

Local impacts of global change:
shifting range limits, advancing phenology, and communicating research

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Abstract

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There are two generalizable biological impacts of climate change: first, species are shifting their distributions (both pole-ward in latitude and up in elevation), and second, species are advancing their spring phenology. Climate change is having these effects because climate controls both where species exist and when species reproduce. However, a significant gap in our knowledge are the causes and consequences of species-to-species variability in their responses to climate change, which I address in my first two chapters. In Chapter One I ask: what role (if any) do pollinators play in establishing the range limits of one subalpine flowering plant (published in *American Journal of Botany*, 2016). I find that in some years, fruit and seed set is limited by pollinators at the high-elevation range limit, suggesting that pollinators may contribute to how far uphill this plant can expand its range, and how rapidly range shifts occur. In Chapter Two I ask: what are the community-wide implications of individual species-specific phenological advance. I find that all species are sensitive to climate (primarily snow disappearance date and growing degree days, and a lesser degree soil moisture draw-down) but that they advance non-uniformly which causes phenological community reassembly in climate change-like conditions. This reassembly causes plants to co-flower in novel ways, and has the potential to alter species interactions both within and between trophic levels. Finally, in Chapter Three, I attempt to make the biological impacts of climate change (like the range shifts and phenological shifts I address in my first two chapters) more relevant to the general public, by asking if it is better to teach (undergraduates) by using local or global examples of climate change's biological impacts (published in *Frontiers in Ecology and the Environment*, 2015). I find that a single in-class activity increased students' content knowledge and leads students to more-strongly believe climate change would alter their lives, to show more willingness to alter their behavior, and to more strongly support government action. Interestingly, I also found a strong gender effect on the influence of local vs. global examples: females learned more if they studied local examples. Together, this work highlights the importance of considering how species interact with their neighbors and environment when assessing the biological impacts of climate change and the importance of implementing evidence-based teaching practices.

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CHAPTER 1:

Lilies at the limit: Variation in plant-pollinator interactions across and elevational range

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Lilies at the limit: Variation in plant-pollinator interactions across an elevational range¹

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PREMISE OF THE STUDY: Many studies assume climatic factors are paramount in determining species' distributions, however, biotic interactions may also play a role. For example, pollinators may limit species' ranges if floral abundance or floral attractiveness is reduced at range margins, thus causing lower pollinator visitation and reduced reproductive output.

METHODS: To test if pollinators influence the altitudinal distribution of *Erythronium montanum* (Liliaceae) at Mount Rainier National Park, we asked whether (1) seed production in this species relies on pollinators, (2) seed production and pollen limitation is greatest at range limits, and (3) pollinator visitation rates (either overall or by individual taxonomic groups) reflect patterns of seed production and pollen limitation.

RESULTS: From this three-year study, we established that this plant does rely on pollinators for fruit set and we found that pollen limitation trended toward being higher at the upper range limit in some years, but not consistently year to year. Insect visitation rates did not mirror spatial patterns of pollen limitation, but annually variable pollinator composition suggested differential importance of some pollinator taxonomic groups (specifically, bumblebees may be better pollinators than syrphid flies).

CONCLUSIONS: Overall, these results suggest that while pollinators are critical for the reproductive success of this high mountain wildflower, plant-pollinator interactions do not obviously drive the distribution of this species. Nonetheless, high spatio-temporal variability in range-wide plant-pollinator dynamics may complicate responses to climate change.

KEY WORDS elevation gradient; global change; mutualisms; plant-pollinator dynamics; pollination; species' distributions

Understanding range limit dynamics requires determining how both abiotic and biotic factors influence species' distributions (HilleRisLambers et al., 2013). It has long been known that abiotic factors influence species' range limits (e.g., Grinnell, 1917; Whittaker, 1956), with factors such as temperature and precipitation affecting species' metabolic performance (Huey et al., 2009; Dillon et al., 2010), survival (Thomas et al., 2004), and ultimately, geographic distributions (Root et al., 2003; Chen et al., 2011). However, biotic factors (i.e., competition, predation, facilitation, and mutualism) are less studied and less understood in their roles in influencing geographic distributions (Davis et al., 1998; Gilman et al., 2010; Wiens, 2011). For example, pollinators may limit a plant's range if plant density is critically low at the range limit, thus not providing sufficient compatible mates or attracting sufficient pollinators (e.g., Kunin, 1997; Stone and Jenkins, 2008; Elliott and Irwin, 2009; and

reviewed in Knight et al., 2005). Alternatively, plants at range limits may be less attractive to pollinators if there are resource limitations that cause plants to be smaller or produce fewer or less attractive flowers (e.g., Klinkhamer and de Jong, 1993; Grindeland et al., 2005; and reviewed in Dafni et al., 1997). Finally, an environmental gradient that limits plant populations may also limit pollinator populations (e.g., Parmesan et al., 1999; Battisti et al., 2006; and reviewed in Hodkinson, 2005). As such, this biotic interaction can strongly influence species' performance, and has been implicated in limiting performance at a few range limits (Stone and Jenkins, 2008; Hillyer and Silman, 2010; Chalcoff et al., 2012; Hellmann et al., 2012; Moeller et al., 2012). Determining how or when pollinators influence range limits is critical to predicting the impacts of climate change on species' distributions (HilleRisLambers et al., 2013), but still represents a critical gap in our knowledge (Sexton et al., 2009).

For pollinators to be important in determining plant distributions, several conditions must be met. First, the plant species must rely on pollinators for sexual reproduction. Ollerton et al. (2011) estimate that 78% of temperate-zone flowering plants rely on animal pollinators, so the potential for pollinators to drive plant fitness is large.

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Second, there must also be evidence of pollen limitation, with focal plants generally performing better at range cores (Sagarin et al., 2006), thus not meeting their full reproductive potential at range limits. Although pollen limitation is common in flowering plants (Knight et al., 2005, 2006), greater pollen limitation at range limits has rarely been demonstrated (but see Chalcoff et al., 2012; Moeller et al., 2012). Finally, patterns of pollen limitation must be attributable to lack of activity of pollinators. Although plant-pollinator interactions are known to play a large role in plant reproductive success (Ashman et al., 2004), and even plant community assembly (Sargent and Ackerly, 2008), few examples outside of studies on invasive species consider the role of pollinators in plant range expansions or contractions (Parker, 1997; Barthell et al., 2001; Stout et al., 2002; Liu et al., 2006; but see Chalcoff et al., 2012; Moeller et al., 2012; Hargreaves et al., 2015). While it is known that pollinator abundance, activity, and metabolic performance may decrease with temperature and elevation (Hodkinson, 2005), our understanding of the role of pollinators in determining species' distributions is limited because it has been hard to demonstrate the link between pollen limitation and pollinator activity (HilleRisLambers et al., 2013).

To address this topic, we monitored plant-pollinator interactions across the altitudinal range of the early flowering plant *Erythronium montanum* S. Wats. (Liliaceae) at Mount Rainier over three years. Specifically, we asked: (1) How critical are pollinators for seed production of this species? (2) Is pollen limitation greatest at range limits? And (3) do visitation rates by pollinators (or pollinator functional groups) match range-specific pollen limitation, validating a role for pollinators in determining range limits? If pollinators are critical for *E. montanum*'s reproductive success, and their contribution is reduced at range limits, plant-pollinator interactions may, at least partly, determine the distribution of this species.

MATERIALS AND METHODS

Study site and species—For three summers (2011–2013) we collected data in the subalpine wildflower meadows of Mount Rainier National Park, a 4392 m tall volcano in the Cascade mountain range in western Washington State, USA (located at 46.8529°N, 121.7604°W). Generally, the region experiences a maritime climate with dry summers and wet winters, with the majority of winter precipitation falling as snow. This climate pattern creates a snow-driven flowering regime in the spring and summer, similar to other high elevation and high latitude regions (Kudo, 1991; Inouye, 2008; Wipf et al., 2009; Hülber et al., 2010). The vegetation on the mountain is dominated by coniferous forests at lower elevations (< 1450 m) giving way to subalpine wildflower meadows at midelevations (1450–1900 m), and bare ground, rock, and ice at the highest elevations (1900 m and above; Franklin et al., 1988).

Erythronium montanum (Liliaceae) is an early flowering geophyte that carpets the subalpine meadows between ~1500–1800 m. *E. montanum* buds through the snow, and immediately after snowmelts individual plants bloom with single stalks displaying one, two, or rarely three flowers. Pendant flowers stand on a single stalk, and have mostly white, eventually reflexed, tepals with a yellow center. Individuals display flowers once per year for approximately 2 wk. After pollination, a capsulate fruit develops for seven to eight weeks, before dispersing no more than 25 seeds.

We selected three (in 2011) or four (2012–2013) sampling sites on the south side of Mount Rainier, evenly spaced along the elevational

gradient (ranging from 1490 m in elevation to 1800 m) to represent different range positions (lower limit, midlow-range (mid-L), mid-high-range (mid-H), and upper limit). Plots within sites were in open subalpine meadows that varied in proximity to forest (details below and in Appendix S1; see Supplemental Data with the online version of this article).

The role of pollinators for plant reproduction: Selfing and pollen limitation—We performed two experiments to assess the role of pollinators in the seed production of *Erythronium montanum* at each of our four range position sites. In the first, we excluded pollinators to determine if seed production in *E. montanum* is dependent on pollinators. In the second, we supplemented pollen to assess if seed production is pollen limited, particularly at range limits. We compared both of these manipulations to unmanipulated controls, as we describe in the paragraphs below.

Selfing—To determine the degree of selfing in *Erythronium montanum*, we excluded pollinators from treatment plants with bags and compared seed production to nearby unmanipulated (open-pollinated) control plants—the same individuals we used as controls for pollen limitation measurements. In each year we identified and tagged between 5 and 63 single-flowered individuals for the bagging treatment at each range position (Appendix S1). Small sample sizes in some site-year combinations resulted from low densities of the plant at lower elevations and high elk herbivory of bagged flowers at one site (mid-L). Individuals were bagged after emergence but before flower buds opened to ensure that pollinators had not yet visited the flowers. Mesh bags were secured to entire plants (with single buds) using plastic twist ties or ribbon drawstrings. In 2013, bumblebees were observed ripping open some closed buds (E. Theobald, personal observation). However, it is unlikely that this greatly affects our estimates selfing rates for two reasons: first, pollen is not readily available and the stigma is not likely receptive before the bud is open; and second, this behavior was extremely rare (relative to the frequency of bumblebee visits that year). Nonetheless, it is possible that our measurements slightly overestimate selfing rates, thereby underestimating the contribution of pollinators to reproductive success.

Pollen limitation—To assess if seed production is pollen limited at our three or four range positions, we supplemented pollen to flowers and compared seed production to paired unmanipulated control flowers. We carried out supplemental pollinations at approximately similar phenological stages each year. At each range position site, we identified between 12 and 81 single-flowered individuals to supplement with pollen; we also identified control plants (receiving no supplemental pollen) no more than 30 cm from each treatment plant, matched for plant size, flower orientation, and proximity to con- and hetero-specifics (Appendix S1; see Supplemental Data with the online version of this article). In 2012 and 2013, pairs were approximately evenly distributed in 15 to 30 1-meter-by-1-meter square plots (1 to 4 pairs per plot, depending on the number of plants available per plot), with plots at least 5 m apart and spread across the meadow at each range position. In 2011, we used a slightly different experimental design, with pairs separated by at least 5 m (but not in plots) and spread across each site.

To supplement pollen we applied pollen with plastic toothpicks to stigmas until they were visibly covered with pollen. To ensure outcrossing, we collected and mixed (in Eppendorf tubes) pollen

from at least 20 individuals in at least five different patches at each site, i.e., donor patches were at least 10 m apart from each other and at least 10 m from the focal individuals. We used pollen within 5 hours of collection.

We measured seed production for all individuals (bagged, supplemental pollination, and control). We harvested fruits once visually mature (28–46 d). Harvested fruits were dried in an oven at 30°C for 7–12 d, after which seeds were counted. If fruits had not been initiated, we recorded these as zero fruit produced. If fruits had clearly aborted, we collected and counted seeds within them. Thus, we were able to determine treatment effects on fruit development (yes/no) as well as seed production (count) contingent on fruits being produced.

To assess if reproduction is also influenced by environmental factors that may vary across the range of *Erythronium montanum*, in 2012 and 2013 we quantified several environmental variables around all focal individuals. Specifically, we quantified canopy cover of trees and shrubs (at the plot level, using a hemispherical densiometer held at flower-height), which we expected to correlate to light availability known to influence plant performance. We additionally quantified soil moisture when individuals were identified, at the pair-level, for hand-pollinated and control plants, and at the individual level for the bagged plants (using a ProCheck reader (Decagon Devices, Pullman, Washington, USA) and a EC-5 Soil Moisture Sensor (Decagon Devices)), which we expected to correlate with water availability, also known to influence plant performance. Finally, we measured conspecific stem density within the meter-square plot, as density could influence plant performance either through competitive or facilitative effects. We only measured these variables in 2012 and 2013, reflecting a slightly different experimental design in those years.

Visitation rates—We measured flower visitation rates in each range position site and year, adjacent to experimental plants, during 15–30 min sampling intervals throughout the course of the 4–6 wk flowering season (Appendix S1; see Supplemental Data with the online version of this article). We watched flowers in ~1 m² patches across sites at roughly similar phenological stages. We characterized a flower visitor as a pollinator if the insect made contact with flower reproductive organs. We identified insect visitors on the wing to gross taxonomic groups (syrphid flies, bumblebees, and other visitors). For each observation period, we also measured a number of biotic and abiotic covariates, specifically: (1) conspecific density (number of flowers and individuals); (2) the dominant flowering species within 5 meters (*Erythronium montanum* or other); (3) percent canopy cover at flower height; (4) air temperature; (5) wind speed; and (6) percent cloud cover (a visual estimate). We also measured the number of days since snow-melt at each site by averaging the date of snowmelt from a network of 10 microclimate sensors (HOBO Pendants (Onset Computer Corporation, Bourne, Massachusetts, USA) or iButtons (Maxim Integrated, San Jose, California, USA) located at each range position site; sensors were systematically distributed at least 20 m apart along ridges (5 sensors) and depressions (5 sensors), to capture within-site variability in snowmelt (Appendix S1; see Supplemental Data with the online version of this article). In 2011 we did not have microclimate sensors at our lowest site, and used the nearest site within this network (~40 m higher in elevation) instead.

Statistical analyses—*Role of pollinators for plant reproduction*—*Selfing*—To evaluate the importance of pollinator access to fruit

(binomial) and seed production (Poisson), we compared plants that were bagged (pollinators excluded) to unmanipulated controls with generalized linear mixed effects models applied to each year of data.

We employed three steps in our data analysis. First, we used a model selection approach to determine whether random effects (of plot) needed to be included in models. We did this by fitting models that varied in random effects but had the same fixed effect (range position, treatment, and their interaction, as recommended by Zuur et al., 2009). We did so for 2012 and 2013 data only, as individual plants were not clustered by plot in 2011. We selected best-fit models based on AIC_c (Akaike's Information Criterion, with a correction for small sample size; Burnham and Anderson, 2002). If AIC_c differed by less than 2.0 units we selected the model with the simpler random effects structure (Burnham and Anderson, 2002). Second, we tested our hypotheses by determining which combination of explanatory variables (range position and/or treatment and their interaction) best explained the data, keeping the random effect structure constant (determined in step 1). We did this by comparing model fit of five models (using the method described previously), ranging from the null model (with only random effects if applicable), to a full model with range position, treatment, and a range position by treatment interaction (and random effects if applicable). We interpreted directionality and strength of coefficients in best fit models to qualitatively evaluate our hypotheses. Third, we verified that including environmental variables (canopy cover, conspecific stem density, and soil moisture, only measured in 2012 and 2013) in the final model did not qualitatively alter our conclusions (i.e., the magnitude and direction of coefficients).

We chose an information-theoretic approach to choose random effects structure, to test our hypotheses (with each model representing an alternative hypothesis), and to verify that environmental covariates were not responsible for patterns (i.e., if environmental variables covary with range position they are likely to influence response variables). We chose this approach over significance testing for all our statistical analyses for consistency, to avoid performing multiple significance tests (given we had multiple possible random effects structures, parameters, and covariates), and to simultaneously evaluate multiple competing hypotheses (models) in terms of their support in the observed data (Johnson and Omland, 2004).

Role of pollinators for plant reproduction—*Pollen limitation*—To determine whether fruit production or seed number in *Erythronium montanum* is pollen limited (i.e., if the pollen supplement treatment increased fruit set and number of seeds per fruit), and whether patterns depend on range position, we compared plants which received our supplemental pollination treatment to unmanipulated controls with generalized mixed effects models applied to each year of data. Our approach was nearly identical to that explained earlier, so we only briefly describe the steps here, noting differences. Our first step was to determine the appropriate random effects structure (Zuur et al., 2009), with possible random effects including pair (in all years) and plot (only in 2012 and 2013). Our second step was to determine whether range position, treatment, and their interaction helped explain the data, keeping the random effect structure constant (from the first step). Third, we tested if environmental variables qualitatively changed our conclusions (only in 2012 and 2013). Again, we interpreted directionality and strength of coefficients in best fit models to qualitatively evaluate our hypotheses.

