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Geographic variation in flowering phenology in an endemic California annual wildflower, Clarkia unguiculata

Author
Haggerty, Brian Paul

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Geographic variation in flowering phenology in an endemic California annual wildflower, *Clarkia unguiculata*

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by

Brian Paul Haggerty

Committee in charge:
Professor Joshua Schimel, Chair
Professor Carla D’Antonio
Professor Susan Mazer
Professor Jonathan Pruitt

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The thesis of Brian Paul Haggerty is approved.

Carla D’Antonio

Susan Mazer

Jonathan Pruitt

Joshua Schimel, Committee Chair

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ABSTRACT

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Deciphering and forecasting seasonal plant activities are key parts of managing natural resources and anticipating natural hazards to ecological communities and human health (Enquist et al. 2014). As a result, understanding species' phenological parameters and their responses to climatic variation has become a pressing objective in ecology, evolution, and natural resource management (Haggerty et al. 2013; Mazer et al. 2015). One way of evaluating species' phenological associations with climate is with a space-for-time approach (Pickett 1989). Elevational gradients, for example, can make steep climatic gradients over short distances, allowing statistical associations to be evaluated between local climatic conditions and population-level mean plant phenology (Korner 2007, Etterson et al. 2016). The recent availability of extensive gridded climatic data through central databases (e.g., PRISM Climate Group) enables researchers to evaluate climatic influences on the phenology of populations and species across complex landscapes. Consequently, assessing geographic patterns of intraspecific phenological variation and its co-variation with climatic conditions is now a viable approach to forming a foundation from which to evaluate future changes in phenology and its potential effects on ecological communities and human health.

A substantial amount of our knowledge on the geographic variation of phenological traits in plants comes from studies on the onset of reproduction, in particular the first

flowering date (FFD) of angiosperms. As a result, FFD is commonly used as a proxy for an individual's entire flowering and reproductive season and has become a standard metric of comparison across studies – so much so that it has been identified by scientists and policy makers as a key indicator by which to assess and compare species' long-term rates of phenological responses to climate change (EPA 2014). Because of this tremendous focus on FFD, the dynamics of the entire flowering season are often overlooked, and as a result far less is known about other parameters characterizing flowering phenology, specifically individual lifetime flowering duration and the overlap (synchrony) of flowering among population members. Thus, while initiating flowering early relative to surrounding conspecifics is widely understood to be of high adaptive value and show predictable geographic variation, far less is understood about whether the duration and synchrony of flowering exhibits similar geographic variation and confers adaptive value.

Understanding the quantitative relationships among these phenological parameters and their associations with climatic conditions is required to forecast the effects of climate change on these ecologically important reproductive attributes. Particularly in semi-arid regions where plant growth and reproduction are limited by both water and temperature, it is critical that we improve our understanding of how multifaceted climatic conditions influence phenological parameters.

Here, I present two chapters examining geographic variation in plant phenology. In the first chapter, I review the literature reporting geographic variation in intraspecific plant phenology as well as the literature reporting on the adaptive significance of flowering time with a focus on flowering onset, duration, and synchrony. In addition, using gridded climatic data, I explore the potential for temperature and precipitation gradients to co-influence

growing season conditions across a 1000m elevational gradient in the semi-arid ecosystem of California's Sierra Nevada. In the second chapter, I run an experiment to investigate whether long-term winter-spring climatic conditions may have influenced the evolution of flowering onset, duration, and synchrony in an annual wildflower, *Clarkia unguiculata*, which is found in these semi-arid habitats. I detected extensive genetically based differences among populations for each phenological parameter. When grown in a common environment, populations originating from low latitudes and elevations characterized by relatively warm and dry winter-spring conditions flowered significantly earlier, for a longer duration, and with lower synchrony than populations originating from higher latitudes and elevations characterized by relatively cool and mesic conditions. Overall, latitudinal and elevational clines in flowering phenology mirrored latitudinal and elevational gradients in long-term climatic conditions. However, variation in flowering duration was best explained by days to flowering, and variation in synchrony was best explained by duration. If these geographic patterns reflect the outcome of adaptive evolution on flowering time, then the warm and dry conditions forecasted for California in the coming decades are likely to exert direct selective pressures on flowering time, which may cause the evolution of earlier onset of flowering, longer potential flowering duration, and lower flowering synchrony.

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Geographic variation in intraspecific plant phenology and the adaptive significance of phenological parameters

Introduction

A fundamental way plants respond to seasonal environmental stressors is by shifting their phenology—changing when they grow, reproduce, and senesce. A species' phenological parameters such as leaf-out or flowering time can be sensitive to environmental conditions (Schwartz 2013) and have strong effects on fitness (Munguía-Rosas et al. 2011; Kingsolver et al. 2012) as well as on ecological communities (Elzinga et al. 2007; Thackeray et al. 2010), forest carbon sequestration (Richardson et al. 2010), and human health (Grewling et al 2012). Discoveries of pervasive phenological changes associated with warming global temperatures (Parmesan & Yohe 2003; Parmesan 2007; Poloczanska et al. 2013) have led scientists and policy makers to identify phenology as a key metric in understanding species' responses to ongoing climate change (IPCC 2014; US-EPA 2014).

As a result, interests in evaluating the links between plant phenology and climate have surged (Pau et al. 2011; Cook et al. 2012; Mazer et al. 2013). Forecasting species' phenological responses to climate change has become a common goal in ecology, evolution, and natural resource management (Enquist et al. 2014). Yet, studies of species' phenological correlations with climate are often hampered by two factors. First, there is a tendency across disciplines to evaluate only mean temperatures as environmental sources of variation, rather than cumulative heat sums, precipitation, and other ecologically-relevant climatic parameters. This limits our understanding of the influence that multifaceted changes in climatic conditions can have on species, particularly in water-limited ecosystems. Second, it is common for studies not to decipher proximate sources of variation (phenotypically plastic responses to local conditions) from ultimate sources of variation (inherited adaptive responses) (cf. Diez et al. 2012). This limits our understanding of the capacity of plant populations and species to shift their phenology to track favorable growing season conditions. Improving the way we characterize climatic conditions and identifying the drivers of phenological correlations with climate are fundamental to predicting how plants will respond to ongoing environmental changes.

A first line of evidence on the potential sensitivity of a species' phenology to *temporal* changes in climatic conditions may be formed by evaluating the current *spatial* distribution of variation in phenological traits across populations. Geographic variation in climate can be a strong force in shaping the differentiation of phenological schedules across a species' range. Where conditions vary across landscapes – such as with gradients of temperature, precipitation, or other growing season conditions – the resulting mosaic of selection pressures can promote phenotypic and genetic divergence among populations (Mayr 1956; Thompson 2005) and result in locally adapted phenological traits within populations (Clausen et al. 1940; Haggerty & Galloway 2011). Thus the sensitivity of a species' phenological parameters to variation in climate can be measured by the relationship between population means of phenological traits and daily, monthly, annual, or long-term climatic factors.

