

TEMPORAL ECOLOGY OF A SUBALPINE ECOSYSTEM:
PLANT COMMUNITIES, PLANT-POLLINATOR INTERACTIONS, & CLIMATE CHANGE

by

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DEDICATION

*To mom, for showing me how to observe the world,
and to Amy, for making it brighter.*

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ABSTRACT

Ecological systems are inherently dynamic, and a primary way in which they are dynamic is through time. Individual organisms, populations, communities, species interactions, and ecosystem functions all follow a temporal progression from the past, to the present, and into the future. This temporal progression can occur over the course of minutes, hours, days, weeks, months, years, decades, or various other timescales. In this sense, temporal dynamics are an intrinsic property of all biological systems. In fact, one of the most prominent signals of recent global climate change is the significant change in the timing of biological events for a diversity of organisms. In light of this widespread pattern, there is a renewed interest in understanding the multifaceted importance of time in ecology. In this dissertation, I investigate the temporal ecology of a subalpine ecosystem, specifically focusing on flowering plant communities and plant-pollinator interactions. I examine the temporal dynamics of this system over multiple decades in response to ongoing climate change as well as over shorter time scales within a growing season. Using a 39-year record of flowering phenology, I show that species-specific shifts in the timing of flowering in response to climate change can substantially reshape a subalpine plant community over this time period. Community phylogenetic analyses reveal that these changes are largely independent of evolutionary history. Using a laboratory experiment, I show that the timing of an important harsh abiotic event – low temperatures that cause frost damage to plants – can differentially affect flowering plant species, with implications for plant demography, community structure, and interactions with pollinators. Finally, I show that plant-pollinator interactions exhibit substantial within-season temporal turnover, and that this temporal flexibility of plant-pollinator interactions from one week to the next is consistent and predictable

across years. Taken together, this dissertation provides a multifaceted investigation of the temporal ecology of plant communities and plant-pollinator interactions, revealing the important consequences of ecological timing at short-term and longer-term scales.

INTRODUCTION

The changing of the seasons has long been of human interest. Indeed, any keen observer can provide an account of the timing of a biological event that is important to her or him, for example, when flowers appear each year in the spring, when certain fruits begin to ripen during the summer, or when leaves start to change color in the autumn. The Greek root word *phainomai* means *to appear*, and phenology is the study of the timing of seasonal events. Nearly 2,000 years ago, the Roman natural historian Gaius Plinius (Pliny the Elder) recognized phenology as a useful indicator of weather conditions and used these phenological observations to guide the planting of certain crop species (Bostock and Thomas 1855). Similarly, Carl Linnaeus favored the phenological observations of leaf and flower budding of certain species as far better indicators of the changing seasons compared to a rigid Western calendar (Linnaeus 1737). Even more recently, farmers' phrases point to an appreciation for species-specific phenological observations, for example, "*plant corn when oak leaves are as large as a squirrel's ear*".

A combination of abiotic and biotic conditions ultimately determine an organism's phenology (Forrest and Miller-Rushing 2010, Post 2013, Wolkovich et al. 2013). In this sense, phenology is a primary axis of the niche, across which organisms can partition resources ranging from light and water to pollinators and seed dispersers. The seasonal timing of life history events is intimately tied to growth, reproduction, and survival (Forrest and Miller-Rushing 2010). From humans, to polar bears, to subalpine plants, failure of any organism to recognize and respond to changing seasonal conditions can have considerable consequences for their growth, reproduction, and survival (Rathcke and Lacey 1985, Inouye 2008, Post and Forchhammer 2008, Miller-Rushing et al. 2010, Cherry et al. 2013).

Temporal dynamics are an intrinsic property of all biological systems. Individual organisms, populations, communities, species interactions, and ecosystem functions all follow a temporal progression from the past, to the present, and into the future. This temporal progression can occur over the course of seconds, minutes, hours, days, weeks, months, years, decades, centuries, millennia, or longer. Now, as global climate change is significantly altering the phenology of many organisms across the globe, there is a renewed interest in understanding the multifaceted importance of time in ecology (Sparks and Menzel 2002, Miller-Rushing and Primack 2008, Forrest and Miller-Rushing 2010, Wolkovich et al. 2013, Post 2013, Wolkovich et al. 2014).

Indeed, phenology is one of the strongest bio-indicators of global climate change (Sparks and Menzel 2002, Parmesan and Yohe 2003). Phenological events are generally occurring earlier in accordance with warmer temperatures under climate change, although several studies have now highlighted the species-specific nature of phenological responses to climate change (Bradley et al. 1999, Menzel et al. 2006, Parmesan 2007). As we make progress to understand how the phenology of organisms is *responding* to rapid changes in climate, we are also learning for the first time about many basic aspects of their phenology and temporal ecology. Why is there so much variation in species' phenological responses to abiotic variation? Are responses similar among closely related taxa? How does phenological variation influence interactions with other species over the short-term (i.e., within a growing season) and over the longer time scales (i.e., across years or decades)? More broadly, how do changes in phenology influence the structure and function of communities and ecosystems? All of these questions can be addressed within a climate change context, but they simultaneously represent gaps in our understanding of basic temporal ecology. For example, understanding the likelihood and potential consequences

of a phenological mismatch between plants and pollinators under climate change requires an understanding of the temporal ecology of plants and pollinators in the first place.

In this dissertation, I investigate the temporal ecology of a subalpine ecosystem, specifically focusing on flowering plant communities and plant-pollinator interactions. I ask how the ecology of this subalpine ecosystem has changed over several decades in response to ongoing climate change, and also how this system naturally fluctuates over relatively short time scales within a season. Throughout this work, I employ long-term phenological records, short-term observations of plant-pollinator interaction networks, community phylogenetic analyses, and manipulative laboratory experiments. The first two components of this research represent the investigation of the temporal ecology of a subalpine plant community from a longer-term, contemporary climate change perspective. Here, I investigated how climate-driven shifts in flowering phenology may affect plant community patterns over the last 39 years in response to local climate change. Next, to further explore these findings, I asked whether the phenological shifts observed in this community reflect evolutionary relationships among taxa. The second two components of this dissertation research represent two different investigations of temporal ecology at shorter time scales (i.e., within a growing season). The first study investigates how harsh episodic frost events can differentially affect plant species, and whether the negative effects of frost damage relate to plant phenology. The second study explores the within-season temporal dynamics of plant-pollinator interactions, asking whether variation in these interactions follows a consistent sequence over the course of the growing season and what ecological factors may ultimately constrain their temporal variation.

All of this dissertation research was conducted at the Rocky Mountain Biological Laboratory (RMBL) in the Colorado Rocky Mountains, USA. This mountain ecosystem has

served as an exceptional study system for the investigation of the temporal ecology of plant communities, plant-pollinator interactions, and the effects of climate change for several reasons. First, the ecosystem can be characterized by rapid seasonal transitions (*sensu* Pau et al. 2011). The growing season is brief, typically extending only 3–5 months, and is otherwise hemmed in on either side by harsh winter conditions. Therefore, not only does the season have a clear start and end, but the bulk of the ecosystem's ecology can be observed over the course of a few months. Second, the RMBL has a rich legacy of long-term records (i.e., several decades) of the ecology of the surrounding areas, including records of plant and animal phenology, as well as detailed local weather observations (Billick and Price 2010). In particular, the 39-year flowering phenology dataset initiated and collected by David Inouye has served as an integral foundation for this research. Not only does this information provide a solid basis for developing detailed ecological questions about the ecosystem, but it also serves as a basis for directly asking how climate patterns have changed and how particular organisms are responding to such changes. Finally, high elevation (and also high latitude) ecosystems are some of the most sensitive ecological systems to climate change. Thus, understanding their temporal ecology is of critical importance as these systems are predicted to continue to change rapidly.

Taken together, this dissertation provides a multifaceted investigation of the temporal ecology of subalpine plant communities and plant-pollinator interactions, revealing the important consequences of ecological timing at both short- and long-term scales.

PRESENT STUDY

The studies discussed here are each presented in the form of manuscripts appended to this dissertation. The following is a summary of the most important findings from each.

2.1 Shifts in flowering phenology reshape a subalpine plant community (Appendix A)

In this study, we employ a uniquely comprehensive 39-year flowering phenology dataset from the Colorado Rocky Mountains to ask how species-specific phenological shifts can reshape plant community structure. We reveal a diversity of species-level phenological shifts that bring into question the accuracy of previous estimates of long-term phenological change. We show that first, peak, and last flowering rarely shift uniformly and instead usually shift independently of one another, resulting in a diversity of phenological changes through time. Shifts in the timing of first flowering on average overestimate the magnitude of shifts in the timing of peak flowering, fail to predict shifts in the timing of last flowering, and underrepresent the number of species changing phenology in this plant community. Ultimately, this diversity of species-level phenological shifts contributes to altered co-flowering patterns within the community, a redistribution of floral abundance across the season, and an expansion of the flowering season by more than one month during the course of our study period. These results demonstrate the substantial reshaping of ecological communities that can be attributed to shifts in phenology. Finally, we proposed the novel idea that climate induced shifts in phenology can lead to temporal no-analogue communities (i.e., novel combinations of species in time, analogous to spatial no-analogue communities) that are likely to precede the loss or gain of species from the community. This research was published in 2014 in the *Proceedings of the National Academy of Sciences of*

the United States of America.

2.2 Phenological responses to climate change do not exhibit phylogenetic signal in a subalpine plant community (Appendix B)

In this study, we explored phylogenetic signal in flowering phenology and in phenological sensitivity to temperature and snowmelt using a 39-year record of flowering from the Colorado Rocky Mountains. Phylogenetic relationships may underlie species-specific phenological sensitivities to abiotic variation and may help to predict these responses to climate change. Although shared evolutionary history may mediate both phenology and phenological sensitivity to abiotic variation, few studies have explicitly investigated whether this is the case. Consistent with other studies, we found evidence in support of phylogenetic signal in first flowering date. However, the strength and significance of that signal were inconsistent across other measures of flowering in this plant community: peak flowering date exhibited the strongest phylogenetic signal, followed by first flowering date; last flowering date and duration of flowering exhibited patterns indistinguishable from random trait evolution. In contrast to first and peak flowering date, phenological sensitivities of all flowering measures to temperature and snowmelt did not exhibit a phylogenetic signal. These findings show that within ecological communities, phylogenetic signal in phenology does not necessarily imply phylogenetic signal in phenological sensitivities to abiotic variation. Furthermore, this suggests that evolutionary relationships among taxa may not be sufficient to predict complex responses to climate change. This study was published in 2015 in *Ecology*.

2.3 Frost sensitivity of leaves and flowers of subalpine plants is related to tissue type and phenology (Appendix C)

In the Colorado Rocky Mountains, warmer temperatures lead to earlier snowmelt, which drives earlier plant phenology. Early phenology can expose plants to a high risk of frost damage because there are more nights with below-freezing temperatures during the early part of the season. Episodic frost events can have strong negative consequences for plant growth, survival and reproduction. Despite the predicted increase in episodic frost events under continued climate change in some ecosystems, our general understanding of the factors associated with frost sensitivity of reproductive and vegetative plant structures in natural plant communities is limited. Variation in the timing of growth and reproduction may be an important strategy by which plants can avoid frost.

In this study, we experimentally investigated the frost sensitivity of eight long-lived perennial herbaceous plant species. The study taxa represent four congeneric pairs from four flowering plant families; within each pair, there is a species with early and late growth and reproductive phenology. Thus, we control for evolutionary history – and therefore additional traits shared through common ancestry – to some degree, while examining the influence of phenology on frost sensitivity. Specifically, we compared frost sensitivity of vegetative and reproductive structures for each species and asked whether frost sensitivity was similar between species within congeneric pairs or, instead, was related to phenology (i.e. differences in the timing of growth and reproduction). For most species (6 of 8), flowers were more sensitive to frost than leaves. Within most congeneric pairs (3 of 4), the leaves of species with later phenology were significantly more sensitive to frost than the leaves of species with earlier phenology. For flowers, the later flowering species were more sensitive in two of the four

congeneric pairs. This study contributes to our general understanding of factors related to interspecific differences in plant sensitivity to episodic frost events of naturally occurring species. The increased frost sensitivity of reproductive structures compared to vegetative structures may be a widespread pattern for long-lived perennial plants. Furthermore, we found evidence for a trade-off between phenology and frost sensitivity, whereby species with later phenology exhibit higher frost sensitivity compared to species with earlier phenology. These results have implications for plant populations, species interactions and ecological communities. This experimental study was published in 2016 in *Journal of Ecology*.

2.4 Within-season temporal dynamics of plant-pollinator interactions: patterns, consistency, and ecological constraints (Appendix D)

In this study, we investigated the within-season temporal dynamics of plant-pollinator interactions using weekly censuses of plant-pollinator interactions over three years in a subalpine ecosystem in the Colorado Rocky Mountains, USA. Specifically, we asked: (1) what are the relative contributions of species turnover and interaction rewiring to the within-season temporal turnover of interactions, (2) how does interaction turnover vary from week to week within a season, and (3) are within-season patterns of interaction turnover consistent from one year to the next? We then employed a series of probability-based simulation models to ask (4) whether within-season interaction turnover and rewiring are constrained by phenology, abundance, morphological size matching, or their joint combinations. We found that the magnitude of within-season interaction turnover was consistently high and primarily driven by rewiring in all three years of the study. Our simulation models suggest that phenology and species abundance appear to be primary

ecological constraints underlying temporal interaction turnover in this system. These results provide clear evidence that plant-pollinator interactions exhibit strong within-season temporal dynamics that appear to be consistently constrained by species phenology and relative abundances. Furthermore, these results underscore the dynamic nature of species interactions at short-time scales and provide useful information for better understanding how complex assemblages of species may respond to dramatic global change. This research is in final preparation for submission to *Ecology Letters*.

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APPENDIX A: SHIFTS IN FLOWERING PHENOLOGY RESHAPE A SUBALPINE
PLANT COMMUNITY

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Shifts in flowering phenology reshape a subalpine plant community

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Phenology—the timing of biological events—is highly sensitive to climate change. However, our general understanding of how phenology responds to climate change is based almost solely on incomplete assessments of phenology (such as first date of flowering) rather than on entire phenological distributions. Using a uniquely comprehensive 39-y flowering phenology dataset from the Colorado Rocky Mountains that contains more than 2 million flower counts, we reveal a diversity of species-level phenological shifts that bring into question the accuracy of previous estimates of long-term phenological change. For 60 species, we show that first, peak, and last flowering rarely shift uniformly and instead usually shift independently of one another, resulting in a diversity of phenological changes through time. Shifts in the timing of first flowering on average overestimate the magnitude of shifts in the timing of peak flowering, fail to predict shifts in the timing of last flowering, and underrepresent the number of species changing phenology in this plant community. Ultimately, this diversity of species-level phenological shifts contributes to altered coflowering patterns within the community, a redistribution of floral abundance across the season, and an expansion of the flowering season by more than 1 mo during the course of our study period. These results demonstrate the substantial reshaping of ecological communities that can be attributed to shifts in phenology.

growing season | no-analogue community | phenological mismatch | phenology curve | species interactions

Phenology, the timing of biological events, is intimately tied to the reproduction and survival of organisms (1). Phenological events generally are occurring earlier in temperate environments in accordance with climate change, although several recent studies have emphasized species-specificity in the direction and magnitude of change (2–5). The great majority of these long-term datasets contain a single measure of phenology for individual species, most often the first day on which a biological event is observed (i.e., “phenological firsts” such as first flowering) (Fig. 1A). In addition to phenological firsts, basic components of an entire phenological response include the timing of the ending of a biological event and details of intermediate stages, such as the timing and magnitude of peak abundance or activity (Fig. 1A). Given that phenological firsts represent the early tail of a population-level response, most assessments of phenological change to date may provide an incomplete view of the magnitude of change, the number of responsive species, and how species-level shifts contribute to change at higher levels of biological organization.

We have amassed a unique long-term record of flowering phenology that allows us to investigate complete phenological responses for a plant community. Over a 39-y period (1974–2012), we have sampled a montane site (2,900 m elevation) in Colorado, USA, counting the total number of flowers of 121 plant species across a series of permanent plots approximately every other day throughout the entire growing season [a map of the 30 permanent 2 × 2 m plots and description of the study site are published elsewhere (6, 7)]. Because flowering phenology is shaped by the abiotic environment as well as biotic

interactions—plant–plant competition, attraction of mutualists, and avoidance of antagonists—changes in flowering phenology have broad implications for ecological interactions and their evolutionary consequences, including those among plants and with higher trophic levels (8). Here we report on 60 common plant species representative of the meadow communities at our site [mostly perennial herbs, excluding less-common species for which data are insufficient (9, 10)]. This portion of the phenology census yields more than 2 million flower counts from which we can assess (*i*) multiple aspects of changes in flowering phenology for individual species, (*ii*) how accurately shifts in first flowering predict shifts in peak and last flowering, and (*iii*) how species-specific shifts in first, peak, and last flowering, as well as changes in floral abundance, contribute to altered patterns of interaction potential among species and changes in community-level distribution of flowers across the season.

Results and Discussion

Here we focus on changes in flowering phenology that have occurred over our 39-y record, a timeframe during which summer air temperatures increased by 0.4 ± 0.1 °C per decade (June–August mean air temperature: $R^2 = 0.32$, $P = 0.0002$) and the date of spring snowmelt advanced by 3.5 ± 2.0 d per decade ($R^2 = 0.07$, $P = 0.10$) (10). Both temperature and the timing of snowmelt are strongly associated with shifts in flowering phenology in this study system (6, 7, 11–13), independently

Significance

Seasonal timing of biological events, phenology, is one of the strongest bioindicators of climate change. Our general understanding of phenological responses to climate change is based almost solely on the first day on which an event is observed, limiting our understanding of how ecological communities may be responding as a whole. Using a unique long-term record of flowering phenology from Colorado, we find that the number of species changing their flowering times likely has been underestimated and the magnitude of phenological change overestimated. In addition to earlier first flowering, we document a diverse assortment of other changes, such as delayed last flowering, as temperatures warm. This variety of species-level phenological shifts has ultimately reshaped various temporal components of the plant community.

Author contributions: D.W.I. designed research; P.J.C., A.M.I., and D.W.I. performed research; P.J.C. and A.M.I. analyzed data; and P.J.C. and A.M.I. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Data deposition: The flowering phenology dataset and metadata are archived at www.rmbi.org and in the Digital Repository at the University of Maryland (<http://drum.lib.umd.edu/>).

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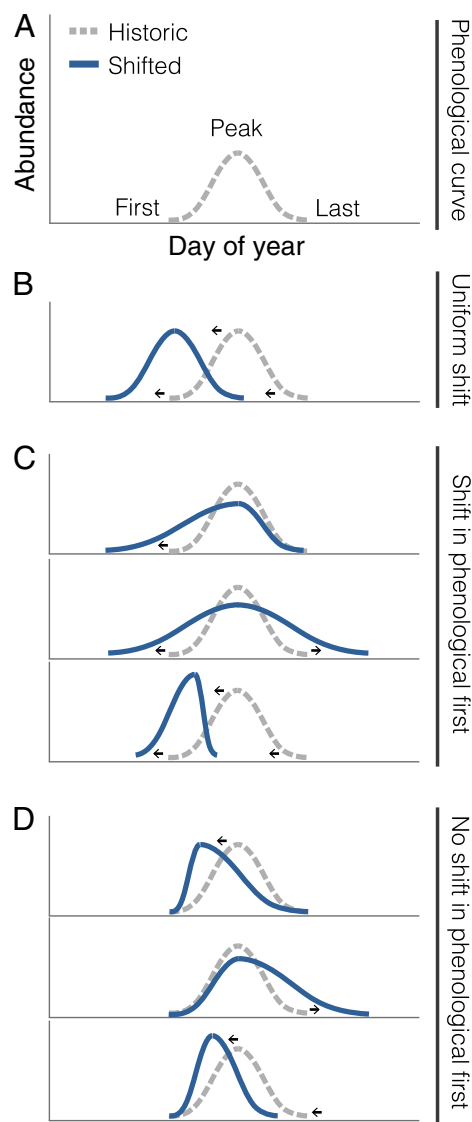


Fig. 1. Conceptual representation of shifts in multiple phenological measures for individual species through time. (A) Multiple measures of flowering phenology available for 60 species from a 39-y study of a plant community in the Colorado Rocky Mountains, USA. (B) If shifts in first flowering change at a rate similar to changes in other measures of phenology, then the distribution shifts forward uniformly through time. (C and D) In contrast, shifts in first flowering may be unrepresentative of both the direction and magnitude of changes in peak and last flowering (C), and peak and last flowering may shift while first flowering remains unchanged (D). Arrows indicate a shift in phenology. For simplicity of conceptual illustration, initial species' distributions are represented as a Gaussian curve, and the area under the curve is held constant.

explaining on average 66% and 68% of the interannual variation in flowering phenology, respectively (10).

We find striking diversity in the phenological shifts of individual plant species through time. First flowering on average advanced by 3.3 ± 0.24 d per decade, peak flowering by 2.5 ± 0.20 d per decade, and last flowering by 1.5 ± 0.42 d per decade; significant shifts were observed in 50%, 38%, and 30% of species' first, peak, and last flowering, respectively, and some form of change occurred in 68% of species (41/60). Thus, basing assessments of phenological change on a single measure of phenology underestimated the number of responsive species by 18–38%, depending on the measure (first, peak, or last

flowering) used. Many species exhibit inconsistent shifts in first, peak, and last flowering (Fig. 2), resulting in changes in flowering duration for 27% of species (Table S1). Of all of the species exhibiting a significant change in flowering, only 17% (7/41) shifted all aspects of their phenology uniformly forward through time, as indicated by significant temporal advancements in first, peak, and last flowering (Figs. 1B and 2). In contrast, 56% (23/41) of these species showed earlier first flowering in combination with disparate changes in peak and last flowering (Figs. 1C and 2). Finally, 27% (11/41) exhibited significant changes in peak or last flowering with no significant change in

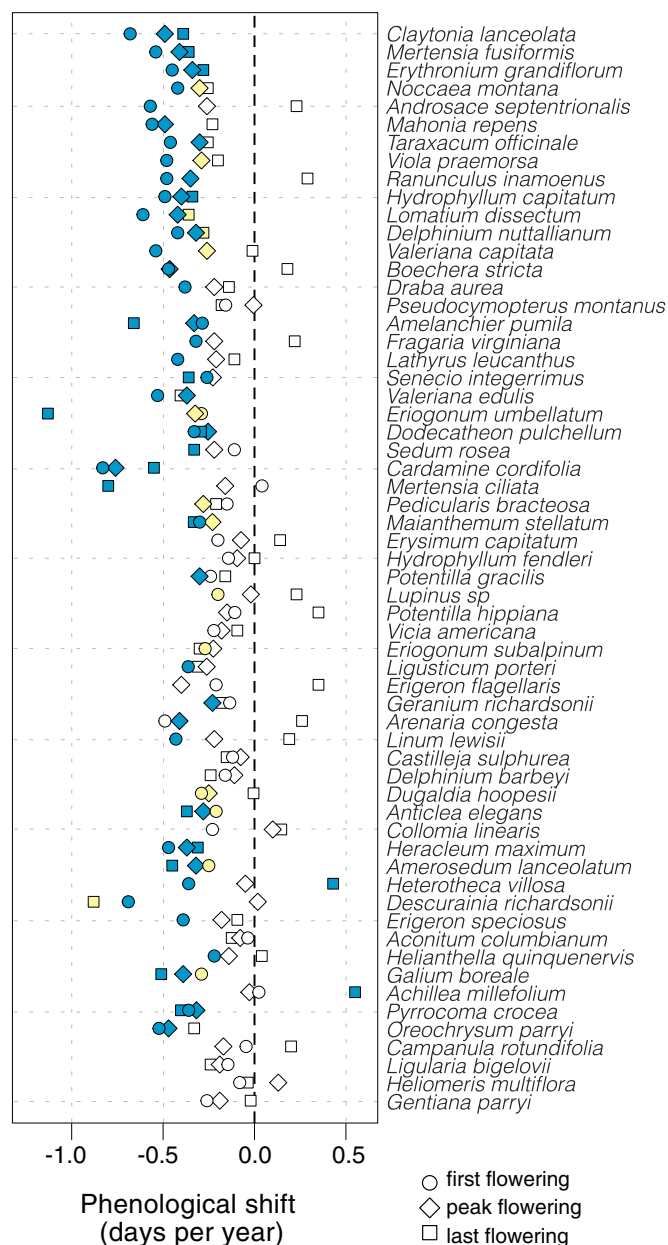


Fig. 2. Shifts in flowering phenology over 39 y (1974–2012). Each symbol represents a phenological shift as the slope of a line from simple linear regressions of first, peak, and last flowering by year (n = no. of years for each species). Significant shifts are represented in blue ($P \leq 0.05$), marginally significant shifts in pale yellow ($0.05 < P \leq 0.10$), and nonsignificant shifts in white ($P > 0.10$). Species are presented in order of mean date of first flowering throughout the growing season.

first flowering (Figs. 1*D* and 2), indicating that classifications of species as responsive or nonresponsive based on phenological firsts can be inaccurate. Intraspecific variation in phenological sensitivity to changing abiotic conditions likely accounts for these independent shifts in first, peak, and last flowering at the population level. For example, the population-level pattern in Fig. 1*C* (Top) could result from the earliest-flowering individuals advancing their flowering at a faster rate than later-flowering individuals; alternatively, this pattern could result from several individuals advancing only their onset of flowering while maintaining open flowers for a longer timeframe. Although very little is known about intraspecific variation in phenological shifts, interspecific variation has emerged as a general pattern across the globe (2–5). Our results demonstrate a more nuanced type of species specificity than has been shown before. Observing this diversity of population-level responses dispels the overly simplistic notion that species' composite phenologies are advancing, delaying, or not changing through time (Fig. 1).

The probability of detecting phenological shifts in long-term data can be affected by changes in abundance (14). Indeed, one benefit of collecting abundance-based phenological data is the ability to examine evidence for biases in estimates of phenological shifts. For example, if a species' floral abundance is increasing through time, its flowers are more likely to be observed both earlier and later in the season simply because there are more flowers to observe. Thus, apparent advances in first flowering and delays in last flowering potentially could reflect increased floral abundance instead of an actual phenological shift; the opposite would be expected with decreasing floral abundance. One third (20/60) of the species in our study exhibited significant changes in peak floral abundance over the timeframe of our study (Table S1). However, in only six cases (first flowering in two species and last flowering in four species) did we detect evidence that advanced phenology could be an artifact of changes in peak floral abundance (Table S2). The great majority of species showing a significant shift in flowering phenology did so independently of a change in peak floral abundance.

A paucity of long-term abundance-based phenological datasets has led to the implicit or explicit assumption that a single phenological measure represents an entire population-level phenological response (Fig. 1*B*) (13–15), but our results do not support this assumption. In this plant community, for every day that species-level first flowering advanced, the timing of peak flowering advanced by only 0.55 ± 0.09 d ($R^2 = 0.42$, $F_{1, 58} = 41.8$, $P < 0.0001$). Furthermore, shifts in species-level first flowering failed to predict shifts in last flowering ($R^2 = 0.05$, $F_{1, 58} = 3.20$, $P = 0.079$, slope = 0.40 ± 0.22 d). The ability of changes in first flowering to predict changes in peak and last flowering is nearly identical when phenological sensitivities to temperature or snowmelt are used in place of change through time (Table S3). Our results for 60 plant species, combined with information on first vs. mean arrival dates of three migratory bird species (16), are suggestive of a general pattern in which phenological firsts change at a faster rate than other measures of the same phenological event. Although additional abundance-based phenological studies will lend insight into the generality of our results, we can make recommendations for refining models of phenological change. Current predictive models based on phenological firsts are likely to exaggerate the magnitude of phenological change; to account for this potential source of bias, models should allow for a dampening of the response of the timing of peak abundance relative to phenological firsts. When modeling the end of life history events, variability in both the direction and magnitude of shifts in phenology should be incorporated.

Species-specific phenological shifts are widely hypothesized to affect patterns of temporal overlap among species, but the extent of such changes for entire communities has remained elusive

(17–19). We contend that the consequences of species-specific phenological change for interactions within trophic levels are generally underappreciated, especially in light of widespread concern about trophic mismatch (20–22). Interactions within trophic levels are important because they can affect community structure and stability (23–25) as well as regulate the response of ecological communities to climate change (26). In this study, 23.2% (725/3,119) of species' pairwise coflowering interactions changed significantly over the 39-y record (of 3,540 possible pairwise coflowering interactions, 3,119 were realized) (Fig. 3). A total of 10.5% (329) of all plant species pairs increased in coflowering, and 12.7% (396) decreased (Fig. 3). A change in coflowering represents altered interaction potential (27), which can affect various ecological processes (19). Increased coflowering between plant species can exacerbate direct interspecific competition for abiotic resources (28) and can affect plant reproductive success indirectly via competition for or facilitation of pollination (29, 30). Because of its effect on plant reproduction, competition for pollination is thought to promote selection for sequential flowering (29, 31–33). Therefore it is probable that the changes in coflowering patterns shown here differ from those that have been shaped over longer timescales by natural selection.

Although climate-induced changes in community composition have been attributed mainly to species loss and colonization in association with shifting geographic ranges (34, 35), these coflowering shifts provide an example of an altered composition in the temporal community in the absence of species' extinction or colonization. No-analog communities are defined as those with no contemporary analog, formed through the dissolution of contemporary species assemblages and the formation of new ones via species-specific range shifts (36, 37). Our results present a similar scenario over modern climate-change time scales in the temporal rather than the spatial dimension. Parallel to the way that species-specific range shifts can lead to novel patterns of

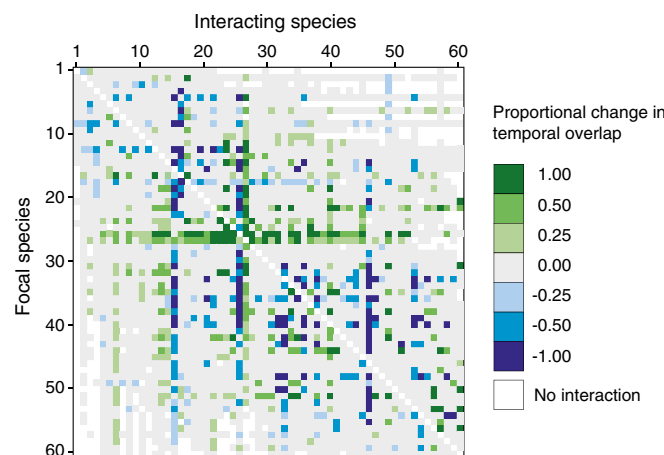


Fig. 3. Community-level change in interaction potential over 39 y. Each cell represents the proportional change in interaction potential, or coflowering overlap, between species pairs over the 39-y study period (1974–2012). Coflowering was calculated annually as the total number of flowers of every species pair that overlap in time, divided by the total number of flowers of the focal species (see main text for an example). To represent change in coflowering visually for all species, we multiplied each rate of change by 39 y; thus, a proportional change of 0.25 indicates a 25% increase in overlap of a focal species with an interacting species over the course of our study period. Proportional changes in overlap values are binned (e.g., $0.25 = 0.01–0.25$). Colored cells indicate significant changes in interaction potential through time ($P \leq 0.05$), gray cells indicate no change, and white cells indicate cases in which species pairs did not coflower in any years of the study. Species are ordered by mean first flowering date (as in Fig. 2).

spatial co-occurrence, species-specific phenological shifts can lead to novel patterns of temporal co-occurrence (Fig. 3).

