Anne Schiffer April 7, 2020

Connecting relative differences in floral phenology to reproductive success

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Environmental Sciences

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Abstract

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Flowering phenology is critical for plant fitness due to both abiotic and biotic drivers of reproductive success. On the abiotic side, an individual's flowering time can influence the potential for frost damage and the soil moisture available for seed development. In terms of the biotic drivers, relative differences in blooming time may impact pollinator visitation rates and availability of pollen donors. However, it is still unclear how individual flowering time and drivers of reproduction impact plant fitness for phenologically distinct species. In this study, I analyzed the impact of relative differences in flowering time on the fecundity of individuals within a population. I collected floral phenology and seed set data for individuals of three subalpine plant species (*Mertensia fusiformis, Delphinium nuttallianum, Potentilla pulcherrima*) in the Colorado Rocky Mountains. To address the abiotic and biotic drivers of reproduction, I calculated changes in soil moisture, included the density of conspecific individuals, and conducted a pollen limitation experiment to isolate pollination function. Additionally, I accelerated snowmelt to simulate the variability of blooming time caused by climate change. My statistical models assessed the effects of relative blooming time, soil moisture, conspecific flower density, and pollination treatment on individual fecundity for the three species. I found that off-peak blooming is beneficial for reproductive success in some species, but the direction of flowering compared to the population peak is species-specific. Off-peak blooming in our earliest-blooming species, *Mertensia*, had marginally significant negative effects on seed production. The flowering time of early season species is constrained by abiotic and biotic factors, which could result in increased seed production for individuals that bloom with the population peak. For individuals of *Delphinium*, our mid-season species, it was reproductively advantageous to flower later than the population peak. However, individuals of *Potentilla*, our late-blooming species, increased seed production when they flowered earlier than the population peak. Blooming earlier or later than the population peak may have enabled individuals to avoid competition while still receiving pollination service. Over time, selection can act on those relative differences in floral phenology and reproductive success, possibly resulting in changes in population size and/or altered community composition.

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Acknowledgements

I’d like to thank Dr. Brosi for being a supportive advisor and a guide through this entire process. I’d also like to thank Loy Xingwen (graduate student, PBEE program) for teaching me everything there is to know about these plants. He also taught me how to hand pollinate and assisted me with statistics of this project. In addition, I’d like to thank the Environmental Sciences Department and Emory College of Arts and Sciences for funding this project through the Lester Grant and an Undergraduate Research Program grant, respectively. I’d like to acknowledge the team of students and scientists both in the field and in the lab that contributed to this project. Finally, I’d also like to acknowledge everyone at the Rocky Mountain Biological Laboratory for helping with the logistics of this study and giving me the opportunity to conduct research in the Rocky Mountains.

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Introduction

The seasonal timing of life history events, or phenology, is important for successful completion of many stages of organismal life cycles (Poulin et al. 1992, Fitter and Fitter 2002, Parmesan 2007). Phenology is particularly important in sessile organisms including plants, because their lack of spatial mobility puts an additional emphasis on proper timing (Cleland et al. 2007, Forrest and Miller-Rushing 2010, Ibáñez et al. 2010). In plants, one key fitness component that is particularly dependent on timing is flowering, the ultimate success of which is affected by both abiotic and biotic drivers (Crone and Lesica 2006, Forrest and Miller-Rushing 2010). In terms of abiotic drivers, an individual plant's flowering time can influence factors such as the risk of frost damage on its flowers or the soil moisture available for floral or seed development (Franks et al. 2007, Thomson 2010, Sloat et al. 2015). In terms of biotic drivers, relative differences in flowering time influence interactions with conspecific pollen donors and heterospecific individuals (Crone et al. 2009, Kehrberger and Holzschuh 2019). Relative flowering time also impacts pollinator visitation rates and the potential for phenological mismatches between plants and their pollinators (Kudo and Ida 2013, Kudo 2014, Rafferty et al. 2015). Understanding the role of flowering phenology on plant fitness is particularly timely in light of rapid climate change, which has been linked to shifts in flowering phenology in recent decades (Dunne et al. 2003, Inouye et al. 2003, Iler et al. 2013, Caradonna et al. 2014). These phenological shifts could be detrimental to the fecundity of wild plant populations (Kudo and Cooper 2019, Pardee et al. 2019), again driven by both biotic and abiotic factors, but much remains unknown about how relative differences in flowering time affect the relative fecundity of individuals within a population.

Abiotic factors affect plant fecundity in a number of ways. Plants require an optimal range for air temperature and water and nutrient availability in order to reproduce successfully (Walker et al. 1995, Vaz et al. 2004, Allen et al. 2010). In most environments, plants increase fecundity by timing life history events to occur within that optimal range (B Rathcke and Lacey 1985, Franks et al. 2007, Thomson 2010). For example, tropical plants avoid flowering during the dry season when water availability is low (Schaik et al. 1993, Engelbrecht and Kursar 2003), and plants in higher altitude or latitude environments flower when they are less likely to freeze (Inouye 2000, Bennie et al. 2010). Consequently, individuals that flower in optimal abiotic conditions are more fecund than other individuals in the population that flower at a different time (Zimmerman and Gross 1984, Mu et al. 2014).

