# The sexual neighborhood through time: competition and facilitation for pollination in *Lobelia cardinalis*

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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 we (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 densities of male- and female-phase flowers) on fruit and seed production for individual flowers presented within the season, and (3) compare the influence of plant 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). By examining temporal and spatial scales we found that (1) total seed production per plant decreased with increasing plant density at the smallest scale but increased with increasing density at all larger scales; (2) at any given time, a female-phase flower benefited from a higher density of surrounding 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 number of 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, implying that the local floral environment may shape how attraction traits like plant height are related to fitness.

Key words: dichogamy; floral traits; Lobelia cardinalis; plant sex ratio; pollinator preference; seed production.

#### 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). Pollen and abiotic resources may simultaneously 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

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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 de Jong 1990) and may experience higher rates of geitonogamous self-fertilization (e.g., Karron 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 Elliott and Irwin 2009). This also holds for stands that are mixtures of heterospecifics (Feldman et al. 2004).

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, 2008, Brys and Jacquemyn 2010). At the patch level, total floral density may influence the rate of pollinator visitation (Feldman et al. 2004). Within a patch, on the other hand, the local density of flowers that are interfertile will determine

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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 femalebiased 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 floral 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, Spigler and Chang (2008) found that the relationship between density of plants and fitness at small scales in Sabatia angularis (i.e., less than 1 m) reflects partitioning of abiotic resources, but that at larger scales (i.e., greater than 1 m), it captures the effects of density on pollinator visitation. 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 between 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 Kolb 2013).

In this study, we examine how fruit and seed production in the dichogamous species Lobelia cardinalis (Campanulaceae) is influenced by natural variation in the surrounding 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). We 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), we explored all relationships at four scales (10, 20, 50, and 100 cm radii). The roots of L. cardinalis are shallow and tend to be shorter than 30 cm (M. P. Bartkowska and M. O. Johnston, personal observations), and the maximum length of leaves on an inflorescence is approximately 20 cm. 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 lasted less than four minutes) and, while visiting a patch, pollinators tended to move among plants that were within 1 m of one another (M. P. Bartkowska and M. O. Johnston, personal observations). Thus, the smallest scales should have captured abiotic resource partitioning, while the larger scales should have captured competition for or facilitation of pollinator service.

#### Methods

### 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 10 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; see Plate 1). 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 usually one (but occasionally two or more) basal rosette(s) for overwintering. In the following season, each rosette is physiologically independent and a single inflorescence may arise from a single rosette. Thus, density at the smallest scales may be determined by environmental factors and by the propensity for clonal reproduction among individuals. We treat all 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.

We 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. The study site was immediately east of the bridge at Poplar Rapids. Flowering occurred from late July to mid-September. Plants produced mature fruit from August to October.



PLATE 1. Lobelia cardinalis approached by a Ruby-throated Hummingbird (Archilochus colubris), its sole pollinator in the eastern portion of its range. Photo credit: M. O. Johnston.

# Mapping plants

In July 2009, we tagged and mapped all plants of L. cardinalis growing along an 80 m length of shoreline. There were very few plants of other species growing within the study site. In addition, there were no other flowering conspecifics at least 500 m west and 100 m east of this site. We 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 of which the majority of the inflorescence was eaten by herbivores, were excluded as focal plants, but were included in estimates of surrounding density.

#### Reproductive and phenotypic traits measured

On each plant, we 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 plant, we recorded the position along the inflorescence and the sex phase of every open flower on 13 dates between 29 July and 14 September, typically every three to four days. We trimmed the data set 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.

# Estimating surrounding individual and floral density

We used the map information to estimate the number of conspecific neighbors surrounding focal individuals at radii of 10, 20, 50, and 100 cm. For each census date, we 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<sup>2</sup>. 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.

# Statistical analysis

*Characterizing surrounding plant densities.*—We 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, 20, 50, and 100 cm), we compared the observed distribution of surrounding plants to a Poisson distribution. For the smallest scale, we binned plants in increments of two, and for all other scales, we 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 surrounding plant density may influence focal plant phenotype and fitness (e.g., plant size and/or seed production may be limited by resource competition), we 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, 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 effects on reproduction.

Two-part mixture analysis to model fruit and seed production at the whole-plant and flower level.-We used a two-part mixture analysis approach to evaluate the effect of plant and floral density on fitness (Cameron and Trivedi 1998). First, we modeled the likelihood of producing fruit given surrounding plant density and focal plant phenotype; this is the binary part of the mixture analysis. Second, for plants that produced at least one fruit, we 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. A two-part mixture analysis can readily take into account separate distributions for fruit and seed production.