However, when spatial variation in population phenology is measured only with an *in situ* approach (e.g., mean first flowering date across natural populations), it is not possible to distinguish ecological or evolutionary mechanisms underlying a phenotypic correlation with climatic factors. On the other hand, if mean first flowering date were measured across populations in an *ex situ* setting (e.g., common garden) and found to correlate with long-term climatic conditions from populations' natal sites, it would indicate that the species may have adapted to geographic variation in climate by shifting flowering time (Schneider & Mazer 2016). This would suggest that first flowering date may be under selection due to climatic factors, something that could be confirmed with additional fitness measures in field studies. Thus, predicting plant responses to ongoing environmental change can be done with a combination of *in situ* and *ex situ* approaches that elucidate the ecological and evolutionary processes underlying species' geographic variation in phenology and their phenological sensitivity to climatic conditions.

The recent availability of extensive gridded climatic data through central databases (e.g., PRISM Climate Group) enables researchers to evaluate climatic influences on the phenology of populations and species across vast landscapes. For example, Mazer et al. (2015) recorded the *in situ* vegetative and reproductive phenology of four woody species across latitude and elevation gradients in California's semi-arid Mediterranean region. They then integrated monthly climatic data from each location and reported strong species-specific and phenophase-specific phenotypic associations with recent winter climatic conditions (temperature, precipitation, and their interaction). Estimates of genetically-based population divergence have also been paired with climate data from populations' natal locations in order to evaluate the extent to which historical climate regimes may have shaped the genetic variation underlying adaptive traits (Joost et al. 2007; Kim & Donohue 2013). Using this approach, Stinchcombe et al. (2004) ran a common garden experiment with European populations of *Arabidopsis thaliana* collected from a broad latitudinal gradient. They reported a positive relationship between genetically-based bolting time and latitude, and attributed this relationship in part to winter precipitation in populations' natal sites (i.e., later bolting in populations from drier winter locations, independent of the effects of latitude). In a similar fashion, clinal variation in flowering time genes also has been associated with latitudinal climatic gradients for this and other model systems (*Arabidopsis* – Chew et al. 2012; *Capsella* – Neuffer 2011; *Populus* and *Acer* – Chuine and Beaubien 2001).

Determining whether intraspecific variation in phenology is the outcome of adaptive evolution is key to understanding whether the phenological parameters that optimize life history schedules in the short term can help populations and species adapt to changing conditions over the long term. Much of what we know about the adaptive significance of plant phenology comes from studies of the onset of flowering. Early flowering within populations is commonly favored by selection (Munguia-Rosas et al. 2011, Kingsolver et al. 2012), making it a key phenological parameter by which species can adapt to spatial variation in climatic conditions over their geographic range (Neuffer 2011; Kooyers et al. 2014). This makes first flowering date (FFD) a natural focus for evaluating and forecasting species' temporal responses to ongoing environmental change. However, FFD is commonly used as a proxy for the subsequent progression of the flowering season, and as a result we know little about the adaptive significance of other parameters characterizing flowering phenology, specifically the duration of the flowering season and the synchrony of flowering among conspecific population members. These reproductive parameters characterize the availability of pollen donors and recipients within populations and provide the basis of predicting patterns of gene flow through assortative mating (Weis et al. 2014). Flowering duration and synchrony also influence the seasonal pulse of resources for pollinators and floral parasites (Elzinga et al. 2007) and the intensity of potential human allergens (Grewling et al 2012). Greater focus must be made on these reproductive attributes if we are to improve our forecasts of species' responses to climate change and their potential consequences for ecological communities and human societies.

Although understanding and forecasting phenological changes has become a priority at regional (Haggerty et al. 2013), national (Schwartz et al. 2012), and global scales (IPCC 2014), currently our ability to evaluate patterns of phenological variation remains limited in three key ways: 1) How can we better characterize ecologically-relevant growing season conditions in regions where temperature and precipitation limit plant productivity?; 2) Can we improve our ability to forecast phenological responses to environmental change by elucidating the drivers of phenological variation within species?; and 3) By improving our understanding of the adaptive significance of flowering time – including flowering onset, duration, and synchrony – can we improve our forecasts of phenological responses to climate change and their consequences for ecological communities?

I address these three questions with a case study of climatic variation and a literature review of intraspecific variation in plant phenology. To begin, I made a case study of climatic variation across a 1000m elevational gradient in the semi-arid ecosystem of California's Sierra Nevada. Using gridded climatic data that are widely available, I explore the potential for temperature and precipitation gradients to co-influence growing season conditions. I then review the literature reporting intraspecific geographic variation in plant phenology, and then review the literature reporting on the adaptive significance of flowering time with a focus on flowering onset, duration, and synchrony. My aim is to improve our understanding of how populations and species respond to climatic conditions across space in order to advance our ability to forecast their temporal responses to climate change.

Geographic gradients in growing season conditions: challenges in measuring meaningful growing season parameters

The drive to understand the links between plant phenology and climatic conditions has long inspired researchers to use geographic gradients as natural laboratories and proxies for climatic gradients (Turreson 1922; Hopkins 1938; Claussen et al. 1940). Whereas favorable growing season conditions and plant phenology were once thought to change in step with elevation and latitude (Hopkins 1938), modern work demonstrates greater complexity in these relationships. For example, the steepness of temperature and precipitation gradients varies seasonally across latitude (reviewed in De Frenne et al. 2013), and complex topography and orographic processes generate a variety of local microclimates across elevation (reviewed in Korner 2007). Regional gradients in plant phenology also can be disrupted by local factors such as snow cover (Molau et al. 2005), precipitation (Kramer et al. 2000), aspect (Peterson 1997), and urban heat islands (Primack et al. 2004). The challenges of studying environmental causes of phenological variation are exacerbated by the numerous ways to calculate the onset and length of growing season conditions, something that has been a problem for ecologists for nearly half of a century (Brinkmann 1979). This has introduced uncertainty in comparing studies reporting variation in growing season length and its covariance with phenological parameters.

Currently, a commonly used metric for characterizing growing season length is the duration of the frost-free season. While the frost-free season may effectively characterize bounds of the growing season in temperate, alpine, arctic, and other biomes where freezing temperatures regulate plant growth, its utility is lost in semi-arid and other water-limited ecosystems where plants often grow vigorously during the coolest and wettest months of the year and senesce with the onset of annual summer drought rather than autumn frosts. This is especially problematic because the length of the frost-free season is becoming a standard of comparison for evaluating the effects of ongoing climate change on species and ecosystems (e.g., IPCC 2014; US EPA 2014). Wilczek et al. (2010) developed a growing season metric that integrates temperature and water availability to predict flowering phenology of the model system *Arabidopsis thaliana*, however it remains limited to just one species and the type and extent of data necessary for these calculations typically exceed the monitoring capabilities of most researchers (e.g., evapotranspiration estimates, whole-basin water availability and loss, and soil and sub-surface water content).

We currently lack an optimal solution for evaluating meaningful growing season parameters in semi-arid regions where plant growth and reproduction are limited by both temperature and precipitation. To address this issue, I used gridded climatic data that are widely available through a centralized online database to explore common and novel methods for quantifying geographic variation in growing season parameters.