Of the biotic factors, interactions with other plants and pollinators impact plant fitness. With regard to plant interactions, conspecific and heterospecific plants influence an individual's reproductive success differently. For example, blooming with individuals of the same species facilitates out-crossing and increases fecundity (Crone et al. 2009, Mu et al. 2014). Blooming with abundant heterospecifics, on the other hand, can interfere with pollen transfer and increase competition for pollinators (Waser 1978, Kehrberger and Holzschuh 2019). In terms of the interactions with pollinators, high pollinator visitation rates are typically beneficial to an individual's reproductive success (Gezon et al. 2016, Kehrberger and Holzschuh 2019). The cues for plant flowering time and pollinator emergence have historically been tightly coupled to ensure the reproductive success of both plants and pollinators (Kudo 2014, Forrest 2015). However, those cues are becoming decoupled due to climate change (Memmott et al. 2007, Forrest and Thomson 2011, Kudo and Ida 2013, Kudo and Cooper 2019). This mismatch between flowering time and pollinator emergence can impact plant fecundity differently between species (Pardee et al. 2019) and over time (Thomson 2019). The effect of relative flowering time on individual plant fitness is important to understand in the context of these phenological shifts.

Within the body of work on floral phenology and plant reproduction, several studies have documented the effects of individual flowering time on reproduction (Gallagher and Campbell , Zimmerman and Gross 1984, Forrest and Thomson 2010, Thomson 2010, Rafferty and Ives 2012, Mu et al. 2014, Gezon et al. 2016, Rafferty et al. 2016, Kehrberger and Holzschuh 2019), but questions remain unanswered about the abiotic and biotic factors that influence individual reproduction for phenologically different species. Compared to studies on population or species differences (Waser 1978, Pardee et al. 2019), studies on the individual level assess an individual's flowering time and reproduction relative to other individuals in the population, which is critical in understanding how selection acts on differences in phenology. Of the individual level studies, some have experimentally induced changes to floral phenology (Gallagher and Campbell , Rafferty and Ives 2012, Gezon et al. 2016), while others have conducted comparative studies (Zimmerman and Gross 1984, Forrest and Thomson 2010, Thomson 2010, Mu et al. 2014, Rafferty et al. 2016, Kehrberger and Holzschuh 2019). The comparative studies examine natural variation in individual flowering time and abiotic and biotic drivers, while the studies that induce phenological change assess the effects of increased variability of flowering time on individual reproduction. This increased variability is important to consider in the context of climate change-induced phenological shifts. To our knowledge, no studies have induced phenological change and assessed how differences in individual phenology affect plant reproduction in multiple co-occurring but phenologically distinct species, while accounting for multiple potential biotic and abiotic drivers of plant fitness.

Here, we connect relative floral phenology to plant fecundity for individuals in populations of three subalpine species in the Colorado Rocky Mountains. We recorded blooming time for each individual and isolated pollination function from resource availability using a pollen limitation experiment (Kearns and Inouye 1993). We experimentally accelerated snowmelt in each site to create greater variability in blooming time when compared with unmanipulated controls. Additionally, we counted the number of flowers for each of the species in our plots every week to incorporate the impact of conspecific density.

To understand the selective pressures on blooming time, we assessed how seed set is affected by *(a)* relative differences in blooming time, *(b)* abiotic drivers such as soil moisture, and *(c)* biotic drivers such as density of conspecific individuals and pollen limitation. We hypothesized that seed set will vary with differences in flowering time because when flowers bloom at different times, they are exposed to different abiotic and biotic factors that influence reproduction. Because early-season species are dependent upon abiotic cues for flowering time, we also predicted that abiotic drivers will have a greater effect on our early-season species. Finally, we predicted that biotic drivers will have a greater effect on our mid- and late-season species because pollinator abundance, conspecific flower density, and heterospecific flower density increase as the season progresses.

Methods

1. Study Site

We conducted this study in and around the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA (38°57.5′ N, 106°59.3′ W) in the 2019 field season. We established eight study sites across two adjacent valleys dominated by subalpine meadows and sagebrush communities. This subalpine region contains a diverse set of flowering plants and pollinators. The growing season in this system extends from late May to September, and pollinators tend to increase in diversity and abundance over the course of much of the growing season, until beginning to taper off in mid-August. Each study site contained paired 10 m × 14 m study plots: one for accelerated snowmelt and one control where snow was allowed to melt naturally (8 pairs, 16 plots total). Study plot pairs had similar aspect and plant community composition. Sites were geographically separated by a minimum of 1 km.

Within each study plot, we established three 1 m × 10 m transects for tracking flowering phenology of the plant community. Each week we recorded the total number of flowers in bloom for every plant species in the community. Along the perimeter of each plot, we designated a one-meter section for tagging individuals of our focal plant species. We visited every site twice per week to track phenological events of tagged individuals.