Visitation rates—To assess whether insect visitation rates varied by range position, we fit generalized linear models using a negative binomial distribution, which accounts for excessive zeros (Lindén and Mantyniemi, 2011). We modeled overall visitation rate and controlled for the duration of the observation period and the number of flowers watched by including an offset, i.e., $\log(\text{flowers watched}/\text{duration of observation period})$. To select the best and most parsimonious model, we again employed a model selection approach but without random effects selection, and with only one explanatory variable of interest (range position). Thus, we simply compared four models in one model selection step: (1) the null model; (2) a model with only range position; (3) a model with only environmental variables; and (4) a model with range position and environmental variables. We fit models to data individually by year because we had quantified more environmental variables in 2012 and 2013 than we had in 2011. The environmental variables we tested included: (1) time into the growing season (days since snow melt) and (2) conspecific dominance within 5 m (yes or no) (2011), and additionally for 2012 and 2013 included (3) conspecific stem density (per m^2), (4) shrub and tree canopy cover, (5) cloud cover, (6) air temperature, and (7) average of the minimum and maximum wind speed recorded during the observation period. We only included environmental variables that were not strongly correlated with each other (Pearson's correlation of <0.6). We selected the best-fit model based on AIC_c scores as above.

To explore how floral visitor communities differed by year, we assessed whether visitation rates by taxonomic groups (syrrhid flies, bumblebees, and other visitors) varied by range position and year. These categories allowed us to distinguish between different foraging behaviors while maintaining group sizes sufficient for analyses. Low visitation rates by some taxonomic groups precluded fitting a range position by year interaction, so we only included year and range position main effects. We used a zero-inflated negative binomial distribution to account for overdispersion (as data included excessive zeros) in visitation rates. Thus, we compared four models: (1) the null model; (2) a model with year; (3) one with range position; and (4) one with year and range position.

Complex models and missing data led to convergence errors for a small subset of models (never best-fitting models). In one case, this necessitated combining data from our two midelevation range positions into one range position; details are in Appendix S2 (see Supplemental Data with the online version of this article). All analyses were conducted in R 3.2.2 (R Development Core Team, 2013). We used the lme4 package for fitting mixed effects models (Bates et al., 2015), the MASS package for fitting negative binomial models (Ripley et al., 2013), and the pscl package for fitting zero-inflated negative binomial models (Jackman et al., 2013). All data are available as online supplemental material (see Supplemental Data with the online version of this article, Appendix S3 for experimental data and Appendix S4 for visitation data).

RESULTS

The role of pollinators for plant reproduction: Selfing and pollen limitation—*Selfing*—Overall we found that pollinators increase fruit set and seed production in *Erythronium montanum*, i.e., bagging reduced fruit set and seed number per fruit each year (Fig. 1; Table 1A). In 2012 and 2013, range position by treatment interactions resulted in better model fits (Table 1A), but range position

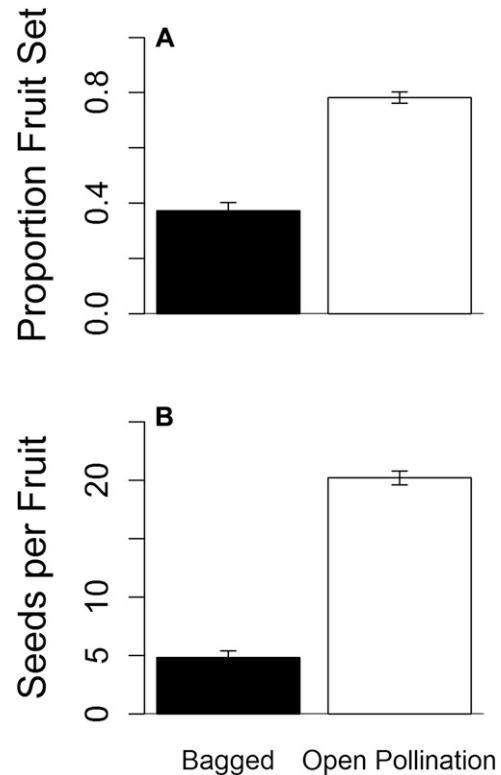


FIGURE 1 The level of pollinator-dependence in fruit and seed production of *Erythronium montanum*. (A) Open pollinated flowers (white bars) set a greater proportion of fruit than bagged flowers, and (B) when they did set fruit, open pollinated flowers (white bars) set more seeds on average than bagged flowers (black). The range position by treatment interaction that was retained in final models in 2012 and 2013 did not differentiate margins from midrange positions, so all range positions were combined; additionally, the patterns were similar for each year so all three years are combined here. Sample sizes for each treatment in each year can be found in Appendix S1, and data are available in Appendix S3; see Supplemental Data with the online version of this article.

differences in treatment effects never differentiated range limits (i.e., lower or upper) from the range center. Across all years, plants that were bagged to exclude pollinators set fruit approximately half as frequently as control plants (Fig. 1A). Furthermore, of those plants that did set fruit, bagged plants produced less than a quarter as many seeds (Fig. 1B).

Pollen limitation—Pollen limitation varied among years, with greater pollen limitation at the highest range position in two of the three years, although best-fitting models only included a range position \times treatment interaction in one of those years (Fig. 2; Table 1B). Specifically, on average, hand-pollinated plants set fruit more often than control plants in 2011 (Fig. 2A), with the effects of the pollen addition treatment higher at the upper range limit, but the interaction between pollination treatment and range position was not retained in the final model (Table 1B). However, seed production did not differ between treatments or range positions in 2011 (Fig. 2D; Table 1B). In 2012, neither range position nor pollen supplementation affected fruit or seed production (Fig. 2B, E). In 2013, all plants (regardless of treatment) set more fruit at both midrange range positions compared to the lower and upper limits (Fig. 2C).

TABLE 1. Parameter estimates from best-fit models generated to explain the degree of pollinator reliance (A—bagging vs. control treatments) and degree of pollen limitation (B—supplemental pollination vs. control) in *Erythronium montanum* on the probability of fruiting and seed count per fruit (contingent on fruits being produced). Models are generalized linear mixed effects or generalized linear models of each year, and were selected based on a stepwise-procedure that compared AIC_c (see text for details). Table shows additive effects. Reference categories and random effects are shown in superscripts. Data are available in Appendix S3; see Supplemental Data with the online version of this article.

Response variable Year	ΔAIC_c^1	Intercept	Range Position ²		Treatment ³	Range Position × Treatment Interaction ⁴		
A) Selfing (bag vs. control)								
P(fruiting)⁵								
2011	19	−0.431			bag	−3.419		
2012	41	2.296			bag	−2.510		
2013 ⁶	24	−0.031	mid-L	2.985	bag	−0.406	mid-L × bag	−1.437
			mid-H	4.101			mid-H × bag	−4.226
			high	1.233			high × bag	−1.478
Seed count^{7,8}								
2011	26	2.707	mid	0.132	bag	−0.866		
			high	0.866				
2012 ⁶	65	2.963	mid-H	0.167	bag	−0.478	mid-H × bag	−1.399
			high	0.177			high × bag	−2.043
2013 ⁶	92	2.773	mid-L	−0.010	bag	−1.654	mid-L × bag	0.730
			mid-H	0.146			mid-H × bag	−0.483
			high	0.067			high × bag	0.456
B) Pollen limitation (supplemental pollination vs. control)								
P(fruiting)⁵								
2011	12	−2.541	mid	2.833	sup. poll.	1.169		
			high	2.136				
2012 ⁹	0	6.942						
2013 ⁶	19	−0.8454	mid-L	NA				
			mid-H	7.1500				
			high	2.6500				
Seed count^{7,8}								
2011 ⁹	0	2.5165						
2012 ^{6,9}	0	3.001						
2013 ⁹	21	2.769	mid-L	0.178	sup. poll.	−0.019	mid-L × sup. poll.	0.016
			mid-H	0.145			mid-H × sup. poll.	0.105
			high	0.049			high × sup. poll.	0.241

Notes: ¹Null model (which includes the appropriate random effect structure) minus the best model, rounded to the nearest whole number; ²Reference group is lower range margin; ³Reference group is control treatment; ⁴Reference group is control treatment at lower range; ⁵Binomial response, proportion fruiting, logit coefficients reported; ⁶Plot random effects included in the final model; ⁷Contingent on fruits being produced; ⁸Poisson response, number of seeds per fruit, log coefficients reported; ⁹Pair random effect included in the final model. NA: All fruits from the supplemental pollination treatment set at the low-mid range position in 2013 so estimating the interaction of treatment by range position for that parameter is not possible. For this analysis, the midlow (mid-L) range position was combined with the midhigh (mid-H) range position to create one single midrange range position.

At low-, midlow-, and midhigh-range positions there was no effect of the pollen addition treatment on seed count, but at the upper range limit there were on average fewer seeds per fruit in control fruits (Fig. 2F), thus the range position × treatment interaction was retained in the final model of seeds per fruit (Table 1B).

Visitation rates—The number of visits to *Erythronium montanum* was generally low, with no difference in overall visitation rates between different range positions in any of the years (Table 2A; Fig. 3), even when controlling for any measured environmental variables. The best fitting models, which never included range position, were either the null model (2011 and 2012) or only included environmental variables (2013; Table 2A).

Separating visitation by taxonomic groups revealed different results (Table 2B). Specifically, for syrphid flies (including large-bodied syrphids in the genus *Volucella*), visitation varied by year (but not range position; Table 2B), indicated by the large variation in pollinator communities shown in Fig. 3. Visitation by syrphid flies was highest in 2011, followed by 2013 and 2012. For bumblebees, the best-fitting model included both range position and year (Table 2B), but model fit was similar for all models (i.e., within 4 AIC_c units). Visitation was greatest for bumblebees in 2012 and 2011,

followed by 2013. There were slightly more bumblebee visits to *Erythronium montanum* in midrange positions than the high-range position and no differences between mid- and low-range positions. There was no clear pattern in visitation for other visitors (including solitary bees, muscid flies, beetles, and the occasional ant) as the null model was selected as the best-fitting (Table 2B).

DISCUSSION

Do pollinators influence the altitudinal distribution of *Erythronium montanum*? We expected to find that (1) *E. montanum* relies on pollinators for seed production, (2) reproductive performance is depressed at range margins, and (3) reduced reproductive output at range margins is matched by low pollinator visitation rates. Our results were more complex thus the interpretation is more nuanced. We did find that pollinators are critical for the reproductive performance of *E. montanum*, i.e., excluding them led both to lower fruit set and a lower number of seeds per fruit (Fig. 1). Additionally, our results also suggested that pollen limitation *may* be greater at range limits, but only at the upper range limit, only in some years, and never overwhelmingly (Fig. 2A, F). Finally, patterns

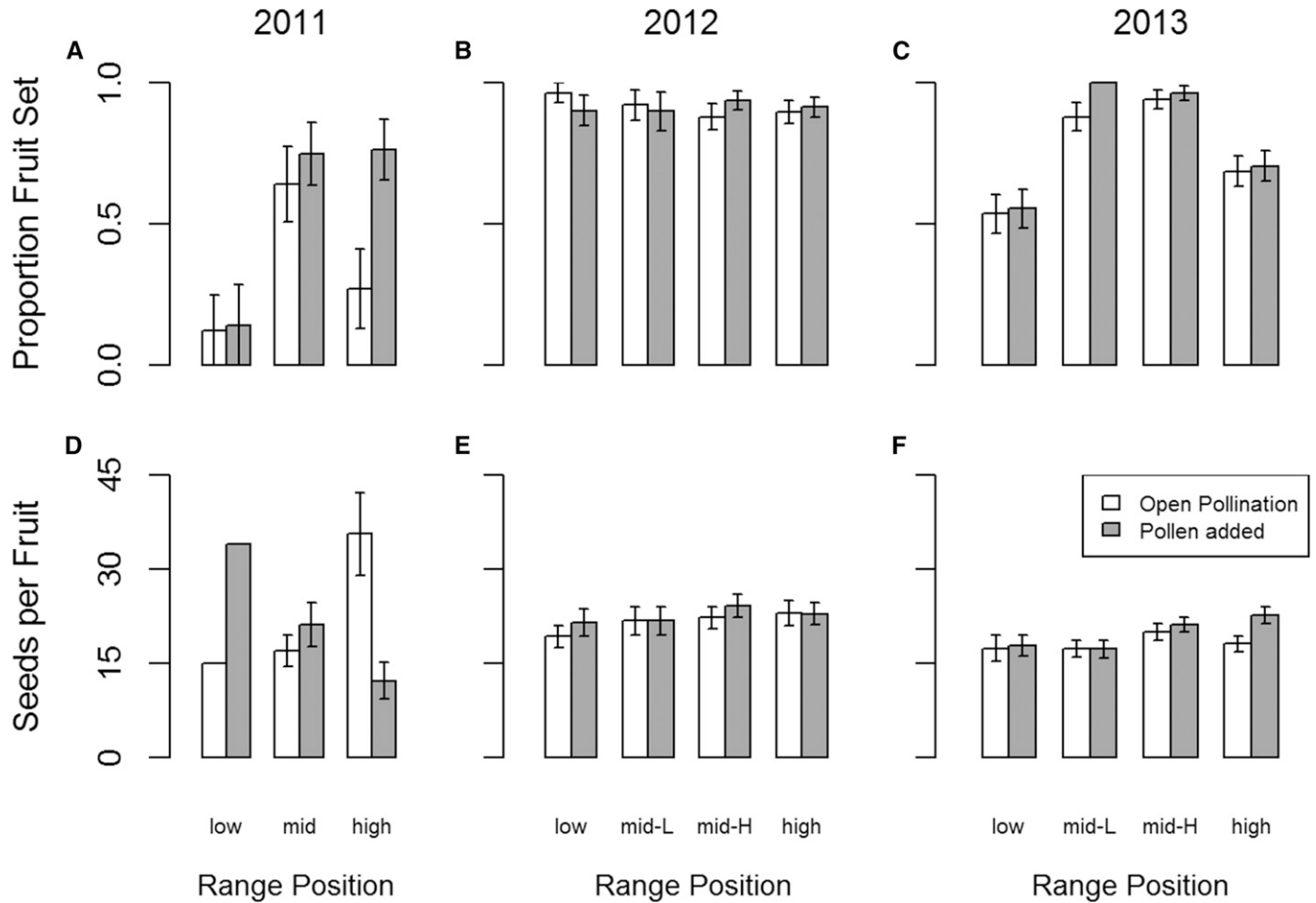


FIGURE 2 The effect of pollen supplementation on the proportion of fruits set (A–C) and the number of seeds in each fruit (D–F) in *Erythronium montanum* over an altitudinal range in 2011 to 2013. Gray bars indicate pollen-supplemented flowers, while white bars indicate control flowers. Difference between bars indicates the degree of pollen limitation. In 2011, there was a trend for pollen limitation at the upper elevation range limit in proportion fruit set (A), but not in seeds per fruit (D). In 2012 (B, E), there was no pollen limitation at any range position. In 2013, there was pollen limitation at the upper elevation range limit in number of seeds set per fruit (F) but not in proportion fruit set (C). In 2013, all hand-pollinated plants set fruit at the midlow (mid-L) range position explaining the lack of error bars (C). Error bars indicate standard error; some error bars are missing either because of 100% fruit set (2013) or low sample size (2011); details in Appendix S2. Sample sizes for each treatment in each year can be found in Appendix S1, and data are available in Appendix S3; see Supplemental Data with the online version of this article.

in pollinator visitation rates did not mirror patterns in pollen limitation and generally were not reduced at range margins (Fig. 3). Below, we discuss evidence for and against the importance of pollinators in determining range limits, and how annual variability could lead to the complex patterns we observed.

It is clear that pollinators strongly influence fitness of *Erythronium montanum* (Fig. 1A, B). In all three years, plants were more likely to set fruit when pollinators were allowed full access to flowers; furthermore, even if a bagged plant did set fruit, it produced fewer seeds than a fruit from a control plant. These results are similar to findings in a congener, *E. americanum* Ker Gawl. (Harder et al., 1985) and illustrate the importance of pollinators. Nevertheless, many plants did produce fruits when pollinators were excluded. Perhaps autonomous self-compatibility in this species has evolved as an adaptive response to low-pollinator environments, as suggested by others (Morgan and Wilson, 2005; Ashman et al., 2004; Busch, 2005; Hargreaves et al., 2015). If so, autonomous selfing may ensure reproductive success for *E. montanum* when outcrossing is

limited (Moeller, 2006; Eckert et al., 2010; Bodbyl Roels and Kelly, 2011; Hargreaves et al., 2015), as it likely is in years when pollinator visits are low in frequency or dominated by less-effective pollinators such as syrphid flies (Fig. 3).