A case study in California's Sierra Nevada

To assess geographic variation in climatic conditions and the potential for temperature and precipitation to jointly constrain growing season conditions, I evaluated gridded climatic data spanning 30 years (1981-2010) for a 1000m elevation gradient in the southern portion of California's Sierra Nevada (Table 1). This semi-arid region has a rich history of botanical and ecological research and is characterized by high species diversity resulting from complex topography and the convergence of valley, desert, foothill, and montane ecosystems. Many herbaceous plants in this region start growing during the mild wet winter and senesce during the springtime transition from mesic to xeric conditions. The annual wildflower *Clarkia unguiculata* is abundant in this region and is unusual in that, wherever it occurs, it is among the last herbaceous species to flower during the transition into annual drought. As such, I characterized spatial variation in this climatic transition for 10 *unguiculata* field sites (Table 1) by using ordinary least-squares regression to analyze the relationship between elevation and the long-term norms for each of 10 climatic parameters for the winter and spring months (January-May). JMP v11 Pro statistical analysis software was used for all analyses.

I obtained all climatic data from PRISM Climate Group's Data Explorer (Oregon State University, http://prism.oregonstate.edu, created 4/7/2016). I obtained 30-year norms for mean monthly climatic conditions at 800m interpolated resolution for each of the following climatic parameters: minimum daily temperature (Tmin); mean daily temperature (Tmean); maximum daily temperature (Tmax); monthly accumulated precipitation (PPT); and daily maximum vapor pressure deficit (VPDmax). Vapor pressure deficit incorporates temperature and relative humidity into one value that describes the dryness of air – as VPD increases, the air becomes drier and evapotranspiration increases in a nearly linear manner. I also obtained daily temperature and precipitation data 1981-2010 at 4km interpolated resolution – the highest available resolution for daily data – in order to derive the following climatic parameters: growing degree day accumulation (GDDsum); growing season start date (GSLstart); growing season end date (GSLend); growing season length (GSL); and the date of the last significant precipitation event (PPTend). These derivative parameters characterize either the cumulative climatic conditions or the timing of climatic milestones that may be useful in evaluating growing season conditions. Definitions for all climatic parameters are provided in Table 2.

Winter and spring climatic conditions as measured by these ten climatic parameters change significantly across the elevation gradient in the southern Sierra Nevada. Whereas mean temperatures and mean accumulated growing degree days decrease significantly with elevation, accumulated precipitation increases significantly with elevation (Figures 1 & 2; Table 2). Maximum vapor pressure deficit also decreases significantly with elevation (Figure 3; Table 2), further indicating that lower elevation sites experience drier atmospheric conditions than higher elevation sites during the winter and spring months. The opposing nature of these temperature and water gradients suggests that both are likely to influence plant productivity, making it a challenge to describe and predict growing season conditions in this region based on these climatic parameters alone.

Given this limitation, the timing of ecologically relevant climatic milestones – the start and length of the growing season, and the onset of annual drought – could help to further characterize geographic variation in growing season conditions in this region. The start of the growing season as indicated by the last spring frost occurs earliest at low elevations and increases significantly with elevation, creating a difference in frost-free conditions of 3-4 weeks between the lower and higher elevation sites (Figure 4a; Table 2). While killing frosts in the spring are known to have strong effects on plant survival and productivity in alpine ecosystems (Inouye 2008), their influence on plants in this region is not understood. Many, if not most, species in the region continue to develop and reproduce after frost events in mid to late spring (personal observation). Whether this is due to a relatively mild frost compared to alpine ecosystems or to high freeze tolerance in species in this region also is not understood.

The length of the frost-free season decreases significantly with elevation in this region, with lower elevation sites experiencing 6-7 weeks of additional frost-free time compared to higher elevations (Figure 4c; Table 2). This means that the end of the growing season indicated by the first autumn frost occurs at low elevations 2-3 weeks after it occurs at higher elevations (Figure 4C; Table 2). However, the length of the frost-free season has little ecological relevance to herbaceous species in this region because the realized growing season ends with severe drought in the late spring. Thus, although there are strong elevational gradients in the start, length, and end of the frost-free season, there remains uncertainty when applying to semi-arid regions the temperature-based growing season metrics that are better suited for temperate, alpine, and high latitude ecosystems (e.g., EPA 2014).

The annual onset of late-spring drought that brings an abrupt end to the growing season for many plants in this region is difficult to pinpoint with the available data. The end of the rainy season – a metric commonly used to evaluate growing seasons in tropical ecosystems – could provide one measure of the curtailing of plant-available water input to ecosystems. For *unguiculata* sites in the southern Sierra Nevada, there is a strong increase with elevation in the date of the last significant precipitation event (i.e., >0.25 inches), though the statistical significance of this relationship depends on the inclusion of a high-elevation outlier site (Figure 4D; Table 2). This outlier site (1198m) is much drier and experiences earlier drought than expected based on its elevation, potentially due to a localized rainshadow effect given its position on the eastern side of a leeward ridge. Even so, the case remains that the 30-year norm for the last rainfall date occurs 2-3 weeks earlier at lower sites than at higher sites. There is a positive correlation among sites between this date and cumulative precipitation (including outlier site: $r^2=0.78$, $F_{1,8}=29.04$, $p<0.001$; excluding outlier site: $r^2=0.93$, $F_{1,7}=91.19$, $p<0.0001$). This suggests that the last rainfall date may provide a useful indicator of cumulative growing season conditions, though the extent to which this metric captures the end of the growing season depends both on edaphic characteristics and on the preceding patterns of precipitation. If severe drought conditions precede the last precipitation event, a late rainfall could be inconsequential to drought-stressed plants that are already finishing their life cycles.

To the extent that the timing of these climatic milestones is ecologically relevant in this region, lower elevation sites are characterized by earlier and greater heat accumulation, lower precipitation sums, drier air, and earlier onset of summer drought than higher elevation sites. This may seem to create more stressful conditions that would result in a shorter growing season. I tested this possibility by evaluating differences in the length of favorable growth conditions by quantifying the number of days between the last spring frost and last precipitation event. According to this index, the length of favorable growth conditions declines significantly with elevation (Figure 4E; Table 2). Whereas plants at lower elevations have a window of approximately 30 days from the last frost to the last precipitation event, plants at higher elevations have a window of 10-15 days (in the outlier site at 1198m, the last precipitation event actually precedes the last spring frost in some years). Whether this index accurately characterizes favorable growth conditions depends on the issues for climatic milestones mentioned previously – that the last spring frost may not be an accurate indicator of growing season start, and that the last precipitation event may be preceded by drought conditions long enough that even a large precipitation event would have little effect on plant growth and reproduction. Testing the ecological relevance of these climatic milestones will be necessary to determine whether this index accurately describes favorable growing season conditions.

