

Effects of clumping of *Delphinium barbeyi* on pollination

Janina Troper

Mentored by Ian Brekheimer

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Abstract

The spatial clustering of plants occurs for a variety of reasons, including the evolution of concentrated floral displays to attract more pollination. Within clusters, traits that attract pollinators may differ from non-cluster conditions— such as increasing floral display size and scent, which can cause an increase in pollinator visitation, which in turn increase the reproductive success of plants. In this study I focused on plant neighborhood density, one potential driver of pollination, to further understand pollination systems in times of change and challenge to these structures. More specifically, I examined the question of whether higher density clumping of *Delphinium barbeyi* increases pollination, and at what spatial scales this effect occurs. I used drone remote sensing, and spatial analysis to choose research sites and measure global densities of *D. barbeyi*, and collected additional data on local density with manual measurements. In order to measure pollination, I used pollen count, determined through pollen smashing. I found that the most significant global density that impacts the pollination of *D. barbeyi* is at a 40 meter to 70 meter scale, when comparing global scales of 20 meters to 80 meters. The relationship between pollen count and global density along with pollen count and local density were both found to be significant across scales of 20 meters to 80 meters. In addition, after adding plant fitness to the regression, the relationship across all scales remained significant.

Introduction

Animal pollination, the transfer of pollen from plant anthers to stigmas by animals, is necessary for sexual reproduction of seed plants in nearly all terrestrial ecosystems. Pollinators have evolved to maximize their foraging success by visiting more flowers on plants or clusters with large floral display sizes to reduce travel times between flowers (Williams 2007). Floral display size can be defined as the number of flowers per inflorescence, plant, or genet (Williams 2007). Floral display size, and therefore quantity and quality of pollination, can be increased with the clustering of plants. The clustering of plants occurs for a variety of reasons, including the evolution to attract more pollination, in addition to other adaptive reasons, such as a higher density of seeds deposited closer to parent plants, higher fitness in patches with suitable environmental conditions, and defense against pathogen attack through producing defense metabolites (Bharadwaj *et al* 2021). Plant clusters vary in size, defined by the total number of individuals in a cluster, and ultimately form a mosaic that together constitutes a community (Watt 1947). Within clusters, traits that attract pollinators may be altered— such as increasing floral display size, scent, and general fitness of plants, which can cause an increase in pollination visitation, which can in turn impact the reproductive success of plants (Williams 2007, Price *et al.* 1980).

While clustering of the same species can be potentially beneficial for plants, it can also create intraspecific competition. This competition can be over essential resources such as water availability, sunlight availability, and pollinator visitation. The differences between species in how they interact with their environments—both abiotic and biotic—are called stabilizing niche

differences. These differences can favor coexistence, yet cause greater harm to the coexistence of plants of the same species (Johnson *et al.* 2022). For instance, when competition for pollination arises, the stabilizing niche differences can allow for greater plant diversity. In addition, below-ground interactions within a competitive environment can have significant negative effects on plot and plant floral display size, flower size, and proportion of viable seeds (Floriane *et al.* 2020). Opposingly, as mentioned above, increased clustering of the same species can also attract more pollinators with their mere abundance impacting floral display size and reducing travel distance. Thus, the intersection that density creates between the potential of increased pollination and increased competition is contradictory and fascinating, creating tension over which impact will triumph the other.

Pollination, while vital for plant reproduction, is facing a disturbing decline globally. Animal pollinators have declined worldwide as a product of climate change, landscape alteration, agricultural intensification, non-native species, and the spread of pathogens. This decline is dangerous as more than 88% of all angiosperm species rely on animals for pollen transfer (Ollerton 2011). Pollination also directly affects the sustainability of global plants and animals, of which include humans (Kevan 2003). Of the major crop species worldwide, 70% are at least partly reliant on animal pollination for yield production, including 35% of global food production (Klein 2007). Hence, a decline in pollination is a hazard to most species on Earth, since, in extreme cases, this could cause extinction of plants and animals which could ultimately lead to significant alterations in the landscape and ecosystem functionality (Kevan 2003).

In this study I examined one potential driver of pollination to further understand pollination systems in times of change and challenge to these structures. More specifically, I examined the impact of clustering of flowers on pollination by looking at the impact of both global and local densities on pollination.

I focused on tall larkspur *Delphinium barbeyi* (hereafter *Delphinium*), a common flowering plant found in Rocky Mountains, Colorado in early July. I used drone remote sensing, and spatial analysis to choose research sites and measure densities of flowers; *D. barbeyi* plants and inflorescences can be detected on this platform, and thus makes it a reasonable experimental flower. In addition, while *D. barbeyi* is self-compatible, it relies significantly more on pollinators for successful reproduction, allowing for the use of fertilization as a metric for pollination visitation (Williams 2001). I did not specify a pollinator to focus on since I am interested in overall pollination.

To address the question of whether higher density clumping of *D. barbeyi* increases pollination, I took data on five potential independent variables per site—density of the focal species determined by remote sensing (hereafter global density), height of the focal plant, width of the focal plant, number of stalks of the focal plant, and local plant density determined by a point quarter system (hereafter local density). These variables combine to create a well-rounded picture of the density of the focal *D. barbeyi* plant as well as *D. barbeyi* fitness. I had one dependent variable to determine pollination—pollen count. This metric is a valid measurement of pollination since bees and other pollinators deposit pollen when visiting a flower. More

pollination will likely yield a higher number of pollen grains. In order to determine pollen count, I will utilize the technique of pollen smashing.

