Research Article

Differences in individual flowering time change pollen limitation and seed set in three montane wildflowers

Annie E. Schiffer[1,2,3,7], Xingwen Loy[2,3,4], Connor Morozumi[2,3,5], Berry J. Brosi[2,3,6]

[1]*Department of Wildland Resources, Utah State University, 5230 Old Main Hill, Logan, UT, 84322, USA*

[2]*Rocky Mountain Biological Laboratory, 8000 County Rd. 317, Box 519, Crested Butte, CO 81224, USA*

[3]*Department of Environmental Sciences, Emory University, 400 Dowman Dr., Atlanta, GA 30322, USA*

[4]*Southeastern Center for Conservation, Atlanta Botanical Garden, 1345 Piedmont Ave NE, Atlanta, GA 30309, USA*

[5]*Department of Biology, University of Louisville, 139 Life Sciences Building, Louisville, KY, 40292, USA*

[6]*Department of Biology, University of Washington, W Stevens Way, Seattle, WA 98195-1800, USA*

[7] For correspondence, email annie.schiffer@usu.edu

Manuscript received _____; revision accepted _____.

Running title: Variation in individual bloom changes seed set in montane flowers

ABSTRACT

Premise of the study

Changes to flowering time caused by climate change could impact plant fecundity, but studies that compare the individual-level responses of phenologically distinct, co-occurring species are lacking. We assessed how variation in floral phenology affects the fecundity of individuals from three montane species with different seasonal flowering times, including in snowmelt acceleration treatments aimed at increasing variability in bloom time.

Methods

We collected floral phenology and seed set data for individuals of three montane plant species (*Mertensia fusiformis, Delphinium nuttallianum, Potentilla pulcherrima*) in the Colorado Rocky Mountains. To examine the drivers of seed set, we measured conspecific floral density and conducted pollen limitation experiments to isolate pollination function. We advanced the phenology of plant communities in a controlled large-scale snowmelt acceleration experiment.

Key Results

We found that differences in individual flowering time relative to the rest of the population impacted fecundity in our focal species, but that effects were species-specific. For our early-season species, individuals that bloomed late relative to the population peak bloom had increased fecundity, while for our mid-season species, simply blooming before or after the population peak bloom period increased individual fecundity. For our late-season species, blooming earlier than the population peak bloom increased individual fecundity. The early- and mid-season species were pollen-limited, and we found evidence that conspecific density impacted seed set only for our early-season species.

Conclusions

Our study shows that variation in individual plant phenology impacts fecundity in three phenologically distinct montane species, and that pollen limitation may be a more influential driver than conspecific density. Our results suggest that individual-level changes in phenology are important to consider for understanding plant reproductive success.

Key words: accelerated snowmelt; *Delphinium nuttallianum*; floral phenology; individual variation; *Mertensia fusiformis*; montane wildflowers; plant reproduction; *Potentilla pulcherrima*; pollen limitation

INTRODUCTION

The seasonal timing of life history events, or phenology, can influence success of many stages in an organism's life cycle (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). A key plant fitness component that is particularly dependent on timing is flowering, the ultimate success of which is affected by both abiotic and biotic conditions (Crone and Lesica, 2006; Forrest and Miller-Rushing, 2010, Hall *et al*., 2018). In terms of abiotic conditions, 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; Hall *et al*., 2018). In terms of biotic conditions, an individual's flowering time relative to its population can affect the availability of pollen donors (Crone *et al*., 2009; Hall *et al*., 2018). Flowering time also impacts pollinator visitation rates and synchrony with pollinator activity, which are likely important for pollinator-dependent plant species (Kudo and Ida, 2013; Kudo, 2014; Rafferty *et al.*, 2015; Robinson and Henry, 2018). 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 (Molau, 1996; Molau, 2005; 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 conditions, but much remains unknown about how individual differences in flowering time affect the fecundity of individuals within a population.

Individual plants often time their life history events to occur within an optimal range of abiotic conditions (Rathcke and Lacey, 1985; Franks *et al.*, 2007; Thomson, 2010). For example, species usually have ideal ranges for air temperature, soil moisture, and nutrient availability for reproduction (Walker et al., 1995; Vaz et al., 2004; Allen et al., 2010), with plants in higher altitude or latitude environments flowering when they are less likely to freeze (Inouye, 2000; Bennie et al., 2010). Consequently, large temporal deviations in flowering from the periods of optimal abiotic conditions can have fitness costs (Zimmerman and Gross, 1984; Mu *et al*., 2014). Similarly, individuals time their fruiting and seed release to maximize seedling establishment in their abiotic conditions (Pearson et al., 2002; Moles and Westoby, 2006). In addition to timing, seed size and weight often determine establishment, with larger seeds germinating and establishing more frequently than smaller seeds (Schaal, 1980; Silvertown, 1981).

Phenology also influences individual fitness through changes in biotic interactions, in particular, plant interactions with one another and their animal pollinators. Within plant interactions, conspecific and heterospecific plants influence an individual's reproductive success differently. For example, blooming gregariously with individuals of the same species increases the pool of potential pollen donors, which can facilitate outcrossing and increase fitness in self-incompatible species (Hall *et al*., 2008; Crone *et al*., 2009; Mu *et al*., 2014; Bogdziewicz *et al*., 2020). Although blooming with more conspecific individuals may lower the probability of being visited by pollinators in some cases (Kehrberger and Holzschuh, 2019), high concentrations of flowers can attract more pollinators that bring in higher quality pollen (i.e., not inbred pollen) from surrounding areas (Bosch and Waser, 1999). Blooming with abundant heterospecifics, however, can interfere with pollen transfer and increase competition for pollinators (Waser, 1978; Kehrberger and Holzschuh, 2019). In terms of pollinator interactions, high pollinator visitation

rates typically benefit an individual's reproductive success (Gezon *et al*., 2016; Kehrberger and Holzschuh, 2019), but this requires temporal synchrony between flowering time and pollinator seasonal abundance. The cues for plant flowering time and pollinator foraging have presumably evolved to be tightly coupled to ensure the reproductive success of both plants and pollinators (Kudo, 2014; Forrest, 2015). However, climate change threatens to decouple the typically distinct cues that plants and pollinators use to time life history events (Memmott *et al*., 2007; Forrest and Thomson, 2011; Kudo and Ida, 2013; Kudo and Cooper, 2019), though the impacts of potential phenological mismatches on plant fecundity can vary between species (Pardee *et al.*, 2019) and over time (Thomson, 2019). Understanding the long-term consequences of phenological shifts on plants requires linking differences in the phenology of individuals to the relative fecundity of individuals within populations.

Previous studies have focused on how variation in individual flowering time affects plant reproduction (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; Pardee *et al*., 2019; Gallagher and Campbell, 2020), but several important knowledge gaps remain. First, to our knowledge all of the studies except two (Rafferty and Ives, 2012; Pardee *et al*., 2019), focus on a single species, thus precluding cross-taxa comparisons in the same site and season. Multi-species studies facilitate a better understanding of taxonomic differences and enable generalization to other species with similar traits. Second, none of these studies included both hand-pollination experiments to distinguish between pollen and resource limitation and measurement of floral conspecific density at bloom time to assess potential pollen donor limitation. Pollen limitation studies are important for assessing pollination effectiveness

and attributing reproductive differences to pollen or resource availability (Kearns and Inouye, 1993). Third, of the studies that experimentally manipulated individual flowering time (Rafferty and Ives, 2012; Gezon *et al.*, 2016; Pardee *et al*., 2019; Gallagher and Campbell, 2020), all have either manipulated single plants or very small plots via small-scale snow removal, thus not changing plant conspecific and heterospecific flowering density simultaneously. To fully understand how shifting phenology impacts individual reproduction, we need to determine the mechanisms that drive fecundity and the different responses to phenological shifts in co-occuring species. To our knowledge, no studies have induced phenological change in individuals and examined the impact on plant reproduction across multiple co-occurring, phenologically distinct plant species, while accounting for potential drivers of plant fecundity.

