Phenology dynamics in California grasslands: Abiotic and biotic influences on the duration of flowering

By

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Abstract

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The timing of plant growth and reproduction in the grasslands of California follows patterns driven by the cool wet and hot dry seasons of the Mediterranean-type climate. However, within this framework there is a great deal of timing complexity. In my dissertation research I explore phenology dynamics in California grasslands, from local to species range scales. Climate change is causing shifts in phenology (the timing of yearly life cycle events). These timing shifts are disrupting interactions between species, and can lead to timing asynchrony between plants and their pollinators. Evidence indicates that a lengthened duration of flowering could buffer some of the negative impacts that occur with timing shifts. Therefore, longer flowering duration may contribute to the adaptive capacity of systems as the climate changes. In Chapter 1, I explore the potential for an extended duration of resources as a potential conservation technique to respond to phenological asynchrony, setting the stage for the three empirical chapters that follow.

In Chapter 2, I examine microclimate influences on the duration of flowering resources in a northern California grassland. To do this, I recorded the flowering time of all species on paired north and south aspects during four successive spring growing seasons (2015-2018). I evaluated flowering time differences of pollinator resource species between paired aspects at the community level, within species, and between genotypes of *Lasthenia gracilis* (Asteraceae). I found that temperature on the landscape was a strong driver of phenology, and that aspect differences resulted in complementary timing of flowering resources across sites. Differences in timing between flowering on north and south aspects served to extend the flowering season by an average of 4-8 days (8-15%), depending on the year. This extension was due to both within-species timing responses as well as species turnover. These findings indicate that both heterogeneous topography and species diversity can extend overall flowering duration.

In Chapter 3, I examine population differentiation for flowering time in common goldfields (*Lasthenia gracilis*, Asteraceae). To do this, I measured variation in flowering time under common growth conditions in a greenhouse. I found that populations of *L. gracilis* exhibit differentiation in flowering time, with earlier flowering in populations from warmer and drier

locations (approximately 1 day earlier per 1 ˚C difference in mean growth season temperature). The differences in population flowering time in the common environment growth conditions were similar to field flowering records in response to site conditions, and were associated with climate variables in the same direction but with a shallower slope. This pattern of response reveals that both environmental and genetic differences influence flowering time and duration, and that these influences are aligned (i.e. co-gradient variation). Due to the existence of population differentiation in flowering traits, planting diverse genotypes may extend flowering duration.

In Chapter 4, I assess how planting date and competition removal influence the timing of flowering. To do this I planted another goldfields species (*Lasthenia californica*, Asteraceae) three times during the wet season (November, January, and March) into plots with and without competition removal treatments, in a serpentine grassland in Northern California. Planting date and competition removal treatments both significantly impacted flowering time, growth, and reproduction. Later planting dates and competition removal delayed flowering time. Later planting resulted in lower inflorescence production, revealing strong abiotic controls on flowering time and fitness in the spring. These results suggest that heterogeneous planting time as well as competition reduction can extend the duration of flowering.

Taken together, these chapters examine the abiotic and biotic influences on flowering time in California grasslands, and provide a conceptual framework for responding to concerns of phenological asynchrony by extending flowering duration. The benefit of conserving and restoring for an extended flowering duration is that longer flowering seasons and increased native floral resources benefit pollinators whether or not a phenological asynchrony occurs. The empirical chapters offer initial tests of techniques that could be applied in landscape restoration contexts to maintain and extend flowering duration, including utilization of abiotic heterogeneity, genetic and species diversity, and alteration of population timing.

To my family –

Thank you for your love,
the joy you bring into my life,
and for your unending support and encouragement

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Introduction

"*By comparison with many other plant communities, the annual grasslands of California are considered phenologically simple and relatively uniform*."
- N. R. Chiarello 1989

"*These ranges are green in winter and golden brown in summer… appears remarkably uniform to the casual observer but is, in fact, extremely variable, being influenced by differences in soils, grazing use, and occasional fires."*
-H. H. Biswell 1956

The timing of plant growth in the grasslands of California follows patterns driven by climate and landscape positioning. At first glance, this timing is uniform and fits into a predictable rhythm with the cool wet and hot dry seasons of California's Mediterranean-type climate (Chiariello 1988). This includes germination with the winter rains, an initial slow growth period during winter, rapid growth and flowering in the spring, then seed set and senescence in rapid succession as the summer drought sets in (Woodmansee and Duncan 1980). However, within this framework, there is a great deal of complexity in the timing of species that generally goes unnoticed. The timing of temperature and precipitation yields a lush green landscape in the winter, a colorful flowering period from March-May, and a dry brown period in the summer once the soil has been mostly desiccated (Evans and Young 1988). These timing dynamics can play out in slightly different ways across the landscape depending on the temperature, moisture and light availability at each point, and the identity of the species present.

Plants respond to abiotic factors in the environment to initiate reproduction, and flowering can be triggered by temperature, moisture and/or photoperiod cues (Rathcke and Lacey 1985). Biotic factors such as plant density, competition, and facilitation alter abiotic factors in the environment, and can play an important role in flowering time (Ellner 1987, Metcalf et al. 2015). Plant timing responses to cues can also vary, both between and within species. This can result in different patterns of timing, which can affect species success due to priority effects, and timing of resource uptake (Leverett et al. 2018). Earlier species or individuals can benefit from longer growing seasons and early access to resources (Lortie and Turkington 2002, Wainwright et al. 2012), but later timing can allow for avoidance of unfavorable conditions early in the season (e.g. frost)(Petru et al. 2006, Donohue et al. 2010, Mercer et al. 2011).

Climate change is causing shifts in phenology (Menzel et al. 2006, Crimmins et al. 2009). Phenology shifts can vary by species, and depend on the direction, magnitude, and variability of the change in abiotic variables (CaraDonna et al. 2014). Timing shifts can alter species interactions (Menzel et al. 2006, Forrest and Thomson 2011, CaraDonna et al. 2014), leading to novel interactions, a change in existing interactions, or novel gaps in time (Wolkovich and Cleland 2011). Shifting community dynamics have implications for invasion ecology, plant-animal interactions, management, and conservation (Elzinga et al. 2007, Wainwright et al. 2012). Documentation of species and community phenology across scales will improve our understanding of community dynamics. In addition, management and conservation practices can

be improved by phenological study, and a better understanding of the controls regulating plant reproductive timing among species, populations and communities (Morellato et al. 2016).

When a species or ecological phase is not concurrent in time with its interacting partner, it is called phenological asynchrony. The extent of asynchrony depends on amount of time both species are present or active in the absence of the other. Phenological asynchrony is evolutionarily unstable in mutualisms, as species are under strong selection to remain in synchrony (Renner and Zohner 2018). However due to the rapid pace of anthropogenic climate change, there may not be enough time available for selection to offset timing shifts. The likelihood of phenological asynchrony in mutualisms will depend on the level of specialization, seasonality and duration of interactions, and the intimacy of the relationship (i.e., symbiotic or free-living; Rafferty et al. 2015). In cases where phenological asynchrony is occurring or expected to occur, reducing impacts may allow for more time for species to adapt. Documenting patterns in species timing and examining the processes behind them may aid in understanding consequences of climate change. In addition, the success of conservation and restoration interventions (e.g. planting, grazing, mowing, burning) are dependent on phenology patterns.

In this dissertation I examine phenology dynamics in California grasslands, from local to species range scales. I observe and test the influence of abiotic and biotic factors on flowering time and flowering season duration, with the lens of developing potential conservation and management strategies to mitigate impacts of climate induced timing shifts. In Chapter 1, I provide a conceptual framework for responding to concerns of phenological asynchrony by extending the duration of phenological activity (e.g., flowering). Lengthened duration of phenological activity may support both animal and plant mutualists by building adaptive capacity to respond to shifts. Animals require resources throughout their lifecycles to maintain stability and function (Russo et al. 2013). Additionally, plant species that bridge temporal gaps and extend resource timing may allow for facilitation of other plant species by supporting mutualist animal populations (Moeller 2004). Recent evidence suggests that extended phenological timing can aid mutualisms (e.g., Hindle et al. 2015; Frankie et al. 2013; Mola & Williams 2018). Therefore, increasing or maintaining availability of partner resources across time may support the survival of species in mutualistic interactions as the climate changes. Techniques that may extend flowering time on the landscape include diversifying species and genotypes, utilizing microclimate heterogeneity, and alteration of population timing. In empirical chapters 2 – 4, I examine facets of each proposed strategy.

Microclimate can strongly influence plant phenology. In Chapter 2, I conduct observations of phenology across paired aspects, to examine how topographic heterogeneity affects the overall duration of flowering time on the landscape. Variation in incident solar radiation (i.e., insolation) due to slope and aspect create energy-load differences that drive temperature and soil moisture dynamics, leading to abiotic gradients across short distances that would otherwise be found across greater latitude or elevational gradients (Bennie et al. 2006). This can influence phenology within species (e.g., Weiss et al. 1988), and result in species turnover between microclimates. In this chapter I pull apart the separate influences of abiotic differences and species turnover on community flowering time and duration. I also explore plasticity influences across topography, with experimental plantings of *Lasthenia gracilis* (Asteraceae) genotypes.

Timing of reproduction can also vary across a across a species range. In Chapter 3, I examine the drivers of phenological differences across the range of *Lasthenia gracilis* (Asteraceae), an important pollinator resource species that is widespread in California grasslands

and frequently included in restoration seed mixes. Differences in flowering time among populations in the field can be a result of genotypic differentiation, responses to environmental conditions, or some combination of the two (Conover and Schultz 1995). Moreover, the influence of both genetic variation and plastic responses on population flowering time may be important to consider for conservation and management practices. To test for population differentiation in phenology, I collected seed from populations across the range of *L. gracilis* in 2017, and examined variation in germination and flowering time under common growth conditions in a greenhouse. I then compared flowering time in the greenhouse to the timing of flowering in the field (sourced from herbarium specimens) to examine the role of plasticity in shaping flowering time variation observed among populations.

Finally, within-year differences in individual timing as well as plant density can influence flowering duration. In Chapter 4, I explore how planting time and competition removal influence flowering time in the species *Lasthenia californica* (Asteraceae). To do this I planted seeds on three different dates during the growing season (November, January, and March). Competition removal plots were also established and compared with control plots to disentangle competition and seasonal priority effects (due to early individuals pre-empting resources) from abiotic influences on growth. These treatments allowed me to examine the effects of differential seeding time on plant growth and flowering time, and elucidate tradeoffs in growth allocation. I explore how differences in biotic and abiotic conditions after germination (due to different planting dates) influence flowering duration with and without competition removal.

In summary, I explore several key influences on flowering time and duration in California grassland systems. By examining the drivers of flowering time at different scales, these studies expand the breadth of knowledge about flowering phenology and influences on flowering duration in this system. This work also explores the potential for maintaining and extending flowering duration, as a possible technique to build adaptive capacity in plant-animal mutualisms to climate changes. It is my hope that this work adds to our understanding of the influences on flowering duration, and will support restoration, conservation and management strategies to maintain and extend flowering resources on the landscape as needed.

Chapter 1: Mismatch managed? Phenological phase extension as a strategy to manage phenological asynchrony in plant–animal mutualisms

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This paper is reproduced here with kind permission from co-authors and the Graduate Division, and serves as an in-depth introduction to the motivation behind the three empirical chapters to follow.

Abstract

Species-specific shifts in phenology (timing of periodic life cycle events) are occurring with climate change and are already disrupting interactions within and among trophic levels. Phenological phase duration (e.g., beginning to end of flowering) and complementarity (patterns of non-overlap), and their responses to changing conditions, will be important determinants of species' adaptive capacity to these shifts. Evidence indicates that extension of phenological duration of mutualistic partners could buffer negative impacts that occur with phenological shifts. Therefore, we suggest that techniques to extend the length of phenological duration will contribute to management of systems experiencing phenological asynchrony. Techniques of *phenological phase extension* discussed include the role of abiotic heterogeneity, genetic and species diversity, and alteration of population timing. We explore these approaches with the goal of creating a framework to build adaptive capacity and address phenological asynchrony in plant-animal mutualisms under climate change.

Conceptual Implications:

- Predictions of phenological asynchrony due to climate change call for novel conservation strategies.
- We propose extending phenological phase duration as one approach for buffering impacts of asynchrony.
- Techniques to extend the duration of plant or animal activity timing include utilizing abiotic heterogeneity, genetic and species diversity, and alteration of population timing.
- Existing biodiversity conservation techniques may have the potential to address mismatch concerns if put into the context of phenological shifts.
- We call on restoration ecologists to propose and test effectiveness of strategies to address mismatch concerns.

Introduction

Considering *phenological shifts* (italicized terms - see Box 1) in management decisions may be critical for conserving species interactions, mitigating invasions, and maintaining ecosystem functions and services (Elzinga et al. 2007). Species-specific shifts in *phenology* are occurring with climate change and are already disrupting interactions within and among trophic levels (e.g., Schmidt et al. 2016). Species vary in their responses to temperature and moisture changes, and one or more environmental cues may influence whether or not timing will shift with climate changes (Cleland et al. 2007). *Phenological asynchrony* between mutualistic species is expected to decrease fitness and yield population declines (Rafferty et al. 2013; van Asch et al. 2007). With predictions of climate-induced asynchrony, and no clear solutions or management principles available for practitioners, we were motivated to explore strategies that may build the capacity of a system to respond to phenological shifts. We propose extending phenological *phase duration* in mutualistic partners as a mechanism to build *adaptive capacity*, and discuss techniques to achieve this goal.

The likelihood and consequences of phenological asynchrony in mutualisms will depend on the level of specialization, seasonality and duration of interactions, and the intimacy of the relationship (i.e., symbiotic or free-living; Rafferty et al. 2015). Phenological asynchrony in mutualisms has been most studied in transportation mutualisms (e.g., seed dispersal [Warren et al. 2011]), but studies have also predicted the potential for asynchrony due to climate change in nutritional and protection mutualisms (Rafferty et al. 2015). Mutualistic species are under strong selection to remain in synchrony, and phenological asynchrony is therefore evolutionarily unstable (Renner & Zohner 2018). On ecological time scales, rapid anthropogenic climate change may increase asynchrony if species respond to different factors and not enough time is available for selection to offset these shifts. Where asymmetry in phenological shifts is occurring or expected to occur, reducing impacts of timing asynchrony may buy time for species to adapt.

Adaptive capacity is defined as the capability of organisms or systems to adjust to potential stress, take advantage of opportunities, or respond and mitigate negative impacts of environmental change (IPCC 2018). Improving and maintaining adaptive capacity provides an ecological buffer that protects the system from collapse when change occurs (Gunderson 2000). In this paper we explore the potential for adaptive capacity of a community to phenological shifts to be improved by extending the phenological phase timing of mutualistic species to allow for adjustments and changes in species interactions, and community restructuring as necessary.

Lengthened duration of phenological activity may allow for adaptive capacity by supporting both animal and plant mutualists. Animal species require sufficient and abundant resources throughout their lifecycles, and continual plant resources over the active season (e.g., flight, foraging, and nesting seasons) are needed for animal populations to maintain stability and function (Russo et al. 2013). If the timing of an animal species becomes out of sync with a plant mutualist resource, other plant species are essential to supplement its needs during the period of time when resources are unavailable (Waser & Real 1979). Plant species that bridge temporal gaps and extend resource timing may also allow for facilitation among plants by supporting mutualist animal populations (Moeller 2004). Recent evidence suggests that extended phenological timing can aid mutualisms (e.g.,Hindle et al. 2015; Frankie et al. 2013; Mola & Williams 2018). Hence, increasing or maintaining availability of partner resources across time may support the survival of species in mutualistic interactions as the climate changes.

We propose that managing ecosystems in the face of mismatch will require

implementing strategies to maintain and extend the duration of phenological activity. An extension in the duration of partner resources may allow for increased survival in species that are undergoing phenological shifts. This duration could be across a community for generalist mutualisms, with species at the beginning of the season starting earlier and those at the end of the season ending later to extend the total season-wide availability of the resource. Increased phenological phase duration may allow for adaptive capacity by maintaining natural patterns of *overlap* and buffering the impacts of timing shifts in the short term

The focus here is on plant-animal mutualisms. However, building adaptive capacity to phenological shifts is not limited to mutualistic interactions. All interactions may experience mismatch with climate shifts, impacting species either positively or negatively depending on the type of interaction. For example, plant–herbivore relationships are dependent on the overlap between plant resources and herbivore timing (van Asch et al. 2007), and asynchrony may benefit plant species while negatively impacting herbivores, while overlap will benefit herbivores and other consumers. Longer phenological duration can benefit non-mutualistic partners, (e.g., deer herbivory [Pettorelli et al. 2005]), and therefore extending phenological phase duration may be a strategy to aid in short-term survival or balancing of ecosystem dynamics for any interactions.

Techniques to extend duration of phenological activity

We propose extending the duration of phenological activity as a management possibility where phenological mismatch is a concern. Phenological activity may be extended via plant resource timing extension, animal partner timing extension, or providing supplemental partners (Figure 1). We propose three techniques that we predict could lead to an increase in phenological phase durations at local- and landscape-scales: (1) diversifying species and genotypes; (2) utilizing microclimate heterogeneity; and (3) alteration of population timing. These techniques are based on the idea that complementarity (patterns of non-overlap) in the timing of mutualistic partner resources will yield overall longer resource availability (Figure 2). In addition, techniques may work synergistically to yield additional extension in timing. The aim should be to maintain the ecosystem's natural patterns of synchrony as much as possible in the face of climate change.

Technique 1: Diversifying species and genotypes

Introduction of species and genotypes, with complementary phenology, can supplement resources during periods of diminished availability that may be created by a phenological shift (Timberlake et al. 2019). Many restoration projects are designed with a diverse array of plant species with complementary traits and hence incorporating phenology into trait selection can extend the duration of resources across the year. In fact, at this time the only strategy that we know has been implemented to extend duration of fruiting and flowering resources is the selection of a palette of species with diverse timing (e.g., early and late flowering species; Figure 2A) in restoration projects (Box 2). This practice may benefit a system in multiple ways, as it may serve to extend timing as well as increase functional redundancy and improve quality of resources (e.g., nutritional value) in a system. While this will be useful for generalist mutualisms, it may also support some specialized mutualisms if specialization occurs at the genus or family level, or on functionally similar species across clades (e.g., bats and *Piper* fruit (Marinho-Filho

1991); thrips and dipterocarps (Appanah 1993)).

A longer window of timing may also be achieved within species by diversifying genotypes. Genotypes with slightly different timing can complement each other, and yield an overall extended phenological duration (Smith et al. 2015). The extent of phenotypic plasticity can also vary by genotype (Pigliucci 2001). Therefore, managing for natural genotypic diversity in timing could extend phenological phases, via diversity in both fixed and plastic timing traits. Planting genotypes from diverse source locations in one place could yield an overall longer resource duration due to the complementarity of both early and late genotypes.

Genotypes with different phenologies may interbreed or adapt to have similar timing based on site conditions (Ware et al. 2019) potentially making the extension of timing short-lived. It may also be difficult to predict the exact timing of genotypes once moved to a new location due to phenotypic plasticity (Monty & Mahy 2009). However, this technique may still aid in increasing adaptive capacity, as the presence of different genotypes may allow for adaptive evolution, or may permit an interacting species to coexist long enough to adapt to new conditions (Millar et al. 2007). Evaluating risks will be important before implementing this technique, as swamping the population with non-adaptive genotypes may be a concern. However, in the case of an uncertain future, increasing the genetic diversity of local populations is likely to be beneficial (Millar et al. 2007).

Technique 2: Utilizing microclimate heterogeneity

Areas with different abiotic conditions within a patch and across a landscape may yield differences in phenological phase timing and duration (Figure 2B). The nature of these areas, their spatial configuration, and scale at which they vary will determine phenological duration at the local and landscape levels. Heterogeneity in microclimates can yield lengthened duration by creating patterns of complementarity in the timing in both plant and animal activity. Abiotic gradients and habitat heterogeneity can impact the timing of both animal and plant species distributed across a site (Hindle et al. 2015; Olliff-Yang & Mesler 2018). Management to conserve, maintain, and restore abiotic heterogeneity, and increasing connectivity across heterogeneous landscapes could extend phenology at both local and landscape scales.

Altering conditions to create microclimate heterogeneity (e.g., watering, shade structures, earth moving) could extend phenological phase duration for both animal and plant species. Microclimate characteristics that can affect phenological timing include landscape positioning like slope, aspect, hilltop, and valley bottom (Weiss et al. 1988), as well as soil moisture and canopy cover (Heinrich 1976). Creating microhabitat heterogeneity can be as straightforward as placing wind shields, which can extend activity timing in alpine environments (Fukuyo et al. 1998). Nesting habitat and positioning can also be manipulated to influence animal timing (e.g., moving bees in trap nests [Forrest & Thomson 2011]). The scale of implementation of this technique will be dependent on the species, and microenvironments must be present within the average foraging range of the animal mutualist to be effective.

Increasing connectivity across microclimates may connect animal and plant mutualists with various timings, effectively increasing the duration of phenological activity on the landscape as a whole. Elevation gradients influence phenology for both plants and animals due to precipitation and temperature gradients (Forrest et al. 2010), and many montane animal species rely on moving to track differences in resource timing due to variation in snowmelt and spring vegetation onset across elevation (Pettorelli et al. 2005). Implementation of this technique would

include providing linkages and corridors, enhancing habitat heterogeneity in closely adjacent locations, or otherwise facilitating the movement of organisms across abiotic gradients (Dunwiddie et al. 2009), with the goal of increasing the chances that suitable partners are within reach of one another at the right time.

Technique 3: Alteration of population timing

Management that increases timing heterogeneity within populations may be used to extend the phenological phase timing of both plant and animal species, as complementarity in timing between individuals in a population will yield extended timing. Techniques to directly alter population timing may include direct manipulation of growth timing, as well as altering biotic and abiotic conditions that affect population timing (Figure 2C).

Manipulating seasonal growth (e.g., via hormone or growth initiation treatments) can alter and extend phenological phase duration as individuals with different growth timing will experience different climactic conditions, which may lead to a variety of timing in one location. Planting on various dates could induce timing complementarity and yield extended duration of phenological activity in one growing season (Iannucci et al. 2008). Effectiveness of directly manipulating seasonal growth on lengthening phenological phase timing would require species that reproduce in the first growing season, and that are not strongly dependent on photoperiod cues. In addition, it is not likely that the effects of such treatments will last for multiple years without continued management.

Heterogeneity in both biotic and abiotic factors may foster a variety of phenological timing, leading to complementarity between patches, and an overall extended season at landscape scale. Grasslands with heterogeneous management practices create a mosaic of timing, supporting successive flowering (Kubo et al. 2009). Competition and disturbance can affect flowering time and duration (Rathcke & Lacey 1985), and density can influence both animal and plant reproductive timing (Ávila et al. 2016; Schmitt 1983). Burning can lengthen flowering time in fire adapted landscapes (Mola & Williams 2018), and heterogeneous fire severity and intensity ('pyrodiversity') across a landscape may further lengthen the flowering season (Tunes et al. 2017). Finally, directly planting or placing nesting habitat across heterogeneous microclimates can also extend timing of populations (see Technique 2).

Concluding Remarks and Future Perspectives

One of the most conspicuous responses of organisms to climate change has been shifts in timing of phenological events (Menzel et al. 2006). These shifts are already causing interacting species to become less synchronous in time than they have been. It will be important to keep the timing of interactions in mind while assessing climate risks and planning for the future, as this will help us envision and plan for instabilities (Russo et al. 2013). Considering novel ways to buffer the impact of climate change on ecosystems is critical for management success. Extending the phenological phase duration of mutualistic partners may be one way to buffer the impacts of timing shifts on asynchronous mutualisms.