Hypothesis

I hypothesized that larger density clumps of *D. barbeyi* will increase visitation of pollinators since higher floral display size would attract more pollinators and clumps will decrease travel time for pollinators.

Methods

Plant Species – *Delphinium barbeyi*, also commonly known as Barbey's Larkspur, Subalpine Larkspur, is a tall, herbaceous perennial flowering plant with widespread presence in the west of the United States, where it occurs in the states of Arizona, Colorado, New Mexico, Utah, and Wyoming. *D. barbeyi* is a species in the Ranunculaceae family (Buttercup family).

This species flowers from July to August, and can grow up to 1.5 meters tall, with 170 flowers on multiple inflorescences open per day (Williams 2001). Its flowers range from an iridescent blue to deep purple in color.

Bumble bees are the most common pollinators of *D. barbeyi*, with hummingbirds, hawkmoths, and flies also common pollinators (Schaeffer 2014). However, *D. barbeyi* can be self compatible (Williams 2001).

Study Site – I conducted this study at and around Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado during the months of June, July, and August 2022. This site is at 2900 meters in elevation. In this area *D. barbeyi* is found in habitats ranging from open dry subalpine meadows to more shaded, moist areas at the boundary of aspen woodlands.

Study Design – In order to identify clumps of *D. barbeyi* for study sites, I utilized remote sensing, i.e. drone imagery, captured with a Micasense Altum multispectral and thermal sensor to capture global densities of *D. barbeyi*. I used *D. barbeyi* classifications from an existing 5 cm resolution vegetation classification developed for the Gothic Townsite area based on data from June, July, and August 2021 (Breckheimer et al. *in prep*). I used this species classification map in QGIS to identify clumps of *D. barbeyi* near RMBL by plotting each plant as a polygon (Figure 1). After identifying polygons for each *D. barbeyi* in the townsite, I uploaded this file into R studio (2022.02.1), to calculate the *D. barbeyi* density of each polygon using the packages “terra” and “sf” (Hijmans 2022, Pebesma 2018). The density of each polygon was determined by the number of *D. barbeyi* plants within a 20 meter radius. After calculating the densities for each plant, I used a stratified random sampling method with 10 density buckets to select my sites. All together, I had selected 80 *D. barbeyi* plants to monitor; however, I ended up cutting 26 of these sites due to reachability and plant flowering success. This left me with 54 total sites, all located in South Gothic (refer to Figure 2). Once the original 80 sites were identified, I transferred this file to FieldMaps, a mobile application for offline access to maps.

I identified five independent variables: (1) density determined by remote sensing, (2) height of focal plant, (3) width of the focal plant, (4) number of stalks of the focal plant, and (5) density determined by a point quarter system.

- (1) As explained above, I used a vegetation classification map and an R script to determine the density of each *D. barbeyi* plant through measuring proportional cover at a global density scale. There was a significant difference between the focal plant's total area and number of stalks (and thus number of inflorescences) and global density. This significance was consistent across five different global density scales (Supp. Table 1 - Supp. Figure 2).

In order to collect data on the other four independent variables, I visited all of my field sites on the week of July 18. I chose my focal plant to be as close to the pre-selected sites as possible, however at times the chosen plant did not have valid inflorescences. In these cases I chose the closest neighboring *D. barbeyi* plant with at least one inflorescence or bud. Once at the sites, I took four pieces of field data, recorded on Google Sheets:

- (2) I took a measurement of the height of the tallest stalk with inflorescences, buds, or successful seeds of the focal plant using a measuring tape.
- (3) I measured the width of the focal plant at its widest point using the same measuring tape. This measurement included the leaves of the plant.
- (4) I counted the number of stalks on the focal plant. I only included stalks that contained either an inflorescence, a promising bud, or a successful seed set (Figure 3a). I excluded all other stalks since they do not pose a competition to surrounding plants.
- (5) In order to determine the local density of the focal *D. barbeyi* plant without remote sensing, I used the point quarter system. In order to do this, I measured the distance between the center of the focal plant and the center of the closest plant in each quadrant, defined by the North to South and East to West plains (Brower 1990). In some cases it was difficult to discern the boundaries between one *D. barbeyi* plant and another, thus I looked at where the stems of the *D. barbeyi* plants grew to determine the different plants. On each of these surrounding four plants I took the same three measurements I did on the focal plant: the height of the tallest stalk with inflorescences, the width of the plant at its largest point, and the number of stalks on the plant. If the *D. barbeyi* site was larger than just one *D. barbeyi* plant, I would mark several focal plants to accurately capture the density of the larger polygon. If I took data on more than one focal plant per site, I averaged each independent variable, including focal plant measurements. Using this information, I applied calculations to determine the total density of each plant (Brower 1990).

After collecting data on my five independent variables per site, I began collecting data on my dependent variable: pollen count. As stated previously, pollen count will likely increase with

high visitation rates, thus it is an important metric for determining pollination rate. In order to determine the pollen count of each plant, I used the technique of pollen smashing.

To begin the pollen smashing procedure, I collected six flowers from every *D. barbeyi* site the week of July 25, as the flowers of the plants were beginning to fruit. I chose to collect six flowers per plant to account for flower-to-flower variability in pollen deposition. At each site, I collected six flowers evenly distributed among the stalks' focal plant; for instance if there were six stalks, I collected one flower from each stalk. The flowers I targeted had the following traits: petals beginning to fall off, exposed stigmas, and fruits with three stigmas (Figure 3b). These traits were important to target as pollen smashing requires stigmas; thus, I was aiming to collect flowers that had intact stigmas. If the sites I visited had no valid stigmas to collect, I discarded the site from my sampling. I collected flowers from roughly 15 sites at a time, and immediately after placed them into a refrigerator to preserve the stigmas. Along with collecting the stigmas during the week of July 25, I also recorded updated GPS points using a Trimble Geo7X unit for each of my sites.

