood of producing fruit given the number of flowers open on a census date) by specifying the structure of the variance-covariance matrix (R-side modeling, Littell et al. 2006). All factors (male- and female-phase flower density, two phenotypic traits and date) in the analyses were treated as fixed effects. For all four scales both types of models were fit using the GLIMMIX procedure in SAS software, Version 9.2 (SAS Institute 2008). Although GLIMMIX was used to fit the model predicting the average number of seeds produced per flower for all female-phase flowers open on a census date, I used a normal distribution and an identity link function. The likelihood of producing fruit was fit using the events/trial syntax in GLIMMIX, where events were the number of fruits produced and trial was the number of flowers open on a census date (Littell et al. 2006, see also "SAS Data Analysis Examples"). This model assumes a binomial distribution with a logit link function. In addition, date was included as a covariate in these models. Seed and fruit production are likely to decline with date because pollinators begin their southerly migration around the time of peak flowering (Bertin 1982).

I also modeled the effects of surrounding floral density (including male and female flowers) and sex ratio using the same method described for models including surrounding female and male floral density. In these models, sex ratio and total surrounding floral density replace surrounding male and female floral density as explanatory variables.

Randomization procedure to account for violation of non-independence of surrounding density.—Our data violate the assumption of statistical non-independence because focal plants were also included in the measure of surrounding density for other focal plants. Thus, for all analyses (excluding the evaluation of the distribution of surrounding plant density) I employed a randomization technique to estimate parameters and confidence intervals. I generated 2000 randomized datasets by randomly choosing focal plants whose area of density did not overlap. All statistical models were fit to each of the randomized datasets. The average parameter estimates and 95% confidence intervals for the randomized datasets were used to assess whether the predictor variables were related to the response. Sample sizes for the randomized datasets varied. The average sample size was 274 for the 10 cm scale, 210 for the 20 cm scale, 126 for the 50 cm scale and 63 for the 100 cm scale.

4.4 Results

4.4.1 The distribution of surrounding individuals and flower sex phases

At the smallest scale (10cm) plants were randomly (Poisson) distributed (χ^2=2.36 *df*=3, *P*=0.50; Figure 4.1) and occurred in aggregations of zero to 12 individuals. At all other scales, compared to a random distribution, there was an excess of focal plants with many neighbors and an excess of focal plants with too few neighbors (at 20cm $\chi^{2=}$ 401.1, *df*=3, *P*<0.0001; at 50cm χ^2= 309.1, *df*=3, *P*<0.0001; at 100cm χ^2= 451, *df*=3, *P*<0.0001; Figure 4.1).

The distribution of the number of surrounding male and female flowers changed throughout the season (Figure 4.2). The highest number of surrounding female flowers occurred on 18 and 24 August, while the highest number of surrounding male flowers occurred on 14 and 18 August. These dates also corresponded to the largest range in surrounding number of male and female flowers.

4.4.2 Effects of surrounding plant density on total seed production and focal plant phenotype

Surrounding plant density was related to the phenotype of focal plants and also to the total number of seeds produced by focal plants (including plants that made no seeds). The total number of seeds per plant decreased with increasing surrounding plant density at the smallest scale (95% CI [-3.20, -1.76]), but increased at larger scales (Figure 4.1; 20 cm 95% CI [0.51, 3.81]; 50 cm 95% CI [2.71, 7.93]; 100 cm 95% CI [4.22, 13.03]). At all scales, plants tended to be shorter as surrounding plant density increased, but the relationship was weaker at higher scales (Table 4.1). There was also a weak negative association between stigma-nectary distance and increasing surrounding plant density at the smallest scale (10 cm).

4.4.3 Whole-plant reproductive success: effects of surrounding plant density and focal plant phenotype

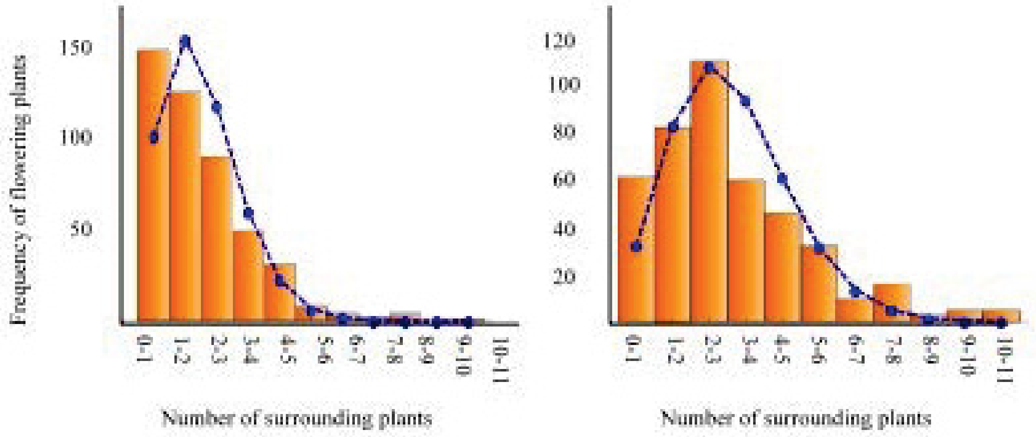
Fruit and seed production per plant were influenced by the surrounding density of plants and plant height, but not by stigma-nectary distance (Table 4.2). At all scales taller plants were more likely to produce at least one fruit, and of those that produced a fruit, taller plants produced more seeds. Increasing density of surrounding plants was associated with an increased likelihood of producing fruit, and the effect increased with scale. In contrast, a high density of surrounding plants was associated with low seed number (for plants that produced at least one fruit), but the relationship was only statistically significantly different from zero at the smallest scale.

4.4.4 Individual flower reproductive success: effects of surrounding male and female flower density, sex ratio, focal plant phenotype and date

The likelihood of producing fruit and the average seed production per flower declined throughout the flowering period (negative coefficient for date in Table 3). For a

a) 10cm radius

b) 20cm radius



c) 50cm radius

d) 100cm radius

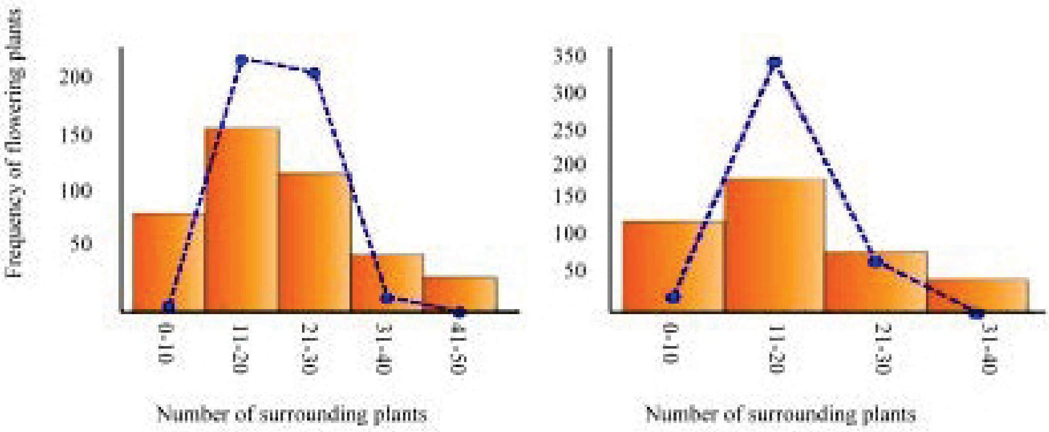
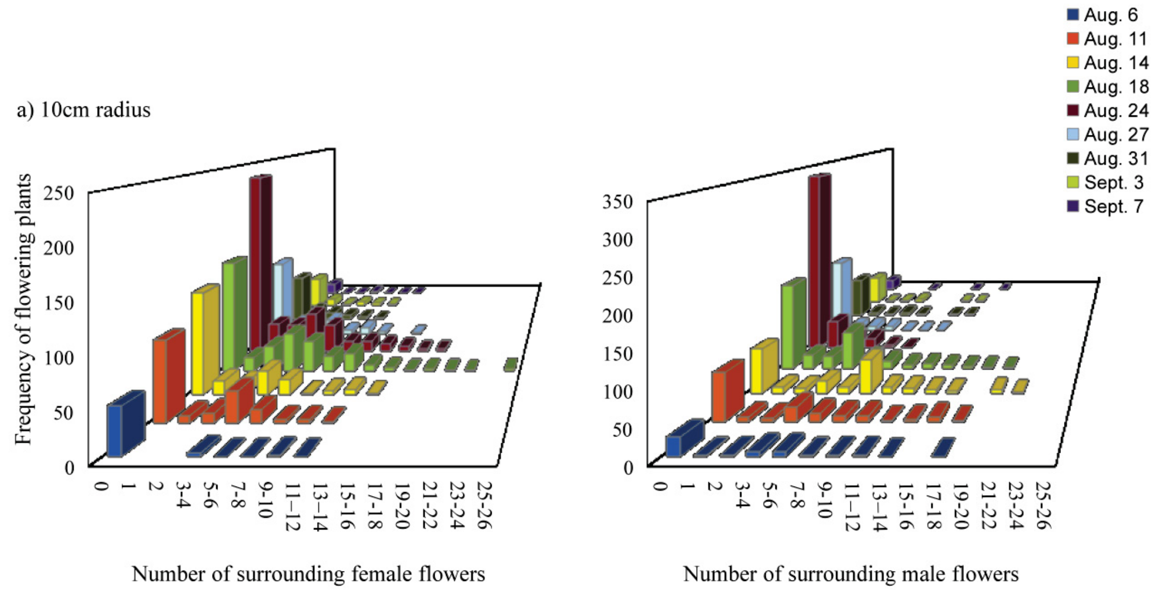
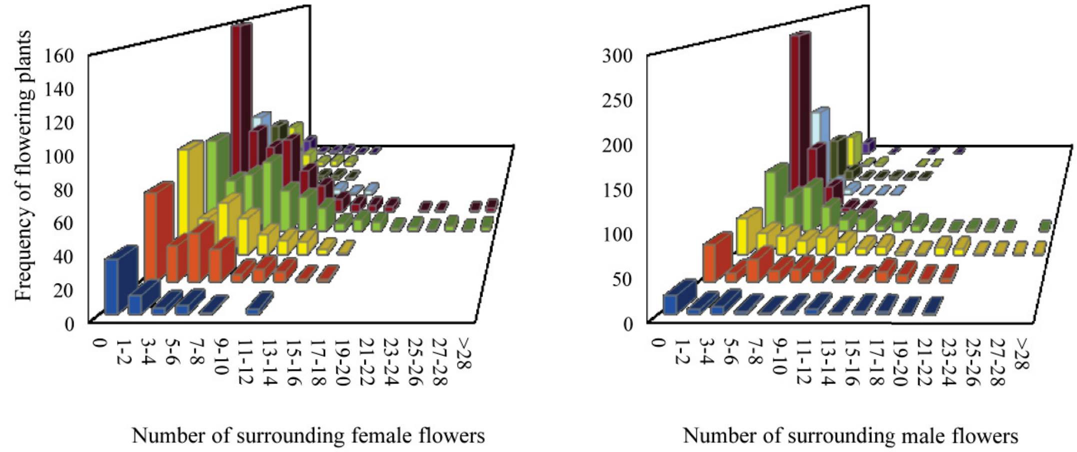


Figure 4.1 Observed distribution of the number of individuals surrounding focal plants at four scales. The distribution predicted for plants randomly distributed in space (based on a Poisson distribution) is overlaid in blue.