We used this approach to model factors influencing the likelihood of producing fruit and the number of seeds produced (provided a fruit was made) at the whole-plant and flower-level. The models for flowerlevel fitness, however, also took into account the repeated measurements taken on the same plant throughout the season. A two-part mixture approach is particularly useful for this type of data because 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. The details of the whole-plant and flower-level fitness models are described in the following sections.

Two-part analysis of fruit and seed production at the whole-plant level.-We analyzed the effects of phenotype and surrounding plant density on total reproductive success at each of the four scales as follows. We used logistic regression to model the likelihood of producing fruit (the binary part of the mixture analysis) and standard multivariate regression to model seed production in plants that produced at least one fruit (the count part of the mixture analysis). In both types of models, surrounding density of plants, plant height, and stigmanectary 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, Version 9.2 of the SAS system for Windows (SAS Institute 2008).

Two-part analysis of fruit and seed production at the flower level.-For all four scales, we analyzed the effects of surrounding male- and female-phase flower density and focal plant phenotype on flower-level fitness. We used linear mixed effects models to predict the average number of seeds produced per day (the count part of the mixture analysis) and generalized linear mixed models to predict the likelihood of producing fruit given the number of flowers open on a census date (the binary part of the mixture analysis). All factors in the analysis (male- and female-phase flower density, two phenotypic traits, and date) were treated as fixed effects. Date was included as a covariate in these models because seed and fruit production are likely to decline with date; pollinators begin their southerly migration around the time of peak flowering (Bertin 1982). The models accounted for non-independence of multiple observations taken of the same plant over time by specifying the structure of the variance-covariance matrix (R-side modeling; Littell et al. 2006). Models were fit using the GLIMMIX procedure in SAS, 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, we 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



FIG. 1. Effect of surrounding plant density on total seed number per plant measured at four scales. The functions extend across the observed range of plant density except at the 10-cm scale, where the observed maximum density was 1000 plants/ $m^2$ .

UCLA Statistical Consulting Group 2013). This model assumes a binomial distribution with a logit link function.

We 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) we employed a randomization technique to estimate parameters and confidence intervals. We generated 2000 randomized data sets by randomly choosing focal plants whose area of density did not overlap. All statistical models were fit to each of the randomized data sets. The average parameter estimates and 95% confidence intervals for the randomized data sets were used to assess whether the predictor variables were related to the response. Sample sizes for the randomized data sets 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.

#### RESULTS

# The distribution of surrounding plants and flower sex phases

At the smallest scale (10 cm) plants were randomly (Poisson) distributed ( $\chi^2 = 2.36$ , df = 3, P = 0.50; Appendix A) and occurred in aggregations of zero to 12 plants. 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 few neighbors (at 20 cm  $\chi^2 = 401.1$ , df = 3, P < 0.0001; at 50 cm  $\chi^2 = 309.1$ , df = 3, P < 0.0001; at 100 cm  $\chi^2 = 451$ , df = 3, P < 0.0001; Appendix A).

The distribution of the number of surrounding male and female flowers changed throughout the season (Appendix B). 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.

# 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 (10 cm; 95% CI, -3.20 to -1.76), but increased at larger scales (Fig. 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 larger scales (Table 1). There was also a weak negative association between stigma–nectary distance and increasing surrounding plant density at the smallest scale (10 cm).

# 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 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

TABLE 1. Influence of surrounding plant density on plant height and stigma-nectary distance.

Scale (cm)	Height to base of last flower	Stigma-nectary distance
10 20 50 100	-0.021 (-0.029 to -0.011) -0.0059 (-0.010 to -0.0015) -0.0020 (-0.0030 to -0.00099) -0.00094 (-0.0014 to -0.00047)	$\begin{array}{c} -0.0012 \ (-0.0025 \ {\rm to} \ -0.00020) \\ -7.57 \times 10^{-6} \ (-0.00050 \ {\rm to} \ 0.00050) \\ 0.000057 \ (-5.99 \times 10^{-5} \ {\rm to} \ 1.80 \times 10^{-4}) \\ -0.000059 \ (-8.92 \times 10^{-5} \ {\rm to} \ 2.91 \times 10^{-5}) \end{array}$

*Notes:* Estimates are means of coefficients from 2000 regressions of independent data points, which were generated by randomly choosing focal plants (from a data set of 430 plants) with nonoverlapping areas of density. Regression coefficients that differ significantly from zero ( $\alpha = 0.05$ ) are shown in boldface type. Values in parentheses are 95% CI.