Between collecting stigmas, I performed the pollen smashing procedure. This typically occurred on the same day the stigmas were collected or a day after collection. For six of the sites, I smashed the stigmas four days after collection, due to time constraints. The smashing procedure occurred as follows: using fine tipped forceps, I removed the three stigmas off the flower and placed them on a glass microscope slide. I then placed two drops of fuchsin jelly, which I had liquified by placing the containing in a beaker of boiling water, onto the three stigmas. I placed a microscope cover over the stigmas, and pressed the jelly into them using a pencil eraser. Not only does pressing the stigmas dye the pollen, but this step also helps make the stigma and pollen grains more planar, rather than dimensional, to better count the grains. I labeled each slide with its corresponding site ID, and stored each slide in a slide box.

Once I smashed six flowers from my 54 sites, I began counting pollen grains. To do this, I placed each slide under a Leica DM750 Microscope at 40x magnification and, with a counter, counted each individual pollen grain. I only counted grains that were touching the actual stigma (including stigma hairs) or at some point in contact with a grain that was touching the stigma (Figure 4a), and that had a clear outline. I included grains that were not stained, but were clearly pollen grains by shape (Figure 4b). If the stigma was split or a piece was broken off, I still counted the pollen grains on this portion of the stigma. I recorded each count in a Google Sheets spreadsheet.

For statistical analysis, I used a negative binomial mixed-effects model implemented in a Bayesian framework with Hamiltonian Monte Carlo computational fitting strategy using the 'rstanarm' R package (Goodrich 2022). This analysis was appropriate due to the lack of normalcy for my dependent variable of pollen count. Using a Bayesian framework I found posterior probabilities between different independent variables and pollen count. In addition, I compared independent variables to one another to further understand the impact of density on plant fitness, in terms of plant height, width, and number of stalks.

Statistical analysis—I analyzed the relationships between pollen count and density measured at seven different spatial scales using a negative binomial mixed-effects model implemented in a Bayesian framework. These models were implemented using the ‘rstanarm’ R package (Goodrich 2022). Density was measured in two distinct manners—one with proportional cover calculated using remote sensing data (global density), and one with point quarter system measurements from the field (local density).

Results

I plotted the relationship between pollen grain count and proportional cover of *D. barbeyi* calculated from remote sensing at five different scales: 20 meters, 30 meters, 40 meters, 50 meters, 60 meters, 70 meters, and 80 meters (Table 1). With each of the analyses, there was a positive linear relationship, with greater than 99% posterior probability (Figure 5). Proportional cover within a 60m radius had the steepest slope between the two variables (mean slope of 16.8867 log grains per unit cover), indicating the strongest landscape effect.

I also plotted the relationship between pollen grain count and the local density, calculated using the point quarter system (Figure 6). Using this metric of density, I also found a significant linear relationship between density and pollen count, with greater than 99% posterior probability. This relationship had a mean slope of 0.8150 log pollen grains per plant * square meter.

While this relationship had significant results, since it is an observational study I also tested for effects of potential confounding variables. For example, higher-density populations might be composed of focal individuals with a larger area and number of inflorescences, potentially attracting more pollinators. To test for this, using a linear regression, I compared density to several measures of plant fitness: total focal plant area and the total number of stalks on each focal plant. The number of stalks is directly proportional to the total number of inflorescences on a plant. When comparing focal plant area with global density, there was a significant relationship consistently across all five scales (Supp. Table 1- Figure 1). Similarly, when comparing focal plant number of stalks with global density, there was also a significant relationship across all five scales (Supp. Table 2 - Figure 2).

In addition to utilizing density based on global densities, I ran two linear regressions comparing plant fitness on local density. Focal plant area and local density did not have a significant relationship ($p\text{-value} = 0.6384$, $\text{Adj. } R^2 = -0.01516$). Similarly, focal plant number of stalks and total density did not have a significant relationship ($p\text{-value} = 0.4669$, $\text{Adj. } R^2 = -0.008979$). Since there was no significance with the relationship between plant fitness and local density, no further regression was necessary to run.

However, due to the significant relationship between plant fitness and global density, I fit multiple linear regressions between pollen count versus global density and focal plant fitness. Scales 20 meters to 70 meters showed a positive linear relationship between pollen count and global density, even after accounting for the effects of focal plant area, with greater than 99% posterior probability. The 80 meter scale also displayed a linear relationship, with greater than a 97.5% posterior probability (Supp. Table 3). The proportional cover at 60 meters had the steepest

slope (15.8846) in relation to logged pollen count, compared to the other scales. The multiple linear regression between pollen count versus global density and focal plant number of stalks demonstrated similar results: scales 20 meters to 70 meters showed a positive linear relationship, with greater than 99% posterior probability, while the 80 meter scale had greater than a 97.5% posterior probability (Supp. Table 4). The global density at 50 meters had the steepest slope (12.5978) in relation to logged pollen count, compared to the other scales.

Discussion

This study used the novel tool of remote sensing to understand the spatial scale at which pollinators detect and respond to floral display clumping in *Delphinium barbeyi*. I found that the most significant global density that impacts the pollination of *D. barbeyi* is at a 40 meter to 70 meter scale, when comparing global scales of 20 meters to 80 meters. The relationship between pollen count and global density along with pollen count and local density were both found to be significant across scales of 20 meters to 80 meters. In addition, after adding plant fitness to the regression, the relationship across all scales remained significant.

