

EFFECTS OF FLOWERING PLANT DENSITY ON POLLINATOR VISITATION, POLLEN RECEIPT, AND SEED PRODUCTION IN *DELPHINIUM BARBEYI* (RANUNCULACEAE)¹

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Variation in flowering plant density can have conflicting effects on pollination and seed production. Dense flower patches may attract more pollinators, but flowers in those patches may also compete for pollinator visits and abiotic resources. We examined how natural and experimental conspecific flowering plant density affected pollen receipt and seed production in a protandrous, bumble bee-pollinated wildflower, *Delphinium barbeyi* (Ranunculaceae). We also compared floral sex ratios, pollinator visitation rates, and pollen limitation of seed set from early to late in the season to determine whether these factors mirrored seasonal changes in pollen receipt and seed production. Pollen receipt increased with natural flowering plant density, while seed production increased across lower densities and decreased across higher flower densities. Experimental manipulation of flowering plant density did not affect pollinator visitation rate, pollen receipt, or seed production. Although pollinator visitation rate increased 10-fold from early to late in the season, pollen receipt and seed set decreased over the season. Seed set was never pollen-limited. Thus, despite widespread effects of flowering plant density on plant reproduction in other species, the effects of conspecific flowering plant density on *D. barbeyi* pollination and seed production are minor.

Key words: *Delphinium barbeyi*; flower density; pollen receipt; pollinator visitation rates; seasonal changes; spatial autocorrelation.

Plant reproductive success varies widely within and among natural populations, and understanding the factors underlying this variation is one of the central goals of plant ecology. One factor associated with reproductive success of flowering plants is flowering plant density (Holland et al., 2002; Ghazoul and Shaanker, 2004; Maron and Crone, 2006). Flowers may benefit from being in dense patches if abundant floral resources attract more pollinators and/or provide an ample supply of compatible pollen donors (Kunin, 1993; Wailes and Ågren, 2004; Hegland and Boeke, 2006). However, flowers in dense patches may also compete with other flowers for pollinator visits or for abiotic resources necessary for seed production (Steven et al., 2003). Natural relationships between flowering plant density and seed production can be deceptive because they may be driven by factors other than pollination (Bosch and Waser, 2001). To disentangle the factors driving the relationships between flowering plant density and plant reproduction, it is necessary to use both observational studies (i.e., natural density variation) and experimental density manipulations and to test specific mechanisms that may be responsible for the patterns. As plant populations become increasingly fragmented, understanding the degree to which pollinator behavioral responses to local flowering plant density drive relationships between plant reproduction and plant density is useful for conserving plant popula-

tions (Cartar, 2005). To understand the effects of flowering plant density on seed production and some of the mechanisms involved, we focused on how natural and experimental flowering plant densities affected multiple steps in the pollination and seed production process: pollinator visitation, pollen receipt, seed production, and predispersal seed predation.

To maximize nectar or pollen acquisition, pollinators can change their foraging behavior in response to flower density (Dreisig, 1995; Cresswell and Osborne, 2004). Dense patches may be more attractive to pollinators because they reduce travel time among multiple sparse patches (Kacelnik et al., 1986). However, while pollinators such as bumble bees often prefer dense flowering patches, they tend to visit a smaller proportion of the flowers in those patches (Goulson, 2003). If seed set is pollinator-limited and if pollinators visit a smaller proportion of flowers in dense patches, then seed set per flower may decline in dense patches (García-Robledo et al., 2005). Alternatively, if seed set is sensitive to inbreeding depression (Williams et al., 2001), then shorter pollinator visits per patch could reduce within-patch pollen movement among more closely related individuals (Field et al., 2005), which could benefit plants, assuming plant patches have strong spatial genetic structuring.

Flower densities may not only affect the frequency of pollinator visits, but also the amount of pollen transferred per visit (Aizen, 1997). For example, bee-pollinated plants often lose a large proportion of their pollen when bees groom, fly, or brush against nonreproductive floral parts (Rademaker et al., 1997; Castellanos et al., 2003). Abundant pollen donors could increase the amount of pollen that reaches stigmas. If pollen donor availability influences pollen receipt, then variation in patch sex ratios might be as important as flower density for pollen transfer and seed production (Lalonde and Roitberg, 1994; Aizen, 1997; Ishihama et al., 2006).

Abiotic resource availability and biotic interactions other than pollination may also influence the relationship between flowering plant density and seed production. If abiotic resources

¹ Manuscript received 26 July 2008; revision accepted 23 January 2009.

The authors thank M. Hamilton for help in the field and the South Gothic Corporation and the RMBL for access to field sites. M. Ayres, J. Bronstein, G. Entsminger, S. Johnson, M. McPeck, D. Peart, C. Williams, and one anonymous reviewer provided valuable comments on the manuscript. This work was supported by funds from the Colorado Mountain Club Foundation, the Botanical Society of America, and the National Science Foundation (DEB-0455348).