Our abundance-based phenology record also allows us to describe change in aggregate community-level phenology (i.e., a seasonal flowering curve for the entire community; Fig. 4A). Aggregate community-level responses are associated with the timing of snowmelt and air temperature, in directions consistent with climate change (Table S4). First flowering at the community level has advanced by 25.0 d over the course of this 39-y study at a rate of 6.4 ± 2.1 d per decade ($R^2 = 0.23$, $F_{1,31} = 9.13$, $P = 0.005$) (Fig. 4B). The timing of the spring peak in floral abundance has advanced by 20.7 d, at a rate of 5.3 ± 1.7 d per decade, whereas the number of flowers composing this peak has remained constant ($R^2 = 0.26$, $F_{1,29} = 10.11$, $P = 0.0035$ and $R^2 = 0.02$, $F_{1,29} = 0.70$, $P = 0.41$, respectively) (Fig. 4B). Similarly, the timing of summer peak floral abundance has advanced by 12.9 d, at a rate of 3.3 ± 1.6 d per decade, with no change in the number of flowers composing this peak ($R^2 = 0.12$, $F_{1,31} = 4.3$, $P = 0.047$ and $R^2 = 0.03$, $F_{1,31} = 0.81$, $P = 0.38$, respectively) (Fig. 4B). In contrast, the date of community-level last flowering has delayed by 12.1 d at a rate of 3.1 ± 1.3 d per decade ($R^2 = 0.13$, $F_{1,35} = 5.37$, $P = 0.026$) (Fig. 4B). The length of the flowering season has expanded by 34.7 d, at a rate of 8.9 ± 1.9 d per decade ($R^2 = 0.42$, $F_{1,31} = 22.72$, $P < 0.0001$) (Fig. 4C), but total floral abundance across the season has remained constant ($R^2 < 0.001$, $F_{1,31} = 0.003$, $P = 0.96$), indicating that the same number of flowers is spread across a longer growing season.

Species-level changes underlie the month-long expansion of the flowering season. Earlier-flowering species advanced their first and peak flowering more rapidly than later-flowering species, a trend not exhibited in last flowering (first: $R^2 = 0.25$,

$F_{1,58} = 19.42$, $P < 0.0001$; peak: $R^2 = 0.17$, $F_{1,58} = 11.97$, $P = 0.001$; last: $R^2 = 0.019$, $F_{1,58} = 1.16$, $P = 0.29$). Although the advancing onset of spring plays a clear role in the expansion of growing seasons in plant communities, the role of end-of-season events is less clear: hypothetically, the end of the season could advance at a slower rate than onset, could not change, or could be delayed (38). In our study, end-of-season flowering is delayed. The two species largely responsible for this delay of end-of-season flowering show significant delays in last flowering with either an advance or no change in first flowering (Figs. 1C and D and 2), again highlighting how phenological firsts can misrepresent overall phenological change. The flowering season is brief in this subalpine plant community (9), so a month-long expansion represents an approximately 30% increase. Redistribution of flowers across this expanded flowering season likely has repercussions for community structure, interactions within and among trophic levels, and ecosystem function (Figs. 3 and 4) (39–41).

Here we show that the classification of species-specific phenological responses to climate change as advancing, delaying, or not changing is an oversimplification when such assessments are based solely on phenological firsts (Figs. 1 and 2). Assuming that phenological firsts represent overall phenological change can lead to inaccurate assessments of the magnitude of change and the number of responsive species within an ecological community, with implications for forecasting phenological shifts under future climate scenarios. We demonstrate that first, peak, and last flowering rarely shift uniformly but instead tend to shift independently of one another, resulting in a wide range of phenological changes through time in individual species. This diversity of species-level shifts in phenology ultimately leads to altered patterns of coflowering (Fig. 3), expansion of the flowering season, and community-level redistribution of floral abundance (Fig. 4). Our results highlight both the importance of considering phenology more broadly than first observations and the substantial reshaping of ecological communities that can be attributed to climate-induced shifts in phenology.

Methods

Study Site and Dataset. This study was conducted at the Rocky Mountain Biological Laboratory (RMBL) in the Colorado Rocky Mountains, USA (38°57.5' N, 106°59.3' W, 2,900 m above sea level). For each of the 121 flowering plant species that occur in our thirty 2×2 m plots, either the number of flowers per stalk or the number of flowering inflorescences (for species with many small flowers) were counted every other day throughout the growing season from 1974–2012. Copies of the flowering phenology dataset and metadata are archived at www.rmb.org and in the Digital Repository at the University of Maryland (<http://drum.lib.umd.edu>). We limited the analysis to species that were present in at least half of the years of the dataset (19 y), leaving a total of 60 species that represent the meadow plant communities in and around the RMBL (see ref. 10 for more information about plant species). There was no census in 1978 and 1990. Thus, there was a maximum of $n = 37$ y for each species, and a minimum of $n = 19$ y because not all species flower in every year. Five of the 30 plots were added in later years: two in 1985 and three in 1998. The addition of five plots should not alter estimates of phenological change, because the magnitude of phenological change generally is not affected by changes in peak floral abundance (Table S2). Furthermore, we find no relationship between changes in peak floral abundance and shifts in the timing of peak flowering for the 60 species studied here ($r = 0.038$, $n = 60$ species). These five plots were excluded from analyses that used floral abundance: species-level change in peak floral abundance, coflowering patterns, and aggregate community-level responses. Records for the annual timing of snowmelt come from a permanent 5×5 m snow plot at the RMBL in which the first day of bare ground is recorded as the date of snowmelt. Mean temperatures used in analyses are the average of the daily minimum and maximum temperatures at the Crested Butte National Oceanic and Atmosphere Administration weather station (ca. 9 km south of the RMBL).

Species-Level Analyses. For each species, the number of flowers was summed across all 30 plots on each census day to create one annual flowering

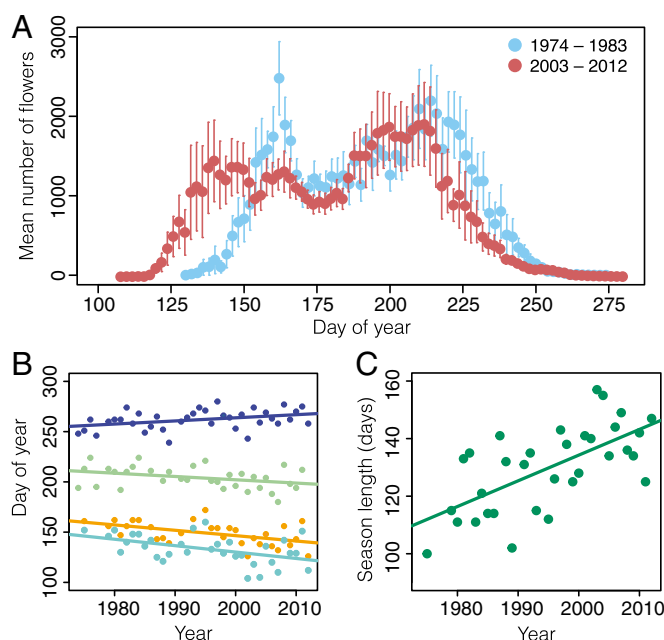


Fig. 4. Aggregate community-level shifts in flowering phenology. (A) Comparison of the season-wide flowering curves for the first and last 10 y of the dataset; 10-y means were used to visualize the amount of change that has occurred in the community flowering curve. Each dot is the 10-y mean number of flowers; error bars are ± 1 SEM. (B) Phenological shifts through time for first flowering of the community (cyan), last flowering for the community (dark blue), and timing of community-level spring peak (orange) and summer peak (green); each dot represents a community-level phenological measure in 1 y. (C) Change in the length of the flowering season; each dot represents the total number of days on which open flowers were present in each year.

distribution per species. First flowering was the first day on which a flower for that species was observed, and last flowering was the last day on which a flower was observed, taken from the across-plot sum. Peak flowering for individual species was the day on which 50% of the flowers were counted (following refs. 9 and 10). Peak floral abundance was the maximum number of flowers counted annually in one census. Years in which the census started late (1976, 1982, 1985, 1992, and 1994) were excluded from analysis when the response variable of interest was affected (first flowering and occasionally peak flowering for the earliest-flowering species). Linear regression was used to analyze change through time, with phenology or peak floral abundance as a response and year as a continuous predictor. We tested for temporal autocorrelation in the time series of species showing significant phenological change through time, using the Ljung-Box test with a lag time of 1 y. We found evidence of significant temporal autocorrelation in only three cases (Table S5). We reanalyzed these three cases with an autoregressive linear model, which allows the error structure to be correlated. The rates of change in these models were very similar to the rates of change in our simple linear regression analysis, and change through time was still significant in all three cases (Table S6). We therefore conclude that temporal autocorrelation in this dataset does not bias our results.

To determine whether phenological shifts could have been an artifact of changes in peak floral abundance (14), we ran correlation analyses for species showing significant shifts in both phenology and peak floral abundance (defined as the maximum number of flowers counted annually in a census for individual species). We looked for increasing peak floral abundance in correlation with earlier first flowering and later last flowering; we also looked for decreasing peak floral abundance in correlation with later first flowering and earlier last flowering. These relationships indicate the possibility of detecting what appears to be a phenological shift that actually is caused by a change in flower abundance (14). We assume that changes in peak floral abundance are indicative of changes in floral abundance because we do not track individual flowers through time. There is no mathematical reason to expect a change in peak abundance to alter the probability of detecting for shifts in the timing of peak flowering.

A thorough analysis of associations of temperature and snowmelt with first, peak, and last flowering has been presented elsewhere; interannual variation in temperature and the timing of snowmelt independently account for a significant amount of variation in first, peak, and last flowering in 93–98% of the species in this study, depending on the flowering response (10). To ensure that our conclusions about phenological predictions based on change through time are not affected by using sensitivities to climate variables in place of year, we used linear regression to assess how well sensitivity of first flowering to climate predicts sensitivity of peak and last flowering to climate (Table S3).

Interaction Potential. For each year, coflowering was calculated as the number of flowers of every pair of species that overlap in time, weighted by the total number of flowers of the focal species. For each pair of species, the minimum number of open flowers of the two species was summed on each census day, representing the total number of flowers for the two species that were open at the same time. We then weighted this minimum value by the total number of flowers for each species in each year, so that coflowering values represent overlap relative to each species' annual floral abundance. For example, the total number of *Claytonia lanceolata* and *Mertensia fusiformis* flowers that overlapped through time in 2012 was 633. A total of 3,909 *C. lanceolata* flowers were counted across all plots in this season, compared with 1,287 flowers of *M. fusiformis*. *C. lanceolata*'s flowering overlap score with

M. fusiformis was 633/3,909 (0.162), and *M. fusiformis*' overlap with *C. lanceolata* was 633/1,287 (0.492). These calculations resulted in 3,540 potential overlap scores for each year (a matrix of 60 species by 60 species, minus the diagonal of same-species interactions). Linear regression was run for each pair of species to examine the amount of change in coflowering overlap through time. We conducted a permutation test of 5,000 runs to obtain *P* values for each regression. Because we already have shown that species-specific phenological shifts are strongly associated with climate (10), we did not analyze the response of coflowering to climate.

Aggregate Community-Level Phenology. Floral abundance was summed across all species and plots on each census day for each year to create an annual community-level phenology curve. Linear regression was used to assess changes in community phenology (first day of flowering, day of spring peak flowering, day of summer peak flowering, last day of flowering, and flowering duration) and community-level floral abundance (spring and summer maximum number of flowers, total number of flowers counted, and average number of flowers counted per census) through time. The onset of the flowering period was missed in 12 y because the census started after flowering had already begun in some of the earliest-flowering plots. In five of these years (1974, 1976–77, 1992, and 1994), peak abundance of the first species to flower and an important component of spring peak flowering, *C. lanceolata* was missed also. These 5 y were excluded from analysis of flowering season length, timing and abundance of spring peak, and start of the flowering season (similar to species-level analyses). For the remaining 7 y (1979, 1982–1983, 1985–1986, 1991, and 1993), we estimated the start of the flowering season based on the slope of a line of flower accumulation from years with known start dates and similar floral abundance. We applied the same procedure to estimate the end of the flowering season for 5 y in which the end of the flowering season was missed (1976–1977, 1984, and 1992–1993). We used ANCOVAs to verify that these estimations did not bias our results by comparing the slopes of regressions using estimated vs. missing values (Table S7).

Two community-level peaks in floral abundance were clearly evident in almost every year, with the exception of 4 y that were excluded from analysis (1985, 1987, 1994, and 2012) (Fig. 4A) (7). Additionally, in 5 y (1989, 1991–92, 2002, and 2007) there was some evidence of a third peak in floral abundance between the spring and summer peaks. We determined the summer peak in these years based on the species that typically compose the summer peak of floral abundance. There was virtually no turnover in species present in the first and last 10 y of the dataset (Fig. 4A), although *Pedicularis bracteosa* was absent in the last 10 y of the dataset. Because this species is relatively rare in this community, we do not expect its absence to affect the community-level patterns shown in Fig. 4A.

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Table S1. Phenological shifts of 60 subalpine plant species in the Colorado Rocky Mountains, USA (1974–2012). Shifts are reported as the slope values from simple linear regressions of flowering time vs. year (days/yr $\pm$ 1 SE); changes in peak floral abundance are shown as percent change over the 39-yr dataset. Species are listed in order of mean first flowering date, with significant changes in bold. Years in which no flowers were observed for a species were included as zeros in the analysis of floral abundance. However, for three species that are difficult to see in the field, we discarded records of years with zero flowers for analysis (*Androsace septentrionalis*, *Collomia linearis*, and *Descurainia richardsonii*). Transformation was required to improve normality of residuals for most species' floral abundance through time; in these cases we added one and then applied a natural log transformation. To report a consistent measure of change in floral abundance, these results are presented as percent change. Outliers were removed to normalize the residuals of floral abundance (15 species) and duration of flowering (10 species). We present results with the outliers removed because this slope should be more representative of long-term change. For only three species did floral abundance results change from significance to marginal significance or vice versa. The trend for *Draba aurea* changed from a significant to a marginally significant increase in peak floral abundance, and *Erysimum asperum* and *Vicia americana* changed from a marginally significant to a significant increase in peak abundance through time. Removal of outliers did not affect the results of changes in duration of flowering through time.

*Response	Species	Shift	R^2	P-value	t_n
first	<i>Claytonia lanceolata</i>	-0.68 $\pm$ 0.22	0.23	0.0053	32
first	<i>Mertensia fusiformis</i>	-0.54 $\pm$ 0.19	0.21	0.0077	32
first	<i>Erythronium grandiflorum</i>	-0.45 $\pm$ 0.16	0.21	0.0065	34
first	<i>Noccaea montana</i>	-0.42 $\pm$ 0.19	0.14	0.034	32
first	<i>Androsace septentrionalis</i>	-0.57 $\pm$ 0.22	0.18	0.0144	32
first	<i>Mahonia repens</i>	-0.56 $\pm$ 0.22	0.19	0.015	31
first	<i>Taraxacum officinale</i>	-0.46 $\pm$ 0.20	0.14	0.029	33
first	<i>Viola praemorsa</i>	-0.48 $\pm$ 0.17	0.19	0.0096	34
first	<i>Ranunculus inamoenus</i>	-0.48 $\pm$ 0.18	0.19	0.011	33
first	<i>Hydrophyllum capitatum</i>	-0.49 $\pm$ 0.15	0.25	0.0027	34
first	<i>Lomatium dissectum</i>	-0.61 $\pm$ 0.23	0.23	0.014	25
first	<i>Delphinium nuttallianum</i>	-0.42 $\pm$ 0.15	0.2	0.0078	35
first	<i>Valeriana capitata</i>	-0.54 $\pm$ 0.17	0.25	0.0034	33
first	<i>Boechera stricta</i>	-0.47 $\pm$ 0.18	0.18	0.018	35
first	<i>Draba aurea</i>	-0.38 $\pm$ 0.17	0.13	0.032	35
first	<i>Pseudocymopterus montanus</i>	-0.17 $\pm$ 0.17	0.036	0.31	30
first	<i>Amelanchier pumila</i>	-0.31 $\pm$ 0.14	0.12	0.038	37
first	<i>Fragaria virginiana</i>	-0.32 $\pm$ 0.14	0.13	0.032	37
first	<i>Lathyrus leucanthus</i>	-0.42 $\pm$ 0.16	0.17	0.011	37
first	<i>Senecio integerrimus</i>	-0.26 $\pm$ 0.15	0.087	0.085	35
first	<i>Valeriana edulis</i>	-0.53 $\pm$ 0.16	0.25	0.0018	37
first	<i>Eriogonum umbellatum</i>	-0.29 $\pm$ 0.15	0.088	0.074	37
first	<i>Dodecatheon pulchellum</i>	-0.33 $\pm$ 0.14	0.14	0.023	37
first	<i>Sedum rosea</i>	-0.11 $\pm$ 0.14	0.024	0.45	26
first	<i>Cardamine cordifolia</i>	-0.83 $\pm$ 0.12	0.6	< 0.0001	34
first	<i>Mertensia ciliata</i>	0.041 $\pm$ 0.12	0.0033	0.73	37
first	<i>Pedicularis bracteosa</i>	-0.15 $\pm$ 0.20	0.028	0.46	22
first	<i>Maianthemum stellatum</i>	-0.3 $\pm$ 0.13	0.16	0.027	30
first	<i>Erysimum capitatum</i>	-0.2 $\pm$ 0.21	0.031	0.35	30
first	<i>Hydrophyllum fendleri</i>	-0.13 $\pm$ 0.18	0.017	0.48	31
first	<i>Potentilla pulcherrima</i>	-0.24 $\pm$ 0.14	0.072	0.11	37

first	<i>Lupinus sp.</i>	-0.2 ± 0.12	0.076	0.099	37
first	<i>Potentilla hippiana</i>	-0.12 ± 0.16	0.02	0.47	29
first	<i>Vicia americana</i>	-0.21 ± 0.15	0.057	0.15	37
first	<i>Eriogonum subalpinum</i>	-0.27 ± 0.16	0.079	0.091	37
first	<i>Ligusticum porteri</i>	-0.35 ± 0.16	0.16	0.035	28
first	<i>Erigeron flagellaris</i>	-0.21 ± 0.21	0.043	0.33	24
first	<i>Geranium richardsonii</i>	-0.16 ± 0.12	0.051	0.18	36
first	<i>Arenaria congesta</i>	-0.49 ± 0.30	0.088	0.12	29
first	<i>Linum lewisii</i>	-0.43 ± 0.16	0.18	0.0098	37
first	<i>Castilleja sulphurea</i>	-0.12 ± 0.17	0.016	0.48	33
first	<i>Delphinium barbeyi</i>	-0.16 ± 0.16	0.03	0.31	36
first	<i>Dugaldia hoopesii</i>	-0.29 ± 0.15	0.1	0.056	36
first	<i>Anticlea elegans</i>	-0.21 ± 0.13	0.076	0.1	36
first	<i>Collomia linearis</i>	-0.23 ± 0.16	0.065	0.15	33
first	<i>Heracleum maximum</i>	-0.47 ± 0.20	0.19	0.025	26
first	<i>Amerosedum lanceolatum</i>	-0.25 ± 0.14	0.094	0.078	35
first	<i>Heterotheca villosa</i>	-0.36 ± 0.13	0.17	0.011	37
first	<i>Descurainia richardsonii</i>	-0.69 ± 0.31	0.2	0.036	22
first	<i>Erigeron speciosus</i>	-0.39 ± 0.14	0.19	0.0085	36
first	<i>Aconitum columbianum</i>	-0.061 ± 0.14	0.0057	0.66	35
first	<i>Helianthella quinquenervis</i>	-0.22 ± 0.13	0.093	0.095	31
first	<i>Galium boreale</i>	-0.29 ± 0.17	0.076	0.098	37
first	<i>Achillea millefolium</i>	0.024 ± 0.14	0.0009	0.87	32
first	<i>Pyrrocoma crocea</i>	-0.36 ± 0.12	0.25	0.0041	31
first	<i>Oreochrysum parryi</i>	-0.51 ± 0.14	0.3	0.0007	35
first	<i>Campanula rotundifolia</i>	-0.046 ± 0.19	0.0019	0.81	35
first	<i>Ligularia bigelovii</i>	-0.17 ± 0.11	0.077	0.11	34
first	<i>Heliomeris multiflora</i>	-0.052 ± 0.20	0.0022	0.8	32
first	<i>Gentiana parryi</i>	-0.26 ± 0.19	0.055	0.19	33
peak	<i>Claytonia lanceolata</i>	-0.49 ± 0.17	0.21	0.0086	32
peak	<i>Mertensia fusiformis</i>	-0.41 ± 0.15	0.18	0.0097	37
peak	<i>Erythronium grandiflorum</i>	-0.34 ± 0.15	0.14	0.029	34
peak	<i>Noccaea montana</i>	-0.3 ± 0.17	0.084	0.091	35
peak	<i>Androsace septentrionalis</i>	-0.26 ± 0.20	0.05	0.2	35
peak	<i>Mahonia repens</i>	-0.49 ± 0.16	0.22	0.0054	33
peak	<i>Taraxacum officinale</i>	-0.3 ± 0.15	0.11	0.053	35
peak	<i>Viola praemorsa</i>	-0.29 ± 0.15	0.096	0.062	37
peak	<i>Ranunculus inamoenus</i>	-0.35 ± 0.16	0.12	0.036	37
peak	<i>Hydrophyllum capitatum</i>	-0.4 ± 0.15	0.16	0.015	36
peak	<i>Lomatium dissectum</i>	-0.42 ± 0.18	0.17	0.031	27
peak	<i>Delphinium nuttallianum</i>	-0.32 ± 0.14	0.14	0.022	37
peak	<i>Valeriana capitata</i>	-0.26 ± 0.15	0.079	0.091	37
peak	<i>Boechea stricta</i>	-0.47 ± 0.17	0.17	0.01	37
peak	<i>Draba aurea</i>	-0.22 ± 0.15	0.061	0.14	37
peak	<i>Pseudocymopterus montanus</i>	-0.006 ± 0.11	0	0.96	30
peak	<i>Amelanchier pumila</i>	-0.33 ± 0.13	0.15	0.017	37
peak	<i>Fragaria virginiana</i>	-0.22 ± 0.14	0.062	0.14	37
peak	<i>Lathyrus leucanthus</i>	-0.21 ± 0.18	0.035	0.27	37
peak	<i>Senecio integerrimus</i>	-0.24 ± 0.15	0.077	0.11	35
peak	<i>Valeriana edulis</i>	-0.37 ± 0.18	0.1	0.051	37

peak	<i>Eriogonum umbellatum</i>	-0.3 ± 0.16	0.097	0.061	37
peak	<i>Dodecatheon pulchellum</i>	-0.34 ± 0.13	0.17	0.012	37
peak	<i>Sedum rosea</i>	-0.22 ± 0.13	0.11	0.11	25
peak	<i>Cardamine cordifolia</i>	-0.76 ± 0.12	0.56	< 0.0001	34
peak	<i>Mertensia ciliata</i>	-0.16 ± 0.12	0.046	0.21	35
peak	<i>Pedicularis bracteosa</i>	-0.28 ± 0.16	0.14	0.09	21
peak	<i>Maianthemum stellatum</i>	-0.23 ± 0.13	0.1	0.096	28
peak	<i>Erysimum capitatum</i>	-0.074 ± 0.20	0.0047	0.72	30
peak	<i>Hydrophyllum fendleri</i>	-0.095 ± 0.18	0.011	0.6	28
peak	<i>Potentilla pulcherrima</i>	-0.30 ± 0.15	0.11	0.05	37
peak	<i>Lupinus sp.</i>	-0.02 ± 0.15	0.0006	0.89	35
peak	<i>Potentilla hippiana</i>	-0.15 ± 0.20	0.021	0.46	29
peak	<i>Vicia americana</i>	-0.18 ± 0.13	0.056	0.16	37
peak	<i>Eriogonum subalpinum</i>	-0.25 ± 0.18	0.054	0.17	37
peak	<i>Ligusticum porteri</i>	-0.29 ± 0.17	0.096	0.11	28
peak	<i>Erigeron flagellaris</i>	-0.40 ± 0.38	0.044	0.31	25
peak	<i>Geranium richardsonii</i>	-0.23 ± 0.12	0.1	0.053	37
peak	<i>Arenaria congesta</i>	-0.41 ± 0.19	0.15	0.045	28
peak	<i>Linum lewisii</i>	-0.22 ± 0.17	0.047	0.2	37
peak	<i>Castilleja sulphurea</i>	-0.10 ± 0.14	0.017	0.47	32
peak	<i>Delphinium barbeyi</i>	-0.11 ± 0.16	0.015	0.48	35
peak	<i>Dugaldia hoopesii</i>	-0.25 ± 0.13	0.1	0.055	36
peak	<i>Anticlea elegans</i>	-0.28 ± 0.12	0.15	0.021	36
peak	<i>Collomia linearis</i>	0.10 ± 0.19	0.009	0.6	32
peak	<i>Heracleum maximum</i>	-0.37 ± 0.14	0.22	0.016	26
peak	<i>Amerosedum lanceolatum</i>	-0.32 ± 0.14	0.13	0.035	34
peak	<i>Heterotheca villosa</i>	-0.05 ± 0.15	0.0038	0.72	37
peak	<i>Descurainia richardsonii</i>	0.018 ± 0.35	0.0002	0.96	21
peak	<i>Erigeron speciosus</i>	-0.18 ± 0.16	0.037	0.26	36
peak	<i>Aconitum columbianum</i>	-0.078 ± 0.13	0.01	0.55	36
peak	<i>Helianthella quinquenervis</i>	-0.14 ± 0.15	0.033	0.33	30
peak	<i>Galium boreale</i>	-0.39 ± 0.16	0.15	0.02	36
peak	<i>Achillea millefolium</i>	-0.028 ± 0.18	0.0009	0.87	32
peak	<i>Pyrrocoma crocea</i>	-0.33 ± 0.13	0.19	0.016	30
peak	<i>Oreochrysum parryi</i>	-0.47 ± 0.15	0.23	0.0038	34
peak	<i>Campanula rotundifolia</i>	-0.17 ± 0.15	0.038	0.26	35
peak	<i>Ligularia bigelovii</i>	-0.19 ± 0.12	0.074	0.12	33
peak	<i>Heliomeris multiflora</i>	0.13 ± 0.15	0.025	0.4	31
peak	<i>Gentiana parryi</i>	-0.19 ± 0.23	0.028	0.42	26
last	<i>Claytonia lanceolata</i>	-0.39 ± 0.16	0.15	0.02	36
last	<i>Mertensia fusiformis</i>	-0.36 ± 0.17	0.11	0.046	37
last	<i>Erythronium grandiflorum</i>	-0.28 ± 0.14	0.11	0.047	36
last	<i>Nocca montana</i>	-0.28 ± 0.19	0.065	0.14	34
last	<i>Androsace septentrionalis</i>	0.23 ± 0.48	0.0068	0.64	35
last	<i>Mahonia repens</i>	-0.23 ± 0.14	0.077	0.11	35
last	<i>Taraxacum officinale</i>	-0.28 ± 0.32	0.022	0.39	37
last	<i>Viola praemorsa</i>	-0.2 ± 0.17	0.04	0.24	37
last	<i>Ranunculus inamoenus</i>	0.29 ± 0.30	0.025	0.35	37
last	<i>Hydrophyllum capitatum</i>	-0.34 ± 0.17	0.11	0.054	35
last	<i>Lomatium dissectum</i>	-0.36 ± 0.19	0.13	0.063	27

last	<i>Delphinium nuttallianum</i>	-0.32 ± 0.17	0.093	0.067	37
last	<i>Valeriana capitata</i>	-0.011 ± 0.17	0.012	0.53	34
last	<i>Boechera stricta</i>	0.18 ± 0.36	0.0074	0.61	37
last	<i>Draba aurea</i>	-0.14 ± 0.18	0.018	0.44	36
last	<i>Pseudocymopterus montanus</i>	-0.18 ± 0.19	0.032	0.33	31
last	<i>Amelanchier pumila</i>	-0.66 ± 0.16	0.32	0.0025	37
last	<i>Fragaria virginiana</i>	0.22 ± 0.20	0.035	0.27	37
last	<i>Lathyrus leucanthus</i>	-0.11 ± 0.20	0.0083	0.59	37
last	<i>Senecio integerrimus</i>	-0.36 ± 0.18	0.11	0.047	35
last	<i>Valeriana edulis</i>	-0.38 ± 0.22	0.079	0.92	37
last	<i>Eriogonum umbellatum</i>	-1.13 ± 0.28	0.32	0.0003	37
last	<i>Dodecatheon pulchellum</i>	-0.33 ± 0.13	0.16	0.014	37
last	<i>Sedum rosea</i>	-0.33 ± 0.14	0.19	0.027	26
last	<i>Cardamine cordifolia</i>	-0.55 ± 0.14	0.33	0.0042	34
last	<i>Mertensia ciliata</i>	-0.8 ± 0.18	0.38	< 0.0001	37
last	<i>Pedicularis bracteosa</i>	-0.21 ± 0.18	0.067	0.26	21
last	<i>Maianthemum stellatum</i>	-0.32 ± 0.13	0.18	0.019	30
last	<i>Erysimum capitatum</i>	0.14 ± 0.20	0.017	0.49	30
last	<i>Hydrophyllum fendleri</i>	0 ± 0	0	0.97	31
last	<i>Potentilla pulcherrima</i>	-0.16 ± 0.19	0.021	0.39	37
last	<i>Lupinus sp.</i>	0.23 ± 0.21	0.033	0.28	37
last	<i>Potentilla hippiana</i>	0.35 ± 0.25	0.07	0.16	29
last	<i>Vicia americana</i>	-0.094 ± 0.22	0.0054	0.66	37
last	<i>Eriogonum subalpinum</i>	-0.3 ± 0.21	0.055	0.16	37
last	<i>Ligusticum porteri</i>	-0.31 ± 0.19	0.094	0.11	28
last	<i>Erigeron flagellaris</i>	0.35 ± 0.48	0.023	0.47	25
last	<i>Geranium richardsonii</i>	-0.19 ± 0.15	0.043	0.22	37
last	<i>Arenaria congesta</i>	0.26 ± 0.43	0.014	0.55	29
last	<i>Linum lewisii</i>	0.19 ± 0.21	0.022	0.38	37
last	<i>Castilleja sulphurea</i>	-0.14 ± 0.17	0.02	0.43	33
last	<i>Delphinium barbeyi</i>	-0.24 ± 0.20	0.042	0.23	36
last	<i>Dugaldia hoopesii</i>	-0.005 ± 0.17	0	0.98	36
last	<i>Anticlea elegans</i>	-0.37 ± 0.14	0.18	0.0095	36
last	<i>Collomia linearis</i>	0.13 ± 0.27	0.008	0.62	33
last	<i>Heracleum maximum</i>	-0.31 ± 0.15	0.15	0.05	26
last	<i>Amerosedum lanceolatum</i>	-0.45 ± 0.19	0.15	0.024	34
last	<i>Heterotheca villosa</i>	0.43 ± 0.17	0.15	0.018	37
last	<i>Descurainia richardsonii</i>	-0.88 ± 0.45	0.16	0.067	22
last	<i>Erigeron speciosus</i>	-0.094 ± 0.20	0.068	0.63	36
last	<i>Aconitum columbianum</i>	-0.1 ± 0.15	0.013	0.51	36
last	<i>Helianthella quinquenervis</i>	0.04 ± 0.23	0.001	0.86	31
last	<i>Galium boreale</i>	-0.51 ± 0.21	0.14	0.022	37
last	<i>Achillea millefolium</i>	0.55 ± 0.22	0.17	0.02	32
last	<i>Pyrrocoma crocea</i>	-0.39 ± 0.19	0.12	0.05	33
last	<i>Oreochrysum parryi</i>	-0.33 ± 0.23	0.059	0.16	35
last	<i>Campanula rotundifolia</i>	0.20 ± 0.20	0.028	0.34	35
last	<i>Ligularia bigelovii</i>	-0.24 ± 0.17	0.06	0.16	34
last	<i>Heliomeris multiflora</i>	-0.049 ± 0.19	0.0021	0.8	32
last	<i>Gentiana parryi</i>	-0.021 ± 0.21	0.0004	0.92	31
duration	<i>Claytonia lanceolata</i>	0.12 ± 0.09	0.057	0.2	31