Due to the significance of the positive relationship between density and pollen count, despite controlling for the confounding variable of plant fitness, I can accept my hypothesis that with increasing density of *D. barbeyi* there is an increase in pollination, leading to a higher number of conspecific pollen grains on stigmas. This finding is supported by the biological rationale that as density of the same flowering species increases, the floral display of that clump also increases, which attracts more pollinators. In addition, pollinators have decreased traveling time between flowers within clumps.

Similar studies investigating the density of *Delphinium* on pollination have been conducted at RMBL. However, unlike my study, these studies found little to no impact of dense or sparse arrays on pollinator visitation (Bosch and Waser 1999, 2001). These studies differed methodically from my study, which could have resulted in these differences. Unlike the two studies, my study used remote sensing data to determine density (at scales ranging 20 meters to 80 meters) along with the point quarter system, while the other two studies determined density with field measurements ranging from 0.3 to 4 meters on average. I suspect that the scale of neighborhood density used in these two studies was likely too small to see an impact on pollination. In addition, changes in climate between 1999/2001 and 2022 have impacted phenology and pollination due to timing changes of snowmelt and bumble bee emergence times (Inouye 2022), which could have resulted in different outcomes to the same research question. Adding my findings into the picture created by these past two studies, we can conclude that pollinator behavior is triggered more by global neighborhood densities (40-70 meter radii) compared to local neighborhood densities (0.3-4 meter radii).

There were several limitations to this study. One limitation included the observational aspect of field measurements as well as pollen counting. Specifically with pollen grain counts, some stigmas had pollen grains that were overlapping, making it difficult to distinguish between separate grains. In addition to observational limitations, I did not track specific pollinators, rather

I looked at overall pollination. Thus, I cannot conclude which species of pollinators are driving this pattern of density on pollination. I also did not perform genetic testing on the pollen grains, making it difficult to discern selfing versus outcrossing pollination rates. Along with this restriction, I performed this study in one general area, South of the Gothic Townsite. Replicating this study in multiple different areas with a greater diversity of environments would be beneficial to test the patterns found. Lastly, due to the size of the remote sensing map used, I was not able to look at global densities larger than 80 meters. However, with a more expansive map, it would be possible to look at scales up to 150 meters, which could provide insight into which special scale is the most impactful for pollination and to which scale this effect minimizes. Therefore, to continue this research, I would like to look at global densities larger than 80 meters, perform this experiment at different sites, and add pollinator species data.

Acknowledgements

Thank you to Charles Williams for teaching me the pollen smashing technique and guiding me through the field methods of this study. Thank you to Ian Breckheimer for mentoring and helping me throughout every stage of this study.