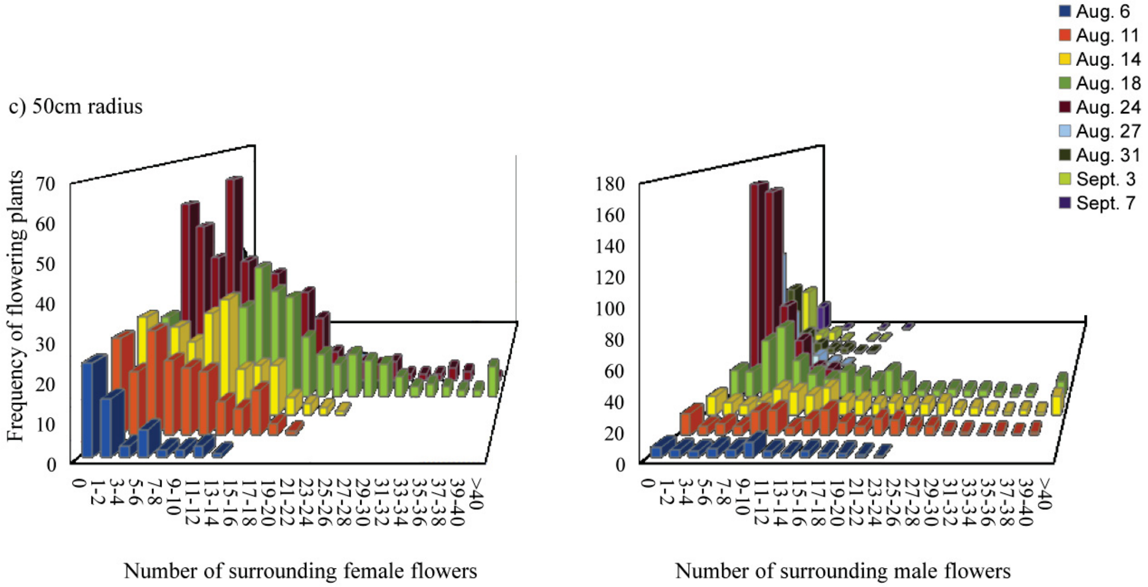
a) 10cm radius



b) 20cm radius

Figure 4.2 Distribution of the number of female (left panels) and male (right panels) flowers surrounding a focal plant (with at least one receptive female flower) across 10 census dates at four scales.

c) 50cm radius



d) 100cm radius

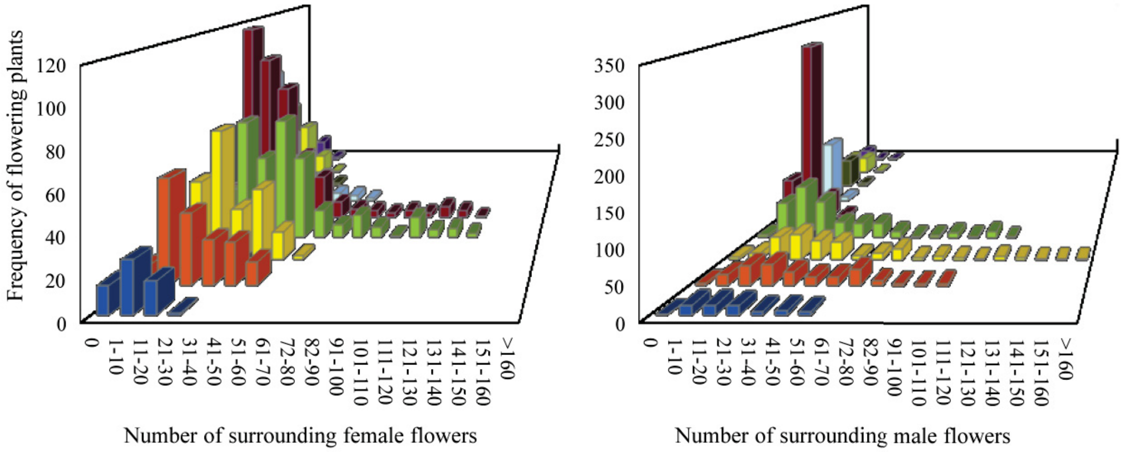


Figure 4.2 cont. Distribution of the number of female (left panels) and male (right panels) flowers surrounding a focal plant (with at least one receptive female flower) across 10 census dates at four scales.

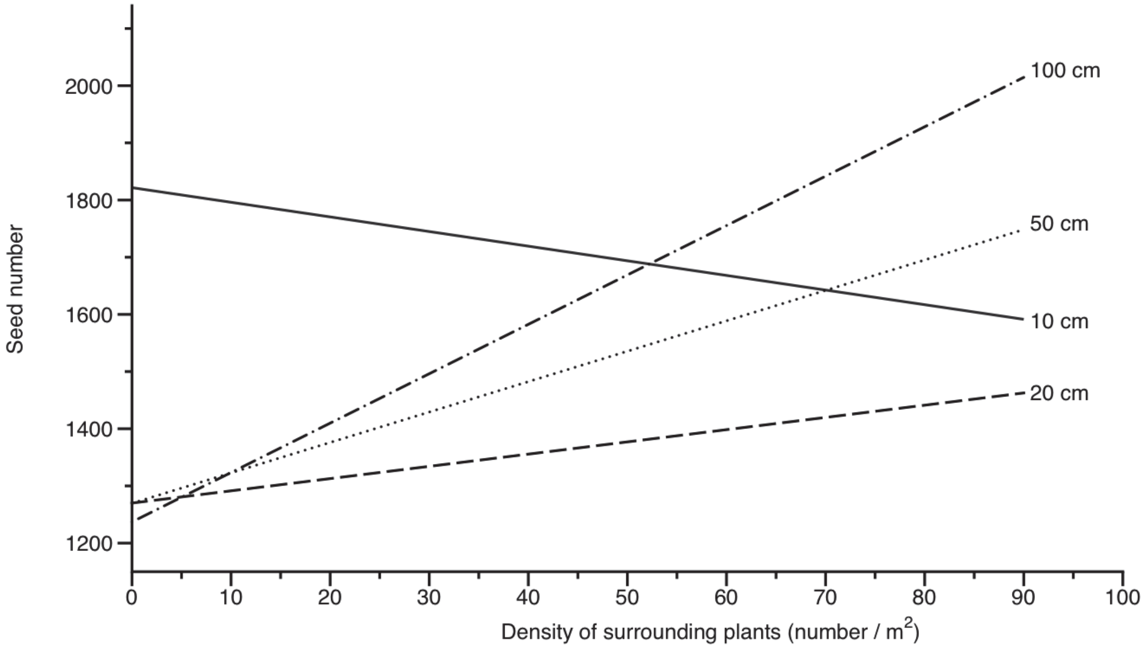


Figure 4.3 Effect of surrounding individual density on total seed number per plant measured at four scales.

given number of female flowers open on a census date, plant height did not influence the likelihood of producing fruit, but was related to increased seed production for plants that produced at least one fruit at the largest scale. At all scales stigma-nectary distance was related to a greater likelihood of fruit production, but did not influence the number of seeds produced.

The likelihood of producing fruit and the average seed production per flower (provided that at least one fruit was produced) declined with higher surrounding female flower density (at all scales; Table 4.3). Although the density of surrounding male flowers did not influence the likelihood of producing fruit, increased male density was associated with a greater average seed production per flower (at all scales; Table 4.3). The effect of surrounding male and female flower density was stronger at larger scales.

The effect of sex ratio was similar to the results of male and female floral density. For a given surrounding floral density, sex ratio did not influence the likelihood of producing fruit (except at the largest scale; Table 4.4). The average number of seeds, however, declined as surrounding sex ratio became more female (Table 4.4).

4.5 Discussion

I examined the influence of local density on fitness at two levels. The whole-plant level reflects seasonal fitness, whereas the flower level measures processes occurring on a shorter time scale. At both levels I also examined fitness-density relationships at four spatial scales. The roots and inflorescence leaves of *L. cardinalis* do not extend beyond 20cm (*personal observations*); thus, the smallest scales will reflect

Table 4.1 Influence of surrounding plant density on plant height and stigma-nectary distance. Estimates are means of coefficients from 2000 regressions of independent data points. Regression coefficients that differ from zero ($\alpha=0.05$) are in bold.

Scale	Height to base of last flower (95% CI)	Stigma-nectary distance (95% CI)
10 cm	**-0.021 (-0.029, -0.011)**	**-0.0012 (-0.0025, -0.00020)**
20 cm	**-0.0059 (-0.010, -0.0015)**	-7.57x10^{-6} (-0.00050, 0.00050)
50 cm	**-0.0020 (-0.0030, -0.00099)**	0.000057 (-5.99x10^{-5}, 1.80x10^{-4})
100 cm	**-0.00094 (-0.0014, -0.00047)**	-0.000059 (-8.92x10^{-5}, 2.91x10^{-5})

Table 4.2 Effects of surrounding plant density and focal plant traits on the likelihood of producing at least one fruit and on the total number of seeds produced (provided that at least one fruit was produced) at four scales. Estimates are means of coefficients from 2000 regressions of independent data points. Estimates that differ from zero (α=0.05) are in bold.

Scale	Parameter	Likelihood of producing at least one fruit (95% CI)	Number of seeds produced provided at least one fruit was made (95% CI)
10 cm	Surrounding plant density	0.0013 (-0.00046, 0.0032)	**-3.66 (-5.02, -2.30)**
	Height of last flower	**0.045 (0.034, 0.056)**	**94.38 (78.43, 107.73)**
	Stigma-nectary distance	-0.015 (-0.051, 0.085	52.07 (-119.57, 12.95)
20 cm	Surrounding plant density	**0.0040 (0.0012, 0.0068)**	-0.61 (-3.47, 1.97)
	Height of last flower	**0.046 (0.035, 0.057)**	**97.13 (81.61, 110.32)**
	Stigma-nectary distance	-0.013 (-0.053, 0.080)	49.58 (-115.77, 14.67)
50 cm	Surrounding plant density	**0.010 (0.0055, 0.015)**	-0.17 (-4.53, 3.88)
	Height of last flower	**0.050 (0.039, 0.060)**	**97.36 (82.39, 110.31)**
	Stigma-nectary distance	-0.0016 (-0.065, 0.070	49.52 (-115.37, 13.65)
100 cm	Surrounding plant density	**0.015 (0.0067, 0.023)**	-2.44 (-10.63, 5.36)
	Height of last flower	**0.048 (0.037, 0.060)**	**97.15 (82.38, 110.05)**
	Stigma-nectary distance	-0.014 (-0.052, 0.082)	49.66 (-116.65, 14.43)

Table 4.3. Effects of surrounding female floral density, male floral density and focal plant traits on the likelihood of producing fruit (given the number of flowers open) and on the average seeds produced per female flower open on a given census date (provided that at least one fruit was produced) at four scales. Estimates that differ from zero are in bold.