Here, we connect differences in the flowering time of individuals within a population to individual seed production as a proxy for per capita fecundity in three montane species in the Rocky Mountains (Colorado, USA). We used both natural variation in individual phenology as well as experimental advancement in the phenology of a subset of individuals (via replicated, controlled snowmelt acceleration manipulations) to create substantial individual variation in flowering phenology. A key difference in our experiments relative to others in this area is that our manipulations were much larger scale (10 x 14 m plots), thus altering not just the bloom time in target individuals but in their pollen donors and competitors as well. To identify the possible selective pressures on blooming time, we assessed how shifts in individual phenology relative to their population blooming affects fecundity through: *(a)* prevailing conspecific floral density during an individual's peak bloom period, and *(b)* pollen limitation, using hand pollination experiments (Kearns and Inouye, 1993). An individual plant may bloom earlier or later than their population's peak bloom, and this may affect the availability of conspecific pollen donors or

pollination, which can ultimately affect the individual's fecundity. We hypothesized that individuals that bloom much earlier or later than neighboring conspecifics will produce lower seed set due to the lack of conspecific pollen donors and pollen transfer by pollinators. Similarly, we also predict that pollen limitation would be greatest in the earliest blooming of the three focal species in this study, relative to the mid- and late-season species, because early season species bloom shortly after snowmelt (Molau, 1993; CaraDonna *et al*., 2014) and, therefore, are susceptible to frost damage and low pollinator visitation (Molau, 1993; Inouye, 2000; Thomson, 2010; Kudo and Ida, 2013).

MATERIALS AND METHODS

Study location–

We conducted this study between May and August 2019 in montane meadows in and around the Rocky Mountain Biological Laboratory (RMBL) in the Gunnison National Forest, western Colorado, United States (38°57.5′ N, 106°59.3′ W, ~2900 m above sea level). This system receives considerable snowfall starting from November to early May (CaraDonna *et al*., 2014). The growing season extends from around mid-May, soon after the ground becomes snow-free, to September (CaraDonna *et al.*, 2014). Soil moisture is highest during and immediately after snowmelt, typically falling steadily until monsoon rains arrive in July (Appendix S1; see Supplemental Data with this article). Pollinators tend to increase in diversity and abundance over the course of the growing season, tapering off in mid-August (Forrest and Thomson, 2011).

Experimental design and phenology manipulation–

We established eight study sites, spaced at least 800 m apart, in meadows across two adjacent valleys. We selected site locations based on their similarity in plant community composition and distance from each other. Each study site contained paired 10 m x 14 m study plots (16 plots total), with the two plots within a site spaced at least 5 m apart and of similar aspect, slope, and plant community composition (Appendix S2; see Supplemental Data with this article). We spaced the paired plots at least 5 m apart to ensure that plant community composition was similar, while allowing buffer space for conducting the manipulation and walking around the plots. At each site, one plot was manipulated with an accelerated snowmelt treatment and the other served as a control in which snowmelt was unmanipulated. We accelerated snowmelt to advance flowering phenology (Price and Waser, 1998; Steltzer *et al.*, 2009; Pardee *et al.*, 2019; Jerome *et al*., 2021). This generated greater variability in individual bloom times, amplifying the phenological range over which we could assess fitness effects (Steltzer *et al*., 2009; Pardee *et al.*, 2019; Jerome *et al*., 2021). We accelerated snowmelt by placing a 14 m x 10 m sheet of black 50% woven plastic shade cloth, which absorbs solar radiation over each snowmelt plot five to six weeks before the anticipated natural snowmelt date (Steltzer *et al*., 2009). Shade cloths were removed when 80% of the snow in plots was completely melted to the ground. Within each study plot, we marked three 1 m x 10 m transects for recording site-level flowering phenology (Fig. 1). We also marked a 1 m wide section within the plot along the perimeter, at least 1 m away from the plot edge, in which we tagged individual plants of the focal species to track individual phenology and seed set (Fig. 1). This perimeter section was inside of the plot boundaries, and in the snowmelt manipulation plot, the snow in the perimeter section melted at a similar rate to the snow in the center of the plot. We measured soil moisture at seven points in the plot every week (Fig. 1).

Selection of focal species–

We selected three native perennial wildflowers as focal taxa: *Mertensia fusiformis* (Boraginaceae)*, Delphinium nuttallianum* (Ranunculaceae)*,* and *Potentilla pulcherrima* (Rosaceae) (hereafter referred to by genus). The wildflowers in this system bloom in the summer growing season, and the flowering phenology of most species is closely linked to the timing of snowmelt, defined hereafter as the first day on which the ground is snow-free (Price and Waser, 1998; Wipf, 2010; Pardee *et al*., 2019). *Mertensia* is one of the first species to bloom, beginning to bloom within two weeks after snowmelt (Inouye *et al.*, 2000). *Delphinium* generally blooms three to four weeks after snowmelt (Wadgymar *et al.*, 2018) and occasionally overlaps in flowering period with *Mertensia* (Miller-Rushing and Inouye, 2009). *Potentilla* blooms about five to six weeks after snowmelt (Stinson, 2004) and its flowering period typically does not overlap with the other two focal species (Pardee *et al.*, 2019). In this study, we consider *Mertensia*, *Delphinium*, and *Potentilla* to be early-, mid-, and late-season blooming species respectively. We selected these focal species to assess how individual variation in flowering time affects fecundity in species with naturally different phenologies, and we maintain this sequence of discussing the focal species in the paper. We also selected these focal species because they are locally abundant and rely on pollinators for maximal seed set (Bosch and Waser, 1999; Burkle and Irwin, 2010; Forrest and Thomson, 2010), allowing us to assess the relative importance in the seasonal availability of pollinators and conspecific pollen donor flowers on fecundity. While *Mertensia* and *Delphinium* are both self-incompatible (Waser, 1978; Forrest and Thomson, 2010), *Potentilla* can self-pollinate, but outcrossing increases seed set (Burkle and Irwin, 2010). *Mertensia* is typically pollinated by bumblebees and solitary bees (Forrest and Thomson, 2010;

Forrest *et al*., 2011), *Delphinium* is pollinated by bumblebees and hummingbirds (Waser, 1978; Schulke and Waser, 2001), and *Potentilla* is pollinated by a wide range of bees and fly species (Burkle and Irwin, 2010).

Tracking phenology and conspecific density–

We tagged individuals of each focal species to assess how their individual flowering phenology impacted their seed set. For each focal species at a site, we selected 16 individual plants per plot —32 plants total per site—that we included in pollen limitation experiments. We used stratified random selection to ensure that these plants were well-spaced along the perimeter section within the plot (Fig. 1). Each selected individual was tagged with soft wire and a colored plastic bead. We visited every tagged plant twice per week to track its flowering phenology, recording the date that we first observed it with an open flower (bloom start) and the date of total flower senescence with no ensuing buds (bloom end). In addition to flowering phenology events, we recorded the total number of flowers that each tagged individual produced. Focal plants were spatially interspersed with conspecific individuals and bloomed with similar timing to the rest of the population in the plot.