It is important to keep potential tradeoffs in mind. For example, techniques to extend phenological overlap may reduce the strength of selection that an asynchrony would cause. While increasing short-term survival may buy time for adaptive evolution, weaker selection pressures would slow the rate of adaptive response. On the other hand, selection cannot act if

either partner in the mutualism is extirpated due to rapidly changing climatic conditions. Decreased overlap is only one of multiple factors that climate changes will impact, and short-term survival may depend on other conflicting factors, both biotic and abiotic, that have stronger impacts and undermine the effectiveness of techniques discussed here (Visser & Gienapp 2019). In addition, extending the duration of an activity may increase the overlap in time with undesirable interactions (e.g. Douglas fir trees and spruce budworm [Chen et al. 2003]).

It is also possible that invasive and weedy species may be facilitated or hindered with extended phenological phase timing, or in the implementation of techniques to extend timing. Any newly introduced species would yield an invasion risk, and introducing or changing disturbance may create opportunities for invasion (Hobbs 1989). Invasive species are typically more phenotypically plastic than non-invasives (Davidson et al. 2011), which may allow for the flexibility to capitalize on empty niches in time (Wolkovich & Cleland 2011). Our strategy to extend phenology may be an effective way to fill empty niches in time with native species rather than allowing invaders to take advantage of timing gaps. As always, focused study of individual systems may be needed to determine costs and benefits of phenological restoration and conservation strategies.

The selection of plant species and genotypes with complementary phenology lies at the heart of farm and garden design, where humans act as consumers seeking to enjoy a colorful display or fresh foods throughout the season. Not surprisingly, gardens can become important resources for animal pollinators and dispersers across the urban to rural gradient, and enhanced resource availability through the year has contributed to species range expansions (Greig et al. 2017). The line between habitat restoration and 'artificial' gardens may become increasingly blurred in the face of climate change, as new species are selected that can manage novel conditions and hence contribute to biodiversity conservation (Dunwiddie & Rogers 2017).

Techniques to maintain and extend resource duration on the landscape are in line with many already used to support adaptive capacity to climate change. Increasing genetic and species diversity in restoration and forestry practices is a top recommendation for conservation of biodiversity under climate change (Heller & Zavaleta 2009). Expanding the number of seed source locations to boost genetic diversity is one strategy suggested for conserving plant populations in an uncertain future (Millar et al. 2007). Restoration projects alter population density and disturbance via seeding density, grazing, mowing, and prescribed burning (Stromberg et al. 2007). Utilizing diverse topography and microtopography on the landscape has also been implemented to support establishment of plant species and aid ecosystem function (Biederman & Whisenant 2011). Incorporating timing as a dimension of conservation and restoration planning can further achieve goals by aiming for adaptive capacity to climate-induced asynchrony. The strategies outlined here may also reduce asynchrony due to factors other than climate change, such as effects of landscape simplification and habitat loss.

The extent of phenological mismatch that will occur with climate change and the resulting impacts on population demography is unclear, as there is still a paucity of evidence for the effects of asynchrony on population fitness (Renner & Zohner 2018). It is likely that the extent and impact of phenological phase shifts and any need for management intervention will be context dependent. Continued research is needed on techniques to extend phenology, the risks and impacts of different techniques, and the relative increase in adaptive capacity with extended phenology in different systems. We consider extending phenological phase duration as a tool to add to the arsenal of strategies being developed with the aim of mitigating climate change impacts. We hope that the ideas proposed here will inspire continued discussion and research on

creative strategies to mitigate the impacts of phenological mismatch with climate change.

Figures

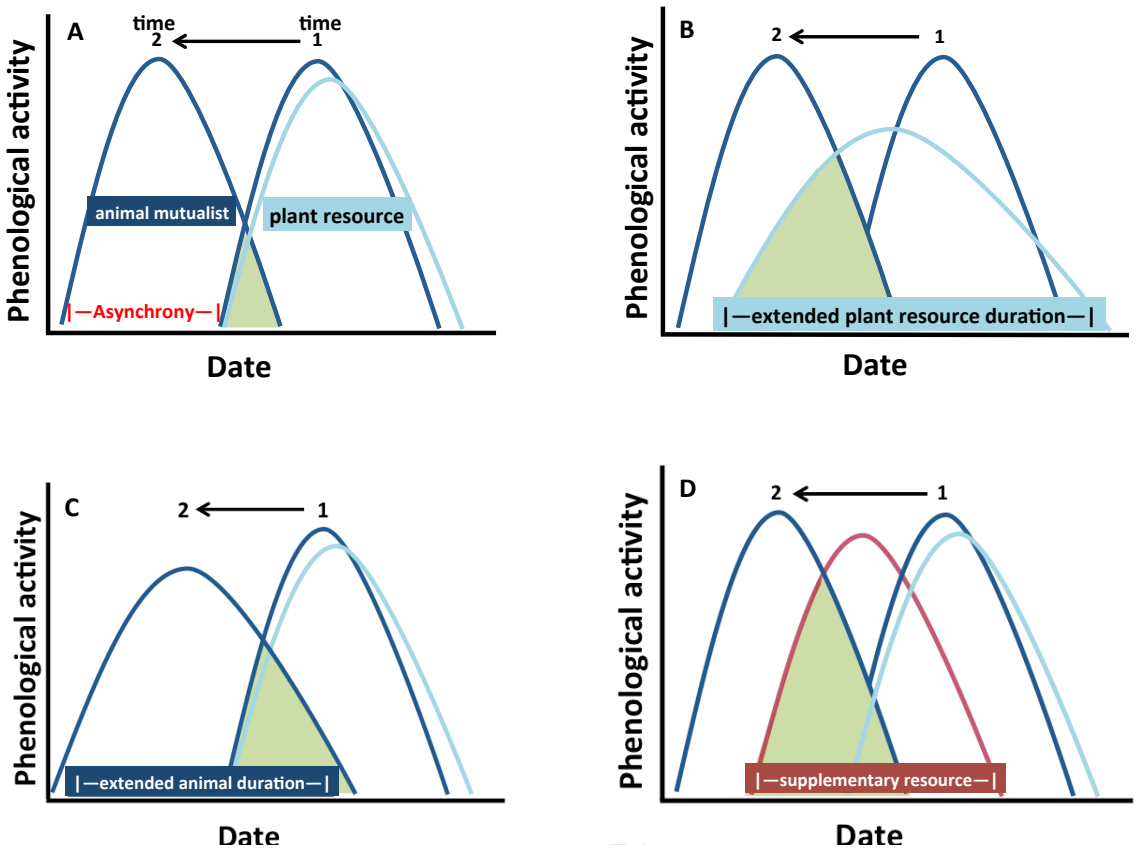

Figure 1. Concept and strategy - extending phenological phase duration. (A) Example timing shift resulting in an asynchrony. If the timing of phenological activity (e.g. flight period, nesting) of a focal animal species (dark blue curves) undergoes a timing shift (from time 1 to time 2), this shift could reduce the overlap (green shading) with the phenological timing (e.g. flowering, fruiting) of its primary plant mutualist in this location (light blue curve). Extending the timing of the plant activity (e.g. flowering, fruiting) may reduce asynchrony with the mutualistic animal partner (pollinator, seed disperser, etc.). (C) Extending the timing of the mutualistic animal species may also reduce asynchrony. (D) Adding other complementary species (plants or animals) into the community as supplemental partners can also reduce asynchrony experienced by a focal species (here animal mutualist is represented as focal species).

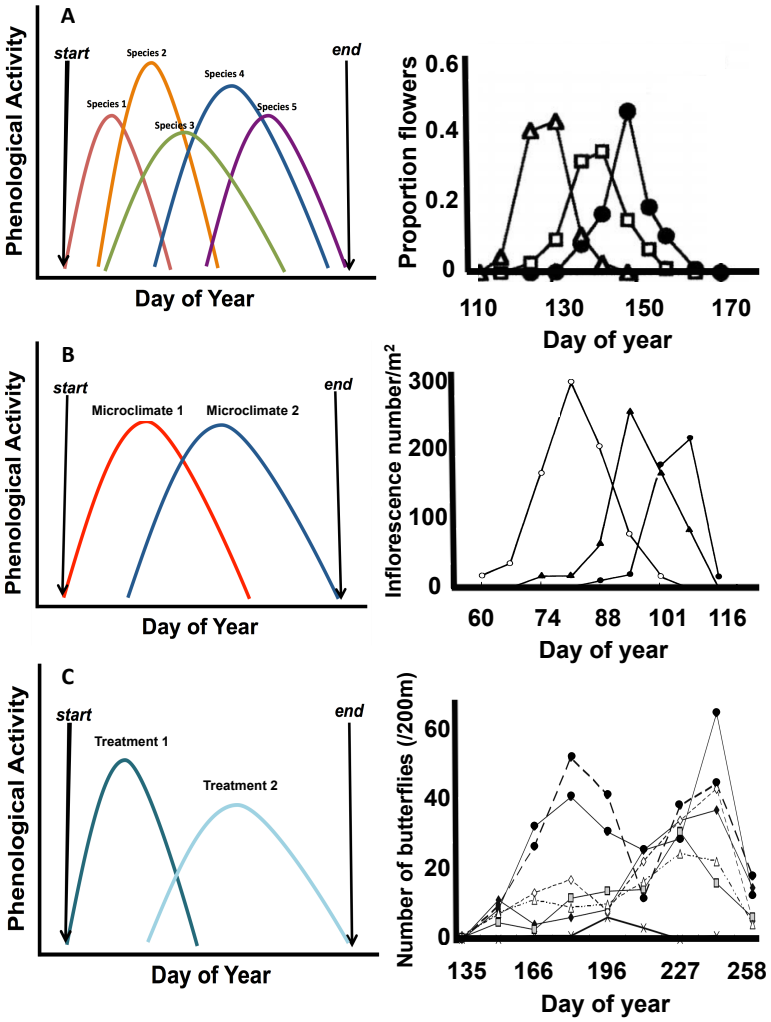

Figure 2. Techniques for extending phenological phase duration. Conceptual diagrams (Left panels), and empirical examples (Right panels). The overall duration (time between "start" and "end" dates) of phenological activity (e.g. flowering, fruiting) will be extended when the timing of these resources are staggered across the season. This can be achieved via: **(A) Left -** Technique 1: diversifying species and/or genotypes, **Right -** Flowering phenology of three different Clarkia species (from Moeller 2004); **(B) Left -** Technique 2: Utilizing microclimate heterogeneity, **Right -** Influence of topography on floral resources (SW facing 15˚ slope [open circles], flat [triangles], and N facing 11˚ slope [filled circles]; from Weiss et al. 1988); and **(C) Left -** Technique 3: Alteration of population timing, **Right -** Seasonal changes in the butterfly abundance, where the number of butterflies is the mean number per year at each study site. Treatments include fire breaks (circles), mowing (filled diamonds), road (open diamonds), abandoned grassland (grey squares), scrub (triangles), and forest (Xs) (from Kubo et al. 2009).

Boxes

Box 1. Glossary of important concepts and definitions.

Adaptive capacity: capability of organisms or systems to adjust to potential stress, take advantage of opportunities, or respond to and mitigate negative impacts of environmental change.

Phenology: the timing of periodic life cycle stages of organisms.

Phase duration: time from start to end of a particular phenological phase (e.g. flowering period).

Phenological complementarity: complementary timing in species growth and reproductive timing (ex: complementary flowering species flower at different times of the year). Complementarity is used here to describe patterns of non-overlap in species of the same functional group or guild.

Phenological overlap: Overlap in species growth and reproductive timing. Extent of overlap depends on amount of time both species are active simultaneously. Overlap in a mutualism occurs when a species or ecological phase is concurrent in time with its interacting partner.

Phenological mismatch/asynchrony: when a species or ecological phase is not concurrent in time with its interacting partner. Extent of asynchrony depends on amount of time both species are present or active in the absence of the other. A complete mismatch occurs when the phenological phases of mutualistic partners are entirely out of sync with each other.

Phenological shift: a change in the timing of life cycle stages, resulting in timing that is earlier or later in the year.

Box 2. Point Blue Conservation Science's Climate Smart Planting design tool

Point Blue Conservation Science in California was an early developer and adopter of "Climate Smart" restoration practices. A planting design tool created by Point Blue Conservation Science allows practitioners to (Figure IA) select candidate plant species for restoration planting and then (Figure IB) view how flowering and fruiting resources will likely be distributed through time once plants are established (Point Blue Conservation Science 2019). This allows project designers to select complimentary flowering and fruiting species to provide resources across the full season. Areas restored using these metrics may reduce the impacts of phenological mismatch by supplementing plant resources for generalist animal species across the season.

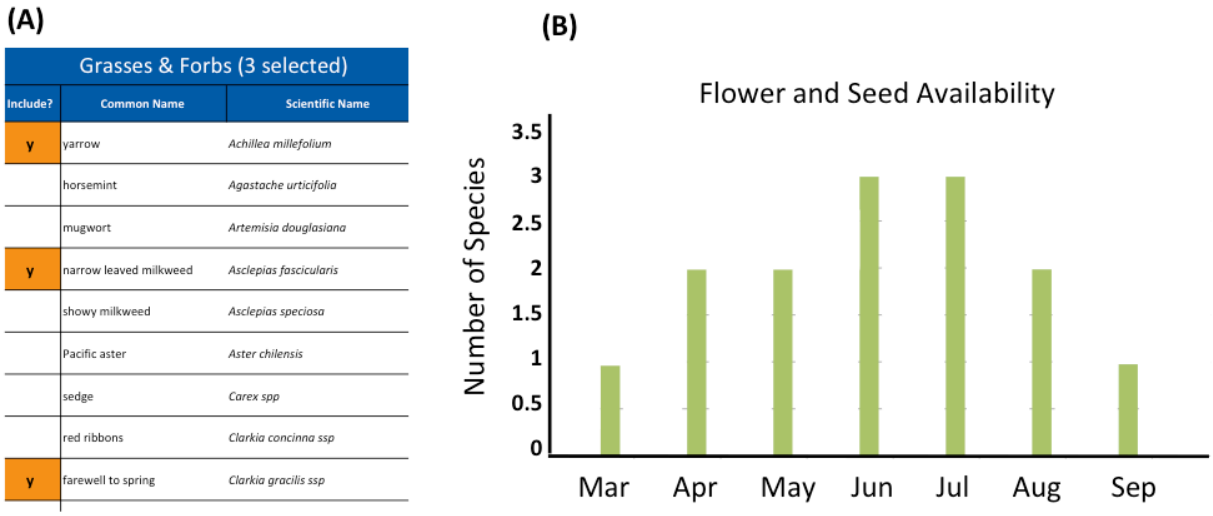

Figure I. (A) List of plant species available for restoration projects. Species are split by functional group, and listed with both common and scientific names. Species are selected using a "Y" in the "Include?" column. (B) Expected diversity and duration of species providing flower and fruit resources in a restoration is shown, based on species selected in list A.

Chapter 2: Topographic heterogeneity lengthens the duration of pollinator resources

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This paper is reproduced here with kind permission from co-authors and the Graduate Division, and is the first of three empirical examinations of abiotic and biotic influences on flowering time and duration in California grasslands.

Abstract

The availability of sufficient and diverse resources across time is important for maintenance of biodiversity and ecosystem functioning. In this study we examine the potential for variation in environmental conditions across topographic gradients to extend floral resource timing. Flowering time on a landscape may vary across topography due to differences in abiotic factors, species turnover, or genotypic differences. However, the extent to which this variation in phenology affects overall flowering duration on a landscape, and the components of diversity that influence flowering duration, are unexplored. We investigate whether differences in flowering time due to topography yield an overall extension in duration of flowering resources in a northern California grassland. We recorded flowering time of pollinator resource species across four successive spring growing seasons (2015-2018) on paired north and south aspects. Flowering time differences were evaluated both at the community level and within species present on both paired aspects. The role of plasticity was examined in an experimental case study using genotypes of *Lasthenia gracilis*. We found that aspect is a strong determinant of phenology, with earlier flowering on warmer south-facing slopes. Aspect differences resulted in complementarity in timing of flowering resources across sites, as aspects that started flowering earlier also ended earlier. Complementarity between north and south aspects served to extend the flowering time of pollinator resources by an average of 4-8 days (8-15%), depending on the year. This extension can be attributed to both within species responses to aspect differences as well as species turnover. Flowering of *L. gracilis* genotypes was distinct across aspects, demonstrating that plasticity can drive the extension of flowering duration. Our findings indicate that heterogeneous topography can extend overall flowering time of pollinator resources, which may support pollinator biodiversity**.** Extension was most pronounced at the community level, which incorporates species turnover as well as plastic and genotypic differences within species.

Introduction

Sufficient and diverse resource availability across time is important for biodiversity and ecosystem functioning. Resource availability is dependent on the phenology (seasonal life cycle timing) of both resources and interacting partners in a system. For pollinators, the presence of pollen and nectar-rich floral resources (e.g. Figure 1) across the entire flight season is critical for maintaining diversity, population stability, and pollination function (Russo 2013). A reduction or change in season duration can have adverse consequences for both pollinator and plant populations (Aldridge et al. 2011).

Anthropogenic climate change may shorten phenological duration (Høye et al. 2013; Prevéy et al. 2019) posing a risk to pollination mutualisms. On the other hand, an extension of flowering time duration can support mutualisms (Hindle et al. 2015), and increase local pollinator biodiversity and pollination efficacy (Morandin & Kremen 2013). Climate refugia (locations on the landscape where the impacts of climate change are buffered) are expected in heterogeneous landscapes, due to the presence of a variety of microclimatic conditions (Morelli et al. 2020). We predict that variations in microclimate created by heterogeneous topography might also aid plant-pollinator mutualisms by serving to extend flowering duration across space. This prediction necessitates a better understanding of the influence of topography on flowering time on the landscape.

The timing of flowering is driven strongly by abiotic cues, including temperature, moisture, and photoperiod (Rathcke & Lacey 1985). As temperature and precipitation have shifted with climate change, so have the timing of life history events, advancing the timing of flowering and pollinator foraging seasons (Parmesan 2006). Climate change has already led to species-specific timing changes in plants and pollinators (CaraDonna et al. 2014). These shifts have been documented to disrupt species interactions (e.g. Schmidt et al. 2016), and can result in pollination asynchronies, especially in free-living mutualistic partners with brief, seasonal interactions (Rafferty et al. 2015).

Phenological responses to abiotic conditions can also lead to timing differences across gradients on a landscape (Ward et al. 2018). Topoclimate, or small scale (10 -100 m) variations in abiotic conditions due to differences in topography (Geiger & Aron 2009; Oldfather et al. 2016), can be used to observe the combined effects of differences in temperature and moisture gradients on phenology. Topoclimate differences can greatly influence individual species phenological timing, and in some cases the magnitude of variation in timing across a landscape is greater than interannual variation due to yearly weather conditions (Weiss, Murphy, & White 1988; Weiss 1993). Slope and aspect temperature and moisture differences can be ecologically significant across even moderate topography, driving vegetation patterns and ecosystem processes (Bennie et al. 2008). These effects may influence community flowering time across the landscape by affecting individual species timing as well as species turnover.

Topography can create short distance gradients in abiotic conditions comparable to those observed across larger latitudinal or elevational gradients. North-facing slopes in the northern hemisphere are pole-facing, and therefore receive lower amounts of incident solar radiation (i.e., insolation). Equator-facing slopes, or south-facing slopes in the northern hemisphere, receive more direct solar radiation, and therefore experience higher temperatures and a faster soil dry down rate (Bennie et al. 2006). These energy load differences between north-and south-facing slopes are greatest in the mid-latitudes, due to planetary geometry (Holland & Steyn 1975). The dynamics of light, temperature, and moisture differences across these two contrasting aspects

allow for the examination of different abiotic environments in close proximity.

Varying abiotic conditions due to small-scale heterogeneity in topography can cause patch differences in flowering time, yielding an overall extension of flowering across the landscape. For example, each aspect on a hill will have a start, middle and end date of flowering time (Figure 2A-B). The duration of flowering on the landscape will be determined by the complementarity (or non-overlap; e.g. Figure 2C, green brackets) between these phenological curves, from the earlier of the start dates to the later of the end dates (see Figure 2). Therefore, the duration and degree of complementarity in flowering time across topographic gradients on the landscape determines the overall flowering time. Just as herbivores can "surf" waves of green-up across the landscape (Merkle et al. 2016), complementarity in flowering time among aspects may allow pollinators to utilize flowering resources available over time in different patches on the landscape.

Extensions in flowering time can be generated by population plasticity, genotypic heterogeneity (Smith et al. 2015), and species turnover (Timberlake et al. 2019; reviewed in: Olliff-Yang, Gardali, & Ackerly 2020). By measuring both community and species-level components of flowering differences across topographic gradients, we can examine the components of diversity that contribute to observed patterns of flowering time. Both plasticity and genotypic variation can contribute to intraspecific differences in phenology across topoclimates (Phillimore et al. 2012; Anderson et al. 2012). Species turnover will yield additional changes in the timing of flowering resources (Wright et al. 2015). The effects of these components may be antagonistic across the landscape, yielding similar timings, or synergistic, yielding an extension in timing when complementary flowering patches are combined together. The combined effect of both community and species-level components will influence the overall flowering time at the community level.

It is valuable to examine the different influences of topographic heterogeneity within and among species, as the components will matter for plant-pollinator mutualisms. Insect species can specialize on specific plant taxa or on plant species with similar traits across clades (Willmer 2011). Specialist pollinator species depend on the flowering time of only the particular taxa or morphological type of plants they visit (Willmer 2011). If topographic gradients result in an extension of flowering duration within species, then specialist pollinators will benefit from complementarity (Olliff-Yang, Gardali, & Ackerly 2020). In contrast, if an extension in flowering time is driven by species turnover, then the benefits will depend on the spectrum of pollinators utilizing the respective plant species. The scale of floral resource availability on the landscape matters, as small pollinators may only move short distances while foraging ($\leq$ 100 m), while larger pollinators can forage over longer distances (e.g. bumblebees up to 1.5 km) (Zurbuchen et al. 2010; Osborne et al. 2008). Patches of floral resources on the landscape must be present within foraging range to benefit pollinators.

Mediterranean-type climates are characterized by cool wet winters and hot dry summers (Köppen 1923). In the grasslands of California, these thermal dynamics and timing of precipitation yield a lush green landscape in the winter, a colourful flowering period from March-June, and a senescent period in the summer as the soil dries out (Dallman 1998). These timing dynamics play out in different ways across the landscape depending on the temperature, moisture, and light available at each point. Species-specific differences in flowering time also contribute to variation across the landscape, leading to complementarity in utilization of soil nutrient and moisture resources across the season (Wolkovich & Cleland 2011; Gross et al. 2007).

In this study, we compare the flowering time of a grassland community (Figure 1) across paired north and south-facing slopes, and examine the components of diversity involved in the flowering responses to topoclimatic conditions. We investigate how heterogeneous topography influences the duration of flowering time across the landscape, and decompose the components that may lead to an extension in timing within and among the grassland species. Specifically, we address: 1. How does microsite variation due to aspect impact the flowering time of pollinator resources? 2. Do differences in flowering time due to topography yield an overall extension of flowering resources on the landscape? 3. What is the contribution of differences within vs. among species to observed patterns at the community level? 4. When controlling for genotypic differences, does plasticity contribute to intraspecific differences across aspect? We explore these questions to assess the importance of topography on community level pollinator resource timing, and to evaluate the potential for topographic heterogeneity to mitigate shifts in phenology with climate change. Adaptive capacity, the ability to respond to climate changes via evolutionary, plasticity, or dispersal events, will increase a species chance of survival into the future (Beever et al. 2016). Extended phenological timing has been proposed as a possible way to buffer some impacts of shifts in phenology with climate change, yielding adaptive capacity (Olliff-Yang et al. 2020). If topographic heterogeneity lengthens flowering time duration on a landscape, it may serve to support species interactions in responding to climate changes.