2. Selection of Focal Species

I selected three perennial subalpine forbs that grow in and around the Rocky Mountain Biological Laboratory: *Mertensia fusiformis* (Boraginaceae), *Delphinium nuttallianum*

(Ranunculaceae), and *Potentilla pulcherrima* (Rosaceae) (hereafter referred to by genus). *Mertensia*, *Delphinium*, and *Potentilla* can be considered early-, mid-, and late-season species respectively. *Mertensia* is one of the first species to flower in this system, blooming about 2 weeks after snowmelt. *Delphinium* blooms about 3-4 weeks after snowmelt, and *Potentilla* blooms about 5-6 weeks after snowmelt. We maintain the order of discussing the species from early- to late-season throughout the paper. We selected focal species based on distribution and abundance, including only self-incompatible species. We selected self-incompatible species because these species rely on pollinators and conspecific plants to set seed. Of the native, pollinator-dependent species, *Mertensia, Delphinium,* and *Potentilla* occurred most frequently across our eight sites. Additionally, we worked with an early-, a mid-, and a late-season species to highlight potential differential impacts of relative differences in blooming time across the growing season.

3. *Flowering Phenology Manipulation*

To advance snowmelt in manipulated plots, we placed a 50% plastic shade cloth over the snowmelt plots 5-6 weeks before the predicted natural snowmelt date. Shade cloths were removed when the snow in plots was completely melted to the ground. Throughout the study period, we measured snow depth and soil moisture once per week in both accelerated snowmelt and control plots. Accelerated snowmelt plots were advanced eight days on average compared to the natural snowmelt plots.

4. *Soil Moisture Measurements*

To include soil moisture as a covariate, we recorded the percent volumetric water content using a FieldScout TDR 150 Soil Moisture Meter with 8-inch probes, manufactured by Spectrum Technologies Inc., in every site each week. We recorded soil moisture in seven locations around the perimeter of the plot: in each of the four corners and in the middle of each of the three lengths of the horseshoe-shaped perimeter.

5. Pollen Limitation

We supplemented pollen for the three focal species in order to examine plant fecundity in the context of pollen availability rather than resource availability. Within each of the sites, we tagged 16 individuals in the control and accelerated snowmelt plots; eight were tagged to receive supplemented pollen and eight were tagged to remain open to pollination. We were able to conduct pollen limitation experiments for *Mertensia* in four sites, *Delphinium* in four sites, and *Potentilla* in seven sites. We visited every site twice a week to hand-pollinate all open flowers on pollen-supplemented individuals. When hand-pollinating, we collected pollen from individuals within the plot, but not from other tagged individuals. Individuals assigned the open treatment were not manipulated in any way. The relative difference in seed set between the open and hand-pollinated treatments indicates the degree of pollen limitation at the site.

6. Plant Reproduction

We collected all seeds from the tagged individuals after ovule expansion. We counted seeds in the lab after the field season ended. I used two different metrics to quantify plant fecundity: 1) the total number of developed seeds and 2) the proportion of developed seeds (vs. undeveloped ovules). The total number of developed seeds is a measure of whole-plant fecundity, which is important when assessing reproduction of individuals. However, this measure is sensitive to sampling and is affected by the number of flowers on the plants selected. The proportion of developed seeds, on the other hand, is representative of pollination function. The proportions may be less sensitive to sampling but are less representative of whole-plant fecundity. I classified seeds as developed or undeveloped based on species-specific measurements of seed length, width, and color. I was unable to calculate the proportion of developed seeds for *Potentilla* because undeveloped seeds were difficult to distinguish from other elements of the carpel.

7. Data Analyses

We worked with multiple plant individuals in each site, which cannot be considered independent samples given environmental homogeneity within sites and heterogeneity among sites, and additionally because of likely genetic similarity of individuals within a site. To address this, I used a generalized linear mixed effects model with site as a random effect and relative bloom timing in the population, conspecific density, and pollen limitation treatment as fixed effects.

a. Relative Differences in Flowering Phenology

To compare the timing and reproduction of individuals relative to other individuals in the population, I first calculated the population peak of flowering using data collected in the transects of the control plots at each site. For each of the three species, the date with the maximum number of open flowers recorded in the transects was considered the population peak in that site. I then converted the peak date to Julian Day, as well as all other date-based variables in our analysis.

For every tagged individual of the three species, the recorded first flowering and last flowering dates were assigned half-week date estimates because we visited every site twice per week. I calculated the date estimates for the first flowering by using the midpoint between the first record of a flower blooming and the most recent record of flower absence (Taylor 2019). To estimate the peak flowering date of the individual, I used the midpoint between the first flowering estimate and last flowering estimate. Using the population peak date and the individual peak estimates, I calculated the relative position of an individual's estimated peak to the population peak at each site. This relative position was represented by a number of days before or after the population peak date.