In addition, we recorded the total number of open, reproductively receptive flowers of each of the three focal species once per week in the three transects of both control and accelerated snowmelt plots. Flowers were considered reproductively receptive when petals were open, anthers had pollen, and stigmas were receptive. These floral abundance measures did not include the tagged individuals, only neighboring conspecific plants within the plots. Tracking flower abundance within plots served two functions. First, we used floral abundance in the control plots

as the baseline for natural phenological progress at a site when calculating the variation in bloom time for tagged individuals (see *Data analysis: Calculating individual phenology*). Second, we used floral abundances in both control and accelerated snowmelt plots to estimate the conspecific floral density during each tagged individual's bloom period (see *Data analysis: Calculating conspecific floral density*). In the snowmelt manipulation plots, the transect plants and focal plants received the manipulation at the same time, and the distance transect and focal plants was 4 m or less.

Pollen limitation experiment–

We conducted hand-pollination treatments to isolate the effect of pollen limitation from resource availability (Kearns and Inouye, 1993). For each focal species, of the 16 tagged individual plants in every plot of every site, we randomly assigned eight to receive only natural pollination ("open" treatment, i.e., unmanipulated) and the other eight received supplemental pollen via hand-pollination ("hand" treatment). When hand-pollinating, we used a clean paintbrush to collect pollen from two or three untagged plants within each plot, transferring pollen onto the stigmas of plants tagged for hand-pollination. As individual plants produced a succession of flowers over time, we visited every site twice per week to hand-pollinate fresh flowers. We used the difference in seed set between the open and hand-pollinated treatment plants to assess the degree of pollen limitation at each plot (see *Data analysis: Evaluating pollen limitation*).

Measuring individual fecundity–

To assess how an individual's phenology affects fecundity, we collected and counted fruits from all tagged individuals of the three focal species. All fruits were collected from each focal plant

when ovules were fully expanded but fruits had not yet released seed. We scored all seeds in all fruits for each plant as developed or undeveloped based on species-specific measurements of seed length, width, and color. While these thresholds for length, width, and color were consistent for scoring all seeds of a species, the thresholds were subjective. We 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). An individual's seed set is dependent upon the flower number, and we found high variation in the number of flowers produced per plant for the three species. The total number of flowers was included in our models and figures to account for the effect on fecundity metrics. While flower number itself is a metric of fecundity, we accounted for flower number to capture the effectiveness of pollination and limit the extent to which genetics determine flower number, and therefore, seed set. The total number of developed seeds is important when comparing individual fecundity and ultimately fitness. The proportion of developed seeds to undeveloped ovules, by contrast, can be used to assess pollination success (Allison, 1990; Slobodník, 2002; Brys *et al*., 2007), though resource availability and genetics influence the proportion as well (Griffin and Barrett, 2002; Holland and Chamberlain, 2007). We initially measured seed mass but found no substantial differences between the seeds measured. We calculated the total developed seeds for all three species, and we calculated the proportion of developed seeds for *Mertensia* and *Delphinium*, but not *Potentilla*. We were unable to calculate the proportion of developed seeds for *Potentilla* because undeveloped seeds were difficult to distinguish from other elements of the carpel.

Data analysis–

All analyses were conducted in R 3.5.1 (R Core Team, 2018). A fully reproducible Rmarkdown report of the analysis is available as supplemental material. Soil moisture was not a driver of plant fecundity, and therefore was not included as an explanatory variable (Appendix S3: see Supplemental Data with this article).

Calculating individual phenology–

To determine if blooming early or late impacts a plant's fecundity, we first needed to establish how much earlier or later a tagged individual bloomed relative to the rest of the population. To create a basis for comparison of individuals to their population at each site, we used the Day of Year at which untagged, unmanipulated plants in the control plot attained the greatest recorded total number of blooming flowers across all three transects (the natural "population peak bloom" for a focal species at a site). We included flower surveys from all three transects to increase sample size and improve accuracy for floral abundance.

We then estimated the peak bloom date for every tagged individual of a focal species, defined as the estimated day with the most active reproductively receptive flowers in an individual's blooming period. We estimated the peak floral abundance for each species and determined *Mertensia* peaked approximately one third through its blooming period, while *Delphinium* and *Potentilla* peaked approximately halfway through their blooming periods (Appendices S4-S6; see Supplemental Data with this article). The individual peak was calculated based on estimated (rather than directly observed) dates of bloom start and bloom end, because it was not logistically possible to observe individuals daily, and data were recorded twice per week. The estimated day for individual phenological events (bloom start or end) was the midpoint between the day of a newly recorded event and the most recent preceding day the event was not recorded. For

example, the first day a tagged individual blooms is estimated to be the midpoint between the first day a bloom was observed and the day of the most recent record of bloom absence (Taylor, 2019).

We calculated the variation in individual phenology at every site by the difference in days between an individual's peak bloom date and the population's peak bloom date at the site. An individual that bloomed earlier than its site's population peak thus had a negative value for its difference in bloom time, while a late-blooming individual had a positive value. An individual that bloomed on the same day as the calculated population peak bloom had an individual phenology value of zero.

Calculating conspecific floral density–

We calculated the mean number of blooming conspecific flowers in the three transects of both control and manipulated plots every week. To calculate the conspecific density during an individual's bloom, we assigned each tagged individual the mean conspecific floral density associated with the week closest to its peak bloom date. Thus, the conspecific density value for an individual was the average number of open conspecific flowers within the plot during the individual's peak bloom date. These values represent the average number of neighboring conspecifics and are not the absolute conspecific density in the meadow. For two weeks during the season, we could not do a *Potentilla* flower survey in some sites due to logistical constraints (we visited three of the seven sites in one week and the other four sites in the following week) and could not calculate mean conspecific density for the week. In those cases, an individual's peak bloom date did not match to a mean conspecific density value and those individuals were removed from the dataset.

Evaluating individual phenology and conspecific density–

We analyzed the relationship between individual phenology and fecundity using generalized linear mixed effects models (GLMMs) from the "glmmTMB" package (Brooks et al., 2017). We used a mixed-effects modeling framework because repeated measurements within research sites led to non-independence of data. The three focal species were modeled separately. For each, we modeled seed set as a function of individual phenology and conspecific density. We included two groups of fixed effects: one for individual blooming time and one for conspecific density. Both groups included an interaction with the number of flowers produced by an individual (henceforth "flowers"). The number of flowers was assessed in both fixed effects terms, but not as a main effect to preserve degrees of freedom. Our response variables for seed set were the total number of developed seeds and the proportion of developed seeds (data available only for *Mertensia* and *Delphinium*). We used negative binomial errors for total developed seeds ("dev.seed" in the model below), as the data were overdispersed relative to a Poisson distribution, and binomial and beta-binomial errors for the proportion of developed seeds. We checked the models measuring total developed seeds for zero-inflation, and we corrected these models as needed using the "DHARMa" package (Hartig, 2022). We included "site" as a random effect (random intercept) in all models to account for site differences. We excluded individuals that received hand-pollination treatments from the individual phenology analysis because the pollen-supplemented individuals could influence the fecundity assessment.

For the individual phenology term, we used an interaction between two main effects because blooming time was non-monotonic, precluding the use of linear models in its unmodified form. Specifically, we split individual phenology into blooming before ("early") vs. after ("late") the

population peak bloom (henceforth “early.late”) and their continuous Day of Year distance from the population peak (henceforth “deviation”) to assess if individual flowering time affected fecundity. This structure allowed us to assess: 1) if earlier and later blooming individuals (relative to the population peak) had different fecundity levels, via the main effect of “early.late”; 2) if temporal deviation from the population peak generally impacted fecundity, via the main effect of “deviation”; and 3) if temporal deviation impacted earlier vs. later blooming individuals distinctly, via the “deviation * early.late” interaction.