In sum, even as gridded climatic data become more widely available through central databases, there remain no optimal solutions for calculating meaningful growing season parameters in semi-arid regions where plant growth and reproduction are limited by temperature and precipitation. Given the challenges presented here for assessing growing season conditions in California's Sierra Nevada, general caution should be exercised when evaluating ecological patterns on geographic gradients that do not also evaluate climatic influences. This suggests that temperature-based definitions of growing season length that are commonly used to quantify and compare the effects of ongoing climate change on ecosystems are inadequate for capturing climatic variation in large portions of the U.S. and other semi-arid regions in the world. In the absence of widely available metrics and analytical tools that evaluate multidimensional components of climatic variation, it will remain a challenge to characterize geographic variation in seasonal climatic conditions in semi-arid regions, making it difficult to decipher the environmental causes of phenological variation for many species.

Geographic variation in phenological parameters

Geographic variation in climate can be a strong force in shaping the differentiation of phenological schedules across species' ranges. Latitudinal and elevational gradients in the frost-free growing season are well characterized, with lower latitudes and elevations generally experiencing earlier and longer annual frost-free seasons than higher latitudes and elevations (Korner 2007; De Frenne et al. 2013). The above case study in California's Sierra

Nevada echoes this pattern. The coarsely predictable nature of temperature gradients associated with latitude and elevation might lead to assumptions that plant phenology matches these patterns, both at phenotypic and genetic scales. If this were the case, populations at low latitudes and elevations would consistently exhibit early phenology with elongated duration to match the long growing season, and populations at high latitudes and elevations would consistently exhibit late phenology that was compressed into a short period of time to match a brief growing season length. While these patterns are, indeed, generally observed at phenotypic scales, they become substantially more complex once the genetically based patterns are investigated. Here, I explore the phenotypic and genetic contributions to phenological patterns across latitudinal and elevational gradients.

Latitudinal gradients in phenological patterns – insight from in situ *approaches*

Evaluating phenotypic patterns of phenological variation across latitudinal gradients presents logistical challenges associated with collecting high-frequency data across broad geographic areas. To circumvent these challenges, patterns of plant phenology can be detected with remote sensing techniques or with networks of coordinated volunteer observers (i.e., citizen science). Remotely sensed measures of plant phenology such as satellite-derived Normalized Difference Vegetation Index (NDVI) are powerful approaches to exploring phenology at large geographic scales. While remote sensing techniques can reveal broad phenological patterns and poise the researcher to evaluate large-scale environmental causes of phenotypic variation, these methods are mostly limited to the landscape scale and therefore to the multi-species plant communities therein. Phenotypic patterns of plant phenology at these scales generally match the spatial pattern of the length of the frost-free season – phenology tends to be later and more compressed at higher latitudes where the growing season starts late and ends early (De Frenne et al. 2013). The annual poleward progression of the "green wave" indicating the onset of vegetative growth (green-up) is readily observable with satellites (Schwartz 1998) and is closely associated with local accumulations of springtime growing degree days (Liang and Schwartz 2014). The end of season "brown-down" is similarly observable across regions (Bradley et al. 2007), although it is generally less conspicuous than green-up.

Remote sensing also provides the capacity to detect phenological changes in response to large-scale climatic patterns such as the steepness of latitudinal temperature gradients (Schwartz and Reiter 2000; De Frenne et al. 2013). The gradient in mean springtime temperature across latitude can range among seasons and years from shallow, defined as little change in temperature across latitude, to steep, defined as a large change in temperature across latitude (De Frenne et al. 2013). The steepness of this gradient is projected to become shallower over the coming century (i.e., becoming more similar at high and low latitude) and is expected to cause faster green-up across North America by significantly advancing green-up at higher latitudes (Jeong et al. 2013). This prediction is consistent with general circulation models (GCMs) that forecast greater rates of warming at higher latitudes (Henry and Molau 1997), and with meta-analyses showing higher rates of phenological change at higher latitudes (Menzel et al. 2006; Parmesan 2007). The accuracy of large-scale phenological projections is important for informing regional and global scale simulations of atmosphere-biosphere interactions (Botta et al. 2000; Levis and Bonan 2004; Jeong et al. 2013), from which predictions of terrestrial carbon and water budgets are derived (Jeong et al. 2012; Migliavacca et al. 2012).

Although remote sensing facilitates the detection of proximate climatic sources of variation on landscape-scale phenology and bio-climatic feedbacks, it offers little information on intraspecific phenological patterns unless large monospecific patches are available for observations. An alternative large-scale approach is to engage networks of volunteer observers (i.e., citizen scientists) to conduct ground-based phenological monitoring at regional (Haggerty et al. 2013) to continental scales (Schwartz et al. 2012). Citizen science is a burgeoning scientific and educational enterprise that is facilitating the engagement of society in conservation and sustainability issues on local to global scales (Shirk et al. 2012). This approach could contribute substantially to our understanding of intraspecific phenological variation across large geographic gradients and its links with climatic parameters. Its success, though, ultimately hinges on the value of the data to resource managers and decision makers (Dickinson et al. 2010; Schwartz et al. 2012). For example, Mazer et al. (2015) coordinated observers across latitudinal and elevational gradients in California to monitor vegetative and reproductive phenology in several woody species. They then calculated phenological sensitivities of each species' phenophases to recent winter climatic conditions, from which forecasts were made for natural resource managers for future climate scenarios. In a compelling analysis of the utility of citizen science to climate change research, Cooper et al. (2014) demonstrated that data from citizen science projects actually contribute to far more of highly-revered research articles containing key findings on climate change response than researchers realize, giving citizen science an "invisible prevalence" in the literature.

Though informative and powerful, the remote sensing and volunteer observer approaches are currently unable to discern the likely contributions of proximate and ultimate sources of variation. This limits our ability to distinguish whether large-scale patterns in phenology are due to phenotypically plastic responses to local climatic conditions, or due to locally adapted genetic responses to the gradients, or a combination of these processes. *Ex situ* approaches such as common gardens and growth chambers are ultimately necessary to achieve these goals.

Latitudinal clines in phenological patterns – insight from ex situ *approaches*

Common garden, growth chamber, and greenhouse experiments can reveal genetic bases to phenological traits by reducing environmental sources of variation, allowing the expression of genetic variation and genetically based population divergence to be revealed. Clinal variation in phenological traits is observed when population differentiation that is observed in *ex situ* studies co-varies with environmental gradients. Co-gradient clinal variation occurs where plant phenology mirrors climatic gradients – populations from northern latitudes exhibit a delay in spring growth and reproduction relative to populations from southern latitudes. This life history strategy presumably helps northern populations match the later onset and shorter duration of the frost-free seasons, and has been observed in common garden studies where populations from higher latitudes exhibit longer pre-reproductive periods (Bocher 1949; Reinartz 1984a, 1984b; Lacey 1986); earlier autumn bud set in preparation for the following year (Brissette & Barnes 1984); and later springtime emergence, bolting, and first flowering date (Kalisz & Wardle 1994) than intraspecific populations from lower latitudes.