Although we found pollen limitation in some years and places (Fig. 2), the relationship between pollen limitation and range position of *Erythronium montanum* is less clear. We never found evidence of greater pollen limitation at the lower range limit, suggesting that other processes constrain population growth there. Direct climatic effects on growth and survival may explain the lower overall fruit set in 2011 and 2013 at the lowest elevation (Fig. 2). For example, frost may be particularly damaging to subalpine plants that flower early in the growing season (Inouye, 2000; Bannister et al., 2005; Inouye, 2008; Thomson, 2010) and may have negatively impacted fruit set here. Alternatively, growth at the lower range limit could be constrained if there is greater competition for light with canopy trees, which could decrease survival and reproduction (e.g., Ettinger et al., 2011), though canopy cover was never retained in

TABLE 2. Parameter estimates from best-fit zero-inflated negative binomial models explaining patterns of overall visitation rates (A) and visitation rates by individual insect functional groups (B). Note that range position was not retained in any year in the best-fitting model but was retained in the model explaining bumblebee visitation. The group syrphid flies includes small- and large-bodied syrphids in the genus *Volucella*. The group “Other Visitors” includes solitary bees, muscid flies, beetles, and ants. Table shows additive effects. Reference categories are shown in superscripts. Data are available in Appendix S4; see Supplemental Data with the online version of this article.

A) Overall visitation rates

Year	ΔAIC_c^1	Intercept	Covariates
2011	0	-0.002	
2012	0	-1.805	
2013	10	-1.006	Environmental Variables Only ²

B) Individual visitation rates by functional group

Group	ΔAIC_c^1	Intercept	Year ³	Range position ⁴		
Syrphid Flies	62	-0.046	2012	-3.117		
			2013	-0.947		
Bumblebees	4	-3.173	2012	0.615	mid	1.694
			2013	-1.454	high	0.667
Other Visitors	0	-3.217				

Notes: ¹Null model minus the best model, rounded to the nearest whole number; ²Environmental variables were the only explanatory variables retained in the model and never impacted our variable of interest (range position). The environmental variables had the following effects on visitation rates (- for negative, + for positive): neighbor (+); canopy (+); density (-); days since snow (-); average wind (-); temperature (+); cloud cover (-); ³Reference group is 2011; ⁴Reference group is lowest range position, mid-elevations were combined.

final models explaining fruit set (Table 2). By contrast, we did find some evidence of greater pollen limitation at the upper range limit (Fig. 2), but only for one year and moderately so for an additional year (Fig. 2A, F; Table 1B; range position \times treatment interaction not retained in the final model likely due to small sample size).

Additionally, we did not find strong evidence that patterns of visitation rates mirror patterns of pollen limitation (our third expectation; Figs. 2 and 3). However, variation in visitor functional groups with differing pollination efficacies might provide a possible explanation for annual differences in pollen limitation. Year-to-year variation in pollinator abundance is common (Herrera, 1988; Price et al., 2005; Alarcón et al., 2008; Burkle and Alarcón, 2011), and has

similar morphology to *E. montanum* (Thomson, 1986; Thomson and Thomson, 1989). More studies would be needed to specifically test these hypotheses of differential pollinator efficacy and the impacts of foraging behavior on community-level pollen quantities (e.g., Alarcón, 2010; King et al., 2013).

CONCLUSIONS

In total, our results suggest that plant-pollinator interactions are important for the success of this species, but do not consistently influence reproductive success at range limits. Our results suggest that annual variability in plant-pollinator interactions is substantial: annual differences in reproductive success were greater than differences among range positions in reproductive success (Fig. 2). Similarly, annual variation in insect visitation rates, both total visitation and visitation by key functional groups, is large (Fig. 3). Pollen limitation is weak and episodic in nature, potentially leading to variable reproductive success across this altitudinal range of *Erythronium montanum*. This variation may in part contribute this plant's distribution, as increasingly variable plant-pollinator interactions could result in increasingly variable reproductive output, but the role of annual variability in pollinator services for species' distributions remains unknown. There is, however, growing evidence that large inter-annual abiotic variability may be as, or more, important than climatic means in influencing species' distributions (Jentsch et al., 2007).

Understanding the role of biotic factors, such as mutualisms, across large climatic gradients is particularly important given the importance and prevalence of climate change

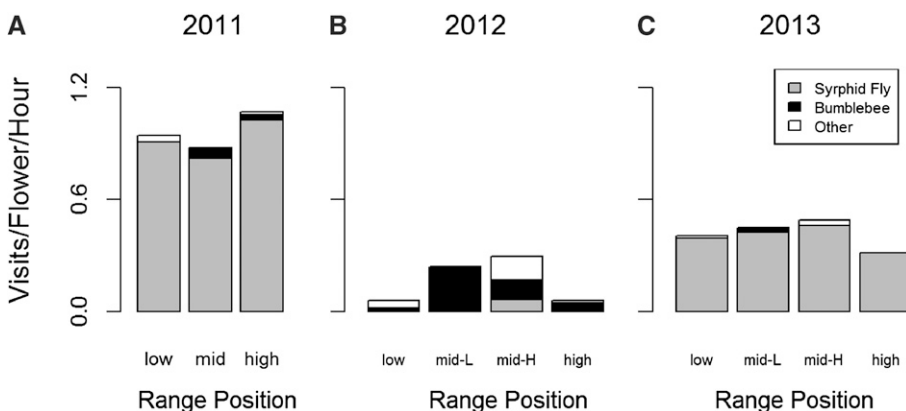


FIGURE 3 Overall insect visitation and visitor composition to flowers of *Erythronium montanum* across an altitudinal range in 2011 to 2013. Overall visitation did not vary by range position in any year. Syrphid flies visited more frequently in 2011 than in 2012 or 2013, but there were more syrphids in 2012 than in 2013. There was no difference in bumblebee visitation between 2011 and 2012 or between 2011 and 2013, but in 2012 bumblebees visited more than in 2013. There was no trend in other visitors (including solitary bees, muscid flies, beetles, and ants) across year or range position. Total hours of observations at each range position in each year are indicated in Appendix S1, and data are available in Appendix S4; see Supplemental Data with the online version of this article.

(Hegland et al., 2009; Sexton et al., 2009; Gilman et al., 2010). Pollinators are critical to reproductive success of this species, which means that direct physiological impacts of climate, as well as indirect effects of climate mediated through plant-pollinator interactions, may both influence climate change responses of this species. We found that pollen limitation may be greater at the upper range limit of *Erythronium montanum*, but that these constraints are episodic in nature and may only potentially influence this species' ability to expand its distribution upwards. More research into the complex interplay of biotic and abiotic range determination is needed as climate-induced range shifts are gaining documentation.

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CHAPTER 2:

Climate change-induced phenological reassembly of a flowering community

Elli J. Theobald, Ian Breckheimer, and Janneke HilleRisLambers

ABSTRACT

Many studies have documented shifts in phenology driven by ongoing climate change, and it is clear that species differ in the degree to which their phenology is shifting. However, there is a general lack of understanding of the community-level implications of these individual phenological shifts. To determine how and whether differences among species in their sensitivities to climate drivers, and thus, species-specific phenological shifts drive the phenological reassembly of floral plant communities, we quantified the relationship between flowering phenology and climate for 48 subalpine wildflower species over six years at 70 climatically variable locations at Mount Rainier National Park. Specifically, we measured flowering presence and absence weekly over each growing season to estimate species-specific relationships between three climate drivers (date of snowmelt, growing degree day accumulation, and soil moisture draw-down) and the timing and duration of flowering. Then, we used this information to assess whether phenological community reassembly ensued, by comparing floral communities in an extremely warm and dry year characterized by early snow melt (2015 – a climate change ‘natural experiment’) to climatically typical years (2010-2014). We found that our species differed in their phenological sensitivities to all climate drivers, with these differences leading to floral community reassembly in early snowmelt years (conditions which are projected to be average in mid-century). Community reassembly was most apparent early and late in the flowering seasons and in locations where snow duration is shortest (e.g., at low elevations and on ridges and slopes in the landscape). Because such floral community reassembly may have implications for plant-pollinator dynamics (e.g. phenological mismatches and plant-plant competition for pollinators), failing to monitor community-level implications of species-specific phenological shifts could underestimate the extent of climate change impacts.

INTRODUCTION

Shifts in phenology, the timing of biological events, are both a symptom and a harbinger of climate change (Walther et al. 2002; Root et al. 2003; Parmesan 2006). Numerous studies have documented shifts in the timing of plant flowering that coincide with warming trends in regional climate (e.g., Fitter and Fitter 2002; Inouye 2008). Despite the generality of this pattern, not all plant species are shifting their reproductive phenologies uniformly (Parmesan and Yohe 2003; Parmesan 2007; Thackeray et al. 2010). Instead, some species are advancing their reproductive timing, some species are delaying it, and a substantial portion are not shifting at all. Such species-specific phenological shifts likely arise because species are differentially sensitive to key climatic drivers (e.g. growing season temperature vs. water availability), each of which are changing at different rates in different places. Regardless of the cause, these species-specific shifts in phenology could influence floral communities, and potentially, the ecosystem functions that depend on them (Diez et al. 2012).

Surprisingly, few studies have explored the community-level implications of species-specific phenological sensitivities to climate change (but see Diez et al. 2012 and CaraDonna et al. 2014). For example, multi-species studies exist, but most attempt to generalize species-specific differences in phenological sensitivity to climate change by examining how trophic levels (Voigt et al. 2003; Parmesan 2007; Thackeray et al. 2010), traits (e.g., Miller-Rushing and Primack 2008; Molnár et al. 2012; Cortés-flores et al. 2015), or identity of climate variables

(e.g., Wolkovich et al. 2012, Diez et al. 2012) drive species-specific phenological responses. Others have attempted to assess community-level responses from the perspective of impacts on a subset of species in communities (e.g. Forrest et al. 2010). The few community-level studies that exist document community changes in first-flower dates (Diez et al. 2012), or pair-wise occurrences within communities (CaraDonna et al. 2014), without determining how the community as a whole is altered – for example if phenological community reassembly is likely (Figure 1).

Climate-change-induced phenological community reassembly is caused and characterized by two conditions, which must be met for phenological community reassembly to occur in a warmer world. First, species must be sensitive to the key climate drivers that are changing. Second, species must differ in their sensitivities to these climate drivers, either by having different sensitivities to the same climate drivers or by being sensitive to different climate drivers (Figure 1). Reassembly is then characterized by species co-flowering in new ways, for example, through increased or decreased overlap between flowering (Figure 1). These changes in overlap are caused by shifts in the timing of flowering or in changes in the duration of flowering, which are dictated by each species' individual sensitivity to climate. In turn, these new co-flowering patterns are measured by changes in richness, similarity, and novelty between typical communities and climate-change communities (Figure 1).

Understanding if and where phenological community reassembly happens is critical, because such shifts are likely to influence the nature and strength of ecological interactions that are shaped by their community context. The majority of plant species rely on animal pollinators for sexual reproduction (Ollerton et al. 2011), implying that changes in community-wide flowering phenology under climate change may result both in the loss and the gain of interactions between plant species and their pollinators. This will also alter plant-plant interactions, both by changing competitive and facilitative interactions mediated by shared pollinators. In essence, these novel plant interactions could negatively impact reproductive success and population persistence of some species while benefiting others. From the pollinator's perspective, differential shifts and novel progressions of the flowering community could alter the available floral resources throughout the growing season (Aldridge et al. 2011), potentially negatively impacting pollinator populations in the long term (Potts et al. 2010; Vanbergen and Initiative 2013). The community-level impacts of individualistic phenological shifts could therefore have important trophic implications and have wide-reaching conservation repercussions (Morellato et al. 2016). Thus, identifying sites and communities with the largest magnitude of reassembly will help target research effort on the ecological consequences and help land managers identify species and sites most at risk.

To better understand the potential for floral community reassembly and its likely causes, we studied the floral phenology of a community of 48 species observed over 6 years at 70 locations in the subalpine meadows of Mount Rainier National Park. Specifically, we 1) estimated species-specific climate sensitivities to three climate drivers (snow, growing degree days, and soil moisture) in terms of the timing of peak flower and the flowering duration, and 2) used these estimates and a 'natural experiment' of climate change (a year mimicking conditions projected to be average in the late 21st century) to calculate the degree to which species-specific climate sensitivities resulted in floral community reassembly. We also dissected this floral community reassembly to determine when (i.e., early or late in the season) and where (i.e., which locations across elevation bands as well as topographic positions in the landscape) floral community reassembly occurs. We hypothesized that species have individualistic sensitivities to

snowmelt, soil moisture, and growing degree days, which would affect both the timing of flowering and the duration of flowering. We expected that these species-specific sensitivities would cause floral community reassembly with warming. However, because the rate and direction of climate change (in key drivers of phenology) *and* local community composition (which may contain species that differ in their sensitivity to climate change) vary spatially and temporally, we expected that community reassembly might also vary spatially and temporally (i.e. with the flowering season).

METHODS

Data Collection –

We collected phenology data in the subalpine wildflower meadows of Mount Rainier National Park for six summers (2010 – 2015). Mount Rainier (located at 46.8529°N, 121.7604°W) is a 4392 m tall volcano in the Cascade mountain range in western Washington State, USA. The region experiences a maritime climate with dry summers and wet winters, with the majority of winter precipitation falling as snow (Franklin et al. 1988). Mt. Rainier is dominated by coniferous forests at lower elevations (< 1450 m), subalpine wildflower meadows at mid-elevations (1450 m – 1900 m), and bare ground, rock, and ice at the highest elevations (1900 m and above; Franklin et al. 1988).

On the southern slope of Mt. Rainier, we identified five sites, spaced approximately 100m apart in elevation. Collectively, these sites spanned the distribution of subalpine meadows at Mt. Rainier (1490 m – 1901 m). At each site we selected and permanently marked 12 – 16 one m² plots within which we sampled floral phenology. Plots were stratified to sample natural topographic variation in the landscape at each site, and an equal number of plots were situated on small-scale ridges, slopes, and depressions at each site. Because snow accumulates in depressions and first melts from ridges (Ford et al. 2013), this sampling design meant that the plots fully captured the natural variation in date of snow melt driven by insolation and topography at each site. In fact, there was as much variation in snowmelt date between plots within sites as there was between sites (Figure S1, Table S1, Ford et al. 2013).

At each plot we measured the date of snow melt, known to be an important control of flowering in snow-dominated ecosystems (Inouye 2008; Hülber et al. 2010; Lambert et al. 2010), as well as two other climate covariates that we suspected influence flowering phenology: post-snow air temperature growing degree days (Pau et al. 2011), and post-snow soil moisture duration (Chaves et al. 2003). We chose these environmental variables because their influence on flowering phenology is accepted and the mechanisms are reasonably well understood. Moreover, they are correlated with other environmental variables at our sites (Figure S2). We monitored snow using data-logging microclimate sensors (HOBO Pendants made by Onset Computer Corporation or iButtons made by Maxim) buried approximately 4 cm below the soil surface and programmed to measure soil temperature in 4-hour intervals. Date of snow disappearance was identified in these time series of soil temperatures as the day where surface soil temperature changes from a near constant measurement of less than 1°C to diurnally fluctuating with measurements above this temperature (Lundquist and Lott 2008, Raleigh et al. 2013). In the six years of data collection, 9.5% of our sensors failed, in which cases date of snow melt was interpolated using a linear regression model applied to all sensors (since plot level differences in snowmelt date were extremely consistent from year to year).

Each site was also instrumented with two air temperature sensors (HOBO Pendants made by Onset Computer Corporation or iButtons made by Maxim) suspended under white plastic

funnels approximately 5m above the soil surface in nearby trees (this height meant that the air sensors were never covered by snow). These sensors recorded air temperatures at 2-hour intervals, and data was downloaded twice yearly. We used these sensors to calculate growing degree days (GDD) as the daily temperature sum above a 0°C temperature threshold that each plot experienced in the 50 days after snow melted there. Because plots differed in the date of snowmelt, this GDD value was unique to each plot

We measured soil moisture in each plot weekly using a ProCheck reader (Decagon Devices, Pullman, Washington, USA) and a EC-5 or GS-3 Soil Moisture Sensor (Decagon Devices). In each week at each plot, we took point measurements at 1 – 3 representative locations within the plot and averaged the readings if multiple were taken. As volumetric soil moisture is not necessarily biologically relevant for plants, raw measurements were converted to seasonal estimates of the number of days between snow melt and soil drying to 5% volumetric water content (considered stressful to plants) at each plot and year. This conversion was done by fitting a log-linear drawdown curve for each site and year in a linear mixed-model with intercept fixed at 1 and plot and year as random slope effects. Estimates were computed using the R package lme4 (Bates et al. 2013).