duration	<i>Mertensia fusiformis</i>	-0.033 ± 0.09	0.005	0.7	31
duration	<i>Erythronium grandiflorum</i>	0.13 ± 0.051	0.19	0.014	32
duration	<i>Nocca montana</i>	0.059 ± 0.10	0.01	0.57	30
duration	<i>Androsace septentrionalis</i>	0.48 ± 0.53	0.03	0.38	31
duration	<i>Mahonia repens</i>	0.25 ± 0.12	0.13	0.046	31
duration	<i>Taraxacum officinale</i>	0.016 ± 0.33	0	0.96	33
duration	<i>Viola praemorsa</i>	0.19 ± 0.13	0.063	0.15	34
duration	<i>Ranunculus inamoenus</i>	0.60 ± 0.31	0.11	0.059	33
duration	<i>Hydrophyllum capitatum</i>	0.22 ± 0.11	0.11	0.061	34
duration	<i>Lomatium dissectum</i>	0.13 ± 0.11	0.064	0.22	25
duration	<i>Delphinium nuttallianum</i>	0.078 ± 0.11	0.015	0.49	35
duration	<i>Valeriana capitata</i>	0.25 ± 0.093	0.2	0.011	31
duration	<i>Boechera stricta</i>	0.77 ± 0.39	0.1	0.059	35
duration	<i>Draba aurea</i>	0.22 ± 0.15	0.066	0.14	34
duration	<i>Pseudocymopterus montanus</i>	-0.026 ± 0.17	0	0.88	30
duration	<i>Amelanchier pumila</i>	-0.35 ± 0.11	0.24	0.0021	37
duration	<i>Fragaria virginiana</i>	0.54 ± 0.11	0.42	< 0.0001	37
duration	<i>Lathyrus leucanthus</i>	0.31 ± 0.14	0.12	0.036	37
duration	<i>Senecio integerrimus</i>	-0.097 ± 0.13	0.017	0.46	35
duration	<i>Valeriana edulis</i>	0.14 ± 0.20	0.014	0.48	37
duration	<i>Eriogonum umbellatum</i>	-0.84 ± 0.20	0.34	0.0002	37
duration	<i>Dodecatheon pulchellum</i>	0 ± 0	0	0.93	37
duration	<i>Sedum rosea</i>	-0.22 ± 0.10	0.17	0.039	26
duration	<i>Cardamine cordifolia</i>	0.29 ± 0.11	0.18	0.013	34
duration	<i>Mertensia ciliata</i>	-0.85 ± 0.18	0.41	< 0.0001	36
duration	<i>Pedicularis bracteosa</i>	0.34 ± 0.15	0.21	0.033	22
duration	<i>Maianthemum stellatum</i>	-0.016 ± 0.07	0.0017	0.83	30
duration	<i>Erysimum capitatum</i>	0.28 ± 0.19	0.072	0.15	30
duration	<i>Hydrophyllum fendleri</i>	0.18 ± 0.15	0.046	0.24	31
duration	<i>Potentilla pulcherrima</i>	0.073 ± 0.12	0.0099	0.56	37
duration	<i>Lupinus sp.</i>	0.43 ± 0.16	0.17	0.011	37
duration	<i>Potentilla hippiana</i>	0.47 ± 0.22	0.14	0.044	29
duration	<i>Vicia americana</i>	0.12 ± 0.21	0.0094	0.57	37
duration	<i>Eriogonum subalpinum</i>	-0.025 ± 0.14	0.001	0.85	37
duration	<i>Ligusticum porteri</i>	-0.014 ± 0.08	0	0.85	27
duration	<i>Erigeron flagellaris</i>	0.86 ± 0.45	0.13	0.082	25
duration	<i>Geranium richardsonii</i>	0.10 ± 0.10	0.027	0.35	35
duration	<i>Arenaria congesta</i>	0.75 ± 0.42	0.11	0.085	29
duration	<i>Linum lewisii</i>	0.62 ± 0.20	0.22	0.0034	37
duration	<i>Castilleja sulphurea</i>	-0.019 ± 0.21	0	0.94	33
duration	<i>Delphinium barbeyi</i>	-0.08 ± 0.16	0.007	0.63	36
duration	<i>Dugaldia hoopesii</i>	0.28 ± 0.19	0.06	0.15	36
duration	<i>Anticlea elegans</i>	-0.16 ± 0.10	0.07	0.12	36
duration	<i>Collomia linearis</i>	0.37 ± 0.32	0.039	0.27	33
duration	<i>Heracleum maximum</i>	0.16 ± 0.19	0.028	0.42	26
duration	<i>Amerosedum lanceolatum</i>	-0.24 ± 0.13	0.09	0.081	33
duration	<i>Heterotheca villosa</i>	0.79 ± 0.17	0.38	< 0.0001	37
duration	<i>Descurainia richardsonii</i>	-0.19 ± 0.48	0.008	0.69	22
duration	<i>Erigeron speciosus</i>	0.10 ± 0.16	0.01	0.53	35
duration	<i>Aconitum columbianum</i>	-0.041 ± 0.14	0.0027	0.76	36
duration	<i>Helianthella quinquenervis</i>	0.26 ± 0.16	0.086	0.11	31

duration	<i>Galium boreale</i>	-0.21 ± 0.18	0.041	0.23	37
duration	<i>Achillea millefolium</i>	0.61 ± 0.19	0.26	0.0034	31
duration	<i>Pyrrocoma crocea</i>	-0.013 ± 0.15	0.0003	0.93	31
duration	<i>Oreochrysum parryi</i>	0.19 ± 0.18	0.03	0.32	35
duration	<i>Campanula rotundifolia</i>	0.24 ± 0.24	0.029	0.33	35
duration	<i>Ligularia bigelovii</i>	-0.067 ± 0.15	0.0058	0.67	34
duration	<i>Helioomeris multiflora</i>	0.10 ± 0.15	0.004	0.73	32
duration	<i>Gentiana parryi</i>	0.24 ± 0.14	0.093	0.084	33
abundance	<i>Claytonia lanceolata</i>	43.1	0.06	0.14	37
abundance	<i>Mertensia fusiformis</i>	-40.9	0.086	0.077	37
abundance	<i>Erythronium grandiflorum</i>	-34.0	0.045	0.21	37
abundance	<i>Noccaea montana</i>	-15.5	0.04	0.23	36
abundance	<i>Androsace septentrionalis</i>	-15.6	0.054	0.19	34
abundance	<i>Mahonia repens</i>	12.4	0.02	0.42	35
abundance	<i>Taraxacum officinale</i>	0.0	0	0.99	37
abundance	<i>Viola praemorsa</i>	-2.2	0	0.94	37
abundance	<i>Ranunculus inamoenus</i>	29.7	0.24	0.0022	37
abundance	<i>Hydrophyllum capitatum</i>	-46.5	0.065	0.13	37
abundance	<i>Lomatium dissectum</i>	-213.4	0.22	0.0038	37
abundance	<i>Delphinium nuttallianum</i>	-39.0	0.036	0.26	37
abundance	<i>Valeriana capitata</i>	11.7	0.037	0.25	37
abundance	<i>Boechera stricta</i>	-20.0	0.085	0.08	37
abundance	<i>Draba aurea</i>	12.3	0.1	0.057	36
abundance	<i>Pseudocymopterus montanus</i>	25.3	0.015	0.47	37
abundance	<i>Amelanchier pumila</i>	-57.1	0.43	<0.0001	37
abundance	<i>Fragaria virginiana</i>	36.6	0.1	0.052	37
abundance	<i>Lathyrus leucanthus</i>	-19.2	0.082	0.086	37
abundance	<i>Senecio integerrimus</i>	3.3	0.0009	0.86	36
abundance	<i>Valeriana edulis</i>	-2.6	0	0.88	34
abundance	<i>Eriogonum umbellatum</i>	-88.8	0.16	0.016	37
abundance	<i>Dodecatheon pulchellum</i>	13.7	0.013	0.49	37
abundance	<i>Sedum rosea</i>	-104.2	0.14	0.021	37
abundance	<i>Cardamine cordifolia</i>	-13.7	0.007	0.61	37
abundance	<i>Mertensia ciliata</i>	-196.5	0.33	0.0002	37
abundance	<i>Pedicularis bracteosa</i>	-307.7	0.52	<0.0001	37
abundance	<i>Maianthemum stellatum</i>	-28.4	0.016	0.46	36
abundance	<i>Erysimum capitatum</i>	61.5	0.14	0.026	36
abundance	<i>Hydrophyllum fendleri</i>	-52.0	0.039	0.24	37
abundance	<i>Potentilla pulcherrima</i>	34.9	0.037	0.26	37
abundance	<i>Lupinus sp.</i>	-6.1	0.0026	0.76	37
abundance	<i>Potentilla hippiana</i>	111.1	0.31	0.0004	37
abundance	<i>Vicia americana</i>	28.3	0.15	0.018	36
abundance	<i>Eriogonum subalpinum</i>	-14.7	0.034	0.27	37
abundance	<i>Ligusticum porteri</i>	116.3	0.3	0.0005	37
abundance	<i>Erigeron flagellaris</i>	67.0	0.11	0.042	37
abundance	<i>Geranium richardsonii</i>	-88.5	0.12	0.036	37
abundance	<i>Arenaria congesta</i>	159.0	0.2	0.013	31
abundance	<i>Linum lewisii</i>	168.1	0.33	0.0002	37
abundance	<i>Castilleja sulphurea</i>	-48.9	0.1	0.056	36
abundance	<i>Delphinium barbeyi</i>	-4.9	0.009	0.59	36

abundance	<i>Dugaldia hoopesii</i>	11.7	0.02	0.42	35
abundance	<i>Anticlea elegans</i>	-43.1	0.18	0.0084	37
abundance	<i>Collomia linearis</i>	-45.4	0.085	0.1	33
abundance	<i>Heracleum maximum</i>	122.5	0.2	0.0049	37
abundance	<i>Amerosedum lanceolatum</i>	22.9	0.023	0.38	37
abundance	<i>Heterotheca villosa</i>	-13.9	0.005	0.69	37
abundance	<i>Descurainia richardsonii</i>	37.9	0.01	0.69	18
abundance	<i>Erigeron speciosus</i>	11.3	0.012	0.52	36
abundance	<i>Aconitum columbianum</i>	-32.9	0.27	0.0011	36
abundance	<i>Helianthella quinquenervis</i>	26.1	0.013	0.5	37
abundance	<i>Galium boreale</i>	-163.6	0.38	<0.0001	37
abundance	<i>Achillea millefolium</i>	30.8	0.014	0.48	37
abundance	<i>Pyrrocoma crocea</i>	31.1	0.012	0.53	37
abundance	<i>Oreochrysum parryi</i>	0.0	0.004	0.72	37
abundance	<i>Campanula rotundifolia</i>	21.2	0.022	0.38	37
abundance	<i>Ligularia bigelovii</i>	-7.3	0.002	0.8	37
abundance	<i>Heliomeris multiflora</i>	10.2	0.093	0.066	37
abundance	<i>Gentiana parryi</i>	-28.8	0.023	0.37	37

* Response variables include first, peak, and last flowering date, duration of flowering (last minus first day of flowering), and peak number of flowers.

^t n = number of years.

Table S2. Effects of floral abundance on detecting phenological shifts. Shown are species that exhibited significant shifts in phenology and significant changes in peak floral abundance through time. A negative correlation between first flowering and peak abundance in species flowering earlier suggests that earlier flowering is a result of increased floral abundance. Conversely, a positive correlation between last flowering and peak abundance in species with earlier last flowering suggests that earlier last flowering is a result of decreased floral abundance. Significant correlations between flowering phenology and peak floral abundance that indicate an effect of changes in floral abundance on detection probability of phenological shifts are highlighted in bold ($n = \#$ years).

Flowering response	Species	<i>r</i>	<i>P</i>
first	<i>Heracleum maximum</i>	-0.44	0.02
first	<i>Ranunculus inamoenus</i>	-0.42	0.014
first	<i>Eriogonum umbellatum</i>	0.48	0.0027
first	<i>Amelanchier pumila</i>	0.24	0.16
first	<i>Ligusticum porteri</i>	0.12	0.55
first	<i>Linum lewisii</i>	-0.28	0.09
first	<i>Lomatium dissectum</i>	0.17	0.41
last	<i>Amelanchier pumila</i>	0.53	0.0008
last	<i>Anticlea elegans</i>	0.56	0.0003
last	<i>Eriogonum umbellatum</i>	0.62	< 0.0001
last	<i>Mertensia ciliata</i>	0.67	< 0.0001
last	<i>Galium boreale</i>	0.31	0.07
last	<i>Heracleum maximum</i>	0.07	0.73
last	<i>Sedum rosea</i>	0.28	0.17

Table S3. The ability of sensitivity in first flowering to climate to predict the sensitivity of peak and last flowering to climate. Climate is expressed as mean June-August air temperature and timing of spring snowmelt. For example, for every day that first flowering advances per 1°C increase in air temperature, peak flowering advances by only 0.54 ± 0.09 days per increase in °C. $n = 60$ species for all tests. Change in first flowering was a better predictor of change in last flowering when sensitivities to snowmelt were used in place of temperature or year, but note that the relationship between first and last is still quite variable, with a general trend of firsts over-predicting other phenological responses.

	Slope $\pm$ SE	R^2	$F_{1, 58}$	P
Shift in peak in response to temperature	0.54 ± 0.09	0.39	36.8	< 0.0001
Shift in peak in response to snowmelt	0.56 ± 0.08	0.46	49.1	< 0.0001
Shift in last in response to temperature	0.40 ± 0.18	0.08	5.0	0.029
Shift in last in response to snowmelt	0.42 ± 0.13	0.14	9.6	0.0029

Table S4. Responses of aggregate community flowering phenology and abundance to climate. Slopes are from simple (if one predictor) and multiple (if two predictors) linear regression. Temperature is mean summer air temperature from June-August ($^{\circ}\text{C}$), and snowmelt is the first day of bare ground in a permanent snowmelt plot (day of year). It is widely supported that temperatures in the months prior to flowering are good predictors of flowering phenology (5, 10), and in subalpine and alpine habitats, the timing of snowmelt is also strongly associated with flowering phenology (11-14). The timing of snowmelt and spring temperatures are negatively correlated (10), as are snowmelt and summer temperatures (June–August mean temperature; $r = -0.41$, $P = 0.014$). However, air temperature after snowmelt occurs in May does not represent a causal effect of temperature on the timing of snowmelt, which is also affected by the amount of winter snowfall and dust deposition from storms in the Western USA. Therefore, for these analyses we used mean summer air temperature from June-August and excluded air temperature from analysis of spring events in light of strong support for snowmelt as a driver of spring phenology from our other work (e.g., 9, 10).

Response	Predictor	R^2	Slope $\pm$ SE	t	df	P
First flowering	Snowmelt	0.79	0.89 ± 0.083	116.5	1, 31	< 0.0001
Spring peak	Snowmelt	0.86	0.74 ± 0.056	172.6	1, 29	< 0.0001
Summer peak		0.60			2, 29	< 0.0001
	Snowmelt		0.53 ± 0.095	5.61		< 0.0001
	Temperature		-2.45 ± 1.77	-1.39		0.18
Last flowering		0.25			2, 33	0.0090
	Snowmelt		2.92 ± 1.93	1.52		0.14
	Temperature		0.37 ± 0.11	3.30		0.0024
Flowering season		0.41			2, 30	0.0004
	Snowmelt		-0.50 ± 0.16	-3.15		0.0037
	Temperature		4.70 ± 2.71	1.74		0.093
# Flowers (Spring peak)	Snowmelt	0.17	31.7 ± 13.2	5.79	1, 29	0.023
# Flowers (Summer peak)		0.19			2, 29	0.051
	Snowmelt		43.9 ± 17.8	2.45		0.020
	Temperature		42.9 ± 331.4	0.13		0.90
Total # flowers		0.16			2, 30	0.079
	Snowmelt		629 ± 296	2.12		0.042
	Temperature		-278 ± 5060	-0.06		0.96
Mean # Flowers per census		0.36			2, 30	0.0011
	Snowmelt		16.1 ± 4.7	3.41		0.0019
	Temperature		-61.6 ± 80.9	-0.76		0.45

Table S5. Tests for temporal autocorrelation in species showing a significant change in flowering phenology. P-values correspond to the Ljung-Box test for autocorrelation in time series with a lag time of one year. *P-values* less than 0.05 indicate significant temporal autocorrelation and are shown in bold (only three cases).

Flowering response	Species	<i>P</i>
first	<i>Amelanchier pumila</i>	0.71
first	<i>Androsace septentrionalis</i>	0.56
first	<i>Arabis drummondii</i>	0.65
first	<i>Cardamine cordifolia</i>	0.56
first	<i>Claytonia lanceolata</i>	0.25
first	<i>Delphinium nuttallianum</i>	0.27
first	<i>Descurainia richardsonii</i>	0.46
first	<i>Dodecatheon pulchellum</i>	0.60
first	<i>Draba aurea</i>	0.68
first	<i>Erigeron speciosus</i>	0.56
first	<i>Erythronium grandiflorum</i>	0.70
first	<i>Fragaria virginiana</i>	0.34
first	<i>Heracleum maximum</i>	0.91
first	<i>Heterotheca villosa</i>	0.31
first	<i>Hydrophyllum capitatum</i>	0.64
first	<i>Lathyrus leucanthus</i>	0.72
first	<i>Ligusticum porteri</i>	0.21
first	<i>Linum lewisii</i>	0.23
first	<i>Lomatium dissectum</i>	0.74
first	<i>Mahonia repens</i>	0.84
first	<i>Maianthemum stellatum</i>	0.23
first	<i>Mertensia fusiformis</i>	0.95
first	<i>Noccaea montana</i>	0.78
first	<i>Oreochrysum parryi</i>	0.27
first	<i>Pyrrocoma crocea</i>	0.56
first	<i>Ranunculus inamoenus</i>	0.16
first	<i>Taraxacum officinale</i>	0.68
first	<i>Valeriana capitata</i>	0.34
first	<i>Valeriana edulis</i>	0.80
first	<i>Viola praemorsa</i>	0.91
peak	<i>Amelanchier pumila</i>	0.61
peak	<i>Amerosedum lanceolatum</i>	0.52
peak	<i>Anticlea elegans</i>	0.27
peak	<i>Arabis drummondii</i>	0.08
peak	<i>Arenaria congesta</i>	0.40
peak	<i>Cardamine cordifolia</i>	0.61
peak	<i>Claytonia lanceolata</i>	0.89
peak	<i>Delphinium nuttallianum</i>	0.34

peak	<i>Dodecatheon pulchellum</i>	0.32
peak	<i>Erythronium grandiflorum</i>	0.84
peak	<i>Galium boreale</i>	0.07
peak	<i>Geranium richardsonii</i>	0.047
peak	<i>Heracleum maximum</i>	0.11
peak	<i>Hydrophyllum capitatum</i>	0.22
peak	<i>Lomatium dissectum</i>	0.32
peak	<i>Mahonia repens</i>	0.68
peak	<i>Mertensia fusiformis</i>	0.31
peak	<i>Oreochrysum parryi</i>	0.13
peak	<i>Potentilla pulcherrima</i>	0.47
peak	<i>Pyrrocoma crocea</i>	0.76
peak	<i>Ranunculus inamoenus</i>	0.52
peak	<i>Taraxacum officinale</i>	0.69
peak	<i>Valeriana edulis</i>	0.99
last	<i>Achillea millefolium</i>	0.22
last	<i>Amelanchier pumila</i>	0.88
last	<i>Amerosedum lanceolatum</i>	0.28
last	<i>Anticlea elegans</i>	0.62
last	<i>Cardamine cordifolia</i>	0.45
last	<i>Claytonia lanceolata</i>	0.54
last	<i>Dodecatheon pulchellum</i>	0.08
last	<i>Eriogonum umbellatum</i>	0.12
last	<i>Erythronium grandiflorum</i>	0.36
last	<i>Galium boreale</i>	0.06
last	<i>Heracleum maximum</i>	0.17
last	<i>Heterotheca villosa</i>	0.64
last	<i>Hydrophyllum capitatum</i>	0.60
last	<i>Maianthemum_stellatum</i>	0.44
last	<i>Mertensia ciliata</i>	0.78
last	<i>Mertensia fusiformis</i>	0.053
last	<i>Pyrrocoma crocea</i>	0.65
last	<i>Sedum rosea</i>	0.033
last	<i>Senecio integerrimus</i>	0.10

Table S6. Comparison of results from simple linear regression models containing significant temporal autocorrelation vs. autoregressive linear models. Results shown are for the three species where a significant phenological shift also exhibited significant temporal autocorrelation ($P < 0.05$; see Table S5). Slope values generated from autoregressive linear models were fit using generalized least squares. $P < 0.05$ for all slopes, indicating significant change through time regardless of model type.

Species	Flowering response	Basic linear model slope $\pm$ SE	Autoregressive linear model slope $\pm$ SE
<i>Geranium richardsonii</i>	peak	-0.23 $\pm$ 0.12	-0.24 $\pm$ 0.10
<i>Mertensia fusiformis</i>	last	-0.36 $\pm$ 0.17	-0.35 $\pm$ 0.14
<i>Sedum rosea</i>	last	-0.33 $\pm$ 0.14	-0.32 $\pm$ 0.11

Table S7. ANCOVAs showing that slopes of change through time in community level flowering phenology are not affected by the use of estimated vs. missing values (Category). Missing values were estimated based on known start dates and similar floral abundances. For example, there were 840 flowers on the first census in 1993. We used flowering data for years with a known onset date and a census period with a similar number of flowers (700-1000 flowers) to determine the average slope or rate of flower accumulation up to 840 flowers. We applied this rate of flower accumulation to estimate the start date of the flowering season. Using the subset of the dataset containing missing values (NA's in place of estimated values), the length of the flowering season has extended by 36.3 days over the course of our record ($R^2 = 0.41$, $F_{1,22} = 15.42$, $P = 0.0007$; 9.3 ± 2.4 days per decade). The start of flowering has advanced at a rate of 6.4 ± 2.7 days per decade ($R^2 = 0.20$, $F_{1,23} = 5.69$, $P = 0.026$), and the end of flowering has delayed at a rate of 3.2 ± 1.5 days per decade ($R^2 = 0.13$, $F_{1,30} = 4.57$, $P = 0.041$). Total flower abundance counted throughout the season has not changed ($R^2 = 0.0$, $F_{1,21} = 0.11$, $P = 0.74$), nor has average flower abundance per census ($R^2 = 0.08$, $F_{1,21} = 1.85$, $P = 0.19$).

Response	Coefficient	Slope $\pm$ SE	<i>t</i>	<i>P</i>
First flowering	Intercept	1405.73 $\pm$ 438.04	3.21	0.0022
	Year	-0.64 $\pm$ 0.22	-2.91	0.0053
	Category	10.50 $\pm$ 674.83	0.016	0.99
	Year*category	-0.005 $\pm$ 0.34	-0.016	0.99
Last flowering	Intercept	-358.25 $\pm$ 271.58	-1.32	0.19
	Year	0.31 $\pm$ 0.14	2.28	0.026
	Category	66.64 $\pm$ 415.48	0.16	0.87
	Year*category	-0.033 $\pm$ 0.21	-0.16	0.87
Flowering season	Intercept	-1652.0 $\pm$ 380.5	-4.34	< 0.0001
	Year	0.89 $\pm$ 0.19	4.68	< 0.0001
	Category	-80.18 $\pm$ 599.9	-0.13	0.89
	Year*category	0.040 $\pm$ 0.30	0.13	0.89
Total # Flowers	Intercept	25,467 $\pm$ 766,466	0.033	0.97
	Year	20.99 $\pm$ 384.17	0.055	0.96
	Category	-282,880 $\pm$ 1,238,752	-0.23	0.82
	Year*category	140.65 $\pm$ 620.14	0.23	0.82
Mean # Flowers per census	Intercept	26,425 $\pm$ 12,969	2.04	0.047
	Year	-12.66 $\pm$ 6.50	-1.95	0.057
	Category	-4171.22 $\pm$ 20,960	-0.20	0.84
	Year*category	2.07 $\pm$ 10.49	-0.20	0.84

APPENDIX B: PHENOLOGICAL RESPONSES TO CLIMATE CHANGE DO NOT
EXHIBIT PHYLOGENETIC SIGNAL IN A SUBALPINE PLANT COMMUNITY

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Phenological responses to climate change do not exhibit phylogenetic signal in a subalpine plant community

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Abstract. Phylogenetic relationships may underlie species-specific phenological sensitivities to abiotic variation and may help to predict these responses to climate change. Although shared evolutionary history may mediate both phenology and phenological sensitivity to abiotic variation, few studies have explicitly investigated whether this is the case. We explore phylogenetic signal in flowering phenology and in phenological sensitivity to temperature and snowmelt using a 39-year record of flowering from the Colorado Rocky Mountains, USA that includes dates of first, peak, and last flowering, and flowering duration for 60 plant species in a subalpine plant community. Consistent with other studies, we found evidence in support of phylogenetic signal in first flowering date. However, the strength and significance of that signal were inconsistent across other measures of flowering in this plant community: peak flowering date exhibited the strongest phylogenetic signal, followed by first flowering date; last flowering date and duration of flowering exhibited patterns indistinguishable from random trait evolution. In contrast to first and peak flowering date, phenological sensitivities of all flowering measures to temperature and snowmelt did not exhibit a phylogenetic signal. These findings show that within ecological communities, phylogenetic signal in phenology does not necessarily imply phylogenetic signal in phenological sensitivities to abiotic variation.

Key words: *Blomberg's K; flowering time; Pagel's Lambda; phenological shifts; phenology; phylogenetic niche conservatism; Rocky Mountain Biological Laboratory; species specificity.*

INTRODUCTION

Shifts in phenology have emerged as one of the strongest bio-indicators of changing abiotic conditions across the globe (Sparks and Menzel 2002, Parmesan and Yohe 2003). Although there is an overwhelming trend for earlier phenological events in many ecosystems, considerable species-specific variation is evident in phenological responses to climate change (Fitter and Fitter 2002, Parmesan 2007, Crimmins et al. 2010, CaraDonna et al. 2014). If closely related taxa within ecological communities resemble each other in their phenological sensitivity to abiotic variation, then relatedness may help to predict species vulnerability, as well as community and ecosystem change, under future climate change scenarios (Willis et al. 2008, Cleland et al. 2012, Wolkovich et al. 2013a).

Investigation of phylogenetic relationships in plant phenology from a wide range of taxa from across the globe has revealed that phenological events in plants generally exhibit nonrandom phylogenetic patterns: that is, closely related taxa tend to resemble each other in the timing of their life-history events (e.g., Wright and Calderon 1995, Davies et al. 2013, Wolkovich et al.

2013b). Accordingly, the hypothesis has emerged that phenological sensitivity to changing abiotic conditions (i.e., climate-driven phenological shifts) likely exhibits phylogenetic signal as well. This hypothesis is based on the idea of conserved physiological responses to environmental cues among closely related taxa that mediate both phenology and phenological change (Davis et al. 2010, Davies et al. 2013). Despite this logical prediction, the little evidence available does not consistently support it. For example, Davis et al. (2010) reported that closely related taxa from two plant communities showed similar phenological sensitivities to temperature, but these same taxa did not show similar shifts in first flowering date through time. Similarly, Wolkovich et al. (2013b) found no evidence of phylogenetic signal for shifts in first flowering for taxa from several different plant communities in North America.

Furthermore, no studies to date have investigated whether phylogenetic patterns exhibited in mean dates of first flowering are consistent with other measures of phenology, in part due to a paucity of long-term records for which multiple phenological measures are available for the majority of taxa in a community. First, peak, and last flowering, and duration of flowering have been shown to shift independently of one another under climate change (CaraDonna et al. 2014), leaving open the potential for phylogenetic signal to vary across these component measures.

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Herein we explore phylogenetic patterns in plant phenology and phenological sensitivity to abiotic factors using a 39-year flowering phenology data set from the Colorado Rocky Mountains, USA that includes information on dates of first, peak, and last flowering, as well as flowering duration. We focus our investigation on 60 representative taxa from this subalpine plant community for which we have sufficient data to assess phenological change accurately. Specifically, we test for phylogenetic signal in flowering time and in phenological responses to climate change. We define flowering time as mean flowering date across years, and we define phenological responses to climate change as shifts in flowering phenology through time, and sensitivity of flowering time to snowmelt date and temperature. We ask two primary questions: (1) are phylogenetic patterns similar between phenology and phenological change within this community, and (2) are phylogenetic patterns consistent among the different component measures of a phenology distribution (first, peak, last, and duration)? Based on previous findings (Davies et al. 2013), we expected flowering time to exhibit nonrandom phylogenetic patterns, in part due to conserved evolutionary responses associated with the appropriate abiotic conditions for optimal timing of reproduction. However, because of the considerable variation in phenological responses to climate change among species in this community (Iler et al. 2013a, CaraDonna et al. 2014), we did not expect closely related taxa to exhibit similar phenological sensitivity to abiotic variation. For this same reason, we expected to observe differences in phylogenetic patterns among first, peak, last, and duration of flowering.