In contrast to these studies, counter-gradient clinal variation occurs where populations from northern latitudes are genetically differentiated for earlier phenology than populations from lower latitudes. This pattern has been demonstrated for several phenophases in a variety of taxa, including: earlier flowering (*Capsella bursa-pastoris* – Neuffer and Hurka 1986; *Capsella bursa-pastoris* – Weber and Schmid 1998; *Arabidopsis thaliana* – Stinchcombe et al. 2004); faster anthesis (*Datura stramonium* - Weaver et al. 1985); earlier increases in stem diameter (*Populus tremula* - Hall et al. 2007); and earlier leaf-out (*Lythrum salicaria* - Olsson and Agren 2002; *Betula papyrifera* - Hawkins and Dhar 2012). That populations from higher latitudes exhibit genetically based earlier phenology in common environments than do those from lower latitudes suggests that, for these species, populations from higher latitudes either respond to earlier cues (e.g., shorter springtime photoperiod) or have reduced sensitivity to cues (e.g., vernalization requirements) that allow them to start growing as soon as conditions are favorable. The mechanism underlying these responses is unknown for most species studied. However in at least one species, *Arabidopsis thaliana*, vernalization requirements decrease at higher latitudes due to dampened expression of alleles that are responsible for detecting springtime climatic conditions, specifically the VERN, FRI, and FLC genes (Lempe et al. 2005; Shindo et al. 2005; Stinchcombe et al. 2005; Hopkins et al. 2008). This continent-scale, genetically-based cline in *Arabidopsis* flowering time is thought to be the outcome of selection, as determined through population genomics analyses (Sterken et al. 2009) and through controlled environment studies that identified covariance among populations of genetically-based flowering time with climatic gradients (Caicedo et al. 2004; Stinchcombe et al. 2004; Lempe et al. 2005).

In a unique example of a common garden study that incorporated a broad latitudinal gradient extending across Europe from high temperate to sub-tropical latitudes, Neuffer (2011) discovered earliest onset of flowering both in the southern-most and the northern-most populations with mid-latitude populations exhibiting the latest flowering times. These southern-most sites are in comparatively arid regions characterized by Mediterranean summer drought that arrests growth and reproduction of many plant species. That populations from this region may have evolved early phenology in response to seasonal drought is analogous to populations in the far north evolving early phenology in response to a short frost-free season. Though potentially driven by different selective forces – drought in the south, frost in the north – intraspecific populations at the latitudinal range margins may have evolved similar phenology in response to shorter growing seasons. This interesting pattern should be investigated in other taxa and regions including California's Mediterranean ecosystems.

While *ex situ* studies of intraspecific plant phenology across latitudinal gradients have demonstrated either co-gradient or counter-gradient clinal patterns, our ability to predict which pattern a given species might exhibit is limited. This limits our ability to interpret phenological patterns that are observed across any climatic gradient. This calls for a stronger focus to be placed on the links not just between phenology and climate but also on the genetic and physiological mechanisms that influence phenological schedules.

Elevational gradients reveal great complexity in climate, phenology, and plant biology

Elevational gradients present fewer logistical constraints than latitude gradients for the study of clinal variation, particularly for field-based studies. Elevational gradients can generate steep climatic changes over short distances and thereby provide exciting natural laboratories with which to investigate patterns of phenological variation within and among intraspecific populations. This setting is particularly useful for studying the co-variance of phenological traits with additional life history, morphological, and physiological traits, facilitating the assessment of whether whole-plant resource allocation tradeoffs might constrain or facilitate responses to environmental change (e.g., Milla et al. 2009).

Climatic gradients across elevation are generally more difficult to predict than across latitude (Korner 2007). As demonstrated in the earlier case study in California's Sierra Nevada – specifically with the variable mid-elevation sites and the rainshadowed outlier site – temperature and other growing season conditions may not scale predictably across all elevations. Variation in landscape physiography such as slope, aspect, and geological features can interact with weather patterns and edaphic characteristics to create complex arrays of microclimates that may disrupt regional gradients in temperature and precipitation; it may even create climatic inversions where, for example, temperatures increase with elevation. These can drive counter-gradient phenological patterns (Fisher et al. 2006). Landscape complexity also moderates microclimatic variation in factors such as elevation-associated decreases in the partial pressure of all atmospheric gasses including CO_2, and increases in solar radiation and the proportion of damaging UV-B radiation (Korner 2007).

Taken together, climatic parameters can interact in complex ways across elevation gradients and introduce a variety of interacting physiological stressors for plants. For example, changes in growing season length may not correlate well with changes in temperature across elevation gradients due to the influence of water availability (Galen and Stanton 1999), potentially preventing trait variation among intraspecific populations to exhibit as smooth a cline as expected from changes in temperature alone. Yet this has been little studied. Establishing relationships between clinal variation in plant phenology and climatic parameters is therefore more challenging than is generally accepted (Korner 2007).

Here, I explore whether general patterns of intraspecific variation are observed across elevational gradients, for phenological parameters as well as other traits that may influence the life history schedule of wild plants.

Elevational gradients in vegetative & reproductive investments, morphology, and phenology – insight from in situ *approaches*

The categories of intraspecific plant traits most commonly observed to change across elevation gradients include phenology, morphology, and resource allocation to physiological, structural, vegetative, and reproductive systems. The most conspicuous intraspecific change with elevation is in plant size – there is a strong tendency for plant habit to become more prostrate and compact (Korner et al. 1989; Milla et al. 2008) with concomitant decreases in body and organ size (Korner 1997). This shift toward a more modest habit can arise several ways, the most commonly documented of which are increased shoot compactness (Blionis and Vokou 2002; Milla et al. 2009); decreased reproductive investment (Milla et al. 2009); and greater investment either in roots (Blionis and Vokou 2002) or in the structural support of reproductive structures relative to vegetative structures (Fabbro and Körner 2004).

This reduction in intraspecific plant size at higher elevations has been associated with lower productivity (Korner 1997) and annual carbon gain (Milla et al. 2009). Compared to low elevation populations, those at higher elevations have been shown to compensate for this deficit with faster growth rates (Galen et al. 1991; Milla et al. 2009); higher photosynthetic capacity (Korner and Diemer 1987); more efficient CO2 fixation (Körner et al. 1991); or increased number of stomata (Korner 2003). Populations at higher elevations also can exhibit higher nitrogen resorption efficiency and be more likely to pre-form the next year's organs at the end of the growing season (Milla et al. 2009), a process consistent with intraspecific clinal variation at higher latitudes (Lempe et al. 2005; Böhlenius et al. 2006).

The second major type of intraspecific change with elevation is reproductive, including the morphology of reproductive organs, phenology, and reproductive effort. Several factors are known to contribute to clinal variation in reproductive strategies, including mating system (Zhigang et al. 2006); soil depth (Torang et al. 2010; but see Blionis and Vokou 2002); nutrient availability (Zhigang et al. 2006); pollination syndrome (Korner 1999; Zhu et al. 2009); changes in the pollinator community (Arroyo et al. 1982); and decreases in pollinator visitation rates (Arroyo et al. 1985; Totland 1993; Bingham and Orthner 1998; Blionis and Vokou 2002; Fabbro and Körner 2004). Among populations distributed across elevation gradients, resource allocation to reproduction is generally observed to decrease at higher elevations in favor of carbon gain and its storage in vegetative structures (Milla et al. 2009; Diggle 1997; Craine and Lee 2003; Korner 2003). The mediation of this allocation away from reproduction may be facilitated by more efficient reproduction at higher elevations (Milla et al. 2009; Guo et al. 2010a; Guo et al. 2010b; Guo et al. 2011).