Finally, at each 1-by-1 m plot we monitored the flowering phenology of all of the animal-pollinated angiosperms present, totaling 48 species across our sites. To monitor flowering, we visited each of the plots weekly, from approximately the time of snow disappearance to the time of fruit dispersal or the time of snow fall (between 9 and 13 total weeks, depending on the year), and recorded the whether the species had flowers (flowers present) or not (flowers absent). For a small number of plot-species combinations (~3%), flowering was also recorded on individuals up to 1m away from the focal plot to increase the number of monitored individuals (targeting rare species), and thus the reliability of estimates. The full observational dataset (2010 – 2015) included 29,420 observations, and can be found in an online repository.

Data Analysis –

Flowering sensitivity to snow melt, soil moisture, and growing degree days –

Model structure –

To quantify species-specific flowering sensitivity to snow melt, soil moisture, and growing degree days we fit flowering observations as a function of these climate covariates for all species simultaneously in a generalized non-linear mixed effects model implemented in a hierarchical Bayesian framework. Specifically, we modeled the relationship between the observed presence/absence of flowers y of species i , in plot j , year k , and on day of year m and plot- and year-specific climate covariates. We treated our flower presence/absence data as a Bernoulli distributed random variable.

$$y_{ijkm} \sim \text{Bernoulli}(\alpha) \tag{1}$$

The Bernoulli mean probability α was related to covariates via a logit link function, and a unimodal curve relating flowering probability to day of year (DOY) described by three parameters that varied by species: the time of peak flowering (opt), the duration of flowering (width) and the maximum probability of observing a flower (height):

$$\text{logit}(\alpha) = \text{width}_{ijk} * (\text{DOY}_{ijkm} - \text{opt}_{ijk})^2 + \text{height}_{ijk} \tag{2}$$

We allowed each phenological curve parameter (height, width, and optimum) to vary as functions of the environmental covariates snow disappearance date (SDD), soil moisture duration (SoilMoist), and growing degree days (GDD) for each species within each plot in the following way:

$$opt_{ijk} = \beta_{1i} * SDD + \beta_{2i} * SoilMoist + \beta_{3i} * GDD + \beta_{0i} + \beta_{0_{jkm}} \quad (3)$$

$$height_{ijk} = \gamma_{1i} * SDD + \gamma_{2i} * SoilMoist + \gamma_{3i} * GDD + \gamma_{0i} + \gamma_{0_{jkm}} \quad (4)$$

$$width_{ijk} = \exp(\delta_{1i} * SDD + \delta_{2i} * SoilMoist + \delta_{3i} * GDD + \delta_{0i}) * -1 \quad (5)$$

In combination, equations 2 – 5 allowed each species to be influenced by each environmental variable in three different ways. Parameters $\beta_{1i} - \beta_{3i}$ represent the sensitivity of peak flowering to climatic covariates, parameters $\gamma_{1i} - \gamma_{3i}$ represent estimates of the sensitivity of flowering duration to climatic covariates, and parameters $\delta_{1i} - \delta_{3i}$ represent estimates of the sensitivity of the maximum probability of flowering to climatic covariates (SDD, soil moisture duration, and GDD). For example, a species experiencing no phenological shifts with changes in snow disappearance date would be indicated by a β_{1i} that is zero, with values of 1 indicating a consistent lag (i.e. number of days) between SDD and peak flowering, regardless of date of snowmelt. Species whose flowering duration lengthened with earlier snowmelt would have a negative δ_{1i} , and species whose flowers are less likely to be observed in early snowmelt conditions would have a negative γ_{1i} . Finally, the β_0, γ_0 and δ_0 , parameters represent plot and species-specific intercepts.

Model fitting –

We simultaneously fit all model parameters using a hierarchical Bayesian approach that treats species-specific parameters (e.g. the duration sensitivity to SDD, γ_{1i}) as drawn from a common Normal distribution with an overall (across species) mean and a variance term that reflects species-to-species variability. This approach allowed us to jointly estimate species-specific parameters of interest as well as community means, ‘borrowing strength’ across species so that species with limited information (for example rare species, or extremely early-flowering species where early season zeros were not always observed) would shrink to the mean and fit biologically plausible curves. Additionally, this acknowledges that the flowering time of all species is likely partly controlled by a common set of biological process, thus making individual species not entirely independent.

We accounted for residual non-independence among measurements as well as unmeasured environmental variability by additionally including normally distributed, zero-centered plot and year random effects for the height intercept ($\beta_{0_{jkm}}$) and the optimum intercept ($\gamma_{0_{jkm}}$) parameters in the model. We did not include a random effect on the width intercept because the addition of this term caused our model to mix poorly and converge extremely slowly. We allowed the height parameter to vary by species because it better allowed for flexible model fitting, but we did not interpret this parameter as having strong biological meaning, since the probability of observing a flower is also strongly linked to its’ abundance within plots (and therefore not necessarily strongly linked to per-individual or across population-flowering

probability). Preliminary model fitting with more complex plot and year random effect structures never qualitatively affected our results.

In each model, we used diffuse Normal priors with a mean of zero and variance of 100 for parameter means and uniform priors with a minimum of 1×10^{-6} and a maximum of 20 for variances. To reduce correlations between parameter estimates and avoid numerical problems, we rescaled each covariate to a mean of 0 and a standard deviation of 1 before model fitting. We estimated parameters using Markov Chain Monte-Carlo (MCMC) simulation techniques, implementing three MCMC chains, discarding the first half of all iterations as burn-in. We assessed model convergence by visual inspection of the chain histories and the Gelman-Rubin statistic (Brooks and Gelman 1998) for each parameter. We then updated the model with an additional 10,000 iterations, and thinned by selecting every 10 draws of each chain to produce 1000 independent posterior samples for each parameter. Models were updated in JAGs 4.0.1 and were run in R version 3.2.3 (R Core Team 2015).

Quantifying floral community reassembly: three metrics

We used three metrics to determine how strongly species-specific phenological sensitivities to climate drivers would result in floral community reassembly in climate change conditions, specifically considering where, within our study system, and when, in the flowering season, floral community assembly was greatest. For each, we capitalized on the climate change-like extremely warm, dry, and low-snow conditions experienced in 2015 (Figure 2 & Figure S1; Mauger et al. 2015), and compared flowering phenology in those conditions to phenology in conditions typical of 2010-2014, collectively near the 20th-century average for temperature, precipitation, and snowpack at our sites. Because 2015 mimicked conditions expected to be average in the mid to late 21st century (Figure 2 & Figure S1; Mauger et al. 2015), any large phenological changes in flowering communities in that year are likely to be analogous to the changes we are likely to see under continued climate change by the late 21st century.

First, we determined how climate change might influence the progression of the number of co-flowering species (floral community richness) across the growing season in 2015 vs. average conditions. We calculated floral richness because it may influence competition for pollinators among plants and the diversity of floral resources available to pollinators. Specifically, we computed mean conditions of SDD, soil moisture duration, and GDD separately for plots within each topographic position (ridges, slopes, and coves) and elevation site (1490m – 1901m) for 2015 and the baseline period 2010 – 2014. Then we used parameter estimates describing species-specific phenologies (and responses to climate change) to estimate the probability of each species flowering in a given topographic position within a given elevation, on every day of the growing season relative to when snow melted in these typical vs. climate change-like years. We estimated the number of species flowering under both scenarios using the model fit to predict the probability of flowering for each species and summing those probabilities. Because each species does not occur at each topographic position or elevation and varies in overall abundance (prevalence), we multiplied the probability of flowering by the probability that a species occurs in a representative plot in that topographic position for all analyses of community reassembly. For example, if a species occurs in 2 out of 5 of the plots in a single topographic position at a single elevation, then all of the flowering probabilities are multiplied by 0.4 (2/5) for that topographic position. Thus, species that don't occur at a topographic position don't contribute to the assessment of reassembly, and rare species contribute less than common species.

Next, we examined climate-driven flowering community structure changes (i.e. dissimilarity in floral communities) by calculating the Bray-Curtis dissimilarity metric between flowering communities in typical-melt and early-melt conditions for each day since snow melt across all elevations and topographic positions. We define the “dissimilarity index” to be the Bray-Curtis distance between these communities on a given day since snow melt, which indicates the magnitude of community shifts relative to the start of the flowering season. This metric could be relevant to pollinators if they encounter a combination of species that are not typical. This measure varies from 0, indicating no change, to 1, indicating that the communities are completely dissimilar and do not share any species. We computed these metrics relative to snow melt date at each site because the calendar date flowering season was shifted in the typical vs. climate change-like year, and we wanted to compare community similarity relative to the start of the flowering season.

Finally, we measured flowering community reassembly as a driver of novel communities by calculating a novelty index for each topographic position within each elevation (i.e. site). The novelty index measures how unprecedented floral communities are in early snowmelt (i.e., climate change like) years compared to typical snowmelt years. Novel floral communities might influence plant-pollinator dynamics from both the plant (now competing with ‘new’ competitors) and pollinator (now making choices between new combinations of floral resources) perspective. We quantify novelty by comparing the species composition of the flowering community on a given day in 2015 to the composition of the flowering community observed at any time point in 2010-2014 at that site/elevation. In other words, it measures how different the flowering community in a climate change-like year is from any floral community observed in a typical year. Like the dissimilarity index described above, we used the Bray-Curtis distance as our measure of novelty, so values near 1 for novelty indicate that the observed combination of flowering species in 2015 are completely novel and weren’t observed at that elevation in any day from 2010 – 2014. Because all three indices of community reassembly (changes in richness, similarity, and novelty) are transformations of the phenology model predictions, we assessed uncertainty in these indices by computing posterior credible intervals of the indices using each of 1000 posterior samples of the parameters of the fit phenology model.

RESULTS

Flowering sensitivity to climate –

Species varied in peak sensitivities such that all species shifted their flowering phenology earlier in climate change-like conditions. Specifically, species shifted the timing of peak flower between 39.3 and 59.0 days earlier (mean=49.5 days; sd=4.52; Figure S3) in response to the warm, early-melt conditions (mean=58 days earlier; range=45 – 63 days earlier; Table S1) in 2015. Although phenological advance was ubiquitous in climate change-like conditions, sensitivities of peak flower to climate drivers were not uniform (Figure 3; peak flower snowmelt sensitivity: mean=0.896, sd=0.0525, peak flower GDD sensitivity: mean=-0.0311, sd=0.00381, peak flower soil moisture sensitivity: mean=0.0109, sd=0.00619). Community-wide, the timing of peak flower was most sensitive to snowmelt (100% of species) and growing degree days (98% of species), and species were overall less sensitive to soil moisture (17% of species; Figure 3). Taken together, this means that species all advanced the timing of peak flower in warm, early melt conditions but did not track climate perfectly.

Similarly, differential species-specific duration sensitivities to climate drivers led to a difference in flowering duration for all species in climate change-like conditions. Overall, 54%

of species (n=26) lengthened their flowering duration whereas 46% (n=22) shortened their duration under warmer, earlier snowmelt conditions. Differences in flowering duration ranged from an 18.7 day shortening of flowering duration to a 15.0 day lengthening of flowering duration (mean lengthening 0.740 days, $sd=6.10$). Flowering duration sensitivities were less consistently related to climatic covariates compared with sensitivity of peak flowering: flowering duration was most sensitive to GDD (35% of species, $mean=0.217$, $sd=0.113$), followed by snowmelt (31% of species, $mean=0.0844$, $sd=1.13$) and soil moisture (8% of species, $mean=0.00388$, $sd=0.105$).

There were several ways in which differences among species in their responses to warming were generalizable across the community. Species whose flowering duration lengthened in warmer, earlier snowmelt conditions tended to also delay peak flower relative to snow melt in 2015 ($F=9.14$, $DF=46$, $P<0.01$; Figure S5). This is likely due to species having experiencing different changes in GDD in 2015, with species at early-melt sites experiencing cooler conditions that delay plant development (Figure S1). The onset of flowering shifted less relative to snow melt for early-flowering species than for late-flowering species ($F=8.40$, $DF=46$, $P<0.01$), but this pattern was not apparent for changes in peak flower or the end of flowering ($P>0.2$ for both relationships). Changes in flowering duration were also related to the average length of flowering duration, with longer-flowering species shortening their flowering period while shorter-flowering species lengthened their flowering period in 2015 ($F=27.64$, $DF=46$, $P<0.01$; Figure S5).

Overall, we found strong evidence for generalizable responses to environmental conditions that closely mirror those we expect in the late-21st century under unabated climate change (early snowmelt, generally warmer temperatures, and drier soils). Earlier snowmelt drives consistently earlier flowering for all species (although the magnitude of the shift does vary), and has large but species-specific influences on flowering durations in this system (Figure 3). Increases in post-snow growing-degree days drive earlier flowering peaks for all species and shorter flowering periods for most species (Figure 3). Decreases in soil moisture duration cause smaller and species-specific changes in phenology for a subset of species (Figure 3).

Community reassembly –

Comparing community richness (Figure 4), dissimilarity (Figure 5), and novelty (Figure 76) trajectories in climate change like conditions (2015; Mauger et al. 2015), to trajectories in 2010 – 2014 indicates that low-elevation ridges and slopes experienced the greatest amount of floral community reassembly in 2015. First, differences in flowering richness was highest at low elevations and at ridges and slopes (Figure 4). Similarly, median dissimilarity was highest at the lowest elevation, and was higher in the ridges and slopes at the lower elevation sites than the higher elevation sites (Figure 5A). There was not a strong location-specific pattern in median novelty (Figure 6A). Overall, coves and high elevation sites appear to reassemble less, likely because they melt in the heart of the growing season when temperatures are peaking, even in the early melt year. In contrast, ridges and sites at lower elevations melted when conditions were cooler and wetter in 2015 than they typically are. These novel combinations of environmental conditions interacted with species-specific sensitivities to climate drivers to produce large differences in community reassembly across the elevation gradient of subalpine meadows.

Reassembly is apparent early in the season, lowest in mid-season, and greatest later in the season (Figure 4 & 5 & 6). This was reflected in differences in flowering richness which were highest early and late in the season (Figure 4), dissimilarity through time which showed variable

patterns but generally most pronounced late and early in the season (Figure 5B), and novelty that was highest early in the season (Figure 6B). Interestingly, late-season dissimilarity didn't result in novel communities (Figure 5 & 6); instead, novelty is the highest early in the season (Figure 6). These patterns in novelty are due to early-flowering species lengthening their flowering duration in 2015, while later-flowering species shortening their flowering duration ($F=6.74$, $DF=46$, $P<0.05$; Figure S4). These asymmetric shifts in duration (likely driven by reduction in late-season soil moisture) mean that early flowering species newly co-flower with mid-season flowers (novel flowering patterns) whereas late-flowering species simply truncate their flowering (not inducing novelty, but altering the temporal progression of the floral community relative to snowmelt).

DISCUSSION

Do species-specific climate sensitivities imply that communities will reassemble phenologically with climate change? Overall, we found that species have differential sensitivities to snowmelt, soil moisture, and growing degree days, both in terms of the timing of peak flower and in terms of flowering duration (Figure 3). In all, these species-specific phenological responses to climate drivers resulted in floral community reassembly (Figure 4 & 5 & 6) in a year that mimicked climate change conditions predicted in the 2080s (Figure 1 & S1; Mauger et al. 2015). Below we elaborate on these results, and speculate about the causes, impacts, and implications of our study for floral community reassembly in a warmer world.

Species-specific responses to climate: Causes

Overall, species-specific climate sensitivities caused species to differentially shift both their timing of peak flower and duration of flowering with changing climate drivers, but the responses were more consistent than we originally expected. For example, although species showed individualistic shifts in peak flower in response to earlier snowmelt, *all* species did shift their phenology earlier in response to changes in this driver. Similarly, species' peak flowering uniformly shifted earlier with greater growing season temperatures (GDD), with most additionally shortening their flowering duration in response to this driver (Figure 3). Other studies have found evidence of similar climate drivers (most often snow, and to a lesser extent a gross treatment of temperature as seasonal or monthly means) as influencing phenology in mountainous regions (Dunne et al. 2003; Inouye 2008; Iler et al. 2013), but few (to our knowledge) have found complex effects of multiple climate drivers (snow, growing degree days, and to a lesser extent soil moisture) on different aspects of phenology, as we did (Figure 3).

It is not surprising that snow melt and growing degree days were the strongest predictors of peak flowering (as opposed to flowering duration), because the potential growing season is very short at in the subalpine meadows of Mount Rainier (sometimes lasting only 2.5 months). Thus, strong phenological sensitivities to snow melt in this system likely reflects strong selection for these high mountain plants to time their reproductive phenology closely to the limited time periods that are climatically favorable (Sheth and Angert 2016). Similarly, the accumulation of growing degrees is known to constrain plant development, and thus strongly influence when a plant is ready for reproduction (Wahid et al. 2007). Sensitivity to growing degree days may also be an adaptive way to reduce the risk of frost in this system (Inouye 2008; Forrest and Miller-Rushing 2010). Thus for these subalpine species, flowering soon after snow melt and when air is warm may be the best way to maximize reproductive opportunity where climatic conditions are harsh (similar to Crimmins et al. 2010).