Methods

Study system

This study was conducted in the grasslands of Pepperwood Preserve (Figure 1; Sonoma Co., California, 38.57˚ N, -122.68˚ W). Four sites were chosen based on presence of paired north (pole-facing) and south (equator-facing) aspects within 100 m of each other (35 - 80 m), and with pollinator resource species present (M. Halbur, pers. comm.). The sites were located in grasslands within 2 km of each other. On each aspect, three 1 m^2 quadrats were placed randomly, and spaced 3 m apart, yielding a total of 24 plots. In 2016 and 2017 an additional site was monitored (n = 30 plots in these years).

Abiotic measurements

Temperature and moisture were recorded at the plots to quantify microsite differences between aspects. Temperature was recorded at each aspect with an iButton (Thermochron, N = 8) placed 10 cm below the soil surface and set to record every hour. Temperature measurements were taken from March through June in all four years to compare aspect temperature differences during the flowering season. Additionally, temperature measurements were collected throughout the year in 2016 to capture full growing degree day accumulation curves. Soil moisture was measured manually in 2016 (on 26 Apr, 26 May, 21 Jun) and 2017 (on 10 Mar, 6 Jun) at all plots with a Hydrosense II soil moisture probe (Hydrosense II (Campbell Scientific, Inc.), 2019). Temperature measurements were taken simultaneously, and moisture measurements were taken within 10-30 minutes of each other at each site on each measurement date. For site temperature comparisons, any missing temperature data (e.g. an ibutton failure in 2017, animal disturbance of an ibutton in 2018) was extrapolated by taking site averages from other years and adjusted based on air temperature differences. Cumulative temperature differences were assessed by visually

comparing growing degree day accumulation (using base temperature of 5˚ C) and tested using a binomial sign test. Differences in maximum temperatures, minimum temperatures, and soil moisture, on north and south aspects were assessed using analysis of variance (ANOVA) models, with site, measurement date, and year as fixed effects. Moisture data was log transformed to meet assumption of residual normality. Significance of aspect was assessed via model comparison with simplified models (with aspect removed).

Phenology measurements

In each plot, we recorded flowering throughout the spring growing season (March-June) in 2015, 2016, 2017 and 2018. Flowering phenology was observed for all pollinator resource species in the plots, including native and non-native species, annuals and perennials. Species status as a pollinator resource was identified by direct observations of animal visitation during the study, together with outside sources, including information provided by the Xerces society (Mader et al. 2011). Richness of pollinator resource species in each plot varied from 1 to 22 species over the entire season. Inflorescences in flower for each species in each plot were counted weekly to determine start, middle, and end flowering, as well as the length of the flowering season. For species with inflorescences that had more than one phenology stage present, an inflorescence was counted as flowering when at least 50% of it was in flower. Not all species were present in all sites, or on both slopes.

Community flowering dates for pollinator resource species were calculated based on cumulative plot flowering over the season, as follows: start date as the date when 5% of the cumulative number of flowers in a plot (summed over the season) had been reached, mid-flowering date as the date when 50% flowering was reached, and end date as the date when 95% of flowering had been reached. Flowering duration was defined as the total number of days between start and end dates (when 5% and 95% flowering had been reached, respectively) for each plot.

Analyses

Q1: microsite variation in resource timing

The relationship of mid-flowering to average temperatures during the flowering season (March-May) was tested using linear regression models, examining both within year and between year trends. Sites (and years in the combined model) were included as fixed factors to account for plot pairing. Flowering dates were then compared across north and south-facing aspects, with aspect, year and site as fixed factors. As it was not monitored in 2015 or 2018, the fifth site (TT, three tree hill) was not included in these inter-annual ANOVA comparisons, to maintain a balanced design. Models were then tested against simplified models (with aspect removed) to determine whether aspect was significant in determining flowering date and compared using AICc information criterion metrics. As the effect of aspect may differ depending on the year and site, interactions with aspect were also tested by comparing full ANOVA models against models with interactions removed.

Q2: phenological extension

Extension in flowering time was calculated by comparing the duration (in number of days) of flowering time on both aspects combined at a site for each year, vs. the duration on the longer of the two slopes (north or south). This is a conservative calculation of flowering time extension, because it is based on extending the longer flowering slope, and duration start and end dates were defined as the date of 5% and 95% flowering, respectively (see above). The percent of flowering resource extension was calculated as:

$$Extension = \frac{\text{Combined} - \text{Longer}}{\text{Longer}} * 100$$

A binomial sign test was performed to examine the influence of aspect on complementarity, testing whether aspects with earlier flowering start dates also end earlier more often than expected by chance.

Q3: diversity components of extension

Absolute turnover at a site was calculated as the total number of species present on only one aspect (i.e. Turnover = [# unique species on N aspect] + [# unique species on S aspect]). This was calculated for each site/year combination. To examine the amount of extension explained by community turnover, absolute turnover was compared with observed flowering time extension using simple linear regression.

Extension in community flowering time was then examined with species turnover removed, to decompose the influences of flowering time differences between aspects. To do this, the community-level analyses were restricted to only include species present on both aspects at a site in a given year. The community flowering dates for pollinator resource species were then recalculated based on cumulative plot flowering over the season. In this analysis, any difference in flowering time observed between aspects is due to differences in within-species responses (plasticity or genetic variation), and not attributed to species turnover at the site.

Aspect influence on flowering time was also examined at finer scales. To assess the extent to which the flowering time of individual species was affected by aspect, the difference in flowering dates was calculated for each species present on both slopes at a site. Species flowering dates were defined as above: start date as the date when 5% of the cumulative number of flowers in a plot (summed over the season) had been reached, mid-flowering date as the date when 50% flowering was reached, and end date as the date when 95% of flowering had been reached for each species. When flowering was only observed for a species on one survey date, the duration of flowering was calculated as 1 day (although it is likely that flowering occurred for two days or longer depending on the species). There were instances of gaps between flowering time on north and south aspects for individual species, and these were removed when calculating site flowering duration. The fifth site (monitored in 2016 and 2017) was included in the species-level comparison of flowering date differences and season extension, as it added 3 new species and additional observations of other species. However, this additional site was not used in any ANOVA model comparisons.

Q4: population plasticity contributions to differences across aspect

Sites were chosen with paired aspects in close proximity (< 100 m), and therefore the genetic differentiation between north and south slopes was expected to be minimal. However, to explicitly examine the role of plasticity in aspect effects, experimental plots of goldfields (*Lasthenia gracilis* (DC.) Greene) were set up just outside of phenology plots in 2017 and 2018, with 3 subplots per aspect at each site (n = 24). These 30x30 cm subplots were planted with 30 seeds each, collected from two grassland locations on Pepperwood Preserve from 10 maternal lines (3 seeds per line per plot). Seeds were planted in the fall and marked with toothpicks to differentiate them from any other *Lasthenia* individuals occurring at the site. Flowering time was recorded for these subplots in the same way as the study phenology plots – with all open inflorescences from all individuals counted each week from March through June. Counts were conducted only on planted and marked *Lasthenia* individuals within each plot. As plot flowering was composed of individuals from the same genetic lines, any differences in timing between north and south aspect plots would therefore reveal population mean plasticity across aspects. Flowering time of *L. gracilis* within each site (due to presence on both aspects) was assessed using ANOVAs. Flowering time and extension metrics were quantified and analysed using the same method as the community and species-level analyses. All analyses were performed in version 3.6.2 of R, using tidyverse packages in RStudio (R Core Team 2019; Wickham et al. 2019; Rstudio Team 2020)).

Results

Q1: microsite variation in resource timing

Aspect was significant in determining both maximum and minimum temperatures ($p < 0.001$). Soil temperatures on north-facing slopes were on average 3.06˚C cooler than south-facing slopes. This led to warmer south aspects overall (Figure 3 – compare overall aspect/year points), and a faster accumulation of growing degree days on south facing slopes (Appendix I Figure 1; S aspects accumulated more growing degree days March-June than N aspects [in 17/18 cases; binomial test, $p < 0.001$]). Paired aspects had significantly different soil moisture content ($p < 0.001$), with north aspects more moist (approximately 1.7% VWC higher on average than south facing slopes on measurement dates). However, one site (BH) tended towards lower volumetric water content on the north-facing slope, likely due to thinner soils on this aspect.

Aspect was a strong determinant of phenology for the start, mid and end of flowering (Table 1). Warmer plots (due to warmer slopes and/or years) resulted in earlier timing of pollinator resources in all years (Figure 3; slope = -3.4, $R^2 = 0.55$, $p < 0.001$). Flowering time differed between aspects, with earlier timing on south-facing slopes (Figure 4A). These phenological responses to landscape position resulted in differences in flowering time leading to complementarity across slopes (Figure 4A), as aspects that started flowering earlier also ended earlier more often than expected by chance (binomial test, $p = 0.021$). Year was also significant in determining start, mid and end dates (all dates $p < 0.001$), likely due to differences in temperature and precipitation each year (Appendix I Figure 2). However, the interaction between year and aspect was not significant, so aspect influenced timing similarly every year. The effect of aspect on flowering time was dependant on the site (Site:Aspect interaction $p <$

0.001), and this site effect was consistent across years (Year:Site:Aspect interaction NS, Appendix I Table 2).

Q2: phenological extension

Where complementarity in community flowering time existed between slopes, it served to extend overall flowering time of pollinator resources by approximately 4-8 days (8-15%), depending on the year [mean 6.8 days, or 12.1%] (Table 2A). Having both aspects present at a site increased the duration of flowering time in most sites (Appendix I Table 1). The coolest and wettest year (2017) yielded the longest overall combined community flowering duration across aspects, mainly due to a lengthened flowering duration on the south aspects (Figure 4A, Table 2A). However, the interaction between year and aspect was not significant.

Q3: diversity components of extension

Species turnover accounted for some of the difference in aspect flowering time. When turnover was removed in the extension calculations, flowering time extension decreased by an average of 5 days (Table 2C). However, there was some variation in the turnover effect, and not all sites exhibited reduced flowering time extension when species turnover was removed (Figure 4C, Table 2C). Additionally, two sites decreased in the extension metric in 2015 with turnover, indicating that intraspecific differences in flowering time between aspects was greater than interspecific differences in these cases. Full tables of site by year extension metrics are included in the supplementary information (Appendix I Table 1).

A total of 32 pollinator resource species were present across both aspects, in one or more plots. These species showed a variety of timing responses to aspect. The mean difference across species was 1.0 days for start dates, 3.3 for mid-flowering dates, and 6.6 for end dates (Figure 5A-C). These differences resulted in a mean extension of 2.6 days averaged across species (Figure 4, Figure 5D). Most (75-81%) of the differences between north and south slope flowering times were positive, revealing later flowering start, mid and end dates on north-facing slopes. However, this was not always the case, and some species-year combinations yielded no difference, or an *earlier* timing on north-facing slopes.

Q4: population plasticity contributions to differences across aspect

Subplots of *Lasthenia gracilis* genotypes also exhibited differences in timing due to aspect ($p < 0.001$, Table 3; Figure 5 - *L. gracilis* points). This difference reveals population mean plasticity in flowering time in response to abiotic differences between aspects. This flowering difference across aspects extended the flowering time of *Lasthenia* an average of 4.5 days (Table 3; Figure 5D – *L. gracilis* points). Unfortunately, no naturally occurring *L. gracilis* occurred on the south facing slopes in the study sites during this experiment, so we could not compare whether the effect of aspect in the experimental plots was different than in the natural communities for *L. gracilis*. However, this pattern of complementarity follows that observed for other species within the main plots. Genotypic differences in phenological phase timing between aspects would increase the extension of flowering time if it led to complementarity in natural communities.

Discussion

Our study establishes that topographic heterogeneity can lengthen flowering time duration on a landscape and may therefore support species interactions in responding to climate changes. Topographic positioning, and the resulting differences in abiotic and biotic conditions, led to complementarity in flowering time of pollinator resources across the landscape. The lengthened duration of resource availability on the landscape reveals the potential of diverse topography to support both pollinator and plant species.

Temperature accounted for approximately 41% of the variation in mid-flowering dates (25– 52% each year), with flowering dates advancing approximately 3.4 days per 1˚C average temperature increase during the March-May flowering season (Figure 3; marginal R^2 of mid-flowering dates x temperature: 0.41). This strong negative relationship is consistent with temperature being a driver of flowering time in temperate regions, and matches trends observed in other systems across both space (e.g. Timberlake et al. 2019) and time (e.g. Miller-Rushing & Primack 2008). The observed sensitivity of the flowering time response to temperatures across space (3.4 days per 1˚C) is congruent with previous reports of plant flowering of ~ 3 days earlier for each 1˚C increase in temperatures over time (e.g. Miller-Rushing & Primack 2008).

The timing of flowering across microsites resulted in complementarity in flowering resources within a site. Our results show an average of 8-15% total extension (depending on the year), yielding an average of 4-8 additional days of pollinator resource availability within small scale landscape features (1000 - 2000 m^2 area). This scale is important as small pollinators may only move very short distances, and the linear distances between aspects in this study (≤ 100 m) were within foraging range of small insect pollinators (Zurbuchen et al 2010). The extension of flowering duration was due, in part, to intraspecific differences across aspects (0-8.6%), with an additional 1-14% extension each year due to turnover (Figure 4B-C, Table 2). Therefore, both intraspecific differences and species turnover are important determinants of resource duration across the landscape.

These calculations of the flowering time extension are conservative estimates, and the benefits of heterogeneous topography on the landscape may be much greater than reported here. If extension calculations were instead based on the shorter flowering period of the two paired aspects, the mean season extension observed at a site would be 12-22 days (26-47%) depending on the year. We also defined start and end dates as the date of 5% and 95% flowering, respectively. If absolute start and end dates of flowering were used instead, the extension estimates may be even greater.

Other properties of landscape patches can work synergistically with heterogeneous topography to extend flowering time. The effect of aspect was dependant on site, a pattern that was consistent across years (Site:Aspect $p < 0.001$ and Year:Site:Aspect interaction NS; Appendix I Table 2). This indicates that there are properties that make some sites more conducive to phenological complementarity and extension. Both abiotic and biotic differences between aspects in a site may determine the magnitude of flowering time complementarity, and therefore overall flowering duration. Turnover between aspects at a site explained 26% of the variation observed in community flowering time extension (Figure 4B). Once turnover was removed in extension calculations, the extension of flowering time with paired aspects was reduced to zero in 8 out of 16 cases (Figure 4). To further explore this finding, we examined site richness and determined that sites with higher overall richness also exhibited higher flowering resource extension (correlation test, $p = 0.02$). In addition, as temperature is related to flowering

time (Figure 3), sites with larger temperature differences between aspects also resulted in the more flowering time complementarity and extension.

Individual species responses were variable (Figure 5), but in a majority of cases (75%) the presence of a species on both aspects within a site led to an extension of flowering time for that species (Figure 5D, Table 2). This indicates that topography can extend the duration of flowering within a species, which is important for more specialized pollinators. Intraspecific extension across aspects is due in part to different abiotic cues across topography, such as degree day accumulation, as warmer plots (e.g. south facing aspects) exhibited earlier flowering in most cases (Figure 3). However, in some cases north facing slopes exhibited earlier flowering times (Figure 5), indicating that the differences in intraspecific flowering across aspects are a bit more complex. This may be due to differences in plant density (e.g. Schmitt 1983), or in soil properties, moisture, shading, or disturbance (e.g. Heinrich 1976). Likely multiple factors are at play in determining flowering extension across topography, and abiotic and biotic factors may interact to determine extension for each species.

Gaps between flowering on paired aspects did occur for individual species and should be taken into consideration. As differences in conditions between patches on the landscape become more distinct, flowering resource timing will be extended, but only up to the point at which a gap in time between the two flowering curves occurs. High heterogeneity in abiotic conditions with a continuum of temperature and moisture environments should maximize the extension of pollinator resources and minimize flowering gaps, as intermediate condition patches can fill floral resource "valleys" between patch type extremes (e.g. Aldridge et al 2011).

The timing differences exhibited by the *L. gracilis* experimental plots indicate phenotypic plasticity for flowering time, in response to different abiotic conditions across aspects (Table 4). However, species may also exhibit genotypic differentiation at this scale, and genotype by environment interactions may account for some of the larger magnitude of extension exhibited by some species (Figure 5D) and complexity in species responses observed. Reciprocal transplant studies are necessary to determine unequivocally whether genotypic variation contributes to intraspecific differences between sites.

The annual timing of life cycle events determines when and how species interact and is important for ecosystem function. Pollinators require readily available resources within an appropriate foraging distance at specific time periods in order to complete their life cycles. Likewise, animal-pollinated plant species need pollinators to be present at the right time for successful reproduction. Lengthened flowering seasons support pollinator biodiversity (Russo et al. 2013), and can improve plant pollination services (Kremen et al. 2007). Therefore, the presence of topographic heterogeneity may serve to support both plant and pollinator biodiversity in natural systems by extending flowering time on the landscape.

Pollinators rely on both presence and abundance of resources throughout the season (Aldridge et al. 2011), and have varying nutritional needs (Vaudo et al. 2015). Our study examines the ability of topoclimate to extend the flowering time of plant communities and species, focusing on the presence of resources across the season, but not the abundance or quality of those resources. The quantity of resources flowering during the season was quite variable, and species and floral abundances differed across aspects. The quality and nutritional content of floral resources can vary due to differences in abiotic conditions and species present (Vaudo et al. 2015). It will therefore be important to consider resource quality, along with abiotic differences and biotic diversity, in determining the potential of topographic gradients to support pollinators across the flowering season.

Our findings reiterate that topographic heterogeneity is important to consider in determining the impacts of climate change. The average temperature difference of 3 °C found between north and south slopes is roughly equivalent to the lapse rate for 500 m elevation difference or about 5˚ latitude in flat landscapes (Barry 2008; Bennie et al. 2008), and to the amount of warming that may occur over the next 50 years (IPCC 2014). Studies have indicated that topography may create important microrefugia for species as the climate changes (Dobrowski 2011). Microclimatic effects on plant phenology (e.g. due to topographic positioning) may allow animals to move across the landscape as resources become available, increasing the duration of resources on the landscape as a whole (Hindle et al. 2015). Our study highlights the importance of topographic heterogeneity as a means of extending flowering time on a landscape, and thus potentially supporting species interactions.

Topographic heterogeneity may serve to buffer some impacts of shifts in phenology with climate change, yielding adaptive capacity. A longer flowering phenology across a landscape may aid both pollinator and plant species to cope with these changes by buffering the magnitude of asynchrony at the landscape level (Olliff-Yang, Gardali, & Ackerly 2020). Recent modelling predicts duration as one of the most important factors in species persistence for plants and pollinators with shifts in phenology (Franco-Cisterna et al. 2020), and topographic diversity has been predicted to reduce the chance of mismatch for some species (Hindle et al. 2015). Conserving, restoring and maintaining high species diversity across topography may therefore support species interactions by buffering the impacts of mutualism asynchronies with climate change.

Figures

Figure 1. A grassland community at Pepperwood Preserve (Sonoma County, California, 38.57˚ N, -122.68˚ W). Our study site was a heterogeneous Mediterranean-type grassland with a rich array of pollinator resource species, both native and non-native. Pictured are the iconic California poppy *(Eschscholzia californica*, Papaveraceae) and common goldfields (*Lasthenia gracilis,* Asteraceae)*,* among other grass and forb species. Photo by Alexander C. Yang.

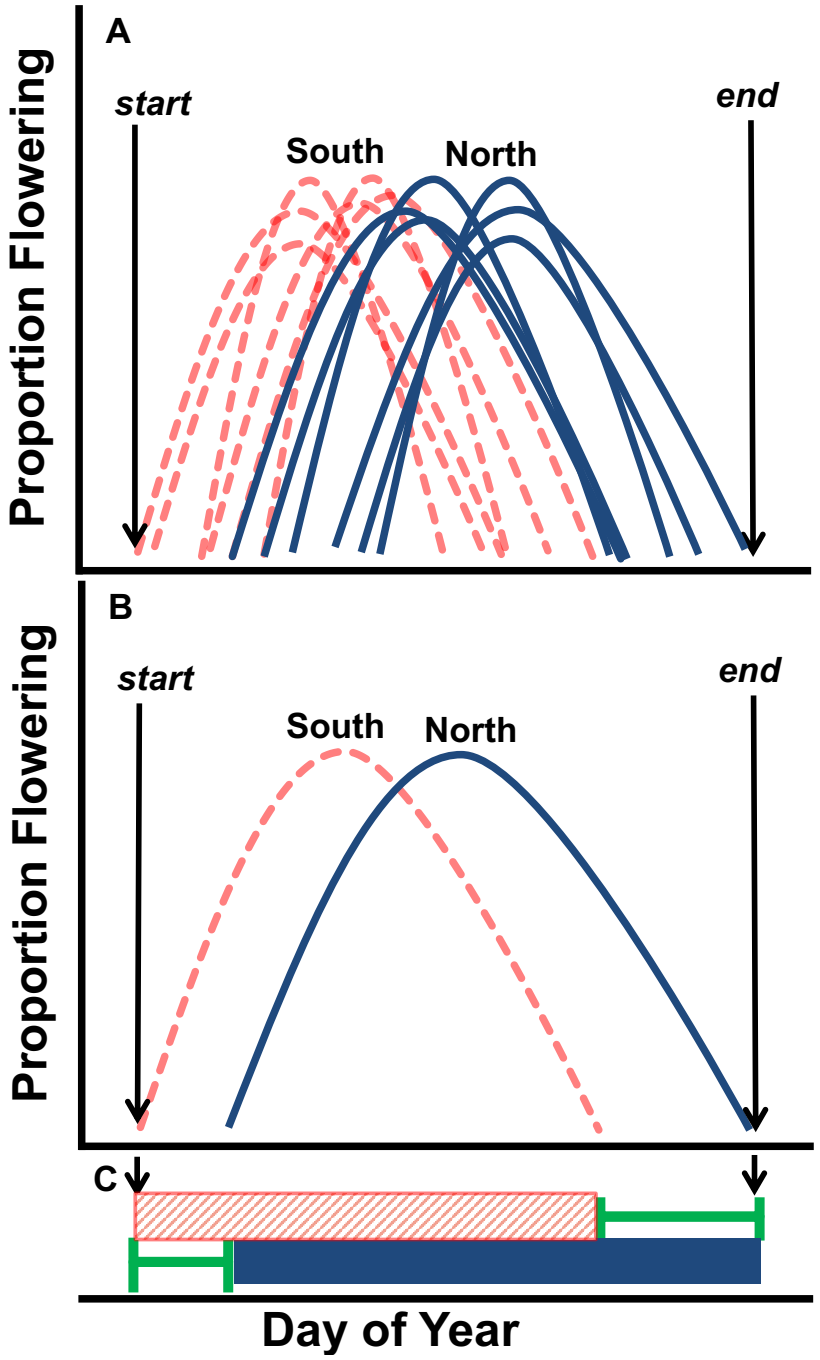

Figure 2. Theoretical overlap of flowering time on two adjacent opposite facing slopes. Proportion of flowering is (# flowers each date)/(total season flowers). Here light red (dashed/patterned) indicates south-facing, and dark blue (solid) indicates north-facing. (A) Individual species flowering curves on each slope, (B) cumulative flowering proportions of all species on each slope, and (C) overall flowering time duration, and extension due to complementarity. North-facing slopes in the northern hemisphere may have a delayed phenology due to lower amounts of incident solar radiation. The flowering time across these two adjacent areas on a landscape may yield a longer overall flowering time on the landscape than in either location individually due to complementarity (non-overlap) in resources. This extension in flowering time duration due to complementarity is shown by the bold green brackets (C).