There is general agreement that intraspecific clinal variation in flower size (i.e., pollinator attraction) depends on pollination syndrome, with wind-pollinated species maintaining similar flower size across elevation gradients and insect-pollinated plants exhibiting a range of responses to elevation (Zhu et al. 2009). Among insect pollinated plants, Zhu et al. (2009) reported increased flower organ size at higher elevations, while Fabbro & Korner (2003) and Blionis et al. (2001) found no change in flower mass, and Blionis & Vokou (2002) found smaller flowers but no change in corolla size. Additionally, Fabbro & Korner (2003) found no change in flower display area, a whole-plant proxy for pollinator attractiveness. Such variation in clinal patterns of flower size suggest that it is a highly plastic and/or adaptable trait that may respond rapidly to changes in ecological context that co-vary with elevation (e.g., pollinator assemblages – Galen 1996). It could also be the case that absolute flower size may show different clinal patterns than relative flower size; given that most species exhibit more prostrate and compact habits at higher elevations, if absolute flower size remains constant with elevation, flowers will be disproportionately large on high-elevation plants.

Reproductive phenology has received a great deal of attention across elevation gradients, with particular emphasis on floral traits. At the level of individual flowers, there is broad agreement that flower longevity increases with elevation (Blionis et al. 2001; Arroyo et al. 1981; Primack 1985; Stenstrom et al. 1992; Bingham and Orthner 1998; Blionis and Vokou 2002; Fabbro and Körner 2004; Makrodimos et al. 2008). There also is evidence that, at least for 8 of 9 dichogamous species assessed on one elevation gradient, the female phase lasts longer than the male phase (Blionis et al. 2001). Bingham and Orthner (1998) also described increased stigma receptivity duration with elevation. Whether clinal variation in flower longevity has been attributed to increased water availability (Galen and Stanton 1999) or cloud cover (Korner 1999), or other factors such as decreased pollinator abundance and activity at higher elevations (Bingham and Orthner 1998; Blionis and Vokou 2002; Fabbro and Körner 2004), taken together, there is strong and cohesive evidence for increasing floral longevity with elevation.

At the individual plant level, where flowering time is comprised of the onset and duration of flower production during the growing season, there is strong consensus that flowering onset for many species is delayed at higher elevations where growing seasons conditions generally are cooler and arrive later than at lower elevations (Vasek and Sauer 1971; Reader 1984; Blionis et al. 2001; Blionis and Vokou 2002; Makrodimos et al. 2008; Méndez-Vigo et al. 2011). Although there is one example of accelerated phenology at higher elevations (Fisher et al. 2006), it was due to a temperature inversion that cause the onset of spring temperatures to occur first at higher elevations. All evidence at the intraspecific scale points to earlier onset of flowering with warmer temperatures, a pattern consistent with field-based experimental warming tests (Alatalo and Totland 1997; Henry and Molau 1997; Price and Waser 1998; Stenstrom et al. 1997; Suzuki and Kudo 1997) even though such tests under-estimate the change in phenology per degree C relative to that observed in natural populations that have been monitored over many years (Wolkovich et al. 2012). Price & Waser (1998) further discovered that while experimental warming induced earlier flowering, it had no effect on the duration of flowering in each of 10 unrelated sympatric species. Flowering duration also has been found to remain constant across elevations for nine sympatric species in the genus *Campanulastrum* (Blionis et al. 2001). Indeed, it has long been thought that the duration of flowering is determined more by internal physiological constraints (Raynal and Bazzaz 1975; Parrish and Bazzaz 1979) than by external environmental cues.

In sum, general expectations of clinal variation in plant traits across elevation gradients include smaller plant size but more efficient physiology and reproduction. For reproduction, while clinal variation in floral morphology seems to be highly variable among species and biomes, clinal variation in the onset and duration of flowering appear to be more consistent. In two studies, clinal variation in both vegetative and reproductive systems have been observed and compared. Milla et al. (2009) found that vegetative and reproductive performance did not uniformly decrease with elevation in two species of *Saxifraga*. Blionis et al. (2001) found that structural and temporal traits act independently within each of nine congeneric species, such that phenological traits (floral longevity, onset of flowering) increased predictably with elevation whereas structural traits (flower mass, floral display area) did not change with elevation. Future work exploring seasonal resource allocation between vegetative and reproductive systems could help to provide additional mechanistic understanding of the factors that contribute to variation in phenological patterns.

Adaptive significance of flowering phenology parameters

Evaluating geographic variation in plant phenology across climatic gradients can help to establish expectations of how phenological and life history traits might respond over time to environmental change. The mechanisms by which traits evolve, however, can only be explored by examining the selective forces on, and the fitness advantages associated with, variation in phenological traits.

The selective forces that influence the timing of plant life cycles have been well-documented and synthesized (Fenner 1998; Elzinga et al. 2007; Munguía-Rosas et al. 2011). Regardless of whether these selective forces arise from abiotic factors (e.g., climate, nutrients, water, and edaphic factors), internal physiological constraints (e.g., resource partitioning), or biotic interactions (including both mutualistic and antagonistic interactions), a substantial amount of our knowledge of geographic variation and adaptive significance of phenological traits in plants comes from studies of the onset of reproduction, in particular the first flowering date of angiosperms.

First flowering date can be highly sensitive to environmental conditions (Menzel et al. 2006), have strong effects on fitness (reviewed by Munguía-Rosas et al. 2011), and exhibit high levels of phenotypic and genetic differentiation among intraspecific populations (Claussen et al. 1940; Haggerty & Galloway 2011). There is broad agreement across hundreds of studies, two meta-analyses (Munguía-Rosas et al. 2011; Kingsolver et al. 2012), and several reviews (Kingsolver et al. 2001; Hoekstra et al. 2001; Kudo 2006; Elzinga et al. 2007; Kingsolver & Pfennig 2007) that natural selection consistently favors the early onset of flowering within populations. Thus, individuals that initiate flowering before their co-occurring conspecifics consistently achieve higher fitness. There can, however, be a limit to the benefits gained with early flowering; in one clear example, early flowering individuals and species were more susceptible to late season killing frosts than those that flowered later (Inouye 2008).

Due to its environmental sensitivity, consistent influence on fitness, and pervasiveness in the literature, first flowering date has become a central focus of research on plant phenology. As a result, FFD has become a standard metric of comparison across studies, so much so that it has been identified by policy makers as a key indicator by which to assess and compare species' long-term rates of phenological change in response to climatic conditions (EPA 2014). However, much of what we know about the adaptive significance of flowering phenology ends with this trait – FFD is commonly used as a proxy for the subsequent progression of reproduction. Indeed, the duration of an individual's flowering season is often overlooked, and as a result far less is known about the adaptive significance of the entire flowering season compared to its onset. For example, while initiating flowering early relative to co-occurring conspecifics is widely observed to confer high relative fitness, far less is understood about the effects on fitness of the progression and overlap of flowering among conspecifics (i.e., duration and synchrony).