Our results highlight the importance of considering all climate variables (or at least more than one) when calculating climate sensitivity, for two key reasons. First, attributing all of the variation in responses to any single climate variable will undoubtedly over-estimate the importance of that variable. We found far less between-species variation in the sensitivity of peak flower to snowmelt and growing degree days than we expected (like CaraDonna et al. 2014), which complement findings in Diez et al. 2012 who note that inter-specific variation decreased with the inclusion of multiple climate variables. Second, climate change will cause changes in many axes of climate (but potentially differentially so). Incorporating multiple divers of change may therefore be essential both to understanding the mechanisms by phenology is cued to climate as well as predicting potential phenological shifts in response to climate change (Diez et al. 2012; Wolkovich et al. 2012).

Species-specific responses to climate: Impacts

Phenological community reassembly requires species-specific differences in climate sensitivities, but is also influenced by the magnitude of difference in climate sensitivities and in the magnitude of change in the climatic drivers of phenology (Crimmins et al. 2010). For example, reassembly was most apparent at low-elevations and along ridges and slopes in the landscape (Figure 4 – 6). Because variation in climate sensitivities among species did not differ much between species occurring on ridges and slopes, this pattern was largely driven by the fact that these sites (low elevation and ridges and coves) experienced larger differences in both snowmelt and growing degree day accumulation in 2015 than did high elevation sites (Figure S1). An additional factor was that the direction of change in snowmelt and growing degree day accumulation differed at these sites. Specifically, snow disappeared from low elevation ridge and slope sites so early in 2015 that they experienced cool, spring-like temperatures; earlier snowmelt in these sites was not correlated with increases in GDD (Figure S1), which is counterintuitive albeit consistent with what is expected as the climate warms (Inouye 2000; Inouye 2008; Mauger et al. 2015). By contrast, cove plots (across all elevations) and high elevation sites experienced both earlier snowmelt and warm and summer-like temperatures (high elevations and coves experienced virtually no change in GDD in 2015 compared to typical years; Figure S1). Thus, both the magnitude of climatic change and covariance among climatic drivers (in change) varied spatially, driving differential reassembly across elevations and topographic positions.

It is also possible that locations experiencing minimal reassembly contained combinations of species that were either not strongly sensitive to climate drivers or that varied less in their phenological sensitivities to climate. For example, at high elevations there is likely even stronger selective pressure for species to time their reproductive phenology closely to climatically favorable environments given that the growing season is extremely short and predictably so (Sheth and Angert 2016). Consistent with this possibility is that species found at low-elevations advanced their peak phenology much more than plants at higher-elevations (Figure S5); and low-elevation plants lengthened their flowering duration more than high-elevation plants (Figure S5). For these high-elevation species, flowering soon after snowmelt is critical for maximizing reproductive opportunity (similar to Crimmins et al. 2010). This suggests that locations where all species are tightly cued to climate may see less overall variation in responses to climate change, minimizing reassembly in climatically variable years.

Reassembly also varied temporally within the flowering season. Specifically, early in the season there was both high reassembly (Figures 4 & 5) and high novelty (Figure 6). This is likely

due to early flowering species shifting their phenologies more than later flowering species (Figure S5; Miller-Rushing and Primack 2008; Miller-Rushing and Inouye 2009; Munguía-Rosas et al. 2011), decreasing the extent to which early flowering species co-flower with other species. Early flowering species also tended to lengthen their flowering duration compared to later flowering species (Figure S5), increasing overlap with mid-season flowers creating novel co-flowering patterns. In contrast, late flowering species tended to decrease their flowering duration (Figure S5), which increased dissimilarity, but not novelty (Figure 5 & 6).

Implications of community reassembly

What are the implications of the floral community reassembly we found? Although beyond the scope of this study, we speculate that floral community reassembly could have large impacts on plant-pollinator dynamics, mediated through a change in the co-occurrence of flowering species available to and competing for pollinators. For example, floral community assembly may disrupt current patterns in plant-pollinator interactions (Burkle et al. 2013), if early-flowering species advance their peak flowering disproportionately compared to late-flowering species. These novel communities may result in periods of floral resource over-loads (early in the season) for pollinators followed by periods of insufficient resources (late in the season; e.g., Aldridge et al. 2011), because plant species provision different amounts of sugar (via nectar) and protein (via pollen; Figure S6 & Figure S7 respectively). The impacts of these differential shifts on nutrient availability throughout the growing season and across locations is largely unknown (but see Figures S6 & Figure S7) but may impact pollinator foraging habits (Burkle et al. 2013) and pollinator survival long term (Potts et al. 2010; Vanbergen and Initiative 2013); on the other hand, the acclimation and adaptation year-to-year variability may so buffer pollinators from long term disruptions (Bronstein et al. 2006; Alarcón et al. 2008).

Furthermore, plants interact with other plants through competition for pollinators or through facilitation of pollination services. If currently co-flowering species shift differentially thus no longer co-flower in a warmer world, these species lose both the competitive drawback of co-flowering and also lose the facilitative benefit of co-flowering. These shifts have the potential to change the dynamics of competition for and facilitation of pollinator services. Changes in the facilitative and competitive interactions between plant species could lead to increased or decreased plant reproduction and warrants further investigation.

Floral community reassembly in the future may be different than we observed in 2015. First, our climate change analog year (2015) mimicked snowmelt conditions expected in the 2080s (Figure 2 & Figure S1; Mauger et al. 2015), but growing season air temperatures predicted in the 2050s (Mauger et al. 2015). Thus, much like the impact of warming experiments on phenology, our findings, based on one extreme year, could under-estimate natural phenological variation (Wolkovich et al. 2012). In addition, our estimates don't account for longer-term processes that might mitigate the causes and consequences of reassembly such as developmental plasticity, adaptations, and changes in species distributions. These factors may make phenological reassembly less dramatic and less disruptive. Thus it is critical to continue assessing the community-level impacts of species-specific phenological shifts.

CONCLUSIONS

Assessing the community-level implications of species-specific phenological shifts is a critical, yet often overlooked, aspect of assessing the biological impacts of climate change. Differences in climate sensitivities can have large impacts on the magnitude and direction of

shifts in peak flower and on the duration of flowering, and in turn, these phenological shifts can have large impacts on the composition and timing of floral communities. Interestingly, we found that species-specific sensitivities to climate, as well as spatial and temporal patterns the magnitude of change in three critical climate drivers jointly influenced the temporal and geographic patterns of community reassembly. In networks of interacting species, such as plant-pollinator networks, this has the potential to alter or disrupt species interactions with implications for both parties. Targeted efforts to determine the ecological consequences of phenological community reassembly may help identify extinction risks for species, and impacts on important ecological processes as the climate warms.

FIGURES

Figure 1: Conceptual figure showing how and why phenological community reassembly may occur. For three representative flowering species, the top panel (A) shows seasonal phenological progressions in typical years and the bottom panel (B) shows seasonal phenological progressions in climate change like years. The three species have differential sensitivities to climate thus are shifting their phenologies differentially: the yellow species advances its peak flower less than the purple species (shown by comparing the slopes of the solid red and the dashed red lines), and the yellow species lengthens its flowering duration while the green species shortens its flowering duration (shown by comparing the relative length of the solid and dashed lines in the top and bottom panels). Evidence of phenological community reassembly is apparent in sequential days from late-May to mid-June. First the community in early-June in the warm (climate change-like) year has higher richness than the community in the same time frame in a typical year: three species instead of one species are flowering. This increased richness also indicates community dissimilarity (as the two communities are different from each other) and is also a novel community because there is no time in the season in a typical year when all three species (yellow, green, and purple) are flowering.

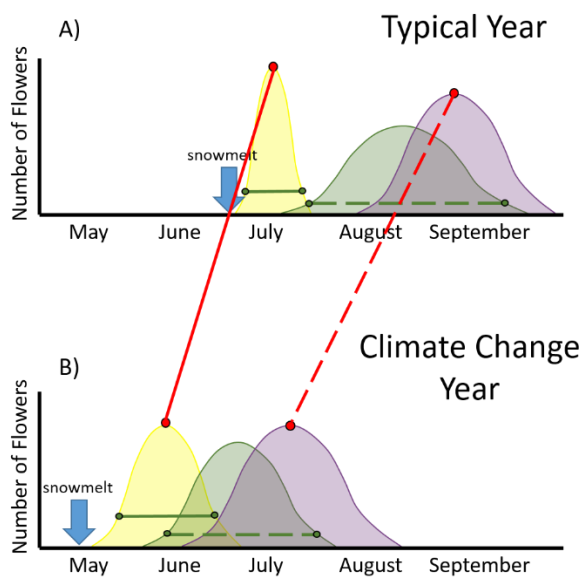
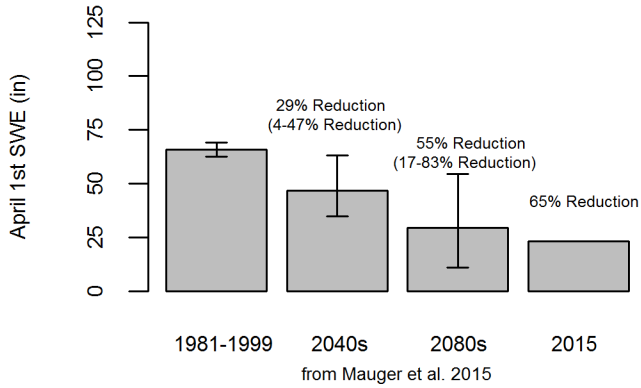


Figure 2: April 1st Snow Water Equivalent (SWE), as measured by the Paradise snow pillow, from 1981-1999 and 2015. Mauger et al. (2015) project April 1st SWE based on climate change scenarios for the Cascade mountain range (of which Mount Rainier is the tallest peak). Bars show projected average conditions and “error bars” show range of conditions. The conditions recorded at Mount Rainier in 2015 are likely to mimic mid-century conditions under moderate climate change scenarios (Mauger et al. 2015).



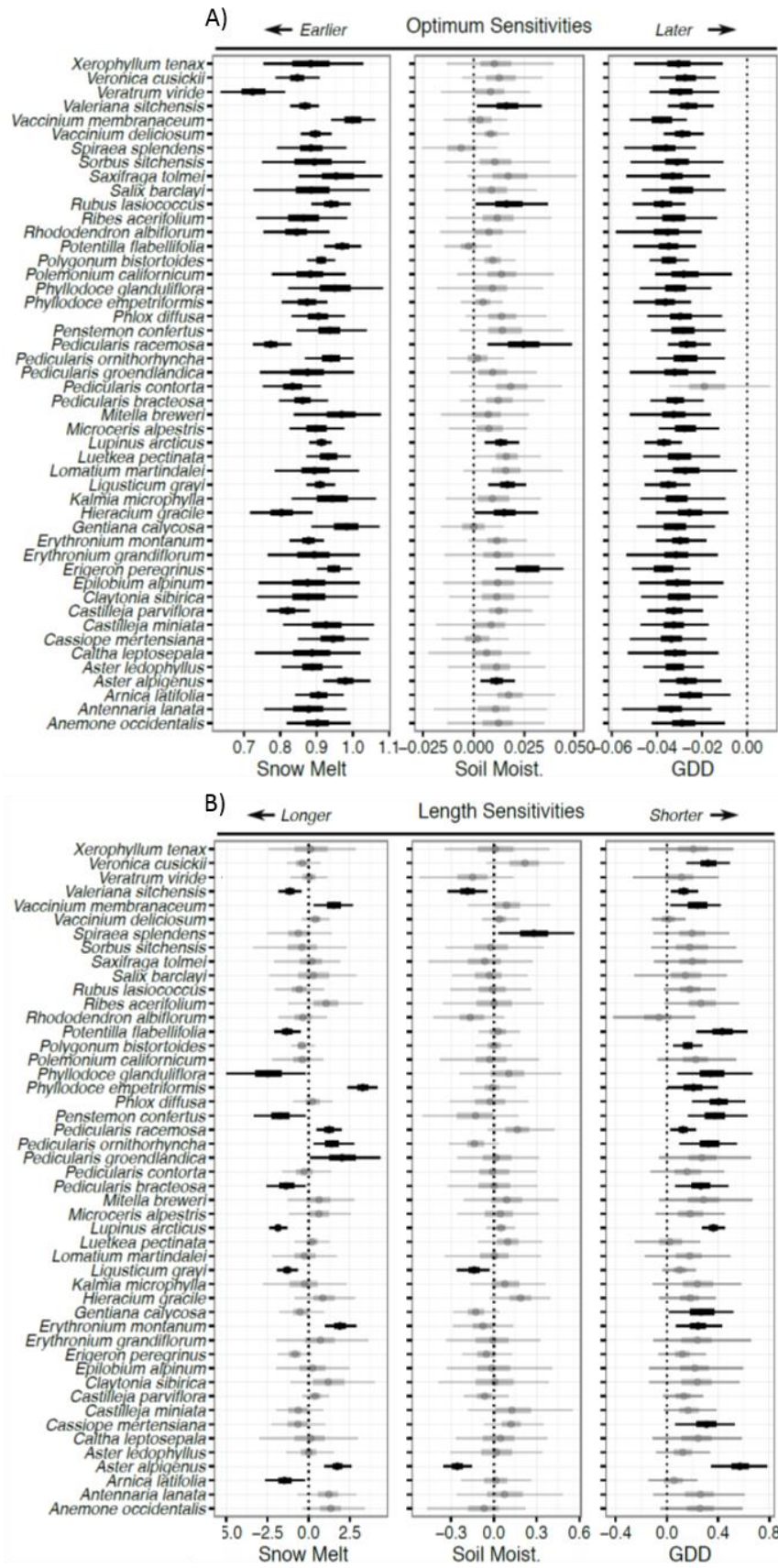


Figure 3: Species vary in their phenological sensitivity to climate change, both in terms of A) peak flower (optimum) and B) flowering duration (length) to snow melt, soil moisture, and growing degree days. Each plot shows parameter values for each species, points are centered at species-specific mean sensitivity, thick bars span 50% and narrow bars span 95% credible intervals, light grey values are not credibly different than zero. Dashed lines at zero show parameter values for no change / sensitivity.

Figure 4: Estimated species richness for each day from snowmelt to 80 days past snowmelt at each topographic position at each elevation in early (pink) and typical (teal) snowmelt years. Shading indicates 80% credible intervals. Reassembly is noticed when curves do not align, primarily at lower elevations along ridges and slopes.

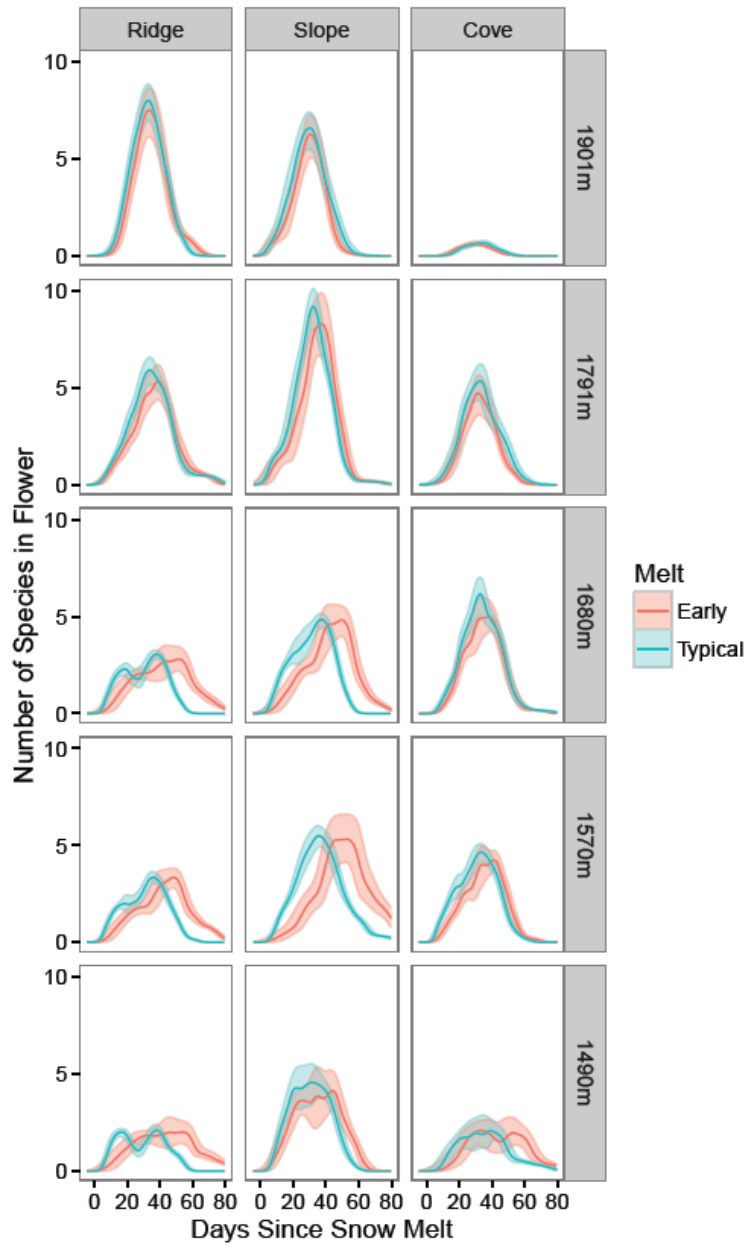


Figure 5: Dissimilarity (Bray-Curtis) of communities in early vs. typical snowmelt years. Reassembly is most apparent when dissimilarity is high (values close to 1). Median dissimilarity (A) is highest at low elevations and at ridge and slope topographic positions and trajectory of dissimilarity (B) shows that dissimilarity is most pronounced early and especially late in the growing season. In A, wide bars span 50% and narrow bars span 95% credible intervals, and in B dashed lines show 95% credible intervals.