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limit seed production, then effects of flower density on pollinator visitation and pollen receipt may not affect seed production (Burd, 1994; Ashman et al., 2004). Or, if abiotic resources promote higher flowering plant density and seed production independently, then natural gradients in water and nutrients could drive positive correlations between flowering plant density and seed production (Bosch and Waser, 2001). Patchiness in abiotic resources for pollinators, such as nest site availability, could also mask or magnify flowering plant density relationships with pollination success (Potts et al., 2003). In addition, biotic interactions such as seed predation (or herbivory more generally) could mask the benefits of higher pollination rates for seed production (Herrera et al., 2002), especially if dense flower patches attract more pollinators as well as more seed predators or other herbivores or florivores (Steffan-Dewenter et al., 2001). In particular, predispersal seed predators not only greatly reduce female fitness, but they may also disproportionately attack flowers with higher pollination, thereby negating the positive effects of higher pollination for female reproduction (Leimu et al., 2002; Cariveau et al., 2004; Lavergne et al., 2005). Given that the relationship between flowering plant density and seed production can be mediated by biotic interactions and abiotic resources, experiments manipulating flowering plant density and pollination concurrently are critical for examining whether a pollination mechanism may drive patterns between flowering plant density and seed set.

In this study, we tested how conspecific flowering plant density affected pollen receipt and seed production of the protandrous, bumble bee-pollinated wildflower, *Delphinium barbeyi* (Ranunculaceae). *Delphinium barbeyi* is naturally patchy, with flowering plant density varying within patches (S. E. Elliott, personal observation). Although heterospecific flowering plant density can also affect pollination in some species (Feldman et al., 2004), we focused on conspecific flowering plant density because *D. barbeyi* is visited by bumble bees who primarily forage on this one flower species while it is in bloom (Elliott, in press).

We asked two questions. (1) Does flowering plant density affect pollination and seed production? We predicted that the number of pollinator visits per flower would vary among plant patches that varied in flowering plant density, resulting in concurrent variation in pollen receipt and seed production. If flowers in dense patches facilitate higher pollinator visitation per flower, then seed production per flower should increase in denser plots. Instead, if flowers in dense patches compete for pollinator visits, then seed production per flower should decrease in denser plots. These predictions assume that pollen receipt increases with the number of pollinator visits per flower and that seed production is limited by pollen receipt. Thus, we measured the relationship between pollinator visits and pollen grains received per flower, and we compared seed set between supplemental hand-pollinated and naturally pollinated flowers. (2) Do the relationships among flowering plant density, pollination, and seed production vary across the flowering season, and could seasonal changes in pollinator visitation and floral sex-phase ratios account for such variation? Because pollinator abundance and male-to-female flower-phase ratios vary over the season, both of these factors could influence the relationship between flowering plant density and seed production.

MATERIALS AND METHODS

Study system—We examined the relationship between flowering plant density and reproduction in the herbaceous perennial wildflower, *Delphinium bar-*

beyi (Ranunculaceae, Huth), in July and August 2005, at the Rocky Mountain Biological Laboratory (RMBL, elevation 2831–3441 m a.s.l.) in Gunnison County, Colorado, USA. *Delphinium barbeyi* grows in moist meadows and forest clearings in distinct clustered patches. Plants bear an average of 13.6 ± 0.5 inflorescences per plant (mean ± 1 SE), with each inflorescence producing 25.4 ± 0.8 flowers (Elliott, 2008). *Delphinium barbeyi* flowers are protandrous and self-compatible, but they produce very few seeds autogamously (autogamous: 1.1 ± 0.6 seeds per flower, $N = 8$ plants; naturally outcrossed: 13.4 ± 4.5 seeds per fruit, $N = 7$ plants) and therefore, require pollinators to maximize seed set (Williams et al., 2001). Individual plants vary in the degree to which their seed production is pollen-limited (Williams et al., 2001).

Around the RMBL, the long-tongued bumble bee, *Bombus appositus*, is the most common pollinator of *D. barbeyi* (Inouye, 1976). *Delphinium barbeyi* flowers are also visited by other bumble bees (*B. flavifrons*, *B. bifarius*, *B. frigidus*, *B. nevadensis*, *B. occidentalis*), hummingbirds (*Selasphorus platycercus*, *S. rufus*, and *Stellula calliope*), and sphinx moths (*Hyles lineata*) (Inouye, 1976; Waser, 1982; Williams et al., 2001). Seed set per visit does not differ between flowers visited by *B. appositus* or *B. flavifrons* (R. E. Irwin, unpublished data), but the relative pollination efficiencies of the other visitors are unknown. Floral visitation by bumble bee pollinators increases over *D. barbeyi*'s 9-week blooming period because bumble bee colonies are hatching new workers (Elliott, 2008). Therefore, if bee density mediates pollinator foraging response to flowering plant density or overall pollinator limitation of seed set, then the effects of flowering plant density on pollination and seed set may vary over the flowering season. Because *D. barbeyi* is protandrous, there should be more male-phase flowers (pollen donors) per female-phase flower early in the blooming period.

Adult flies also visit *D. barbeyi* flowers. The flies are in the Anthomyiini tribe of the Anthomyiidae. In a nearby site, flies contributed to 0.3–0.6% of flower visits to *D. barbeyi* (R. E. Irwin, unpublished data). Seed production of *D. barbeyi* flowers visited only by flies typically does not differ significantly from plants with no visitors, suggesting that the flies are not important pollinators of *D. barbeyi* (Elliott, 2008). The female flies deposit eggs singly or in groups on the carpels prior to fruit expansion, and at our study site approximately 10% of all seeds are lost to these seed predators (Elliott, 2008).