This gap in our knowledge is somewhat surprising since reproductive duration and synchrony have been a topic of research for several decades (e.g., Primack 1980; Augspurger 1981). While the duration of flowering is calculated simply as the number of days from the first flower to the last, flowering synchrony can be calculated a variety of ways. Methods for calculating flowering synchrony were initially developed by Primack (1980) and improved by Augspurger (1983). Augspurger’s calculation has become the most commonly used method for quantifying flowering synchrony, and calculates the number of days that each individual overlaps in flowering with every other individual in the population. An individual’s lifetime overlap of flowering with its population is expressed as:

$$\left(\frac{1}{n-1}\right)\left(\frac{1}{f_i}\right)\sum_{j=1}^{n} w_j$$

where n is the number of flowering plants; f_i is the number of census days that plant i flowers; and w_j is the number of census days that plant i and j flower simultaneously. This equation yields values that range from 0 (low synchrony) to 1 (high synchrony) and is effective for calculating the overlap of flowering days, but is blind to the extent to which the magnitude of flower production overlaps among individuals during the season. Thus, an individual at its peak flowering with 100 open flowers is treated equal to an individual finishing its flowering with 1 open flower.

To improve this methodology, four modifications of this equation have been proposed to incorporate the magnitude of flowering (see Table 1 in Elzinga et al. 2007). One of these modified equations incorporates flower number of the focal plant, but not for the other plants in the population. The other three modifications incorporate flower number of both focal and co-occurring population members, but two of these are not amenable to comparing flowering synchrony among populations since they involve rank-ordering individuals within a population. The remaining equation, developed by Elzinga et al. (2007), incorporates flowering intensity of focal and surrounding plants, and is suitable for population comparisons. In this equation, an individual's lifetime flowering synchrony with its population is expressed as:

$$\sum_{t=1}^{k}\left[\left(\frac{x_t}{\sum_{t=1}^{k} x_t}\right)(f_t)\right]$$

where k is the maximum number of census days; x_t is the total number of flowers a plant produces on census day t; and f_t is the proportion of the total number of flowers in the population produced on census day t. Similar to previous equations, this equation yields values that range from 0 (low synchrony) to 1 (high synchrony). This equation can be translated as follows:

$$\sum_{t=1}^{k}(individual's\ proportional\ flower\ production)_t * (population's\ proportional\ flower\ production)_t$$

This equation allows for the calculation of flowering synchrony for each individual at time t, as well as each individual's lifetime flowering synchrony summed across the entire season. This method of calculating the intensity of flowering overlap also allows for population-level means and variances to be calculated, both at the daily scale and at the scale of the entire flowering season, allowing for quantitative comparisons of flowering synchrony among populations.

The majority of research on reproductive synchrony has been focused on ecological interactions as they pertain to pollination, predator satiation, or masting events. Indeed, a substantial amount is known about the ecology of plant reproductive synchrony within populations, and it has long been understood that flowering synchrony can influence reproductive success (Augspurger 1983). This, however, has not led to an equal understanding of the *evolution* of plant reproductive synchrony.

Comparatively few studies have focused on evolutionary processes underlying flowering synchrony, and where they have there are conflicting outcomes and predictions for how flowering synchrony should evolve. For example, one study indicates that high levels of synchrony are advantageous by allowing individuals to achieve efficient pollinator attraction (Pettersson 1994), whereas two others indicate that low levels of synchrony are advantageous by allowing individuals to reduce competition for pollination service (Rathcke 1983; Devaux & Lande 2009). Yet another study demonstrates that pollinators and herbivores can impose opposite patterns of selection on synchrony resulting in no net selection (Gomez 1993). Although slightly stronger agreement can be found among conceptual models that suggest synchrony should evolve as a bet-hedging strategy in unpredictable environments (Iwasa & Levin 1995; Fagan et al. 2010), it remains unclear whether high or low levels of synchrony are generally more advantageous for particular species or in any particular environment or disturbance regime. These discrepancies are evident in a recent meta-analysis of 105 species that found no net phenotypic selection on flowering synchrony, even though selection on synchrony was detected within many of the studies that were reviewed (Munguía-Rosas et al. 2011 and citations therein).

It also remains unclear and untested whether there is a relationship between the onset and synchrony of flowering and whether that relationship might influence fitness. This is an important step to take in better understanding the adaptive significance of flowering phenology. It may be the case that flowering early within a population confers fitness advantages not just due to *absolute* timing but also due to *relative* timing. Early flowering may in fact be a mechanism for individuals to reach peak flowering when more surrounding conspecifics also are in flower (i.e., increasing synchrony). Consequently, phenotypic selection analyses that include both the onset and synchrony of flowering may help to discern the independent and combined contributions of these traits to fitness (Haggerty, unpublished data). This will further help to improve forecasts of population responses to ongoing climate change as well as potential ecological consequences of changes in flowering time.

Conclusions

Evaluating the links between plant phenology and climate has become a common goal in ecology and evolution. A species' phenotypic and genetically based phenological sensitivities to climatic variation can be evaluated by analyzing the relationship between mean population phenology and climatic conditions. Spatial correlations between phenological and climatic parameters can provide fundamental information for forecasting temporal changes in phenology in response to environmental change. While these predictions may be fairly straight-forward in regions where growing seasons are constrained by temperatures alone, they will be more challenging in semi-arid and other water-limited ecosystems where combinations of climatic factors limit plant growth and reproduction. Even as gridded climatic data become more widely available through central databases, there remain no optimal solutions for calculating meaningful growing season parameters in semi-arid regions. Consequently, it will remain a challenge to characterize geographic variation in seasonal climatic conditions in these regions without evaluating phenological sensitivities to multiple climatic parameters.

The complex nature of growing season conditions in California's Sierra Nevada serves as an excellent natural laboratory with which to evaluate geographic variation in phenology and its relationship to complex climatic conditions. In my research I aim to use this setting to evaluate the climatic influences on the geographic variation in flowering phenology in the annual wildflower *Clarkia unguiculata*. In doing so my goal is to begin to evaluate the adaptive significance of flowering phenology by discerning the proximate and ultimate sources of variation in flowering onset, duration, and synchrony. In my next chapter I run a common garden experiment with seeds collected from 29 *unguiculata* populations (including the 10 field sites) representing the entire latitudinal and elevational range of the species. In this experiment I investigate whether long-term winter-spring climatic conditions may have influenced the evolution of flowering onset, duration, and synchrony. Overall, my research expands our current knowledge on the adaptive significance of flowering phenology and the ways in which wild plant populations and species may respond to ongoing environmental change.

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Table 1. Names and locations of 10 field research sites for which daily climatic data were obtained from PRISM Climate Group at 800m & 4km spatial resolution for the period 1981-2010.