Scale	Parameter	Likelihood of producing at least one fruit (95% CI)	Average seeds produced provided at least one fruit was made (95% CI)
10 cm	Surrounding female flower density	**-0.0027 (-0.0041, -0.0011)**	**-0.0026 (-0.0044, -0.00082)**
	Surrounding male flower density	0.00058 (0.00055, 0.0016)	0.0021 (-0.00015, 0.0046)
	Height of last flower	0.0030 (-0.0025, 0.0080)	0.0052 (-0.0020, 0.011)
	Stigma-nectary distance	**0.057 (0.018, 0.097)**	-0.038 (-0.011, 0.093)
	Date	**-0.10 (-0.11, -0.091)**	**-0.11 (-0.14, -0.083)**
20 cm	Surrounding female flower density	**-0.0055 (-0.0087, -0.0022)**	**-0.010 (-0.014, -0.0068)**
	Surrounding male flower density	0.0014 (-0.0011, 0.0037)	**0.0073 (0.0020, 0.013)**
	Height of last flower	0.0027 (-0.0027, 0.0079)	0.0065 (-0.00011, 0.013)
	Stigma-nectary distance	**0.051 (0.012, 0.091)**	-0.027 (-0.022, 0.083)
	Date	**-0.10 (-0.11, -0.09)**	**-0.11 (-0.14, -0.082)**
50 cm	Surrounding female flower density	**-0.016 (-0.025, -0.0063)**	**-0.041 (-0.054, -0.027)**
	Surrounding male flower density	0.0030 (-0.0027, 0.0089)	**0.025 (0.010, 0.041)**
	Height of last flower	0.0020 (-0.0035, 0.0071)	0.0052 (-0.00099, 0.013)
	Stigma-nectary distance	**0.061 (0.021, 0.102)**	-0.035 (-0.0097, 0.085)
	Date	**-0.10 (-0.11, -0.090)**	**-0.11 (-0.14, -0.086)**
100 cm	Surrounding female flower density	**-0.024 (-0.036, -0.012)**	**-0.065 (-0.080, -0.050)**
	Surrounding male flower density	0.0013(-0.0078, 0.0094)	**0.043 (0.021, 0.064)**
	Height of last flower	0.0031 (-0.0022, 0.0081)	**0.0072 (0.00066, 0.013)**
	Stigma-nectary distance	**0.061 (0.020, 0.102)**	-0.037 (-0.010, 0.090)
	Date	**-0.10 (-0.11, -0.091)**	**-0.11 (-0.13, -0.084**

Table 4.4. Effects of surrounding floral density, sex ratio (proportion female) and focal plant traits on the likelihood of producing fruit (given the number of flowers open) and on the average seeds produced per flowers open on a given census date at four scales. Estimates are means of coefficients from 2000 regressions of independent data points. Estimates that differ from zero (α=0.05) are in bold.

Scale	Parameter	Likelihood of producing at least one fruit (95% CI)	Average seeds per flower provided at least one fruit was made (95% CI)
10 cm	Surrounding floral density	**-0.001 (-0.0020, -4.8x10^{-5})**	-0.00126 (-0.0028, 0.00022)
	Surrounding sex ratio	-0.378 (-0.99, 0.204)	-0.307 (-1.67, 1.03)
	Height of last flower	-0.0035 (-0.018, 0.0090)	0.0139 (-0.0084, 0.029)
	Stigma-nectary distance	0.0594 (-0.073, 0.208)	0.0638 (-0.079, 0.23)
	Date	**-0.125 (-0.160, -0.087)**	**-0.170 (-0.26, -0.055)**
20 cm	Surrounding floral density	**-0.00310 (-0.0055, -0.00085)**	-0.0019 (-0.0044, 6.25x10^{-5})
	Surrounding sex ratio	-0.0874 (-0.38, 0.19)	**-0.631 (-1.28, -0.22)**
	Height of last flower	0.00449 (-0.0034, 0.012)	**0.0174 (0.0092, 0.027)**
	Stigma-nectary distance	**0.0684 (0.0025, 0.13)**	0.0592 (-0.010, 0.13)
	Date	**-0.134 (-0.16, -0.11)**	**-0.147 (-0.19, -0.094)**
50 cm	Surrounding floral density	**-0.0080 (-0.013, -0.0033)**	**-0.0132 (-0.019, -0.0071)**
	Surrounding sex ratio	-0.113 (-0.35, 0.14)	**-0.851 (-1.58, -0.14)**
	Height of last flower	-0.00336 (-0.0097, 0.0025)	**0.00787 (0.0018, 0.014)**
	Stigma-nectary distance	**0.0671 (0.021, 0.11)**	0.0391 (-0.022, 0.10)
	Date	**-0.117 (-0.13, -0.10)**	**-0.125 (-0.16, -0.095**
100 cm	Surrounding floral density	**-0.0123 (-0.019, -0.0055)**	**-0.0191 (-0.026, -0.011)**
	Surrounding sex ratio	**-0.453 (-0.77, -0.14)**	**-1.043 (-1.80, -0.17)**
	Height of last flower	0.000463 (-0.0052, 0.0057)	**0.00929 (0.0032, 0.015)**
	Stigma-nectary distance	**0.0636 (0.020, 0.11)**	0.0415 (-0.018, 0.11)
	Date	**-0.106 (-0.12, -0.092)**	**-0.122 (-0.16, -0.092)**

competition for below- and above-ground resources. The influence of local density on pollination, however, will extend beyond the smallest scales. In *L. cardinalis* fruit and seed production is expected to closely match pollinator movement, as seed production is strongly pollen limited in this species (Johnston 1993, Bartkowska and Johnston 2012), and there is no evidence of inbreeding depression at the seed stage (Johnston 1992), thus making *L. cardinalis* an excellent study subject for pollination studies.

I found that over the course of the season plants benefited from having many neighbors, but within the season female-phase flowers competed for pollen, indicating that surrounding floral density and sex ratio are important determinants of seed production. Furthermore, I found evidence of resource competition at the smallest scale and facilitation for pollination at larger scales at the whole-plant level.

4.5.1 **Whole-plant reproductive success: scale-dependent effects of local plant density**

At the smallest scale (10 cm) increased surrounding density of plants was associated with a reduction in total seed production. Plants in dense aggregations also tended to be shorter, and (at the smallest scale) flowers on plants in dense patches tended to have smaller stigma-nectary distances. These observations suggest that at the smallest scale plants were competing for abiotic resources. At scales greater than 10 cm, however, I found that the density of surrounding plants had a positive effect on total seed production and the effect was stronger at larger scales. Similarly, after accounting for plant height, the likelihood of a plant producing at least one fruit also increased with surrounding plant density. A positive relationship between fitness and surrounding plant density could be caused either by pollinators preferentially visiting high-density patches or by larger plants growing in high-density patches. Experimental manipulations of density and resource availability would allow one to partition the influence of microsite quality from the effect of density. Because plants tended to be shorter in high-density patches, it is more likely that pollinators preferentially visiting high-density patches caused the positive relationship between fitness and surrounding plant density. Previous studies have also reported strong scale-dependent effects of local density. In a recent study of *Sabatia angularis*, Spigler and Chang (2008) found that the density of neighboring plants within 1m reduced reproductive success of focal plants, but beyond that distance (up to 4m) reproductive success increased (see also Gunton and Kunin 2009).

4.5.2 The sexual environment and competition for pollinator service

Pollinator preference for high-density patches may result in facilitation at the whole-plant level, but on a given day female-female competition and mate availability may be more important in determining whether a particular flower sets seed and how many seeds are produced. At the flower level the likelihood of producing fruit and the average number of seeds produced per open female flower both declined with increasing surrounding female flower density (at all scales), suggesting that female flowers competed for pollen. This observation is also consistent with Feldman (2006), where she found that pollinators visited more plants in dense patches, but visits per plant decreased in dense patches (see also Rathcke 1983). Although competition for abiotic resources could also account for a negative relationship between surrounding female flower density and seed production, competition for abiotic resources cannot account for the positive relationship between male flower density and seed number (Table 3). Furthermore, when I explicitly removed the influence of total local floral density in our sex ratio models, I found that female-biased surrounding sex ratio reduced seed production at high densities (at the flower level), again consistent with female-female competition for pollen. Thus, female-female competition and availability of mates are likely to be the causal factors underlying the relationship between seed production and floral density. Our results are consistent with predictions of models of pollen transfer where the amount of pollen carried by a pollinator increases with every male flower visited (the density of male flowers) and declines with every female flower visited (Price and Waser 1982, Aizen 2001, Bianchi and Cunningham 2012).

Despite the prevalence of dichogamy among angiosperm species (3151 of 4200 angiosperms, 75%, exhibit intrafloral dichogamy; Bertin and Newman 1993), little is known about the consequences of temporal separation of sex phases within flowers for pollination and subsequent reproductive success of individuals. In studies of *Alstroemeria aurea*, Aizen (2001) found that the quantity of pollen deposited by bumblebees per visit decreased as the population became more female biased. Surrounding sex ratio, however, had little effect on total fitness, most likely because plants were not pollen limited (Aizen 1997, 2001). Other studies have found that seed set declines overtime as a population becomes female-biased (reported for *Delphinium barbeyi* in Elliott and Irwin 2009) and that the likelihood of fruit set increases when the population is male-biased (reported for *Stylidium armeria* in Brookes and Jesson 2010). These two results, however, could also be caused by other unmeasured factors. Fully dichogamous flowers are especially interesting because the optimal longevity of male and female phases should be affected by surrounding density and sex ratio, and these change over space and time.

4.5.3 Floral traits and pollinator attraction against a background of plant and floral density

Understanding the role of density in pollination and reproductive success is challenging, not only because multiple processes may underlie a relationship between density and fitness, but also because density may influence plant phenotype (Wyszomirski and Weiner 2009). Because I included phenotype and density in the same models, I were able to examine how a character (plant height) known to experience pollinator-mediated selection and a character that is not a direct target of pollinator-mediated selection (stigma-nectary distance) influenced reproduction while accounting for density. At the whole-plant level, I found a positive association between plant height and the likelihood of producing at least one fruit and a positive association between plant height and seed production, suggesting pollinator preference for tall plants. A relationship between plant height and fitness might also be caused by the correlation of plant height with flower number because taller plants with more flowers have more opportunities to produce fruit. I therefore modeled the likelihood of producing fruit given the number of flowers produced by a focal plant using events/trial syntax in the logistic procedure (Bernoulli distribution) in SAS software, Version 9.2 of the SAS system for Windows (SAS Institute 2008). This analysis again revealed a positive association (results not shown). Thus, the increased likelihood of producing fruit with increasing surrounding plant density most likely reflects more frequent pollinator visits to high-density patches.

Although I also found an association between plant height and seed number at the whole-plant and per flower level, I cannot rule out the possibility that these relationships were caused by differences in pant vigor or by phenotypic plasticity associated with difference in microsite quality. In a previous study (Bartkowska and Johnston 2012), however, I found that plant height experienced pollinator-mediated selection and had a significant nonlinear relationship with seed number (positive nonlinear selection gradient, gamma, for stem diameter, which is used as a proxy for height in Bartkowska and Johnston 2012). Our current findings coupled with our previous study suggest that pollinators may be drawn to high-density patches and taller plants, but that the local density of male and female flowers further influences successful pollen transfer. Thus, taller plants and those in low surrounding female flower density but high male flower density achieve the greatest fitness.

Although stigma-nectary distance was associated with an increased likelihood of fruit production, this relationship is more likely caused by floral position than plant-pollinator fit or pollinator preference. Stigma-nectary distance is positively related to flower size and position (data not shown). Larger flowers are more likely to be located towards the base of the inflorescence, and possibly have more resources available to mature fruit. Seed production is also known to vary systematically with floral position in this species; seed number per flower is lowest in terminal flowers (Devlin 1989).

4.5.4 **Consequences for natural selection**

Our results suggest that dichogamous and other sexually polymorphic populations may experience pollen limitation caused in part by the availability of mates and female-female competition. In such populations the sexual neighborhood will determine the reproductive success of individual male- and female-phase flowers. Because the sexual neighborhood—the surrounding sex ratio and floral density—changes within a season, the optimal longevity and investment in sex phases of individual flowers will not be constant.

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CHAPTER 5 SURVIVAL AND FITNESS CONSEQUENCES OF ROSETTE SIZE AND NUMBER MEASURED AT THE RAMET AND GENET LEVEL

5.1 Abstract

Most perennial angiosperms are capable of clonal reproduction. Allocation to clonal reproduction can affect survival and fitness within and between seasons. Because clonal plants typically have several clonal offshoots (ramets), studies of fitness should ideally be conducted at the genet level. Few studies, however, have quantified variation among individuals in allocation to clonal reproduction and have assessed the fitness consequences at the ramet and genet level. In this study, I used three consecutive years of phenotypic measurements coupled with estimates of survival into the following year to explore how variation in rosette size and number among ramets and genets of *Lobelia cardinalis* influence ramet and genet survival and fecundity during the following year. Genet and ramet survival increased with increasing size and number of rosettes produced by ramets during the previous year. Although survival increased with greater rosette size and number, the shape of the functions differed among years. Furthermore, the fitness (measured as seed production) consequences for the genet associated with the numbers of rosettes produced in the previous season differed among years. Variability in survival and seed production among years suggests that selection for rosette number and size may vary through time. Possible limits to rosette size and number are also discussed.