In all three species, fecundity relative to bloom timing was non-monotonic and thus incompatible with standard statistical modeling frameworks. To address this issue, I used the number of days deviated from the population peak and added a categorical variable to distinguish measurements that were before vs. after the population peak. I assessed the statistical interaction between deviation and before/after population peak to allow for different responses (slopes) between early vs. late blooming plants.

b. Conspecific Density

To understand the effect of blooming with conspecifics on plant fecundity, I assigned the number of conspecifics in bloom to every individual of every species. Using the flower counts from the transects, I averaged the total number of flowers in bloom in the transects in each plot of every site. For every week of the field season, I had an average conspecific density for each species, site, and plot (natural snowmelt or accelerated snowmelt). The conspecific densities were representative of plot densities during the individual's blooming time (i.e. individuals in the accelerated snowmelt plot were associated with an average conspecific density of the accelerated snowmelt plot).

c. *Soil Moisture*

To assess soil moisture as a covariate and abiotic limitation to seed set, I calculated soil moisture variables for each site using our weekly moisture measurements. We missed two weeks of soil moisture measurements in our earliest melting site. To compensate for this, I used the soil moisture measurement that was recorded two weeks after a site's melt-out date as the starting moisture value in the soil moisture variables. The soil moisture variables included the mean soil moisture over the course of the season, the rate of change of moisture over the season, the effective moisture minimum, the moisture at the end of the season, and the moisture range between the beginning of the season and the effective moisture minimum. The effective moisture minimum was the soil moisture measurement in the seventh week of my study. In this week, all sites reached their minimum soil moisture values. Relative to other measures of soil moisture, these soil moisture variables were the least intercorrelated.

I ran a generalized linear mixed effects model for the soil moisture variables. The total developed seed counts of all three species were combined for the response variable. I listed the soil moisture variables as fixed effects and site, plot treatment, and species as random effects. I then used model selection on this full model to select the best combinations of these soil moisture variables, which I intended to include in my final mixed effects models.

d. Mixed Effects Models

I ran models for the three species separately, and also separately for the two fecundity variables (total number of developed seeds and the proportion of developed seeds). The total number of developed seeds fit a negative binomial distribution, and the proportion of developed seeds fit a binomial distribution. I included site as a random effect (random intercept) in all models. I included three classes of fixed effects: for relative differences in flowering phenology, as mentioned previously, I included an interaction between deviation from the population peak and the early/late classification. For conspecific density, I used an interaction between the number of conspecific individuals and the plot treatment (natural snowmelt or accelerated snowmelt), which allowed for potentially differential slopes in accelerated snowmelt vs. control plots. Finally, I included pollen limitation treatment (open vs. hand-pollinated). I did not include soil moisture in my final models because the null model was selected when I ran the model selection for soil moisture variables.

Results

I present my results organized by the three classes of fixed effects that I considered: 1) relative differences in flowering phenology; 2) conspecific density; and 3) pollen limitation (i.e. hand-pollinated vs. open treatments). Within each of these classes, I discuss results from the three focal species, organized from early to late season species (*Mertensia*, *Delphinium*, *Potentilla*). Finally, within each species I first report results on total seed production, and then results on proportions of developed seed (though again, I was unable to calculate proportions for

Potentilla). Throughout, I use "marginally significant" to indicate *p*-values between 0.05 and 0.10.

1. Relative Differences in Flowering Phenology

a. *Mertensia*

For the flowering time of *Mertensia*, my early-season species, I did not detect an effect of an individual's relative position in population phenology on the total number of developed seeds. I did not distinguish a main effect of deviation from the population peak ($p = 0.560$), a main effect of early/late distinction ($p = 0.300$), or the interaction between deviation and early/late distinction ($p = 0.450$) on developed seed counts. However, deviation from the population peak had a marginally significant negative effect on the proportion of developed seeds ($p = 0.076$; Figure 2a). I did not find an effect of the early/late distinction ($p = 0.179$) or the interaction between the deviation and early/late distinction ($p = 0.909$) on the proportion of developed seeds.

b. Delphinium

I did not detect an effect of relative differences in flowering time on developed seed counts in my mid-season species, *Delphinium*. I did not detect a main effect of deviation from the peak ($p = 0.270$), a main effect of early/late distinction ($p = 0.750$), or an interaction between the two variables ($p = 0.530$). For the proportion of developed seeds, while I did not detect a main effect of deviation from the population peak ($p = 0.131$), individuals that flowered late compared to the population peak produced higher proportions of developed seeds (main effect, $p = 0.015$; Figure 2c), and I also found a significant interaction between deviation from peak bloom and early/late distinction ($p < 0.001$; Figure 2c); specifically, the later an individual bloomed, the higher its seed set, with an increased positive effect (greater slope) of deviation on late-blooming individuals.

c. *Potentilla*

In my late-season species, *Potentilla*, I found a marginally positive main effect of deviation from the population peak on the total number of developed seeds (p = 0.079; Figure 1e). I did not detect a main effect of the early/late variable on seed set (p = 0.602), but I found a marginally significant interaction between deviation and the early/late distinction (p = 0.080; Figure 1e). The earliest blooming *Potentilla* individuals produced a higher number of total developed seeds, with a negative effect of late blooming (negative slope) on seed set and a positive effect of early blooming (positive slope) on seed set.