1. Does flowering plant density affect pollination and seed production?—

In natural (observational study) and experimental plots, we measured pollen receipt and seed production as a function of flowering plant density. In the experimental plots, we also measured pollinator visitation, and we measured pollinator visitation and pollen receipt at two time points, early (19–23 July) and late (29 July–4 August) during the flowering season. These time intervals represent the midpoints of the first and second halves of *D. barbeyi*'s blooming period. Due to time constraints, we did not monitor pollinator visits or seasonal relationships in natural plots.

Study plots—Because bumble bees are more likely to respond to flowering plant density in plots ranging in size from 100 to 2000 m² and to restrict foraging bouts to areas within 18 m² (Osborne and Williams, 2001; Johnson et al., 2003), we used circular 100-m² plots. The perimeter of each plot was separated by 20 m. We placed 148 natural plots throughout a 10-km section of the East River Valley near the RMBL and in adjacent drainages. We positioned natural plots in *D. barbeyi* patches that ranged from 0.01 to 0.68 flowering plants per m² (mean ± 1 SE = 0.26 ± 0.01 flowering plants/m²). We measured flowering plant density as the number of *D. barbeyi* plants with buds or flowers within each plot. There was no relationship between inflorescences per plant and flowering plant density ($r = 0.10$, $P = 0.2$, $N = 148$ plots).

We manipulated flowering plant density in 31 patches in one meadow area in the East River Valley. These patches initially had medium to high densities of flowering plants (0.33–0.77 flowering plants/m²). We randomly assigned plots to density treatments, which consisted of 4, 8, 12, 14, 16, 18, 22, 28, or 32 unclipped plants remaining per 100-m² plot (3–4 replicates per treatment). These experimental densities spanned the lower 67% density range found in the natural patches (median natural density = 0.24 plants/m²). We used a range of experimental densities so we could detect potential nonlinear relationships between flowering plant density, pollination, and seed production (Goulson, 2000; Feldman, 2006). We clipped inflorescences, rather than removing entire plants, to avoid altering competition for water or nutrients that might fuel pollinator rewards or seed set. We also clipped inflorescences from a 10-m buffer around each plot to ensure that pollinator responses to experimental densities were not confounded by neighboring flower densities (Osborne and Williams, 2001). In the year prior to this study, bee density was comparable in the natural and experimental study areas (Elliott, 2008).

Pollinator visitation rate—We monitored pollinator visitation rate in experimental plots that spanned the full range of density treatments (16 plots early in the season and 14 plots late in the season). In each part of the season, we monitored pollinator visits to four focal plants per plot for three to four 30-min intervals (1.5–2 h of observation per plot) between 0900–1700 hours (peak hours of bumble bee activity). In each time interval, we recorded each pollinator (species, plus caste for bumble bees: queen, worker, or male) that visited a focal plant and the number of focal plant flowers it visited before leaving. We counted the number of male- and female-phase flowers open on each focal plant to calculate pollinator visitation rate as the proportion of flowers visited per minute of observation (and to calculate floral sex ratios, described later).

Pollen receipt—We quantified average stigmatic pollen receipt per flower per plot using a subsample of flowers in each plot. In natural plots, we collected stigmas from 12 flowers per plot (three flowers per inflorescence from two inflorescences per plant on two focal plants per plot) during peak bloom. In two plots, each with only one plant, we sampled four inflorescences per plant. In experimental plots, we collected stigmas from 16 flowers per plot (four flowers per plant from four focal plants per plot) after each pollinator observation period. We collected stigmas after petals had fallen off, suggesting that stigmas were no longer receptive. We marked sampled flowers with a dot of paint on their pedicels so we could revisit those flowers when the fruits had matured. In another perennial wildflower, *Ipomopsis aggregata* (Polemoniaceae), collecting stigmas at this stage does not affect fruit or seed production (Waser and Fugate, 1986), and we noticed no visible differences in fruit maturation between those that we did and did not collect stigmas from. We mounted stigmas on microscope slides, used basic fuchsin dye to stain the pollen (Kearns and Inouye, 1993), and counted the number of conspecific and heterospecific pollen grains with a compound microscope. We only present analyses of conspecific pollen because heterospecific pollen was rare ($4.6 \pm 0.4\%$ of grains, median = 0.0%, $N = 1128$ flowers). Each flower has three unfused carpels; thus, we summed pollen receipt across all three stigmas as a measure of pollen receipt per flower. We averaged pollen receipt per flower per plot.

Seed production—We quantified seed production from the same flowers from which we collected stigmas. In natural plots, we collected fruits in all of the plots. In the experimental plots, we only collected fruits from eight plots that spanned the full experimental density range because the remaining plots were destroyed by free-range cattle. If flowers aborted, we included them in the analyses as producing zero seeds per flower. For flowers that produced fruits, we counted the number of seeds surviving and seeds consumed by fly larvae. Seed coats of consumed seeds remain intact after larvae destroy the endosperm. To determine how many ovules developed into mature seeds, we summed surviving and consumed seeds. Unless there were qualitative differences in effects on developed seeds, we only reported surviving seed production per flower.