Figures and Tables

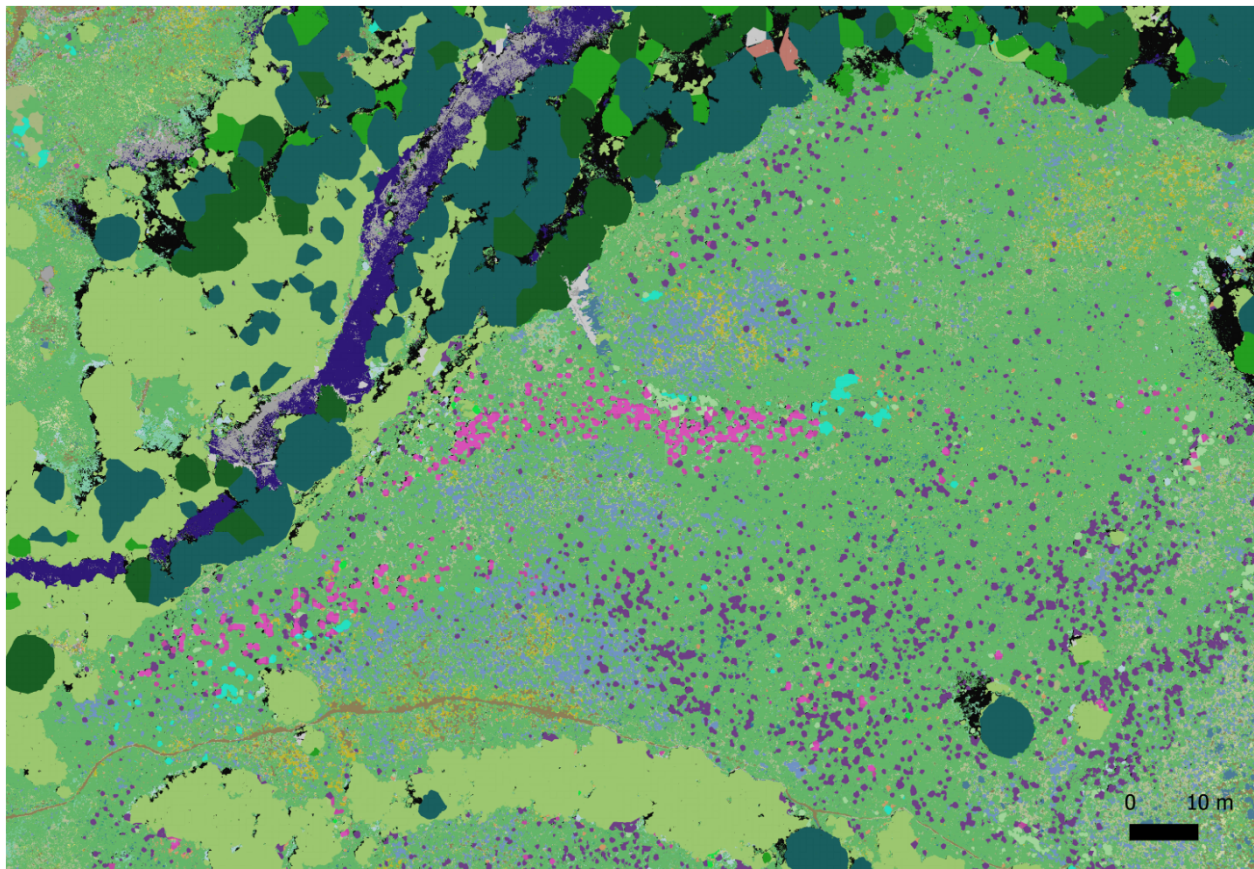


Figure 1. A subset of the vegetation classification map used. Pink represents *D. barbeyi*.



Figure 2. Maps from QGIS of all 54 *Delphinium barbeyi* sites. Bright green represents sites. All sites were located in South Gothic.



Figure 3a. Examples of appropriate flowers (left), buds (middle), and seeds (right) to count a stalk.



Figure 3b. Examples of appropriate stigmas to collect for pollen smashing, including exposed stigmas with petals falling over or already fallen off (left), or a seed set with three stigmas (right).



Figure 4a. Pollen grains that are touching one another toward the bottom of the stigma are still counted as there is a physical connection to at least one pollen grain and the stigma. The pollen grain right of this lower clump not in focus would not be counted, as it does not touch the stigma or another grain touching the stigma.

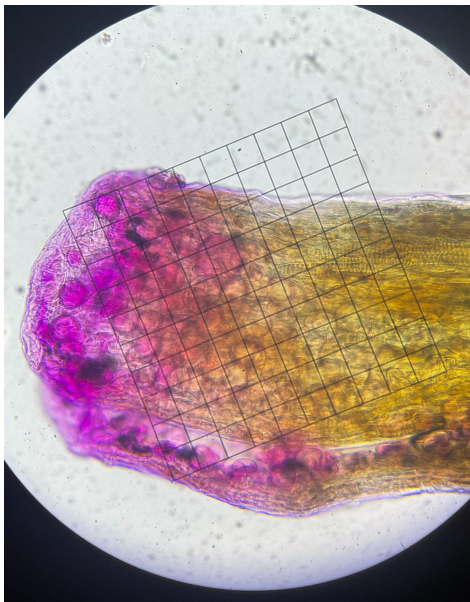


Figure 4b. Example of stigma with unstained pollen grains during pollen smashing. Pink circles are stained pollen, yellow/green circles are unstained pollen grains, identifiable by shape. Image on the right is a stigma with only stained pollen grains.

Results of Pollen Grains (logged) versus Proportional Cover at Five Distinct Scales

Pollen Grains vs.	Intercept	Slope Estimate (posterior mean)	Slope (1% credible interval)	Slope (99% credible interval)
Proportional cover at 20m	2.2175	4.6463	2.7064	6.5211
Proportional cover at 30m	1.8765	7.3932	4.8890	9.9315
Proportional cover at 40m	1.7068	10.5886	6.6089	14.4917
Proportional cover at 50m	1.4286	14.8018	9.4347	20.7007
Proportional cover at 60m	1.4315	16.8867	9.5454	23.8521
Proportional cover at 70m	1.9211	14.8906	6.4657	23.6346
Proportional cover at 80m	2.1971	12.1617	2.8536	20.9627

Table 1. This table compares the mean, 1% credible interval, and 99% credible interval slopes for five different distances for proportional cover, derived from drone imagery. Slope represents the number of pollen grains logged per unit of proportional cover. Proportional cover has the steepest slope with proportional cover being within 60 meters.