2. *Conspecific Density*

a. *Mertensia*

I did not distinguish a main effect of plot treatment (p = 0.950), conspecific density (p = 0.190), or the interaction between plot treatment and conspecific density on *Mertensia* individual developed seed counts (p = 0.850). However, for the proportions of developed seeds, while I did not detect a main effect of plot treatment (p = 0.127), I found a marginally significant negative main effect of conspecific density. Specifically, individuals blooming with many conspecifics tended to have lower proportions of developed seeds (p = 0.057; Figure 2b). I also found a marginally significant interaction between plot treatment and conspecific density, with a reduced negative effect (lower slope) of conspecific density for those individuals in the control plot (p = 0.074; Figure 2b).

b. *Delphinium*

For *Delphinium* individuals, I did not see a main effect of plot treatment ($p = 0.870$), conspecific density ($p = 0.660$), or the interaction between the variables on the total number of developed seeds ($p = 0.480$). The proportions of developed seeds, on the other hand, were significantly higher in the accelerated snowmelt plot (main effect; $p = 0.022$; Figure 2d) and were positively affected by conspecific density (main effect; $p = 0.029$; Figure 2d). I also found a significant interaction between conspecific density and snowmelt acceleration, with a slight positive effect of conspecific density (positive slope) for those individuals in the control plot and a negative effect of conspecific density (negative slope) for individuals in the accelerated snowmelt plot ($p < 0.001$; Figure 2d).

c. *Potentilla*

Conspecific density had significant effects on the seed set of *Potentilla* individuals. The total number of developed seeds was significantly higher in the accelerated snowmelt plot (main effect; $p = 0.016$; Figure 1f), and for individuals that bloomed with many conspecifics, the total number of developed seeds increased (main effect; $p = 0.019$; Figure 1f). I found an interaction between plot treatment and conspecific density, with a positive effect of conspecifics (positive slope) on individual seed production in the control plot and a slight negative effect (negative slope) of conspecifics on individuals the accelerated snowmelt plot ($p = 0.049$; Figure 1f).

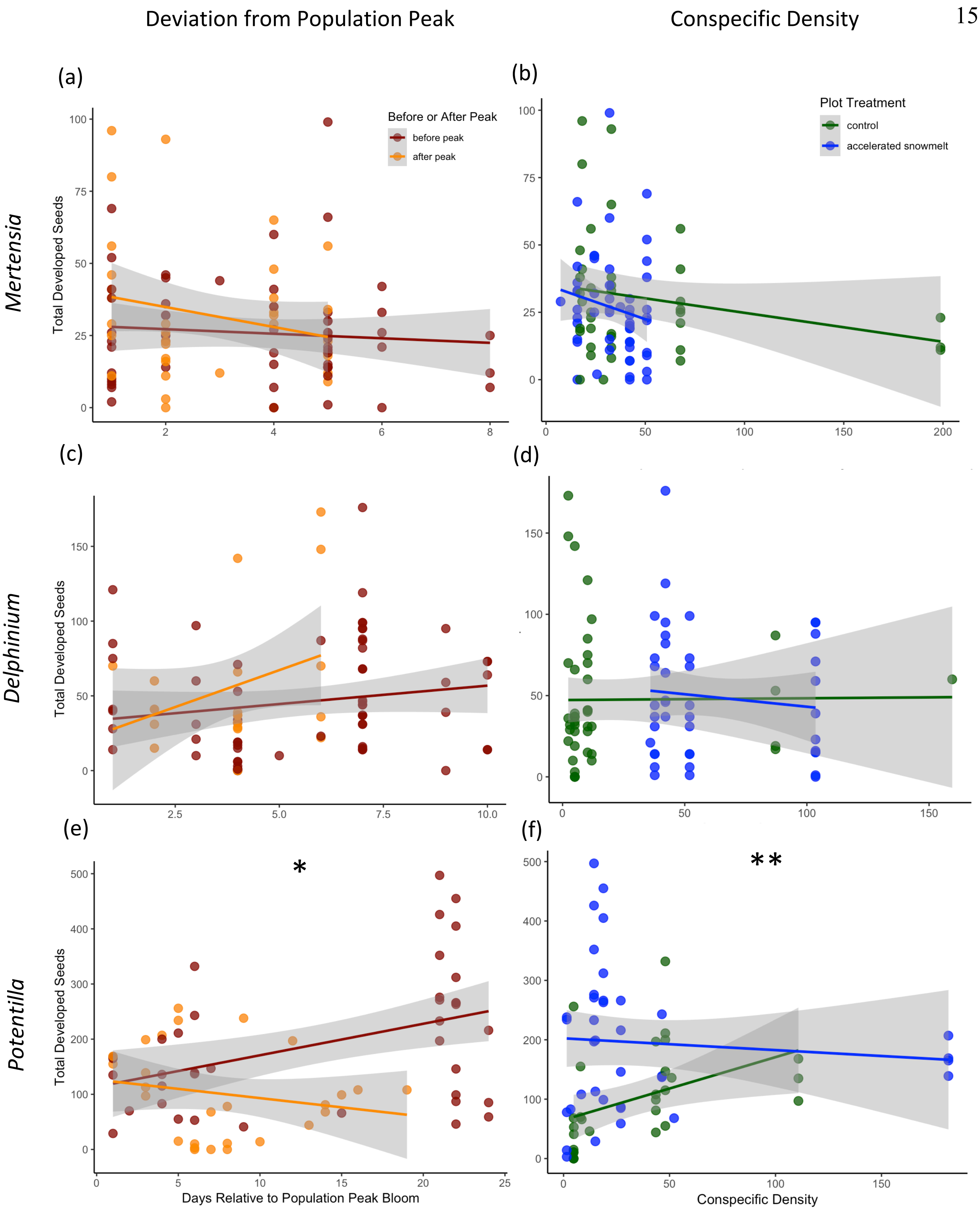

Figure 1. The effects of relative flowering time and conspecific density on total number of developed seeds for *Mertensia fusiformis* (a,b), *Delphinium nuttallianum* (c,d), and *Potentilla*