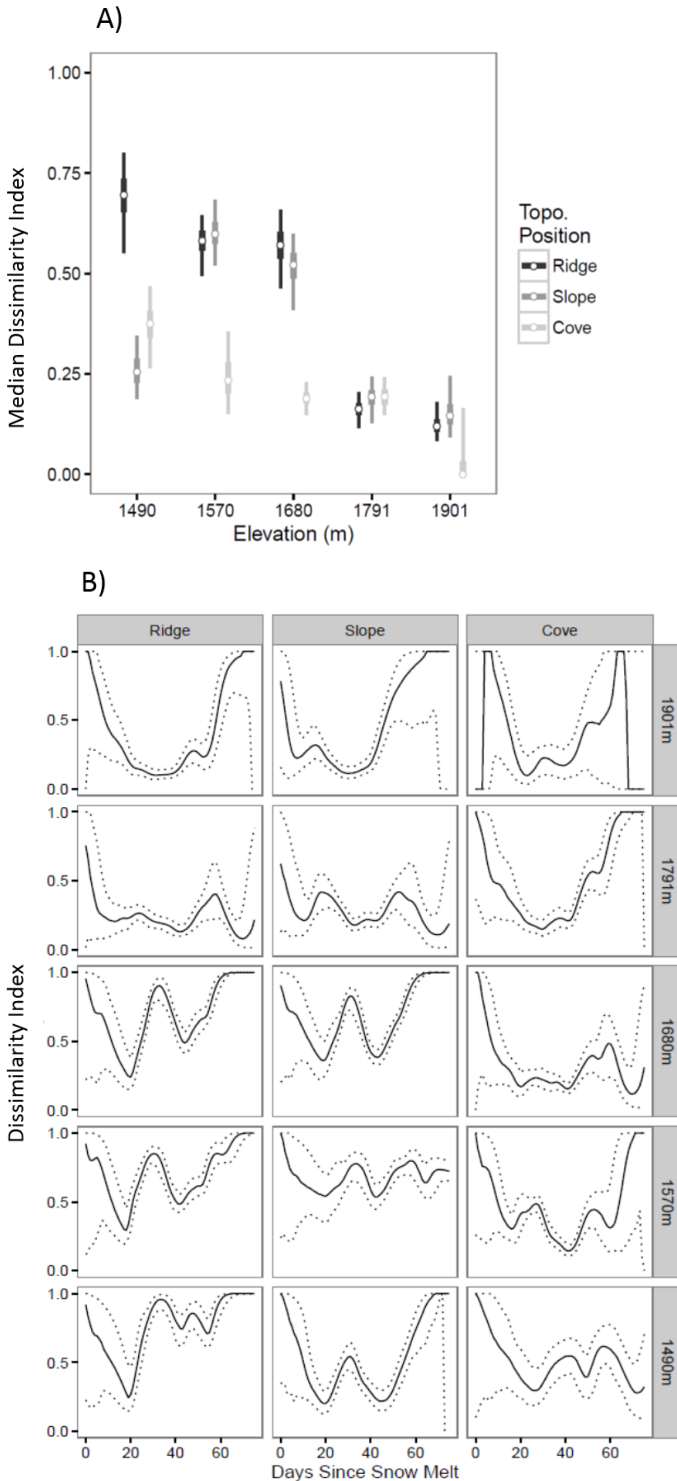
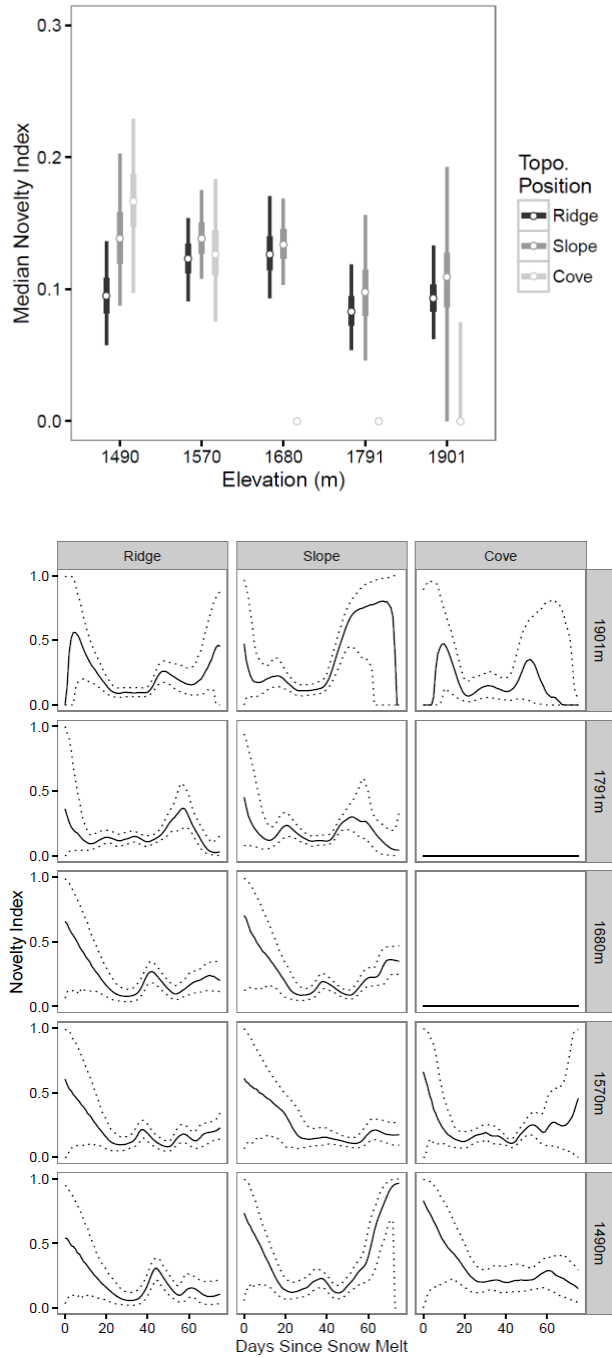


Figure 6: Novelty of communities in early vs. typical snowmelt years. Median novelty (A) varies less than dissimilarity (Figure 6) and is generally lowest on ridges. Interestingly, trajectory of novelty (B) shows that novelty is most pronounced early in the season despite reassembly being most pronounced late in the season (Figure 6). In A, wide bars span 50% and narrow bars span 95% credible intervals, and in B dashed lines show 95% credible intervals.



SUPPLEMENTAL MATERIALS

Supplemental Figures:

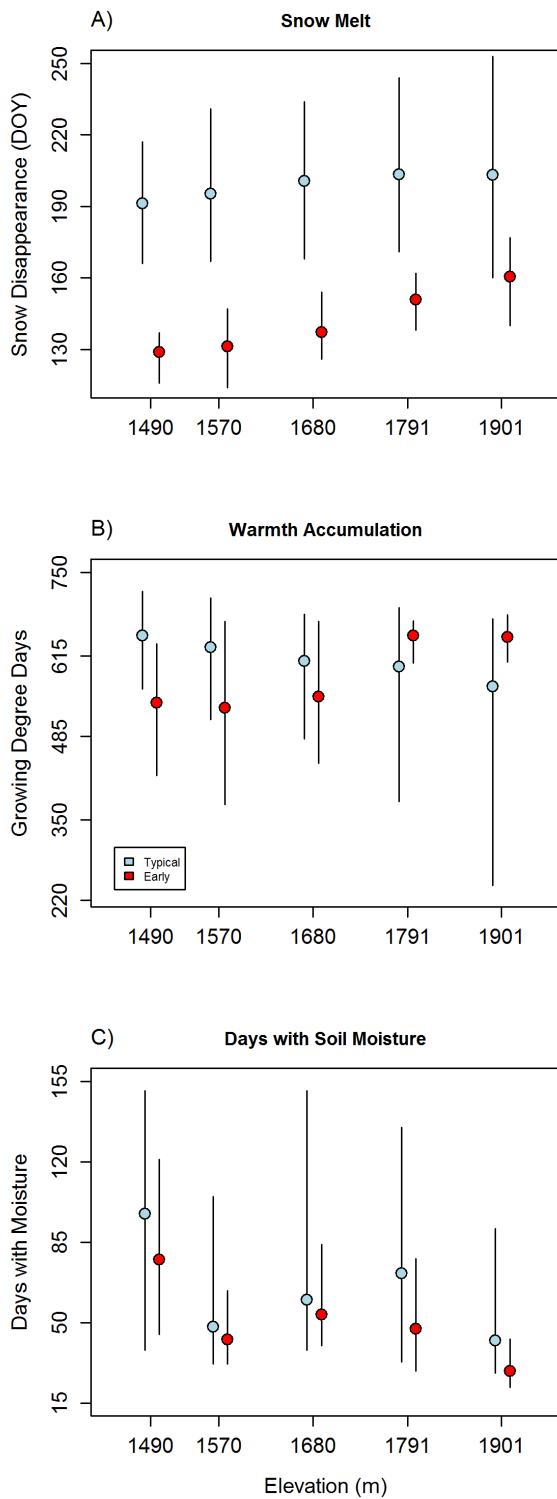


Figure S1: A) Distribution of snow melt days at each site in typical- and early-melt years. The point is centered over the mean of the 15 temperature sensors and the bars span the range from minimum to maximum snow disappearance date measured at that site in those years. For reference, DOY 130 is May 10th, DOY 190 is July 9th in non-leap years. B) Growing degree days accumulated for 50 days after snowmelt at each site in typical- and early-snowmelt years. The point is centered over the mean growing degree days accumulated and the bars span the range from minimum to maximum at each site in those years. C) Number of days where soil moisture is greater than 5% of total recorded at each site in typical- and early-snowmelt years. The point is centered over the mean moisture from the 15 plots at each site and the bars span the range from minimum to maximum days of moisture at that site in those years.

Figure S2: A – C) Snow disappearance is correlated with average canopy cover (measured with a hemispherical crown densitometer on the four sides of each of the plots, then averaged), but not aspect of the plot or slope of the plot. D – F) Not surprisingly, growing degree day accumulation is not correlated with canopy, aspect, or slope. Growing degree days is a site-level measurement (see text) whereas canopy, aspect, and slope are each plot-level measurements. G – I) Soil moisture is correlated with average canopy cover and with slope.

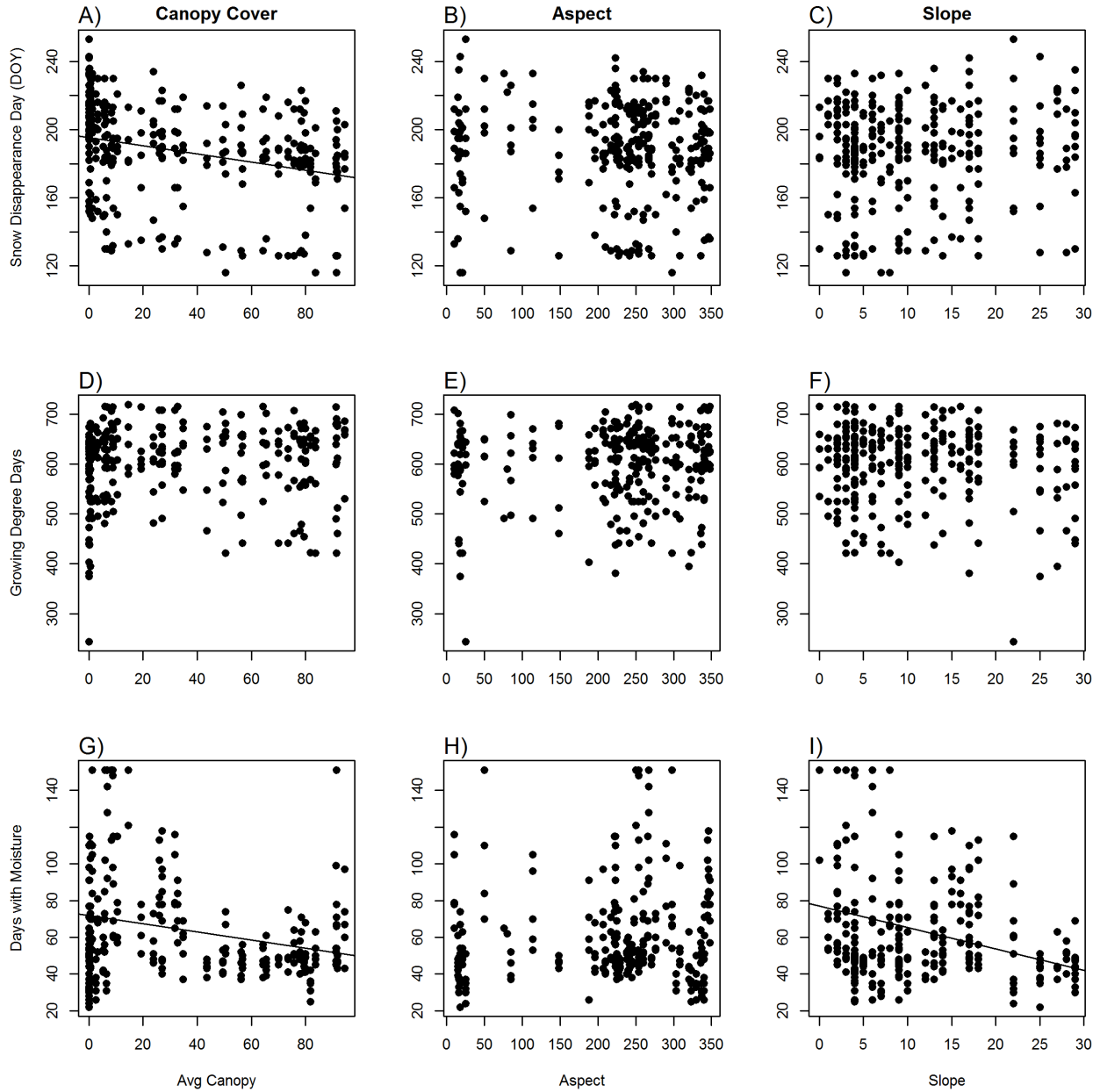


Figure S3: Flowering phenology relative to the time that has elapsed since snow disappearance in typical years (dashed line, 2011 – 2014) and a climate change-like year (solid line, 2015). Points indicate peak flower for each species, lines span the duration of flowering. Species that track snow melt perfectly (i.e. have highly sensitive peak flower, with a slope of 1) will have small differences in flowering time relative to the number of days which have elapsed since snowmelt, species that are not sensitive to snowmelt (i.e., and have a slope of <1) will have points that are farther apart and the point on the solid line will fall to the right of the point on the dashed line. Finally, species with peak flower highly sensitive to snowmelt (and have slopes >1) will have the point on the solid line to the left of the point on the dashed line. Species whose widths are very sensitive to snowmelt will have flowering durations that are very different in early vs. late years thus their lines will be very different lengths.