Statistical analyses—In the statistical analyses described next, we used plot as the unit of replication (i.e., all variables were measured on a per-flower basis and averaged per plot). For the experimental plots, the analyses were separated into early and late time periods. We used linear regression to test the prediction that pollinator visitation rates, pollen receipt, and seed production increase with flowering plant density. To analyze the relationship between seed production and natural flowering plant density, we included a quadratic term in the regressions because a bivariate scatter plot suggested a unimodal relationship (Fig. 1). For natural plot seed production, we chose between the linear and polynomial models based on the model with the higher adjusted R^2 and lower Akaike's information criterion (AIC). Models that minimize AIC by at least two units provide a better fit to the data (Sakamoto et al., 1986). We also tested for correlations among pollinator visitation rates, pollen receipt, and seed set because failure of density to affect seed set could occur if pollen receipt did not affect seed set and/or if pollinator visitation rates did not affect pollen receipt.

Because spatial location may affect flowering plant density, pollination, and reproduction (Koenig and Knops, 1998; Williams et al., 2001; Kuhn et al., 2006), the spatial location of our plots might have influenced the relationships among these variables. Thus, we evaluated spatial patterns in response variables by calculating Moran's I values with regression residuals (Legendre, 1993), and we used simultaneous autoregressive models to analyze the relationships between flowering plant density and pollen receipt and seed production. We compared models with and without accounting for spatial autocorrelations in the response variables using model fit (highest R^2) and AIC (Lichstein et al., 2002; Kissling and Carl, 2008). Spatial analyses were performed with the program SAM (Spatial Analysis in Macroecology version 3.0; Rangel et al., 2006). All other analyses were performed using the program JMP version 4.04 (SAS Institute, 2001).

2. Do the relationships among flowering plant density, pollination, and seed production vary across the flowering season, and could seasonal changes in pollinator visitation and floral sex-phase ratios account for such variation?—We explored seasonal variation in floral sex ratios, pollinator visitation rates, and pollen limitation of seed set, all of which might mediate *D. barbeyi* seed set because (1) flowers are protandrous so male-to-female phase floral sex ratio should be higher early in the season, (2) bumble bees increase in abundance over *D. barbeyi*'s blooming period so pollinator visitation rates should be higher late in the season, and (3) if abiotic resources necessary for seed production are depleted over the season, then pollen limitation of seed set should be stronger early in the season.

We used one-tailed paired t tests to test the predictions that male-to-female phase flower ratios decreased and per flower pollinator visitation rates increased from early to late in the season. We used two-tailed paired t tests to test whether pollen receipt and seed production changed over the season. Increases in pollen receipt and seed set throughout the season could suggest that higher pollinator visitation rates benefit seed set. Decreases in pollen receipt and seed set throughout the season could suggest that costs of lower male-to-female phase flower ratios, or some other factor, outweigh the benefits of higher pollinator visitation rates.

Pollen limitation—We compared seed set between supplementally hand-pollinated flowers and open-pollinated control flowers. On each focal plant in 10 experimental plots that spanned the full experimental density range, we assigned one inflorescence to a hand-pollination treatment and a second inflorescence to a control treatment. Once during each pollinator-observation period, we hand-pollinated any open female-phase flowers on the hand-pollination inflorescences. We collected dehiscing anthers from at least 10 plants growing >5 m away to avoid potential pollen incompatibility and because plants in nature may receive pollen from multiple donors. We added pollen by brushing dehiscing anthers onto receptive stigmas. To control for flower handling, we handled a similar number of flowers in the control treatment. Combining both observation periods, the 29 treated hand-pollination inflorescences each had an average of 14.3 ± 1.4 hand-pollinated flowers (4.1% of all flowers). Given that we hand-pollinated a small proportion of the flowers, it is unlikely that there was competition for resources among flowers on the same plant in the hand-pollinated and control treatments (but see Knight et al., 2006). To test whether hand-pollination increased stigmatic pollen receipt, we collected stigmas from 230 early flowers (111 hand-pollinated and 119 control) and 120 late flowers (75 hand-pollinated and 45 control), and we counted pollen grains (as described).

In the early period, we treated 29 and 28 inflorescences in the hand-pollination and control treatments, respectively. In the late period, we treated 28 and 26 inflorescences from the hand-pollination and control treatments, respectively. The sample sizes varied because we could only treat assigned inflorescences that had open female-phase flowers. The number of open female-phase flowers per inflorescence ranged from 2–22, with averages of ten and nine open female-phase flowers per inflorescence for early hand-pollination and control treatments, respectively, and an average of five open female-phase flowers per inflorescence for late hand-pollination and control treatments. We used two-tailed t tests to compare pollen receipt and seed production between flowers in control and hand-pollination treatments. We used plot as the unit of replication for seed set. For pollen receipt, we used flower as the unit of replication because we did not have sufficient flower samples to use plot as the unit of replication. In addition, we used a fully crossed two-way ANOVA with pollination treatment, density treatment, and their interaction to test whether hand-pollinating flowers altered density effects on seed set.

RESULTS

1. Does flowering plant density affect pollination and seed production?—In natural plots, pollen receipt per flower increased linearly with flowering plant density ($r^2 = 0.06$, $P = 0.005$, Fig. 1A). For seed production per flower, a polynomial model describing an increase in seed set over a low density range and decrease in seed set over a high density range provided a better fit to the data than a linear model (adjusted $R^2 = 0.07$, $P = 0.001$; Fig. 1B, Table 1). Seed production increased in plots with higher pollen receipt, but this relationship was dampened by accounting for seed predation (Table 2).