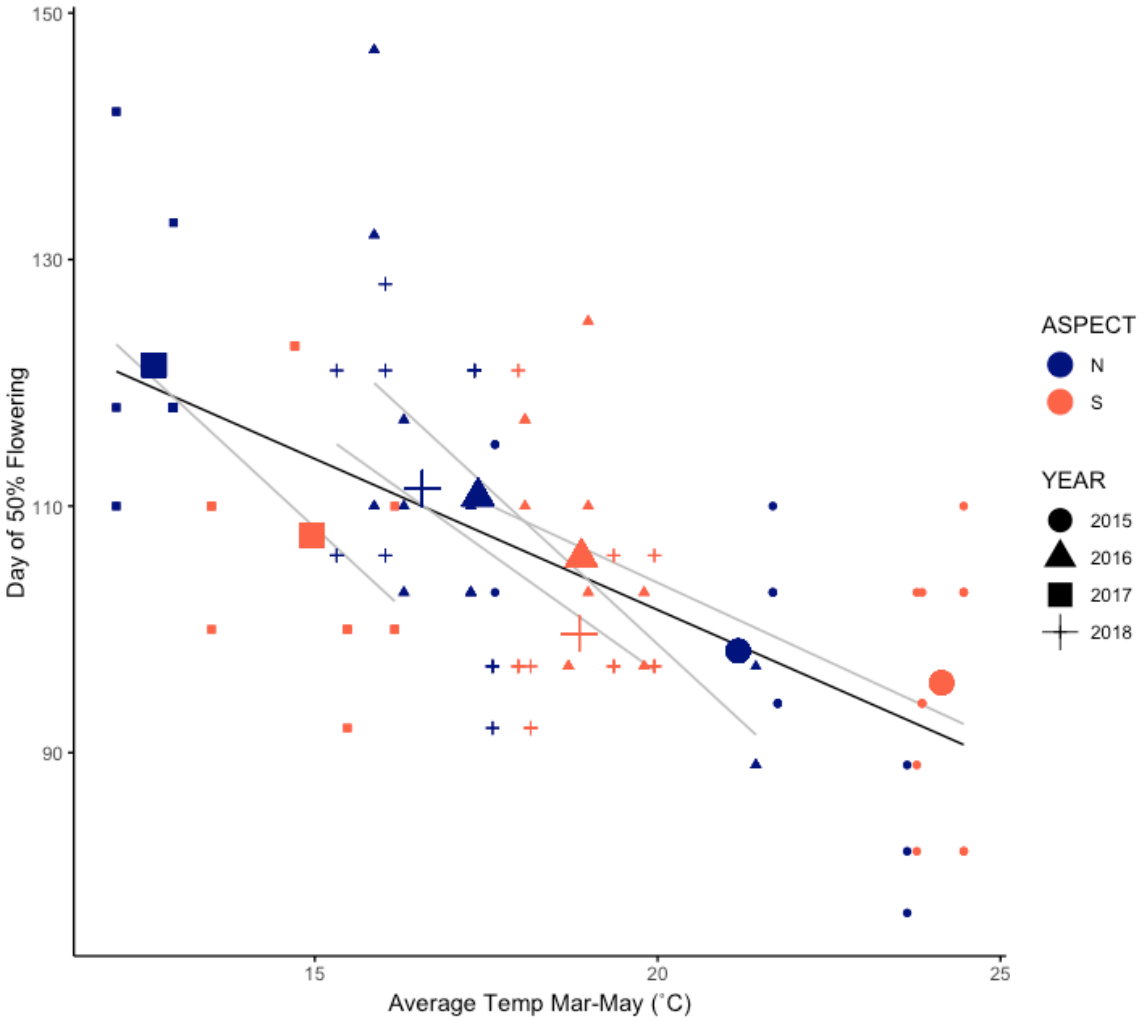

Figure 3. Date of mid-flowering by average temperature during the flowering season. Colors indicate the plot aspect: North (dark blue) and South (light red). Symbols indicate years: 2015 (circles), 2016 (triangles), 2017 (squares), and 2018 (crosses). Grey lines of fit are from simple linear regression of mid-flowering date by average March-May temperature in each year. Black line shows the overall relationship between the mid-flowering date of pollinator resources to average plot temperature during the flowering season across years (slope = -3.4, $R^2 = 0.55$, $p < 0.001$, [from full linear model with year and site included as covariates], marginal R^2 of temperature: 0.41). Larger points show overall means of each aspect(color)/year(shape) combination (e.g. blue triangle is the overall mean of North aspects in 2016) for visual comparison of overall aspect differences each year.

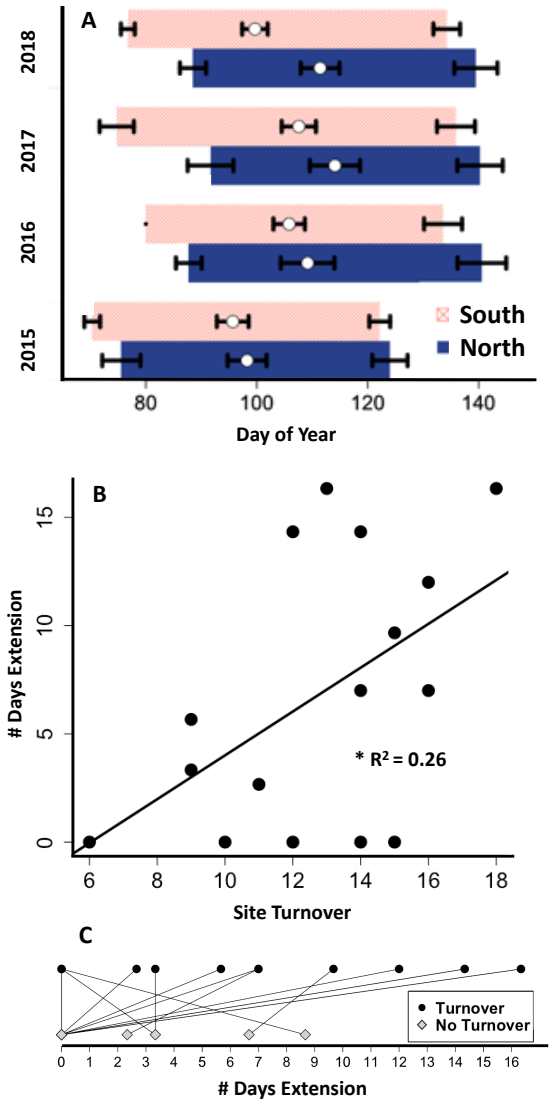

Figure 4. Topographic influence on flowering duration. A) Start (date of 5% flowering, left end), mid-flowering (date of 50% flowering, white middle points), and end (date of 95% flowering, right end) of flowering time of pollinator resources in plots on south (light red patterned) and north (dark blue solid) facing slopes in 2015-2018. Day of year (DOY): DOY 80 = 21 March, DOY 100 = 10 April, and DOY 140 = 20 May [+1 DOY for 2016 leap year]. Black bars show standard error of the mean. B) Number of days extension of pollinator resource flowering time by absolute site turnover. Turnover was calculated as the total number of species found on only one of the two aspects at a site in a given year (Turnover = # unique species on N aspect + # unique species on S aspect). Points show the number of days extension of pollinator resource timing at a site in the full community (all species, with turnover). C) Black points show the number of days extension of pollinator resource timing at a site in the full community (all species, with turnover), and grey filled diamonds show community flowering time extension calculated without species turnover (restricted to only pollinator resource species present on both aspects at a site). Line segments connect site/year pairs with vs. without turnover calculations. The number of days of extension was reduced in 10/16 sites, 4/16 remained the same, and 2/16 increased when turnover was removed from extension calculations.

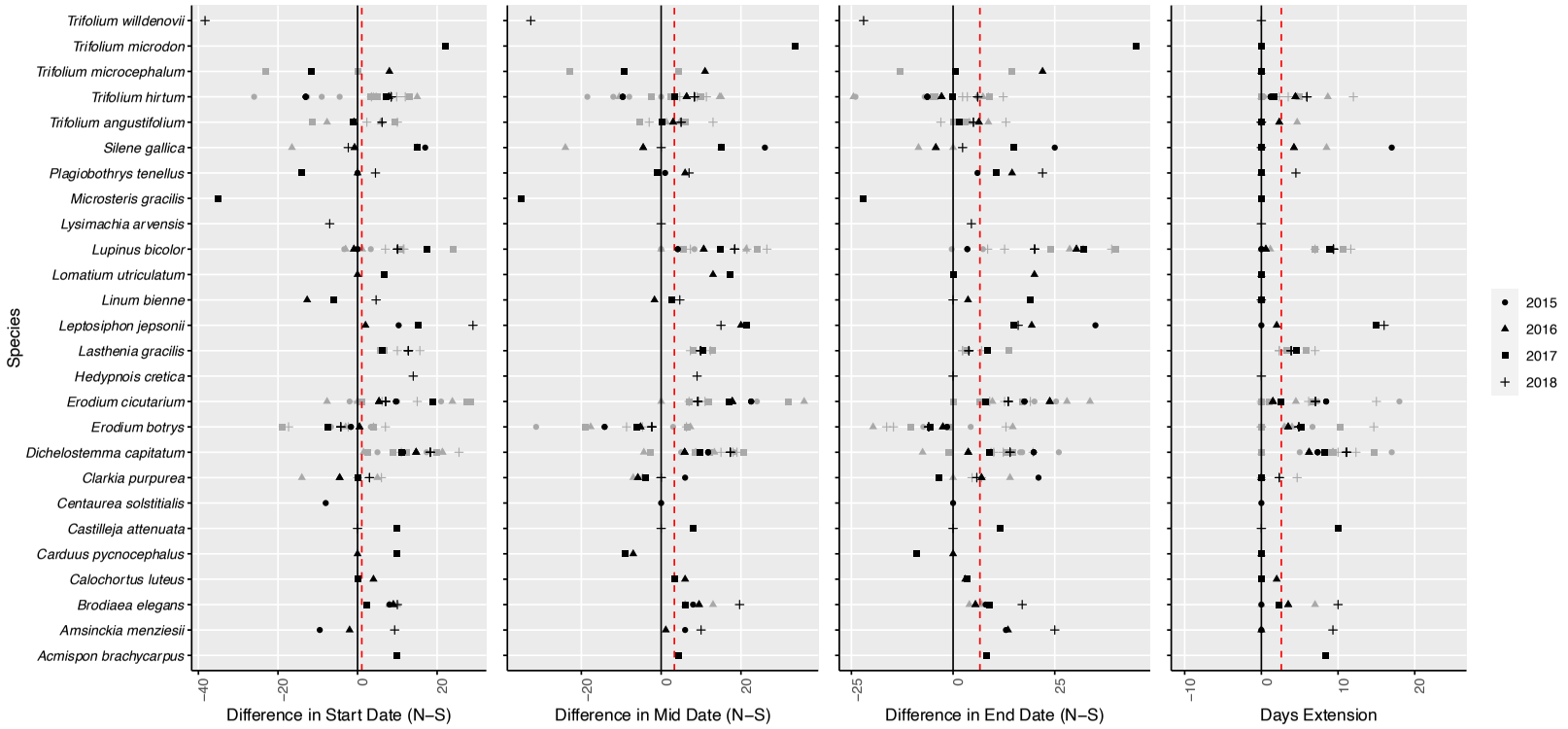

Figure 5. Species differences and flowering extension by site and year. Difference in mean (a) start (5% flowering), (b) mid (50% flowering), and (c) end (95% flowering) dates between North and South facing slopes (North date – South date), and (d) Number of days of flowering extension (Combined duration - longer slope duration) for pollinator resource species present on both slopes at a site. Grey points represent difference within each site within a given year, and Black points are mean difference among sites each year. Symbols indicate years: 2015 (circles), 2016 (triangles), 2017 (squares), and 2018 (crosses). Red dashed lines indicate the mean across species (weighted 1 value (mean across years) per species). In panels A-C positive values indicate earlier timing on south facing slopes, and later timing on north facing slopes. Values from *Lasthenia gracilis* are from 2017 & 2018 experimental plots where genotypic variation was controlled.

Tables

Table 1. Testing aspect influence on flowering time. Testing the inclusion of aspect as a fixed effect in the models, against the simplified model with aspect removed. Models include dates (Start [date of 1st 5% flowering], Mid-flowering [date of 50% flowering], and End [date of last 5% flowering]) as dependant variables, with year (Y) and site (S) as fixed effects. F ratios, P value, bold font, and asterisks ($p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *) indicate significance of including aspect in the model, as determined by testing the full model against the null model with aspect removed.

Phenology Measure	Model	Df	AICc	R^2m	R^2c	F Ratio	p value	
Start	null (Y+S)	8	714	0.335	0.290			
	Y+S+**Aspect**	9	679	0.552	0.516	42.7	**< 0.001**	***
Mid	null (Y+S)	8	729.7	0.445	0.408			
	Y+S+**Aspect**	9	722.6	0.497	0.457	9.1	**0.003**	**
End	null (Y+S)	8	750.6	0.303	0.256			
	Y+S+**Aspect**	9	748.8	0.333	0.280	4.0	**0.047**	*

Table 2. Overall community duration of season and extension metrics. Average number of days of flowering in plots on south facing, north facing, and combined slopes at a site. Days and percentage extension in flowering time (calculated as: (Combined-Longer)/Longer) averaged across all sites for (A) all pollinator resources, as well as (B) after removal of species turnover (limited to only including species present on both aspects of a site each year). (C) Difference in the number of days of flowering extension at each site every year ([All resources] – [Resources without turnover]). Negative values reflect cases where overall community flowering time extension across aspects was lower than the average extension due to within species differences.

(A) Community pollinator resources

Year	Mean South	Mean North	Mean Combined	Mean Days Extension	Range of Days Extension	Mean Extension
2015	51.8	48.4	58.4	4.42	0 – 14.3	7.9%
2016	53.6	52.8	64.6	7.25	0 – 16.3	12.7%
2017	61.2	48.6	70.1	8.33	0 – 14.3	15.2%
2018	57.5	51	65.3	7.17	0 – 16.3	12.6%

(B) Community pollinator resources, no turnover

Year	Mean South	Mean North	Mean Combined	Mean Days Extension	Range of Days Extension	Mean Extension
2015	40.58	45.67	50	3.83	0 – 8.7	8.63%
2016	51.67	50.83	54.58	0	0	0.00%
2017	40.83	42.5	53.08	0.58	0 - 2.3	1.00%
2018	49.58	48.33	56.33	1.67	0 – 6.7	2.72%

(C) Extension difference

Year	Site	Extension Difference (days)
2015	BH	0.00
2015	DP	-8.67
2015	FR	-3.33
2015	TP	12.67
2016	BH	0.00
2016	DP	9.00
2016	FR	2.00
2016	TP	16.33
2017	BH	0.00
2017	DP	4.67
2017	FR	14.33
2017	TP	12.00
2018	BH	0.00
2018	DP	3.00
2018	FR	2.67
2018	TP	16.00

Table 3. Duration of season and extension metrics split by species. Average number of days of flowering for species on south facing, north facing, and combined slopes at a site. Duration is defined as number of days from mean start dates (at least 5% in flower) to mean end dates (at least 95% in flower) within each species. Percentage extension in flowering time is calculated as: ((Combined-Longer)/Longer) for each species.
Pollinator resource species

Year	Mean South	Mean North	Mean Combined	Mean Days Extension	Range of Days Extension	Mean Extension
2015	17.7	25.9	30.9	3.77	1.6 - 5.8	14.1%
2016	16.2	21.9	28.2	2.37	0.7 - 3.5	14.1%
2017	16.9	19	26.6	3.59	0.8 - 6.3	16.0%
2018	21.2	22.5	31.7	5.35	4.5 - 6.7	19.6%

Table 4. Duration of season and extension with fixed genotypes. Average number of days of flowering in *Lasthenia gracilis* plots on south facing, north facing, and combined slopes at a site. Duration is defined as number of days from mean start dates (at least 5% in flower) to mean end dates (at least 95% in flower) for experimental plots of *L. gracilis*. Percentage extension in flowering time was calculated as: (Combined-Longer)/Longer) for all sites, in both years with *L. gracilis* experimental plots.

Lasthenia gracilis

Year	Mean South	Mean North	Mean Combined	Mean Days Extension	Mean Extension
2017	16.2	20.2	25.6	5.4	28.3%
2018	23.7	14.8	27.6	3.9	16.8%

Data Accessibility

Data are archived and publicly available on Dryad, DOI https://doi.org/10.6078/D1KX30
Data analysis code R scripts are available at: https://github.com/rlolliff/Flowering-time-across-topography

Chapter 3: Population differentiation in flowering time in *Lasthenia gracilis*, a widespread annual forb

Abstract

The timing of cyclical life history events (phenology) is dynamic, and phenological traits can vary across a species ecological and/or geographical range. Differences in phenology among populations in the field can be a result of genetic differentiation, variation in environment, or some combination of the two. Moreover, the influence of both genetic variation and plastic responses on population flowering time may be important to consider for conservation and management practices. In this study, I examine the drivers of phenological differences across a species range, focusing on common goldfields (*Lasthenia gracilis*, Asteraceae), a species that is widespread in California grasslands, provides an important pollinator resource, and is used frequently in restoration seed mixes. To test for population differentiation in phenology, I collected seed from 21 populations across the range of *L. gracilis* and examined variation in germination and flowering time under common growth conditions in a greenhouse. I recorded the germination date, the number of days from germination to flowering, longest leaf at first flower, and the total number of inflorescences across the growing season. I determine that populations of *L. gracilis* exhibit differentiation in flowering time, with earlier start of flowering in populations from warmer and drier locations (approximately 1 day earlier per 1 ˚C difference in mean growth season temperature). I then compared population flowering response in the common environment to field flowering records sourced from herbarium specimens. Population differences in flowering time in the common environment growth conditions were similar to field flowering records in response to site conditions, and were associated with climate variables in the same direction but with a shallower slope. This pattern of response reveals that both environmental and genetic differences influence flowering time in the field, and that these influences are aligned (i.e. co-gradient variation). Due to the existence of population differentiation in flowering traits, phenology may be important to consider in the design of conservation and land management plans, especially when sourcing *L. gracilis* seeds for restoration plantings.

Introduction

Phenology is a dynamic trait that can vary across a species range. Differences in phenology among populations result from genetics, the environment, and interactions between genotype and environment (Rathcke and Lacey, 1985; Elzinga et al., 2007). The balance of these responses is critical as suitable life cycle timing is vital for a species success in its environment (Rathcke and Lacey 1985). The timing of growth determines the environmental conditions experienced during a plant's lifetime, the availability of potential mates, the overlap of facilitative and competitive interactions between species, and interactions with other trophic levels (e.g., pollinators) (Pilson, 2000; Inouye, 2008), as differences in germination and flowering time can affect fitness (Inouye, 2008; Wolkovich and Cleland, 2011; Anderson et al.,

2012; Wainwright et al., 2012). Because of this, plants have evolved cuing mechanisms to determine adaptive flowering time (Grillo et al., 2013).

Plants initiate reproduction in response to temperature, precipitation, and photoperiod cues (Rathcke and Lacey 1985). Species vary in which variables trigger reproduction, and can respond to a combination of temperature, moisture or light in the environment (Rathcke and Lacey 1985). In addition, genetic responsiveness to changes in cues can vary within and among species (CaraDonna et al., 2014). Cuing mechanisms have been well studied in some crops and model plant systems, but for most plant species we know relatively little about the specific cues (or combination of cues) regulating phenological events (Davis et al. 2015).

The pattern and extent of phenotypic variation is determined by both genetic and environmental influences (Conover et al. 2009). Therefore, variation in flowering time across a species range reflects both plastic and genetic variation in response to environmental factors. Species and populations can exhibit plasticity in timing responses to environmental cues and/or be genetically differentiated in flowering time. Gaining an understanding of the mechanisms behind flowering time variation (i.e. the relative contributions of genetic variation and plasticity) can aid in interpreting phenology measures and observations (Inouye et al., 2019), and elucidate evolutionary responses of flowering time (and its plasticity) to climate and other environmental gradients.

Genetic differentiation within species is widespread in grassland species (Bucharova et al., 2017). Population differentiation in phenological events has been found in some cases, in both germination (e.g., Mummey et al 2016), and flowering time (Eckhart et al. 2004). Species can also vary in which life history events become differentiated across a gradient (Prendeville et al., 2013). Temperature and precipitation are important environmental drivers of phenology (Rathcke and Lacey 1985). In the Mediterranean-type climate of California, both temperature and moisture can play a strong role in cuing flowering time (Mazer et al 2015). Studies have found that both higher spring temperatures and drier winters can advance reproductive phenology of spring-flowering species (e.g., Love et al., 2019) In addition, patterns of variation in phenological traits among populations can be more complex than morphological characteristics in response to climate (e.g. Eckhart et al. 2004).

Plant species can alter reproductive timing to track environmental conditions, and phenological shifts in response to climate changes have been observed as alterations in the seasonal timing of growth, flowering, fruiting, and leaf drop (Menzel et al. 2006, Forrest and Thomson 2011, CaraDonna et al. 2014). Species that shift their timing as conditions change have increased in abundance and superior performance (e.g., growth and flower production) compared to less responsive species (Willis et al., 2008; Cleland et al., 2007). Intraspecific variation in phenological shifts is also likely; across a species range, differences have been documented in phenological cueing (Olmsted, 1944; Potvin, 1986; Olsson and Ågren, 2002; Mummey et al., 2016), shifts in response to climate (Morin et al., 2009), and responses to selection (Sheth and Angert, 2016) .

Gaining a better understanding of the phenological variation observed in the field is important, as differences may result in priority effects and altered community or ecosystem dynamics (Mummey et al., 2016; Rudolf, 2019). Research strategies to pull apart the influence of plasticity vs genetic variation on differences in field populations include a comparison of field observations with common garden studies, as well as reciprocal transplants. Plasticity, in the broadest sense, is environmentally induced phenotypic variation (Stearns 1989, Conner and Hartl 2004, Gianoli and Valladares 2012). Experimental studies can aid in determining whether

variation observed in the field is plastic or genetically fixed (Gianoli and Valladares, 2012). While it would be ideal to compare common garden data with field observations from across the range, this method is logistically difficult for phenological traits. Herbarium specimens have proven to be a powerful method for conducting analyses on field flowering time across species ranges (Willis et al. 2017; e.g., Hereford et al., 2017; Love et al., 2019). Herbarium collections can expand studies across time and space, giving unique understanding of responses to climate change, and enough statistical power to assess responses to multiple timing cues (Davis et al., 2015). Comparison of common garden data with herbarium data trends can reveal phenotypic plasticity of flowering time in response to environmental conditions in the field. Population differentiation data from common environment gardens can elucidate estimates of plasticity by establishing the presence and strength of population differentiation.

In this study I test for population differentiation in flowering time across the range of *Lasthenia gracilis* (Figure 1), explore contributions of plasticity behind flowering variation, and the possible implications for restoration and land management. Specifically, I address the questions: 1) How does source environment (provenance) influence days to flowering under common garden conditions? If there is genetic differentiation for flowering time across the species range, I expect to see a strong relationship between days to flowering in a common garden and the long-term average climatic conditions of the population source locations. 2) What is the relative contribution of genetic variation to the variation in flowering time observed in nature? If there is a contribution of plasticity, then I expect to see a greater range of flowering dates across climate gradients under field conditions than can be explained by genetic differentiation alone.

Predictions

Changes in flowering time across a gradient (e.g., temperature) can be due to phenotypic plasticity, genotypic differentiation, or both (Conover & Schultz 1995). If there is no genotypic differentiation, all variation in timing along a gradient will be due to plasticity. Where genotypic differentiation for a trait occurs, populations will exhibit differences in that trait when grown in the same environment. If populations of *L. gracilis* are differentiated in flowering time with respect to seed source location (provenance) climate, I will observe either co-gradient or counter-gradient variation (Figure 2). If genetic and environmental influences on flowering time are aligned, it will result in co-gradient variation, and result in accentuated variation across the driving climate gradient(s). In this case mean trait values will exhibit the same slope direction but less difference between populations when observed in common conditions, compared to variation in the field (Figure 2B). On the other hand, if genetic and environmental influences on phenotypes oppose one another, a phenomenon called counter-gradient variation, the change in mean trait expression will be diminished across the gradient in the field, compared to the common garden (Conover et al. 2009). Counter-gradient variation can occur for a number of reasons; for example, in cooler locations where metabolic rates are typically slow, it can be adaptive to have faster metabolic rates to counteract environmental drivers (Conover & Schultz 1995). Counter-gradient variation in flowering time would result in a larger difference between population mean trait values, and either a steeper or an opposite direction slope observed in common environment growth conditions (Figure 2D). Further, if environment has no influence on phenotype (e.g., no phenotypic plasticity), all phenotypic variation observed across the gradient would be due to genotypic differentiation, and the relationship of flowering time

between populations would be the same in the field as in common environment conditions. I have no a priori expectations about the patterns of differentiation in this species, and flowering time responses could be either co-gradient or counter gradient.