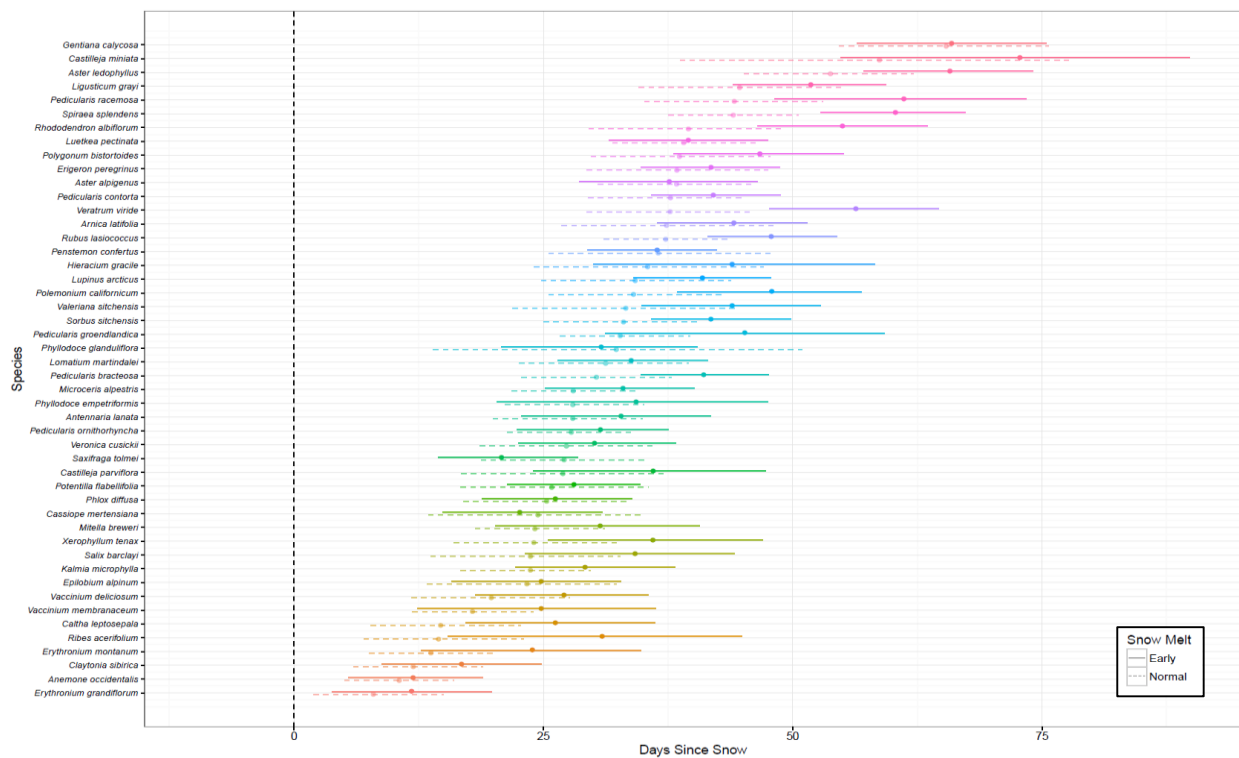


Figure S4: Species which lengthened flowering duration advanced peak flower more than species which shortened flowering duration. Differences (peak shift and duration change) are the climate change-like year (2015) compared to typical years (2011 – 2014).

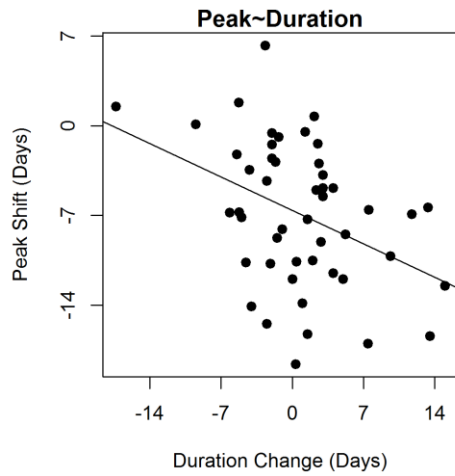
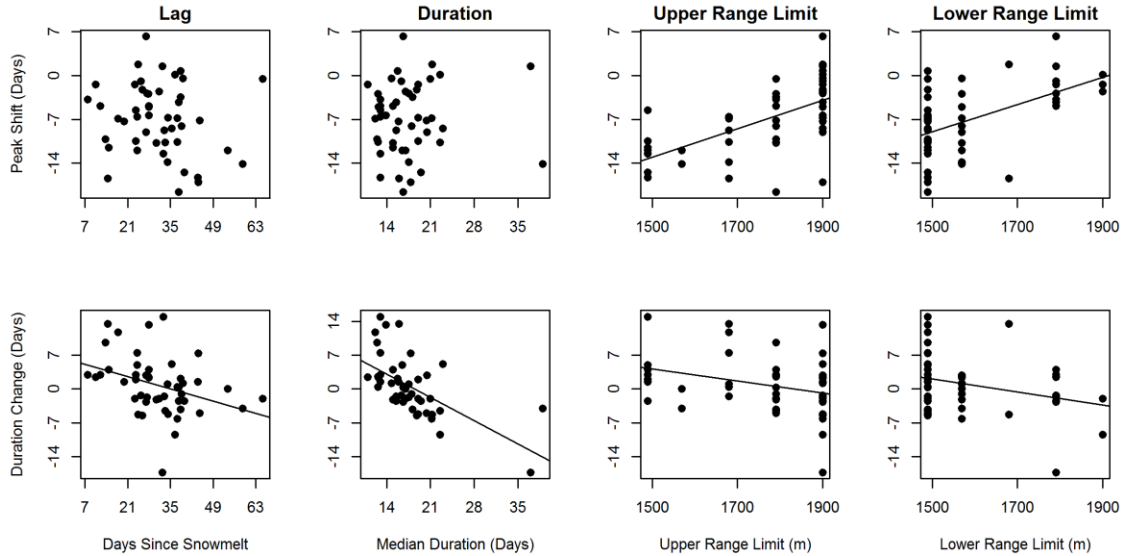


Figure S5: Trait correlations showing the number of days shift in peak flowering (top) and the number of days of change in duration (bottom). Differences (peak shift and duration change) are the climate change-like year (2015) compared to typical years (2011 – 2014). Early-flowering species lengthen flowering duration while late flowering species shorten flowering duration (column: Lag), short flowering species lengthened their flowering duration while long flowering species shortened their flowering duration (column: Duration), Low elevation plants advanced peak flower and lengthened flowering duration more than high-elevation plants (column: Upper Range Limit and Lower Range Limit).



There was no detectable difference between abundant species and rare species or between woody and herbaceous species in terms of their phenological response to climate. Differences (peak shift and duration change) are the climate change-like year (2015) compared to typical years (2011 – 2014).

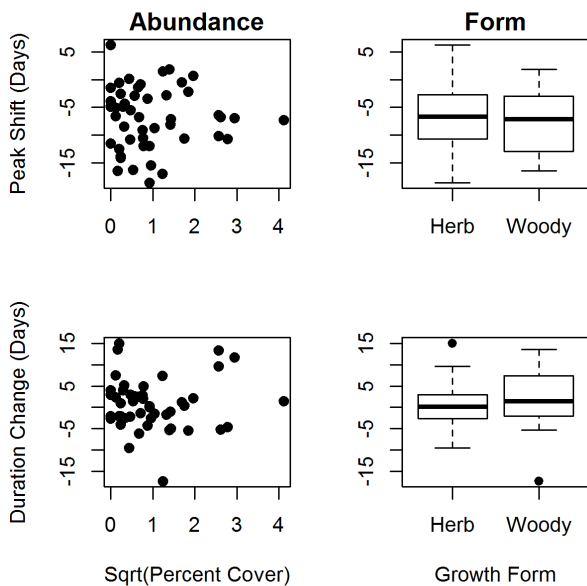


Figure S6: At their upper range limit and lower range limit, plant species provision different amounts of nectar per flower (A), which translates to a net difference in the amount of sugar available (B), largely because the nectar has different concentrations (C). The species sampled are referred to by their 4-letter code (first two letters of genus and first two letters of species) and include *Anemone occidentalis*, *Erythronium montanum*, *Phlox diffusa*, *Phyllodoce empetrififormis*, *Pedicularis bracteosa*, *Valeriana sitchensis*, *Lupinus arcticus*, and *Gentiana calycosa*.

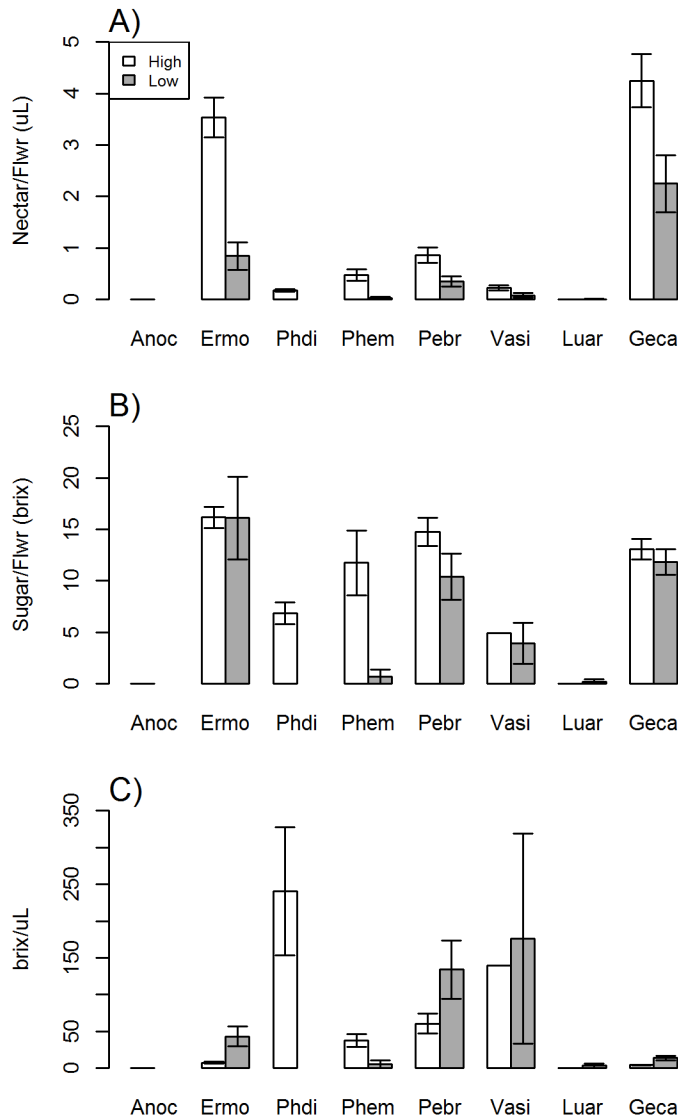


Figure S7: At their upper range limit and their lower range limit, plant species have different amounts of pollen per anther (A) and per flower (B). This translates to offering different amounts of protein per anther (C) and per flower (D). The species sampled are referred to by their 4-letter code (first two letters of genus and first two letters of species) and include *Anemone occidentalis*, *Erythronium montanum*, *Phlox diffusa*, *Phyllodoce empetriformis*, *Pedicularis bracteosa*, *Valeriana sitchensis*, *Lupinus arcticus*, and *Gentiana calycosa*.

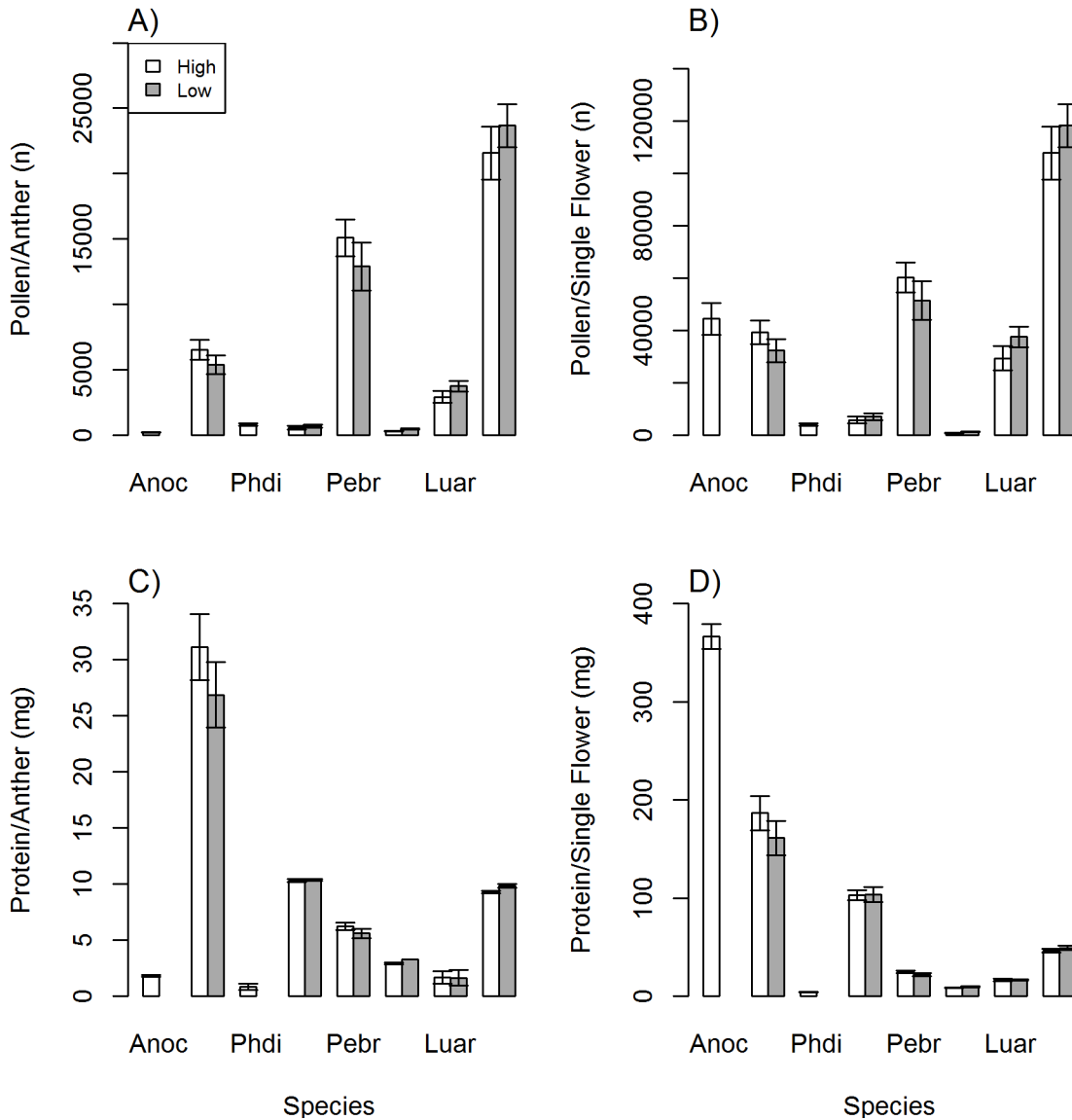


Table S1: Date of snow melt varied considerably throughout the study, with 2015 being the earliest snow melt we recorded. Numbers reported are Day of Year. For reference, DOY 200 is July 19th in non-leap years.

	Year						Mean 2010-2014	Mean Difference from 2015
	2010	2011	2012	2013	2014	2015		
Overall	204	220	201	186	191	142	201	58
1490	NA	211	194	177	182	129	191	62
1570	NA	217	197	181	187	132	196	64
1680	197	221	201	187	192	137	200	63
1791	NA	224	204	192	197	151	204	53
1901	201	230	208	193	200	163	208	45

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CHAPTER 3:

Women learn more from local than global examples of the biological impacts of climate change

Elinore J. Theobald, Hrach Gabrielyan, and Janneke HilleRisLambers

Citation:

Theobald, E. J., Crowe, A., HilleRisLambers, J., Wenderoth, M. P., & Freeman, S. (2015). Women learn more from local than global examples of the biological impacts of climate change. *Frontiers in Ecology and the Environment*, 13(3), 132-137.

Women learn more from local than global examples of the biological impacts of climate change

Elinore J Theobald, Alison Crowe, Janneke HilleRisLambers, Mary P Wenderoth, and Scott Freeman*

Are students influenced more by analyzing local or global examples of the biological impacts of climate change? Using a randomized trial in a large, introductory undergraduate biology course, we found that a single in-class activity led to a 45% increase in the frequency of correct answers on a test of conceptual understanding. Additionally, after completion of the activity, students more strongly believed that climate change would alter their lives, were more willing to modify their own behavior, and indicated more support for government action to address climate change. There was also a robust gender effect on the influence of local versus global examples: females learned better if they studied local examples. Women reported greater willingness to alter their behavior than men, and students with higher university grades were more likely to support government action to mitigate climate change. Our findings support the use of local examples in curricula and illustrate the power of large, randomized trials in determining effective methods in climate-change education.

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Policy leaders and educators have established three important points of consensus regarding public understanding of climate change (NRC 2011, 2012; McCright *et al.* 2013):

- (1) the need for education is urgent;
- (2) curricula should meet specific learning goals, such as addressing known misconceptions and training for quantitative literacy; and
- (3) the teaching materials themselves should use actual data, employ active learning (wherein students methodically develop their understanding of course material as opposed to solely being lectured to by the professor; Freeman *et al.* 2014), and be evidence-based.

Although some published curricular materials are consistent with one or more of these recommendations (eg CLEAN 2014), little has been done to test the efficacy of these materials. The exceptions have been studies that used assessments designed by the study authors (a necessity given the lack of third-party tests), which tested students before and after instruction and revealed statistically significant learning gains (eg Nam and Ito 2011). To our knowledge, however, none of those studies used a large, randomized trial to test alternative hypotheses regarding which types of activities best support student learning about the biological impacts of climate change. Moreover, few assessed changes in conceptual understanding in addition to changes in student affect (ie student attitudes and opinions about climate-change issues) and almost no studies focused on student learning about

the biological consequences of climate change.