For the conspecific density term, we included an interaction between the number of conspecifics at the time of blooming (henceforth “conspecifics”) and the plot treatment (snowmelt acceleration vs. control; henceforth “plot.treat”). This was modeled as an interaction term because the abundance of conspecific flowers was on two different scales for the snowmelt acceleration vs. control plots. In the snowmelt acceleration plots, we advanced the phenology of the plants in the plot and created “islands” of early blooming individuals, where conspecific density was likely close to the absolute number of individuals in the manipulated plot. In the control plots, by contrast, plants both inside and outside of the plot bloomed simultaneously, and the conspecific density was thus functionally higher than that in the accelerated snowmelt plot.

Our individual phenology and conspecific density models shared the basic form of:

dev.seed ~ flowers:(deviation * early.late) + flowers:number.conspecifics/plot.treat + (1 | site)

Where “dev.seed” is the number of developed seeds, the “*” term indicates an interaction including the main effects, the “:” indicates an interaction without main effects, and the “/” indicates an interaction with only one main effect (“plot.treat” is not assessed as a main effect). The first group of fixed effects is the three-way interaction term for individual phenology: “deviation”, “early.late”, and “flowers”. The second group of fixed effects is the three-way interaction term for conspecific density: “number.conspecifics”, “plot.treat”, and “flowers”. Again, “flowers” was included in both interaction groups because the number of flowers on a plant determines seed set. The “1|” indicates a random intercept.

Evaluating pollen limitation–

We assessed pollen limitation in separate GLMMs to differentiate pollination function from resource availability and include our hand-pollinated individuals in the analysis. We again analyzed fecundity in two separate models for each species, using the total number of developed seeds and the proportion of developed seeds as response variables. We included “plot treatment” nested within “site” as a random effect (random intercept). We set our fixed effects as an interaction between pollination treatment (open vs. hand-pollinated; henceforth “treat”) and the number of flowers produced by the individual (“flowers”). Thus, pollen limitation would be detectable if the slope (interaction) of seed production relative to the number of flowers were steeper in the hand pollinated relative to the open flower treatments. The *Mertensia* and *Potentilla* models (but not the *Delphinium* model) measuring the total number of developed seeds were zero-inflated, and thus we corrected for zero-inflation in those two species.

Our pollen limitation models share the basic form of:

dev.seed ~ treat/flowers + (1 | site/plot.treat)

Where "dev.seed" is the response variable for number of developed seeds. The fixed effect is an interaction between pollination treatment and number of flowers, excluding the main effect of "flowers" on seed set. The random effect (random intercept) is plot treatment nested within site, indicated by "1|".

RESULTS

Across the eight study sites, we examined *Mertensia* populations in four sites, *Delphinium* in four sites, and *Potentilla* in seven sites. We tagged 512 individuals in total and collected seeds from 463 individuals. We could not collect seeds from some tagged individuals due to damage from herbivory or disease. Of those 463 collected individuals, 124 were *Mertensia* individuals, 118 were *Delphinium* individuals, and 221 were *Potentilla* individuals. Accelerated snowmelt plots melted out earlier than unmanipulated control plots by 5-17 days, with a mean of eight days. The 2019 growing season had relatively late snowmelt (our lowest elevation site melted at the end of May, and highest melted in mid-June) and a late June snowstorm, which caused frost damage in multiple early-season species. Soil moisture gradually decreased until late July, when late summer monsoons arrived and persisted through August (Appendix S1; see Supplemental Data for this article). The first snow after the growing season occurred in October (barr, 2020).

All relevant statistics from model summaries are listed in Table 1.

Individual phenology and conspecific density–

For all focal species, we found relationships between individual phenology and fecundity. When flower number per individual is accounted for, all three species had statistically significant relationships between deviation from the population peak bloom and fecundity. Again, in *Mertensia* and *Delphinium* we separately measured both total developed seeds and the proportion of developed ovules, so there are two effects reported for each of those species. In *Potentilla*, we only measured the total developed seeds.

Individual phenology—positive effects–

We found positive relationships with deviation (increased fecundity with increasing bidirectional departure from the population peak) in the total developed seeds in *Delphinium* (main effect of "deviation"; P = 0.025; Fig. 2C) and the proportion of developed ovules in *Mertensia* (main effect of "deviation"; P = 0.019; Fig. 3A). We also found marginal evidence that deviation positively impacted total developed seeds in *Mertensia* (main effect of "deviation; P = 0.088). In two of the three species, we detected directional effects, though in opposite directions. One of these was positive: in *Mertensia*, blooming later than the population peak had a marginally significant positive effect on the total developed seeds (main effect of "early.late"; P = 0.075; Fig. 2A).

Individual phenology—negative effects–

We detected a negative directional effect and found a negative relationship with deviation (reduced fecundity with increasing departure from the population peak) in the total developed seeds in *Potentilla* (main effect of "deviation"; P = 0.006; Fig. 2E). Blooming later than the population peak had a negative effect on total developed seeds relative to blooming earlier (main effect of "early.late"; P = 0.005; Fig. 2E), with a more severe effect on later blooming individuals (significant "deviation*early.late" interaction; P = 0.002; Fig. 2E).

Individual phenology—no effect–

For the proportion of developed ovules in *Delphinium*, we did not detect an effect of deviation (main effect of "deviation"; P = 0.158; Fig. 3C), early or late blooming (main effect of "early.late"; P = 0.387; Fig. 3C), or the interaction between deviation and early vs. late (P = 0.827; Fig. 3C).

Conspecific floral density–

We detected an interaction effect of conspecific density and plot treatment on the proportion of developed seeds in *Mertensia* individuals (P = 0.005), with a more negative effect of conspecific density in the accelerated snowmelt plot (Fig. 3B). We did not detect an effect of conspecific floral density in *Delphinium* or *Potentilla*. Neither of the main effects of conspecific density and plot treatment, nor the interaction between the two, appeared to have an effect on their flowers and their seed set.

Pollen limitation–

We detected pollen limitation in *Mertensia* and *Delphinium*. The interaction between pollination treatment and flower number was significant for the developed seed count in *Mertensia* (P < 0.001) and *Delphinium* individuals (P < 0.001). Pollen limitation was also evident in the proportion of developed seeds for *Mertensia* (P = 0.005) and *Delphinium* individuals (P = 0.028). We did not find evidence of pollen limitation in *Potentilla*.

DISCUSSION

We investigated whether individual differences in flowering time impact the fecundity of three montane forbs through two specific mechanisms, pollen donor availability (conspecific density)

and pollen limitation. We accelerated snowmelt at the plot level to generate greater variability in flowering phenology and tracked the phenology and fecundity of individual plants. We found that differences in individual flowering time from the population peak impacted fecundity in our focal species, with species-specific effects. In *Mertensia*, our earliest flowering focal species, we found that individuals with a peak bloom after the population peak had increased fecundity. For the mid-season species, *Delphinium*, individuals with a peak bloom that simply deviated from the population peak (either early or late) had increased fecundity. However, in *Potentilla*, the late-season species, individuals that peaked later than the rest of the population had reduced fecundity. Differences in flowering time relative to the rest of the population affected fecundity mainly via pollination rather than availability of pollen donors (conspecific density). In other words, when individuals bloomed earlier or later relative to the population peak bloom, effective pollination was likely limited and this drove changes in individual fecundity. This work underscores how individual-level variation in flowering phenology can affect reproductive success.