MATERIALS AND METHODS

Flowering phenology data

Flowering phenology data were collected at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (38°57.5' N, 106°59.3' W, 2900 m above sea level). The subalpine landscape surrounding the RMBL is a mosaic of wet and dry meadows, and aspen and spruce forest. For each flowering plant species that occurs in our 30 2 × 2 m plots, either the number of open flowers per stalk or the number of flowering inflorescences (for species with many small flowers) were counted every other day throughout the entire growing season from 1974 to 2012. This allowed us to examine multiple components of a phenological distribution: first, peak, and last day of flowering, as well as flowering duration. Although 121 species occur in these permanent plots, we limit our analyses of phylogenetic signal in flowering traits to the 60 species for which we have sufficient data to assess phenological shifts accurately. This sampling of 60 species includes 54 genera representing 80% (24 out of 30) of the vascular plant families present in this plant community (see Appendix A: Table A1 for details). For additional details about the data set, study site, and quantification of phenological change,

see Inouye et al. (2002), Inouye (2008), Iler et al. (2013a), and CaraDonna et al. (2014).

Climate data

Over the course of our study period (1974–2012), summer air temperatures have increased by $0.4^{\circ} \pm 0.1^{\circ}\text{C}$ (mean $\pm$ SE) per decade, and the date of spring snowmelt has advanced by 3.5 ± 2.0 days per decade (Iler et al. 2013a). These changes in abiotic conditions are consistent with global patterns of climate change that have occurred since the 1970s (IPCC 2007). We use both mean summer temperature and the date of snowmelt in our analyses because they represent two biologically independent abiotic factors important for flowering phenology in this ecosystem. Mean summer temperatures used in analyses (June–August) are the average of the daily minimum and maximum temperatures at the Crested Butte National Oceanic and Atmosphere Administration weather station, Colorado (~9 km south of the RMBL). Furthermore, June, July, and August temperatures are all positively correlated with one another; thus, they represent a composite measure of summer temperature. Records for the annual timing of snowmelt in this ecosystem come from a permanent 5 × 5 m plot at the RMBL, in which the first day of bare ground is recorded as the date of snowmelt (snowpack measurements are recorded daily).

Quantifying flowering phenology and phenological responses to climate change

We quantified mean date of flowering for first, peak, last, and duration of flowering in two ways: (1) averaged across the first 10 years of the data set (1974–1984), and (2) averaged across all 39 years of the data set (1974–2012). Averaging flowering time values across all 39 years, a time period when phenological shifts are known to have occurred (CaraDonna et al. 2014), introduces more error into our measures of flowering time for each taxon, which can influence estimates of phylogenetic signal (Blomberg et al. 2003). Furthermore, flowering time values averaged across the first 10 years of the data set more likely represent a pre-climate-change baseline (sensu Iler et al. 2013b). The date of first flowering reported here is the first day on which a species is observed in flower in any of our permanent plots; peak flowering was calculated as the day on which 50% of flowers of a given species were recorded as open; last flowering is the last day on which a species was observed in flower in any plot; and duration of flowering is the number of days a species was recorded as in flower (CaraDonna et al. 2014).

We quantified phenological responses of flowering time to climate change in three ways: (1) shifts in flowering phenology through time (1974–2012), (2) shifts in flowering phenology in response to snowmelt date (snowmelt sensitivity), and (3) shifts in flowering phenology in response to summer temperature (temperature sensitivity). The timing of snowmelt date and

temperature are strongly associated with shifts in flowering phenology in this study system (Inouye et al. 2002), independently explaining on average 68% and 66% of the interannual variation in flowering phenology across species, respectively (Iler et al. 2013a). Compared to phenological sensitivities to snowmelt date and summer temperature, shifts in flowering phenology through time provide the most conservative measure of phenological change and the measure with the highest variation. We include phenological shifts through time in our analyses because such shifts are a widely used metric of phenological change, especially when information on local abiotic factors is unavailable or unreliable. Trait values for shifts in flowering phenology through time for each plant species were quantified as the slope of a linear regression model of flowering date vs. year; trait values for phenological sensitivity to snowmelt were quantified as the slope of a linear regression model of flowering date vs. snowmelt date; and trait values for phenological sensitivity to temperature were quantified as the slope of a linear regression model of flowering date vs. June-to-August mean temperature. All flowering time data used in analyses are reported in Appendix A: Table A1 and Appendix B: Table B1, and are available elsewhere, i.e., Iler et al. (2013a) and CaraDonna et al. (2014).

Testing for phylogenetic signal

To conduct phylogenetic analyses, we used a pruned molecular phylogeny that is fully resolved down to genus for 1246 taxa and includes the vast majority of the taxa in this study (see Davies et al. [2013] for the complete phylogeny and the Supplement for our community phylogeny). The two taxa from our community that were not included in the molecular phylogeny were placed within the nearest clade. We tested for phylogenetic signal in flowering phenology and phenological responses to climate change using two commonly employed metrics: Blomberg's K and Pagel's λ (Pagel 1999, Blomberg et al. 2003). Because Blomberg's K and Pagel's λ differ in the way they test for phylogenetic signal, which can generate some degree of inconsistency in results, we use both metrics to ensure appropriate interpretation of patterns in our plant community (Münkemüller et al. 2012). Our intention was not to explicitly compare inconsistencies between methods, but instead, to reveal cases in which we obtained inconsistent results, and to treat them with caution. To test the robustness of our results to variation in phylogenetic branch length and topology, we compared our results from the molecular genus tree (Table 1; Supplement) to those generated from a less well-resolved phylogeny (Wikström et al. 2001, following Davies et al. 2013); the two phylogenies differ in branch lengths and topology above the genus level (see Appendix D: Table D1 for details).

Both Pagel's λ and Blomberg's K compare the observed distribution of trait values with the analytical

expectation based on the topology of the phylogeny and assume trait evolution under Brownian motion (Pagel 1999, Blomberg et al. 2003, Ackerly 2009). K values that are approximately equal to 1 match trait evolution under the Brownian motion expectation, and indicate phylogenetic signal; K values much less than 1 and closer to 0 indicate little or no phylogenetic signal associated with random trait evolution or convergence; K values greater than 1 indicate strong phylogenetic signal and conservatism of traits. We evaluated the statistical significance of phylogenetic signal in Blomberg's K by comparing the observed patterns of phylogenetic independent contrasts of each trait value relative to a null model of randomly shuffling trait values across the phylogeny, as implemented in the R package Picante (Kembel et al. 2010, R Development Core Team 2014). In contrast, Pagel's λ is a scaling parameter that ranges from 0 to 1. Lambda values of 0 indicate no phylogenetic signal, whereas values of 1 indicate perfect phylogenetic signal, matching trait evolution under Brownian motion (Pagel 1999). We estimated the statistical significance of phylogenetic signal using Pagel's λ by comparing our observed trait values to those expected on a phylogeny with λ values of 0 (i.e., no phylogenetic signal); thus statistical significance indicates that trait values are nonrandomly distributed across the phylogeny. Calculations of Pagel's λ and significance tests were conducted using the R package Geiger (Harmon et al. 2008, R Development Core Team 2014).

RESULTS

Overall, our tests of phylogenetic signal using Blomberg's K and Pagel's λ were consistent: in only one case did we detect significant phylogenetic signal using one metric and not with the other (first flowering time; Table 1). Similarly, phylogenetic signal was quantitatively and qualitatively consistent using either mean flowering time values across the first 10 years of the data set (1974–1984) or mean values across the entire 39 years of the data set (1974–2012; Table 1 and Appendix C: Table C1); additionally, phylogenetic signal in flowering time (peak) did not vary directionally across the 39-year study period ($R^2 = 0.037$, $P = 0.253$). We therefore focus further discussion of flowering time on the mean date of flowering calculated over the first 10 years of the data set because less error is associated with this measure, and because it more likely represents a pre-climate change baseline compared to the 39-year mean over which phenological shifts are known to have occurred (CaraDonna et al. 2014).

The strength and significance of phylogenetic signal in flowering phenology varied across each of our different measures of flowering time (first, peak, last, and duration of flowering; Table 1). Peak flowering exhibited the strongest phylogenetic signal using either Blomberg's K or Pagel's λ (Fig. 1); first flowering exhibited a similarly strong nonrandom phylogenetic pattern, but

TABLE 1. Phylogenetic signal of flowering phenology traits for 60 species from a subalpine plant community in the Colorado Rocky Mountains, USA.

Phenological trait	Blomberg's <i>K</i>		Pagel's λ	
	Value	<i>P</i>	Value	<i>P</i>
Flowering time				
First flowering	0.683	0.041	0.434	0.120
Peak flowering	0.748	0.016	0.587	0.041
Last flowering	0.498	0.514	0.011	0.952
Duration of flowering	0.449	0.692	<0.001	0.999
Shift through time				
First flowering	0.342	0.907	<0.001	0.999
Peak flowering	0.351	0.894	<0.001	0.999
Last flowering	0.467	0.482	<0.001	0.999
Duration of flowering	0.445	0.563	<0.001	0.999
Snowmelt sensitivity				
First flowering	0.512	0.370	0.147	0.597
Peak flowering	0.413	0.701	<0.001	0.999
Last flowering	0.511	0.366	<0.001	0.999
Duration of flowering	0.633	0.060	<0.001	0.999
Temperature sensitivity				
First flowering	0.480	0.490	<0.001	0.999
Peak flowering	0.441	0.591	<0.001	0.999
Last flowering	0.485	0.393	<0.001	0.999
Duration of flowering	0.569	0.157	0.279	0.565

Notes: *K* significant from random at $P < 0.05$ and λ significant from zero at $P < 0.05$ are shown in bold. Flowering times shown here are calculated from the mean date of flowering for each species over the first 10 years of the data set (1974–1984); results for flowering time across all 39 years (1974–2012) are quantitatively similar and shown in Appendix C: Table C1. Values for shifts in flowering phenology through time, and phenological sensitivities to snowmelt date and temperature, are based on slopes of linear regression models from Caradonna et al. (2014); all values used in analyses are reported in Appendix A: Table A1 and Appendix B: Table B1.

only when calculated with Blomberg's *K* (values for Pagel's λ were qualitatively similar but marginally significant; Table 1). Last flowering and flowering duration exhibited no phylogenetic signal (Table 1). In contrast to first and peak flowering date, shifts in flowering phenology through time, phenological sensitivity to snowmelt date, and phenological sensitivity to temperature did not show phylogenetic signal for any measure of phenology (Table 1, Fig. 1b–d). Both Blomberg's *K* and Pagel's λ values were low, and in all cases were not significantly different from a random pattern of trait evolution (Table 1). In general, our estimates of phylogenetic signal were robust to variation in branch length and tree topology (Appendix D: Table D1).

DISCUSSION

Although phylogenetic signal in phenological responses to climate change may be present in some communities, even among those that are geographically distinct (Davis et al. 2010), our results provide evidence that this is not necessarily a ubiquitous phenomenon present in all plant communities. Shifts in flowering phenology through time and phenological sensitivities to snowmelt

date and temperature did not exhibit phylogenetic signal, indicating that more closely related taxa in this subalpine plant community do not share similar phenological sensitivities to abiotic variation (Table 1, Fig. 1). However, consistent with other studies (e.g., Davies et al. 2013, Wolkovich et al. 2013b), our results reveal that more closely related taxa do tend to commence flowering at more similar times during the growing season in this community than would be expected by a random pattern of trait evolution. It is important to note that our results pertain to phylogenetic patterns within an ecological community, rather than for species within a well-sampled clade; a community phylogeny inevitably represents a subsample of taxa from multiple clades, the results of which can provide insight into ecological patterns (e.g., species vulnerability, community change) rather than mechanisms underlying trait evolution.

The strength and significance of evidence for phylogenetic signal varied across different measures of flowering phenology. Mean date of peak flowering showed the strongest and most consistent pattern of phylogenetic signal (Table 1). Peak flowering may be the most robust measure of phylogenetic signal in flowering time because peak abundance represents an aggregate population response (Miller-Rushing et al. 2008). Although mean date of first flowering showed qualitatively similar patterns to peak flowering, only Blomberg's *K* detected significant phylogenetic signal in first flowering (Table 1). This discrepancy between first and peak flowering using Pagel's λ is likely attributed to two factors. First, unlike peak flowering, the date of first flowering is often represented by only a few individuals instead of representing an aggregate property of the population; this can introduce more variability into this measure of flowering time (sensu Miller-Rushing et al. 2008), which can decrease the likelihood of detecting phylogenetic signal (Blomberg et al. 2003). Second, simulation studies have shown that Pagel's λ is a more conservative measure of phylogenetic signal when compared to Blomberg's *K* (Münkemüller et al. 2012).

In contrast to first and peak flowering, we found no evidence for phylogenetic signal in mean date of last flowering or mean duration of flowering (Table 1). Unlike first and peak flowering, last flowering and flowering duration are affected by a suite of abiotic and biotic factors occurring after flowering onset, which can affect floral longevity (Primack 1985). For example, while warmer temperatures may advance the onset of flowering, resource availability (e.g., water and nutrients) can alter floral production, delay the end of flowering, and extend overall flowering duration (Campbell and Halama 1993). Similarly, biotic factors such as pollination may extend or contract the end of the flowering period (Primack 1985, Bolmgren et al. 2003). Furthermore, similar to first flowering date, last flowering, and by logical extension, flowering duration, is also often represented by a few individuals, rather

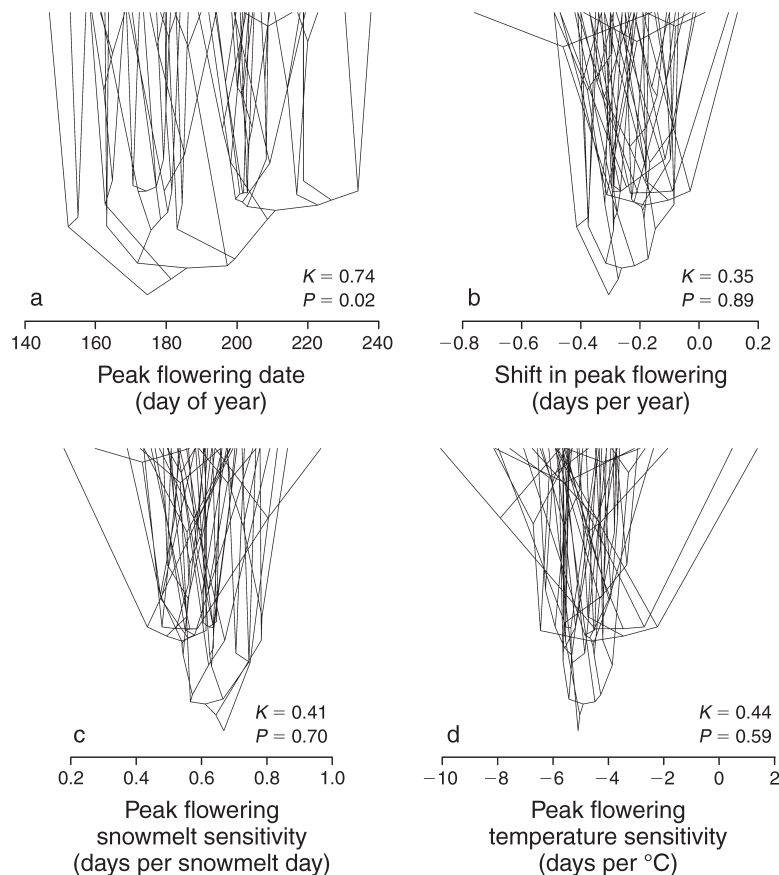


FIG. 1. Traitgrams illustrating phylogenetic signal in (a) mean date of peak flowering, (b) shift in peak flowering date through time, (c) shift in peak flowering in response to snowmelt date, and (d) shift in peak flowering in response to temperature. Blomberg's K and P values are shown for each signal. Tips on the tree are arranged by trait values; more crossover among branches within the tree indicates less phylogenetic signal. Mean date of peak flowering shown here is calculated from the first 10 years of the study period (1974–1984). This figure is presented for illustrative purposes and is not intended for interpretation of ancestral states.

than an aggregate property of the population. These results suggest that different component measures of a phenological distribution do not necessarily exhibit identical phylogenetic patterns. Therefore, measures of phenology that best capture aggregate population-level patterns (e.g., peak flowering) may provide the most reliable signal of phylogenetic patterns in phenological traits, compared to measures that represent only the early and late tail-ends of a population-level response (e.g., first and last flowering).

For all measures of changes in flowering phenology, more closely related taxa did not resemble each other in their phenological responses to climate change in this subalpine plant community (Table 1, Fig. 1b–d). Measurement error in both rates of phenological change and sensitivity to abiotic factors can influence the ability to detect phylogenetic signal. However, this is unlikely here because approximately 70% of the variation in flowering phenology in this ecosystem is independently explained by the timing of snowmelt and summer temperatures (Iler et al. 2013a). Furthermore, in all cases, tests of phylogenetic signal in phenological change

using either Blomberg's K or Pagel's λ revealed consistently low values that were indistinguishable from random patterns of trait evolution (Table 1, Fig. 1b–d). The only near exception was the sensitivity of flowering duration to snowmelt, which was marginally significant (using Blomberg's K ; Table 1). Snowmelt date is correlated with snowpack and hence the amount of water entering this ecosystem in the spring (Inouye et al. 2002). The ability of a species to flower for more or less time during the growing season may be influenced by floral longevity and other physiological mechanisms related to drought stress (Primack 1985), which may be relatively conserved among some taxonomic groups.

The hypothesis that conserved evolutionary responses to abiotic conditions mediate both phenology and phenological responses to local abiotic variation is logical; however, the evidence presented in this study does not support it. Our results provide a clear counterpoint to the expectation that phenological responses to climate change show strong phylogenetic patterns within plant communities. We propose two non-mutually exclusive explanations that may underlie

the discrepancy between phylogenetic signal in phenology vs. the absence of phylogenetic signal in phenological sensitivity to abiotic factors.

First, the presence of phylogenetic signal in flowering time does not imply strong phylogenetic conservatism (i.e., phylogenetic niche conservatism; Losos 2008), nor does it preclude closely related taxa from occupying different abiotic environments via differences in seasonality or habitat even if they share similar physiological pathways related to phenology. Differences in seasonality or habitat can act as environmental filters by which a phenological response can be mediated. In this subalpine ecosystem, the growing season is brief and changes rapidly within a year, thus slight differences in flowering time can represent considerably different abiotic environments, which have been shown to contribute to differences in phenological change among closely related taxa in this plant community (Miller-Rushing and Inouye 2009, Iler et al. 2013a, CaraDonna et al. 2014). Similarly, closely related taxa flowering at very similar times can occupy microhabitats that represent different abiotic environments, which can also mediate phenological shifts. For example, *Potentilla pulcherrima* and *P. hippiana* (Rosaceae) flower at virtually the same time during midsummer (peak flowering: 22 July $\pm$ 11 days and 23 July $\pm$ 10 days, respectively), but occupy wet meadows and dry rocky meadows, respectively, and do not exhibit similar phenological shifts (Iler et al. 2013a, CaraDonna et al. 2014).

Second, because phenology is highly heritable and closely tied to survival and reproduction, it plays a major role in determining the geographic ranges of plants (Rathcke and Lacey 1985, Chuine and Beaubien 2001, Etterson 2004, Franks et al. 2007, Forrest and Miller-Rushing 2010). Phenological sensitivity to local abiotic variation is likely shaped by interactions between local and regional climate conditions experienced by a species across its range. The strength of local vs. regional effects will in part depend on the balance between local adaptation and gene flow among populations. Assuming that all of the closely related, co-occurring species in a community do not have identically overlapping ranges, their phenological sensitivities likely differ, as do the climate conditions experienced across their ranges; thus, closely related taxa may exhibit different phenological sensitivities to local abiotic variation, despite similar physiological mechanisms that may underlie flowering time.

In sum, we hypothesize that local and regional climate conditions, combined with processes occurring during community assembly, will mediate phylogenetic patterns of both phenology and phenological responses to climate change within ecological communities. In contrast to previous work, the findings presented in this study show that within a plant community, phylogenetic signal in phenology does not necessarily imply phylogenetic signal in phenological sensitivities to abiotic variation. Assuming that phenology represents pheno-

logical change could lead to potentially inaccurate conclusions about the effects of shared evolutionary history on phenological traits and their responses to future abiotic change.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–D and the Supplement are available online: <http://dx.doi.org/10.1890/14-1536.1.sm>

Appendix A (Table A1). Taxa list and flowering time data for the 60 plant species used in phylogenetic analyses. Flowering time data shown here include the mean date of flowering averaged over the course of the first ten years of the data set (1974–1984) and mean date of flowering averaged over the entire 39-yr study period (1974–2012; when phenological shifts are known to have occurred). First, peak, and last flowering time measurements are recorded as day of year; flowering duration is recorded as the number of days a species is in flower. This 60 species subsample of the plant community represents 54 genera and 24 vascular plant families, compared to the full 121 species community, which contains 97 genera and 30 plant families.

Family	Species	Mean flowering time (1974–1984)				Mean flowering time (1974–2012)			
		First	Peak	Last	Duration	First	Peak	Last	Duration
Asteraceae	<i>Achillea millefolium</i>	196	226	243	46.8	201	223	247	51.8
Ranunculaceae	<i>Aconitum columbianum</i>	198	211	232	33.6	195	209	228	37.9
Rosaceae	<i>Amelanchier pumila</i>	169	176	194	24.8	163	170	183	24.8
Crassulaceae	<i>Amerosedum lanceolatum</i>	196	204	235	21.6	192	199	213	27
Primulaceae	<i>Androsace septentrionalis</i>	165	175	226	67.8	150	168	225	83.3
Melanthiaceae	<i>Anticlea elegans</i>	193	205	222	28.7	189	201	215	31.3
Brassicaceae	<i>Arabis drummondii</i>	169	178	213	41.1	161	174	216	59.7
Caryophyllaceae	<i>Arenaria congesta</i>	188	206	231	51.4	184	199	236	59.3
Campanulaceae	<i>Campanula rotundifolia</i>	205	223	241	36.1	207	219	237	36.2
Brassicaceae	<i>Cardamine cordifolia</i>	187	193	194	17	175	183	195	26.3
Orobanchaceae	<i>Castilleja sulphurea</i>	192	204	224	31.7	187	202	219	37
Montiaceae	<i>Claytonia lanceolata</i>	146	156	171	27.3	136	147	163	34.2
Caryophyllaceae	<i>Collomia linearis</i>	195	200	234	39	190	205	239	55
Ranunculaceae	<i>Delphinium barbeyi</i>	192	208	232	40.8	188	204	225	42.3
Ranunculaceae	<i>Delphinium nuttallianum</i>	163	174	178	27.3	156	169	183	32.4
Brassicaceae	<i>Descurainia richardsonii</i>	204	212	251	31	193	202	220	37
Primulaceae	<i>Dodecatheon pulchellum</i>	179	186	196	16.3	174	180	189	19.9
Brassicaceae	<i>Draba aurea</i>	170	181	210	39.6	162	177	207	49.7
Asteraceae	<i>Dugaldia hoopesii</i>	195	213	235	40.1	188	209	234	51.5
Asteraceae	<i>Erigeron flagellaris</i>	193	207	215	46.8	183	200	233	58.6
Asteraceae	<i>Erigeron speciosus</i>	200	222	251	50.7	193	216	242	54.8
Polygonaceae	<i>Eriogonum subalpinum</i>	185	198	214	28.9	181	192	206	30.9
Polygonaceae	<i>Eriogonum umbellatum</i>	176	190	234	58.6	171	184	214	47.8
Brassicaceae	<i>Erysimum asperum</i>	179	188	199	24.7	176	186	202	31.6
Liliaceae	<i>Erythronium grandiflorum</i>	155	161	167	13.7	148	153	161	18.8
Rosaceae	<i>Fragaria virginiana</i>	169	174	184	15.5	164	169	184	24.3
Rubiaceae	<i>Galium boreale</i>	201	223	242	41.3	199	217	234	40.3
Gentianaceae	<i>Gentiana parryi</i>	235	242	251	15.6	231	238	246	22.7
Geraniaceae	<i>Geranium richardsonii</i>	189	208	239	41.8	183	203	227	48.6
Asteraceae	<i>Helianthella quinquenervis</i>	200	210	225	24.9	195	207	223	33.3
Asteraceae	<i>Heliomeris multiflora</i>	214	229	251	36.5	217	231	245	34.3
Apiaceae	<i>Heracleum maximum</i>	204	212	224	20	190	203	218	34.9
Asteraceae	<i>Heterotheca villosa</i>	198	222	218	53.1	193	219	254	67.4
Boraginaceae	<i>Hydrophyllum capitatum</i>	162	171	178	17.9	154	163	171	22.2
Boraginaceae	<i>Hydrophyllum fendleri</i>	178	185	237	18.6	177	183	193	21.9
Fabaceae	<i>Lathyrus leucanthus</i>	173	184	214	41.1	164	179	207	47.9

Apiaceae	<i>Ligularia bigelovii</i>	213	223	238	24.7	208	219	233	30.8
Apiaceae	<i>Ligusticum porterii</i>	188	200	240	27.5	182	194	208	32.6
Linaceae	<i>Linum lewisii</i>	193	202	231	38.1	186	199	230	50.1
Apiaceae	<i>Lomatium dissectum</i>	169	170	190	15.3	155	162	169	20.3
Fabaceae	<i>Lupinus sericeus</i>	181	190	198	28.1	178	189	208	35.4
Berberidaceae	<i>Mahonia repens</i>	160	168	175	18.1	152	159	169	23.9
Asparagaceae	<i>Maianthemum stellatum</i>	182	184	203	8.8	176	178	184	14.1
Boraginaceae	<i>Mertensia ciliata</i>	178	193	224	46.5	175	190	215	43.8
Boraginaceae	<i>Mertensia fusiformis</i>	151	164	185	37.7	142	157	178	43
Brassicaceae	<i>Nocca montana</i>	157	165	179	25.4	149	159	176	33.2
Asteraceae	<i>Oreochrysum parryi</i>	213	224	239	26.1	207	218	234	33.1
Orobanchaceae	<i>Pedicularis bracteoasa</i>	182	187	191	17	176	183	194	26.6
Rosaceae	<i>Potentilla gracilis</i>	182	208	209	55.7	178	202	234	61.8
Rosaceae	<i>Potentilla hippiana</i>	187	215	240	53	178	203	231	59.1
Apiaceae	<i>Pseudocymopterus montanus</i>	163	179	210	47.2	163	181	206	49.6
Asteraceae	<i>Pyrrocoma crocea</i>	210	218	232	21.4	202	211	224	27.7
Ranunculaceae	<i>Ranunculus inamoenus</i>	163	170	192	33	153	164	200	54.2
Crassulaceae	<i>Sedum rosea</i>	178	184	203	15.7	175	180	188	19.9
Asteraceae	<i>Senecio integerrimus</i>	169	179	193	23.6	166	175	188	27.3
Asteraceae	<i>Taraxacum officinale</i>	161	174	177	60.9	152	168	215	68.2
Caprifoliaceae	<i>Valeriana capitata</i>	165	176	203	41.4	157	172	198	47.5
Caprifoliaceae	<i>Valeriana edulis</i>	181	200	239	59	170	194	230	65.3
Fabaceae	<i>Vicia americana</i>	183	198	223	39.7	181	194	218	42.6
Violaceae	<i>Viola praemorsa</i>	161	167	217	19	153	161	172	25.7

Appendix B (Table B1). Phenological change and phenological sensitivity to snowmelt date and summer temperature data for the 60 plants species used in phylogenetic analyses. All values are the slope of a linear regression with year, snowmelt date, or summer temperature, as predictor variables.