Materials and Methods

Study species

In this study I examine the flowering time in *Lasthenia gracilis* (Asteraceae; sensu Chan et al., 2002), or common goldfields, a widespread annual wildflower in the grasslands of California (Figure 1). This species is obligately pollinated, requiring cross pollination to set seed (Ornduff, 1966). Both flowers and fruit are important resources for wildlife, providing nectar and pollen for invertebrates, as well as seeds for harvester ants and kangaroo rats (Olney, 2008). This species also has cultural value as a nutrition source for Native American tribes (Bean and Saubel., 1972), and is commonly used as a groundcover in restoration plantings and land mitigation projects across California (Newton and Claassen, 2003; Montalvo et al., 2017).

It is important to note that the taxonomy of *L. gracilis* and close relatives has received careful study, and the group does present some challenges. In particular, *L. californica* and *L. gracilis* (sensu Chan et al., 2002) are difficult to distinguish morphologically, greatly overlap in their range in California, and produce fertile hybrids (Chan et al., 2002; Rajakaruna et al., 2003). Both species are often labeled as "*L. californica*" and are combined in seed mixes used in restoration projects (Montalvo et al., 2017). I was able to distinguish *L. gracilis* from congeneric *L. californica* collected from non-hybrid zones using a dissecting scope, as long as pappus was present on the seed. Dr. Bruce Baldwin (Integrative Biology, UC Berkeley), who was involved in resolving the *Lasthenia* phylogeny (Chan et al., 2002; Rajakaruna et al., 2003), helped confirm any unclear species ID as necessary. Populations without pappus could not be identified morphologically and were excluded from this study.

Initial assessment of flowering responses to climate – herbarium record data

I examined the flowering time of field specimens from herbarium records of *Lasthenia gracilis.* The species *L. gracilis* requires flowers or fruit to be identified to species, and plants are senescent/desiccated once fully in fruit. All herbarium specimens present in the University of California Berkeley and Jepson Herbaria (UCJEPS), as well as all imaged specimens available online through the Consortium of California Herbaria (CCH2), had flowers present, so I considered all herbarium records as an occurrence of "in flower".

Collection data were cleaned and georeferenced using methods described in Baldwin et al., 2017. The date of specimen collection was converted into a day of year of collection (from 1 to 365), and specimens with only year or month of collection recorded (e.g., collected in 1999 or April 1999) were excluded from the final dataset. Duplicate collections (same location and date) were removed. For comparison analyses with the 30-year climate normals, data were further restricted to the years included in the climate dataset (1981-2010).

I examined trends in collection date and temperature over time, as well as the trend of collection date against site temperature and precipitation. I then used multiple regression models to examine the relationship between date of collection and temperature and precipitation records from collection year. I examined responses to mean annual temperature and precipitation in the

year of collection. Precipitation data were log transformed for analysis. Temperature trends over time were also examined for each collection site.

I also examined the relationship of collection date with long-term site means (30 year normals 1981-2010) of using growth season (December- April) temperature and precipitation. Multiple regression models were fit with both temperature and log precipitation. Slope estimates from the multiple regressions were then used to compare trends with common environment flowering data (described below). Climate data used were downscaled gridded temperature and precipitation records from PRISM (Parameter-elevation Regressions on Independent Slopes Model) at 4 km resolution (PRISM Climate Group, 2020).

Seed collection

Seed from 21 populations of *Lasthenia gracilis* were collected from across its range in California (Figure 3), though only 20 were used in the final analyses due to greenhouse mortality. Populations were chosen to span temperature and precipitation gradients, both early and late in the growing season (Figure 3). The coastal to inland gradient in California influences temperature seasonality, with coastal areas exhibiting reduced seasonality with warmer winters and cooler summers. This seasonality can be observed as the triangle shape in the climate space spanned by temperature (Figure 3A). I attempted to collect seed from both coastal and inland populations across the range, in order to span the gradient in seasonality as well.

Infructescences were collected haphazardly from throughout each site to maximize collection of seeds from different genetic individuals. Seeds from 20 individuals were collected and maternal lines were kept separate. Seeds were stored inside at room temperature until being placed into cold and wet stratification for germination the next year. Voucher specimens of individuals from each population were collected and are located in the University of California Berkeley Herbarium (Specimen IDs: UC2061402-6, 8-9, 11, 14-15, 17-18, 20-22, 26, 30).

Test of population differentiation - Common garden study

All populations were grown in the greenhouse from seed, using 13 maternal lines from each population (seed from 13 different individual plants in the provenance site). Seeds were first germinated in a dark cold room (temperature 5 ˚C) on wet filter paper in petri dishes (using deionized water). These were checked daily for germination, and germinated seeds were transferred into wet soil in the greenhouse on the date of germination. To reduce bias, germinated individuals were chosen for planting at random on each date, and randomly replaced if germination occurred across multiple dates.

Three germinated individuals from each maternal line were transferred into the soil, then reduced to one randomly selected individual from each line after two weeks. Plants were grown in a greenhouse in bottom-watered trays to mimic wet spring soil conditions. Temperatures in the greenhouse were kept between 18 and 35 ˚C. I used a soil combination of Peat Moss, Coarse Perlite, and Dolomite Lime (Sunshine Mix #4; Sun Gro Horticulture, 2005), with cotton in the bottom of the planting cones to wick up water and prevent soil leakage from the bottom of the cone. The level of water was kept at the lower 1/8 - ¼ of the planting cones, high enough for the water to wick up into the soil, but not waterlog the soil. Bottom water was reduced to tip of the cone (bottom 1 cm) on April 15th and then removed entirely May 1st to simulate summer dry down. Populations were randomly assigned to trays, and trays were rotated weekly. Plants

germinated during 9 February – 6 March and were grown in the greenhouse until all plants had senesced in the end of May.

Inflorescences were scored every 2-3 days to quantify the number of days from germination to first flower, peak (max # inflorescences) flowering, and end of flowering for each replicate. Longest leaf at first flower was also measured (to the nearest 1 mm) using a ruler. Not all maternal lines survived to flower in the greenhouse, so only populations with at least 8 maternal lines were included in the analyses (n = 20 populations). To maintain a balanced design, if more than 8 maternal lines survived to flowering in the greenhouse, only 8 randomly selected lines were used in the analyses.

Days to 1st flower was counted as the number of days from germination date to the date of anthesis of the first flowers. Number of days to peak flowering time was counted as the number of days from germination until the maximum number of inflorescences were in flower, and end dates as the number of days from germination until all inflorescences were senescent. Leaf length at first flower was also measured.

I used random-effects models to test differences among source populations in flowering time (days to start and days to peak flowering). Because maternal lines were representative random samples from the source populations, and I were interested in the variance among lines and not the absolute differences in their means, I used an F test of the source population term (following Bennington and Thayne, 1994).

Multiple regression models were used to test differences in mean number of days from germination to first flower and peak flowering date, in relation to provenance temperature and precipitation. Since end of flowering was imposed by reducing watering, differences between population end dates were not analyzed. The relationship between provenance climate and leaf length at first flower was also tested. I used a Bonferroni correction for multiple comparisons to reduce the Type I error rate. I found no evidence of maternal effects in tests of seed weight or during a second-generation common garden experiment (Appendix II; E. Cox & R. Olliff-Yang, unpublished data), thus validating that the results of this study are due to population differentiation.

Comparison of greenhouse timing to responses of field flowering records

The timing of flowering herbarium specimens was compared to peak flowering in the greenhouse, based on the slope of the relationships between day of flowering and provenance climate. Herbarium specimen data, reflecting variation in the field, reflect both genotypic and phenotypic differences, while the common garden differences are only genetic, so any differences in slopes will reveal plasticity.

Results

Initial assessment of flowering responses to climate – herbarium record data

Flowering time of *L. gracilis* recorded in herbarium collections has advanced slightly over time, by an estimated 5 days on average over the past 100 years ($p = 0.006$; Figure 4A). Temperature and precipitation in year of collection were both predictors of flowering collection date. Flowering collection date was negatively correlated with provenance temperatures, with flowering specimen collection dates occurring approximately 4.7 days earlier in warmer years

and locations ($p < 0.001$; Figure 4B). Precipitation was positively correlated with flowering collection date ($p < 0.001$; Figure 4C). Within this varied temporal and spatial dataset, spring mean temperature records from site and year of the herbarium collections has increased moderately in most provenance locations over the past 100 years (Figure 5).

Flowering collection date was significantly associated with both temperature and precipitation (Table 1). However, the R^2 value was still quite low, explaining only 11% of the variation in flowering time (Table 1). Flowering was negatively associated with temperature, with approximately 4.3 days earlier flowering per 1 ˚C difference in yearly mean temperature ($p < 0.001$; Table 1), with variation in mean temperatures across the range of about 8 ˚C. Flowering collection dates were positively associated with provenance precipitation, indicating earlier flowering specimen collection in drier locations and years ($p = 0.00102$; Table 1). This indicates that populations respond to both site temperature and precipitation.

Test of population differentiation - Common garden study

I found that days to first flower differed significantly among populations grown in the greenhouse, but days to peak flowering was not significantly different (Table 2). Population differentiation in start of flowering was strongly related to provenance temperature and precipitation, with populations from warmer and drier provenance environments exhibiting earlier flowering (Table 3). Both timing of start and peak flowering were responsive to provenance temperature and precipitation; however, temperature was not a significant predictor of peak timing (Table 3). Longest leaf at first flower was also differentiated by population ($Chisq = 16.118$, $p < 0.001$), and exhibited significant relationships with long-term mean provenance temperature and precipitation, with shorter leaves in populations from more xeric sites ($p < 0.001$, Table 2).

Comparison of greenhouse timing to responses of field flowering records

Both herbarium flowering records and common garden start of flowering were significantly related to provenance temperature and precipitation (Table 3, Figure 6). Herbarium records of flowering time were approximately 2.97 days more sensitive to warmer average conditions than the timing of populations in common garden in response to provenance temperature (Table 3, field slope is 2.97 greater). Common garden population differences were similar to specimen collection dates to provenance precipitation (Table 3, 0.21 difference in slope estimates). Peak flowering time was significantly related to precipitation, but exhibited less response (shallower slope) when compared to herbarium collection precipitation trends.

Discussion

This examination of flowering time reveals that both population differentiation and plasticity have roles in shaping flowering time variation observed among populations of *Lasthenia gracilis*. Population differentiation in start of flowering time was related to provenance temperature and precipitation (Table 3, Figure 6), revealing the importance of both (or other correlated aspects of climate) in the evolution of flowering time in this species. Comparison of the common garden flowering time with herbarium data reveals that plastic differences likely play a role in differences in flowering time on the landscape. While a quantification of plasticity

would require an examination of flowering differences in response to yearly temperatures, the presence of a steeper trend in herbarium flowering response to provenance temperature qualitatively reveals that plasticity is involved in accentuating the differences between populations in the field. In addition, the regression slopes observed in herbarium record flowering time are in the same direction as the common environment flowering responses (Table 3, Figure 6), indicating co-gradient variation among this species.

The observed co-gradient response to temperature and precipitation, indicate that genetic and environmental influences on flowering time are aligned in *L. gracilis*. Co-gradient responses have been observed in other common garden studies. For example, in some species, populations from cooler regions exhibit longer growth periods before flowering (e.g. Bocher 1949; Reinartz 1984), and later emergence and first flowering dates (Kalisz & Wardle 1994) than populations from lower warmer provenance locations. However, this observation differs from studies that have reported counter-gradient variation. The species *Erythranthe cardinalis* exhibited fewer number of days to first flowering in from populations from cooler and wetter sites than populations from warmer and drier sites in common environment conditions (Sheth & Angert 2016). There have also been cases where flowering time exhibits both co-gradient and counter-gradient variation depending on the garden conditions, such as in the California endemic *Clarkia xantiana* subsp. *Parviflora* in wet vs. dry years (Eckhart 2004).

Differences in longest leaf at first flower also exhibited population differentiation, with smaller leaves at first flower in warmer and drier locations (Table 2). While I did not compare to length in herbarium collections, *Lasthenia* species exhibit differences in leaf morphology based on their ecohydrological niche (Forrestel et al. 2015). The pattern of smaller leaves in genotypes from more xeric habitats follows a similar trend in other species (e.g. Abrams et al. 1990). In addition, because both early flowering and smaller leaf length exhibit the same trend, this may reflect selection for early flowering favoring trait values associated with resource acquisition (e.g. high SLA) (Sheth and Angert, 2016). However, if smaller leaves indicate smaller plants at first flowering (e.g. *Circium vilgare* - Klinkhamer et al. 1987), this may also reflect a difference in carbon allocation strategies, and possibly in the point at which individuals switch from vegetative to reproductive growth (e.g. *Polygonum arenastrum* Geber and Dawson, 1990). In a test of individuals from six of the study populations, plant height was 62% correlated with longest leaf length at first flower (N = 72 [12 individuals per population]), but ranged from 8% to 79% depending on the population (E. Cox, unpublished data). Further examination of plant size allometry is needed to parse out these relationships.

The results of this study reveal that differentiation and plasticity play an important role in flowering time in *L. gracilis,* which may be true for other California species, in particular early spring annual wildflowers, and grassland species. Temperature and precipitation play an important role in the timing of annual species in California, as the dry hot summer of the Mediterranean-type climate imposes limits on the growing season (Dallman 1998). However, it is important to keep in mind that the degree of population differentiation can be species and trait specific (Bucharova et al 2017). There are also accounts of counter-gradient variation (Sheth & Angert 2016), as well as a combination of both co- and counter-gradient variation (Eckhart 2004), in phenological response to environmental conditions in other California wildflower species. Therefore, the trends and patterns of differentiation in this species should not be generalized.

Variation in herbarium specimen data is due to the combination of genotypic and plastic responses to environmental conditions. In this study, herbarium records were obtained from

many years of data collection and therefore also reflect any ongoing evolution in flowering responses during the collection years. It is likely, especially in annual species like *L. gracilis*, that some genetic change through time is also incorporated in the slope differences between field and common environment data.

Specimens are collected opportunistically, and collection date may not reflect ideal flowering time for the local population, but there is no reason to expect collectors would be systematically biased towards earlier or later dates. While herbarium specimen dates are not perfect phenology records, these data are robust indicators of field flowering time responses to provenance characteristics. Previous studies have found that herbarium records are good proxies for both start and peak flowering dates observed in the field, and relationships with temperature and precipitation are highly similar (Bolmgren & Lönnberg 2005, Miller-Rushing et al. 2006, Robbirt et al. 2011).

Management implications

Common garden experiments can aid in assessing the role that genetic diversity may play in climate change adaptation strategies (Harris et al., 2006; Guerin et al., 2012). Incorporating phenology differences into management and restoration practices may be essential for sustaining species interactions (i.e. pollination and seed dispersal), mitigating invasions, and retaining ecosystem function (Elzinga et al. 2007, Wainwright et al. 2012, Olliff-Yang et al 2020), and may influence the success of restoration projects (Mummey et al., 2016). Phenology may also be important to consider during planning for movement of species or genotypes. Assisted migration and targeted gene flow are increasingly proposed conservation methods to aid in populations in ability to adapt to novel climates (Aitken and Whitlock, 2013; Kelly and Phillips, 2016). These techniques have been suggested where there is concern that a species will not be able to adapt or disperse to new locations quickly enough for persistence (Hoegh-Guldberg et al., 2008; Early and Sax, 2011). Success of these strategies will depend on the performance of the introduced individuals, and phenology can make an important contribution to performance.

Provenance location influences flowering time and should be taken into consideration when choosing genotypes for restoration. Genetic differences in flowering time between populations may extend the duration of flowering time, as a mix of genotypes with different timings in one location will result in offset flowering and result in overall longer flowering (Figure 7). In this study, the greatest difference in mean start timing was approximately 17 days and the greatest difference in mean peak timing was approximately 15 days. Lengthened resource availability can support pollinator biodiversity, buffering possible phenological mismatch with pollinators (Olliff-Yang et al., 2020). Planting multiple genotypes in order to extend flowering duration may allow for system resilience by buffering the impacts of timing shifts, allowing for interspecific interactions to continue in the face of novel climates. In addition, as more genetic diversity (e.g., for reproductive timing) is present upon which natural selection can act, the ability of a species to adapt to new situations will increase.

Seed sourcing from non-local populations may be an issue if risks of outbreeding depression, genetic swamping, or novel genotypes leading to weediness are high (Wilson et al. 2016, Gross et al. 2016). Combining genotypes in one location also poses a risk of inadvertently combining cryptic species, which may reduce viability and disrupt genetic lineages (St. Clair and Howe 2007), so careful taxonomic work should be done before combining populations. However, gene flow can also have positive effects on fitness (Bontrager & Angert 2018), and

restoration (Gray et al. 2011) or revegetation of degraded lands (Sexton et al. 2013) can be opportunities to experiment with assisted gene flow in relatively low risk ecosystems.

Increased genotypic diversity may further enhance possibilities for synchrony, and therefore help to maintain ecosystem functioning. Restoration practices are already being developed to maintain floral and fruiting resources throughout the year to mitigate impacts of phenological shifts as the climate changes (Gardali et al. *unpublished*). These practices are aimed at increasing the probability that a generalist plant or animal will survive negative impacts if its timing becomes offset from the rest of the community. The research presented here suggests that this strategy may be possible to achieve for more specialized species via planting a diverse set of genotypes.

Figures

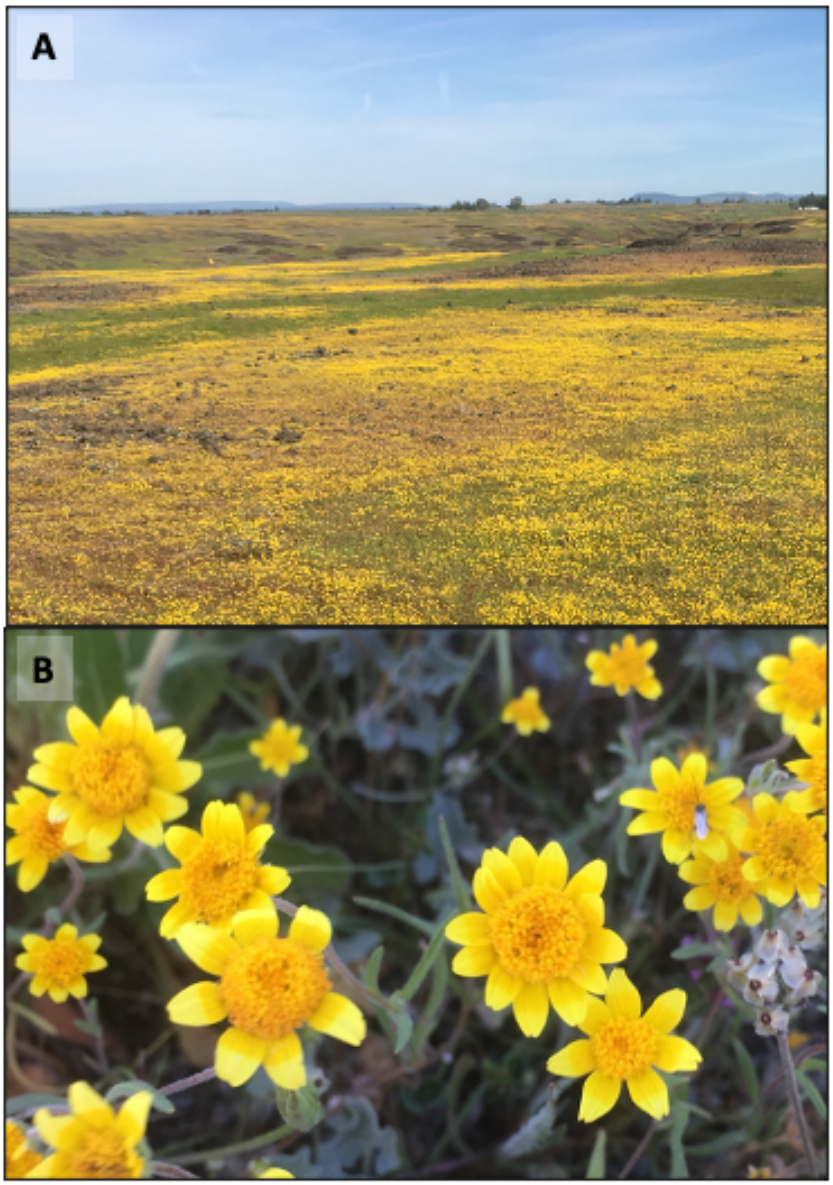

Figure 1. ***Lasthenia gracilis***. A) A golden grassland covered with flowering *L. gracilis* in the spring (North Table Mountain CA, April 2017). An example of how the common name "Goldfields" for the *Lasthenia* genus was inspired. B) close up of *L. gracilis* inflorescences. Photos by Alexander C. Yang.

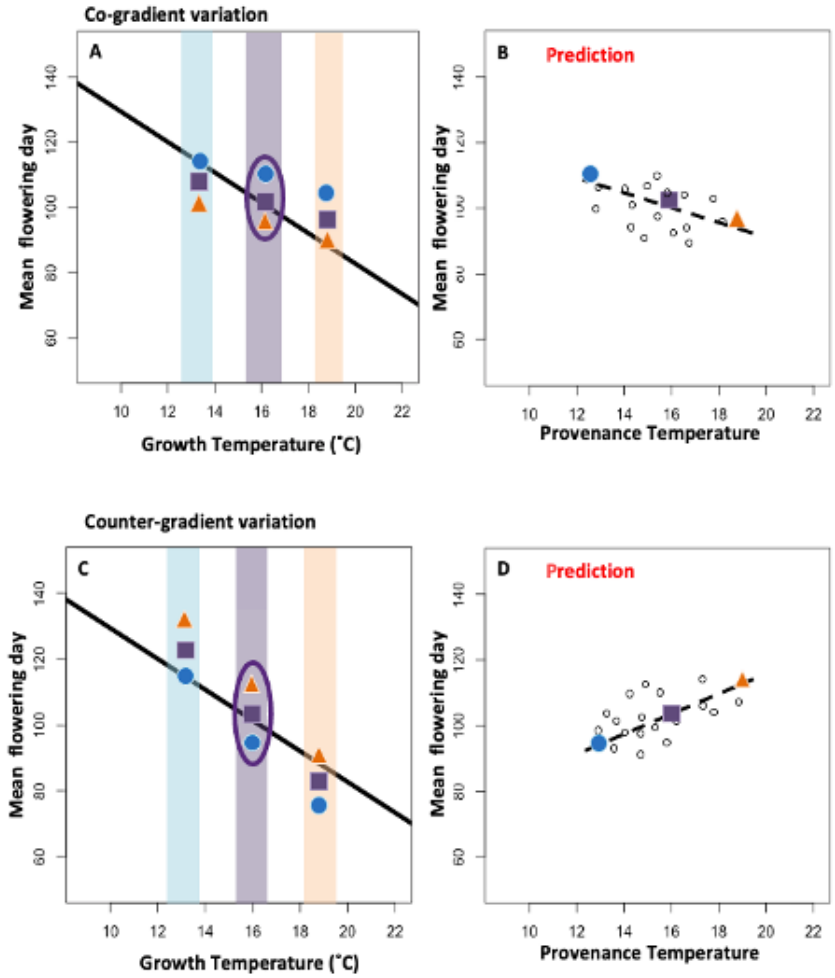

Figure 2. Co-gradient and counter-gradient variation predictions. Shapes indicate theoretical populations from different source environments (indicated by shaded boxes), and colors indicate provenance climate for each of the three seed sources. These include a blue circle from the coldest location (blue shading), purple square from the median temperature location (purple shading), and orange triangle from the warmest location (orange shading). Trend line in Left panels (A & C) show actual field indicates observed relationship between mean annual temperature (in the year of collection) and day of year of Lasthenia gracilis flowering from herbarium collections (See Figure 4B). Points on left panels (A & C) show predictions for the pattern of timing if populations were grown in each respective environment. If co-gradient differentiation exists (A & B), populations grown in the same environment will exhibit a similar pattern of timing as the field. If counter-gradient differentiation is present (C & D), populations grown in the same environment will exhibit the opposite pattern of timing than the field. In this example the common environment depicted in the right panels is the median temperature environment (purple), circled on the left panels. Right panels (B & D) show predicted pattern timing from coldest, median, and warmest temperature provenances for populations grown in common environment conditions among other seed sources (unfilled points) for both (B) co-gradient and (D) counter-gradient variation. Dashed line (right panels) indicates the association of genotypic differences with the gradient. Note: If there is no plasticity, the slope in both left and right panels would be identical. Alternatively, if no genotypic differentiation is present, the common environment will not show a trend (slope of zero).