In *Mertensia*, our earliest flowering focal species, we found that deviation from the population peak, specifically blooming after the peak, increased the seed set. Our finding is consistent with other studies that have suggested late blooming in *Mertensia* can increase fecundity (Forrest and Thomson, 2010; Pardee *et al*., 2019). Additionally, we found that *Mertensia* individuals were pollen-limited and were affected by conspecific density. A variety of factors can limit the ratio of developed seed and undeveloped ovules, including pollen limitation, resource limitation, and genetic factors (Allison, 1990; Slobodník, 2002; Griffin and Barrett, 2002; Brys *et al*., 2007; Holland and Chamberlain, 2007). While these factors are still poorly understood, some pollen limitation and resource limitation can be phenologically driven. For many early-flowering

temperate plants, late-spring frosts may strongly limit plant fitness due to damage to flowers and ovules (Thomson, 2010; Gezon *et al*., 2016). Also, early season species like *Mertensia* may begin to flower while pollinator abundance is low, thereby impacting visitation rates and reproductive success of early-blooming individuals (Forrest and Thomson, 2010*;* Pardee *et al.*, 2019). Our pollen limitation and conspecific density results indicate that early-blooming *Mertensia* individuals likely endure lower pollinator visitation and greater competition with conspecific individuals for those early-season pollinators. Individuals that bloom later than the rest of the population may increase fecundity by avoiding frost damage, benefiting from higher pollinator abundance, and reducing competition with conspecifics.

The seed set of our mid-season species, *Delphinium*, significantly increased with deviation from the population peak bloom, both early and late. Unlike early-season species like *Mertensia*, mid-season species may be able to avoid the dangers of frost (Dunne *et al.*, 2003; Pardee *et al*., 2019). Furthermore, *Delphinium* flowers have been shown to be remarkably tolerant to frost (Forrest *et al*., 2010*a*; CaraDonna and Bain, 2016). Thus, the phenology of *Delphinium* individuals may be less abiotically constrained, allowing for greater deviation in blooming time within a population (CaraDonna *et al*., 2014). Our results show that this deviation from the population peak increased individual fecundity (Fig. 2C), which is consistent with a recent study in the same system that used a snow removal treatment and found that this species produces more seeds when it blooms earlier than unmanipulated plants (Pardee *et al*., 2019). We suspect that pollinator visitation, rather than the availability of conspecific pollen donors, drove this increase in fecundity with deviation because we found that *Delphinium* was pollen-limited in the studied year, but we did not detect an effect of conspecific floral density. This suggests that there were

ample pollen donors for outcrossing, but pollinator visitation was insufficient. Although flowering during the population peak bloom could hypothetically increase genetic diversity, it may be detrimental to pollination due to increased intraspecific competition for pollinators. For *Delphinium*, blooming much earlier or later than the population peak bloom may reduce competition for pollinators, and therefore, increase seed set. This trend could indicate that *Delphinium* is experiencing disruptive selection, in which phenological shifts away from the peak bloom are selected through pollination (Sabat and Ackermen, 1996; Chen and Pannell, 2022). Also, it is important to note that while we did not detect an effect of conspecific floral density in our plots, *Delphinium* densities outside of the study plots were not measured. This is important because landscape-level conspecific floral density may be a greater driver for highly mobile pollinators, such as bumblebees and hummingbirds (Waser and Price, 1981; Bosch and Waser, 1999). Because *Delphinium* is less abiotically constrained than early season species, selection may act on individuals that deviate from the population peak bloom and potentially reduce the intraspecific competition for pollinators.

In *Potentilla*, our late-season species, variation in individual phenology created differences in fecundity, but unlike *Mertensia* and *Delphinium*, these individuals were not pollen-limited. More specifically, in *Potentilla* populations, early-blooming individuals produced a higher seed set relative to late-blooming individuals, and blooming much later than the population peak was detrimental to fecundity (Fig. 2E). Our results are consistent with previous studies which showed that individuals of late-season species with artificially advanced phenology were more reproductively successful (Price and Waser, 1998; Pardee *et al*., 2019). Pardee *et al*. (2019) also found that early-blooming *Potentilla* individuals received higher pollinator visitation. We know

that pollinators are typically abundant later in the growing season in montane regions (Kudo, 2014; Gallagher and Campbell, 2020), so pollen limitation may be less likely in this late-season species. By blooming early, individuals received higher pollinator visitation and produced more flowers and seeds (Pardee *et al*., 2019). For *Potentilla,* it may be advantageous to bloom early while pollinators are abundant in the late season, though other factors may limit how early or late this species can bloom. For example, interspecific competition with earlier season species or physiological constraints could weaken selection pressure for blooming much earlier (Iler *et al.*, 2013; Faust and Iler, 2021). Though late season snow in the 2019 season could have limited how early *Potentilla* bloomed, our *Potentilla* individuals likely benefited from blooming earlier than the population peak and receiving higher pollinator visitation.

We found mixed evidence that the availability of conspecific pollen donors during blooming drives individual-level fecundity in our three study species. We hypothesized that low conspecific pollen donor density would decrease fecundity in our early-season species, but our results do not support this. In fact, high conspecific density caused decreased fecundity in our early-season individuals, potentially due to intraspecific competition for early-season pollinators. Furthermore, we did not find evidence for an effect of conspecific density in our other focal species. This is surprising because other studies suggest that the conspecific pollen donor density can be an important predictor of fecundity in herbaceous plants (Gibbs and Talavera, 2001; Waal *et al*., 2014; Kehrberger and Holzschuh, 2019; Towers *et al*., 2020). We measured conspecific density at the plot level, and it is possible that landscape-level conspecific density is more influential for fecundity, particularly for species that are dependent on highly mobile pollinators. Additionally, our snowmelt acceleration experiment advanced the phenology of the floral

community at the plot level. Therefore, the conspecific density count is a very accurate numerical representation in the early snowmelt plot (when other individuals surrounding the plot are not yet in bloom, though also when pollinator presence is low), while in the control plot, the count is a less accurate estimate of the conspecific density in the surrounding meadow. A separate issue is that conspecific density and variation in individual phenology could interact. Specifically, higher conspecific density could be beneficial when other conspecific pollen donors are rare, and detrimental due to intraspecific competition for pollinators when conspecific individuals are common. Ultimately, our interaction term of "conspecific density*plot treatment" does not take the differing effects of conspecifics on early vs. late individuals into account, and the effect could be canceled out by combining the potential benefits and detriments of conspecific pollen donor availability. Unfortunately, we did not have the statistical power to include a three-way interaction between conspecific density, plot treatment, and flowering time in our models to assess this issue directly. Thus, while we did not see consistent evidence of pollen donor availability on fecundity in all of our species, we also cannot rule out that it could be an important mediator of fecundity.

Individual differences in blooming time affect individual fecundity, and therefore species may experience selection pressure on floral phenology over time (Forrest *et al.*, 2010*a*; Anderson *et al*., 2012; CaraDonna *et al*., 2014; Wadgymar *et al.*, 2018). Our study shows that shifts in blooming relative to the rest of the population can be advantageous for fecundity via pollination. These phenological differences impact reproduction and may be selected for over other blooming times (Stinson, 2004; Faust and Iler, 2021). Depending on the species, selection on blooming time could be directional (O'Connell and Johnston, 1998; Elzinga *et al.*, 2007; Koenig *et al*., 2012) or disruptive (Sabat and Ackerman, 1996; Chen and Pannell, 2022). In the context of

climate change, selection on individual phenological shifts could drive population-level phenology, which may facilitate climate "tracking" in these plant populations (Cleland *et al*., 2012). Furthermore, as climate change alters the abiotic and biotic cues for floral phenology, selection may act on the species that are particularly sensitive to those cues. For example, selection could act on the early-season species that are sensitive to abiotic cues, or on the mid-season species that are sensitive to biotic cues (Pau *et al*. 2011). However, despite the advantages of deviating from the population's peak bloom, we cannot say for certain that directional selection is acting on our focal species. First, if deviating from the population peak bloom is beneficial for reproduction, selection pressure would weaken as other individuals shift their phenology in tandem. Second, our focal species are perennials with very long lifespans, and selection pressures may not be consistent for an entire generation (Forrest *et al*., 2010*a*; Thomson, 2019). Third, abiotic and biotic variation across years could weaken selection pressure (Forrest *et al.*, 2010*a*; Iler *et al*., 2013; Faust and Iler, 2021). For instance, variation in the phenology of co-flowering species could increase interspecific competition through heterospecific pollen transfer and conspecific pollen loss, which could interfere with selection pressure for an individual's blooming time (Forrest *et al*., 2010*a*; Faust and Iler, 2021). Further studies that assess the effect of individual variation in phenology on fecundity are needed to understand the potential barriers to directional selection in this system.