Pollen receipt and seed production regression residuals showed minor spatial autocorrelation in natural plots (pollen:

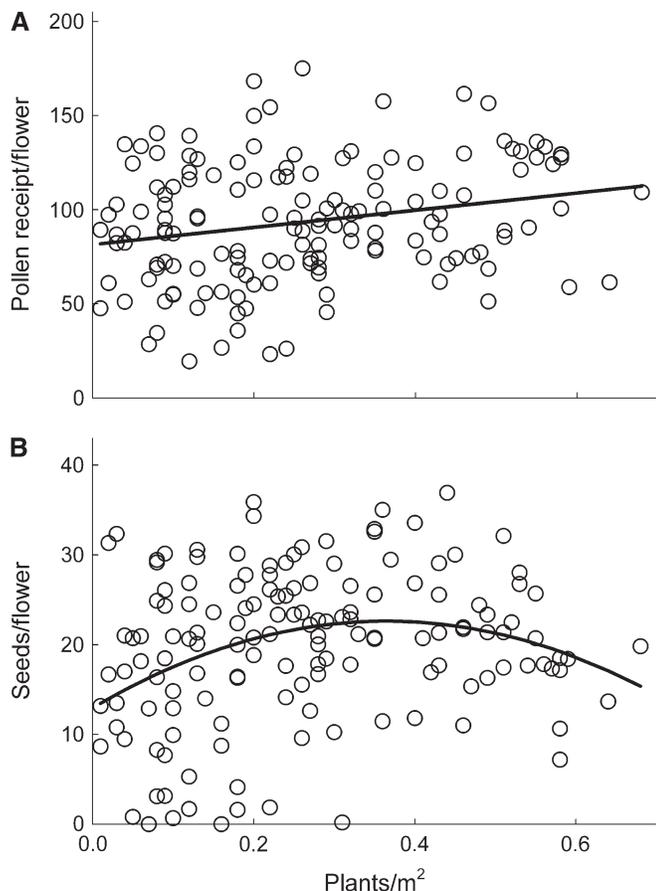


Fig. 1. Relationship between natural *Delphinium barbeyi* flowering plant density (flowering plants/m² in 100-m² plots) and (A) pollen receipt per flower (pollen receipt = 81.5 + 45.6 × density) and (B) surviving seeds per flower (seeds = 18.0 + 14.7 × density – 72.8 × density).

maximum Moran's $I = 0.13$; seeds: maximum Moran's $I = 0.22$). Models including density and spatial coordinates explained more of the variation in pollen receipt and seed production than models that did not include spatial components (highest R^2 and lowest AIC; Table 1). For seed production, a polynomial density model with spatial coordinates provided a better fit than the linear density model with spatial components (Table 1).

In contrast to natural plots, we found no statistically significant effects of experimental flowering plant density on pollination or seed production. Early in the season, pollinator visitation rates increased with experimental flowering plant density ($\beta = 0.82 \pm 0.45$), but this relationship was not statistically significant ($r^2 = 0.19$, $P = 0.09$; Fig. 2A). Late in the season, the relationship between pollinator visitation rate and flowering plant

TABLE 1. Comparison of models incorporating density and spatial location to predict *Delphinium barbeyi* pollen receipt and seed production in 100-m² plots.

Response	Predictors	R^2	AIC
Pollen receipt	Density + space	0.07	1445.7
	Density	0.06	1448.8
Seeds per flower	Density + Density ² + space	0.11	1045.5
	Density + space	0.08	1048.1
	Density + Density ²	0.09	1049.2
	Density	0.03	1055.6

density disappeared ($r^2 < 0.01$, $P = 0.90$; Fig. 2A). Flowering plant density had no effect on early season pollen receipt ($r^2 < 0.01$, $P = 0.85$; Fig. 2B). Late in the season, pollen receipt increased with flowering plant density, but this relationship was not statistically significant ($r^2 = 0.01$, $P = 0.13$; Fig. 2B). Experimental flowering plant density did not affect seed production early ($r^2 = 0.01$, $P = 0.79$; Fig. 2C) or late in the season ($r^2 = 0.10$, $P = 0.38$; Fig. 2C).

In the experimental plots, neither pollen receipt nor seed production per flower varied with pollinator visitation rate per flower (Table 2). Bumble bees (*Bombus* spp.) were the primary visitors to *D. barbeyi* flowers. *Bombus appositus* workers contributed to >75% of all visits during both parts of the season (Table 3). Early in the season, seed production increased with pollen receipt, but this effect disappeared after accounting for seed predation (Table 2). In contrast, late in the season, seed production did not increase with pollen receipt (Table 2).

2. Do the relationships among flowering plant density, pollination, and seed production vary across the flowering season, and could seasonal changes in pollinator visitation and floral sex-phase ratios account for such variation?

In experimental plots, the male-to-female phase flower ratio decreased by 87% from early to late in the season ($t_{11} = 2.04$, $P = 0.03$), and pollinator visitation rates increased by an order of magnitude over this time period ($t_{11} = 4.10$, $P = 0.002$, Fig. 3A, B). Because flower production did not differ from early to late in the season (95% confidence interval for mean difference in open flowers per plant between early and late time periods was –61.4–55.2 open flowers per plant, $N = 12$ plots), changes in pollinator visitation rate per flower were not driven by decreases in available flowers. Pollen receipt decreased by 36% from early to late in the season ($t_{24} = 3.56$, $P = 0.002$, Fig. 3C).