5.2 Introduction

Investment in different functions or traits supported by common resources that contribute to fitness is expected to reflect a compromise that maximizes fitness (Stearns 1992, reviewed in Obeso 2002). In iteroparous organisms that reproduce both sexually and asexually, the optimal allocation of resources to each mode of reproduction is likely to reflect a trade-off between current and future reproduction. This relationship becomes more complex in perennial plants that produce clonal offshoots that themselves become sexually reproductive. In plants, clonal offspring are referred to as ramets, and all ramets produced by a genotype are collectively referred to as a genet (Harper 1977). The number of clonal offshoots produced may influence the size and spatial distribution of ramets and genets in future years. Thus, competition for abiotic and pollinator resources may be determined in part by allocation to clonal reproduction by parental plants during the previous season. In this manner, allocation to clonal growth will affect ramet and genet reproductive output during subsequent years.

Most perennial plants invest in some form of clonal reproduction (approximately 70% of species, Klimes et al. 1997). Clonal life history traits such as the size and number of clonal offshoots, the degree of physical integration among clones (clones can be physiologically independent or integrated through rhizomes, stolons or bulbs) and the spatial distribution of clones within a population (ramets can be clumped or widely dispersed with intermingling among genets) may have important consequences for genet fitness (Fischer and van Kleunen 2002, Vallejo-Marín et al. 2010). The distribution of ramets, for example, has been shown to have fitness consequences; species that produce a clumped distribution of ramets (“phalanx”) tend to have high survival, and high birth rates, whereas those with highly dispersed and intermingled clones (“guerilla”) have higher spreading ability, allowing them to exploit open spaces more readily (reviewed in

Fischer and van Kleunen 2002). Clonal reproduction can also shape the spatial distribution (number, density and genotype) of individuals within populations (Vallejo-Marín et al. 2010). In the extremes, a population can comprise a single clone or each flowering stem represents a unique multilocus genotype (reviewed in Vallejo-Marín et al. 2010). Thus, clonal growth also shapes the genetic structure of populations.

Within populations, the local density of flowering ramets and genets may also affect the intensity of competition for abiotic and pollinator resources and patterns of pollen dispersal and subsequent mating opportunities of individual plants (Chapentier 2001). For plants that produce physiologically independent ramets in a phalanx architecture, producing many ramets may limit the size and fitness of individual ramets due to competition. Flowering stems in high-density patches are often smaller and produce fewer flowers, likely due to greater competition for abiotic resources (reviewed in Weiner et al. 2001).

Mating opportunities and the quality of pollen transferred are also likely to be affected by the local number and density of ramets and genets. In dioecious populations of *Sagittaria latifolia*, northern populations may be male-biased (and thus have limited mating opportunities) because male plants allocate more resources to clonal reproduction than female plants (van Drunen and Dorken 2012). In hermaphroditic species, the density and distribution of ramets and genets may influence pollination and self-fertilization. For instance, in a study of *Decodon verticillatus*, nearly 25% of selfing results from pollen movement among ramets (Eckert 2000). Unlike autogamous self-fertilization, geitonogamy does not provide reproductive assurance (as a pollen vector is required) and has associated costs of ovule and pollen discounting (Lloyd and Schoen 1992, see also

Barrett 2002). Furthermore, in self-incompatible species, geitonogamous self-fertilization may prevent legitimate fertilization by clogging stigmas with incompatible pollen (e.g. Araki et al. 2007, see also Barrett 2002).

Although environmental factors govern the energy available to individuals for clonal and sexual propagation, a genetic component can also underlie clonal reproduction. Rosette (ramet) formation in *Ranunculus reptans* varies heritably among individuals (van Kleunen et al. 2002), suggesting that ramet formation has the potential for adaptive evolution. How individual variation in clonal characters (such allocation to size and number of clonal offspring) contributes to individual fitness has largely been overlooked (although see Geber 1992).

Using three years of field observations, I quantify the fitness consequences of rosette size and number at the ramet and genet level in a natural population of *Lobelia cardinalis* (Campanulaceae). Rosettes are produced after investment in floral structure has been made; however, rosettes may partly compete with developing fruit for photosynthate. Thus, within a season rosette size and number may be determined by the progenitor plant size and reproductive output. Because each surviving rosette becomes a flowering plant in the following year, decisions made by parental plants regarding resource allocation between size and number of rosettes is likely to influence subsequent size and fitness of ramets and genets in following years.

5.3 Methods

5.3.1 Study species and site

Lobelia cardinalis is an herbaceous short-lived perennial that is widely distributed throughout North America. It grows in moist habitats, and is usually found near the edges of rivers, streams and lakes. Seeds germinate during spring and subsequently form rosettes. Few rosettes flower during their first year (Johnston 1992, see also Devlin et al. 1984). Rosettes grow and eventually bolt, producing an inflorescence of two to > 50 flowers (Devlin 1984, *pers. obs.*). Flowering occurs from late July to mid-September. The flowers open acropetally (from bottom upward) along the single inflorescence and are protandrous with no overlap of sexual phases within a flower. Although self-compatible, *L. cardinalis* produces self-fertilized seeds only through geitonogamous transfer of pollen from male to female flowers. Plants produce fruit from August to October. Inflorescences begin producing basal rosettes as early as mid-August (or sooner if the individual was eaten or damaged). Flowering ramets typically produce zero to four basal rosettes for overwintering, although plants with up to six rosettes have been observed in the study population (*pers. obs.*). During the following season, each surviving rosette may produce a single physiologically independent inflorescence.

I studied plants growing along the Petawawa River and Lake Travers in the northeastern section of Algonquin Provincial Park in Ontario, Canada, from 15 July to 14 October 2009, July 20th to October 15th 2010, July 18th to October 10th 2011 and August 2nd to October 10th 2012.

5.3.2 **Field methods**

During 2009, I tagged all ramets growing along an 80m length of the northern shoreline of Lake Travers. During the subsequent three years, all plants growing on this site were tagged and included in this dataset. Thus, in each year new genets and ramets were added to the study. During autumn 2009, 2010 and 2011, plastic stakes were placed in the ground beside each individual. Thus, ramets could be identified during the following year based on proximity to the plastic tag. Recovery rate of the tags was approximately 70% in 2010, approximately 86% in 2011 and 84% in 2012. Survival to 2010, 2011 and 2012 was assessed during mid to late-July. By this time, all surviving individuals had produced an inflorescence and were easily identified.

5.3.3 **Assignment of ramets to genets**

During 2009 most ramets were treated as genets during 2009, although there were a few exceptions. There were five groupings of flowering ramets with inflorescences clustered within 5 cm, which were each assumed to be components of individual genets. During all subsequent years, ramets were assigned to genets based on their progenitor ramet during the previous season. The number of ramets that comprise a genet is likely underestimated for all years.

5.3.4 **Phenotypic traits measured**

Plant size and flower number were estimated for all tagged plants during 2009, 2010 and 2011. During 2009 plant size was estimated by stem diameter. I measured the diameter of the stem (at 5 cm from the ground) to the nearest 0.5 mm with digital calipers. During all other years, plant size was estimated as the length of the stem from the ground to the base of the lowers flower, measured to the nearest 0.5 cm.

During October of 2009, 2010 and 2011, I counted the number of rosettes initiated by each ramet and I measured the length of the longest leaf on each ramet to the nearest 0.5 cm. In the sister species *L. inflata*, the length of the longest leaf correlates strongly and positively with rosette dry mass (Simons and Johnston 2000).

During 2012, the site was highly disturbed by fishermen. Thus, apart from survival, no other plant traits were measured.

5.3.5 **Fitness traits measured**

During 2009, 2010 and 2011 all fruits from each individual were collected as they matured. Each fruit was dissected and the seeds photographed. From these images, the number of seeds per fruit were counted using IMAGEJ 1.43 (NIH, Bethesda, MD, USA).

5.3.6 **Statistical analysis**

All tests were conducted using JMP 10 software (SAS Institute, Cary, NC)
I used Mann-Whitney Wilcoxon test (Sokal and Rohlf 1981) to compare the mean plant size (stem diameter in 2009 and length to first flower in 2010 and 2011), flower number, fruit number and seed number (for plants that produced fruit) between plants that produced different number of rosettes. The assumptions of ANOVA were violated; specifically, the variance in predictor variable was heteroskedastic. Thus, non-parametric statistics were more appropriate.

Linear regression was used to examine both how ramets' phenotypic and reproductive traits influenced the size of rosettes they produced during a growing season and the effect of autumn rosette size on phenotypic and fitness traits during the subsequent year. For ramets that produced multiple rosettes, I assessed the similarity in size between rosettes produced by a plant using Pearson product-moment correlations. When more than one rosette was produced by a ramet, rosettes were labeled as rosette 1, rosette 2 etc according to size; the largest rosette was labeled rosette 1 and so forth.

The relationship between the size of a rosette produced in the fall and the phenotypic and fitness traits of the inflorescence that is produced in the following year was explored using a standard linear regression.

Logistic regression was used to explore how the size of the rosette size influenced over-winter survival and probability of producing a flowering stem during the following year at the ramet and genet level. At the genet level, I tested how the average size of the rosettes initiated by a genet influenced the likelihood of at least one ramet surviving to flower during the following year. Because most ramets could not be assigned to genets in 2009, the relation of genet survival to 2010 to rosette size and number during 2009 could not be explored.

The difference in seed production between genets that produced one, two, or more than two rosettes was explored using an ANOVA and the Tukey's honestly significant difference *post hoc* tests (Sokal and Rohlf 1981). During 2009, most flowering stems were considered to be genets and very few plants successfully matured fruits during 2010. Although some ramets did produce more than 2 rosettes during 2009, none survived and produced mature seeds in 2010. Very few genets survived to 2011 and produced seeds during 2011. All genets that had produced more than 3 rosettes during 2010 were combined into a single category (>2).

The relationship between the phenotype of ramets (mean ramet length to first flower and the mean ramet flower number) and the number of ramets in a genet was explored using an ANOVA and the Tukey's honestly significant difference *post hoc* tests (Sokal and Rohlf 1981). ANOVA and the Tukey's honest difference *post hoc* tests were also used to explore how the total fruit and seed number produced by a genet depended on the number of ramets per genet.

5.4 Results

5.4.1 **Rosette production**

During 2009 and 2011 most ramets produced one rosette, whereas during 2010 half of all ramets failed to produce any rosettes (Figure 5.1). During all years, plants that produced more rosettes tended to be larger (estimated as stem diameter and height to first flower during 2009, and as length to first flower during 2010 and 2011), and to produce more flowers, fruits and seeds; however, means differed significantly only for stem diameter and flower number during 2009 (Figure 5.2).

Average rosette size (length of logest leaf) did not differ between years (Table 5.1). Rosettes produced by the same flowering stem were highly correlated in size (Table 5.2). During 2009, larger plants produced more flowers, fruits and larger rosettes (Figure 5.3 left panels). However, very little of the variation in rosette size was explained by the phenotypic traits or fruit number (Stem diameter: rosette size = 2.6 + 0.21*stem diameter, R^2=0.05, P<0.0001; Flower number: rosette size = 2.2 + 0.03*flower number, R^2=0.02,

P=0.01; Fruit number: rosette size = 3.4+0.05*fruit number, R^2=0.01, P=0.02). During 2010 and 2011, the size of daughter ramets did not vary significantly with any measured characteristics of the parent ramet (Figure 5.3 middle and right panels).