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

TABLE 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.

*Notes:* Estimates are means of coefficients from 2000 regressions of independent data points, which were generated by randomly choosing focal plants (from a data set of 430 plants) with nonoverlapping areas of density. Estimates that differ significantly from zero ( $\alpha = 0.05$ ) are shown in boldface type. Values in parentheses are 95% CI.

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.

# 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 given number of female flowers open on a census date, plant height did not influence the likelihood of producing fruit. At the largest scale, however, plant height was related to increased seed production for plants that produced at least one fruit. At all scales, larger 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 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 number of seeds produced per flower (at all scales; Table 3). The effect of surrounding male and female flower density was stronger at larger scales.

When accounting for local floral density (including male and female flowers together), we found that, at all scales, the likelihood of producing fruit declined with increasing total floral density (Table 4). At the two largest scales (50 cm and 100 cm), the average number of

seeds produced per female flower also declined with increasing total floral density. The effect of sex ratio was similar to the effects 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). The average number of seeds, however, declined as the surrounding sex ratio became more female biased (Table 4).

### DISCUSSION

We examined the influence of surrounding plant and floral density on female fertility at two temporal levels (whole-plant and individual flower) and four spatial scales. At the whole-plant level, we found evidence of resource competition at the smallest scale and facilitation for pollination at larger scales. At the flower level, seed production increased with a higher density of malephase flowers and a lower density of female-phase flowers, indicating that female-phase flowers competed for pollen. Thus, surrounding floral density and sex ratio are both important determinants of seed production.

# 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, we found that the density of surrounding plants had a positive effect on total seed production and the effect was stronger at larger scales. Similarly,

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

TABLE 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 number of seeds produced per female flower open on a given census date (provided that at least one fruit was produced) at four scales.

*Notes:* Estimates are means of coefficients from 2000 regressions of independent data points, which were generated by randomly choosing focal plants (from a data set of 430 plants) with nonoverlapping areas of density. Estimates that differ significantly from zero ( $\alpha = 0.05$ ) are shown in boldface type. Values in parentheses are 95% CI.

after accounting for plant height, the likelihood of a plant producing at least one fruit also increased with increasing 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 higher density of neighboring plants within 1m reduced reproductive success of focal plants, but beyond that distance (up to 4 m), reproductive success increased with increasing plant density (see also Gunton and Kunin 2009).

# 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 density of surrounding female flowers (at all scales), suggesting that female flowers competed for pollen. 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 we explicitly removed the influence of total local floral density from the sex ratio models, we found that seed production decreased with a female-biased surrounding sex ratio (at scales larger than 10 cm at the flower level), again consistent with female-female competition for pollen. Thus, female-female competition and availability of mates are likely to be important 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 and declines with every female flower visited (Price and Waser 1982, Aizen 2001, Bianchi and Cunningham 2012).

Fully dichogamous flowers are especially interesting because the optimal allocation to male and female

TABLE 4	<ol> <li>Effects of surro</li> </ol>	unding floral densi	ty, sex ratio (prop	ortion female), ar	nd focal plant trai	ts on the likelihoo	d of producing
fruit	(given the number	of flowers open) a	and on the average	ge seeds produced	l per flowers oper	n on a given censu	is date at four
scale	es.						

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

*Notes:* Estimates are means of coefficients from 2000 regressions of independent data points, which were generated by randomly choosing focal plants (from a data set of 430 plants) with nonoverlapping areas of density. Estimates that differ significantly from zero ( $\alpha = 0.05$ ) are shown in boldface type. Values in parentheses are 95% CI.

function within flowers, including the longevity of male- and female-floral phases, should be affected by surrounding density and sex ratio, and these change over space and time (Brunet and Charlesworth 1995, Ishii and Harder 2012). There are few empirical data, however, concerning the consequences of temporal separation of sex phases within flowers for pollination and subsequent reproductive success of individuals. In studies of Alstroemeria aurea, surrounding sex ratio 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 over time 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 such as decreasing pollinator visits later in the season. The strongest evidence supporting the hypothesis that frequency-dependent selection in dichogamous populations can shape floral form comes from Delphinium glaucum. In this species, Ishii and Harder (2012) found that late-flowering plants produced larger flowers with more anthers per ovule and a longer male phase than early-flowering plants, suggesting that correlations between phenology and sex allocation can arise due to

frequency-dependent selection favoring the less common sex role during particular times of the season.