We found no difference in developed seeds produced by hand-pollinated and control flowers early ($t_9 = 1.75$, $P = 0.11$) or late in the season ($t_9 = 0.23$, $P = 0.83$; Fig. 3D), suggesting that plants were not pollen-limited for seed set. Experimental densities did not alter the effect of hand-pollinating flowers on seed set in either part of the season (early: $F_{1,16} = 0.53$, $P = 0.48$; late $F_{1,16} = 0.21$, $P = 0.65$). One reason that hand-pollinated and control flowers did not differ in seed set may be because pollen

TABLE 2. Correlations among *Delphinium barbeyi* per-flower pollinator visitation rates, pollen receipt, and seed production for natural plots and for experimental plots (sampled early and late in the blooming period). Correlation coefficients are reported with P -values in parentheses, then sample size (number of plots).

Response	Natural pollen	Early visitation	Late visitation	Early pollen	Late pollen
Pollen		–0.27 (0.36) $N = 14$	–0.04 (0.90) $N = 14$		
Developed seeds	0.31 (0.0001) $N = 148$	0.64 (0.24) $N = 5$	–0.38 (0.35) $N = 8$	0.72 (0.04) $N = 8$	0.47 (0.17) $N = 10$
Surviving seeds	0.22 (0.01) $N = 148$	–0.21 (0.73) $N = 5$	–0.38 (0.35) $N = 8$	0.39 (0.34) $N = 8$	0.47 (0.17) $N = 10$

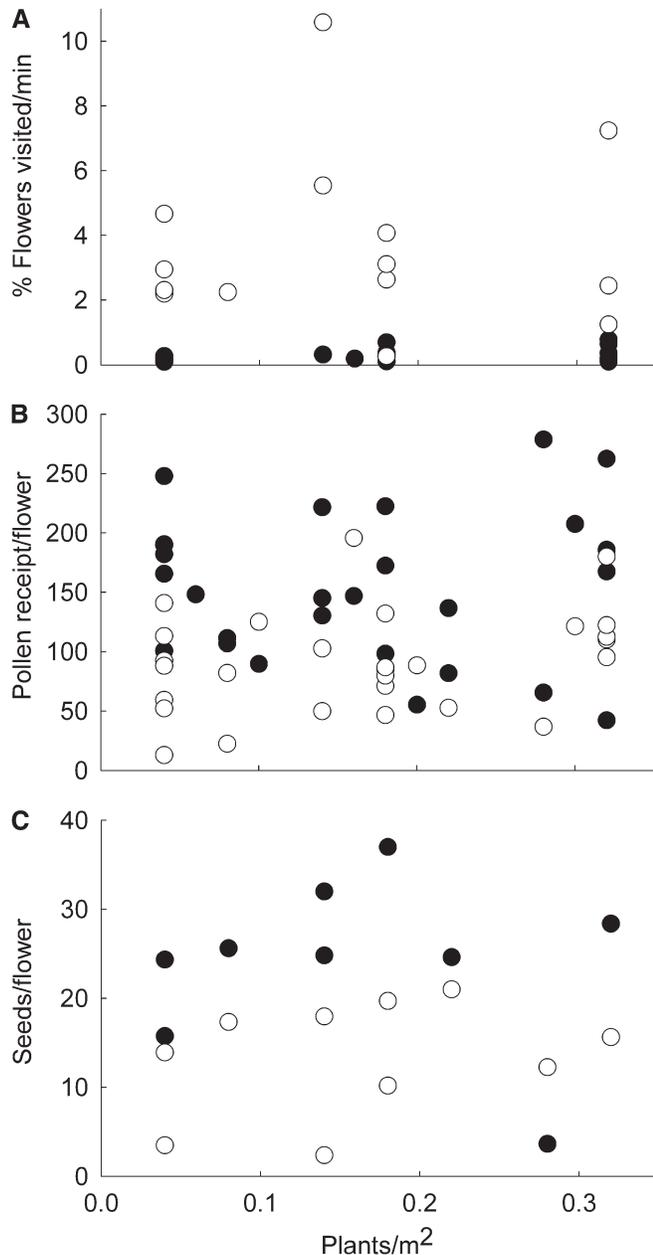


Fig. 2. Relationship between experimental *Delphinium barbeyi* flowering plant density (plants/m² in 100-m² plots) and (A) percentage of open flowers visited by pollinators per minute, (B) pollen receipt per flower, and (C) surviving seeds per flower for flowers blooming early (closed circles) and late (open circles) in *D. barbeyi*'s blooming period.

receipt did not differ between control and hand-pollinated flowers early ($t_{228} = 0.79$, $P = 0.43$; mean pollen grains per flower \pm 1 SE: hand-pollinated = 140 ± 10 pollen grains, control = 131 ± 9 pollen grains) or late in the season ($t_{118} = 0.71$, $P = 0.48$; hand-pollinated = 97 ± 9 pollen grains, control = 86 ± 12 pollen grains). Seed production of hand-pollinated flowers decreased by 12% over the season, although this decrease was not statistically significant ($t_8 = 1.89$, $P = 0.10$; Fig. 3D). Similarly, natural seed production decreased by 44% over the season ($t_8 = 2.72$, $P = 0.03$, Fig. 3E).