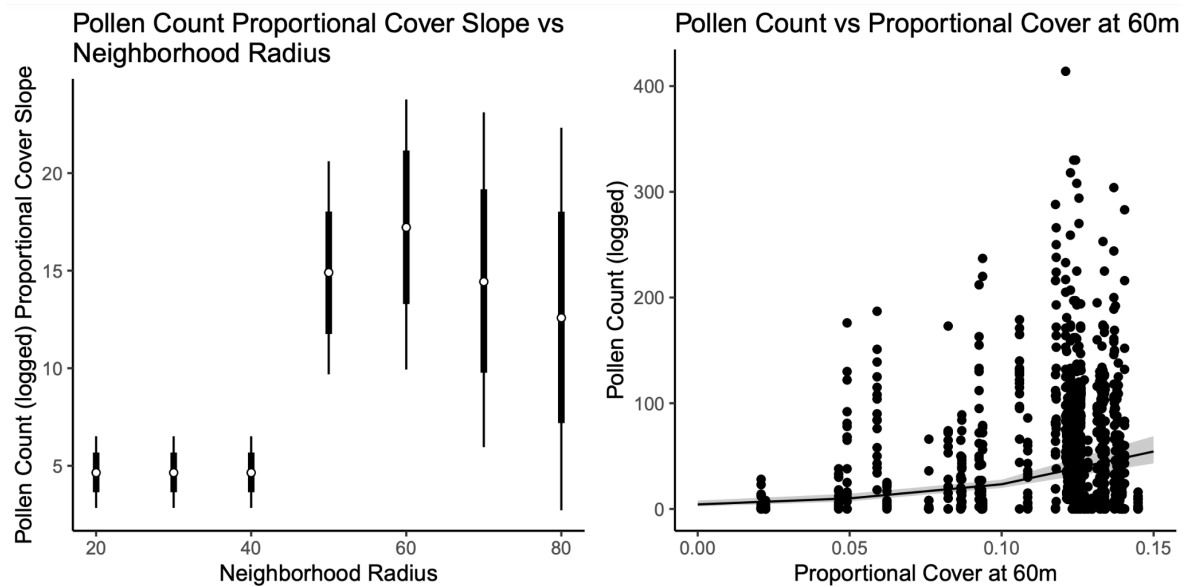


Figure 5. The graph on the left shows a strip plot of pollen count versus proportional cover slopes at seven different neighborhood radii of 20m, 30m, 40m, 50m, 60m, 70m, and 80m. The graph on the right shows a mixed-effects model of pollen count versus the neighborhood radius with the steepest slope (60m, with the best fit line showing a 90% credible interval).

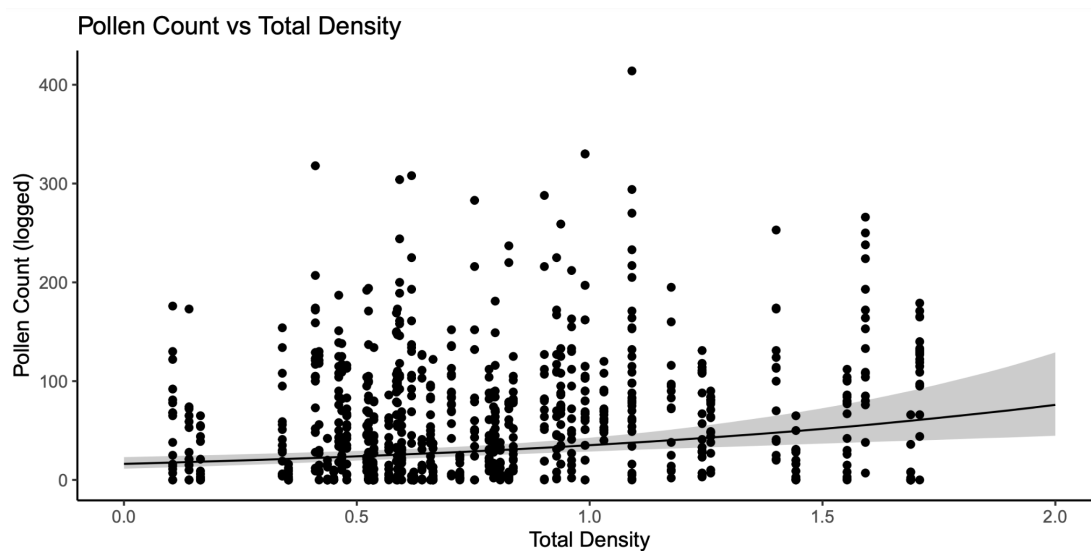


Figure 6. This mixed-effects model shows a significant positive correlation between pollen count (logged) and total density, derived from the point quarter system using field measurements. The best fit line shows 90% credible interval. The mean slope for the best fit line is 0.8150 logged pollen grains per unit of proportional cover. The 1% credible interval for slope is 0.1820, and the 99% credible interval for slope is 1.3396. The mean intercept is 2.7878.

References

- Bharadwaj R, Kumar SR, Sharma A, Sathishkumar R. Plant Metabolic Gene Clusters: Evolution, Organization, and Their Applications in Synthetic Biology. *Front Plant Sci.* 2021 Aug 13;12:697318. doi: 10.3389/fpls.2021.697318. PMID: 34490002; PMCID: PMC8418127.
- Brower, James E, Jerrold H. Zar, and Carl . Ende. *Field and Laboratory Methods for General Ecology*. Dubuque: Wm. C. Brown Publishers, 1990. Print.
- Floriane Flacher, Xavier Raynaud, Amandine Hansart, Benoît Geslin, Eric Motard, Séléné Verstraet, Manon Bataille, Isabelle Dajoz, Below-ground competition alters attractiveness of an insect-pollinated plant to pollinators, *AoB PLANTS*, Volume 12, Issue 4, August 2020, plaa022, <https://doi.org/10.1093/aobpla/plaa022>
- Goodrich B, Gabry J, Ali I, Brilleman S (2022). “rstanarm: Bayesian applied regression modeling via Stan.” R package version 2.21.3, <https://mc-stan.org/rstanarm/>.
- Hijmans, R. J., Bivand, R., Forner, K., Ooms, J., Pebesma, E., & Sumner, M. D. (2022). Package ‘terra’.
- Inouye, D. W. (2022). Climate change and phenology. *WIREs Climate Change*, 13(3), e764. <https://doi.org/10.1002/wcc.764>
- Johnson, C.A., Dutt, P. & Levine, J.M. Competition for pollinators destabilizes plant coexistence. *Nature* 607, 721–725 (2022). <https://doi.org/10.1038/s41586-022-04973-x>
- Klein, A.M., *et al.* Importance of pollinators in changing landscapes for world crops *Proc. R. Soc. Lond. B*, 274 (2007), pp. 303-313
- Kevan, Peter G. & Viana, Blandina F. (2003) The global decline of pollination services, *Biodiversity*, 4:4, 3-8, DOI: 10.1080/14888386.2003.9712703
- Ollerton J., *et al.* How many flowering plants are pollinated by animals? *Oikos*, 120 (2011), pp. 321-326
- Pebesma, E. J. (2018). Simple features for R: standardized support for spatial vector data. *R J.*, 10(1), 439.
- Price, Peter W., et al. “Interactions Among Three Trophic Levels: Influence of Plants on Interactions Between Insect Herbivores and Natural Enemies.” *Annual Review of Ecology and*

Systematics, vol. 11, 1980, pp. 41–65. *JSTOR*, <http://www.jstor.org/stable/2096902>. Accessed 27 Jun. 2022.