pulcherrima (e,f) individuals. Each point represents an individual plant, and the lines are simple linear regression lines. In the relative flowering time plots, red represents flowers that bloomed before the population peak and orange represents flowers that bloomed after. In the conspecific density plots, green represents the control plot and blue represents the accelerated snowmelt plot. Note the differences in scales on the *x*- and *y*-axes. Standard error is included in the shaded regions. I did not detect an effect of relative differences in flowering time or conspecific density on *Mertensia* or *Delphinium* developed seed counts. For *Potentilla* individuals, blooming earlier than the population peak had a marginally significant positive effect on seed production, and conspecific density had a significant positive effect on seed production. Asterisks represent significance at ** $p < 0.05$ and * $p < 0.1$.

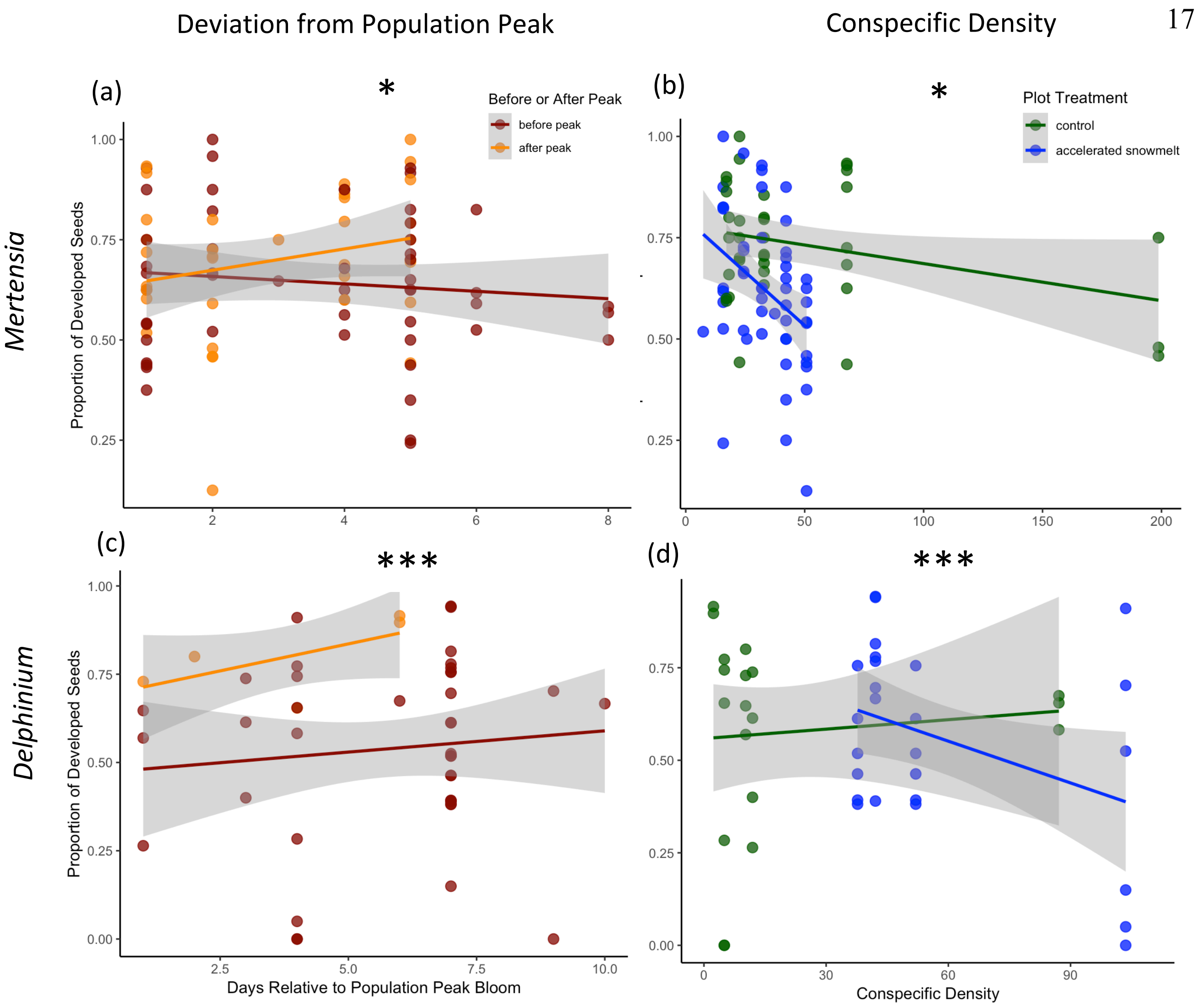

Figure 2. The effects of relative flowering time and conspecific density on the proportions of developed seeds for *Mertensia fusiformis* (a,b) and *Delphinium nuttallianum* (c,d) individuals. *Potentilla pulcherrima* was not included in this analysis because I did not have undeveloped seed counts for the proportion calculations. Each point represents an individual plant, and the lines are simple linear regression lines. In the relative flowering time plots, red represents flowers that bloomed before the population peak and orange represents flowers that bloomed after. In the conspecific density plots, green represents the control plot and blue represents the accelerated snowmelt plot. Note the differences in scales on the x- and y-axes. Standard error is included in the shaded regions. Deviation from the population peak had a slight negative effect on seed set

for *Mertensia* individuals. For *Delphinium*, the later an individual bloomed relative to the population peak, the higher its proportions of developed seeds. For both *Mertensia* and *Delphinium*, conspecific density had an increased negative effect on the proportions of developed seeds of individuals in the accelerated snowmelt plot. Asterisks represent significance at *** $p < 0.001$ and * $p < 0.1$.