Species	Shift through time (1974–2012)				Snowmelt sensitivity				Temperature sensitivity			
	First	Peak	Last	Duration	First	Peak	Last	Duration	First	Peak	Last	Duration
<i>Achillea millefolium</i>	0.02	-0.03	0.55	0.61	0.45	0.61	0.53	0.08	-2.38	-4.73	-1.23	1.16
<i>Aconitum columbianum</i>	-0.06	-0.08	-0.10	-0.04	0.47	0.53	0.58	0.10	-3.00	-3.73	-5.22	-2.76
<i>Amelanchier pumila</i>	-0.31	-0.33	-0.66	-0.35	0.62	0.58	0.70	0.08	-4.46	-4.33	-6.67	-2.50
<i>Amerosedum lanceolatum</i>	-0.25	-0.32	-0.45	-0.24	0.56	0.58	0.65	0.10	-4.76	-5.60	-7.78	-2.84
<i>Androsace septentrionalis</i>	-0.57	-0.26	0.23	0.48	0.99	0.86	1.05	0.00	-5.25	-5.65	-4.82	2.52
<i>Anticlea elegans</i>	-0.21	-0.28	-0.37	-0.16	0.54	0.54	0.55	0.00	-4.94	-5.89	-6.12	-1.72
<i>Arabis drummondii</i>	-0.47	-0.47	0.18	0.77	0.77	0.61	0.37	-0.32	-5.67	-7.98	-2.47	3.83
<i>Arenaria congesta</i>	-0.49	-0.41	0.26	0.75	0.69	0.69	0.64	-0.06	-4.15	-3.73	-2.61	3.21
<i>Campanula rotundifolia</i>	-0.05	-0.17	0.20	0.24	0.49	0.61	0.48	-0.01	-3.48	-5.06	0.41	3.61
<i>Cardamine cordifolia</i>	-0.83	-0.76	-0.55	0.29	0.66	0.66	0.62	-0.04	-7.18	-8.09	-7.59	-0.61
<i>Castilleja sulphurea</i>	-0.12	-0.10	-0.14	-0.02	0.49	0.47	0.39	-0.11	-2.76	-1.86	-3.13	0.34
<i>Claytonia lanceolata</i>	-0.68	-0.49	-0.39	0.12	0.94	0.83	0.78	-0.14	-8.08	-6.16	-5.98	0.34
<i>Collomia linearis</i>	-0.23	0.10	0.13	0.37	0.48	0.42	-0.15	-0.63	-2.77	0.48	-0.12	6.07
<i>Delphinium barbeyi</i>	-0.16	-0.11	-0.24	-0.08	0.57	0.63	0.71	0.14	-4.52	-5.55	-7.99	-4.35
<i>Delphinium nuttallianum</i>	-0.42	-0.32	-0.32	0.08	0.62	0.63	0.78	0.16	-3.81	-5.65	-6.30	-2.70
<i>Descurainia richardsonii</i>	-0.69	0.02	-0.88	-0.19	0.73	0.28	0.39	-0.34	-13.22	-5.52	-8.84	3.06
<i>Dodecatheon pulchellum</i>	-0.33	-0.34	-0.33	0.00	0.63	0.61	0.56	-0.07	-5.37	-5.66	-5.93	-0.37
<i>Draba aurea</i>	-0.38	-0.22	-0.14	0.22	0.81	0.68	0.53	-0.22	-5.96	-4.47	-6.26	-0.44
<i>Dugaldia hoopesii</i>	-0.29	-0.25	-0.01	0.28	0.53	0.50	0.44	-0.08	-5.14	-3.70	-1.00	4.32
<i>Erigeron flagellaris</i>	-0.21	-0.40	0.35	0.86	0.51	0.97	0.64	0.13	-10.53	-10.06	-2.21	10.71
<i>Erigeron speciosus</i>	-0.39	-0.18	-0.09	0.10	0.58	0.56	0.44	-0.14	-5.05	-2.86	-2.89	1.08
<i>Eriogonum subalpinum</i>	-0.27	-0.25	-0.30	-0.03	0.71	0.76	0.82	0.11	-5.63	-7.06	-7.17	-2.02
<i>Eriogonum umbellatum</i>	-0.29	-0.30	-1.13	-0.84	0.73	0.71	1.08	0.35	-5.70	-5.89	-12.07	-6.40
<i>Erysimum asperum</i>	-0.20	-0.07	0.14	0.28	0.81	0.81	0.63	-0.18	-5.84	-4.33	0.99	6.71
<i>Erythronium grandiflorum</i>	-0.45	-0.34	-0.28	0.13	0.75	0.68	0.59	-0.09	-5.02	-4.06	-3.98	0.44
<i>Fragaria virginiana</i>	-0.32	-0.22	0.22	0.54	0.67	0.66	0.71	0.04	-5.25	-5.32	-2.54	1.70
<i>Galium boreale</i>	-0.29	-0.39	-0.51	-0.21	0.64	0.62	0.75	0.11	-6.86	-7.56	-10.89	-3.21
<i>Gentiana parryi</i>	-0.26	-0.19	-0.02	0.24	0.74	0.69	0.50	-0.24	-5.23	-5.84	-3.12	3.14
<i>Geranium richardsonii</i>	-0.16	-0.23	-0.19	0.10	0.27	0.42	0.55	0.29	-2.50	-3.83	-4.99	-3.12
<i>Helianthella quinquenervis</i>	-0.22	-0.14	0.04	0.26	0.39	0.51	0.60	0.21	-4.61	-3.60	-5.47	-2.27
<i>Heliomeris multiflora</i>	-0.05	0.13	-0.05	0.10	0.30	0.18	0.10	-0.20	-1.21	1.38	4.06	5.82
<i>Heracleum maximum</i>	-0.47	-0.37	-0.31	0.16	0.33	0.45	0.42	0.08	-3.93	-4.80	-5.35	-1.92
<i>Heterotheca villosa</i>	-0.36	-0.05	0.43	0.79	0.59	0.37	0.37	-0.22	-6.39	-2.37	3.47	10.17
<i>Hydrophyllum capitatum</i>	-0.49	-0.40	-0.34	0.22	0.70	0.77	0.72	0.02	-4.29	-5.48	-5.66	-2.02
<i>Hydrophyllum fendleri</i>	-0.13	-0.10	0.00	0.18	0.40	0.53	0.60	0.11	-4.93	-4.08	-3.30	0.51
<i>Lathyrus leucanthus</i>	-0.42	-0.21	-0.11	0.31	0.68	0.77	0.61	-0.08	-5.27	-6.47	-3.44	0.96
<i>Ligularia bigelovii</i>	-0.17	-0.19	-0.24	-0.07	0.34	0.50	0.55	0.21	-2.01	-4.42	-4.01	-2.49
<i>Ligusticum porterii</i>	-0.35	-0.29	-0.31	-0.01	0.59	0.64	0.64	0.06	-5.35	-5.75	-6.03	-1.05
<i>Linum lewisii</i>	-0.43	-0.22	0.19	0.62	0.66	0.70	0.42	-0.24	-6.79	-6.75	-2.05	3.92
<i>Lomatium dissectum</i>	-0.61	-0.42	-0.36	0.13	0.85	0.71	0.65	-0.13	-4.44	-4.41	-5.41	-1.00
<i>Lupinus sericeus</i>	-0.20	-0.02	0.23	0.43	0.49	0.48	0.57	0.08	-4.42	-2.94	-0.21	3.87

<i>Mahonia repens</i>	-0.56	-0.49	-0.23	0.25	0.94	0.78	0.55	-0.39	-8.06	-6.39	-2.96	4.20
<i>Maianthemum stellatum</i>	-0.30	-0.23	-0.32	-0.02	0.52	0.50	0.47	-0.05	-4.53	-3.87	-3.10	1.46
<i>Mertensia ciliata</i>	0.04	-0.16	-0.80	-0.85	0.38	0.41	0.60	0.22	-1.47	-3.06	-8.63	-8.00
<i>Mertensia fusiformis</i>	-0.54	-0.41	-0.36	-0.03	0.86	0.73	0.76	-0.02	-7.58	-5.65	-6.87	-2.16
<i>Nocca montana</i>	-0.42	-0.30	-0.28	0.06	0.83	0.78	0.73	-0.08	-7.49	-5.41	-7.38	-0.07
<i>Oreochrysum parryi</i>	-0.51	-0.47	-0.33	0.19	0.51	0.64	0.79	0.28	-7.60	-8.14	-9.16	-1.27
<i>Pedicularis bracteosa</i>	-0.15	-0.28	-0.21	0.34	0.43	0.58	0.60	0.09	0.34	-2.67	-2.87	0.42
<i>Potentilla gracilis</i>	-0.24	-0.30	-0.16	0.07	0.66	0.66	0.69	0.04	-4.49	-5.92	-6.74	-2.36
<i>Potentilla hippiana</i>	-0.12	-0.15	0.35	0.47	0.45	0.51	0.36	-0.09	-2.71	-3.72	-0.03	1.14
<i>Pseudocymopterus montanus</i>	-0.17	-0.01	-0.18	-0.03	0.69	0.53	0.53	-0.11	-4.28	-3.91	-5.75	-2.30
<i>Pyrrocoma crocea</i>	-0.36	-0.33	-0.39	-0.01	0.49	0.59	0.58	0.12	-3.64	-5.30	-6.48	-2.33
<i>Ranunculus inamoenus</i>	-0.48	-0.35	0.29	0.60	0.80	0.72	0.75	-0.12	-6.27	-5.79	-4.78	3.01
<i>Sedum rosea</i>	-0.11	-0.22	-0.33	-0.22	0.53	0.56	0.59	0.06	-2.72	-3.69	-4.68	-1.98
<i>Senecio integerrimus</i>	-0.26	-0.24	-0.36	-0.10	0.60	0.59	0.54	-0.06	-4.84	-5.57	-6.29	-1.59
<i>Taraxacum officinale</i>	-0.46	-0.30	-0.28	0.02	0.89	0.68	0.34	-0.61	-5.42	-5.13	-3.26	3.59
<i>Valeriana capitata</i>	-0.54	-0.26	-0.01	0.25	0.81	0.66	0.56	-0.26	-6.24	-5.44	-3.87	-1.86
<i>Valeriana edulis</i>	-0.53	-0.37	-0.38	0.14	0.55	0.55	0.60	0.05	-6.49	-6.56	-6.50	-0.14
<i>Vicia americana</i>	-0.21	-0.18	-0.09	0.12	0.52	0.46	0.65	0.14	-5.28	-4.24	-3.96	0.90
<i>Viola praemorsa</i>	-0.48	-0.29	-0.20	0.19	0.77	0.70	0.65	-0.10	-3.06	-4.93	-4.38	-2.06

Appendix C (Table C1). Strength and significance of phylogenetic signal in flowering time for 60 species from a subalpine plant community in the Colorado Rocky Mountains. Flowering time values reported here are averaged across the entire duration of the 39-year study period (1974–2012).

Flowering phenology trait	Blomberg's K	P-value	Pagal's λ	P-value
First flowering (1974–2012)	0.668	0.038	0.405	0.153
Peak flowering (1974–2012)	0.697	0.044	0.512	0.052
Last flowering (1974–2012)	0.635	0.162	0.456	0.069
Duration of flowering (1974–2012)	0.538	0.310	< 0.001	> 0.999

Appendix D (Table D1). To test the robustness of our results to variation in branch length and topology, we compared our results from the molecular genus tree (Table 1; Appendix E) to those generated from a less well-resolved phylogeny (Wikström et al. 2001, following Davies et al. 2013); the two phylogenies differ in branch lengths and topology above the genus level. Values reported in this table are from phylogenetic signal tests from the less well-resolved phylogeny (Wikström et al. 2001). Overall, phylogenetic signal results were qualitatively similar across the two different phylogenetic trees, indicating that our results on the molecular tree are robust to variation in branch length and topology. In general, the strength and significance of phylogenetic patterns was similar, especially using Blomberg's K . However, we note that results for Pagel's λ for flowering time were somewhat sensitive, but this is not surprising, and is expected to some degree. Pagel's λ tends to be a more conservative test (Münkemüller et al. 2012), and the results presented here are generated from a phylogeny where branch length and topology estimates are less reliable as compared to the molecular tree. Thus, because the results of Pagel's λ are qualitatively similar across both trees, we argue they are reasonably robust to variation in phylogenetic branch length and topology.

Phenological trait		Blomberg's K	P	Pagel's λ	P
Mean flowering time (1974–1984)	first	0.470	0.042	0.181	0.267
	peak	0.518	0.020	0.285	0.107
	last	0.470	0.049	< 0.001	0.999
	duration	0.461	0.167	< 0.001	0.999
Mean flowering time (1974–2012)	first	0.479	0.028	0.222	0.221
	peak	0.529	0.011	0.310	0.086
	last	0.543	0.023	0.279	0.117
	duration	0.469	0.171	< 0.001	0.999
Shift through time	first	0.362	0.437	< 0.001	0.999
	peak	0.309	0.897	< 0.001	0.999
	last	0.366	0.487	< 0.001	0.999
	duration	0.363	0.539	< 0.001	0.999
Snowmelt sensitivity	first	0.403	0.215	0.106	0.586
	peak	0.289	0.945	< 0.001	0.999
	last	0.449	0.069	< 0.001	0.999
	duration	0.443	0.056	< 0.001	0.999
Temperature sensitivity	first	0.329	0.719	< 0.001	0.999
	peak	0.291	0.916	< 0.001	0.999
	last	0.399	0.201	< 0.001	0.999
	duration	0.372	0.383	< 0.001	0.999

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Supplement

Data files for the community phylogeny used in analyses.
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File list

caradonna_supplement_gothic_community_phylogeny.txt

Description

The caradonna_supplement_gothic_community_phylogeny.txt file is a text file that contains the raw data for the community phylogeny used in analyses. Each name within the file corresponds to a species within the plant community, and numbers correspond to branch lengths.

APPENDIX C: FROST SENSITIVITY OF LEAVES AND FLOWERS OF SUBALPINE
PLANTS IS RELATED TO TISSUE TYPE AND PHENOLOGY

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

Frost sensitivity of leaves and flowers of subalpine plants is related to tissue type and phenology

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Summary

1. Harsh abiotic conditions – such as low temperatures that lead to spring and summer frost events in high-elevation and high-latitude ecosystems – can have strong negative consequences for plant growth, survival and reproduction.

2. Despite the predicted increase in episodic frost events under continued climate change in some ecosystems, our general understanding of the factors associated with frost sensitivity of reproductive and vegetative plant structures in natural plant communities is limited. The timing of growth and reproduction may be an important strategy by which plants can avoid frost.

3. In this study, we experimentally investigated the frost sensitivity of eight long-lived perennial herbaceous plant species from a subalpine ecosystem in the Colorado Rocky Mountains, USA. The study taxa represent four congeneric pairs from four flowering plant families; within each pair, there is a species with early and late growth and reproductive phenology. Thus, we control for evolutionary history – and therefore additional traits shared through common ancestry – to some degree, while examining the influence of phenology on frost sensitivity. Specifically, we compared frost sensitivity of vegetative and reproductive structures for each species and asked whether frost sensitivity was similar between species within congeneric pairs or, instead, was related to phenology (i.e. differences in the timing of growth and reproduction).

4. For most species (6 of 8), flowers were more sensitive to frost than leaves. Within most congeneric pairs (3 of 4), the leaves of species with later phenology were significantly more sensitive to frost than the leaves of species with earlier phenology. For flowers, the later flowering species were more sensitive in two of the four congeneric pairs.

5. *Synthesis.* This study contributes to our general understanding of factors related to interspecific differences in plant sensitivity to episodic frost events of naturally occurring species. The increased frost sensitivity of reproductive structures compared to vegetative structures may be a widespread pattern for long-lived perennial plants. Furthermore, we find evidence for a trade-off between phenology and frost sensitivity, whereby species with later phenology exhibit higher frost sensitivity compared to species with earlier phenology. These results have implications for plant populations, species interactions and ecological communities.

Key-words: climate change, freezing, frost resistance, phenology, phylogeny, plant–climate interactions, Rocky Mountain Biological Laboratory

Introduction

‘Low temperature represents, together with drought and salt stress, one of the most important environmental constraints limiting the productivity and distribution of plants on Earth’. –Sakai & Larcher (1987)

In many ecosystems, the potential for plants to be exposed to unfavourable abiotic conditions that can have negative consequences for their survival, growth and reproduction is high (Sakai & Larcher 1987; Inouye 2000). This is particularly the case in high-elevation and high-latitude ecosystems with the occurrence of episodic spring and summer frost events. Historically, many studies have investigated frost damage and

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freezing sensitivity of economically important plant species, with an emphasis on understanding freezing tolerance during winter (e.g. Sakai & Larcher 1987). Only more recently have studies explicitly focused on episodic frost events that occur during the growing season for plants in natural communities (e.g. Inouye 2008; Augspurger 2009; Lenz *et al.* 2013; Vitasse *et al.* 2014). These episodic frost events – despite their infrequent nature – have considerably different ecological and evolutionary implications than freezing winter temperatures (Inouye 2000). For example, a single frost event during the growing season can have strong detrimental effects on plants, including the destruction of vegetative and reproductive structures (Inouye 2000; Augspurger 2009; Lenz *et al.* 2013; Vitasse *et al.* 2014), with implications for reproduction (Forrest & Thomson 2010), demography (Inouye 2008) and species interactions (Nixon & McClain 1969). Under recent climate change, the probability of plant exposure to extreme episodic frost events has increased in many high-elevation and high-latitude ecosystems, including our own study site in Colorado (e.g. Hänninen 1991; Inouye 2008; Augspurger 2012; Wheeler *et al.* 2014; but see Scheifinger *et al.* 2003 and Bennie *et al.* 2010 for counter examples), making it increasingly important to understand the ecological consequences of frost. This seemingly paradoxical phenomenon occurs when late winter and early spring conditions are particularly warm, or spring snowmelt is early, which advances the timing of plant growth and reproduction; the probability of frost damage is high under these conditions because plants are growing earlier in the season at a time when night-time temperatures can dip well below freezing (Inouye 2000, 2008; Augspurger 2012). For example, in our study system in Colorado, substantial frost damage to plants is associated with early snowmelt dates (Inouye 2008). In particular, there is still much to be learnt about how frost sensitivity may differ among vegetative and reproductive structures, interspecific differences in frost sensitivity within natural communities, and the potential population and community-level consequences of episodic frost events.

It is generally assumed that reproductive structures are more sensitive to frost than vegetative structures (Sakai & Larcher 1987). However, available evidence from observa-

tions in natural plant communities is limited and relatively ambiguous: some studies find evidence for higher sensitivity in flowers compared to leaves (e.g. Inouye 2000; Inouye, Morales & Dodge 2002), others find no difference (e.g. Nixon & McClain 1969; Augspurger 2009), and yet another finds the opposite pattern (Augspurger 2011). It is possible that episodic frost events occurring during the growing season represent such an extreme abiotic condition that both vegetative and reproductive structures are damaged equally. Still, it remains unclear how vegetative and reproductive structures differ in response to episodic frost events occurring throughout the growing season.

When the negative consequences of episodic frost events are severe, plants may invest in a phenologically mediated frost avoidance strategy over a tolerance strategy (Sakai & Larcher 1987; Cannell 1997; Lenz *et al.* 2013). It has been proposed that optimal timing of life-history events (e.g. growth and reproduction) in temperate ecosystems should be a balance between avoiding the negative effects of abiotic extremes – which are more likely early in a growing season – and biotic competition, which should increase the later a species becomes active (Iwasa & Levin 1995; Post 2013). Under this logic, plant traits, as well as evolutionary history, will constrain this optimum: plants with the greatest ability to withstand harsh abiotic conditions should have earlier phenological events that relieve the effects of biotic competition to some degree and provide a longer growing season. However, if evolutionarily conserved physiological limitations constrain frost sensitivity (*sensu* Zanne *et al.* 2014), then related taxa may show no difference in frost sensitivities despite differences in phenology.

To explore the roles of phenology (timing of growth and reproduction) and shared evolutionary history as they relate to the ability of plants to cope with episodic frost events, we experimentally investigated frost sensitivity of leaves and flowers of eight long-lived perennial herbs from a subalpine ecosystem in the Colorado Rocky Mountains, USA. These eight taxa comprise four congeneric pairs from four flowering plant families; each of these pairs includes a species with early and late growth and reproductive phenology within the season (Table 1). This sampling of taxa controls for evolutionary his-

Table 1. Taxonomic information, habitat preference, mean flowering date (1974–2014), peak flowering date for the 2014 study season and frost trial number for the eight subalpine plant species used in this study. For each species, mean date of flowering is the average of peak flowering date across the 41-year study period (1974–2014); standard error (SE) in all cases is standard error of the mean. Mean flowering date is significantly different between each early and late pair ($P \ll 0.05$). Frost trial number corresponds to the date range for each experimental frost trial (see Table 2 for specific dates)

Species	Family	Habitat	Mean flowering date $\pm$ SE	Peak flowering date, 2014	Frost trial
<i>Mertensia fusiformis</i>	Boraginaceae	Wet and dry meadows	06 June $\pm$ 1.8	09 June	1
<i>Mertensia ciliata</i>	Boraginaceae	Wet meadows	09 July $\pm$ 1.3	13 July	2
<i>Hydrophyllum capitatum</i>	Hydrophyllaceae	Wet and dry meadows	11 June $\pm$ 1.8	13 June	1
<i>Hydrophyllum fendleri</i>	Hydrophyllaceae	Wet meadows, aspen forest	01 July $\pm$ 1.8	27 June	2
<i>Delphinium nuttallianum</i>	Ranunculaceae	Wet and dry meadows	18 June $\pm$ 1.5	19 June	1
<i>Delphinium barbeyi</i>	Ranunculaceae	Wet meadows	23 July $\pm$ 1.6	29 July	3
<i>Erigeron flagellaris</i>	Asteraceae	Dry meadows	17 July $\pm$ 4.1	12 July	2
<i>Erigeron speciosus</i>	Asteraceae	Wet and dry meadows	03 Aug $\pm$ 1.7	06 Aug	3

tory – and therefore other traits shared through common ancestry – to some degree, while investigating how phenology relates to frost sensitivity. Specifically, we examined the temperatures at which vegetative (leaves) and reproductive (flowers) structures are sensitive to frost and asked two primary questions: (i) Are frost sensitivities similar among vegetative and reproductive structures within species, and (ii) are frost sensitivities similar among closely related species (congeneric pairs), or do closely related species with early vs. late phenology exhibit differences in frost sensitivity? We discuss the implications of our findings from the perspectives of population dynamics, species interactions and community ecology.

Materials and methods

To determine temperatures at which species exhibit signs of frost damage and to relate interspecific variation in frost sensitivity to ecological and evolutionary patterns and processes, we selected eight subalpine plant species that varied in phenology (timing of growth and reproduction) and evolutionary relationships (Table 1). Our experimental procedure for examining the frost sensitivity of these taxa had three stages: (i) plant tissue collection in the field, (ii) exposure of plant tissue to experimental temperature treatments in the laboratory and (iii) assessment of frost damage following each treatment.

STUDY SITE AND STUDY SPECIES

All plant specimens used in this study were collected at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (38°57.5'N, 106°59.3'W, 2900 m above sea level). The area surrounding the RMBL is a subalpine ecosystem consisting of a mosaic of wet and dry meadows, and aspen and spruce forest. This ecosystem is snow-dominated for much of the year; thus, the growing season is relatively brief, lasting approximately 3–5 months (CaraDonna, Iler & Inouye 2014). Snowmelt date in this ecosystem is highly variable from year to year, ranging from mid-April to mid-June, although the date of snowmelt is getting earlier in accordance with climate change (Anderson *et al.* 2012; Iler *et al.* 2013a). Freezing temperatures during springtime are common in this plant community: from 1973 to 2005, minimum temperatures in June ranged from –1.7 to –8.3 °C (mean = –4.3 °C; Inouye 2008). The frequency of freezing temperatures has not changed over the course of this time period (1973–2005; Miller-Rushing & Inouye 2009); instead, there is an ecologically relevant interaction between earlier snowmelt dates, plant phenology and frost damage. When snowmelt is early, flowering phenology is advanced (Iler *et al.* 2013b), and as a result, plants are exposed to a greater occurrence of freezing temperatures during growth and reproduction (Inouye 2008; Miller-Rushing & Inouye 2009). For example, at our study site in Colorado, Inouye (2008) found that when snowmelt date is early, a substantial proportion of flowers of three herbaceous species experience severe frost damage; such frost damage does not occur when snowmelt date is later. As snowmelt dates are predicted to become earlier and earlier in this ecosystem, the severity and frequency of frost damage to plants are also likely to increase (Inouye 2008).

Our eight taxa represent four congeneric pairs from four flowering plant families: *Erigeron flagellaris* and *E. speciosus* (Asteraceae); *Mertensia fusiformis* and *M. ciliata* (Boraginaceae); *Hydrophyllum capitatum* and *H. fendleri* (Hydrophyllaceae); and *Delphinium nuttallianum* and *D. barbeyi* (Ranunculaceae). Each of these congeneric

pairs includes a species with early and late-flowering phenology (Table 1), which allows us to explore the relationship between phenology and frost sensitivity while controlling for evolutionary relationships to some degree. Each early and late pair has significantly different flowering dates (*t*-test, *Mertensia* spp. $t = -14.85$, $P < 0.001$; *Hydrophyllum* spp. $t = -7.9$, $P < 0.001$; *Delphinium* spp. $t = -16.17$, $P < 0.001$; *Erigeron* spp. $t = -4.10$, $P < 0.001$; Table 3, Fig. 2). All eight taxa are native and widely distributed across western North America in subalpine and montane ecosystems. Furthermore, all taxa are long-lived perennial herbs, which is the dominant life-history strategy of the plant taxa in this subalpine community. For these species, at the end of each growing season, the above-ground biomass senesces. All species overwinter below-ground and upon snowmelt at the start of each new growing in the spring, the growth of vegetative and reproductive structures occurs once again.

PLANT TISSUE COLLECTION

Vegetative (leaves) and reproductive (flowers) plant tissues were collected from approximately 100–120 randomly chosen plant individuals around the RMBL. The total number of samples collected for each plant species varied based on the availability of plant tissue during each collection period (see Tables S1 & S2 in Supporting Information); for one plant species, we had difficulty collecting this amount of plant tissue, and the sample size is reduced (*Hydrophyllum capitatum*: $n = 62$). For all species, plant tissue was collected at the same developmental stage. Specifically, for leaves, only mature, fully expanded leaf tissue was collected, and for flowers, only newly opened floral tissue was collected. From each individual plant, a single undamaged leaf and a single undamaged flower were collected and then immediately placed inside a plastic bag with a damp paper towel and placed inside a cooler. For consistency, and so we did not damage leaf tissue or floral tissue, all leaves were cut near the base of the petiole, below the leaf blade, and flowers were cut near the base of the pedicel, below the receptacle. Within 2 h of collection, all detached leaf and flower tissues were transferred from the field cooler to a laboratory refrigerator and stored at 5 °C until prepared for an experimental temperature treatment. The treatments were applied within 24 h of field collection. We collected fresh plant tissue each day prior to each experimental treatment to control for plant tissue senescence that may occur after collection from the field. In addition, the temperatures experienced by herbaceous plants during the growing season may influence their frost sensitivity (e.g. Sakai & Larcher 1987; Rapacz & Janowiak 1998). In our subalpine ecosystem, temperatures fluctuate between warm daytime (i.e. > 15 °C) and cool nighttime temperatures (i.e. < 5 °C) for the vast majority of the growing season (see Fig S1). Thus, all of our study taxa experienced relatively similar temperature regimes, and it is therefore unlikely that temperatures experienced prior to field collection would strongly bias our results. Air temperatures at the RMBL before and during plant sampling and frost trials are reported in Figure S1.

FROST EXPERIMENT

We used a Sigma M26-C4 programmable temperature chamber (Sigma Systems Corporation, Mansfield, MA, USA) to expose plant tissue to five independent temperature treatments: 0, –2.5, –5.0, –7.5 and –10 °C. Our selection of these temperature treatments encompasses the range of night-time freezing temperatures that occur at our study site in Colorado during early spring after snowmelt (minimum temperature range from 1973 to 2005: –1.7 to –8.3 °C;

Inouye 2008); our lowest temperature treatment was meant to represent an extreme condition that lies just beyond what is observed in our ecosystem. The actual target temperatures reached during each treatment are reported in Table 2 and are the temperatures used in all subsequent analyses. For each complete experimental frost trial – that is, all five temperature treatments – approximately 100 samples of flowers and leaves were used for each species; approximately 20 samples were used for each individual temperature treatment (i.e. 20 samples at five temperature treatments = 100 samples per species). Across the growing season, three complete experimental frost trials were conducted that coincided with the availability of vegetative and reproductive tissue for our focal taxa at our field site (Table 1). Because we had only one temperature chamber, individual temperature treatments within a complete frost trial occurred on separate days (or at night) during this time period, assignment of each treatment was random within each complete frost trial to prevent any biases that may arise as a result of experimental treatments being conducted on separate days. Because liquid CO₂ is the cooling agent for the temperature chamber, all plant tissues were wrapped in aluminium foil to prevent CO₂ poisoning and to prevent excess water loss and desiccation during the experiment (Sakai & Larcher 1987; Wheeler *et al.* 2014). Each leaf and flower sample was wrapped individually in its own foil packet.

Temperature treatments were programmed to model the rate and duration of night-time frost events at our field site, based on temperature data from a weather station at the study site (see Fig. S2). All frost simulations began at 5 °C. Plant tissue was placed inside the temperature chamber, and over the course of 6 h, the temperature ramped down from 5 °C to one of the five target temperatures (0, –2.5, –5.0, –7.5 or –10 °C; Table 2). Once the target temperature was reached, it was held for 2 h and then brought back up to 5 °C in 1 h. The total time for each temperature treatment was 9 h. Thus, we controlled for time, rather than cooling rate, because this better matches what occurs in our ecosystem during a frost event (Fig. S2).

ASSESSMENT OF FROST DAMAGE

Once each temperature treatment was complete, individual plant specimens were removed from the temperature chamber and visually examined for frost damage. Frost damage to leaves and flowers is generally obvious due to discoloration and wilting associated with oxidation of polyphenols, or a characteristic odour caused by the decompartmentalization and autolysis of the protoplast (Sakai &

Larcher 1987). For consistency, assessment of frost damage was always conducted by the same three researchers (P.J.C., J.A.B. and R.M. Brennan). In addition, all experimentally manipulated plant tissue was compared to freshly collected plant tissue for reference. Frost damage was scored on a binary scale where 1 = frost damage, and 0 = no frost damage. For flowers, we examined frost damage to the entire flower (i.e. all floral parts were assessed simultaneously without distinction among specific floral parts). We used a binary damage scale over a continuous damage scale, because in the vast majority of cases, if frost damage occurred, it was either complete or extensive throughout the plant tissue (e.g. Fig. 1). Finally, visual inspection of frost damage has been shown to correspond with electrolyte leakage methods (Obrist 2001; Vitasse *et al.* 2014).

ASSESSMENT OF PLANT PHENOLOGY

Data on plant phenology for our study species come from a long-term study of flowering phenology at the RMBL (1974–2014). In this long-term study, the number of open flowers per stalk was counted every other day from 1974 to 2014 for all flowering plant species that occur in 30 2 × 2 m plots. In our study of frost sensitivity, we define phenology for each species as the mean date of peak flowering averaged across the 41-year study period (Table 1) (Inouye 2008; Iler *et al.* 2013b; CaraDonna, Iler & Inouye 2014). Specific peak flowering dates for each species during the 2014 study year are reported in Table 1. Peak flowering date is the day on which 50% of the flowers were counted as open for each species. We do not have species-level data on vegetative phenology; thus, we use flowering phenology as an approximate measure for overall vegetative and reproductive phenology for each species. Reproductive and vegetative traits are often highly correlated (Primack 1987),

Table 2. Target and actual temperatures reached during each of the three frost trials. During each treatment, the temperature in the chamber began at 5 °C and then over the course of 6 h was brought down to the each target temperature, held for 2 h and then brought back up to 5 °C in 1 h; with this design, we controlled for time, rather than cooling rate, because this more closely resembles what occurs at our study site in the Colorado Rocky Mountains, USA (Fig. S2). ‘Frost trial 3’ occurred over fewer days compared to the other frost trials because some of the individual temperature treatments were conducted at night

Frost trial	Treatment temperature (°C)					Frost trial date range
Target	0.0	–2.5	–5.0	–7.5	–10.0	NA
1	0.0	–2.5	–4.9	–7.1	–9.8	19–26 June 2014
2	0.0	–2.3	–4.8	–6.9	–7.4	02–09 July 2014
3	0.0	–2.5	–4.8	–7.4	–9.4	28–31 July 2014

Delphinium barbeyi (Ranunculaceae)



Erigeron speciosus (Asteraceae)



Fig. 1. The flowers of *Delphinium barbeyi* and *Erigeron speciosus* after the warmest (0 °C) and the coolest (–10 °C) experimental temperature treatments. Plant tissue was collected from a subalpine ecosystem near the Rocky Mountain Biological Laboratory in the Colorado Rocky Mountains, USA (2900 m above sea level).

and indeed, flowering and leaf-out times appear to be highly correlated in many species (*sensu* Schwartz & Reiter 2000; Post *et al.* 2008 Polgar, Gallinat & Primack 2014; Zohner & Renner 2014). Based on our (P.J.C. and J.A.B.) personal observations for our study taxa, plants that flower early tend to be plants that begin vegetative growth earlier in the season; additionally, because the growing season is brief, the duration between emergence of vegetative structures and the timing of reproduction is relatively short. Therefore, in this study, we are assuming that flowering phenology and vegetative phenology are correlated to some degree.