Schaeffer, Robert & Phillips, Cody & Duryea, M. & Andicoechea, Jonathan & Irwin, Rebecca. (2014). Nectar Yeasts in the Tall Larkspur *Delphinium barbeyi* (Ranunculaceae) and Effects on Components of Pollinator Foraging Behavior. *PloS one*. 9. e108214. 10.1371/journal.pone.0108214.

Watt, Alex S. “Pattern and Process in the Plant Community.” *Journal of Ecology*, vol. 35, no. 1/2, 1947, pp. 1–22. *JSTOR*, <https://doi.org/10.2307/2256497>. Accessed 27 Jun. 2022.

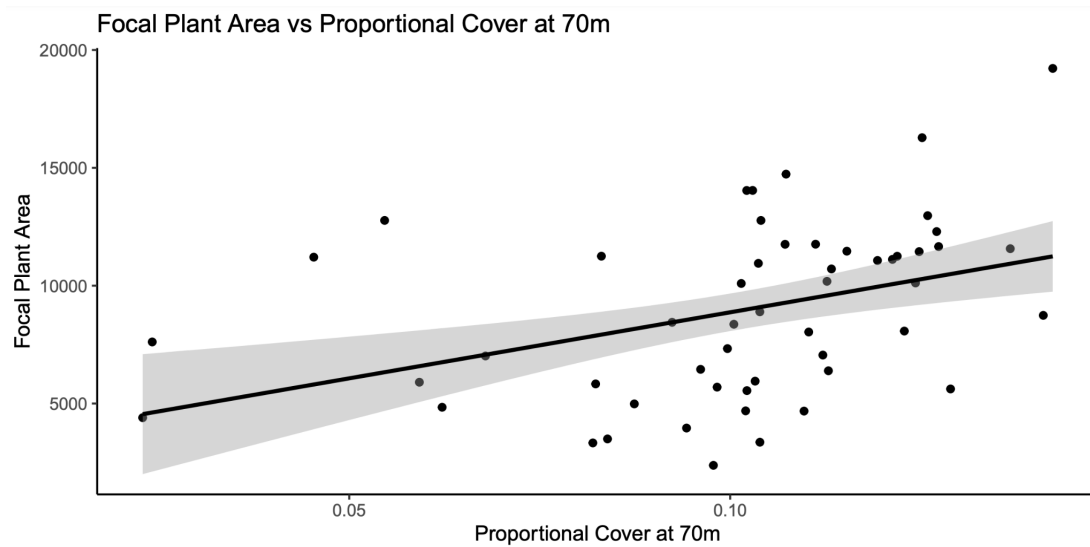
Williams, Charles F. “Effects of Floral Display Size and Biparental Inbreeding on Outcrossing Rates in *Delphinium Barbeyi* (Ranunculaceae).” *American Journal of Botany*, vol. 94, no. 10, 2007, pp. 1696–705. *JSTOR*, <http://www.jstor.org/stable/27733342>. Accessed 27 Jun. 2022.

Williams, Charles F., et al. “Pollination, Breeding System, and Genetic Structure in Two Sympatric *Delphinium* (Ranunculaceae) Species.” *American Journal of Botany*, vol. 88, no. 9, 2001, pp. 1623–33. *JSTOR*, <https://doi.org/10.2307/3558407>. Accessed 27 Jun. 2022.

Supplemental Results**Focal Plant Area (cm²) versus Proportional Cover at Five Distinct Scales**

FP Area vs.	Intercept	Mean Slope	P-value	Adjusted R Squared
Proportional cover at 20m	6291	10545	0.01656	0.08997
Proportional cover at 30m	6067	14225	0.0231	0.07937
Proportional cover at 40m	5495	21763	0.0147	0.09375
Proportional cover at 50m	4548	33519	0.008542	0.1109
Proportional cover at 60m	3832	45057	0.00678	0.1181
Proportional cover at 70m	3266	56048	0.003681	0.1372
Proportional cover at 80m	3748	55014	0.00813	0.1124

Supp. Table 1. This table compares the significance of the linear positive relationship between the *D. barbeyi* focal plant area (calculated using height * width) and the proportional cover of neighboring *D. barbeyi* at five different scales. Slope represents change in area (cm²) of focal plant per unit of proportional cover. All five scales show significant relationships.