DISCUSSION

While natural variation in *D. barbeyi* flowering plant density was correlated with pollen receipt and seed set, experimental densities had little to no effect on pollinator visitation rates, pollen receipt, or seed set. In experimental plots, floral sex ratios, pollinator visitation rates, pollen receipt, seed set, and density trends with these factors varied from early to late in the season. Overall, effects of flowering plant density on *D. barbeyi* reproduction were minor.

That we only found significant effects of flowering plant density in natural plots and not in experimental plots suggests the importance of both observational (natural densities) and experimental (manipulated densities) studies to assess causality (Power et al., 1998; Underwood et al., 2000; Abrams, 2001). Although positive effects of plant density and population size on seed set and outcrossing appear to be common across a diversity of plant species with different breeding systems and growth forms (Ghazoul, 2005), only 21 of 123 species reviewed by Ghazoul (2005) included experimental manipulations of flowering plant density. Thus, the underlying mechanisms driving the relationships between natural density and seed set remain unclear in most systems. In our system, if underlying variation in abiotic resources caused the joint increase in seed production and flowering plant density over the lower natural density range, then such covariation could explain why we did not see similar trends when we manipulated flowering plant density (Bosch and Waser, 2001). In addition, because pollen receipt increased with natural flowering plant density, abiotic resources may have also affected per-flower nectar and pollen rewards used to attract pollinators, creating spurious positive correlations between pollen receipt and seed production (Carroll et al., 2001). Only 3–9% of the variation in pollen receipt and seed production was explained by flowering plant density in natural plots, and some of the variation in pollen receipt and seed production was explained by fine-scale spatial autocorrelation, suggesting that patchiness in abiotic resources may contribute to density–pollen and density–seed relationships. Given the effect sizes in the experimental study, detecting statistically significant density effects ($\alpha = 0.05$) would have required 40 plots for effects of flowering plant density on early-season pollinator visitation rate and 92 plots for effects of density on late-season pollen receipt.

Two caveats are important to the interpretation of this study. First, the outcomes of this study may have changed had we manipulated flowering plant density at a larger spatial scale. For example, in 2007 at the whole meadow scale, *D. barbeyi* seed set increased with pollinator visitation rates; however, visitation rate was not higher in meadows with more flowers (Elliott, 2008). Work that addresses how the relationships between flower density and pollination success vary with natural and experimental variation in bee densities will provide additional insights. Future studies could also manipulate flower density per patch and patch size to disentangle their effects on pollinator behavior and plant reproduction (Cresswell and Osborne, 2004; Heard et al., 2007). Second, density relationships may vary considerably among years because seed production can vary drastically among years and may be strongly linked to snowmelt and frost dates (Inouye et al., 2002; Elliott, 2008).

For density to affect pollen receipt through increases in per-flower pollinator visitation rate, there needs to be a strong relationship between pollinator visitation rates and pollen receipt. In other flower species, the number of pollen grains or

TABLE 3. Percentage of visits to *Delphinium barbeyi* flowers by different species (and castes for bumble bees), early and late in the blooming period.

Species	Caste	Early		Late	
		By caste	Total	By caste	Total
<i>Bombus appositus</i>	worker	75.5	77.5	76.9	79.9
	queen	2.0		0.9	
	male	0		2.1	
<i>B. flavifrons</i>	worker	7.2	9.7	13.3	15.3
	queen	2.5		2.0	
	male	0		0	
<i>B. nevadensis</i>	worker	2.8	5.5	<0.1	<0.1
	queen	2.7		<0.1	
	male	0		0	
<i>B. californicus</i>	worker	4.1	4.1	4.5	4.8
	queen	0		0.3	
	male	0		0	
<i>Hyles lineata</i>			2.1		0
<i>Selophorus platycerus</i>			0.9		0
<i>S. rufus</i>			0.1		0

unique sires per flower increase with pollinator visitation rate (Engel and Irwin, 2003; Karron et al., 2006). However, in this study, pollinator visitation rates to *D. barbeyi* flowers were not correlated with pollen receipt in either part of the season. While pollinator visitation rate is inherently related to pollen receipt at some level in *D. barbeyi* (e.g., pollen receipt decreases by 71% when all pollinators are excluded from flowers; S. E. Elliott, unpublished data), either our snap-shot estimate of pollinator visitation rate was too coarse to detect a relationship between visitation rate and pollen receipt, or pollen receipt was saturated with surplus pollinator visits (Brown and Kephart, 1999). For example, despite an order of magnitude increase in pollinator visitation rates across the season, pollen receipt decreased. In addition, when we hand-pollinated flowers, they did not receive more pollen than open-pollinated control flowers, suggesting that stigmatic surface area was saturated. It was unlikely that added pollen grains fell off due to stigmas being unreceptive because hand-pollinated stigmas still received an order of magnitude more pollen grains than seeds produced per flower. To determine whether flowers received multiple pollinator visits, we multi-

plied per-flower pollinator visitation rates by the length of time that pollinators were active each day (i.e., 8 h). Assuming that all flowers were visited equally, we calculated that open flowers would receive 1.5 and 17.8 visits per day, early and late in the season. Thus, all flowers probably received multiple visits, and pollen receipt may saturate at low visitation rates.