5.4.2 **Ramet survival and performance**

Overwinter survival to flower during the subsequent year varied extensively among years: 2009, 50%; 2010, 11%; 2011, 80%. Larger rosettes in autumn were more likely to survive to flower during the following season; however, the relationship was statistically significant only for survival to 2010 (P<0.0001; top panel Figure 5.4). Autumn rosettes size during 2009 positively affected flower production during 2010 (Figure 5.5), and larger rosettes in 2010 tended to grow into taller ramets with more flowers in 2011 (Figure 5.5). However, rosette size during the preceding autumn explained little variation in flower number in 2010, and first flower height and flower number in 2011 (Figure 5.5; 2010 flower number= 11.2 + 1.9*rosette size 2009, *df*=40 , R^2=0.03, P=0.012; 2011 first flower height = 47.5 - 0.46* rosette size 2010, *df*=87, R^2=0.003, P=0.62; 2011 flower number =8.32 + 0.45* rosette size 2010, *df*=86, R^2=0.004, P=0.56).

5.4.3 **Genet survival and seed production**

Genets that produced large rosette(s) tended to survive to the following year better than those with small rosettes; however, the relationship was statistically significant only for 2009 genets (Figure 5.6 left panels). The likelihood of genet survival to flower in the following year increased with the number of rosettes initiated during the previous fall (Figure 5.6 right panels).

Seed production differed between plants that produced one or more rosettes during the previous year. During 2010, genets represented by a single rosette during 2009 produced more seeds than genets that had initiated multiple rosettes (Figure 5.7 top panel). Genets that produced more than two rosettes in 2010 produced the most seeds on average compared to genets that produced two or one rosette in 2010 (Figure 5.7).

5.4.4 **Ramet phenotype and genet fitness**

2010 and 2011 genets with more ramets tended to produce shorter inflorescences with fewer flowers than smaller genets, although these relations were not statistically significant (Figures 5.7 and 5.8). In 2010, most ramets failed to reproduce sexually as nearly all plants were consumed by herbivores, primarily slugs. In 2011, genets comprised of two ramets produced statistically significantly more fruits, on average, than single-ramet genets (Figure 5.8). Seed production did not differ statistically among any genet class (Figure 5.8).

5.5 Discussion

Genet and ramet survival generally varied positively with the size and number of rosettes produced by ramets during the previous year, despite considerable inter-year variation. The consequences for genet seed production similarly varied with rosette number among years. Ramets that initiated two rosettes in 2009 produced groupings of ramets (considered to be genets) with fewer seeds in 2010 than ramets that produced a single

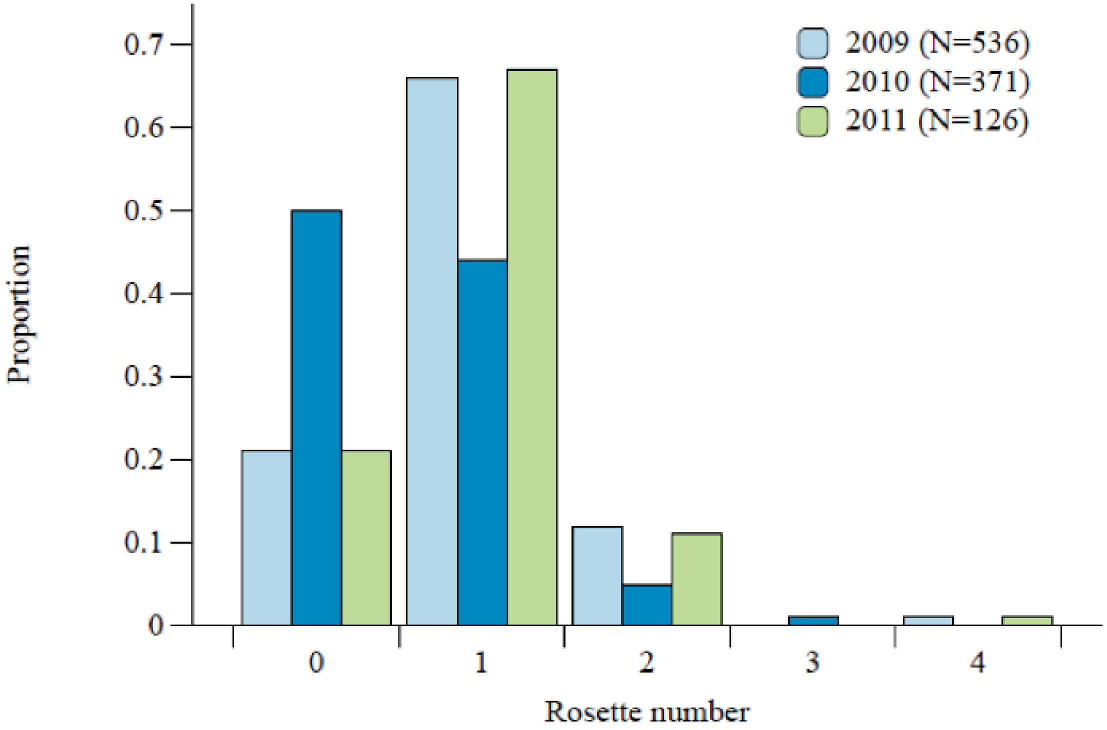

Figure 5.1 Observed distribution of the proportion of individuals producing zero to four rosettes in three years

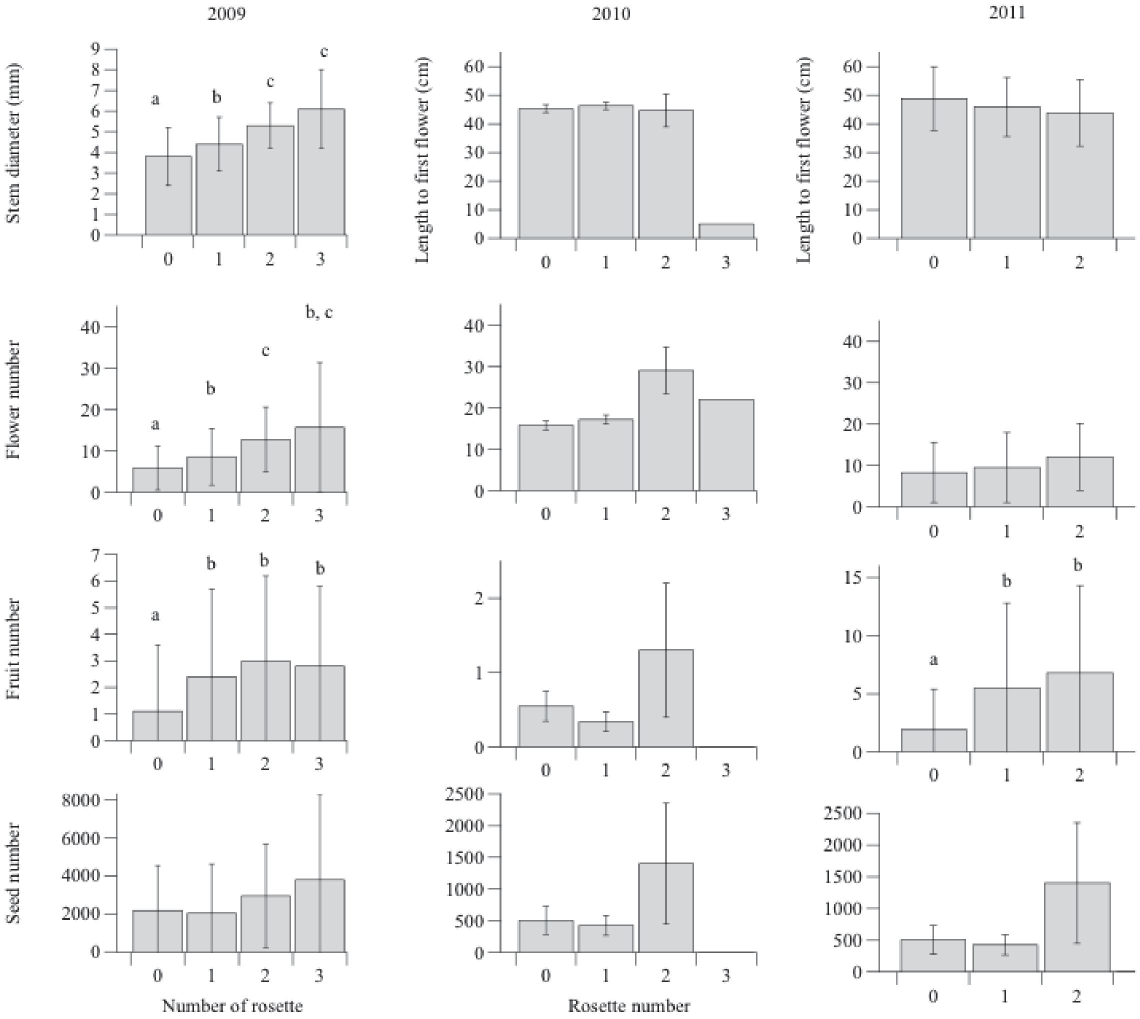

Figure 5.2 Means (± SE) of plant size, flower number, and fruit and seed number for ramets producing different number of rosettes during three years. Different letters above distinguish groups with statistically significantly different means based on Mann-Whitney Wilcoxon test. Plant size was measured as stem diameter in 2009 and length to first flower in 2010 and 2011.

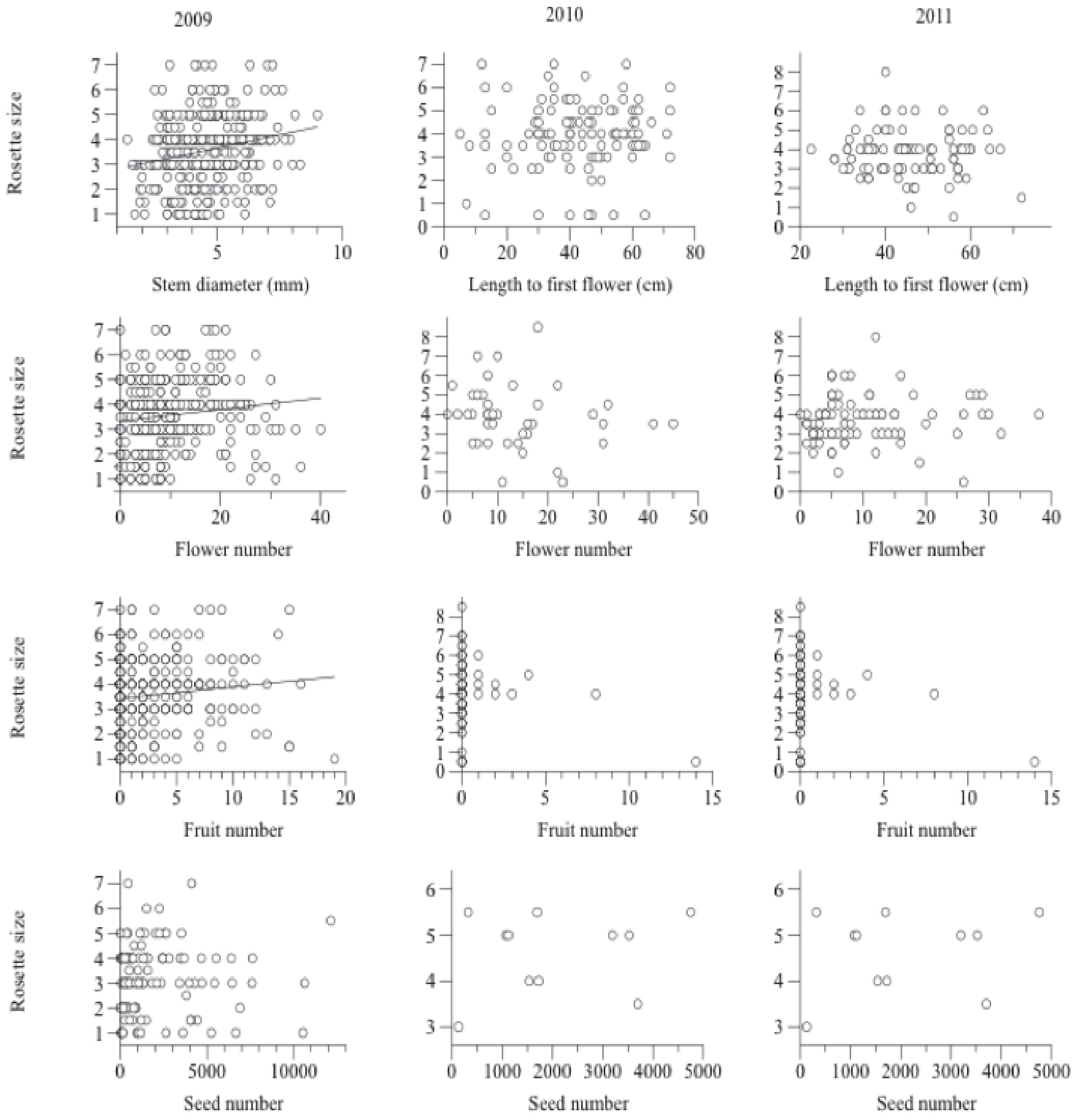

Figure 5.3 Relationship of the size (of longest leaf) of largest rosette to plant size, flower number, fruit and seed production during each of three seasons. The line of best fit is displayed only for statistically significantly regressions.