3. *Pollen Limitation*

a. *Mertensia*

In my early-season species, I did not detect an effect of pollen supplementation on individuals. Both developed seed counts ($p = 0.590$) and proportions of developed seeds ($p = 0.395$) were unaffected in individuals that received the hand pollination treatment.

b. *Delphinium*

For my mid-season species, *Delphinium*, I did not detect an effect of pollen supplementation on the total number of developed seeds ($p = 0.520$), but individuals that received the pollen supplementation treatment produced marginally higher proportions of developed seeds ($p = 0.059$).

c. *Potentilla*

I did not detect an effect of pollen supplementation on the total number of developed seeds in individuals of my late-season species ($p = 0.196$).

4. *Soil Moisture*

I did not detect an effect of soil moisture on the three species. In my model selection, the null model was the best compared to any combination of the following soil moisture variables: mean, rate, effective minimum, end measurement, and range.

Effect	***Mertensia fusiformis***		***Delphinium nuttallianum***		***Potentilla pulcherrima***
Flowering Time	*Total*	*Proportion*	*Total*	*Proportion*	*Total*
Deviation	0.560	0.076 *	0.270	0.131	0.079 *
Early/Late	0.300	0.179	0.750	0.015 **	0.602
Deviation × early/late	0.450	0.909	0.530	<0.001 ***	0.080 *
Conspecific Density					
Number of conspecifics	0.190	0.057 *	0.660	0.029 **	0.019 **
Plot treatment	0.950	0.127	0.870	0.022 **	0.016 **
Conspecifics × plot treat	0.850	0.074 *	0.480	<0.001 ***	0.049 **
Pollen Limitation	0.590	0.395	0.520	0.059 *	0.196

Table 1. A summary of the effects of flowering time, conspecific density, and pollen limitation on the total developed seeds and proportion of developed seeds for *Mertensia fusiformis*, *Delphinium nuttallianum*, and *Potentilla pulcherrima*. Values are *p*-values from the mixed effects models. Seed production for each species is divided into total number of developed seeds and proportion of developed seeds. The effects of flowering time and conspecific density are divided into main effects and interactions. Asterisks represent significance at * <0.1, ** <0.05, and *** <0.001.

Discussion

My study investigated the effects of relative differences in flowering time, along with abiotic and biotic drivers of reproduction, on the seed production of three subalpine forbs. After manipulating and tracking phenological events of my three focal species, I found that relative flowering time influences plant reproduction for these subalpine forbs. Off-peak blooming is beneficial for reproductive success in some cases, but the direction of flowering compared to the population peak is species-specific. Off-peak blooming (i.e., either early or late) in my earliest-blooming species, *Mertensia*, had marginally significant negative effects on seed production. For individuals of *Delphinium*, my mid-season species, it was reproductively advantageous to flower later than the population peak. However, individuals of *Potentilla*, my late-blooming species, increased in fecundity when they flowered earlier than the population peak. My findings suggest that, depending on the species, selection may act on individuals that bloom earlier or later than the population peak, which could have subsequent consequences for plant community composition and plant-pollinator interactions.

Contrary to my hypothesis, I did not detect any strong effect of flowering time on seed set in our early-season species, *Mertensia fusiformis*. While I found a marginally significant trend that off-peak blooming negatively affects seed set in *Mertensia*, a number of abiotic and biotic constraints may cause selection to constrict the flowering duration of early-season species. Early-season species are more sensitive to the abiotic conditions than later season species (Dunne et al. 2003, Caradonna et al. 2014, Pardee et al. 2019). In many cases, abiotic conditions must be within a narrow range for flowers of early-season species to set seed (Walker et al. 1995, Crone and Lesica 2006, Gezon et al. 2016). In addition, these species must receive adequate pollination in the early season to increase seed set (Gezon et al. 2016, Pardee et al. 2019). For those early-season species, individuals face a trade-off between frost damage when blooming early and low pollinator visitation due to competition with other flowers when blooming late (Thomson 2010, Gezon et al. 2016). While I did not find strong relationships between fecundity and soil moisture, relative blooming time, conspecific density, or pollen limitation, these factors likely still affect early-season species like *Mertensia*. Despite my large true sample size, my data contained high variation, and more samples are needed to distinguish the drivers of *Mertensia* reproduction.