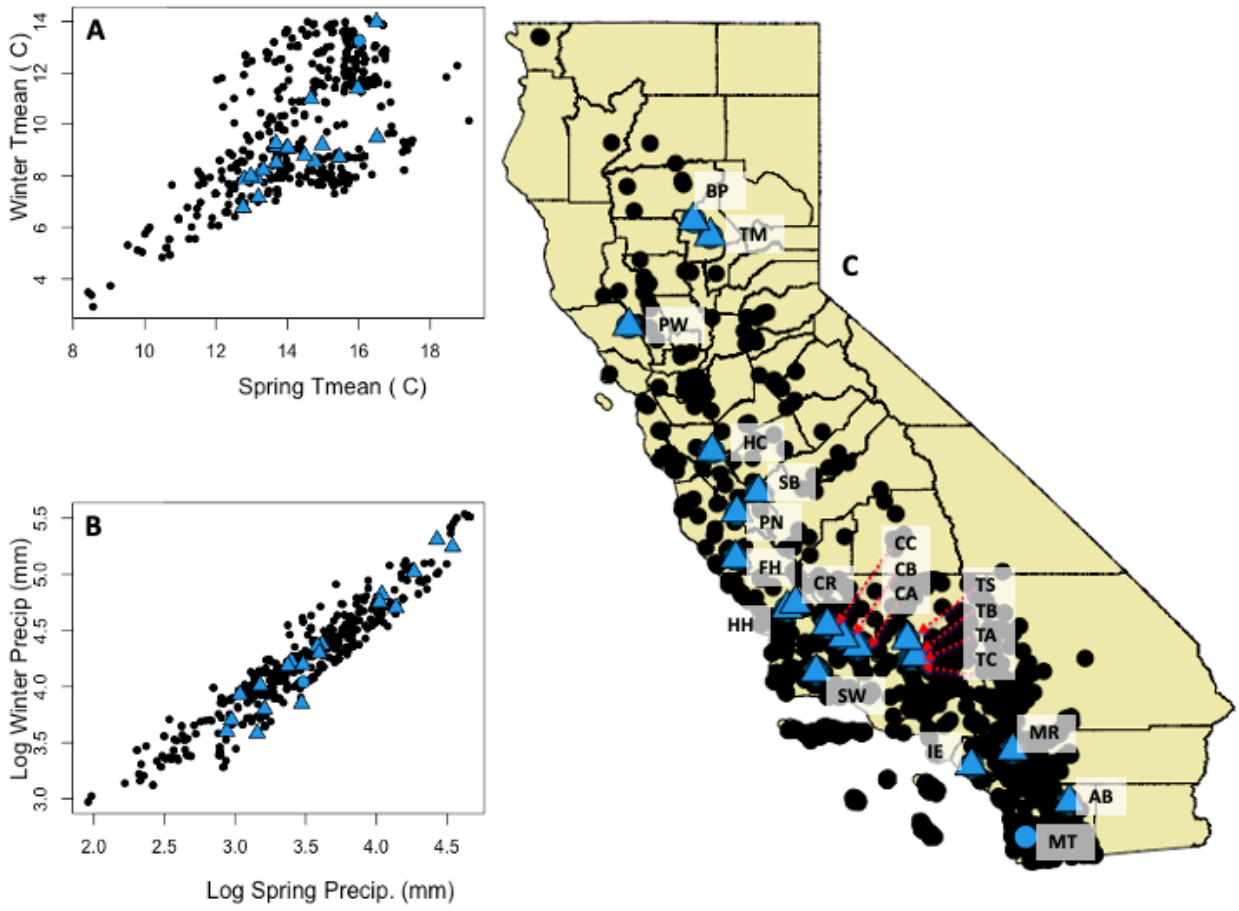

Figure 3. Provenance climate characteristics and species range in California. A) Temperature and B) log precipitation climate space of all Consortium of California Herbaria (CCH) collection records of *Lasthenia gracilis* (black), with provenance locations for the common garden in blue. Populations included in the analyses (with 8 lines surviving to flowering in the common environment) are shown as blue triangles. Winter indicates Dec-February and Spring indicates March-May long term climate averages over the 1981-2010 period. C) All CCH records of *Lasthenia gracilis*. Black points are herbarium specimen collection locations, blue points are seed collection locations (N = 21), with triangle points included in the analyses (N = 20).

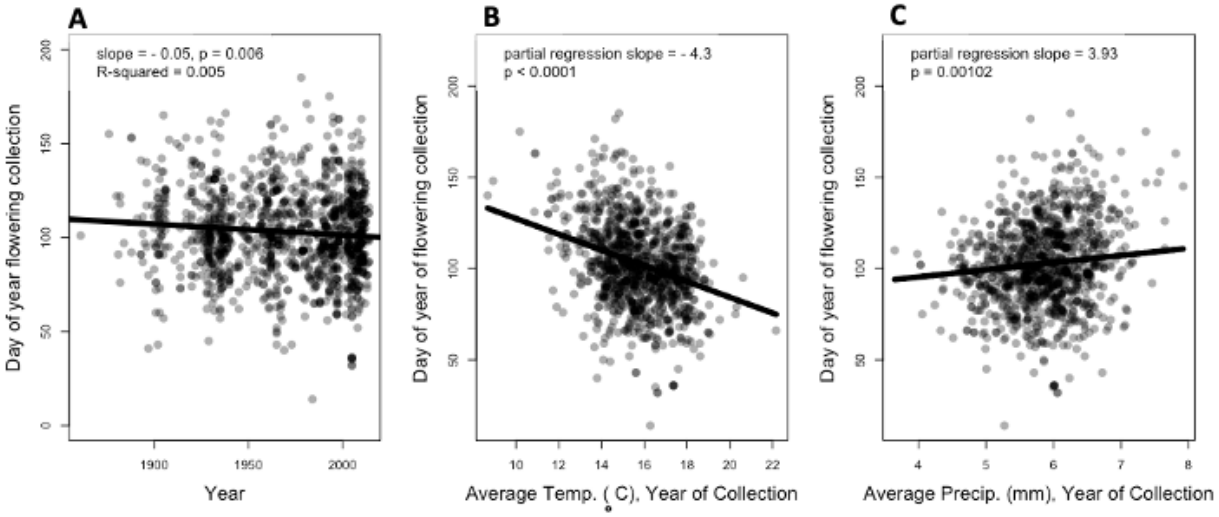

Figure 4. ***Lasthenia gracilis*** **flowering trends over time and in response to abiotic conditions.** Day of year of flowering from herbaria records A) over time, B) with mean temperature (˚C), and C) log of mean precipitation (mm) in the year of collection.

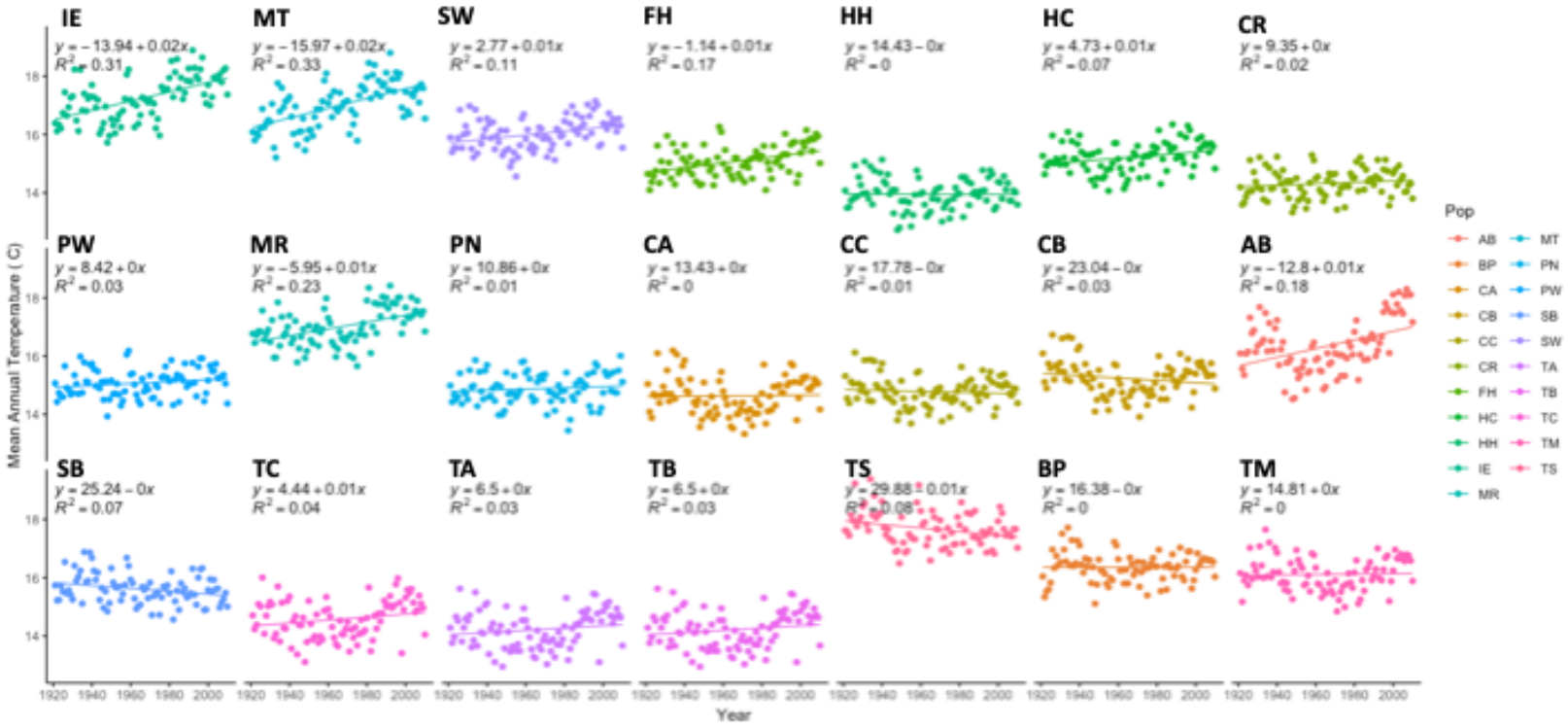

Figure 5. Temperature change in the provenance location of each collected population over 90 years (1921-2010). Color corresponds to population (also labeled with two letter code on each panel). Panels ordered by distance from the coast (closest to coast in top left panel, furthest in bottom right).

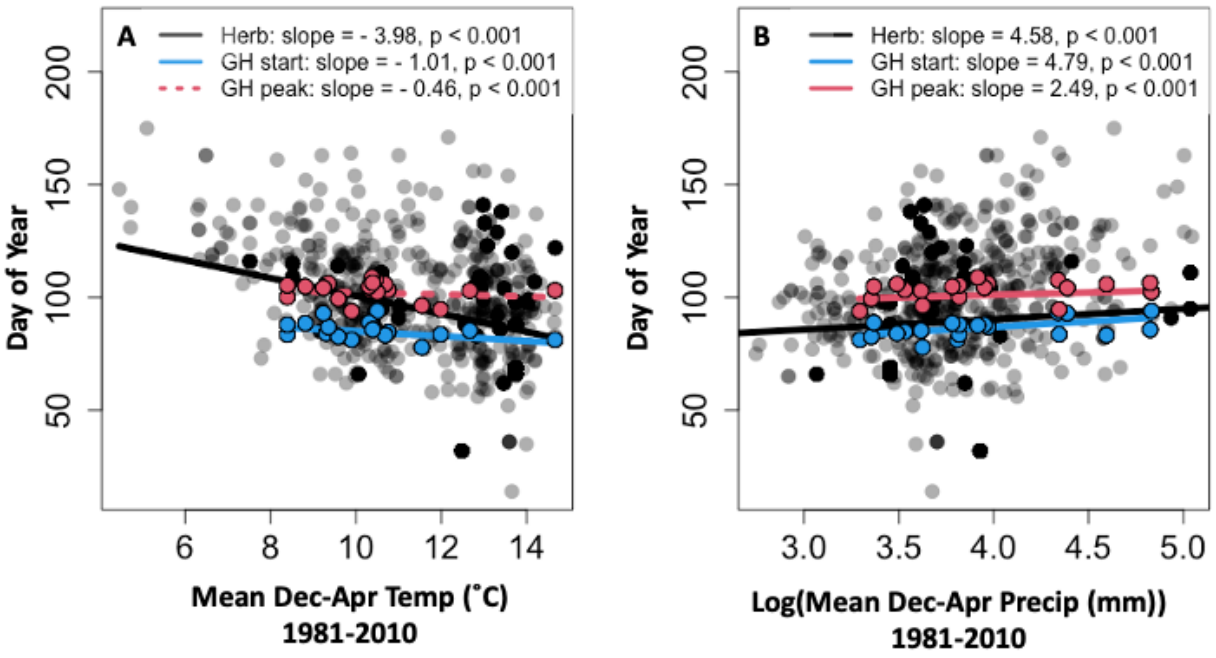

Figure 6. Common environment flowering time comparison to herbarium record timing. Average number of days to start (blue) and peak (red) in common growth conditions, and herbarium record flowering collection dates (black) related to average growing season (Dec-Apr) provenance (A) temperatures and (B) log of precipitation (30 year normals 1981-2010). Solid lines indicate significance at Bonferroni corrected alpha ($\alpha = 0.02$). Slope and intercepts for the lines in B & C plotted from the multiple regression model with both temperature and log of precipitation variables included (slopes and significance values presented in Table 3).

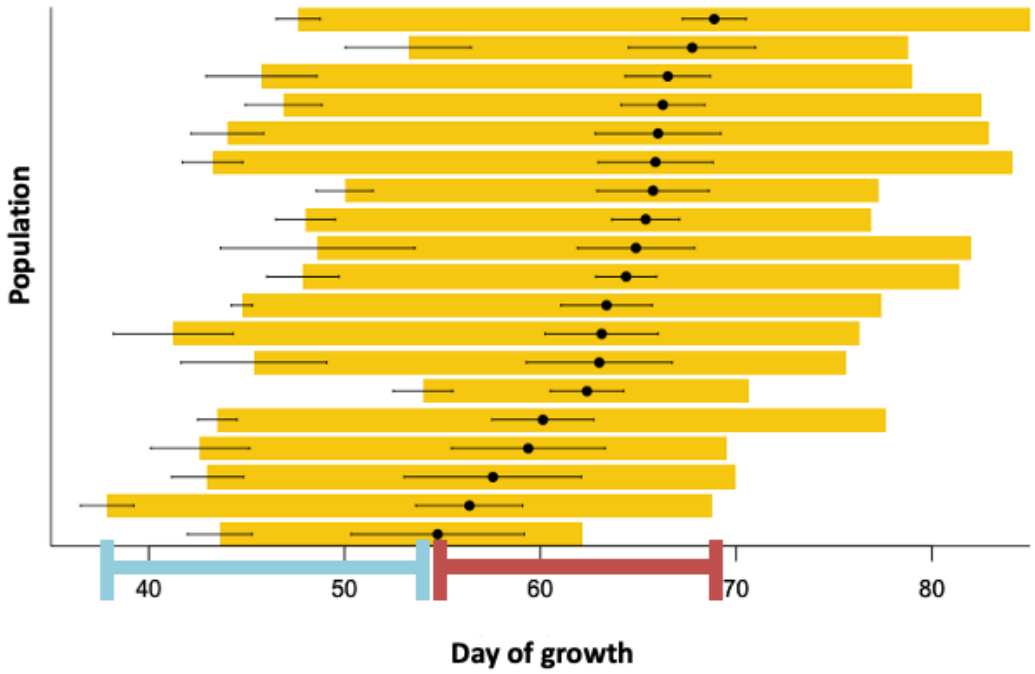

Figure 7. Genetic differences in flowering time between populations may extend duration of common garden flowering time. Start (left bar end) peak (black points), and end (right bar end) mean flowering dates. Date means are across 8 lines per population with standard error whiskers shown for start and peak dates. Populations arranged by average peak date. Light blue bracket indicates the greatest number of day difference in mean start timing (approximately 17 days) and dark red bracket indicates the greatest difference in mean peak timing (approximately 15 days) between populations.

Tables

Table 1. The relationship of herbarium collection flowering date to climate variables in the year of collection. Regression variables include annual mean temperature ("temp") and log of precipitation ("precip") in the year of collection. Model $R^2 = 0.11$, F-statistic: 66.6 on 2 and 1066 degrees of freedom, p-value: < 0.001.

Variable	Estimate	Std. Error	t value	p value
(intercept)	147.50	10.94	13.49	< 0.001
temp	-4.31	0.44	-9.91	< 0.001
log (precip +1)	3.93	1.19	3.29	0.00102

Table 2. Population differentiation in flowering time and leaf length at first flower. Differences in number of days to start and peak flowering by population, as well as length of longest leaf at first flower. Models with and without population as a random effect are compared. Bolded p values indicate significance of population at Bonferroni corrected alpha ($\alpha = 0.02$, 3 tests); (n = 20 populations).

Response	Model	AIC	BIC	Log Lik	deviance	Chisq	p value
Start							
	null	1052.9	1059	-524.45	1048.9		
	(1|Population)	1030.9	1040	-512.43	1024.9	24.05	**< 0.001**
Peak							
	null	1131.2	1137.4	-563.62	1127.2		
	(1|Population)	1129.8	1139	-561.88	1123.8	3.4623	0.063
longest leaf at 1st flower							
	null	664.77	670.9	-330.39	660.77		
	(1|Population)	650.65	659.84	-322.33	644.65	16.118	**< 0.001**

Table 3. Field vs. common environment flowering response to provenance climate. Multiple regressions of flowering record day of year (DOY) and common environment (CE) flowering time (# days to START and PEAK flowering; [n = 20 populations]) with average growth season (Dec-Apr) provenance temperatures (30 year normals 1981-2010). Bolded p-values indicate variable significance at Bonferroni corrected alpha (α = 0.02, [3 models]). See Figure 6 for visual slope comparisons.

Model/ Response:	**R^2**	**variable**	**Slope Estimate**	**Std. Error**	**t value**	**p value**
Flowering collection DOY	0.12	**intercept**	124.07	6.17	20.10	**< 0.001**
		Temp	-3.98	0.2333	-17.05	**< 0.001**
		log(Precip+1)	4.58	1.12	4.10	**< 0.001**
CE START	0.40	**intercept**	77.71	2.85	27.24	**< 0.001**
		Temp.	-1.01	0.18	-5.46	**< 0.001**
		log (Precip+1)	4.79	0.56	8.62	**< 0.001**
CE PEAK	0.13	**intercept**	97.73	3.33	29.37	**< 0.001**
		Temp.	-0.46	0.22	-2.15	0.033
		log (Precip+1)	2.49	0.65	3.84	**< 0.001**

Chapter 4: Time to flowering shortens with later planting dates, but at a cost to fitness

Abstract

Modifications in the timing of life-history events can alter the constraints experienced by organisms. Individuals can compensate for a change in timing of germination by modifying their growth and flowering time. However, biotic and abiotic factors may affect these compensatory responses. In this study I assess how biotic and abiotic differences due to planting date influence the timing of flowering, survival, and reproduction. To do this I manipulated the timing of the annual common goldfields (*Lasthenia californica*) in a serpentine grassland in Northern California, by seeding three times during the growing season (November, January, and March). Competition removal plots were compared with control plots to disentangle competition and seasonal priority effects (due to early individuals pre-empting resources) from abiotic influences on growth. Both planting date and competition treatments significantly impacted flowering time, growth, and reproduction. Later planting dates did delay flowering time, but this delay was minimal as flowering time was constrained within set biotic and abiotic boundaries. Competition removal moderately reduced the fitness cost of later planting dates, but only to a certain extent, revealing strong abiotic controls on flowering time and fitness in the spring. This study has implications for the timing of restoration projects, as planting time influences both the timing of flowering as well as the overall reproductive success of planted individuals. Our results suggest that practitioners should aim to plant earlier in the season. In addition, I find that heterogeneous planting time and competition levels can extend the timing of flowering on the landscape.

Introduction

Timing of growth and reproduction is critical for individual success. This is especially true for annual plants, as lifetime reproductive output of an individual is dependent on the conditions experienced over a single growing season (Cohen 1976, Schmitt 1983). In annual species, germination timing dictates both the biotic and abiotic conditions experienced throughout the lifetime of an individual, and therefore shapes an individual's fitness. Plants go through an initial juvenile phase of vegetative growth to acquire sufficient carbon reserves before flowering (Simpson and Dean 2002). Therefore, germination timing will also influence reproductive timing, with implications for community interactions.

Earlier germinating individuals benefit from a longer growing season and early access to resources (Lortie and Turkington 2002, Wainwright et al. 2012). Later germinating individuals may benefit from avoiding unfavorable conditions early in the season (e.g. frost)(Petru et al. 2006, Donohue et al. 2010, Mercer et al. 2011), and from temporal resource partitioning (Leverett et al. 2018). However, later individuals may experience harsh end of season conditions, such as early frosts or, in Mediterranean-type climates, drought and heat extremes. In locations with unpredictable season duration, annual plants are expected to exhibit a graded allocation strategy, where both vegetative and reproductive growth occurs during an intermediate period. This graded allocation strategy allows for bet-hedging and is predicted to yield optimal reproductive allocation when season duration is unpredictable (Wong and Ackerly 2005).

Plants respond to abiotic factors in the environment to initiate reproductive timing, and flowering can be triggered by temperature, moisture and/or photoperiod cues (Rathcke and Lacey 1985). With sufficient resource availability, an annual plant will initiate reproductive

growth after the abiotic cue(s) triggering flowering are experienced. Because nearby plants modify the temperature, moisture, and light in the surrounding environment, as well as available resources (Rathcke and Lacey 1985, Simpson and Dean 2002), biotic factors such as plant density, competition, and facilitation also play an important role in the timing of reproduction and can have strong effects on the evolution of life history timing (Ellner 1987, Metcalf et al. 2015). Therefore, abiotic and biotic factors can both influence flowering time, and can interact to shift the balance of facilitation and competition depending on the timing of germination (Leverett 2018).

In grasslands of Mediterranean-type climates, season duration is relatively unpredictable. In Northern California, precipitation is highly variable, and expected to become more so as the climate changes. Annual plant germination in California grasslands occurs after rainfall events of at least 15 mm (Heady 1977); these may come as early as October or as late as March, and the onset of the summer dry season is variable. This unpredictability suggests that plants in these systems should exhibit a graded allocation strategy, and a capacity to respond adaptively to variation in germination or planting dates.