Table 5.1 Mean (± SE) lengths of the longest leaf, ranges and sample sizes for first through fourth rosettes produced by a flowering ramet during three years. Leaf length did not differ significantly among years or among rosette order within years

	2009			2010			2011		
Rosette order	*N*	Mean ± SE (cm)	Range (cm)	*N*	Mean ± SE (cm)	Range (cm)	*N*	Mean ± SE (cm)	Range (cm)
1	419	3.6 ± 1.3	1.0-7	182	4 ± 1.5	0.5-8.5	99	3.8 ± 1.3	0.5-8
2	67	3.3 ± 1.2	1.0-7	20	4.7 ± 1.5	2.5-8	15	3.5 ± 1.3	1-6
3	6	3.8 ± 1	3.0-5	2	6 ± 0.7	5.5-6.5	1	3	
4							1	3	

Table 5.2 Pairwise correlations of rosette size for the first to the third rosettes produced by flowering ramets from 2009-2011.

	2009		2010	2011
	Rosette 1	Rosette 2	Rosette 1	Rosette 1
Rosette 2	0.48^{***}		0.84^{***}	0.80^{**}
	N=67		N=20	N=15
Rosette 3	0.80^{*}	0.85^{*}		
	N=6	N=6		

$^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.0001$

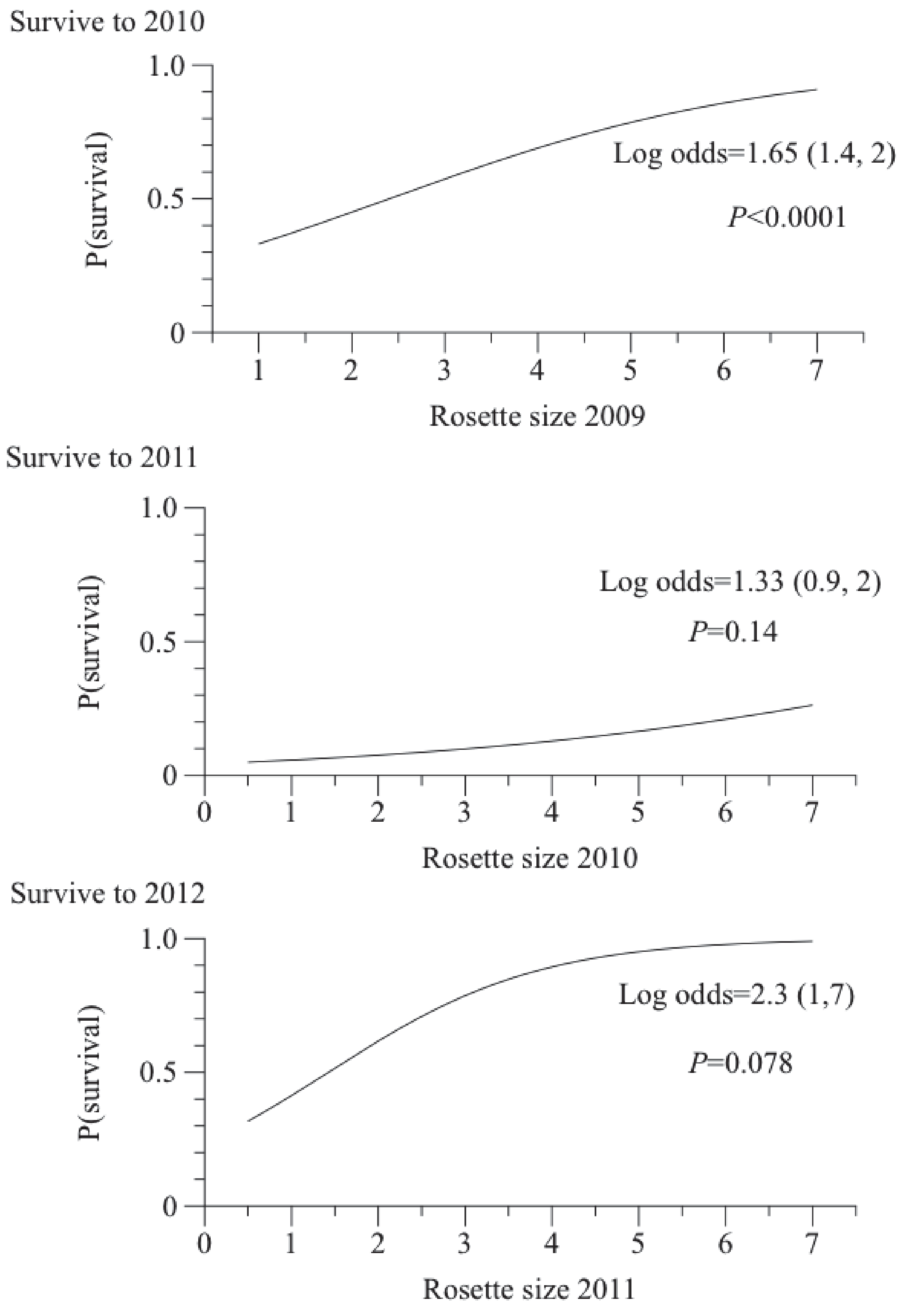

Figure 5.4 Relations of the probability of over-winter ramet survival to rosette size for 2010-2012. The log odds (and 95%CI presented in brackets) and the *P* value for each relationship is presented for each function.

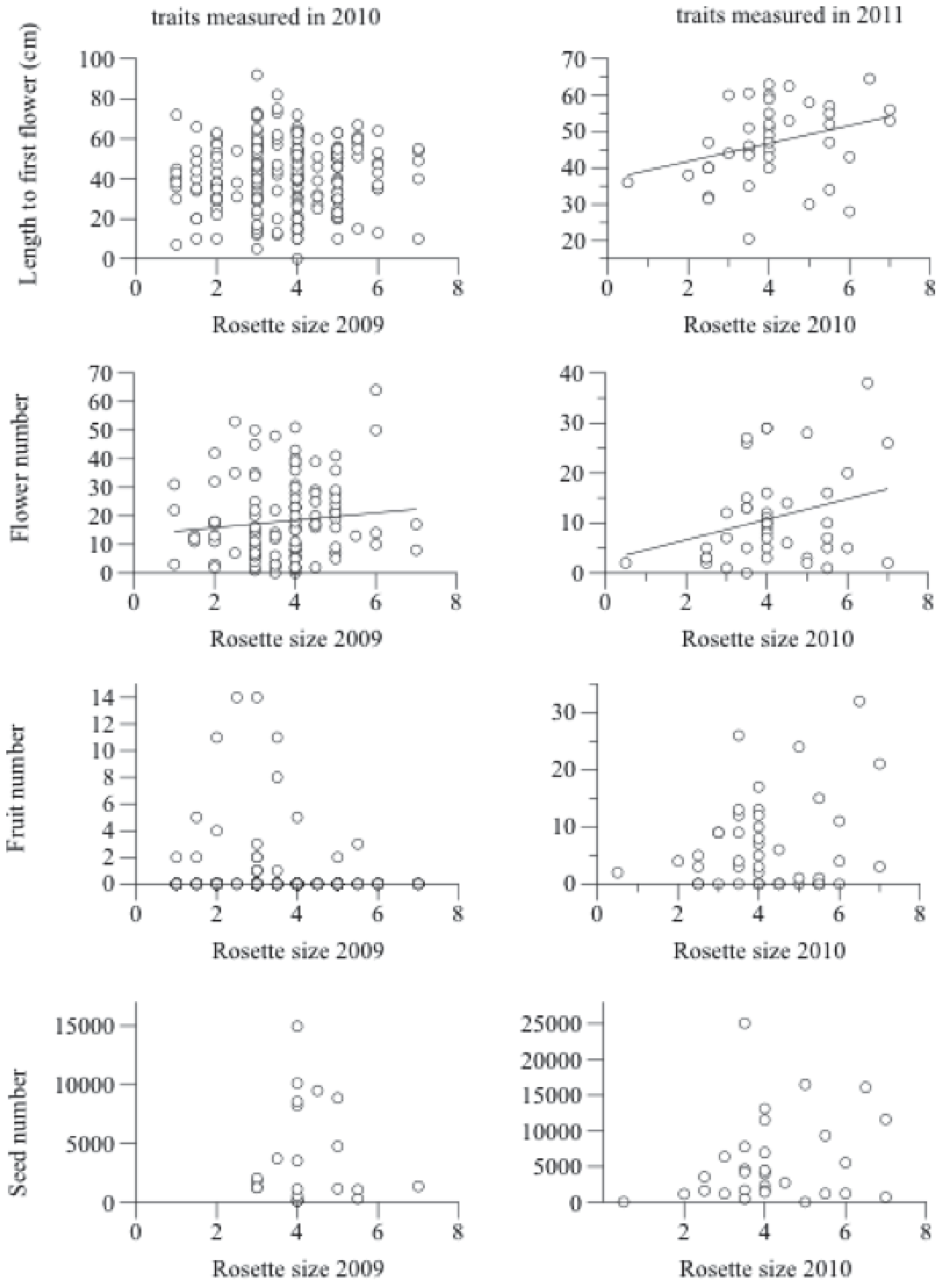

Figure 5.5 Relations of subsequent first flower height, flower number, and fruit and seed numberto autumn rosette size for individuals measured during 2009 and 2010. Only statistically significant regression relations are illustrated.