Individuals of my mid-season blooming species, *Delphinium nuttallianum*, showed an increase in fecundity when they bloomed later than the population peak. In the middle of the growing season in subalpine communities, species are less likely to experience frost damage or limited abiotic resources (Dunne et al. 2003, Pardee et al. 2019). However, fecundity in mid-season species may be strongly influenced by pollinator visitation (Gallagher and Campbell , Pardee et al. 2019) and conspecific density (Brown and Mitchell 2001, Mu et al. 2014). My findings suggest that for mid-season species like *Delphinium*, blooming with conspecifics may reduce seed set through competition for pollinators, rather than increasing seed set through the availability of pollen donors. Despite a marginal effect of pollen limitation on seed production, seed set decreased when conspecific density increased. This could indicate that individuals were pollen-limited not due to a lack of pollen donors, but possibly by lower pollinator visitation rates. By blooming later than the population peak, individuals may have increased the proportion of developed seeds due to lower conspecific competition for pollinators. In addition, more pollinator species emerge later in the season in subalpine regions, including bumblebee species, an important taxon for pollination of many mid-season plant species (Gallagher and Campbell , Thomson 2010) and particularly for *Delphinium* (Schulke and Waser 2001). The combination of off-peak blooming and higher pollinator abundance later in the season could have positively impacted the seed set for my mid-season species. My data are consistent previous studies that found increased seed set in later blooming individuals of other mid-season species in seasonal environments (Zimmerman and Gross 1984, Mu et al. 2014), but are inconsistent with a recent study that suggests *Delphinium* produces more seeds when the species blooms earlier (Pardee et al. 2019). A critical nuance in my data is that I detected an effect of blooming time on the proportion of developed seeds but not the total number of developed seeds. Although these two metrics measure slightly different aspects of plant fecundity, my findings still indicate that plant fecundity was affected by blooming time in *Delphinium* individuals. Overall, as communities are reshaped by climate change, competition for pollinators may be a growing concern for the fitness of individuals of mid-season species in subalpine communities.

In contrast to mid-season *Delphinium*, individuals of my late-season species, *Potentilla pulcherrima*, had increased fecundity when they bloomed earlier than the population peak. Late-season species are less constrained by the abiotic environment than earlier species (Caradonna et al. 2014, Pardee et al. 2019), and pollinators are typically abundant later in the growing season in subalpine regions (Gallagher and Campbell , Kudo 2014). However, late-season species can still experience competition for pollinators with conspecific and heterospecific plants (Brown and Mitchell 2001, Kehrberger and Holzschuh 2019). My results indicate that individuals that bloom earlier than the population peak may avoid competition for pollinators. As with my mid-season species, I found a negative effect of blooming with conspecifics on seed set, but unlike my mid-season species, early-blooming individuals produced more developed seeds. One potential mechanism for this result is that if pollinators are abundant later in the season, blooming earlier than the population peak lengthens the duration of flowering time, which could allow for more pollinator visits (Pardee et al. 2019). Additionally, my findings support other snowmelt manipulation studies, in which individuals of late-season species that bloomed in an experimentally warmed or early snowmelt plot produced a higher seed set (Price and Waser 1998, Pardee et al. 2019). Thus, individuals of late-season species in subalpine communities could benefit from climate-induced changes in snowmelt and growing season length.

Although I incorporated many components into my study design, my study was limited in several ways. First, I could have improved my study by counting the undeveloped seeds and calculating a proportion of developed seeds for *Potentilla* individuals. To count the number of undeveloped seeds, a microscope would likely be necessary to distinguish the unfertilized ovules from other components of the carpel. This would have provided a better understanding of how relative blooming time affects a *Potentilla* individual's seed production. Second, while I include species that are classified as early-, mid-, and late-season species, different species could produce different results than the species that I selected, and further studies with more focal species are needed. Third, my snowmelt acceleration experiment is limited by creating "islands" of early bloomers. These islands of early flowers are the only flowers in bloom in the area and may differentially attract pollinators, which could inflate the pollination services they receive (Forrest 2015). Finally, the observed effect of flowering time on fitness differs based on the duration of the study (Thomson 2019), and a longer study is needed to understand the long-term effects of blooming time on fitness.

Several future research directions could stem from this study. For example, a study that incorporates pollinator abundance or visitation rates would better quantify the degree of pollen limitation. Additionally, connecting phenology to fitness on the landscape level is necessary to understand the broader changes in a study system. A study could increase the number of sites and assess the impact of the abiotic conditions and elevation on phenology and fitness across sites. Also, my data contained a lot of variation, and future studies should work with a more substantial sample size. While it is challenging to increase the sample size when including several species, pollen limitation experiments, and snowmelt manipulation, a larger sample size would more clearly reflect the effects of flowering time on fitness.

The positive effect of off-peak blooming on plant fecundity could have implications for plant community composition, plant-pollinator interactions, and population persistence in a changing climate. As individuals in populations begin to shift earlier or later in the season, new combinations of co-flowering species may emerge in temperate systems. Shifts in plant community composition will consequently affect pollinator visitation to flowers in bloom, and chances of pollen limitation may change for certain overlapping species. Changes to pollinator visitation and conspecific density, along with climate-induced changes to the abiotic conditions, could affect the persistence of a plant population. This is especially concerning for subalpine populations that are not resilient to changes in abiotic or biotic conditions. An individual's blooming time may affect not only individual fecundity, but also the fitness and persistence of the population.

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