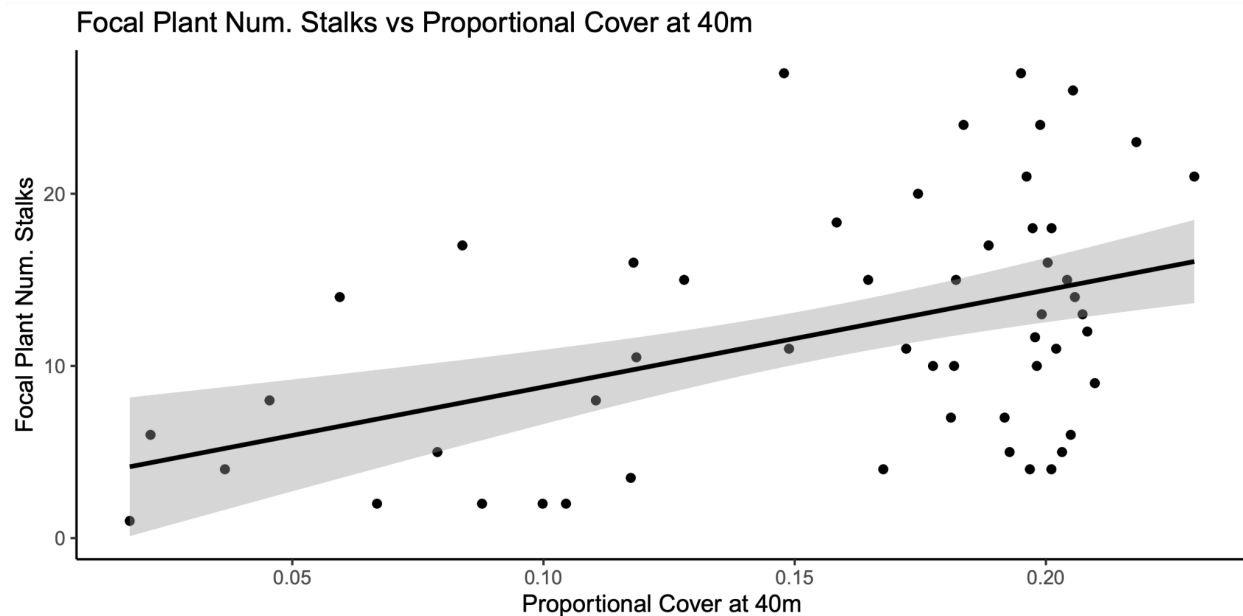


Supp. Figure 1. This linear regression model shows the significant linear relationship between focal plant area and proportional cover at 70 meter, with a 90% credible interval. The proportional cover scale of 70 meters was chosen for this graph as it has the smallest p-value compared to the other proportional cover scales (p-value = 0.003681, Adj R^2 = 0.1372, Intercept = 3266 cm^2 , Est. Slope = 56048 cm^2).

Focal Plant Num. Stalks versus Proportional Cover at Five Distinct Scales

FP # stalks vs.	Intercept	Mean Slope	P-value	Adjusted R Squared
Proportional cover at 20m	7.389	18.580	0.03020	0.07095
Proportional cover at 30m	5.028	34.800	0.00358	0.1381
Proportional cover at 40m	3.154	56.249	0.000851	0.182
Proportional cover at 50m	1.493	80.628	0.000868	0.1814
Proportional cover at 60m	0.3676	103.1220	0.00678	0.1181
Proportional cover at 70m	0.565	113.528	0.00231	0.1517
Proportional cover at 80m	2.718	98.954	0.0145	0.09409

Supp. Table 2. This table compares the significance of the linear positive relationship between the *D. barbeyi* focal plant number of stalks and the proportional cover of neighboring *D. barbeyi* at five different scales. Slope represents change in number of stalks per unit of proportional cover. All five scales show significant relationships.



Supp. Figure 2. This linear regression model shows the significant linear relationship between focal plant number of stalks and proportional cover at 40 meter, with a 90% credible interval. The proportional cover scale of 40 meters was chosen for this graph as it has the smallest p-value compared to the other proportional cover scales. The p-value of this relationship is 0.000851, with an adjusted R^2 of 0.182, an intercept of 3.154, and a slope of 56.249 stalks per proportional cover unit.

Pollen Count vs Proportional Cover and Focal Plant Area

Pollen Count vs FP Area vs.	Intercept	Estimated Slope	Slope (1% credible interval)	Slope (99% credible interval)
Proportional cover at 20m	2.0678	3.9592	1.7221	6.2947
Proportional cover at 30m	1.8597	7.3330	4.5596	10.0007
Proportional cover at 40m	1.7021	10.5981	6.6846	14.8458
Proportional	1.4141	13.3724	6.6128	20.2904

cover at 50m				
Proportional cover at 60m	1.4198	15.8846	7.1847	24.8317
Proportional cover at 70m	1.8503	14.1087	3.4162	24.2285
Proportional cover at 80m*	2.1662	11.3437	-0.3682	23.0818

* Slope at 2.5% credible interval is 1.2957.

Supp. Table 3. This table shows the results of a multiple linear regression run between pollen count and proportional cover at five different scales, holding focal plant area constant. Slope represents change in pollen count by unit of proportional cover holding focal plant area constant.

Pollen Count vs Proportional Cover and Focal Plant # Stalks

Pollen Count vs Focal Plant # Stalks and	Intercept	Estimated Slope	Slope (1% credible interval)	Slope (99% credible interval)
Proportional cover at 20m	2.1023	3.6721	1.3552	5.9309
Proportional cover at 30m	1.8496	7.2482	4.1759	2.4755
Proportional cover at 40m	1.6949	10.5078	6.4619	14.6755
Proportional cover at 50m	1.4116	12.5978	5.3548	19.7380
Proportional cover at 60m	1.4002	5.9263	14.8753	24.0322
Proportional cover at 70m	1.8841	12.4062	1.4018	23.4946
Proportional cover at 80m*	2.1398	10.1692	-1.1253	20.8799

*Slope at 2.5% credible interval is 0.8647

Supp. Table 4. This table shows the results of a multiple linear regression run between pollen count and proportional cover at five different scales, holding focal plant number of stalks constant. Slope represents change in pollen count by unit of proportional cover holding focal plant area constant.