In environments with limiting physical stresses, such as systems with ultra-mafic soils (e.g., serpentine), the importance of competition vs facilitation in neighboring plants can vary. Competition may be strong between species because resources are scarce (Tilman 1988), but facilitation may also play a role if neighboring vegetation can improve survival (Bertness and Callaway 1994). Because endemics to low-fertility soils are often poor competitors, these systems are generally thought to be refuges from competition (Tansley 1917, Sharitz and McCormick 1973, Kruckeberg 1984). Facilitation is predicted to be stronger in harsh environments, but the balance of competition and facilitation may vary over time, as seen in more productive systems (Leverett 2018).

In this study I examine the effects of differential seeding time on plant growth and flowering time, to elucidate tradeoffs in growth allocation, with the goal of informing restoration management practices. I explore how differences in biotic and abiotic conditions after germination (due to different planting dates) and influence the growing season, and influence the timing of the shift to flowering, and reproductive success in a widespread annual plant used commonly in restoration practices. Specifically, I address: 1) Does delayed planting time delay individual plant flowering time? 2) How does heterogeneous planting time influence population flowering time and duration? and 3) How does germination timing influence survival and reproduction with and without neighboring plant removal?

Material and Methods

Study site & species

The study species, common goldfields (*Lasthenia californica,* Figure 1), is an annual forb that is widespread in northern California. This species is commonly used in restoration projects, which occur throughout the winter and early spring. As an obligate outcrossing species (Rajakaruna and Bohm 1999), *L. californica* is dependent on pollinators for successful fruit set. This species also germinates quickly after exposure to cool wet conditions, which was ideal for establishing different germination cohorts. Populations of *L. californica* are found in a variety of different environments and habitats, including serpentine and nonserpentine grasslands.

This study occurred in a serpentine grassland at the UC Davis McLaughlin Natural Reserve in California (Lake Co. CA, 38.86007° N, 122.40806° W, 632 m elevation). Serpentine soils are formed from the metamorphosis of ultra-mafic mantle crust, yielding substrate that is high in heavy metals, low in essential nutrients, and low in calcium-to-magnesium ratios (Safford et al. 2005). Site location was chosen based on the proximity to the source seed collection, and knowledge of areas where the focal species, *L. californica,* is known to do well (C. Koheler, *pers. comm*.). This experiment was conducted during the 2016 water year, from November 2015 through June 2016. During this study the average daily temperature was 12˚C (minimum temperature -3.9˚C, maximum temperature 32.8˚C) and cumulative precipitation received was 268 mm (Western Regional Climate Center, Knoxville Creek), with a mean annual temperature of 15.9˚C in 2016 (Climate WNA, 2020). At this site, the average mean annual temperature was 15.2˚C and cumulative precipitation was 765 mm for the 1981-2010 time period (generated with ClimateWNA v4.62, based on methodology in Hamann et al. 2013), so the conditions during the year of this study were relatively typical but slightly drier than the long-term mean.

Seed was collected for this study in 2011 and 2015 from many (> 100) individuals in an adjacent meadow. Seeds were stored together in paper bags in at room temperature in a low humidity room until they were planted.

Treatments

I set up 18 randomized blocks of 6 30x30 cm plots. Each plot had a 10 cm buffer between itself and the next plot and the edge of the block. Blocks were placed in the meadow using stratified random sampling, with 9 plots being placed randomly on the north facing slope, and 9 on the south facing slope of the meadow to evenly account for aspect variation. One subplot of each treatment was included in randomized order within each block. Thermochron ibuttons were deployed in November into the center of each block to determine the differences in degree-days experienced by plants. These were set to record temperature every hour.

Two plots in each block were seeded on November 1st 2015, two on January 1st 2016, and two on March 1st 2016. Based on the growing season, the November planting date is referred to as “early” seeding and the March date as “late” seeding. In each plot 20 seeds were planted, and location of planted seeds was marked with pins. All plots contained 8 seeds from 2011 collection and 12 seeds from 2015 seed collection. During planting, small holes were made with sewing pins and seeds were placed into the holes using tweezers. The pins had large colorful heads and were left in place to ID planted seedlings. All three seeding cohorts were planted just before a rain event to stimulate germination.

To determine whether the change in flowering time was due to date of planting or to a change in priority effects or competition, each planting date had one plot that was clipped every two weeks before and after planting, and one control plot. Clipping at the soil surface did not eliminate all competition, but removed as much of the competition as possible without disturbing the soil. No plots needed to be clipped before the November planting date, as the meadow had burned in August 2015 and no germination had yet occurred. Treatment plots were set up in a randomized block design. Vertical plant height (to tip of tallest leaf) was measured, and number of inflorescences counted every two weeks. Survival to reproduction was calculated as the percent of germinated individuals that made it to flowering.

During survey dates the inflorescence counts were categorized as “flowering”, “recently flowering”, and “fruiting”. The “recently flowering” inflorescences were beginning to senesce (wilting and drying, with darker golden flowers and spent anthers) but could be assumed to be flowering during the week prior to the survey. Therefore, these inflorescences were given a flowering date of 1 week prior for the flowering time analyses.

The Asteraceae family produces inflorescences with few to many individual flowers on a head (Keil 2017). In *Lasthenia californica*, each flower has the potential to produce one seed, although this is dependent on successful pollination. The number of seeds per inflorescence is therefore dependent on both number of flowers produced (which can range from 3 – 50+) and on successful pollination. In this study the number of inflorescences was counted to estimate a proxy of reproduction and infer differences in fitness between treatments. I observed that individuals that were able to produce many inflorescences also often produced more flowers per inflorescence. Individuals with more inflorescences also had longer overall flowering duration, and therefore an increased likelihood of successful pollination events. Hence, I believe that counting inflorescences to approximate difference in reproduction is conservative.

There was some difficulty with seedling ID as *L. californica* seedlings can look very similar to other species (e.g. *Plantago erecta* before leaf hairs emerge). Although seedlings were marked by pins, if a similar species germinated very close to the pin, seedlings could have been confused. At the time of data collection, unclear species ID was noted. In addition, other *L. californica* individuals were present in ambient plots, and in some cases it was unclear which individuals were growing from the planted marked seed. All plants with uncertain ID or origin were removed for analyses.

Analyses

The effects of seeding date, competition reduction, and their interaction on flowering time, and flowering duration were assessed using linear mixed models, using block as a random factor (n = 18 blocks). Residuals were examined to verify that model assumptions were met. Importance and significance of the fixed effects (seeding date, competition reduction, and their interaction) in the models were determined using ANOVA model comparison in R, as well as AIC and BIC metrics. The effect of all treatments on the overall flowering on the landscape was also examined by looking at the differences in start and end dates and assessing complementarity between treatment plots.

The effects of seeding date, competition reduction, and their interaction on inflorescence production were also assessed using linear mixed models, using block as a random factor. Data for inflorescence production was log transformed to meet ANOVA assumptions of residual normality and homoscedasticity. Fixed effect significance and importance in the models were determined using ANOVA model comparison in R, as well as AIC and BIC metrics. All analyses were performed in version 3.31 of R, and mixed models were fit using the lme4 package (R core 2016, Bates et al. 2015).

Results

Treatment effects on growth and reproduction

Planting date and competition removal both significantly influenced growth rate.

Initiation of first flower occurred faster in plots with later planting dates, as the number of days from germination to first flower was significantly reduced ($p < 0.0001$, Figures 2 & 3). Maximum height was reached at a similar time of year in November and January cohorts, but delayed in the March cohort (Figure 2). Competition also shortened the number of days to first flower ($p = 0.03$), but there was a significant interaction between planting date and competition ($p = 0.002$), and the effect of competition was most pronounced in the March cohort (Figure 3A). The initiation of flowering seemed to require some minimum height threshold around 3 cm, as both January and March cohorts began to flower around this height, while November cohorts began flowering at a slightly taller height on average (Figure 3B).

Reproductive output was affected by both planting date and competition level. Later planting dates reduced the number of inflorescences produced ($p < 0.0001$), and the main difference occurred between the November and January/March cohorts in ambient plots. In competition removal plots, planting date only reduced fitness in the March cohort (Figure 3C). Competition had a strong effect on reproduction across all planting dates ($p < 0.0001$, Figure 3C). The survival of germinated individuals to flowering was not different between planting cohorts, but there seems to be a mild facilitative effect in ambient plots.

Treatment effects on flowering time and duration

Planting date and competition removal both significantly influenced the timing of flowering in the season. Later planting dates yielded later start, peak, and end dates of flowering in the year ($p < 0.0001$, Table 1). Competition removal plots also exhibited later flowering start ($p = 0.03$), peak ($p<0.0001$), and end ($p<0.0001$) dates. The effect of planting date on flowering start date was dependent on the competition treatment (interaction $p = 0.002$), and the main influence of competition reduction was observed in the March cohort (Figure 4A).

Later planting dates reduced the duration of average individual plant flowering. Both later planting time & competition shortened flowering duration ($p < 0.0001$), and the effect of planting date on flowering duration was dependent on the competition treatment (interaction $p = 0.03$). Examining the pattern of influence, the effect of competition reduction on flowering duration was most pronounced in the November cohort (Figure 4B).

Examining the combined effect of treatments on flowering duration revealed that planting both early and late cohorts led to longer flowering on the landscape overall, due to complementary flowering time between plots. Having staggered germination timing increased the duration of flowering on the landscape by approximately 11 days in ambient plots, due to staggered dates of flowering between cohorts (Figure 4A). Flowering duration was further increased when competition removal plots were also considered, with an increase of up to 26 days of flowering on the landscape with the staggered timing between both later planting and competition removal plots (Figure 4A).

Although the topographic treatment was not replicated, aspect did influence survival and reproduction in the treatments. Plots on the north-facing slope exhibited higher reproduction (Figure 5), which interacted with the effect of planting time. Inflorescence production was lower in general on the south aspect, and competition removal did not seem to ameliorate the effects of late planting.

Discussion

Planting time and competition both influence the flowering season of *Lasthenia californica*. I conclude from this work that flowering time in this species adjusts based on planting date, and that flowering duration can be extended by planting at various times in one location. However, later planting dates had a detrimental impact on both the survival and reproduction of individuals, resulting in lower overall planting success of later cohorts. Reduced competition treatments also extended duration of flowering, mainly by increasing the number of inflorescences produced by individual plants. Earlier plantings with competition reduction treatments are recommended for the most successful individuals, and extremely late planting should be avoided unless it is critical to extend flowering at the end of the season.

The strength of biotic and abiotic constraints on growth, flowering time, and reproduction varied across the season. Patterns observed in days to flower, date of maximum height reached, height at first flowering, start date of flowering, and inflorescence production (a proxy for fitness) all revealed nuances of the influence of abiotic and biotic constraints on plant allocation timing. However, despite the extreme differences in germination time, flowering time was not shifted as dramatically as might be expected. From this finding it can be inferred that abiotic constraints limit the capacity for flowering time shifts.

Delayed planting greatly reduced fitness, but this pattern occurred between different planting cohorts depending on competition removal treatment. Fitness decline occurred between November and later cohorts in ambient plots, but did not decline until the March cohort in competition removal plots. This pattern indicates that competition removal can ameliorate some loss of fitness due to late planting. Early growth was best for reproductive output, and therefore early planting time should be prioritized in restoration projects. It is important to remember that "fitness" differences here are based on total inflorescence production, and full reproductive fitness will depend on total seed set. The genus *Lasthenia* is obligately outcrossing (Rajakaruna and Bohm 1999), and fluctuations in the presence of pollinators throughout the season might further influence reproductive success. It should also be noted that there was less germination overall in the March planting cohort, but seeds planted late may survive in the soil and yield successful individuals in the next growing season. Tracking cohorts over multiple years is needed to determine any long-term impacts of planting time.

Later planting time shortened individual flowering duration, but this effect was dependent on the level of competition. Competition removal lengthened plant flowering duration, but not in the March cohort (Figure 4B). These interactions between planting time and competition treatments suggest that flowering at this location may be more constrained by abiotic factors at the end of the season (high temperature and low moisture) than at the beginning of the season. Flowering duration was cut short due to abiotic constraints at the end of the season regardless of competitive environment. This reflects the stress of elevated temperatures and drought as summer approaches in Mediterranean-type systems, which is the main constraint for survival and reproduction of annual plants in these regions (Larcher 2000).

Conversely, competition removal delayed flowering, an effect that was most pronounced in the March cohort (Figures 3A & 4A). This is contrary to the expectation that reduced competition will allow for larger plant sizes earlier in the season and an earlier switch from vegetative to reproductive growth (Rathcke and Lacey 1985). However, this supports the prediction that selection should favor individuals that accelerate flowering in the face of increasingly scarce resources (Callahan and Pigliucci 2002). Previous studies have also found

earlier flowering with increased density as a response to changes in light quality (Simpson and Dean 2002).

Plants compensated for the germination delay by speeding up development. Later planting dates shortened the number of days between germination and first flower (Figure 3A). A similar phenomenon has been noted in frog development with later egg hatch cohorts exhibiting faster development in the absence of priority effects (Murillo-Rincón et al. 2017). The shortened timing to reproduction reveals the tradeoff between vegetative growth and reproduction at the end of the season, likely due to increasing water limitation as summer approaches. Competition removal seemed to ameliorate this tradeoff slightly, as plants were able to begin flowering slightly later and produce more inflorescences in competition removal treatment plots (Figure 3). However, despite faster growth rates, there seemed to be a height threshold at the time of first flower, and flowering in the March cohort was delayed until individuals reached a height of approximately 3 cm (Figure 3B). This suggests that the switch to flowering is not entirely dependent on environmental cues, and that there is some genetically determined height threshold that must be reached.

Theory predicts that longer flowering duration can potentially ameliorate impacts of phenological mismatch on pollination mutualisms with climate change (Olliff-Yang et al. 2020). Therefore, based on our results, staggered timing in one location due to seeding time and competition reduction may also be a valuable conservation technique. Variable seeding time extended the flowering time in *L. californica* by an average of 11 days in ambient plots. Competition reduction yielded an average extension of 13 days. The extension from competition removal occurred mainly due to a lengthened end of season flowering duration, as individuals continued to produce inflorescences. Combining the two techniques in this location resulted in an average overall flowering time extension of 26 days. Therefore, these techniques may aid in increasing the overlap between flowering and pollinator presence, extending pollen and nectar resources for mutualistic flower visitors and enhancing reproductive assurance for the plants. However, the tradeoff of reduced survival and reproductive effort of later planted individuals should be taken into consideration.

During the study I observed additional heterogeneity in flowering time and reproduction due to block aspect. The blocks were evenly split between a north-facing and south-facing slope, and abiotic differences likely led to additional extension in the duration of the flowering season (e.g. Olliff-Yang and Ackerly 2020). Plots on the north-facing slope exhibited later and longer flowering on average. Aspect also influenced inflorescence production (Figure 5), interacting with the effect of planting time. Survival and inflorescence production were lower in general on the south aspect, and competition removal did not seem to ameliorate the effects of the March planting time. Although the topographic treatment was not replicated, this observation suggests that the success of later planting will depend on the abiotic conditions of a site. There may be a longer window of time to plant in cooler and wetter habitats, and planting in hotter drier parts of the landscape should be prioritized earlier in the season.

This study has implications for the timing of grassland management and restoration practices, as planting time and density changes (e.g., due to grazing) may influence both the timing of flowering and overall fitness of individuals. Restoration practices in California annual grasslands include planting seed from October through March. These results suggest that later planting may result in an unsuccessful growth season. The impacts of late planting may be mitigated by density removal treatments, but earlier planting dates should be prioritized. Grazing and mowing to reduce density should be approached with caution, as these treatments will not

have the same effect as neighbor removal, and this species and other natives may be adversely affected by grazing and clipping (Kimball and Schiffman 2003).

Figures

Figure 1. Close up of *Lasthenia californica* inflorescence and insect visitors. Ray and disc flowers visible on an individual in a serpentine meadow. *L. californica* is visited by many invertebrates for nectar and pollen including flies (left) and solitary bees (right). Photo by Alexander C. Yang.

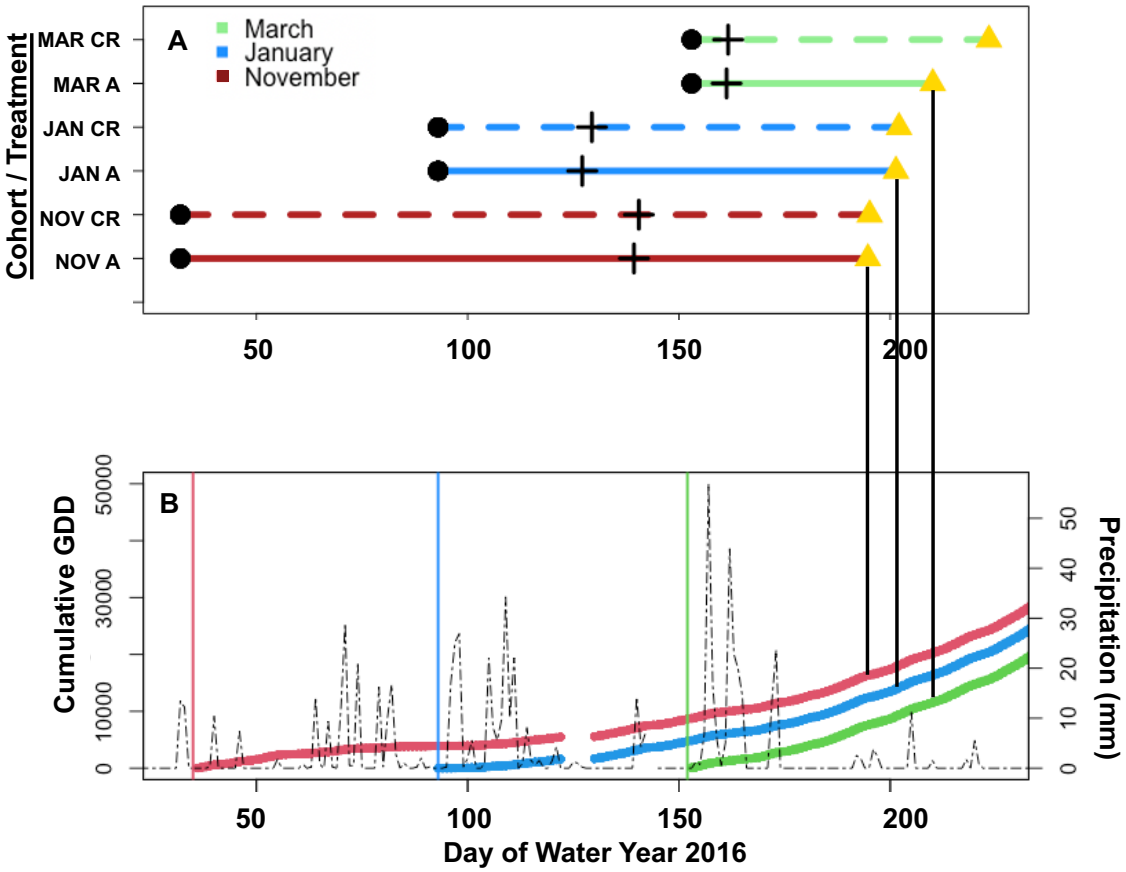

Figure 2. Timing of biotic and abiotic events. A) Growth and flowering time of *Lasthenia californica* in experimental plots. Circle points indicate planting dates for each cohort, plus signs note average date of maximum height reached, and golden triangles indicate average start of flowering . B) Growing degree day accumulation for each cohort. C) Precipitation events during the experiment. Vertical lines at the beginning of each accumulation curve indicate planting dates in November, January, and March. In all panels colors indicate planting time (Red = November, Blue = January, and Green = March). Black lines connecting graphs show where flowering start corresponds with growing degree days. Note lower accumulation of degree days in both later planted cohorts.

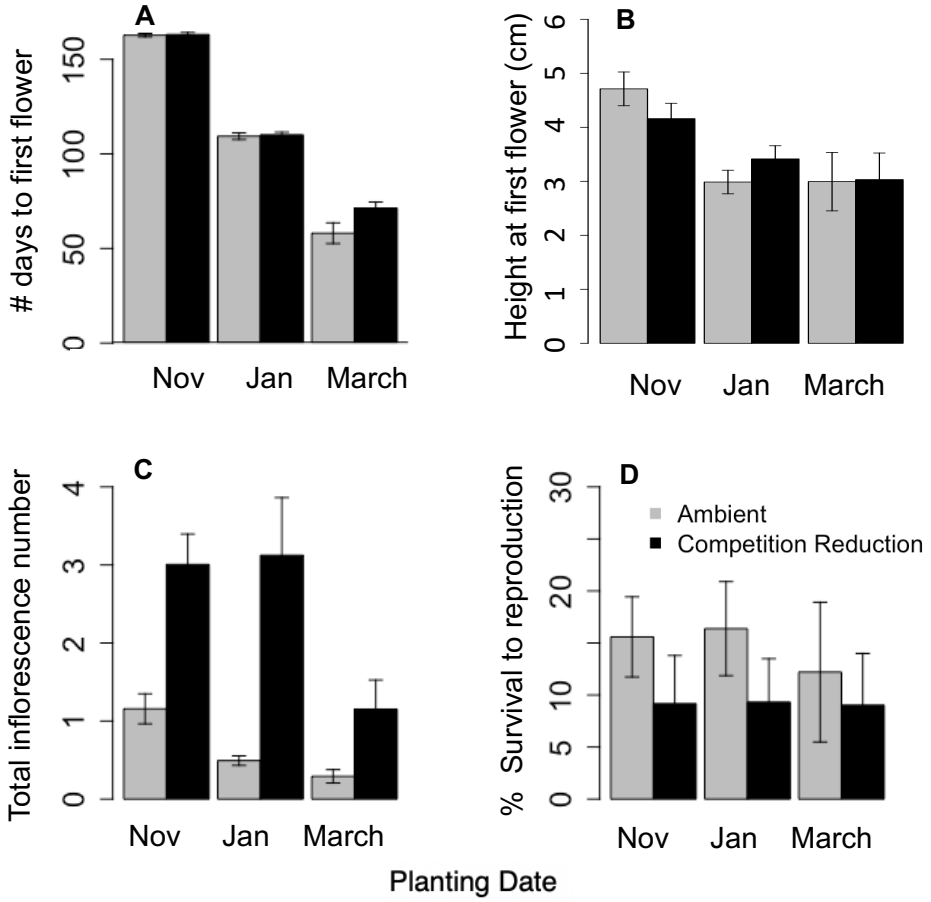

Figure 3. Treatment effects on flowering initiation and reproduction. Planting date and competition treatment by (A) number of days to first flower, (B) height at first flower, and (C) total inflorescence numbers. Grey bars show ambient plots, while black bars are competition removal plots. Whiskers show standard error.