Changes in floral sex ratios and pollinator behavior could have contributed to the decrease in pollen receipt per flower throughout the season. For example, late in the season bumble bees may have groomed more of the pollen to their corbiculae to take back to the hive to feed their growing colony (Cartar, 1992; Weinberg and Plowright, 2006). In a system similar to the one reported here, pollinator visits to flowers of the protandrous perennial herb, *Alstroemeria aurea* (Alstroemeriaceae), increase late in the season when there are fewer male-phase flowers available, and consequently, bumble bees deliver an order of magnitude fewer pollen grains per visit (Aizen, 2001). Also, if bees had preferentially visited male- or female-phase flowers (as in Carlsson-Graner et al., 1998), then we may not have adequately described visitation rates to

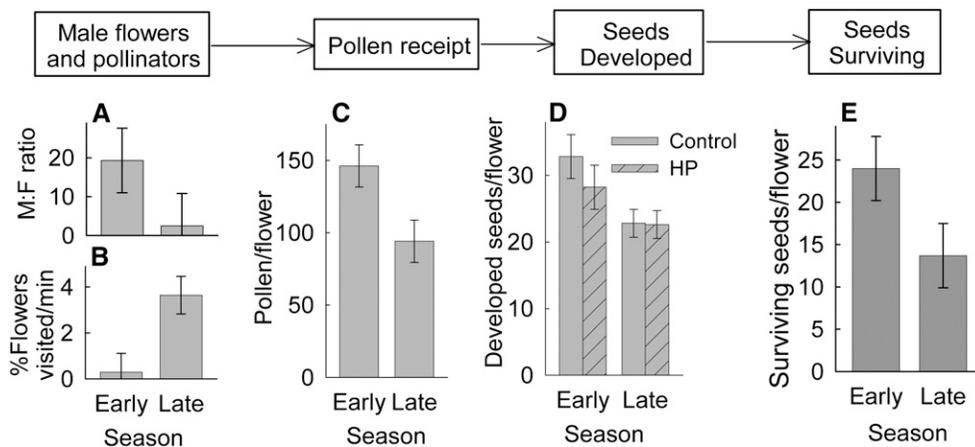


Fig. 3. Variation in *Delphinium barbeyi* seed production and factors associated with seed production measured in 100-m² experimental plots. (A) Male-to-female phase flower ratio ($N = 12$ plots), (B) percentage of open flowers visited per minute ($N = 12$ plots), (C) pollen receipt per flower ($N = 25$ plots), (D) developed seeds per flower (solid = open control flowers, hatched = hand-pollinated flowers, $N = 9$ plots), and (E) surviving seeds per flower ($N = 9$ plots) early and late in the blooming period. Error bars represent ± 1 SE around plot means.

female-phase flowers, which we ultimately hoped to link to pollen receipt. If bees were primarily collecting pollen, they might have preferentially visited male-phase flowers. If bees were preferentially collecting nectar, then they might have preferentially visited female-phase flowers because female-phase flowers contain 22% more nectar per flower than male-phase flowers (mean nectar volume per female-phase flowers ± 1 SE: 0.61 ± 0.04 μ L per flower, male-phase flowers: 0.50 ± 0.03 μ L per flower; $t_{452} = 2.3$, $P = 0.02$).

The late season decrease in *D. barbeyi* seed production was probably not due to increased pollen limitation. For example, in the herbaceous perennial, *Lithophragma parviflorum* (Saxifragaceae), seed set of hand-pollinated, late-blooming flowers was lower than early-blooming, hand-pollinated flowers (Pellmyr and Thompson, 1996). *Delphinium barbeyi* could have had fewer resources for late-blooming flowers if their early-blooming flowers depleted available resources. While shortages of male-phase flowers late in the season may have contributed to the decrease in *D. barbeyi* pollen receipt, because hand-pollinated plants also produced fewer seeds late in the season, pollen supply probably did not limit late-season seed set. Instead, limited abiotic resources may have influenced the decrease in *D. barbeyi* seed set.

Redispersal fly seed predators dampened or masked the relationships between *D. barbeyi* pollen receipt and seed production in natural plots and in early blooming flowers in experimental plots. However, seed predators had little to no effect on the relationship between flowering plant density and seed production, suggesting that female flies did not preferentially oviposit in dense flower patches. Similarly, beetle fruit predators of the terrestrial aroid, *Xanthosoma daguense* (Araceae), masked the benefits of increased pollinator visitation rates for fruit production but had little to no effect on pollinator-mediated benefits of plant density (García-Robledo et al., 2005).

Our results support the growing evidence that interaction outcomes are highly contingent on the surrounding biotic and abiotic environment (Thompson, 1999; Strauss and Irwin, 2004; Tscharntke and Brandl, 2004). The consequences of variation in interaction rates alone, such as pollinator visitation rates, did not translate into interaction outcomes, such as pollen receipt and seed production. In plant species that produce many flowers over a long blooming period and that separate male- and female-phase flowers spatially or temporally, fluctuations in factors external to the plant–pollinator interaction, such as abiotic resources, sex ratios, and seed predation, may mask the fitness effects of variation in species interactions.

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