Our work has some limitations worth highlighting. First, we did not ascertain the viability of developed seeds in this study and future studies could include germination trails. Second, while we included species that have distinct phenologies, different species from the same community could produce different results and further studies with more focal species are needed, in

particular to generalize our results related to the differences between early-, mid-, and late-season species. Third, our snowmelt acceleration experiment created "islands" of early bloomers. These islands of early flowers can be—particularly in the early season—the only flowers in bloom in the area and may differentially attract pollinators, which could inflate the pollination services they receive (Forrest, 2015), and the pollen limitation results should be interpreted with caution, particularly for *Mertensia*, our earliest blooming species. Fourth, by conducting plot-level flower counts once per week, our peak bloom estimates may not be completely accurate estimates of population phenology. Finally, the observed effect of flowering time on fecundity may differ over long periods of time (Thomson, 2019), and the long-term effects of blooming time on population fitness are unknown.

We show that variation in individual plant phenology is an important driver of fecundity, and that phenological shifts impact early-, mid-, and late-season species differently. By inducing early snowmelt, we increased variability in floral phenology and found that differences in phenology impacted seed production in our three focal species. Our findings suggest that an individual's reproductive success is sensitive to small changes in phenology, and individual-level differences are important to consider when examining climate-induced phenological shifts. Understanding the relationship between individual phenology and fecundity is critical, as flowering time can affect species interactions and the abiotic environment in complex ways. These interactions could potentiate multiple, opposing drivers of plant fecundity that act simultaneously. While in a given year the net effect of such drivers may be impactful, we need more long-term studies across several environmentally-variable years, along with studies focused on the "downstream" impacts of pollination (i.e., gene flow, demography) to come to a fuller

understanding of how flowering phenology change will impact plant pollination, seed production, reproduction, population growth, and evolution.

ACKNOWLEDGEMENTS

The authors thank the Rocky Mountain Biological Laboratory, and in particular Jennie Reithel, for providing logistical support for this study. We would like to thank Heidi Steltzer, C. Rick Williams, and Rebecca Dalton for their advice on setting up this study and the focal species. We thank Victoria Reynolds, Caleb Sowers, Kaysee Arrowsmith, Aidan Fife, Micah Sharer, Selena Perrin, Andrea Keeler, Erin Paulson, Chelsea Wilmer, Samantha Siegfried, Kristi Haner, and Alex Tiberio for significant contributions in the field, and Eden Nitza, Lindsey Kapel, Albert Liu, Bijia Wang, and Triston Charleston for assistance in the lab. This work was supported by the National Science Foundation [DEB-1834497] to B.J.B.; the US Army Research Office [W911NF-19-1-0231] to B.J.B.; the Emory University Department of Environmental Sciences Lester Grant to A.E.S.; the Emory University Undergraduate Research Program grant to A.E.S.; and the Lewis and Clark Fund of Exploration and Field Research (2017-2018) from the American Philosophical Society to L.X.

AUTHOR CONTRIBUTIONS

A.E.S., L.X., and B.J.B. conceived and designed the study. A.E.S., C.M., and L.X. conducted the fieldwork. A.E.S. performed the analyses with assistance from L.X. and B.J.B. A.E.S. drafted the manuscript, and L.X. and B.J.B. critically revised all drafts of the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data are archived in a GitHub public repository: https://github.com/Brosi-Lab/Annie_plant_fitness.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: Soil moisture in percent volumetric water content (%VWC) during the growing season (May – August 2019) in control and accelerated snowmelt plots.

Appendix S2: Table of site information, including GPS coordinates, elevation, and aspect.

Appendix S3: Model selection results for soil moisture variables: effective minimum soil moisture, soil moisture at end of season, mean soil moisture, range of soil moisture, and rate of soil moisture decline over the course of the growing season. When seed set was analyzed as a function of these variables, the selected model (delta = 0) was not significantly different from the null model (delta = 0.03).

Appendix S4: Floral abundance over the blooming period for *Mertensia fusiformis* across all sites and both plot treatments. The dashed line indicates one third through the blooming period. Solid line indicates halfway through blooming period.

Appendix S5: Floral abundance over the blooming period for *Delphinium nuttallianum* across all sites and both plot treatments. For *Delphinium*, we only display the solid line as there is good congruence between the bloom peak and the halfway point through the blooming period of the species.

Appendix S6: Floral abundance over the blooming period for *Potentilla pulcherrima* across all sites and both plot treatments. For *Potentilla*, we only display the solid line as there is good congruence between the bloom peak and the halfway point through the blooming period of the species.

LITERATURE CITED

Allison, T.D. 1990. Pollen Production and Plant Density Affect Pollination and Seed Production in *Taxus Canadensis*. Ecology 71:516-522.

Allen, C.D., A.K. Macalady, H. Chenchouni *et al*. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259:660-684.

Anderson J.T., D.W. Inouye, A.M. McKinney, R.I. Colautti and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc. R. Soc. B. 279:3843–3852.

barr, billy. 2020. Gothic Weather: Long term weather data. https://www.gothicwx.org/.

Bennie, J., E. Kubin, A. Wiltshire, B. Huntley, and R. Baxter. 2010. Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: the implications of local adaptation to climate. Global Change Biology 16:1503-1514.

Bogdziewicz, M., M. Pesendorfer, E.E. Crone, C. Pérez-Izquierdo, and R. Bonal. 2020. Flowering synchrony drives reproductive success in a wind-pollinated tree. Ecology Letters 23:1820-1826.

Bosch, M., and N.M. Waser. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). American Journal of Botany 86:871-879.

Brooks, M.E., K. Kristensen, K.J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H.J. Skaug, M. Maechler and B.M. Bolker (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal, 9(2), 378-400.

Brown, B.J., and R.J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129:43-49.

Brys, R., H. Jacquemyn, L.D. Bruyn, and M. Hermy. 2007. Pollination Success and Reproductive Output in Experimental Populations of the Self-Incompatible *Primula vulgaris*. International Journal of Plant Sciences 168:571-578.

Burkle, L.A., and R.E. Irwin. 2010. Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. Journal of Ecology 98: 705-717.

CaraDonna, P.J., A.M. Iler, and D.W. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. Proceedings of the National Academy of Sciences 111:4916-4921.

CaraDonna, P.J., and J.A. Bain. 2016. Frost sensitivity of leaves and flowers of subalpine plants is related to tissue type and phenology. Journal of Ecology 104:55-64.

Chen, K.-H., and J.R. Pannell. 2022. Disruptive selection via pollinators and seed predators on the height of flowers in a wind-dispersed alpine herb. American Journal of Botany 109:1–13.