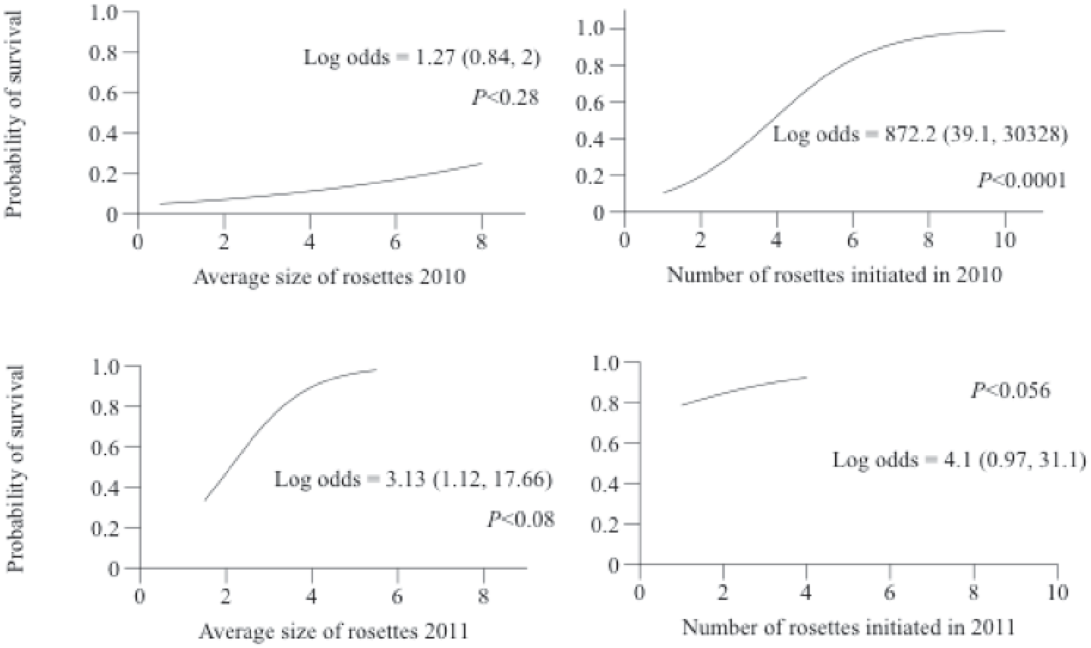

Figure 5.6 Relations of the probability of over-winter genet survival to the average size (left panels) and the total number of rosettes (right panels) initiated during the previous season. The logistic function is drawn only over the observed range of rosette number. The log odds (and 95% CI presented in brackets) and the *P* value for each relation are presented for each function.

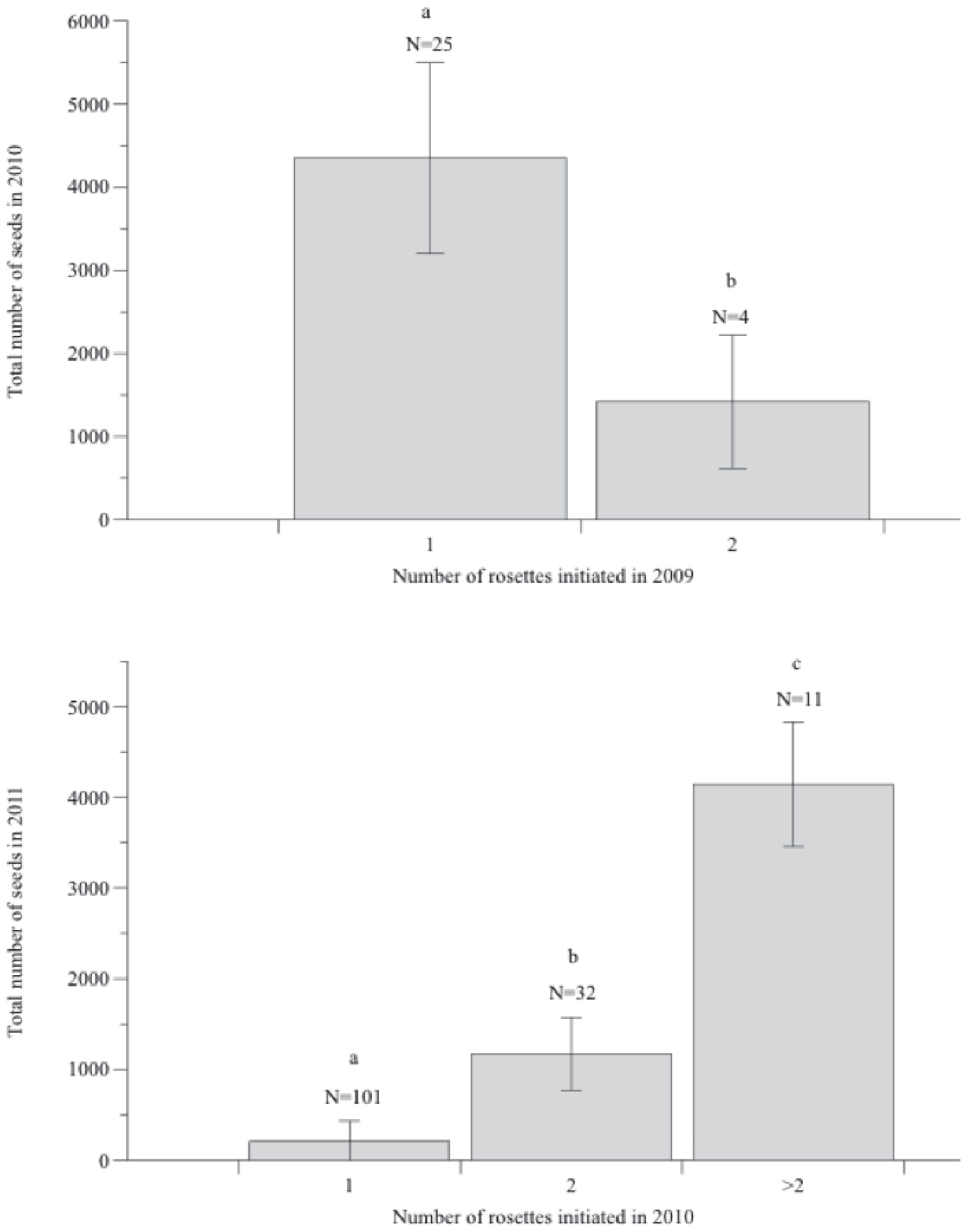

Figure 5.7 Relations of mean (± SE) genet fitness during 2010 and 2011 to number of rosettes initiated during the previous year. Bars with different letters above indicate groups for which the difference in mean was statistically significantly different based on Tukey's honestly significant difference test.

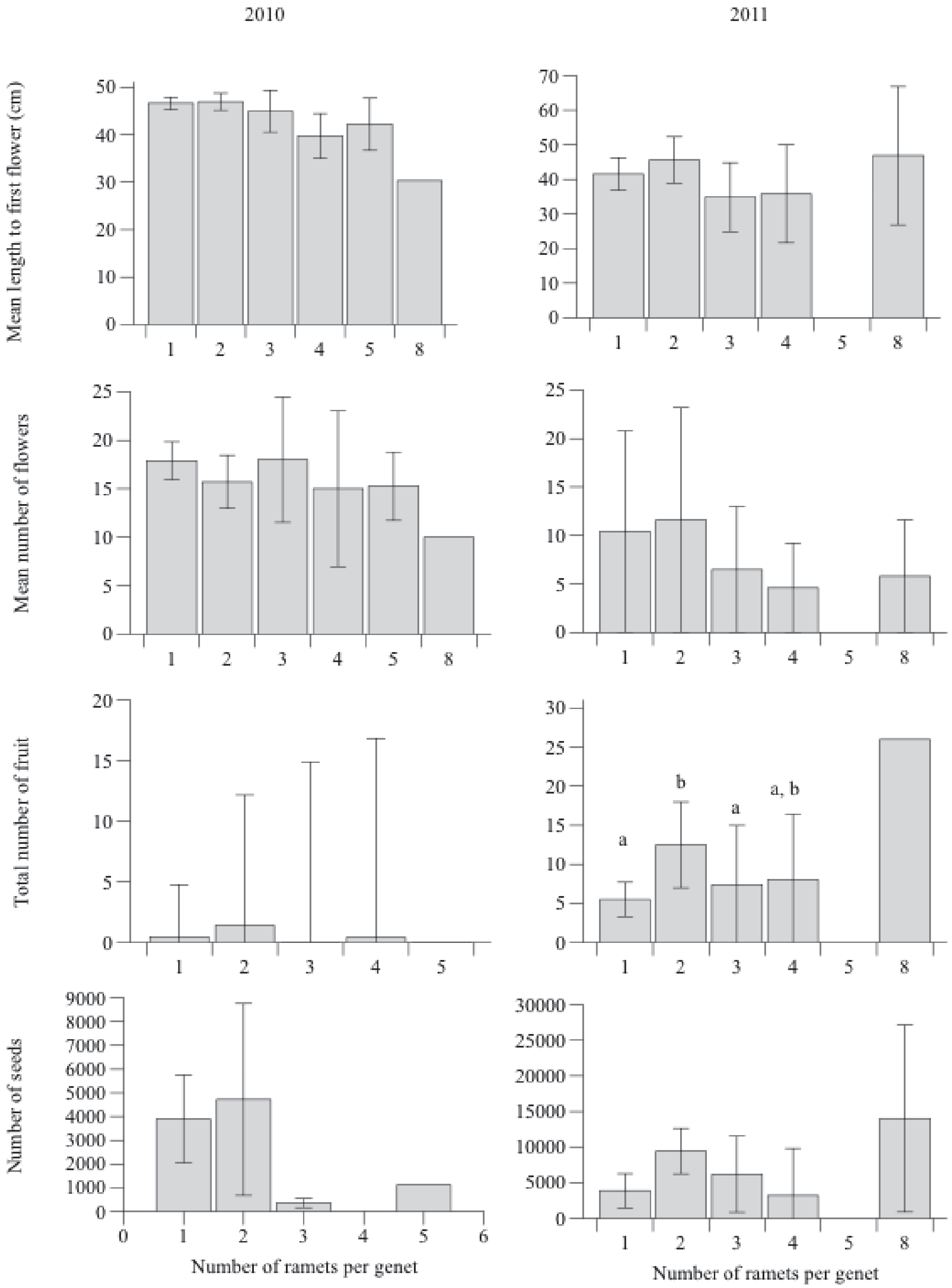

Figure 5.8 Relations of mean (± 95% CI) ramet phenotype and genet female fitness during 2010 and 2011 to ramet number. Bars with no CI represent single genets. Letters distinguish significantly different groups based on Tukey's honestly significant difference test.

rosette in 2009, whereas the opposite pattern occurred during the next year. Hence, selection on rosette number likely also varies among years. In addition, rosette size positively affected ramet and genet survival, but not ramet or genet fecundity during the following year. Because the number of ramets within a genet may influence the quantity and quality of seeds produced in subsequent years, the factors that shape allocation to rosette number will have important consequences for population persistence and plant reproductive success in *L. cardinalis*.

5.5.1 **Causes of variation in rosette size and number**

Within a growing season, many factors influence the resources available to a particular ramet to produce rosette(s), such as the genetic quality of the ramet, the local density of individuals and flowers, and availability of abiotic nutrients (reviewed in Weiner et al. 2001). In non-clonal plants and single ramets, allocation to sexual reproduction tends to increase with plant size (discussed in Kleunen et al. 2002). Many studies of clonal species have reported positive allometric relations between plant size and allocation to clonal biomass (e.g. Schmid et al. 1995, Kleunen et al. 2002, Sandring et al. 2007), but others have not (e.g., Thiele et al. 2009).

I found little support for an allometric association between parental ramet traits and the size and number of rosettes, and no evidence of a trade-off between current investment in reproduction and allocation to rosette size and number. Within years, rosette size and number did not vary strongly with the parental ramet size (significant only during 2009). However, *L. cardinalis* rosettes often begin growing during mid-August, when most ramets have opened roughly half of their flowers. Thus, rosette formation likely competes with developing fruit for photosynthate. However, in *L. cardinalis*, resources available for seed maturation are mostly unused as individuals are highly pollen limited in this population.

Herbivory may also cause rosette size to not scale proportionally with the size of the parental ramet. In some instances, ramets that were eaten survived to produce rosette(s), and individuals that were eaten early in the season often produced very large rosettes (*pers. obs.*). Differences among individuals in pollination, local density, and herbivory could create variation in the resources invested in rosettes, even among ramets that have the same intrinsic resource availability. As a result, a relation of rosette traits (size and number) to parental plant phenotype or reproductive output is likely to be obscured in field studies based on phenotypic associations. Experimental manipulations of either nutrients or allocation to sexual reproduction (e.g., by supplemental hand-pollination) would help elucidate such relations.