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

Because we included phenotype and density in the same models, we were able to examine how a character known to experience pollinator-mediated selection (plant height) and a character that is not a direct target of pollinator-mediated selection (stigma-nectary distance) influenced reproduction while accounting for density. We found an association between plant height and seed production at both the flower and whole-plant level. This could reflect pollinator preference for taller plants, but could also be caused by (1) the correlation of plant height with flower number because taller plants with more flowers have more opportunities to produce fruit, (2) phenotypic plasticity associated with differences in microsite quality, or (3) differences in plant vigor. In a previous study (Bartkowska and Johnston 2012), we found a significant positive relationship between plant height and seed number (independent of flower number) for naturally pollinated but not for handpollinated plants (Bartkowska and Johnston 2012). Thus, at least part of the relationship between plant height and seed number is caused by pollinators. At the whole-plant level, we also found a positive association between plant height and the likelihood of producing at

least one fruit. We modeled the likelihood 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, Version 9.2 of the SAS system for Windows (SAS Institute 2008). This analysis again revealed a positive association between plant height and the likelihood of producing fruit independent of flower number (results not shown). Although this is further evidence for pollinator preference for taller plants, in the absence of pollinator observations we cannot distinguish pollinator preference for taller plants from the other factors listed here.

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. Stigmanectary distance is positively related to flower size and negatively related to flower position (data not shown). Larger flowers are more likely to be located toward 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).

#### Consequences for natural selection

We found that the sexual neighborhood, that is, the surrounding floral density and sex ratio, influences the seed production of individual female flowers. In particular, seed number increases as the neighborhood becomes more male and less female. By influencing successful pollen transfer, the sexual neighborhood will influence competition for pollinator service among plants, thereby shaping selection on traits related to pollinator attraction and pollen transfer. Because the sexual neighborhood changes within a season, the optimal longevity and investment in sex phases of individual flowers will also change (Brunet and Charlesworth 1995, Ishii and Harder 2012). Our results suggest that this occurs for selection via female fitness, but the influence of the sexual neighborhood on male fitness is wholly unknown. In general, one expects that male fitness will be driven more by competition among male flowers, and so changes in the sexual neighborhood will have opposing effects on male and female success. Future studies estimating both male and female fitness would provide valuable insight into what role the sexual neighborhood plays in shaping the evolution of floral characters.

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#### LITERATURE CITED

- Aizen, M. A. 1997. Influence of local floral density and sex ratio on pollen receipt and seed output: empirical and experimental results in dichogamous *Alstroemeria aurea* (Alstroemeriaceae). Oecologia 111:404–412.
- Aizen, M. A. 2001. Flower sex ratio, pollinator abundance, and the seasonal pollination dynamics of a protandrous plant. Ecology 82:127–144.
- Bartkowska, M. P., and M. O. Johnston. 2012. Pollinators cause stronger selection than herbivores on floral traits in *Lobelia cardinalis* (Lobeliaceae). New Phytologist 193:1039– 1048.
- Bertin, R. I. 1982. The Ruby-throated Hummingbird and its major food plants: ranges, flowering phenology, and migration. Canadian Journal of Zoology 60:210–219.
- Bianchi, F. J. J. A., and S. A. Cunningham. 2012. Unravelling the role of mate density and sex ratio in competition for pollen. Oikos 121:219–227.
- Brookes, R. H., and L. K. Jesson. 2010. Do pollen and ovule number match the mating environment? An examination of temporal change in a population of *Stylidium armeria*. International Journal of Plant Sciences 171:818–827.
- Brunet, J., and D. Charlesworth. 1995. Floral sex allocation in sequentially blooming plants. Evolution 49:70–79.
- Brys, R., and H. Jacquemyn. 2010. Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). Plant Biology 12:597–603.
- Cameron, A. C., and P. K. Trivedi. 1998. Generalized count regression. Pages 96–138 in A. C. Cameron and P. K. Trivedi, editors. Regression analysis of count data. Cambridge University Press, Cambridge, UK.
- de Jong, T. J., J. C. Batenburg, and P. G. L. Klinkhamer. 2005. Distance-dependent pollen limitation of seed set in some insect-pollinated dioecious plants. Acta Oecologica 28:331– 335.
- Devlin, B. 1989. Components of seed and pollen yield of *Lobelia cardinalis*: variation and correlations. American Journal of Botany 76:204–214.
- Devlin, B., and A. G. Stephenson. 1984. Factors that influence the duration of the staminate and pistillate phases of *Lobelia cardinalis* flowers. Botanical Gazette 145:323–328.
- Elliott, S. E., and R. E. Irwin. 2009. Effects of flowering plant density on pollinator visitation, pollen receipt, and seed production in *Delphinium barberyi* (Ranunculaceae). American Journal of Botany 96:912–919.
- Feldman, T., W. F. Morris, and W. G. Wilson. 2004. When can two plant species facilitate each other's pollination? Oikos 105:197–207.
- Ghazoul, J. 2005. Pollen and seed dispersal among dispersed plants. Biological Reviews 80:413–443.
- Grindeland, J. M., N. Sletvold, and R. A. Ims. 2005. Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. Functional Ecology 19:383–390.
- Gunton, R. M., and W. E. Kunin. 2009. Density-dependence at multiple scales in experimental and natural plant populations. Journal of Ecology 97:567–580.
- Ishii, H. S., and L. D. Harder. 2012. Phenological associations of within- and among-plant variation in gender with floral morphology and integration in protandrous *Delphinium glaucum*. Journal of Ecology 100:1029–1038.
- Jakobsson, A., A. Lázaro, and O. Totland. 2009. Relationships between the floral neighborhood and individual pollen limitation in two self-incompatible herbs. Oecologia 160: 707–719.
- Karron, J. D. 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity 75:175–180.
- Klinkhamer, P. G. L., and T. J. de Jong. 1990. Effects of plant size, plant density and sex differential nectar reward on

pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57:399–405.

- Leimu, R., P. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94:942–952.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberer. 2006. SAS for mixed models. Second edition. SAS Institute, Cary, North Carolina, USA.
- Morisita, M. 1962. I-Index, a measure of dispersion of individuals. Researches on Population Ecology 4:1–7.
- Ohashi, K., and T. Yahara. 2002. Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. Functional Ecology 16:492–503.
- Price, M. V., and N. M. Waser. 1982. Experimental studies of pollen carryover: Hummingbirds and *Ipomopsis aggregata*. Oecologia 54:353–358.
- Rathcke, B. J. 1983. Competition and facilitation among plants for pollination. Pages 305–338 *in* L. Real, editor. Pollination biology. Academic Press, New York, New York, USA.
- Sandring, S., and J. Ågren. 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. Evolution 63:1292–1300.
- SAS Institute. 2008. SAS for Windows, version 9.2. SAS Institute, Cary, North Carolina, USA.

- Sletvold, N., J. Grindeland, and J. Ågren. 2010. Pollinatormediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. New Phytologist 188:385–392.
- Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Third edition. Freeman, New York, New York, USA.
- Spigler, R. B., and S.-M. Chang. 2008. Effects of plant abundance on reproductive success in the biennial *Sabatia* angularis (Gentianaceae): spatial scale matters. Journal of Ecology 96:323–333.
- Stehlik, I., J. P. Caspersen, and S. C. H. Barrett. 2006. Spatial ecology of mating success in a sexually polymorphic plant. Proceedings of the Royal Society B 273:387–394.
- Stehlik, I., J. Friedman, and S. C. H. Barrett. 2008. Environmental influence on primary sex ratio in a dioecious plant. Proceedings of the National Academy of Sciences USA 105:10847–10852.
- UCLA Statistical Consulting Group. 2013. SAS data analysis example: logit regression. UCLA Statistical Consulting Group. http://www.ats.ucla.edu/stat/sas/dae/logit.htm
- Weber, A., and A. Kolb. 2013. Local plant density, pollination and trait-fitness relationships in a perennial herb. Plant Biology 15:335–343.
- Zorn-Arnold, B., and H. F. Howe. 2007. Density and seed set in a self-compatible FORB, *Penstemon digitalis* (Plantaginaceae), with multiple pollinators. American Journal of Botany 94:1594–1602.

#### SUPPLEMENTAL MATERIAL

# Appendix A

A figure showing the observed distribution of surrounding focal plants at four scales (Ecological Archives E095-076-A1).

# Appendix B

The observed distribution of female and male flowers surrounding a focal plant (with at least one receptive female flower) across 10 census dates at four scales (*Ecological Archives* E095-076-A2).