Cleland, E.E., I. Chuine, A. Menzel, H.A. Mooney, and M.D. Schwartz. 2007. Shifting plant phenology in response to global change. Trends in Ecology & Evolution 22:357-365.

Cleland, E.E., J.M. Allen, T.M. Crimmins, J.A. Dunne, S. Pau, S.E. Travers, E.S. Zavaleta and E.M. Wolkovich. 2012. Phenological tracking enables positive species responses to climate change. Ecology 93:1765-1771.

Crone, E.E., and P. Lesica. 2006. Pollen and Water Limitation in *Astragalus scaphoides*, a Plant That Flowers in Alternate Years. Oecologia 150:40-49.

Crone, E.E., E. Miller, and A. Sala. 2009. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. Ecology Letters 12:1119-1126.

de Waal, C., B. Anderson, and A.G. Ellis. 2014. Relative Density and Dispersion Pattern of Two Southern African Asteraceae Affect Fecundity through Heterospecific Interference and Mate Availability, Not Pollinator Visitation Rate. Journal of Ecology 103:513-525.

Dunne, J.A., J. Harte, and K.J. Taylor. 2003. Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. Ecological Monographs 73:69-86.

Elzinga, J.A., A. Atlan, A. Biere, L. Gigord, A.E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology & Evolution 22: 432-439.

Faust, M.N., and A.M. Iler. 2021. Pollinator-mediated reproductive consequences of altered co-flowering under climate change conditions depend on abiotic context. Climate Change Ecology 100043.

Fitter, A.H., and R.S.R. Fitter. 2002. Rapid Changes in Flowering Time in British Plants. Science 296:1689-1691.

Forrest, J.R.K. 2015. Plant–pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? Oikos 124:4-13.

Forrest, J., D.W. Inouye, and J.D. Thomson. 2010. Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? Ecology 91:431-440.

Forrest, J., and A.J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3101-3112.

Forrest, J., and J.D. Thomson. 2010. Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower. American Journal of Botany 97:38-48.

Forrest, J.R.K., and J.D. Thomson. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecological Monographs 81:469-491.

Forrest, J.R.K., J.E. Ogilvie, A.M. Gorischek, and J.D. Thomson. 2011. Seasonal change in a pollinator community and the maintenance of style length variation in *Mertensia fusiformis* (Boraginaceae). Annals of Botany 108:1–12.

Franks, S.J., S. Sim, and A.E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences 104:1278-1282.

Gallagher, M.K., and D.R. Campbell. 2020. Pollinator visitation rate and effectiveness vary with flowering phenology. American Journal of Botany 107:445-455.

Gezon, Z.J., D.W. Inouye, and R.E. Irwin. 2016. Phenological change in a spring ephemeral: implications for pollination and plant reproduction. Global Change Biology 22:1779-1793.

Gibbs, P.E., and S. Talavera. 2001. Breeding System Studies with Three Species of *Anagallis* (Primulaceae): Self-incompatibility and Reduced Female Fertility in *A. monelli* L. Annals of Botany 88:139-144.

Griffin, S.R., and S.C.H. Barrett. 2002. Factors Affecting Low Seed:Ovule Ratios in a Spring Woodland Herb, *Trillium grandiflorum* (Melanthiaceae). International Journal of Plant Sciences 163:581-590.

Hall, E.S., L.R. Piedrahita, G. Kendziorski, E. Waddle, D.F. Doak, and M.L. Peterson. 2018. Climate and synchrony with conspecifics determine the effects of flowering phenology on reproductive success in *Silene acaulis*. Arctic, Antarctic, and Alpine Research 50:e1548866.

Florian Hartig. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6. https://CRAN.R-project.org/package=DHARMa.

Holland, J.N., and S.A. Chamberlain. 2007. Ecological and Evolutionary Mechanisms for Low Seed. Ecology 88:706-715.

Ibáñez, I., R.B. Primack, A.J. Miller-Rushing et al. 2010. Forecasting phenology under global warming. Philosophical Transactions of the Royal Society B: Biological Sciences 365:3247-3260.

Iler, A.M., T.T. Høye, D.W. Inouye, and N.M. Schmidt. 2013. Nonlinear flowering responses to climate: are species approaching their limits of phenological change? Philosophical Transactions of the Royal Society B: Biological Sciences 368:20120489.

Inouye, D.W. 2000. The ecological and evolutionary significance of frost in the context of climate change. Ecology Letters 3:457-463.

Inouye, D.W., B. Barr, K.B. Armitage, and B.D. Inouye. 2000. Climate change is affecting altitudinal migrants and hibernating species. Proceedings of the National Academy of Sciences 97:1630-1633.

Inouye, D.W., F. Saavedra, and W. Lee-Yang. 2003. Environmental influences on the phenology and abundance of flowering by *Androsace septentrionalis* (Primulaceae). American Journal of Botany 90:905-910.

Kearns, C.A., and D.W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Boulder, CO.

Kehrberger, S., and A. Holzschuh. 2019. How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? Scientific Reports 9:15593.

Koenig, W.D., K.A. Funk, T.S. Kraft, W.J. Carmen, B.C. Barringer and J.M.H. Knops. 2012. Stabilizing selection for within-season flowering phenology confirms pollen limitation in a wind-pollinated tree. Journal of Ecology 100: 758-763.

Kudo, G. 2014. Vulnerability of phenological synchrony between plants and pollinators in an alpine ecosystem. Ecological Research 29:571-581.

Kudo, G., and E.J. Cooper. 2019. When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. Proceedings of the Royal Society B: Biological Sciences 286:20190573.

Kudo, G., and T.Y. Ida. 2013. Early onset of spring increases the phenological mismatch between plants and pollinators. Ecology 94:2311-2320.

Memmott, J., P.G. Craze, N.M. Waser, and M.V. Price. 2007. Global warming and the disruption of plant–pollinator interactions. Ecology Letters 10:710-717.

Miller-Rushing, A.J. and D.W. Inouye. 2009. Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. American Journal of Botany 96:1821-1829.

Molau, U. 1993. Relationships between Flowering Phenology and Life History Strategies in Tundra Plants. Arctic and Alpine Research 25:391-402.

Molau, U. 1996. Climatic impacts on flowering, growth, and vigour in an arctic-alpine cushion plant, *Diapensia lapponica,* under different snow cover regimes. Ecological Bulletins 45:210-219.

Molau, U., U. Nordenhall, and B. Eriksen. 2005. Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. American Journal of Botany 92:422-431.

Mu, J., Y. Peng, K.J. Niklas, and S. Sun. 2014. The Optimization of Seed Yield across the Flowering Season of *Gentiana leucomelaena* (Gentianaceae), an Herbaceous Tibetan Annual. Arctic, Antarctic, and Alpine Research 46:548-557.

O'Connell, L.M. and M.O. Johnston. 1998. Male and female pollination success in a deceptive orchid, a selection study. Ecology 79: 1246-1260.

Pardee, G.L., I.O. Jensen, D.W. Inouye, and R.E. Irwin. 2019. The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs. Journal of Ecology 107:1970-1981.

Parmesan, C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology 13:1860-1872.

Pau, S., E.M. Wolkovich, B.I. Cook, T.J. Davies, N.J.B. Kraft, K. Bolmgren, J.L. Betancourt and E.E. Cleland. 2011. Predicting phenology by integrating ecology, evolution and climate science. Glob. Change Biol. 17:3633-3643.

Pearson, T.R.H., D.F.R.P. Burslem, C.E. Mullins and J.W. Dalling. 2002, Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. Ecology, 83: 2798-2807

Poulin, B., G. Lefebvre, and R. Mcneil. 1992. Tropical Avian Phenology in Relation to Abundance and Exploitation of Food Resources. Ecology 73:2295-2309.