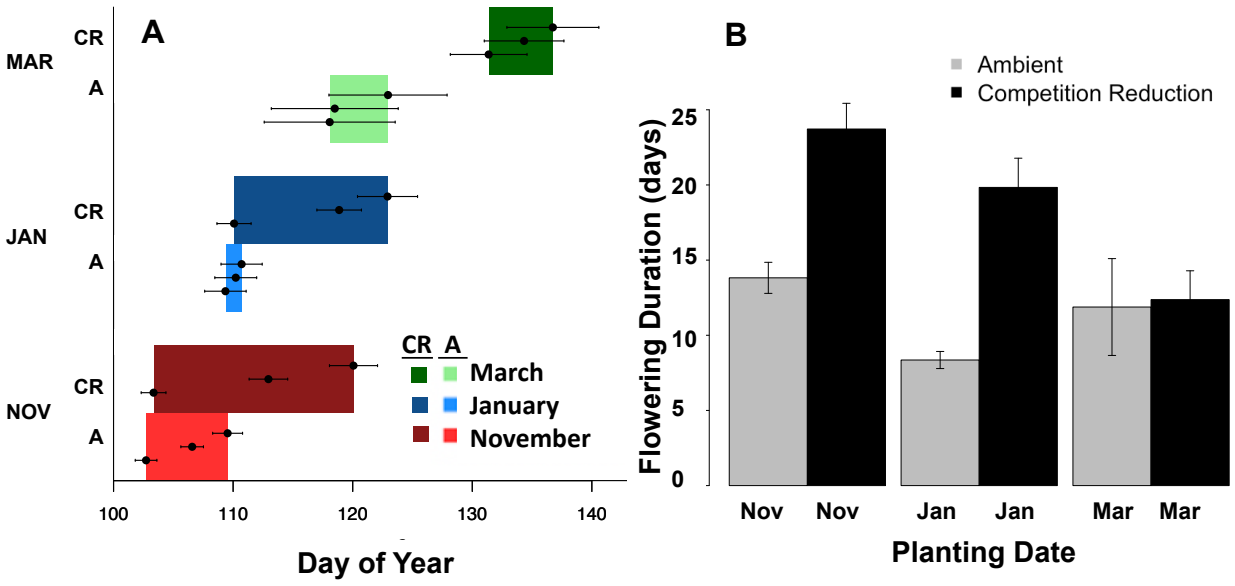

Figure 4. Flowering duration and season extension. (A) Timing of start (left points) peak (mid points) and end (left points) of flowering in treatment plots. Colors indicate planting date (red – 1 Nov, blue – 1 Jan, green – 1 Mar), and shading indicates competition treatment (ambient (A) – light, competition removal (CR) – dark). (B) Planting time and competition effect on season duration. Grey bars show ambient plots, while black bars are competition removal plots. Whiskers show standard error.

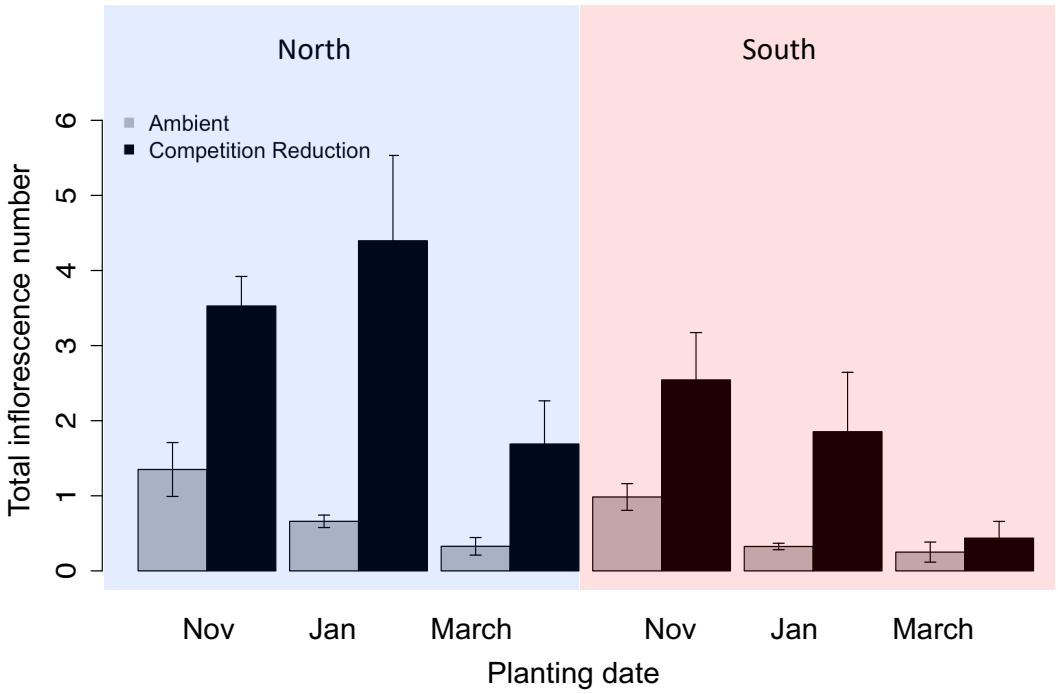

Figure 5. Effect of competition and planting time on fitness depends on aspect. North facing (blue) and south facing (red) slope effect on (A) total inflorescence numbers, and (B) percent of germinated individuals that survived to flower. Grey bars show ambient plots, while black bars are competition removal plots. Whiskers show standard error.

Tables

Table 1. Planting time and competition influences on flowering time - mixed effects model testing. Testing models of A) Start Dates and B) End Dates of flowering with and without fixed effects (1) Interaction of cohort and treatment effects, (2) treatment and (3) cohort explanatory variables. Block is included as a random effect in all models. Model comparisons (2) and (3) do not have an interaction in the model. P value indicates significance of including each fixed effect in the model, as determined by testing the full model against a model with the fixed effect removed.

A) START DATES

	Df	AIC	BIC	logLik	dev	Chi^2	Df	p	
1.	6	650.1	665.2	-319.0	638.1				
Interaction	8	642.0	662.1	-313.1	626.0	12.1	2	**0.00232**	**
2.	5	652.7	665.2	-321.4	642.7				
Treatment	6	650.1	665.2	-319.1	638.1	4.6	1	**0.03256**	*
3.	4	711.6	721.7	-351.8	703.6				
Cohort	6	650.1	665.2	-319.1	638.1	65.5	2	**< 0.0001**	***

B) END DATES

	Df	AIC	BIC	logLik	dev	Chi^2	Df	p	
1.	6	663.0	678.1	-325.5	651.0				
Interaction	8	666.6	686.7	-325.3	650.6	0.41	2	0.8154	NS
2.	5	701.5	714.0	-345.7	691.5				
Treatment	6	663.0	678.1	-325.5	651.0	40.4	1	**< 0.0001**	***
3.	4	694.6	704.7	-343.3	686.6				
Cohort	6	663.0	678.1	-325.5	651.0	35.6	2	**< 0.0001**	***

Conclusions

In this dissertation I examined different facets of the influences on flowering time and duration in California grasslands. First, I discussed the potential for maintaining and extending flowering duration as a possible technique to build adaptive capacity in plant-animal mutualisms to climate changes (Chapter 1). Then, I assessed how topographic influences of flowering time might impact flowering duration, and found that flowering time differences between north and south aspects served to extend the flowering season by an average of 4-8 days (8-15%), depending on the year (Chapter 2). This extension was due to both within-species timing responses as well as species turnover. Results from a greenhouse study (Chapter 3) revealed that both population differentiation and plasticity play a role in shaping flowering time variation observed among populations of *Lasthenia gracilis*. I found differences of up to 17 days in mean start timing in the common environment, indicating that genotypic diversity for flowering time could result in longer flowering duration. Finally, in a field experiment, I found that planting time and competition influence flowering time and duration in the species *Lasthenia californica* (Chapter 4). Variable seeding time extended the flowering time in *L. californica* by an average of 11 days, and competition reduction resulted in an average extension of 13 days. Including all planting dates and both competition levels led to an average 26 day extension of flowering duration at the site. These findings indicate that heterogeneous topography, inter- and intraspecific diversity, planting time and competition levels can extend overall flowering duration. In addition, each empirical study reveals how multiple factors can combine to further accentuate differences in timing.

This work bolsters our understanding of basic grassland ecology, and has implications for grassland conservation, management, and restoration practices. By examining the drivers of flowering time at different scales, these studies expand the breadth of knowledge about flowering phenology and influences on flowering duration in this system. In addition, these findings motivate conservation of diverse topography, species, and genotypes to maintain flowering time duration (and therefore animal resources) on the landscape.

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Appendix I. Figures and tables supplemental to Chapter 2

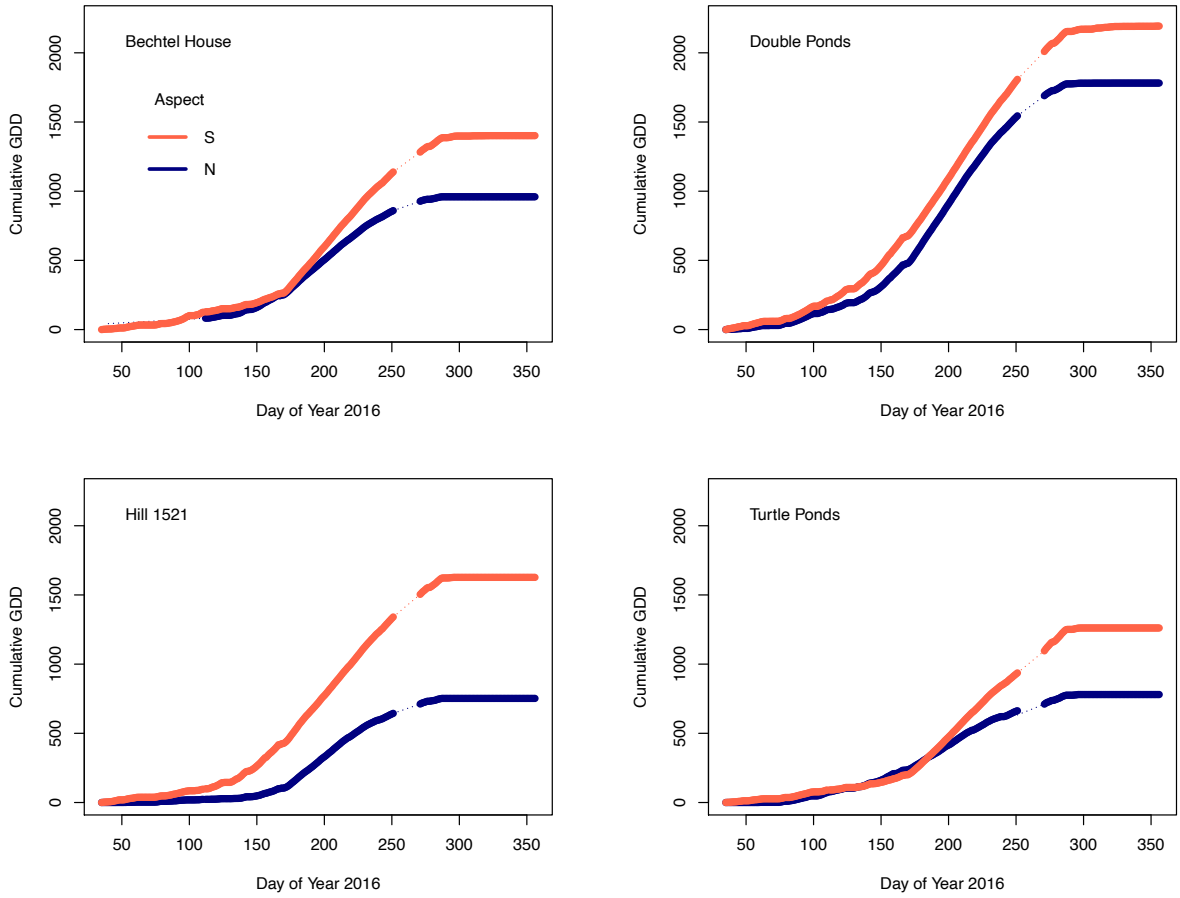

Figure 1. Growing degree day accumulation differences between south (light red) and north (dark blue) facing aspects in 2016. Faster increase (steeper slopes) reveal warmer slopes. Panel titles indicate site: Bechtel House, Double ponds, Hill 1521, and Turtle ponds. DOY 100 = 9 April, DOY 200 = 18 July, DOY 300 = 26 Oct. Degree days calculated with base temp of 5˚C; as ((Max T + Min T)/2) – 5)). Data presented is from 2016, as temperature data was only recorded from March - June in other years. Across all years south aspects accumulated more growing degree days during the growing season than N aspects more often than expected by chance (binomial test, $p < 0.001$).

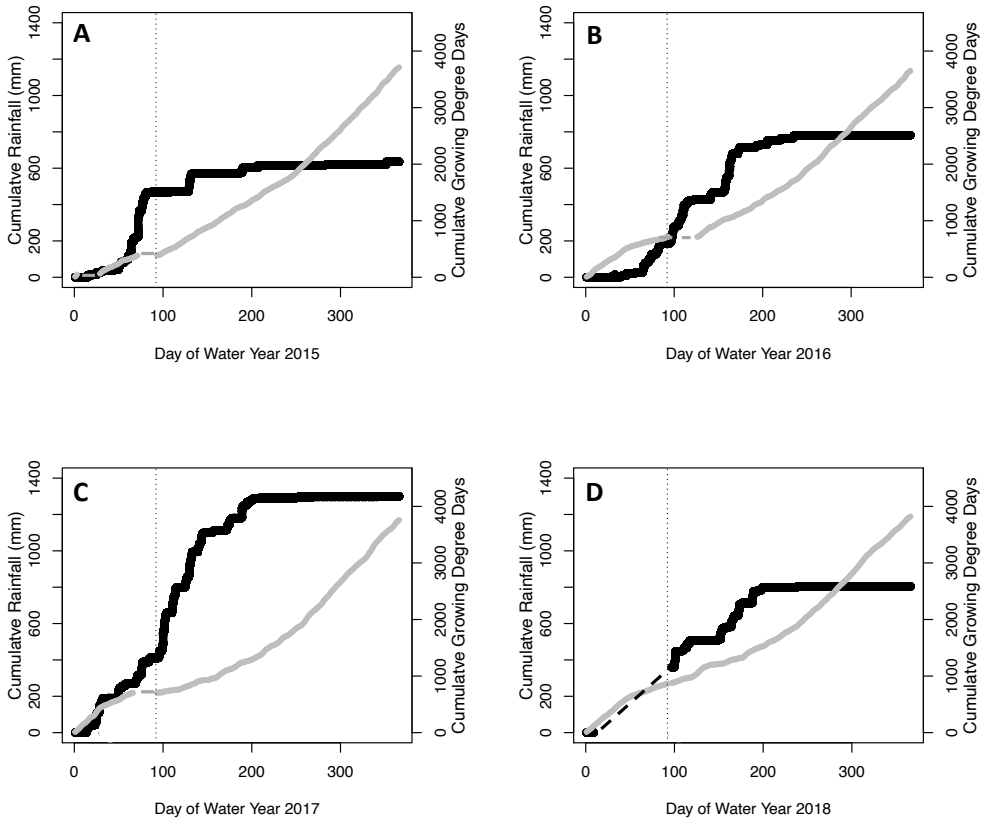

Figure 2. Temperature and rainfall accumulation in (A) 2015, (B) 2016, (C) 2017, and (D) 2018. Day of water year indicates days since 1 October of the previous year (e.g. 1 Oct 2015 begins the 2016 water year), as defining the water year in California (mediterranean- type climate with wet season beginning in October). Black line is rainfall accumulation in mm, and grey line is temperature accumulation in growing degree days (thermal accumulation above base temperature of 5 ˚C). Steeper slopes indicate periods of faster accumulation. Dashed connecting lines show periods of missing data. Missing rainfall data in 2018 water year was extrapolated based on total rainfall for the year. Vertical dotted line indicates DOY = 1 (January 1st) of each year, for reference to other figures.

Table 1. Site by year extension metrics. Average number of days of flowering in plots on south facing, north facing, and combined slopes at each site in every year. Days and percentage extension in flowering time (calculated as: (Combined-Longer)/Longer) at all sites for (A) all pollinator resources, as well as (B) after removal of species turnover (limited to only including species present on both aspects of a site each year). (C) Metrics split by species. Average number of days of flowering for species on south facing, north facing, and combined slopes at a site. Duration is defined as number of days from mean start dates (at least 5% in flower) to mean end dates (at least 95% in flower) within each species (averaged cross species for each site/aspect combination). Percentage extension in flowering time is calculated as: ((Combined-Longer)/Longer) for each species.

A) Community - all resources				**Mean**	**Mean Days**	**Mean**
YEAR	**SITE**	**Mean South**	**Mean North**	**Combined**	**Extension**	**Extension**
2015	BH	57.00	46.33	60.33	3.33	5.85%
2015	DP	54.00	42.33	54.00	0.00	0.00%
2015	FR	47.67	49.67	49.67	0.00	0.00%
2015	TP	57.00	55.33	69.67	12.67	22.22%
2016	BH	65.67	49.67	65.67	0.00	0.00%
2016	DP	54.33	54.67	63.67	9.00	16.46%
2016	FR	49.67	46.00	51.67	2.00	4.03%
2016	TP	56.67	63.33	79.67	16.33	25.79%
2017	BH	76.67	47.67	76.67	0.00	0.00%
2017	DP	65.00	44.33	72.00	7.00	10.77%
2017	FR	53.00	50.00	67.33	14.33	27.04%
2017	TP	50.00	52.33	64.33	12.00	22.93%
2018	BH	60.00	42.67	60.00	0.00	0.00%
2018	DP	58.33	54.00	68.00	9.67	16.57%
2018	FR	60.67	51.67	63.33	2.67	4.40%
2018	TP	56.00	55.67	72.00	16.00	28.57%

(Appendix I Table 1B & C on next page)

B) Community - No Turnover

YEAR	SITE	Mean South	Mean North	Mean Combined	Mean Days Extension	Mean Extension
2015	BH	48.67	48.00	52.00	3.33	6.85%
2015	DP	41.33	40.00	50.00	8.67	20.97%
2015	FR	49.00	49.67	53.00	3.33	6.71%
2015	TP	23.33	45.00	45.00	0.00	0.00%
2016	BH	54.33	56.67	56.67	0.00	0.00%
2016	DP	64.33	55.33	64.33	0.00	0.00%
2016	FR	49.67	43.67	49.67	0.00	0.00%
2016	TP	38.33	47.67	47.67	0.00	0.00%
2017	BH	37.00	64.00	64.00	0.00	0.00%
2017	DP	58.33	35.67	60.67	2.33	4.00%
2017	FR	53.00	35.67	53.00	0.00	0.00%
2017	TP	15.00	34.67	34.67	0.00	0.00%
2018	BH	40.33	52.67	52.67	0.00	0.00%
2018	DP	61.33	52.33	68.00	6.67	10.87%
2018	FR	60.67	44.33	60.67	0.00	0.00%
2018	TP	36.00	44.00	44.00	0.00	0.00%

C) SPECIES

YEAR	SITE	Mean South	Mean North	Mean Combined	Mean Days Extension	Mean Extension
2015	BH	23.04	39.25	42.33	2.92	7.62%
2015	DP	14.06	21.61	33.72	9.56	36.87%
2015	FR	30.33	36.55	39.02	1.57	2.85%
2015	TP	16.50	21.38	32.38	10.38	72.78%
2016	BH	22.67	25.31	33.25	3.67	17.30%
2016	DP	17.92	24.72	34.08	6.36	33.53%
2016	FR	26.71	34.48	40.71	1.31	6.56%
2016	TP	15.43	21.00	27.73	3.30	15.88%
2017	BH	24.43	25.40	34.83	4.67	19.97%
2017	DP	18.80	20.65	33.52	9.78	44.27%
2017	FR	29.57	29.50	42.47	4.80	17.14%
2017	TP	12.56	19.41	26.85	4.93	46.00%
2017	TT	29.68	25.97	34.93	5.25	18.69%
2018	BH	24.19	23.50	37.83	8.36	30.04%
2018	DP	32.42	25.77	39.54	6.65	20.33%
2018	FR	30.81	35.08	43.21	5.65	15.36%
2018	TP	15.38	21.88	28.03	4.92	19.51%

Table 2. Site, aspect, and year interactions

Testing aspect, site, and year interaction effects on flowering time. Testing the inclusion of interactions (3 way and pairwise) as a fixed effects in linear regression models, against simplified models with interactions removed. Models include dates (Start [day of 1st 5% flowering], Mid-flowering [day of 50% flowering], and End [day of last 5% flowering]) as dependant variables, with Aspect (A), year (Y), and site (S) as fixed effects. F ratios, P value and asterisks indicate significance (at $p < 0.05$) of including aspect in the model, as determined by testing the full model against a the null model with aspect removed. Residual degrees of freedom (Res.Df), Residual sum of squares (RSS), Degrees of freedom (Df), Sum of Squares (SS) are also reported. Bold model variables are those being tested, and bold p values indicate significance at $p < 0.05$.

Phenology Measure	Interaction test	Model	Res.Df	RSS	DF	SS	F Ratio	p value
Start	3 way	null (A+Y+S+A:Y+S:A+ S:Y)	64	2868				
		A+Y+S+A:Y+S:A+S:Y + **S:A:Y**	73	3173.7	-9	-306	0.76	0.65
	Site x Year	null (A +Y+S)	88	5366.6				
		A+Y+S + **S:Y**	79	4229.8	9	1137	2.36	**0.02**
	Apect x Year	null (A +Y+S)	88	5366.6				
		A+Y+S + **A:Y**	85	4899.6	3	467	2.70	0.05
	Apect x Site	null (A +Y+S)	88	5366.6				
		A+Y+S + **A:S**	85	4777.5	3	589	3.49	**0.02**
Mid	3 way	null (A+Y+S+A:Y+S:A+ S:Y)	64	3794.7				
		A+Y+S+A:Y+S:A+S:Y + **S:A:Y**	73	4248.2	-9	-454	0.85	0.57
	Site x Year	null (A +Y+S)	88	8473.6				
		A+Y+S + **S:Y**	79	7112.6	9	1361	1.68	0.11
	Apect x Year	null (A +Y+S)	88	8473.6				
		A+Y+S + **A:Y**	85	8161.1	3	313	1.08	0.36
	Apect x Site	null (A +Y+S)	88	8473.6				
		A+Y+S + **A:S**	85	5921.8	3	2552	**12.21**	**< 0.001**
End	3 way	null (A+Y+S+A:Y+S:A+ S:Y)	64	5051.3				
		A+Y+S+A:Y+S:A+S:Y + **S:A:Y**	73	5397.2	-9	-346	0.49	0.88
	Site x Year	null (A +Y+S)	88	11124				
		A+Y+S + **S:Y**	79	9189.1	9	1935	1.85	0.07
	Apect x Year	null (A +Y+S)	88	11124				
		A+Y+S + **A:Y**	85	11041	3	83.5	0.21	0.89
	Apect x Site	null (A +Y+S)	88	11124				
		A+Y+S + **A:S**	85	7415.8	3	3708	14.17	**< 0.001**

Appendix II. Maternal effects test supplemental to Chapter 3

Methods

To examine possible maternal effect influences on phenology, the average population seed weight was measured and influence on phenology dates tested. Because pappus (seed wings) added weight to the seeds that was non-nutritional the pappus was gently removed from all seeds using tweezers prior to weight measurements. The average weight of three seeds was taken from each maternal line, and the mean of all lines was used as the average population seed weight. A pilot study determined that pappus removal reduced germination rates (likely due to seed damage), so seeds used for weight measurements were different than those used in the experiment.

A subset of populations representing a range of climates were also grown for a second generation the next year to further parse out potential maternal effects on population differentiation. Since *Lasthenia gracilis* is an obligate outcrossing species, individuals grown in the first generation were crossed within population by rubbing open inflorescences together, with each individual outcrossed to two different lines. This was done for each day the inflorescences were open. Pots were separated to prevent accidental pollen transfer between populations. Populations for the second-generation study were selected from those with at least 50 seeds from the original study. Seeds from 5 maternal lines from each population were pooled. Populations represented a range of climates, including included Anza Borrego (AB), Carrizo Plain 2 (CB), Henry Coe (HC), Pinnacles (PN), Tejon Mojave 3 (TC), Table Mountain (TM). Twelve individuals from each of the six populations were grown in a common thermal environment but with varying watering treatments testing plasticity of flowering time to watering treatments for a separate study (Cox 2019). Flowering time differences were again assessed using a linear regression of flowering time by provenance location. Watering treatments were included as fixed effects to remove variation in flowering time due to treatment.

Results

Seed weight was not significantly correlated with any of the phenological variables, and did not change model outcomes when included in the models testing for population differentiation. Population differentiation in days to start of flowering was significant in the subset of six populations grown for a second common garden generation (start $F_{(1,70)} = 3.16$, $p = 0.013$).