5.5.2 **Rosette size and future ramet phenotype and fitness**

In iteroparous plants, investment in reproduction within a season may be affected by previous investments in reproduction and growth (Obeso 2002) or from environments experienced early in life (reviewed in Miller et al. 2013). Past growth and reproduction can affect current reproduction indirectly through their effect on current available plant resources (reviewed in Aragón et al 2010). Iteroparous organisms must ‘allocate reproductive effort over their entire lifespan (discussed in Miller et al. 2013). Negative associations between current performance and past allocation of resources to reproduction, termed “cost of reproduction”, have been reported for many plant species

(Obeso 2002). For example, Snow and Whitham (1989) found fruit production in an orchid species caused smaller plants in the next year. Studies of polycarpic plants have also found positive associations between years in plant size, reproduction and survival, plant size and survival and plant size in the following year (discussed in Aragón et al 2010). For example, Horvitz and Schemske (2002, 1995) found that plant size in *Calathea ovandensi* affected future inflorescence production more consistently than plant growth. Positive correlations suggest that certain individuals may consistently outperform others in terms of growth and fitness. Selection has been shown to favor large size through survival and fecundity (reviewed in Crosby and Latta 2013), suggesting that plants that acquire more resources allocate more resources to both components of fitness.

The results of this study suggest that certain individuals consistently outperform others. Within a season neither the phenotypic nor the fitness traits of the parental ramet were associated with the size of rosette(s); although in 2009, ramets that produced more than one rosette tended to be on average taller and produced more flowers. When multiple rosettes were formed, however, rosette size was highly correlated, suggesting that multiple rosettes were formed simultaneously. Thus, plants that were capable of forming multiple rosettes initiated them at the same time. Although rosette size did not vary with ramet traits within a year, it positively affected survival, height and number of flowers produced by the flowering ramet during the following year. An association between rosette size and future seed production may have been overwhelmed by environmental factors causing extensive variation in plant phenotype and reproduction. Nonetheless, larger individuals may consistently outperform others, as indicated by positive associations between (1) ramet size and rosette size during 2009, (2) rosette size and survival to the following year, (3) rosette size in 2009 and the number of flower produced by the ramet in 2010, and (4) rosette size during 2010 and the height and flower number of the ramet produced in 2011.

5.5.3 Genet level fitness consequences (for survival and seed production in following years) of rosette size and number

In species capable of clonal reproduction, genets are the appropriate units of selection and for measuring fitness (reviewed in Pan and Price 2002). Although the main goal of this study was to assess the optimal rosette size and number, the data were too limited to thoroughly explore fitness relationships at the genet level. To search for evidence of an optimal size and number of rosettes, one ideally would look for evidence of curvature in a relationship between rosette size (or number) in one year and fitness at the genet level in the following year. Curvature in the relationship would be indicative of stabilizing selection (Lande and Arnold 1983, see also Stinchcombe et al. 2008). Our sample sizes for ramets and genets that survived winter and that subsequently produced seeds were too small to explore these relationships; in 2010 there were 26 genets with more than one ramet (but not all of these were present in 2009) and in 2011 there were only 11 genets with more than one ramet. Furthermore, for rosette number, there was insufficient variation in the number of rosettes produced for regression-based analysis.

My results, however, suggest that selection on rosette number may have differed among years. The relationship between seed production and the number of rosettes produced in the previous season varied between time periods. Ramets that produced more than one rosette in 2009 may have done so at a cost of reduced seed production in 2010. Ramets that produced one rosette in 2009 produced more seeds on average than those that produced two (those that produced three rosettes made no seeds), suggesting that a greater number of rosettes initiated in 2009 may have had an associated costs of producing fewer seeds the following season. This pattern, however, did not hold in the following time period. It should be noted, in this analysis genet membership was based on the ramet progenitors in the previous year and not the entire genet; this was done to be consistent with the way genets were defined in 2009. Ramets that produced more rosettes in 2010 became clusters of ramets that produced more seeds in 2011. Within a given year, however, genets with three and four ramets produced fewer seed than those with two (Figure 5.8), suggesting that genets with the highest density of ramets may experience reduced seed production.

Survival functions also differed among years, suggesting that optimal allocation may change through time or that my ability to measure how survival related to phenotype differed among years. At the ramet and genet level, larger rosettes were associated a greater likelihood of surviving to the following year and the functions differed among years. The number-survival function measured at the genet level also differed among years. The survival of genets from 2010 to 2011 increased with greater number of rosettes initiated in 2010. In 2011, genets produced fewer rosettes and the number-survival function did not reach an asymptote over the observed number of initiated rosettes. Nonetheless, genet survival to 2012 was positively related to the number of rosettes produced.

Differences in levels of herbivory between years may be one reason why fitness functions (measured as seed production) and survival functions differed between years. In all years plants suffered damage caused by herbivores. By September 2010, only 15 of the 371 ramets tagged in 2010 still had an intact inflorescence that had not been severely grazed by herbivores. Herbivory was less intense in other years; approximately 15% of ramets were grazed in 2009 and 20% of ramets were grazed in 2011. The timing and extent of herbivore damage may influence the size and number of rosettes initiated. The obscene level of herbivory in 2010 may have obscured any relationship between rosette size, number and survival to 2011.

Evaluating the fitness consequences of variation in the size and number of rosettes produced could be approached using optimality models such as the classic Smith-Fretwell model (1974) that describes optimal parental fitness given investment per offspring. Optimality models, however, make many assumptions that are biologically unreasonable thereby limiting their usefulness. In the Smith-Fretwell (1974) model variation among individuals in the amount of energy available to allocate to offspring can vary but the model does not account for variability within an individual. Ramets that start off with the same amount of energy to invest in rosettes may eventually differ in how much energy is allocated to rosettes due to differences in pollination, herbivory or random environmental perturbations. For example, one plant may have been visited more often by hummingbirds and thus allocates greater amounts of resources to fruit and seed maturation. Similarly, the timing and extent of herbivore damage will affect energy invested in tissue repair, and subsequently rosettes. Furthermore, rosette size might not be a property of the parent but also of changing microenvironment; as rosettes grow they may draw resources from the soil and cause density dependence in the size and number of rosettes that can grow and survive in a patch.

5.5.4 **Factors shaping optimal rosette size and number**

Our data suggest that investment in rosettes has important consequences for ramet and genet survival, and for genet fitness (measured as seed production). Producing multiple rosettes may be important for population persistence as sexual reproduction at the ramet and genet level is highly variable and most individuals fail to produce the maximum numbers of fruits and seeds. Higher investment in clonal growth when the opportunity for sexual reproduction is low has been suggested in other species. For example, in *Ipomopsis aggregata* (Paige and Whitham 1987), individuals produced basal rosettes when pollination was scarce, thus shifting their normal semalparous mode of reproduction to iteroparous. Thus, pollen limitation may be one factor involved in shaping investment in clonal growth.

Optimal rosette size and number will also depend on how ramet density influences ramet phenotype and fitness, including the quality of seeds produced. Because ramets are physiologically independent in *L. cardinalis,* as ramet density increases ramets may begin to compete for space, nutrients, and pollinators. In addition, greater numbers of ramets within a genet increases geitonogamous selfing between ramets (e.g., Eckert 2000, Wilson et al. 2005, see also Vandepitte et al. 2013). Unlike autonomous self-fertilization, gieotogamy does not provide reproductive assurance and is also associated with pollen and ovule discounting (Lloyd and Schoen 1992). Competition for abiotic resources and lower offspring quality (caused by geitonogamous selfing) will limit the benefit of producing multiple rosettes.

5.5.5 **Conclusions**

Characterizing the variation in rosettes size and number within and between years and linking the variation to fitness at the ramet and genet level establishes the basic survival and fitness functions that provide a basis for future investigations into how allocation to clonal offshoots affects pollination, reproductive success and plant mating system. I found that genet and ramet survival were related to the size and number of rosettes produced by ramets in the previous year. The size-survival and rosette number-survival functions differed among years. Furthermore, the fitness (measured as seed production) consequences for the genet associated with the numbers of rosettes produced in the previous season differed among years. Beyond some level of ramet density, however, fitness at the genet level fitness is expected to decline as the result of an increased rate of geitonogamous self-fertilization and higher local competition among ramets. Further studies, exploring how investment in clonal growth varies among populations would help establish the extent of life history flexibility and whether difference in allocation represent adaptations to local environments.

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CHAPTER 6 CONCLUSION

During the past three decades many studies have assessed selection in plants, with a particular emphasis on identifying the direct targets of selection (Kingsolver et al. 2001; Kingsolver et al. 2011). However, understanding how adaptive evolution shapes the diversity of organisms also required characterization of the mechanisms that underlie fitness variation among individuals. Therefore, I have evaluated the evolutionary consequences of factors causing fitness variation among individuals of *L. cardinalis*.

Studies similar to that presented in the second chapter of this thesis have shown that many floral characters assumed to have evolved solely in response to pollinator-mediated selection are either not currently under pollinator-mediated selection (e.g. (Caruso et al 2010) or subject to the influences of other selective agents, such as herbivores, that cause fitness variation among individuals according to phenotype (e.g. Parachnowitsch and Caruso 2008). Thus, by quantifying selection caused by pollinators and herbivores the work in my second chapters furthers our understanding of how various agents of selection may shape selection on traits.

The effect of pollinator-mediated selection on floral traits relative to that of herbivore-mediated selection has been expected to depend on the severity of pollen limitation (references). In contrast, in the third chapter, I showed that the expectation that pollen limitation influences selection intensity holds only for selection acting on characters for which pollen limitation varies with phenotype. Any difference in slope between hand- and naturally pollinated plants depends entirely on how pollen limitation scales with trait(s). This underappreciated condition should be considered more widely in studies of pollinator-mediated selection.

Because plants are sessile, individual fitness is also likely to depend on the physical location of individuals within populations. Most studies of density have not considered how changing floral neighborhoods, such as results from dichogamy, may influence individual fitness. To assess this effect, in the fourth chapter I explored how the dynamics of total floral density and the density of male- and female-phase flowers during the flowering season influenced total individual seed production and the success of individual female flowers. I found evidence that: (1) plants in dense patches produce more fruit and seeds, consistent with pollinator preference for high density patches and (2) that the success of female-phase flower open on a given day depended strongly on the local density of surrounding male- and female-phase flowers. My results suggest that local density influences female fitness by determining the role of female-female competition and availability of mates in limiting seed production. Because local density may dictate the nature of interactions among individuals, variation in local density may influence the strength and shape of selection on plant traits.

The local density of flowering individuals within a population depends on environmental factors (such as available space, and nutrients limiting growth) and also by variation among individuals in the size and number of clonal offspring produced. The fifth chapter of this thesis describes the survival and fitness consequences of variation in rosette size and number measured at the ramet and genet level. Most studies of allocation patterns in clonal plants have focused on trade-offs at the ramet level, but in clonal species the genet should be considered the unit on which selection is measured. In contrast, I explored variation among plants in rosette size and number at the ramet and genet level and found that ramets and genets that produce more and larger rosettes have a higher likelihood of survival. Furthermore, rosette production influenced seed production the following year. Plants that produced one rosette in 2009 produced more seeds the following year, but plants that produced more rosettes in 2010 produced more seeds the following year.

Establishing the mechanisms underlying fitness variation is necessary to understand how and why organisms change (or not) through time. The studies presented here help fill a gap in our understanding of the factors shaping selection in natural populations and how temporal variation in floral neighborhood influences pollination and fitness. By evaluating how allocation to rosette formation influences individual fitness components, this work also clarifies the link between life-history strategies and fertility.

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