Price, M.V., and N.M. Waser. 1998. Effects of experimental warming on plant reproductive phenology in a subalpine meadow. Ecology 79:1261-1271.

R Core Team. 2018. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/

Rafferty, N.E., C.D. Bertelsen, and J.L. Bronstein. 2016. Later flowering is associated with a compressed flowering season and reduced reproductive output in an early season floral resource. Oikos 125:821-828.

Rafferty, N.E., P.J. CaraDonna, and J.L. Bronstein. 2015. Phenological shifts and the fate of mutualisms. Oikos 124:14-21.

Rafferty, N.E., and A.R. Ives. 2012. Pollinator effectiveness varies with experimental shifts in flowering time. Ecology 93:803-814.

Rathcke, B., and E.P. Lacey. 1985. Phenological Patterns of Terrestrial Plants. Annual Review of Ecology and Systematics 16:179-214.

Robinson, S.V.J. and G.H.R. Henry. High Arctic plants show independent responses to pollination and experimental warming. Botany 96:385-396.

Sabat, A.M. and J.D. Ackerman. 1996. Fruit set in a deceptive orchid: The effect of flowering phenology, display size, and local floral abundance. American Journal of Botany 83:1181-1186.

Schaal, B.A. 1980, Reproductive capacity and seed size *Lupinus texensis*. American Journal of Botany, 67: 703-709.

Schulke, B., and N.M. Waser. 2001. Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. Oecologia 127:239-245.

Silvertown, J.W. 1981. Seed Size, Life Span, and Germination Date as Coadapted Features of Plant Life History. The American Naturalist 118:860-864

Sloat, L.L., A.N. Henderson, C. Lamanna, and B.J. Enquist. 2015. The Effect of the Foresummer Drought on Carbon Exchange in Subalpine Meadows. Ecosystems 18:533-545.

Slobodník, B. 2002. Pollination success and full seed percentage in European larch (*Larix decidua* MILL.). J. For. Sci 48:271-280.

Steltzer, H., C. Landry, T.H. Painter, J. Anderson, and E. Ayres. 2009. Biological consequences of earlier snowmelt from desert dust deposition in alpine landscapes. Proceedings of the National Academy of Sciences 106:11629-11634.

Stinson, K. A. 2004. Natural selection favors rapid reproductive phenology in *Potentilla pulcherrima* (Rosaceae) at opposite ends of a subalpine snowmelt gradient. American Journal of Botany 91:531-539.

Taylor, S.D. 2019. Estimating flowering transition dates from status-based phenological observations: a test of methods. PeerJ 7:e7720.

Thomson, J.D. 2010. Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 365:3187-3199.

Thomson, J.D. 2019. Progressive deterioration of pollination service detected in a 17-year study vanishes in a 26-year study. New Phytologist 224:1151-1159.

Towers, I.R., C.H. Bowler, M.M. Mayfield, and J.M., Dwyer. 2020. Requirements for the spatial storage effect are weakly evident for common species in natural annual plant assemblages. Ecology 101:e03185.

Vaz, A.P.A., R.D.C.L. Figueiredo-Ribeiro, and G.B. Kerbauy. 2004. Photoperiod and temperature effects on in vitro growth and flowering of *P. pusilla*, an epiphytic orchid. Plant Physiology and Biochemistry 42:411-415.

Wadgymar, S.M., J.E. Ogilvie, D.W. Inouye, A.E. Weis, and J.T. Anderson. 2018. Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. New Phytol, 218: 517-529.

Walker, M.D., R.C. Ingersoll, and P.J. Webber. 1995. Effects of Interannual Climate Variation on Phenology and Growth of Two Alpine Forbs. Ecology 76:1067-1083.

Waser, N.M. 1978. Competition for Hummingbird Pollination and Sequential Flowering in Two Colorado Wildflowers. Ecology 59:934-944.

Zimmerman, M., and R.S. Gross. 1984. The Relationship Between Flowering Phenology and Seed Set in an Herbaceous Perennial Plant, *Polemonium foliosissimum* Gray. The American Midland Naturalist 111:185-191.

TABLES

Fixed effects
Deviation
Early.late
Deviation*early.late
Number.conspecifics
Number.conspecifics *plot.treat
Treat

Mertensia								*Delphinium*								*Potentilla*		
Total seeds (dev.seed)				Proportion				Total seeds (dev.seed)				Proportion				Total seeds		
coeff	SE	n	P	coeff	SE	n	P	coeff	SE	n	P	coeff	SE	n	P	coeff	SE	n
0.002	0.002	47	0.114	-0.002	9.71 e-4	47	**0.045**	0.026	0.012	41	**0.023**	0.016	0.011	41	0.158	0.012	0.004	36
0.019	0.010	47	**0.056**	-0.006	0.006	47	0.349	0.137	0.168	41	0.415	0.135	0.156	41	0.387	0.076	0.027	36
-0.003	0.003	47	0.358	3.98 e-4	0.002	47	0.817	-0.011	0.036	41	0.767	-0.007	0.034	41	0.827	-0.014	0.005	36
-2.36 e-5	8.77 e-5	47	0.787	7.32 e-5	7.29 e-5	47	0.315	0.001	0.002	41	0.505	3.52 e-4	0.002	41	0.845	1.63 e-5	3.24 e-4	36
2.97 e-4	1.94 e-4	47	0.125	4.78 e-5	9.48 e-5	47	0.614	-0.002	0.002	41	0.250	-0.001	0.002	41	0.428	4.60 e-4	3.23 e-4	36
0.024	0.005	100	**<0.001**	-0.002	0.003	100	0.505	0.267	0.077	84	**<0.001**	0.193	0.088	84	**0.028**	0.009	0.029	67

0.745	0.154	0.960	**0.002**	**0.005**	**0.006**	**P**		

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*. Fecundity for each species is divided into the 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: deviation from the population peak bloom ("deviation"), blooming before or after the population peak ("early.late"), and their interaction (denoted with *), and conspecific density and its interaction with plot treatment ("number.conspecifics*plot.treat"). We included the coefficients, standard error, sample size, and *p* values for each result. Statistically significant results are in bold.

FIGURE LEGENDS

Fig. 1. The plot design for control and manipulated plots. We established three 1 x 10m transects for tracking the phenology of the floral community and a 1 m perimeter section within the plot for tagging individual plants of our focal species. We included a 1 m buffer zone between the phenology perimeter section and the edge of the plot. Individual plants were randomly selected and tagged in quadrats 1-30 in the perimeter section. The circles represent locations of soil moisture measurements.

Fig. 2. The effects of individual 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 individual flowering time plots, red circles represent flowers

that bloomed before the population peak and orange triangles represent flowers that bloomed after. In the conspecific density plots, green circles represent the control plot and blue triangles represent the accelerated snowmelt plot. Solid lines represent significant trends and dashed lines represent non-significant trends. Note the differences in scales on the *x*- and *y*-axes. Standard error is included in the shaded regions.

Fig. 3. The effects of individual 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 we did not have undeveloped seed counts for the proportion calculations. Each point represents an individual plant, and the lines are simple linear regression lines (i.e., not following the binomial model predictions). In the individual flowering time plots, red circles represent flowers that bloomed before the population peak and orange triangles represent flowers that bloomed after. In the conspecific density plots, green circles represent the control plot and blue triangles represent the accelerated snowmelt plot. Solid lines represent significant trends and dashed lines represent non-significant trends. Note the differences in scales on the x- and y-axes. Standard error is included in the shaded regions.