DATA ANALYSIS

We analysed the relationship between experimental temperature and frost damage to leaves and flowers using standard logistic regression models (implemented in R using the *glm* function; R Development Core Team 2014). The lethal temperature at which 10%, 50% and 90% of the individuals across temperature treatments experienced frost damage (LT_{10} , LT_{50} , LT_{90}) and the standard error (standard error of inverse prediction) were calculated from each logistic regression model. In one case (leaf frost sensitivity analyses of *Hydrophyllum fendleri*), a standard logistic regression model could not be fit due to perfect separation of data; here, we used a Bayesian logistic regression model that accounts for this standard logistic model violation (implemented in R using the ARM package).

We analysed differences in frost sensitivity between leaves and flowers within species, and between species within congeneric pairs, by comparing the intercepts of each logistic regression model fit via a Wald test. Specifically, different logistic regression intercepts indicate

different relationships between temperature and the probability of frost damage (which is conceptually analogous to comparing slopes in a linear regression); in other words, a significantly different intercept means that plant tissue types, or species within congeneric pairs, differ in their frost sensitivity. In the few cases where one species in a comparison did not exhibit any frost damage within the experimental treatments (i.e. frost sensitivity occurred below -10°C), we used a one-sided *t*-test to examine whether LT_{50} of the damaged species was significantly less than -10°C . Furthermore, we limit our analyses of frost sensitivity to within species comparisons (leaf vs. flower) or within congeneric pair analyses (early- vs. late-flowering species) because our selection of taxa is necessarily a biased sample from the community. Thus, we did not analyse data in a continuous framework using a linear regression model because our selection of species does not represent independent samples.

Results

Frost sensitivity varied considerably among the eight study species. For leaves, frost damage ranged from -5.1°C to below -10.0°C , and for flowers, frost damage ranged from -1.9°C to below -10.0°C (Figs 2 and 3; Table 3). (A complete list of logistic regression model fit coefficients is reported in Tables S1 & S2). For the majority of species, leaf and flower frost sensitivity (LT_{10} , LT_{50} and LT_{90}) occurred within the experimental temperature range. However, in four cases, frost damage did not occur within the experimental temperature range (leaves and flowers of *Erigeron flagellaris*,

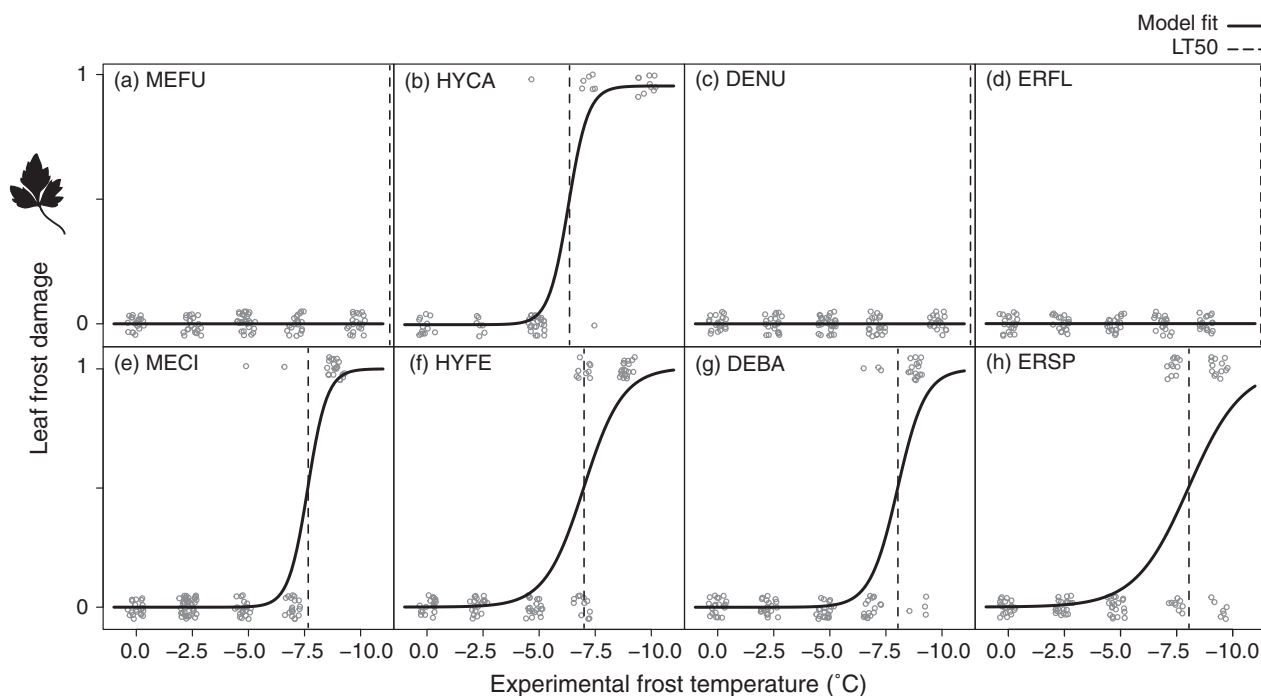


Fig. 2. Leaf frost sensitivities of subalpine plant species from the Colorado Rocky Mountains, USA. (a–h) Logistic regression model estimates showing the relationship between leaf frost damage and experimental temperatures. Black lines represent logistic regression model fits; points represent actual data points, which are jittered for clarity and visualization purposes; dashed lines indicate LT_{50} (dashed lines are shown at the end of the x-axis in cases where LT_{50} did not occur prior to -10°C). Species are ordered by relative phenology (Table 1), and congeneric pairs are stacked on top of each other. Four letter species abbreviation codes are as followed: MEFU = *Mertensia fusiformis*, HYCA = *Hydrophyllum capitatum*, DENU = *Delphinium nuttallianum*, ERFL = *Erigeron flagellaris*, MECI = *Mertensia ciliata*, HYFE = *Hydrophyllum fendleri*, DEBA = *Delphinium barbeyi* and ERSP = *Erigeron speciosus*. Note that the x-axis has been reversed so that an increase in freezing temperature is shown from left to right.

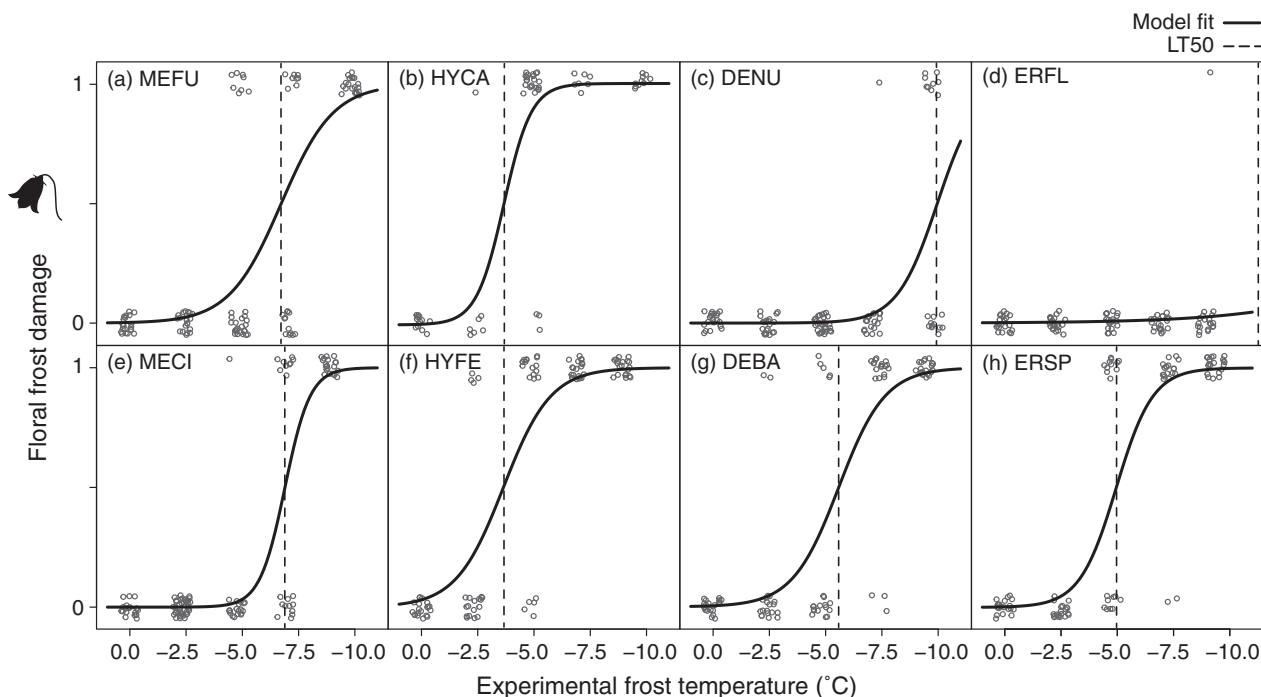


Fig. 3. Floral frost sensitivities of subalpine plant species from the Colorado Rocky Mountains, USA. (a–h) Logistic regression model estimates showing the relationship between floral frost damage and experimental temperatures. Black lines represent logistic regression model fits; points represent actual data points, which are jittered for clarity and visualization purposes; dashed lines indicate LT₅₀ (dashed lines are shown at the end of the x-axis in cases where LT₅₀ did not occur prior to -10°C). Four letter species abbreviation codes are the same as in Fig. 2. Note that the x-axis has been reversed so that an increase in freezing temperature is shown from left to right.

Table 3. Leaf and flower frost sensitivities of eight subalpine species from the Colorado Rocky Mountains. Standard error (SE) in all cases is standard error of the mean. Temperature values for species that did not experience leaf or flower frost damage within the experimental treatments are reported as occurring below -10°C (see Materials and Methods for details). For *Delphinium nuttallianum*, LT₉₀ for flowers falls outside of the experimental treatments and is thus an extrapolated model estimate

Species	Leaf frost sensitivity ($^{\circ}\text{C}$)			Floral frost sensitivity ($^{\circ}\text{C}$)		
	LT ₁₀ $\pm$ SE	LT ₅₀ $\pm$ SE	LT ₉₀ $\pm$ SE	LT ₁₀ $\pm$ SE	LT ₅₀ $\pm$ SE	LT ₉₀ $\pm$ SE
<i>Mertensia fusiformis</i>	< -10.0	< -10.0	< -10.0	-5.7 ± 0.5	-6.9 ± 0.4	-8.1 ± 0.7
<i>Mertensia ciliata</i>	-6.5 ± 0.3	-7.7 ± 0.2	-8.8 ± 0.4	-4.2 ± 0.4	-6.7 ± 0.2	-9.3 ± 0.2
<i>Hydrophyllum capitatum</i>	-5.1 ± 0.7	-6.3 ± 0.4	-7.6 ± 0.4	-2.3 ± 0.7	-3.7 ± 0.4	-5.0 ± 0.4
<i>Hydrophyllum fendleri</i>	-5.8 ± 0.5	-7.0 ± 0.3	-8.12 ± 0.5	-1.9 ± 0.5	-3.7 ± 0.3	-5.5 ± 0.5
<i>Delphinium nuttallianum</i>	< -10.0	< -10.0	< -10.0	-8.0 ± 0.6	-10.0 ± 0.4	-11.9 ± 0.9
<i>Delphinium barbeyi</i>	-6.4 ± 0.6	-8.0 ± 0.4	-9.7 ± 0.6	-3.3 ± 0.6	-5.6 ± 0.4	-7.8 ± 0.6
<i>Erigeron flagellaris</i>	< -10.0	< -10.0	< -10.0	< -10.0	< -10.0	< -10.0
<i>Erigeron speciosus</i>	-5.4 ± 0.7	-8.0 ± 0.4	-10.5 ± 0.8	-3.2 ± 0.5	-5.0 ± 0.3	-6.7 ± 0.5

and leaves of *Mertensia fusiformis* and *Delphinium nuttallianum*), indicating frost resistance even at the most extreme treatments; in these cases, frost sensitivity values are reported as less than -10.0°C (Table 3). In one case (flowers of *Delphinium nuttallianum*), LT₉₀ only did not occur within the experimental temperature range, and the reported LT₉₀ value is based on extrapolated logistic regression model estimates (Table 3).

FLORAL & LEAF FROST SENSITIVITY AMONG SPECIES

Floral tissue was significantly more sensitive to frost than leaf tissue in six of the eight study species (Table 4, Figs 2 and

3). For the remaining two species (*Delphinium nuttallianum* and *Erigeron flagellaris*), little or no frost damage occurred within the experimental treatments, making it unclear in these cases whether leaves and flowers differ in frost sensitivity.

COMPARISON OF LEAF AND FLOWER FROST SENSITIVITY WITHIN CONGENERIC PAIRS

There was a significant difference in leaf frost sensitivity between species within three out of the four congeneric pairs (Fig. 2). In these three cases, the later flowering species was more sensitive to frost (one-sided *t*-test, *Mertensia* spp. $t = 114$, $P < 0.0001$; *Delphinium* spp. $t = 49.5$, $P < 0.0001$;

Table 4. Logistic regression analyses results comparing frost sensitivity between leaf tissue and floral tissue for each species. For all cases where there is a significant effect, floral tissue is more sensitive to frost than leaf tissue (Table 3, Figs 2 and 3). Significant comparisons are indicated in bold. We did not compare leaf and flower frost sensitivity for *Delphinium nuttallianum* and *Erigeron flagellaris* because LT_{50} occurred outside of the experimental temperature range (See Materials and methods for details)

Species	Wald test coefficients		
	χ^2	d.f.	<i>P</i>
<i>Mertensia fusiformis</i>	23.50	1	< 0.0001
<i>Mertensia ciliata</i>	5.40	1	0.02
<i>Hydrophyllum capitatum</i>	22.20	1	< 0.0001
<i>Hydrophyllum fendleri</i>	25.70	1	< 0.0001
<i>Delphinium nuttallianum</i>	NA	NA	NA
<i>Delphinium barbeyi</i>	18.50	1	< 0.0001
<i>Erigeron flagellaris</i>	NA	NA	NA
<i>Erigeron speciosus</i>	21.10	1	< 0.0001

Erigeron spp. $t = 50$, $P < 0.0001$; Table 3, Fig. 2), suggesting a relationship between plant phenology and frost sensitivity independent of evolutionary history. However, there was no statistical difference in sensitivity of leaves to frost between the two *Hydrophyllum* species (Wald test, $\chi^2 = 2.5$, d.f. = 1, $P = 0.11$; Table 3, Fig. 2), suggesting that at least some closely related species exhibit similar leaf frost sensitivity.

For two sets of congeneric pairs, flowers of the later flowering species were more sensitive to frost than the earlier flowering species (Wald test, *Delphinium* spp.: $\chi^2 = 30.00$, d.f. = 1, $P < 0.0001$; *Erigeron* spp. $\chi^2 = 27.60$, d.f. = 1, $P < 0.0001$; Table 3, Fig. 3). In contrast, for the remaining two congeneric pairs, there was no difference in floral frost sensitivity between species (Wald test, *Mertensia* spp. $\chi^2 = 0.40$, d.f. = 1, $P = 0.53$; *Hydrophyllum* spp. $\chi^2 = 0.85$, d.f. = 1, $P = 0.77$; Table 3, Fig. 3).

Discussion

In this study, we experimentally investigated the frost sensitivity of vegetative and reproductive structures for a series of eight long-lived perennial herbs from a subalpine ecosystem in the Colorado Rocky Mountains, USA. Specifically, we explored two primary questions: (i) whether frost sensitivity differed between leaves and flowers within species, and (ii) whether phenology (timing of growth and reproduction) or evolutionary history was related to interspecific variation in frost sensitivity. Overall, frost sensitivity varied considerably among species and plant structures. Within species, flowers were consistently more sensitive to frost than leaves (Tables 3 and 4, Figs 2 and 3). Within congeneric pairs, the leaves of species with later-season phenological events were generally more sensitive to frost than the species with early-season phenological events. For flowers, the relationship between phenology and frost sensitivity was less clear: in two of the

congeneric pairs, the species with later-season phenological events were more sensitive to frost than the species with early-season phenological events, as predicted; for the remaining two congeneric pairs, frost sensitivity was virtually identical despite differences in the timing of phenological events. Thus, our results provide evidence in support of a phenology-frost sensitivity trade-off, but also show that, in some cases, more closely related taxa may exhibit physiological constraints related to frost sensitivity.

Our assessment of frost sensitivity was within controlled experimental conditions and was conducted on detached plant tissues. Detachment of plant tissues may prevent recovery after minor frost damage, especially if physiological processes involved in recovery are inhibited. However, in this study, plant tissue was most often severely damaged (e.g. complete discoloration and wilting) or unaffected (Fig. 1); in these cases, recovery of destroyed plant tissue is unlikely. Frost sensitivities of plants under naturally occurring conditions are likely to vary with cooling and warming rates, microclimate and other habitat heterogeneities (e.g. slope, aspect and humidity) that may buffer or further expose plants to freezing temperatures (Inouye 2000). Thus, our findings from our controlled experimental temperatures are not directly applicable to natural settings but still have important implications for natural plant populations and communities. Furthermore, in a few cases, we were unable to determine specific frost sensitivity values for some species because frost damage did not occur within our experimental temperature treatments (Table 3). However, this limitation does not influence the interpretation of our results because we can infer that frost sensitivity for these species is below -10.0 °C, a freezing temperature that is beyond what is observed in our ecosystem during spring (Inouye 2008).

FLOWERS ARE MORE SENSITIVE TO FROST THAN LEAVES

Reproductive structures are assumed to be more sensitive than vegetative structures, in part because rapidly differentiating and actively growing plant tissues tend to be most sensitive to frost events (Sakai & Larcher 1987). However, the limited evidence for plants from natural communities does not consistently support this assumption (e.g. Nixon & McClain 1969; Inouye 2000; Augspurger 2009, 2011). In our experimental study, we found that flowers were generally more sensitive to frost than leaves (Table 3, Figs 2 and 3). We propose that our finding of greater frost sensitivity in flowers compared to leaves is consistent with demographic life-history trade-offs for long-lived perennial plant species (Franco & Silvertown 2004). All eight of the study species are long-lived iteroparous herbs. Such plants should invest in strategies that ensure survival and growth over short-term reproductive success in order to increase overall population growth (λ). This is because survival and growth characteristically contribute more strongly to population growth for long-lived perennial herbs compared to reproduction (Franco &

Silvertown 2004). In other words, there is less incentive to invest in protection of ephemeral reproductive structures compared to vegetative structures because when vegetative structures survive, another reproductive cycle is possible in the following year.

VARIATION IN FROST SENSITIVITY WITHIN CONGENERIC PAIRS: EVIDENCE FOR THE IMPORTANCE OF PHENOLOGY

Recent work has highlighted a trade-off between plant phenology and frost sensitivity (e.g. Lenz *et al.* 2013; Vitasse, Lenz & Körner 2014; Vitasse *et al.* 2014). Plants that are sensitive to freezing temperatures should be selected to have later phenological events (e.g. leaf-out time and flowering time) that decrease the probability of experiencing a detrimental frost event (Lenz *et al.* 2013). We hypothesize that selection should be more extreme for vegetative structures compared to reproductive structures because the destruction of vegetative structures via frost damage may partially or completely retard growth, which can subsequently lead to partial or complete reproductive failure. Furthermore, this hypothesized relationship between plant phenology and frost sensitivity is consistent with theoretical expectations for optimal timing in seasonal environments. Plant species with earlier phenological events should receive a fitness benefit because they can withstand unfavourable abiotic conditions, and as a result, can avoid more intense biotic competition (Iwasa & Levin 1995; Post 2013) and simultaneously take advantage of a longer growing season (Lockhart 1983). However, this general hypothesis does not account for the possibility that related taxa may have evolutionarily conserved physiological constraints that prevent them from coping with harsh abiotic conditions despite flexibility in their phenological events. In this study, we found strong evidence for a relationship between plant phenology and frost sensitivity, and only limited evidence that, in some cases, closely related taxa may exhibit physiological constraints related to frost sensitivity (Tables 1 and 3, Figs 2 and 3). These results suggest that the related taxa in our study may not have strong physiological constraints related to frost sensitivity, or the benefits of earlier growth and reproduction are strong enough to override any physiological constraints shared among related taxa. Additional research on species representing broader taxonomic coverage and from different ecosystems will be important to further evaluate these findings. Nonetheless, our results from four congeneric pairs suggest that within many clades, taxa may show divergence in phenological traits that mediate frost sensitivity, supporting the phenology–frost sensitivity relationship, whereas in some cases in other clades, related taxa may exhibit no difference in frost sensitivity, despite variation in the timing of phenological events, which may be related to evolutionarily conserved physiological constraints. Our study adds to a growing body of evidence that highlights the relationship between ecological timing and harsh abiotic events (e.g. Inouye 2000, 2008; Augspurger 2009; Forrest & Thomson

2010; Lenz *et al.* 2013; Vitasse, Lenz & Körner 2014; Vitasse *et al.* 2014).

IMPLICATIONS FOR SPECIES INTERACTIONS AND COMMUNITY DYNAMICS

The differences in frost sensitivities between vegetative and reproductive structures we report here have implications for species interactions and community dynamics. Our findings suggest that a single episodic frost event may not be detrimental for plant growth and survival, but can severely reduce floral production via frost damage and reduce reproduction in these subalpine plant species (e.g. Forrest & Thomson 2010; Thomson 2010). Frost sensitivity of flowers among our eight species was highly variable, with some species experiencing frost damage at relatively mild freezing temperatures (-1.9°C), while others remained undamaged by freezing temperatures exceeding -10.0°C (Table 3). This means that a frost event at any given time during the growing season is likely to have variable effects on species, depending on their specific frost sensitivity and phenology, as well as evolutionary history. Assuming that plants under natural conditions exhibit a similar range of frost sensitivity to those observed in our experiment, this variation in frost sensitivity has the potential to alter flowering plant community composition and hence the floral resource landscape available to pollinators following an episodic frost event. Changes in flowering plant community composition can affect pollinator visitation patterns (Waser 1978; Ghazoul 2006), as well as plant–plant competition for abiotic resources (Veresoglou & Fitter 1984), both of which can influence plant reproductive output. Importantly, changes in floral resource composition and abundance are also likely to have consequences for pollinator population dynamics (e.g. Roulston & Goodell 2011; Burkle, Marlin & Knight 2013). Indeed, episodic frost events have been linked to subsequent population declines in squirrels due to reductions in food resources (Nixon & McClain 1969), and a similar scenario is possible for pollinator populations. In this subalpine plant community, coflowering patterns have been shown to be reshuffled under climate change conditions (Forrest, Inouye & Thomson 2010; CaraDonna, Iler & Inouye 2014). As the climate continues to warm in this subalpine ecosystem, and snowmelt and plant phenology continue to become earlier, detrimental frost events are likely to increase, especially for plants commencing growth early in the season (Inouye 2008; Miller-Rushing & Inouye 2009; Forrest & Thomson 2010). The findings presented here are consistent with the interpretation that at least some of the reshuffling observed within this flowering plant community may be attributed to the effects of frost damage.

Conclusions

Harsh abiotic conditions, such as freezing temperatures, are a key challenge limiting the distribution and abundance of plants (Sakai & Larcher 1987; Zanne *et al.* 2014). As climate

change continues to alter the frequency and magnitude of extreme abiotic events, it is increasingly important to understand interspecific variation in species' responses to these events, and ultimately, how these responses may influence both population and community patterns. This study contributes to our general understanding of factors related to interspecific differences in plant sensitivity to episodic frost events of naturally occurring species. The increased sensitivity of reproductive structures to frost compared to vegetative structures may be a widespread pattern for long-lived perennial plants, as is evidenced here, but this hypothesis needs to be experimentally examined in more species and in more ecosystems. As we hypothesized, interspecific variation in plant frost sensitivity may represent different strategies employed by different clades. Our results suggest that the timing of important life-history events (e.g. growth and reproduction), more so than evolutionary history, is likely to influence how plant species respond to harsh abiotic conditions.

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Data accessibility

Frost sensitivity data used in this study are archived at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad/v4cv6> (CaraDonna & Bain 2015). Long-term flowering phenology data and metadata are archived at www.rmbll.org and at the Digital Repository at the University of Maryland: <http://drum.lib.umd.edu/>.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Snowmelt date, daily maximum and daily minimum air temperatures before and during plant sampling and temperature manipulation.

Figure S2. Examples of night-time cooling rates that occur during the spring after snowmelt at the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA.

Table S1. Logistic regression model coefficients for the relationship between experimental temperature and leaf frost damage for the eight plant taxa in this study.

Table S2. Logistic regression model coefficients for the relationship between experimental temperature and floral frost damage for the eight plant taxa in this study.

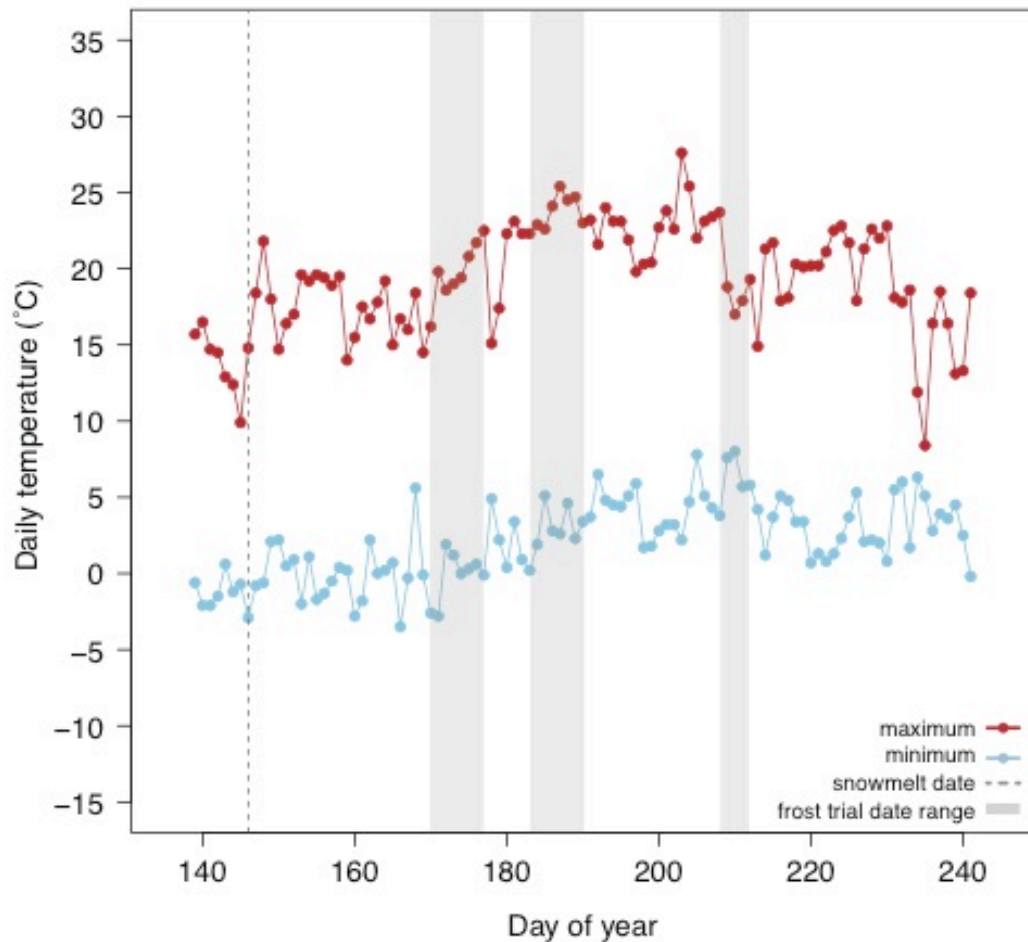


Figure S1: Snowmelt date, daily maximum and daily minimum air temperatures before and during plant sampling and frost manipulation for the 2014 field season at the Rocky Mountain Biological Laboratory (RMBL), Gothic, Colorado, USA. Frost trial date range is the time period over which plant tissue was collected and each individual frost treatment was applied (See Materials and methods for details). The third frost trial occurred over fewer days compared to the other frost trials because some of the individual temperature treatments were conducted at night. Daily maximum and minimum temperature data come from the Billy Barr Weather Station at the RMBL and are available at: <http://www.wrcc.dri.edu/cgi-bin/rawMAIN.pl?corbil>.

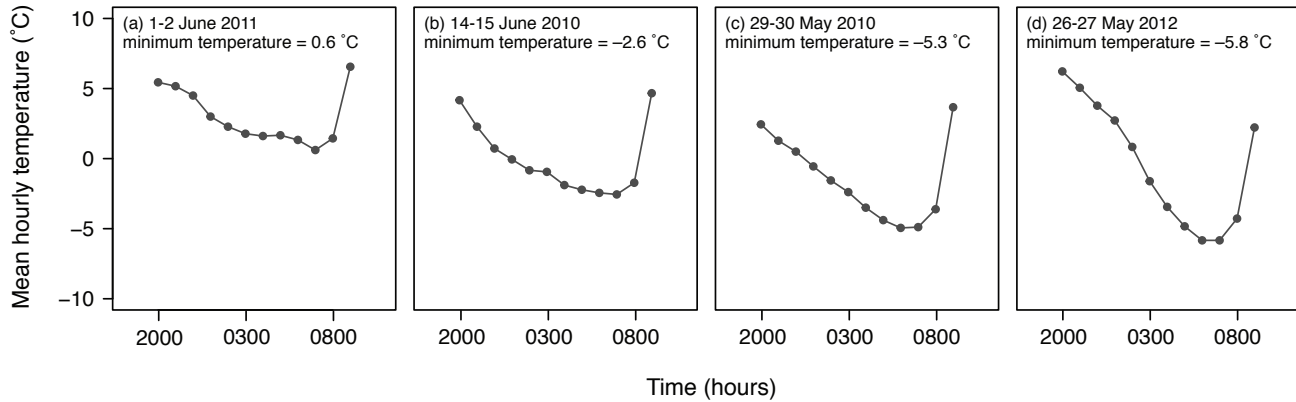


Figure S2: (a–d) Examples of nighttime cooling rates that occur during the spring after snowmelt at the Rocky Mountain Biological Laboratory (RMBL), Gothic, Colorado, USA. These examples begin at approximately the same temperature as our frost trials (ca. 5°C) and reach a similar minimum temperature as our different frost treatments (0.0, -2.5, -5.0, -7.5, and -10.0°C). Hourly temperature data at our field site are only available from 2010 and during this time, no nighttime temperatures that occurred after snowmelt and began near 5°C dropped to our most extreme temperature treatments (our lowest temperature treatment was meant to represent an extreme condition that is beyond what is observed in our ecosystem). Hourly temperature data come from the Billy Barr Weather Station at the RMBL and are available at: <http://www.wrcc.dri.edu/cgi-bin/rawMAIN.pl?corbil>.

Table S1: Logistic regression model coefficients for the relationship between experimental temperature and leaf frost damage (lethal temperature, LT) for the eight plant taxa in this study. *Notes:* (1) slope and intercept values are not reported in cases where the logistic regression model was non-significant; (2) due to perfect separation of the data, *Hydrophyllum fendleri* is analyzed using a Bayesian generalized linear model, which accounts for this logistic regression model violation.