We therefore asked: “will student attitudes toward global warming and their understanding of its biological consequences be more influenced by local or by non-local (‘global’) examples of its impacts?” Our interest in studying student attitudes and opinions was inspired by two seemingly contradictory findings. First, now-classical polling data collected from the general adult population in the US indicate a high level of awareness of climate change but a low level of concern (Leiserowitz 2006). However, more recent data from the US and elsewhere show that personal experience with extreme weather events is triggering increased concern (Myers *et al.* 2012; Akerlof *et al.* 2013). Together, these findings suggest that while climate change is a global phenomenon, understanding, and therefore concern, may be increased most effectively through the use of local examples. On the other hand, concern about climate change may correlate with the respondents’ level of understanding about the most severe impacts. If so, then global examples – where more people, wider geographic regions, or more species are affected – may further increase knowledge and concern.

We were also interested in examining whether different student subpopulations responded differently to the two types of examples of the biological impacts of climate change: local examples or global examples. For instance, there is evidence that women are more willing to modify their behavior based on climate-change research (Hunter *et al.* 2004) and that citizens with a greater understanding of climate change express more concern about it (McCright 2010). The latter observation motivated our interest in studying changes in student conceptual understanding as well as affect.

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Figure 1. (a) Students from the large undergraduate biology class ($n = 481$ students) turn to the people sitting near them to work in informal small groups on the active learning activity. (b) S Freeman circulates the room and answers student questions during active learning activities.

responses on a 1–5 scale, where 1 = relatively little, 3 = a moderate amount, and 5 = a great deal; thus, there was no single correct answer.

During the first 10 minutes of the 50-minute class, the instructor (author JHRL) reviewed the prior day's material on carbon cycling and human impacts on the carbon cycle, and posed two questions that students answered with personal response devices, or "clickers". Teaching assistants then passed out worksheets, which students were asked to complete in informal small groups (Figure 1a). This in-class activity had two versions: one with local examples (hereafter "Local" treatment) and one with global examples (hereafter "Global" treatment) of the biological impacts of climate change. The biological impacts highlighted included trophic mismatch, agricultural output, positive and negative feedbacks, responses to heat stress, range changes, and negative effects of ocean acidification on marine organisms with calcium carbonate shells or skeletons. The activities focused on interpreting figures and drawing conclusions by answering questions. During the 30 minutes allotted to the exercise, the instructor and teaching assistants moved around the lecture hall answering questions (Figure 1b). Students in both treatment groups (Local and Global) had equal access to instructional staff.

After the worksheets were completed and collected, the instructor ended the class with a whole-class wrap-up of the topic and posed one final, survey-type clicker question, asking students where else they had learned about the biological impacts of climate change; thus, there was no single correct answer. A post-test, the content of which was identical to that of the pre-test, was available online immediately after class until 6:00 a.m. the following morning; students electing to take the post-test were awarded "participation points" for completing it (ie students were awarded points regardless of the correctness of their answers). The clicker questions,

Methods

Teaching sequence and data collection

We designed the materials for undergraduates and implemented the experiment in an introductory biology course required of biology majors at the University of Washington. The class meets four times each week for 50 minutes and includes a weekly 2-hour laboratory component. Total enrollment was 481 for the academic quarter analyzed here; for detailed information on the course and the general student population at the university, see Freeman *et al.* (2011).

Prior to the single class session on climate change, students were asked to read sections of a textbook that introduced global patterns in climate and basic information about global warming. Before class, students also completed an 11-item author-designed pre-test, consisting of eight questions relevant to conceptual understanding and three questions gauging affect, via an online course management system. The questions on conceptual understanding were multiple choice, with a single best answer; they addressed the impact of climate change on agricultural productivity, water availability for human use, phenological events, geographic range shifts, consequences of ocean acidification, impact of heat waves on organisms, and positive and negative feedbacks on global warming. The remaining three questions on affect asked students how much they thought climate change would influence their life, how willing they were to change their lifestyle to reduce the impact of climate change, and how much they supported government action to reduce greenhouse-gas emissions. Students answered affect-related questions by ranking their



Figure 2. Impact of active learning activities on student conceptual understanding and affect. (a) Active learning increases student understanding. (b–d) Student attitudes changed on all three affect questions, which were answered on the scale: 1 = relatively little, 3 = a moderate amount, and 5 = a great deal (see WebPanel 2). The P values reported are from two-tailed, paired *t* tests comparing pre- and post-scores. Sample sizes were $n = 218$ for the local treatment and $n = 251$ for the global treatment. Error bars indicate standard error.

worksheets, and pre- and post-tests are presented in Web-Panels 1 and 2.

Data analysis

For both sets of analyses – conceptual and affect – student scores were included only if individuals completed all activities associated with the class session and signed a consent form agreeing to participate in the study; total sample size was 469. All analyses were performed in R version 3.0.2 (R Core Team 2013).

To test for the effect of the Local versus Global worksheets on learning gains, we analyzed the total number of correct responses to the eight questions focused on conceptual understanding. Specifically, we compared pre-versus post-tests using two-tailed, paired *t* tests. To explore how student characteristics might influence changes in conceptual understanding, we fit linear models with normal distributions. Models predicted the change in student score (post-score minus pre-score) as a function of worksheet version (corresponding to Local or Global treatment), and a suite of student characteristics: gender, ethnicity, college grade-point average (GPA), and SAT verbal score (WebFigure 1).

We included interactions of treatment by gender and treatment by ethnicity to test for differential effects of the module on different student groups. We used backwards selection, starting with the most complex model and using likelihood ratio tests, to select the simplest model that contained only covariates that explained significant variance (Bolker 2008).

To explore how student demographic and academic characteristics altered affect, we fit proportional odds models to the responses for each affect question separately – as opposed to the conceptual understanding por-

tion of the test, where we assessed overall changes in knowledge – because we were interested in the separate responses to these questions. Proportional odds models, implemented with the function *polr* in the MASS package (Ripley *et al.* 2013) in R, were appropriate because they allow for ordinal categorical response variables (Agresti and Lang 1993).

We modeled post-score on each affect question independently as a function of pre-score, change in knowledge (post-score minus pre-score on the conceptual questions), activity type (Local versus Global), and the same suite of student characteristics used in the linear models. We included interactions of treatment by gender and ethnicity to test for differential effects of the module on different student groups. We used backwards model selection, starting with the most complex model, and compared candidate models using likelihood ratio tests (Bolker 2008). Reported significance values used a *t* statistic of 1.96 for statistical significance ($P < 0.05$), as that was the appropriate cut-off for 95% confidence for our sample size. We also compared pre- and post-test scores on each of the three affect questions separately, using two-tailed paired *t* tests.

Results

Both treatments of the active learning module – Local and Global examples of climate change – increased student conceptual understanding (Figure 2a) and had significant impacts on student affect (Figure 2, b–d). There was a 45% increase in the frequency of correct answers on the eight-item assessment of conceptual understanding, and students indicated (1) an increased awareness that global warming would affect their lives, (2) a greater willingness to change their personal behavior, and (3) a higher level of support for government action.

Linear models indicated that students performed equally well overall, whether they studied local or global examples (Tables 1 and 2; WebFigure 2), but female students who analyzed the local examples outperformed females who analyzed the global ones (Figure 3a) as well as males who analyzed either example (Figure 3c). In contrast, there was no difference in performance between males who studied the local examples and males who studied the global ones (Figure 3b); there was also no difference in performance between males and females who analyzed the global examples (Figure 3d).

Proportional-odds models showed that student attitudes changed in the direction of increased concern or support in response to all three questions, but were not affected by Local or Global treatments (see WebTables 1–6). In addition:

- For the question “How much do you expect that climate change will affect your life in the future?”, the best and only predictor of final score was affect score on the pre-test: students who had higher affect before the module had higher affect after the module.
- For the question “How willing are you to pay a gasoline tax, drive a smaller car, eat less meat, drive/travel less, or make other changes in the way you live in order to reduce the impact of climate change?”, the best predictors were pre-score and gender: controlling for pre-score, females had higher affect scores than males (Figure 4a). On average, the odds of answering one

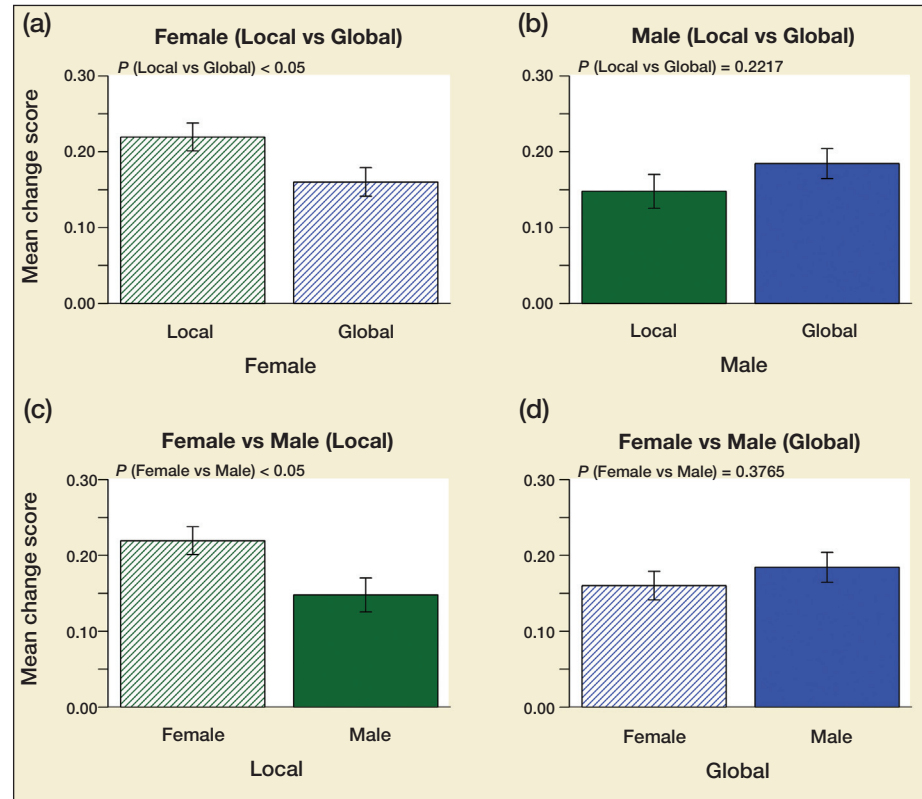


Figure 3. Impact of gender on changes in conceptual understanding. (a) Females who received the local treatment ($n = 129$) outperformed females who received the global treatment ($n = 133$). (b) There was no difference in performance between males who received the local treatment ($n = 89$) and males who received the global treatment ($n = 118$). (c and d) Females who received the local treatment outperformed males who received the local treatment, but there was no significant difference in performance between males and females who received the global treatment. Each bar depicts the raw change in score (post minus pre); data are redrawn in panels to show relevant comparisons: local is always green, global is always blue; females are always hashed, males are always solid. The P values reported are from two-tailed, paired t tests comparing pre- and post-scores. Error bars indicate standard error.

point higher on our five-point scale were 60% greater for females than males.

- For the question “How much do you support US Government ratification of the Kyoto Protocol – an international treaty that calls for a reduction in greenhouse gases – and other government action in order to reduce the impact of climate change?”, the best predictors were pre-score and GPA: controlling for pre-score,

Table 1. The best-fit model predicting the change in student conceptual understanding on the knowledge questions

	Estimate
(Intercept)	0.1676065
GPA	0.0429474
SATVerbal	−0.0001998
trtA	−0.0392748
female	−0.0319976
trtA:female	0.0980291

Notes: Boldface estimates indicate significance at < 0.05 . GPA = college grade-point average; SATVerbal = SAT verbal test score; trtA = Local treatment.

Table 2. Comparing the null and final models of student conceptual understanding on the knowledge questions

Model	Residual degrees of freedom	Residual sum of squares	Sum of squares	Pr(>Chi)
1 [†]	468	22.266		
2 [‡]	463	21.697	0.56914	<0.05

Notes: [†]Model 1: ChangeScore ~ 1; [‡]Model 2: ChangeScore ~ GPA + SATVerbal + trtA + female + trtA * female.

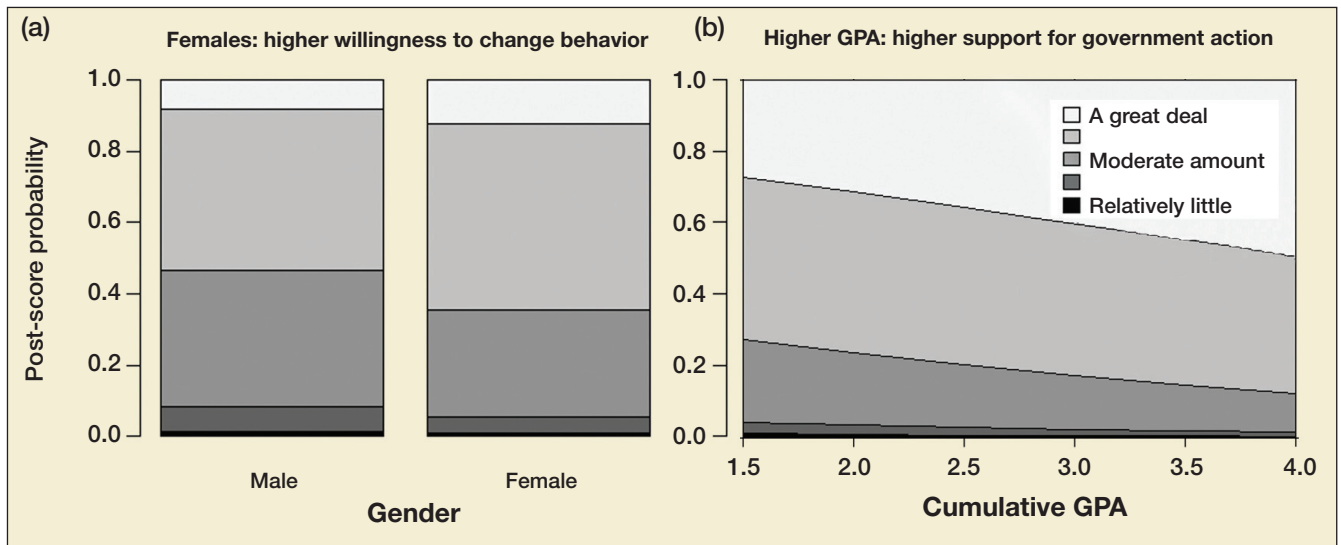


Figure 4. Impact of gender and academic performance on student affect. (a) Controlling for pre-score, females had higher affect scores than did males on one question about personal behavior. (b) Controlling for pre-score, students with higher GPAs had higher affect scores on one question about support for government action. All affect questions were answered on the scale: 1 = relatively little, 3 = a moderate amount, and 5 = a great deal.

students with a higher GPA responded more favorably to government involvement (Figure 4b). A one-unit increase in the four-point GPA scale was correlated with 48% higher odds of students thinking more favorably of government intervention.

■ Conclusions

Although this is the first study to demonstrate a disproportionate increase in conceptual understanding by women in response to local examples of the effects of climate change, the result is consistent with work by McCright (2010), who found that women generally know more about climate change than men, and with data from Myers *et al.* (2012), which suggest that place-based climate-change education strategies are critical for people to understand and accept climate change. The increased willingness among female students to change their personal behavior is also consistent with survey data analyzed by Hunter *et al.* (2004), indicating that women were more likely than men to act in response to climate change, and McCright (2010), who reported that women are more concerned than men about this issue.

Our results indicate that instructors should prioritize local examples in climate-change curricula for undergraduates, given that such examples increase learning gains for women without reducing learning gains for men. Furthermore, because the worksheets used in this study produced statistically significant learning gains for both women and men in just a single day of instruction, our results are consistent with calls for wide implementation of evidence-based, active-learning strategies in climate-change education (McCright *et al.* 2013). These points are relevant to recently launched education initiatives, such as the National Aeronautics and Space Administration's Earth Systems, Technology and Energy

Education for Minority University Research and Education Project (NASA 2014) and the US National Science Foundation's Climate Change Education Partnership program (NSF 2010), and to repositories for classroom materials, such as the National Oceanic and Atmospheric Administration's Education Resources collections (NOAA 2014).

Although our study highlights the importance of using local examples in climate-change education, it also points to the need for additional research on at least two fronts. First, further work is required to confirm the patterns documented here in other student populations, in other course contexts, and with other aspects of climate-change science. For instance, given the influence of political persuasion and religious beliefs on attitudes about climate change (McCright 2010; McCright and Dunlap 2011), do student populations from areas that are more politically and socially conservative than the sample of the urban, West Coast population we studied exhibit the same patterns as documented here? Do women learn more from local examples in teaching situations outside a college classroom? And do the gender differences reported here occur in curricula focused on climate-change research from the physical sciences, or only when considering biological impacts? Second, more research is needed to understand which conditions enhance or reduce linkages between increased knowledge about climate change and behavioral modifications (Malka *et al.* 2009; McCright 2010; McCright and Dunlap 2011). We must understand the conditions under which people are most likely to change their behavior to mitigate the negative impacts of climate change.

Will the widespread use of local examples inspire women to learn more, and lead to personal and public changes in behavior relevant to climate change? What

types of instruction or experiences motivate learning and attitude changes in men? Time, along with further research and educational efforts, will tell.

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