Species	Logistic regression model coefficients					Leaf frost sensitivity (°C)		
	<i>n</i>	Slope ± SE	<i>P</i>	Intercept ± SE	<i>P</i>	LT ₁₀	LT ₅₀	LT ₉₀
<i>Mertensia fusiformis</i>	110	<i>na</i>	1.000	<i>na</i>	1.000	< -10.0	< -10.0	< -10.0
<i>Mertensia ciliata</i>	120	-2.33 ± 0.58	< 0.0001	-17.81 ± 4.33	< 0.0001	-6.70	-7.65	-8.59
<i>Hydrophyllum capitatum</i>	62	-2.27 ± 0.67	0.001	-14.30 ± 4.04	< 0.0001	-5.34	-6.31	-7.27
<i>Hydrophyllum fendleri</i>	100	-1.15 ± 0.21	< 0.0001	-8.06 ± 1.52	< 0.0001	-5.10	-6.99	-8.90
<i>Delphinium nuttallianum</i>	110	<i>na</i>	1.000	<i>na</i>	1.000	< -10.00	< -10.00	< -10.00
<i>Delphinium barbeyi</i>	100	-1.62 ± 0.39	< 0.0001	-13.03 ± 3.14	< 0.0001	-6.67	-8.024	-9.38
<i>Erigeron flagellaris</i>	100	<i>na</i>	1.000	<i>na</i>	1.000	< -10.00	< -10.00	< -10.00
<i>Erigeron speciosus</i>	100	-0.84 ± 0.19	0.001	-6.75 ± 1.51	< 0.0001	-5.41	-8.04	-10.66

Table S2: Logistic regression model coefficients for the relationship between experimental temperature and floral frost damage for the eight plant taxa in this study. Slope and intercept values are not reported in cases where the logistic regression model was non-significant.

Species	Logistic regression model coefficients					Floral frost sensitivity (°C)		
	<i>n</i>	Slope ± SE	<i>P</i>	Intercept ± SE	<i>P</i>	LT ₁₀	LT ₅₀	LT ₉₀
<i>Mertensia fusiformis</i>	110	-0.86 ± 0.17	< 0.0001	-5.81 ± 1.09	< 0.0001	-4.18	-6.72	-9.27
<i>Mertensia ciliata</i>	120	-1.79 ± 0.48	0.0002	-12.32 ± 3.35	0.0002	-5.66	-6.89	-8.12
<i>Hydrophyllum capitatum</i>	62	-1.64 ± 0.48	0.0007	-5.99 ± 2.15	0.0053	-2.32	-3.66	-5.01
<i>Hydrophyllum fendleri</i>	100	-1.22 ± 0.24	< 0.0001	-4.47 ± 0.99	< 0.0001	-1.86	-3.66	-5.47
<i>Delphinium nuttallianum</i>	110	-1.11 ± 0.37	0.0028	-11.09 ± 3.51	0.0016	-7.99	-9.96	-11.94
<i>Delphinium barbeyi</i>	100	-0.97 ± 0.19	< 0.0001	-5.43 ± 1.09	< 0.0001	-3.32	-5.58	-7.84
<i>Erigeron flagellaris</i>	100	<i>na</i>	0.9965	<i>na</i>	0.9963	< -10.00	< -10.00	< -10.00
<i>Erigeron speciosus</i>	100	-1.23 ± 0.27	< 0.0001	-6.09 ± 1.38	< 0.0001	-3.17	-4.95	-6.74

APPENDIX D: WITHIN-SEASON TEMPORAL DYNAMICS OF PLANT-POLLINATOR
INTERACTIONS: PATTERNS, CONSISTENCY, AND ECOLOGY CONSTRAINTS.

CaraDonna, P. J., W. K. Petry, R. M. Brennan, J. L. Cunningham, J. L. Bronstein, N.M.
Waser, and N. J. Sanders. Within-season temporal dynamics of plant-pollinator
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Within-season temporal dynamics of plant-pollinator interactions: patterns, consistency, and ecological constraints

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ABSTRACT

Interactions between species are ubiquitous in nature and play a fundamental role in ecological and evolutionary processes that generate and maintain biodiversity. Despite the fact that ecological systems are inherently dynamic through time, our current understanding of this aspect of species interactions is lacking. In this study, we investigate the within-season temporal dynamics of plant-pollinator interactions over three years in a subalpine ecosystem in the Colorado Rocky Mountains. We find strong evidence that species interactions exhibit predictable within-season turnover that is consistent across years. These temporal patterns are in large part driven by interaction rewiring and appear to be a product of changes in the relative abundances of species from one week to the next. These results have direct implications for understanding how species interactions will respond to continued disturbances related to global change.

INTRODUCTION

Interactions between species are ubiquitous in nature and play a fundamental role in ecological and evolutionary processes that generate and maintain biodiversity (Bascompte *et al.* 2006; Bascompte 2009; Thompson 2009). Interspecific interactions influence population dynamics, community stability, species ranges, and ecosystem functions (Brooker 2006; Ives & Carpenter 2007; Suttle *et al.* 2007; Brosi & Briggs 2013; Burkle *et al.* 2013; Afkhami *et al.* 2014; Ehrlén *et al.* 2016). Interactions link species together within communities, forming complex ecological networks, and a major goal of community ecology is to understand how and why species interact.

Most empirical work on species interaction networks has come from a static perspective, either describing networks as a snapshot in time or as a single network pooled across time (Polis 1991; *reviewed in* Bascompte & Stouffer 2009). Yet, it is broadly recognized that ecological systems are inherently dynamic through time. Individuals, populations, and communities all follow a progression of events from the past, to the present, and into the future (Wolkovich *et al.* 2014). Despite the fact that time is a fundamental axis of all ecological systems, few studies have explicitly investigated the basic seasonal progression of species interactions—especially at fine time scales such as from day-to-day or week-to-week within a season (Olesen *et al.* 2008; Bascompte & Stouffer 2009; Simanonok & Burkle 2014). Therefore, our current understanding of this basic aspect of ecological interactions is lacking, which impedes our ability to make reliable predictions about how and when species will interact, how interactions will respond to natural variation, and their flexibility and resilience to natural and

anthropogenic perturbations (Kaiser-Bunbury *et al.* 2010; Burkle *et al.* 2013; Poisot *et al.* 2015).

Interaction turnover, or interaction beta-diversity, provides a framework for examining the temporal dynamics of species interactions (Poisot *et al.* 2012). Unlike the turnover of species, the turnover of interactions is ultimately driven by two different components (Poisot *et al.* 2012). The first is species turnover: interactions are lost or gained because of the loss or gain of species (Fig. 1). The second is interaction rewiring: interactions are lost or gained because of changes in who is interacting with whom (i.e., the same species interact in different combinations across time or space) (Fig. 1). To date, however, we have a poor understanding of the relative contributions of these two components in explaining how species interactions change through time and space (Burkle *et al.* 2015; Poisot *et al.* 2015). Co-occurrence is an obvious pre-requisite for the formation of interactions but is insufficient for understanding who interacts with whom (Poisot *et al.*, 2015); indeed, interaction rewiring has been documented in several ecological communities (Kaiser-Bunbury *et al.* 2010; Olesen *et al.* 2011a; Poisot *et al.* 2012; Carstensen *et al.* 2014; Simanonok & Burkle 2014). Teasing apart these two contributions to interaction turnover will provide insight into how and why species interactions change through time.

If few ecological constraints exist, patterns of interaction turnover through time may ultimately be related to a neutral-based process whereby species simply interact proportionally to their abundance (Vazquez *et al.* 2009a; Poisot *et al.* 2015). In contrast, species traits such as phenology and morphological size matching may act as ecological constraints on who interacts with whom (i.e., a trait-based process). Phenology is an

obvious trait that constrains which interactions can occur, because species must overlap in time and space to interact (Vazquez *et al.* 2009a, b; Olesen *et al.* 2011a). Once temporal co-occurrence is accounted for, additional factors such as abundance and morphological size matching may further determine which interactions are realized within temporally cumulative networks (Vazquez *et al.* 2009b; Poisot *et al.* 2015). Therefore, neutral and trait-based processes may act independently or together to explain the temporal dynamics of species interactions, but to our knowledge this has never been empirically addressed.

In this study, we investigated the within-season temporal dynamics of plant-pollinator interactions over three years in a subalpine ecosystem in the Colorado Rocky Mountains. Specifically, we asked: (1) how does interaction turnover vary from week-to-week within a season, (2) what are the relative contributions of species turnover and rewiring, and (3) are seasonal patterns of interaction turnover consistent from one year to the next? Additionally, (4) we used probability-based simulation models to ask whether interaction turnover and rewiring depend on phenology, abundance, or morphological size matching. Our study provides strong evidence that plant-pollinator interactions exhibit predictable within-season turnover that is consistent across years. These temporal dynamics were in large part driven by interaction rewiring and appear to be a product of both trait-based (phenology) and neutral-based (abundance) processes.

METHODS

Study system. This study was conducted at The Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (38°57.5'N, 106°59.3'W, 2900 m a.s.l.). RMBL is

surrounded by a mosaic of wet and dry meadows, and aspen and conifer forest. The area is snow-dominated for much of the year, resulting in a relatively short summer growing season of 3–5 months (CaraDonna *et al.* 2014).

Plant-pollinator observations. We observed plant-pollinator interactions at weekly intervals across most of the growing season in 2013, 2014, and 2015. All observations took place in two adjacent dry meadows that measured approximately 2800 m² and 3015 m², respectively, and were separated by ca. 100m of forest. Observations began about one week after snowmelt in each year (Table S1). Within each week, we conducted 32 15-minute plant-pollinator interaction observations (8 hours per week). Each complete weekly interaction census (i.e., all 32 observation periods) occurred over the course of 2–3 consecutive days and, weather depending, was separated from the start of the next weekly census by 3–5 days. We assigned each observation period to one of four quadrants within each meadow in a random fashion. Within each meadow quadrat, a permanent 25 × 1m transect was set up to monitor floral abundance (see below) and served as a reference point for interaction observations. During each observation period, we walked around the meadow quadrant and recorded the frequency of all observed plant-pollinator interactions. We define an interaction as any pollinator species clearly contacting the reproductive structures of flowers. (Nectar robbing was not observed during the three-year study period). All interaction observations took place between 0900–1700 during favorable weather conditions for pollinator activity (ambient temperature > 8°C, not snowing or raining). Our observations spanned 11 weeks in 2013, 15 weeks in 2014, and 16 weeks in 2015 (sampling did not occur during the second week

of the 2015 growing season due to poor weather conditions). From each complete set of weekly observations (i.e., all 32 observation periods) we constructed a single plant-pollinator interaction matrix. Interaction rarefaction curves and abundance-based richness estimators showed that this level of sampling was consistent across weekly observation periods and sufficient to document the majority of pairwise plant-pollinator interactions occurring in each week (Fig. S1; Table S2).

All flowering plants were identified to species. All pollinators were identified to species or to the lowest taxonomic level possible. During observations, pollinators were identified to either species or morphospecies on the wing. A reference specimen was collected for insect morphospecies and later identified using the RMBL Entomology Reference Collection (RMBL_ENT). Otherwise, pollinators were not collected during observations to prevent any effects of destructive sampling on patterns of interaction turnover. A list of all plant and pollinator taxa present in the study is included in Table S3.

Plant and pollinator abundance. Within each meadow quadrant, a permanent $25 \times 1\text{m}$ transect was established to monitor floral abundance and flowering phenology. Once per week, all open flowers or flowering heads (for species with small clustered flowers) were counted for all species present within each transect (*following* CaraDonna *et al.* 2014). The number of observation sampling periods in which a pollinator species was observed interacting with any plant species was used as a proxy for pollinator abundance (e.g., if a species was present in 10 of 32 observation periods in a given week, its abundance value was 10). We opted to use this as our estimate of pollinator abundance instead of

interaction frequency, because it is less dependent upon floral abundance (Vazquez *et al.* 2009b).

Plant and pollinator morphology. Plant and pollinator morphology were measured in 2015 for all species present during the three-year study period (Table S3). For plants, functional nectar depth (*following* Stang *et al.* 2009) was measured on 10–20 individual flowers in the field. For insect pollinators, tongue length was measured on 2–15 individuals using the RMBL Entomology Reference Collection (sample sizes for pollinators varied depending on specimen availability). For the one hummingbird pollinator (*Selasphorus platycercus*) in our dataset, bill length was measured in 1978 on specimen from the RMBL, University of Arizona, and University of Utah vertebrate collections (N.M. Waser *unpublished data*). Because hummingbirds can extend their tongues beyond their bill to reach nectar (Paton & Collins 1989), we estimated tongue length by adding one-third to the bill length (*following* Vizentin-Bugoni *et al.* 2014).

Quantification of interaction turnover. Our approach for quantifying interaction turnover (i.e., interaction dissimilarity or interaction beta diversity) builds upon classic community dissimilarity metrics, but explicitly focuses on the compositional differences among interactions instead of on species (Poisot *et al.* 2012). Specifically, we used Whittaker’s (1960) dissimilarity index to quantify absolute turnover of interactions from week to week across the growing season:

$$\beta_w = \frac{a + b + c}{(2a + b + c)/2} - 1$$

where a is the number of pairwise interactions shared between two successive weekly networks, and b and c are the number of pairwise interactions unique to each of these networks, respectively (therefore, this index ultimately uses binary plant-pollinator interaction information). Values for this index range from 0 to 1; higher values indicate higher interaction turnover. High interaction turnover means that there is a lot of change from one week to the next in who is interacting with whom. We focus on the absolute turnover of interactions (as opposed to frequency based dissimilarity), because we are interested in changes in the presence and absence of interactions from week-to-week across the season (still, however, interaction turnover using a frequency based metric of dissimilarity yields qualitatively similar results in our plant-pollinator community).

A unique aspect of interaction turnover (β_{INT}) is that it can be partitioned into two additive components: species turnover (β_{ST}) and interaction rewiring (β_{OS}) (Fig. 1; Poisot *et al.* 2012). In other words, $\beta_{\text{INT}} = \beta_{\text{ST}} + \beta_{\text{OS}}$. By partitioning plant-pollinator interaction turnover into these two additive components we can directly assess why interactions are changing from week-to-week across the season: simply because the composition of species is changing (i.e., the effect of species turnover, β_{ST}), because of interaction rewiring (β_{OS}), or a combination of both.

Simulation models. We constructed a series of probability based simulation models to explore potential ecological drivers of within-season interaction turnover. First, we built a series of plant-pollinator interaction probability matrices in which the probability of pairwise interactions was determined by different ecological constraints (*following* Vazquez *et al.* 2009b). These ecological constraints included plant-pollinator phenology

(weekly temporal co-occurrence), abundance, morphology, and abundance $\times$ morphology (described in detail below). Because our plant-pollinator observations occurred in the same general location, we do not explore spatial constraints. Second, we used these probability matrices to simulate interactions for each week in each year. We simulated 1000 binary interaction matrices based on the underlying probability matrix for each ecological constraint. For example, if the probability of interaction between plant i and pollinator j is 0.5, this means that these two species will interact in approximately 500 of the 1000 simulated matrices. For all simulations we held constant the total number of observed links between species (i.e., network connectance is always the same), as well as the presence (and absence) of plants and pollinators in each week. Therefore, our simulations do not deviate from actual species composition, but allowed for changes in interactions among temporally co-occurring species. Last, we calculated expected interaction turnover using these simulated interaction matrices and compared these expected values to the observed values from our actual interaction matrices.

We created four different simulation models based on the following ecological constraints:

- (i) *Phenology* – the probability of interaction was equal among all temporally co-occurring plants and pollinators (i.e., completely randomized interactions, or a null model).
- (ii) *Abundance* – the probability of interaction was constrained according to the relative abundance of temporally co-occurring species. Specifically, the probability of interaction between plant i and pollinator j was the product of their weekly relative abundances.

(iii) *Morphological size-matching* – the probability of interaction was constrained by whether or not a pollinator could gain access to a nectar reward for temporally co-occurring species. Specifically, the probability of interaction between plant i and pollinator j is a 1 (i.e., a match) if the length of the pollinator tongue is as long as the functional nectar depth of the flower (± 1 mm) and 0 if the length of the pollinator tongue is less than the nectar depth.

(iv) *Abundance $\times$ morphology* – the probability of interaction was constrained by both abundance and morphological size matching of temporally co-occurring species. The abundance and morphology probability matrices were multiplied together to create this probability matrix. Specifically, the probability of interaction between temporally co-occurring plant i and pollinator j was proportional according to their abundance, given that they also exhibited morphological size-matching.

Data analysis & model comparisons. Week-to-week patterns of turnover within each year were analyzed using linear regression or non-linear regression (when appropriate) for all turnover metrics (β_S , β_{INT} , β_{OS} , and β_{ST}). Because time is directional, we analyzed interaction turnover across successive weeks within each growing season (week-to-week). Therefore, our predictor variable is each successive week-to-week temporal transition and our response variable is the specific turnover metric. We tested for temporal autocorrelation among the residuals of turnover variables within each season to ensure our time series data met the assumption of independence for each regression model; in all cases, temporal autocorrelation was not detectable among the residuals. We

compared the average contribution of species turnover (β_{ST}) and interaction rewiring (β_{OS}) in each year of the study using a two-sided t-test.

We compared the simulated interaction turnover patterns from each model to the observed turnover patterns using a standardized effect size (SES) approach (*following* Sanders *et al.* 2003). The SES provides a measure of the number of standard deviations that the observed interaction turnover value (β_{obs}) is above or below the mean index of the simulated model value (β_{sim}) for each week-to-week transition in each year. Specifically, the standardized effect size is calculated as:

$$SES = \frac{\beta_{obs} - \mu(\beta_{sim})}{\sigma(\beta_{sim})}$$

Assuming a normal distribution of deviations, approximately 95% of the SES values should fall between -2.0 and $+2.0$. Therefore, for each week-to-week transition, values that fall outside of this range indicate that the observed interaction turnover values differ significantly from those generated in the model simulation; values that fall within this range indicate that the observed values are consistent with the model simulation. In other words, points that fall within this range provide support for the hypothesis that the ecological constraint included in the simulation model explains some aspect of interaction turnover.

RESULTS

Over the three annual growing seasons, we observed a total of 566 unique pairwise interactions between 45 flowering plant species and 74 pollinator species, representing 28,959 individual plant-pollinator visitation events (Table S4). Interaction turnover from week-to-week within each growing season was consistently high (Fig. 2).

In all three years of the study, the relative effect of rewiring on total interaction turnover was greater than the relative effect of species turnover (2013: $t = 2.77$, $d.f. = 18$, $P = 0.012$; 2014: $t = 3.91$, $d.f. = 26$, $P < 0.001$; 2015: $t = 5.65$, $d.f. = 26$, $P < 0.001$). On average, rewiring accounted for about 64% of the turnover in interactions within the season, whereas species turnover accounted for about 36% (Table S4). Furthermore, all patterns were qualitatively similar even when rare interactions (i.e., those occurring only once within a week) were removed (Table S5), or when sampling periods were pooled at a broader temporal interval (Table S6), indicating that these results were robust to various sampling artifacts.

Temporal dynamics of interactions within the growing season – The magnitude of week-to-week interaction turnover varied predictably across the growing season, and these temporal patterns were largely consistent across all three years of the study (Fig. 3). Plant and pollinator species turnover (β_s) declined across the growing season (2013: $R^2 = 0.54$, $P = 0.016$; 2014: $R^2 = 0.50$, $P = 0.005$; 2015: $R^2 = 0.69$, $P = 0.002$); in 2015, this general pattern was accompanied by a slight increase in species turnover at the end of the season (i.e., a non-linear decline across the season). Similarly, total interaction turnover (β_{INT}) declined across the season; in 2013, probably because sampling ended earlier in the season, this pattern was weak and non-significant (2013: $R^2 = 0.10$, $P = 0.376$; 2014: $R^2 = 0.65$, $P < 0.001$; 2015: $R^2 = 0.77$, $P < 0.001$). The effect of species turnover (β_{ST}) on total interaction turnover declined across the season (2013: $R^2 = 0.62$, $P = 0.006$; 2014: $R^2 = 0.31$, $P = 0.039$; 2015: $R^2 = 0.89$, $P < 0.001$); in 2015, as was the case for species turnover itself, there was a slight increase in the effect of species turnover on interaction turnover

at the end of the season. In contrast, the effect of rewiring (β_{OS}) on total interaction turnover exhibited a consistent, non-linear pattern across the growing season with a peak during mid-season in all three years (2013: $R^2 = 0.52$, $P = 0.07$; 2014: $R^2 = 0.50$, $P = 0.02$; 2015: $R^2 = 0.62$, $P = 0.005$). A full list of all regression model coefficients is provided in Table S7.

Simulation models – Phenology simulation models (i.e., fully randomized interactions for temporally co-occurring species) produced interaction turnover values that were a poor match to observed values. In general, the phenology simulations over-predicted total interaction turnover and rewiring (Figs. 4, Fig. S2). This indicates that simple temporal co-occurrence is insufficient to predict interaction turnover and rewiring. Morphological size matching simulation models also yielded a poor match to observed patterns of interaction turnover (Fig. 4, Figs. S2).

In contrast, abundance simulations yielded interaction turnover (β_{INT}) and rewiring (β_{OS}) values that matched the observed patterns reasonably well in all years (Fig. 4, Fig. S2). Abundance $\times$ morphology models also matched the observation patterns, but not substantially better than abundance models alone. This can likely be explained by the overwhelming effect of species abundance and the relatively weak effect of species morphology.

All models provided a moderate fit to the observed patterns of the contribution of species turnover (β_{ST}) to overall interaction turnover (Fig. 4, Fig. S2); however, this result is inherent in the way we specified the simulations, because species composition in all models was set to be identical to observed species composition.

Year-to-year turnover – Species compositions of the meadow flora and pollinator fauna were similar across years. On average, 76% of plant and pollinator species were present from year-to-year (Table S8). The identity of plant-pollinator interactions varied more strongly across years: on average only about 42% of interactions were observed from year-to-year. As a result, only about 20% of interaction turnover was due to differences in species composition; the remaining 80% reflected year-to-year rewiring of interactions.

DISCUSSION

A primary means by which ecological systems are dynamic is through time (Wolkovich *et al.* 2014). Yet, our basic understanding of the temporal ecology of species interactions is lacking, impeding our ability to make predictions about how and why species interact, as well as how interactions respond to natural variation and anthropogenic global changes (Bascompte & Stouffer 2009; Poisot *et al.* 2015). Substantial amounts of variation in plant-pollinator interactions across months and years (Medan *et al.* 2006; Alarcon & Waser 2008; Petanidou *et al.* 2008; Olesen *et al.* 2011a, b; Cuartas-Hernández & Medel 2015) has led to the implicit notion that these interactions may be even more variable and difficult to predict at fine time scales within a season. Here we show that, indeed, plant-pollinator interactions exhibit considerable temporal variation from one week to the next. However, this variation follows a predictable pattern of turnover from week-to-week across the growing season that is consistent across years (Fig 3), despite interannual variation in interactions and abiotic conditions (Tables S1 & S7). Not only was interaction turnover predictable from week-to-week, but so too were

the two components of interaction turnover (rewiring and the effect of species turnover). Both trait-based (phenology) and neutral-based (abundance) processes appeared to give rise to the temporal dynamics of interaction turnover and rewiring in this subalpine plant-pollinator community (Fig. 4). Below we discuss how a fine-scale temporal perspective on plant-pollinator interactions improves our understanding of the drivers of interaction variation, patterns of interaction flexibility, and the resilience of ecological networks to loss of species under global change.

Our results demonstrate that interaction rewiring is an important driver of fine-scale temporal turnover of plant-pollinator interactions in this subalpine community. Changes in species composition from one week to the next were insufficient to explain all of the changes in interactions; instead, the extent to which interactions changed over the season were in large part due to rewiring, especially during the middle of the season (Figs 2 & 3). Rewiring accounted for nearly two-thirds of the observed changes plant-pollinator interactions from week-to-week across all three years of the study. There is currently no clear expectation regarding the degree to which changes in species turnover versus rewiring should contribute to interaction turnover. Changes in the presence and absence of species will always influence interaction turnover to some degree: when species turnover is high, the contribution of species turnover to interaction variation will also be high. If species turnover contributes to most of the changes in interaction turnover within a season (Simanonok & Burkle 2014), this suggests that the same mechanisms underlie turnover of both species and their interactions. Alternatively, if rewiring accounts for an equivalent or greater amount of interaction turnover, as we find here, then

other ecological processes unique to the formation of interactions are likely to drive their turnover (Poisot *et al.* 2012; Carstensen *et al.* 2014; Poisot *et al.* 2015).

Indeed, results from our simulation models suggest that both trait-based (phenology) and neutral-based (abundance) processes underlie within-season interaction turnover and rewiring in this system (Fig. 4, Fig. S2). That is, if plant i and pollinator j temporally co-occur and are in high abundance, they are more likely to interact in week t than two species that occur at low abundance. Declines in the abundance of pollinator j and/or the floral abundance of plant i in week $t + 1$ are likely to bring about a change in their interactions, even without their complete absence from the community. This implies that beyond phenology and abundance, additional traits are unnecessary for predicting who interacts with whom in this plant-pollinator community. However, other traits are likely important for predicting the frequency of particular interactions that do occur, such as morphology, nectar concentration, flower color, and floral scent. The influence of flower color and scent on interactions at the community-level remains poorly explored and represents an area of promising future research.

The importance of neutral- versus trait-based processes underlying interaction turnover should depend on the degree of specialization and the amount of variation in traits within a given community. Our subalpine plant-pollinator community is relatively generalized, and most species interact with at least a few other species across the season (Tables S3 & S4). Among all possible plant-pollinator pairs studied in this community, 83% meet the criteria of a morphological size match based on functional nectar depth and pollinator tongue length. Therefore, temporally co-occurring plants and pollinators have few ecological barriers in the way of their interactions, and as a result, once species have

overlapping phenologies, neutral processes predominate (i.e., who interacts with whom is proportional to species abundance). In contrast, in plant-pollinator communities with a higher degree of specialization, such as Brazilian plant-hummingbird networks, there are strong morphological constraints (Maruyama *et al.* 2014; Vizentin-Bugoni *et al.* 2014). Here, once species have overlapping phenologies, trait-based processes like morphological size matching may predominate in accounting for interaction turnover through time. However, neutral processes may come into play within the constraints of phenology and morphological size matching, whereby species interact based on the abundances of with whom they match. Further study is needed to elucidate how trait-based and neutral-based processes may act jointly or independently to explain how and why interactions change in time, but our study suggests that phenology and abundance may predominate in highly generalized interaction networks.

The within-season temporal dynamics of plant-pollinator interactions we report here have direct implications for our general understanding of interaction flexibility, community resilience (and stability), and plant-pollinator responses to global change. If strong temporal dynamics are a regular component of the seasonal progression of interactions, then this suggests that these ecological systems are inherently flexible to within-season perturbations of gains and losses of species, as well as changes in species relative abundance. Many plant-pollinator systems are highly generalized (Waser *et al.* 1996; Petanidou *et al.* 2008; Ollerton *et al.* 2009; Burkle *et al.* 2013) and our results provide one mechanism by which interactions can be generalized—via fine-scale temporal changes in who interacts with whom. Furthermore, if rewiring is a key component to changes in interactions within a season, as we show here, then models of community

resilience likely under-predict community stability (Kaiser-Bunbury *et al.* 2010). This is because most models of community resilience assume that interactions between species are inflexible and do not change during perturbations, which does not capture the regular process of interaction turnover and rewiring that may be common to many ecological systems.

The prevalence of rewiring and neutral-based processes is also important for understanding the potential consequences for phenological shifts of plants and pollinators under climate change (e.g., Miller-Rushing *et al.* 2010; Rafferty & Ives 2011; McKinney *et al.* 2012; Kudo & Ida 2013). According to the phenological mismatch hypothesis, species-specific changes in the phenology of plants and pollinators may ultimately result in temporal decoupling of preferred food resources for pollinators and pollination services for plants, with potentially severe negative consequences (Memmott *et al.* 2007; Hegland *et al.* 2009; Burkle *et al.* 2013). However, if plants and pollinators regularly switch with whom they interact based on a constantly fluctuating resource landscape, they may be able to cope with climate driven changes in temporal overlap and species abundance. Indeed, Burkle *et al.* (2013) found that many novel interactions were formed between plants and pollinators as a result of climate-induced phenological shifts, and generalist plants and pollinators appeared to be buffered from extinction. However, it is critical to note that specialized pollinators—which may not have the same degree of interaction flexibility—are more likely to experience negative consequences of a change in temporal synchrony with flowering plants (Burkle *et al.* 2013). Although complete temporal decoupling of plants and pollinators has yet to be clearly demonstrated, this

would obviously have negative implications for plants and pollinators regardless of specialization level.

Conclusions

There has been a general call for more studies that explicitly investigate fine-scale temporal dynamics of species interactions (Bascompte & Stouffer 2009; Burkle & Alarcon 2011; Poisot *et al.* 2015). This study of plant-pollinator interactions from a subalpine ecosystem provides strong evidence that species interactions exhibit predictable within-season temporal dynamics. This is of particular importance as we consider how communities will respond to continued disturbances related to global change (Tylianakis *et al.* 2008; Bascompte & Stouffer 2009). In order to make accurate predictions about how and why species interact, we must keep in mind that interactions are in constant flux.

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Figure 1. Conceptual diagram illustrating the two components of interaction turnover.

The gain or loss of interactions (interaction turnover, β_{INT}) can be the result of the gain or loss of species (i.e., the effect of species turnover, β_{ST}). Or, the gain or loss of interactions can be the result of changes in who is interacting with whom (i.e., interaction rewiring, the same species interact in different combinations across time or space). Both of these processes can occur across time or space, but are shown here separately for clarity.



Figure 2. Comparison of the two components of plant-pollinator interaction turnover—species turnover (β_{ST}) and rewiring (β_{OS})—across all three years of the study period. Asterisks indicate statistical significance ($P < 0.05$) between β_{ST} and β_{OS} via a two-tailed t-test.

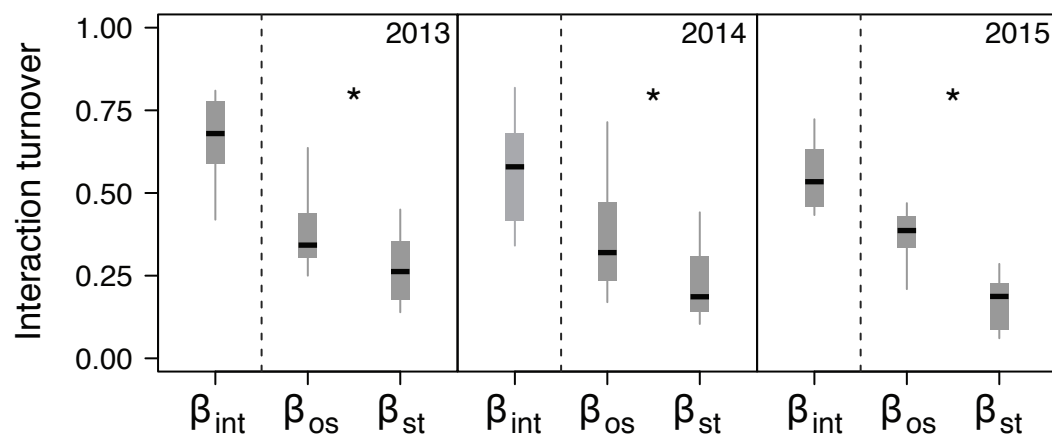


Figure 3. Within-season temporal dynamics of plant-pollinator species turnover (β_W), interaction turnover (β_{INT}), the effect of species turnover on interaction turnover (β_{ST}), and the effect of rewiring on interaction turnover (β_{OS}) across all three years of the study period. Each panel illustrates the relationship between each successive week-to-week temporal transition and each metric of species or interaction turnover. Each grey point indicates a single week-to-week turnover value. Solid black lines indicate significant relationships ($P < 0.05$), and dashed black lines indicate marginally significant relationships ($P < 0.09$), using linear or non-linear regression.

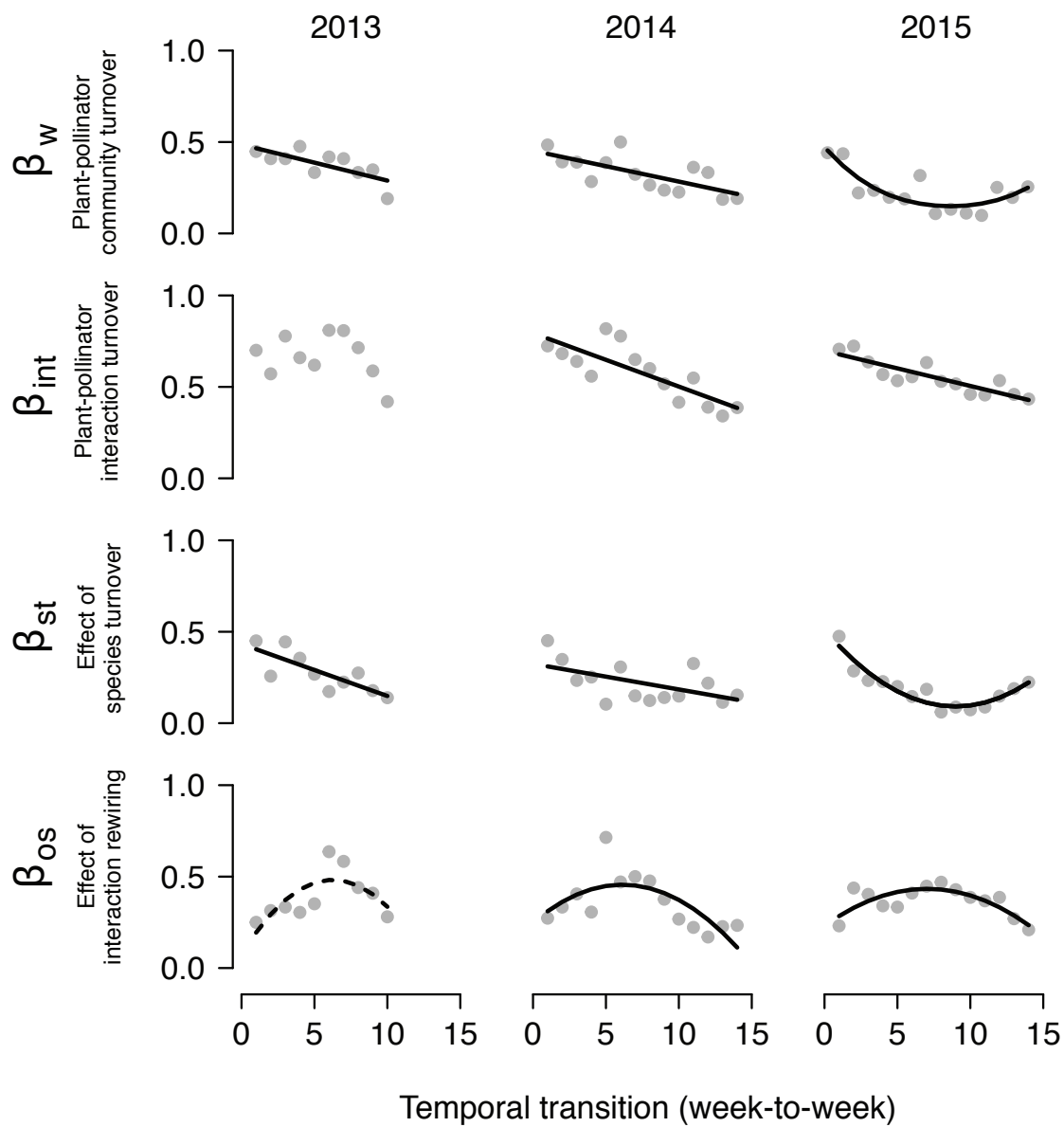
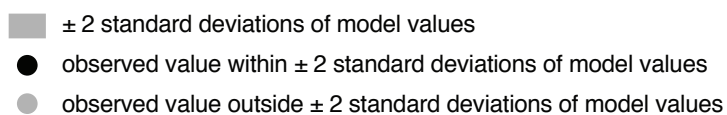


Figure 4. Standardized effect sizes (SES) showing how well the observed interaction turnover values match the expected values under each probability-based simulation model for the 2015 growing season (results for 2013 and 2014 are qualitatively similar and are shown in Fig. S2). The grey shaded region represents ± 1.96 standard deviations, the approximate level of statistical significance ($P < 0.05$). Points that fall within the grey region indicate that the simulation model values and the observed values are statistically indistinguishable.



Supplementary Materials

Tables S1–S8

Figures S1–S2

Table S1. The date of snowmelt and the start date of plant-pollinator interaction observations in each year of the study at the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA.

Year	Snowmelt date	Observation start date
2013	15 May	24 May
2014	26 May	03 June
2015	07 May	12 May

Table S2. Observed and estimated interaction richness for weekly plant-pollinator observations. We assessed our weekly plant-pollinator interaction sampling consistency and completeness by comparing the observed interaction richness with estimated interaction richness according to two non-parametric abundance based richness estimators, Chao 1 and ACE (abundance-based coverage estimator) (Lee & Chao 1994; Chao 2005). We used the Chao 1 and ACE richness estimators over other presence/absence richness estimators because our interaction data are inherently abundance based (Colwell & Coddington 1994; Gotelli & Colwell 2001; Chao 2005).

Year	Week	Observed	Chao 1	Proportion sampled	ACE	Proportion sampled
2013	1	10	12	0.833	11	0.952
	2	30	30	0.993	31	0.979
	3	26	28	0.920	28	0.921
	4	19	23	0.826	20	0.943
	5	28	30	0.940	30	0.947
	6	35	38	0.929	38	0.923
	7	28	31	0.897	31	0.900
	8	24	25	0.947	25	0.957
	9	25	25	0.984	26	0.980
	10	38	39	0.987	39	0.980
	11	24	27	0.885	25	0.950
2014	1	15	19	0.781	17	0.879
	2	13	26	0.510	16	0.839
	3	31	35	0.883	33	0.926
	4	30	55	0.550	32	0.936
	5	47	63	0.745	53	0.894
	6	30	31	0.962	32	0.950
	7	51	58	0.887	55	0.921
	8	46	58	0.793	50	0.915
	9	64	74	0.861	71	0.899
	10	56	72	0.775	61	0.923
	11	33	35	0.936	35	0.951
	12	29	30	0.954	30	0.954
	13	43	53	0.811	46	0.929
	14	45	53	0.847	47	0.950
	15	30	36	0.824	32	0.935
2015	1	10	10	1.000	10	1.000
	2	28	39	0.725	31	0.906
	3	24	29	0.842	25	0.968
	4	41	45	0.911	44	0.936
	5	69	71	0.976	72	0.965
	6	71	82	0.867	81	0.872
	7	84	96	0.875	90	0.929
	8	78	90	0.869	85	0.917
	9	80	89	0.903	88	0.913
	10	86	104	0.827	95	0.907
	11	96	105	0.914	101	0.952
	12	89	103	0.868	95	0.936
	13	69	73	0.943	72	0.962
	14	47	55	0.861	51	0.923
	15	38	46	0.826	39	0.977
	16	22	35	0.638	24	0.936

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Table S3. Plant and pollinator species list for all species present in the three year study at the Rocky Mountain Biological Laboratory, Gothic, CO, USA. Interaction degree indicates the number of interaction links a particular plant or pollinator has with other pollinators or plants.

Plants				Pollinators			
species	Family	Interaction degree	functional nectar depth (mm)	species	Family	Interaction degree	tongue length (mm)
achillea millefolium	Asteraceae	9	0.80	andrena costillensis	Andrenidae	3	2.09
agocerus aurantiaca	Asteraceae	2	0.90	andrena cyanophila	Andrenidae	8	2.41
agocerus glauca	Asteraceae	1	0.90	andrena sp 02	Andrenidae	5	2.40
androsace septentrionalis	Primulaceae	18	1.84	andrena transnigra	Andrenidae	5	2.92
arenaria congesta	Caryophyllaceae	35	1.38	andrena vicinoides	Andrenidae	7	2.24
boechera stricta	Brassicaceae	6	3.41	anthidium emarginatum	Megachilidae	1	5.09
campanula rotundifolia	Campanulaceae	14	1.77	anthophora terminalis	Apidae	5	6.75
castilleja sulphurea	Orobanchaceae	1	9.52	arctophila flagrans	Syrphidae	11	2.52
claytonia lanceolata	Montiaceae	19	1.49	bombus appositus	Apidae	12	9.50
delphinium nuttallianum	Ranunculaceae	19	9.17	bombus bifarius	Apidae	24	8.16
draba aurea	Brassicaceae	13	2.34	bombus californicus	Apidae	11	10.17
erigeron flagellaris	Asteraceae	46	1.07	bombus flavifrons	Apidae	18	8.42
erigeron speciosus	Asteraceae	41	2.76	bombus mixtus	Apidae	3	7.17
erigonum subalpinum	Polygonaceae	16	0.63	bombus nevadensis	Apidae	5	11.19
erigonum umbellatum	Polygonaceae	21	0.90	Bombus occidentalis	Apidae	4	5.7
erythronium grandiflorum	Liliaceae	4	3.42	bombus sylvicola	Apidae	10	8.44
fragaria virginiana	Rosaceae	2	1.25	callophrys affinis	Lycaenidae	1	5.69
galium boreale	Rubiaceae	3	0.81	cartosyrphus sp 02	Syrphidae	3	2.06
gentiana parryi	Gentianaceae	4	8.04	cartosyrphus tarda	Syrphidae	14	2.06
helianthella quinquenervis	Asteraceae	17	4.27	chrysotoxum ventricosum	Syrphidae	10	1.85
heliomeris multiflora	Asteraceae	43	2.49	coelioxus funeraria	Megachilidae	4	4.51
heterotheca villosa	Asteraceae	54	3.38	coenonympha ochracea	Nymphalidae	2	6.32
hydrophyllum capitatum	Hydrophyllaceae	8	5.53	colias alexandra	Lycaenidae	3	11.00
hydrophyllum fendleri	Hydrophyllaceae	1	5.53	colletes kincadilii	Colletidae	8	2.25
ipomopsis aggregata	Polemoniaceae	3	20.15	colletes nigrifrons	Colletidae	5	1.74
lathyrus leucanthus	Fabaceae	9	4.35	cryptopogon sp 01	Asilidae	2	1.39
linum lewisii	Linaceae	4	2.76	eristalis latifrons	Syrphidae	9	1.80
lomatum dissectum	Apiaceae	4	1.32	eupeodes lapponicus	Syrphidae	8	1.25
mahonia repens	Berberidaceae	2	2.99	eupeodes volucris	Syrphidae	14	0.89
mertensia ciliata	Boraginaceae	2	6.50	euphydras anicia	Nymphalidae	4	7.92
mertensia fusiformis	Boraginaceae	7	4.36	glaucopteryx lygdamus	Lycaenidae	11	6.40
phacelia heterophylla	Hydrophyllaceae	4	5.00	halictus confusus	Halictidae	11	1.99
potentilla gracilis	Rosaceae	17	0.49	halictus rubicundus	Halictidae	15	2.70
potentilla hippiana	Rosaceae	36	0.79	halictus virgatellus	Halictidae	10	2.09
pseudocymopterus montanus	Apiaceae	9	1.32	hoplitus fulgida	Megachilidae	8	3.76
ranunculus inamoenus	Ranunculaceae	1	0.00	hoplitus robusta	Megachilidae	3	3.17
rosa woodsii	Rosaceae	3	0.00	lasiglossum inconditum	Halictidae	29	1.64
sedum lanceolatum	Crassulaceae	12	1.43	lycaena cupreus	Lycaenidae	5	4.99
senecio integerrimus	Asteraceae	48	3.72	megachile frigida	Megachilidae	5	5.88
senecio serra	Asteraceae	5	4.57	megachile melanophea	Megachilidae	5	6.26
senecio sp. 2	Asteraceae	2	3.72	megachile pugnata	Megachilidae	7	5.50
solidago multiradiata	Asteraceae	22	2.22	megachile relativa	Megachilidae	12	4.75
taraxacum officinale	Asteraceae	30	0.81	melanostoma kelloggi	Syrphidae	1	1.38
vicia americana	Fabaceae	6	6.58	melanostoma caerulea	Syrphidae	2	1.88
viola praemorsa	Violaceae	7	3.33	melissodes confusa	Apidae	1	5.83
				mesembrina latreillei	Muscidae	4	2.61
				muscid spp 01	Muscidae	17	1.76
				ochlodes sylvanoides	Hesperiidae	10	7.72
				osmia bucephala	Megachilidae	19	6.65
				osmia coloradensis	Megachilidae	11	4.37
				osmia grindelliae	Megachilidae	5	4.32
				osmia iridis	Megachilidae	6	4.81
				osmia lignaria	Megachilidae	1	5.26
				osmia montana	Megachilidae	11	5.44
				osmia proxima	Megachilidae	2	4.18
				osmia sp. 6	Megachilidae	3	3.70
				osmia subaustalis	Megachilidae	6	5.46
				osmia tristela	Megachilidae	2	3.64
				panurginus inepus	Andrenidae	12	1.46
				papilio gothica	Papilionidae	4	14.81
				pieris rapa	Pieridae	8	7.19
				polygonia zephyrus	Nymphalidae	1	10.38
				psithyrus insularis	Apidae	6	9.35
				rhaphomyia sp 01	Empididae	3	1.25
				scathophagidae sp 01	Scathophagidae	5	1.48
				selasphorus platycercus	Trochilidae	4	31.91
				speyeria mormonia	Nymphalidae	7	11.27
				sphaerophoria robusta	Syrphidae	17	1.32
				sphecodes sp 01	Halictidae	6	1.66
				syrphidae 02	Syrphidae	2	1.32
				systoechus sp	Bombyliidae	16	5.40
				tachinidae sp 01	Tachinidae	7	2.19
				tephritidae 01	Tephritidae	1	0.56
				thricops septentrionalis	Muscidae	23	1.11
				villa eumenes	Bombyliidae	7	0.94

Table S4. General summary of plant-pollinator interactions at the Rocky Mountain Biological Laboratory, Gothic, CO, USA. For “All years”, the number of visitation events, pollinator species, flowering plant species, and pairwise interactions are summed across all three years of the study. Network metrics are calculated for all years combined and for each year separately.

Year	Visitation events	Pollinator spp.	Flowers spp.	Pair-wise interactions	Connectance (season-wide)	Network generalization (H2)	Links per species (mean)
2013	3496	42	35	192	0.131	0.330	3.4
2014	12037	56	36	266	0.132	0.433	4.3
2015	13426	73	38	433	0.124	0.415	4.2
All years	28959	76	45	547	0.140	0.400	4.5

Table S5. Correlation coefficients for the relationship between week-to-week turnover values when rare interactions are included versus when rare interactions are excluded. For these analyses, rare interactions are those that occurred only once within a given week (i.e., a singleton within a week). These same interactions may have been observed more frequently in other weeks. Given that all turnover values are highly correlated, this strongly suggests that rare interactions on their own are not accounting for all of the rewiring or overall interaction turnover patterns. Put another way, these results suggest that the observed patterns robust to sampling artifacts associated with rare interactions. For all correlations, $P < 0.05$.

Year	Turnover metric	Correlation coefficient
2013	β_S	0.781
	β_{INT}	0.850
	β_{OS}	0.949
	β_{ST}	0.863
2014	β_S	0.821
	β_{INT}	0.927
	β_{OS}	0.895
	β_{ST}	0.846
2015	β_S	0.953
	β_{WN}	0.890
	β_{OS}	0.599
	β_{ST}	0.935

Table S6. Comparison of season wide averages for each turnover metric between pooled one week and pooled two week sampling periods. All turnover values are qualitatively similar between pooled one week and pooled two week sampling periods and in all cases are statistically indistinguishable (T-test, $P > 0.20$)

	β_S	β_{INT}	β_{ST}	β_{OS}
2013				
week	0.3662	0.6635	0.2681	0.3954
two week	0.4172	0.7276	0.2795	0.4480
2014				
week	0.3288	0.5817	0.2223	0.3593
two week	0.3535	0.6377	0.2220	0.4157
2015				
week	0.257	0.544	0.183	0.361
two week	0.292	0.613	0.203	0.410

Table S7. Regression model coefficients for all turnover metrics. A linear and a non-linear regression model was run for each turnover metric (i.e., the relationship between turnover values and time). Linear and non-linear models were compared using AIC. Lower AIC values indicate a better fitting model and are highlighted in bold. A Δ AIC of 2 was used as a cutoff for model comparisons; if models were within a Δ AIC of 2, linear model (the more simple model) was selected as the best fit.

Year	turnover metric	model	Linear term	Linear standard error	Quadratic term	Quadratic standard error	R ²	P	AIC
2013	β_{OS}	linear	0.016	0.014	na	na	0.135	0.2962	-9.0
2013	β_{OS}	non-linear	0.131	0.050	-0.010456	0.004	0.5188	0.07727	-12.9
2013	β_S	linear	-0.020	0.006	na	na	0.5392	0.0156	-24.7
2013	β_S	non-linear	-0.024	0.254	-0.004008	0.002	0.6825	0.01894	-26.4
2013	β_{ST}	linear	-0.028	0.008	na	na	0.6229	0.006632	-20.8
2013	β_{ST}	non-linear	-0.036	0.037	0.0007042	0.003	0.6254	0.03217	-18.8
2013	β_{INT}	linear	-0.013	0.014	na	na	0.09883	0.3763	-9.7
2013	β_{INT}	non-linear	0.095	0.050	-0.009752	0.004	0.4712	0.1076	-13.1
2014	β_{OS}	linear	-0.015	0.009	na	na	0.1921	0.1169	-13.6
2014	β_{OS}	non-linear	0.074	0.031	-0.005503	0.002	0.5443	0.01326	-19.6
2014	β_S	linear	-0.017	0.005	na	na	0.5013	0.00461	-29.6
2014	β_S	non-linear	-0.015	0.023	-1.12E-04	0.022	0.5016	0.02246	-27.6
2014	β_{ST}	linear	-0.015	0.006	na	na	0.3745	0.02002	-26.0
2014	β_{ST}	non-linear	-0.058	0.023	0.002689	0.001	0.5328	0.01521	-28.0
2014	β_{INT}	linear	-0.029	0.006	na	na	0.6367	0.000626	-21.9
2014	β_{INT}	non-linear	0.016	0.027	-0.002813	0.002	0.7116	0.001071	-23.1
2015	β_{OS}	linear	-0.004	0.005	na	na	0.03932	0.4968	-26.4
2015	β_{OS}	non-linear	0.056	0.019	-0.003671	0.001	0.4922	0.02406	-37.4
2015	β_S	linear	-0.014	0.006	na	na	0.3039	0.04101	-23.7
2015	β_S	non-linear	-0.078	0.018	0.0043	0.001	0.6852	0.00173	-32.8
2015	β_{ST}	linear	-0.015	0.006	na	na	0.3598	0.02335	-24.0
2015	β_{ST}	non-linear	-0.093	0.011	0.0052009	0.001	0.8855	6.66E-06	-46.1
2015	β_{INT}	linear	-0.019	0.003	na	na	0.7645	4.31E-05	-42.4
2015	β_{INT}	non-linear	-0.036	0.013	0.0011	0.001	0.7967	0.00015	-42.4

Table S8. Year to year turnover of plant-pollinator communities and interactions at the Rocky Mountain Biological Laboratory in Gothic, CO, USA.

Comparison	β_S	β_{INT}	β_{ST}	β_{OS}	β_{ST} / β_{INT}	β_{OS} / β_{INT}
2013 v. 2014	0.220	0.579	0.093	0.486	0.161	0.839
2014 v. 2015	0.291	0.565	0.143	0.422	0.191	0.809
2013 v. 2015	0.221	0.584	0.112	0.472	0.253	0.747
Mean	0.244	0.576	0.116	0.460	0.202	0.798

Figure S1. Plant-pollinator interaction rarefaction curves for each week in each year of the study. Rarefaction analyses were conducted in R using the package *vegan* (see Table S2 for details). Dotted lines indicate the estimated interaction richness according to the ACE estimator (abundance-based coverage estimator; Chao and Lee 1992). Grey shading indicates 95% confidence interval.

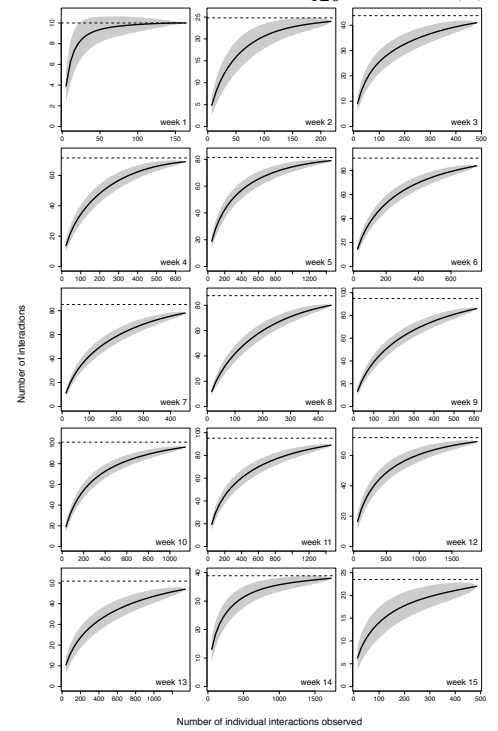
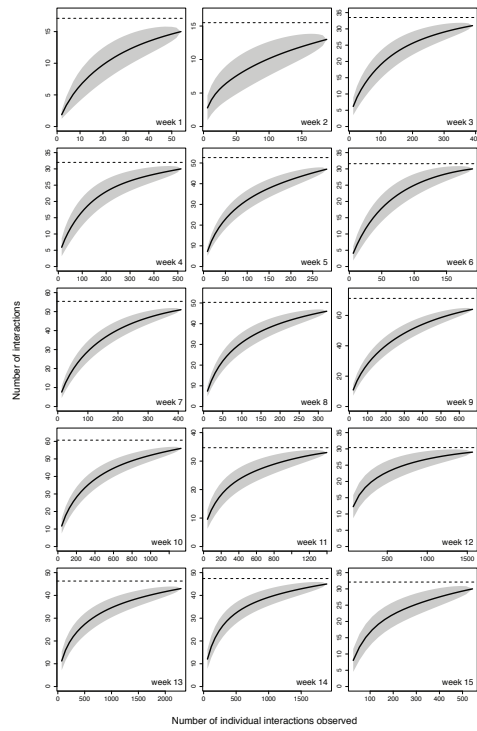
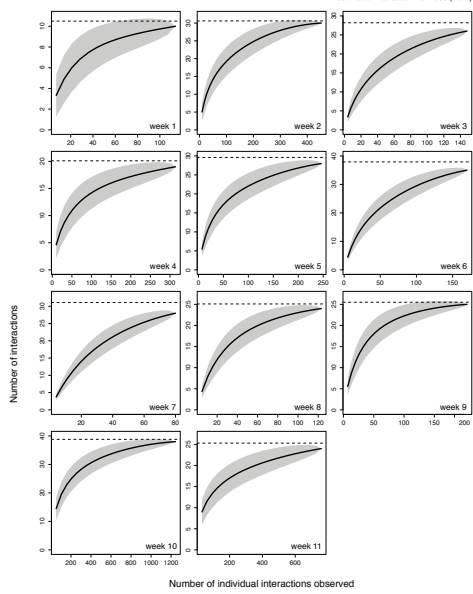
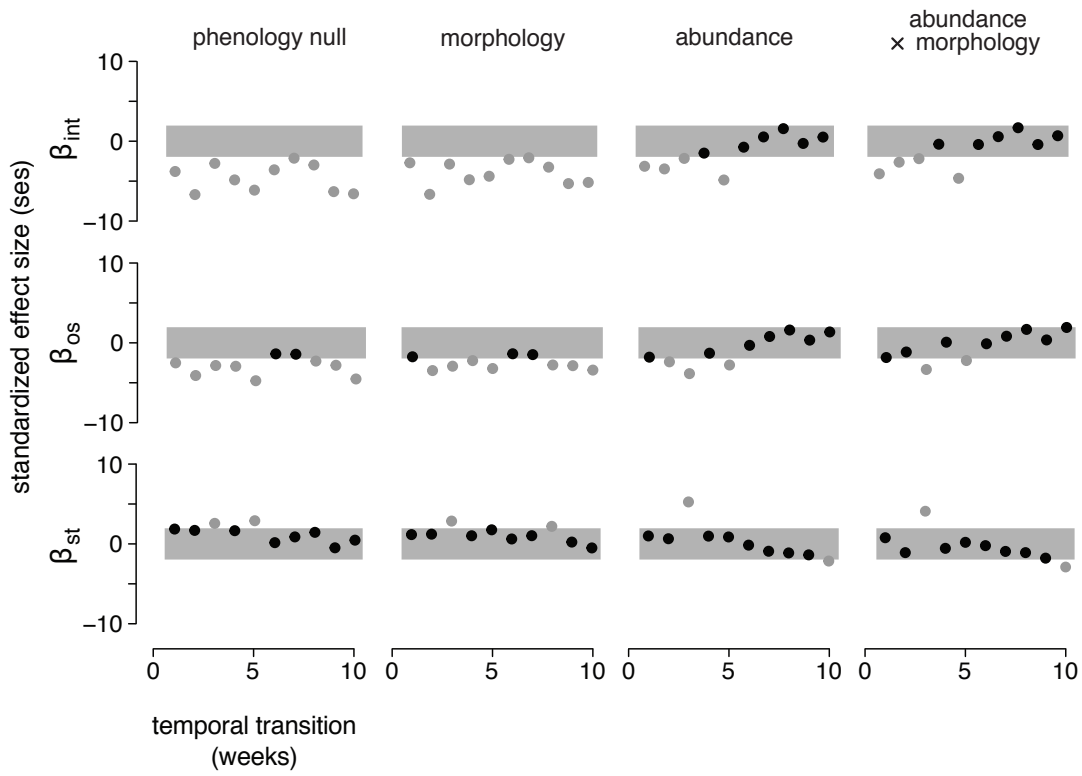
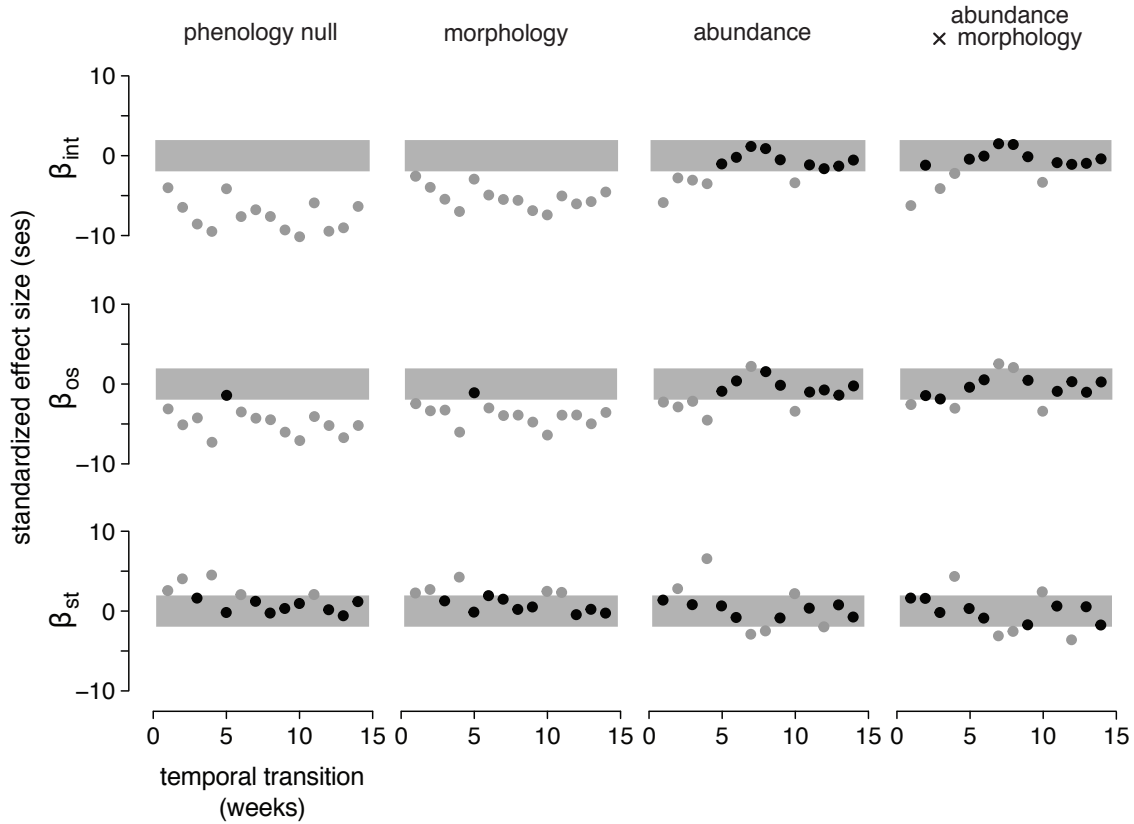


Figure S2. Standardized effect sizes (SES) showing how well the observed interaction turnover values match the expected values under each probability-based simulation model for 2013 (A) and 2014 (B). The grey shaded region represents ± 1.96 standard deviations, the approximate level of statistical significance ($P < 0.05$). Points that fall within this region indicate that the observed values do not differ significantly from the model simulation values.



B. 2014



- ± 2 standard deviations of model values
- observed value within ± 2 standard deviations of model values
- observed value outside ± 2 standard deviations of model values

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