Site Name	Elevation (m)	Latitude	Longitude
Powerhouse	365	35° 28’ 00.46”	-118° 45’ 16.85”
Live Oak	429	35° 28’ 48.66”	-118° 44’ 52.78”
Stark Creek	460	35° 28’ 29.35”	-118° 43’ 37.15”
China Garden	654	35° 31’ 01.21”	-118° 38’ 48.58”
Cow Pie	712	35° 34’ 00.03”	-118° 34’ 03.26”
Mill Creek	733	35° 32’ 15.96”	-118° 36’ 48.64”
Granite Road	863	35° 41’ 26.45”	-118° 43’ 54.04”
Jack & Stage	1000	35° 47’ 45.00”	-118° 42’ 08.80”
Greenhorn	1198	35° 43’ 16.82”	-118° 30’ 03.09”
Sugarloaf	1251	35° 48’ 21.40”	-118° 39’ 54.80”

Table 2. Results of regression analyses evaluating elevational gradients in eleven climatic parameters that characterize 30-year means from January-May across ten sites (N=10). Regression coefficients are reported as the change in climatic parameter per 100m increase of elevation.

Climatic parameter	Definition	Regression coefficient	R^2	P-value
Tmin	Average daily minimum temperatures	-0.28	0.83	0.0003
Tmean	Average daily mean temperatures	-0.28	0.78	0.0008
Tmax	Average daily maximum temperatures	-0.28	0.63	0.0061
GDDsum	Accumulated heat, calculated as the number of degrees that mean daily temperature exceeds 10°C	-22.16	0.77	0.0009
PPTsum	Mean monthly sum of precipitation values	1.73	0.62	0.0065
GSLstart	Day of year of last spring frost	3.70	0.76	0.0008
GSLend	Day of year of first autumn frost	-2.19	0.75	0.0012
GSL	Days between last spring frost and first autumn frost	-5.89	0.80	0.0005
VPDmax	Maximum vapor pressure deficit	-8.09	0.89	<0.0001
PPTend	Day of year of last precipitation event exceeding 0.25 inches	0.88	0.22	0.1664
	Outlier excluded	*1.73*	*0.88*	*0.0002*
GSLstart-PPTend	Days between last spring frost and last precipitation event	-2.82	0.67	0.0040

Figure 1. Temperatures decrease and precipitation increases with elevation in the southern Sierra Nevada Mountains. Each point is a 30-year mean from January-May, ±1 SE. See Table 2 for regression slopes and statistical parameters. Closed circles: Tmin. Closed diamonds: Tmean. Closed squares: Tmax. Open circles: Precipitation.

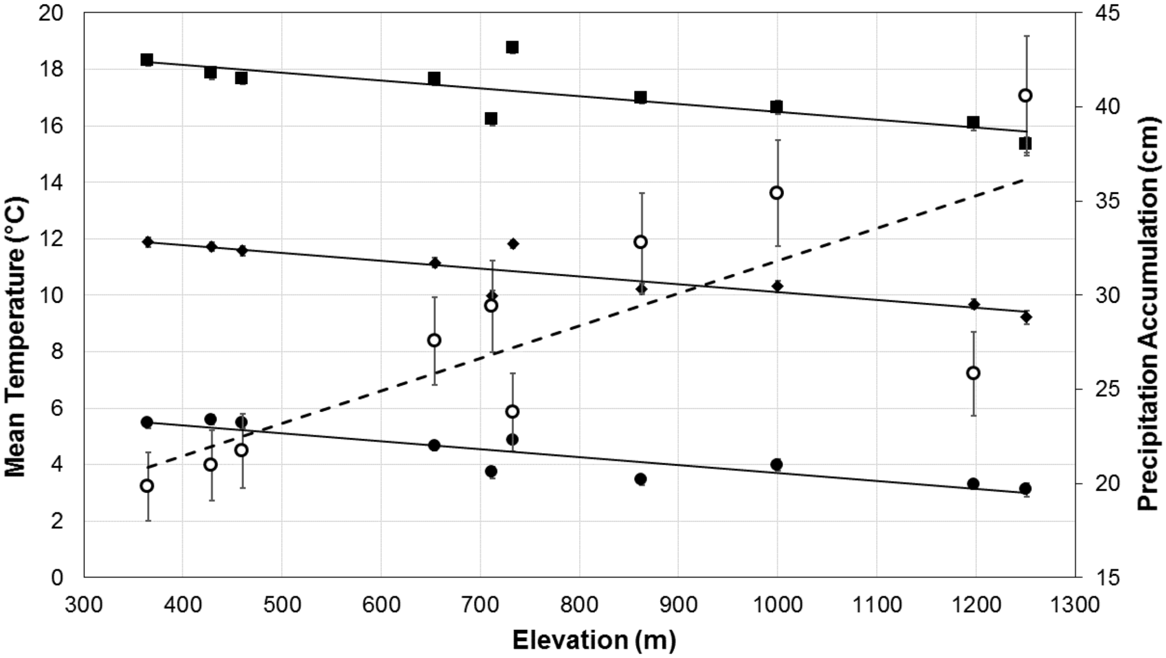


Figure 2. Heat accumulation is greatest at low elevations and declines with elevation. Each point is a 30-year mean from January-May, ±1 SE. See Table 2 for regression slope and statistical parameters.

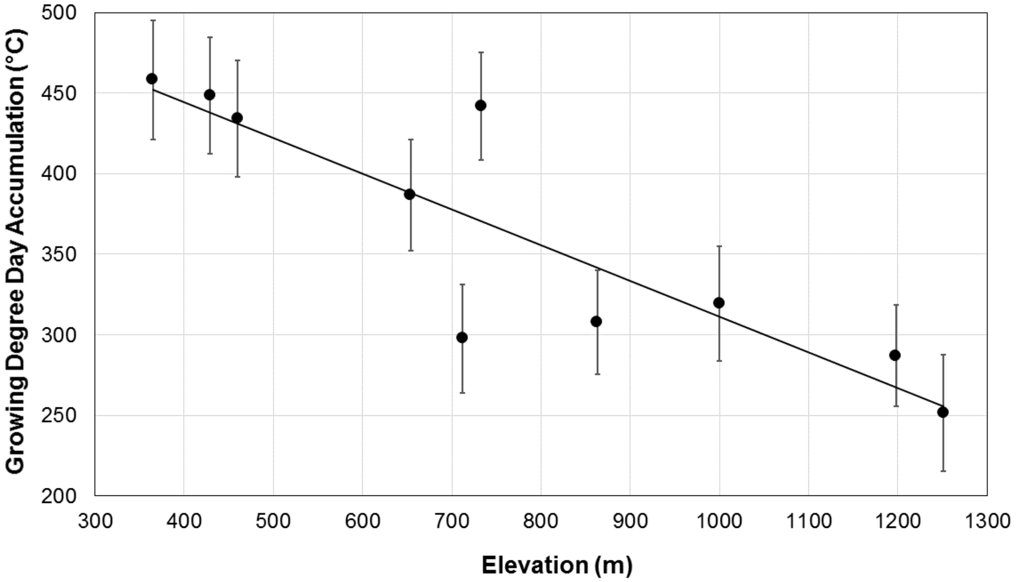


Figure 3. Mean maximum vapor pressure deficit decreases with elevation. Each point is a 30-year mean from January-May. See Table 2 for regression slope and statistical parameters.

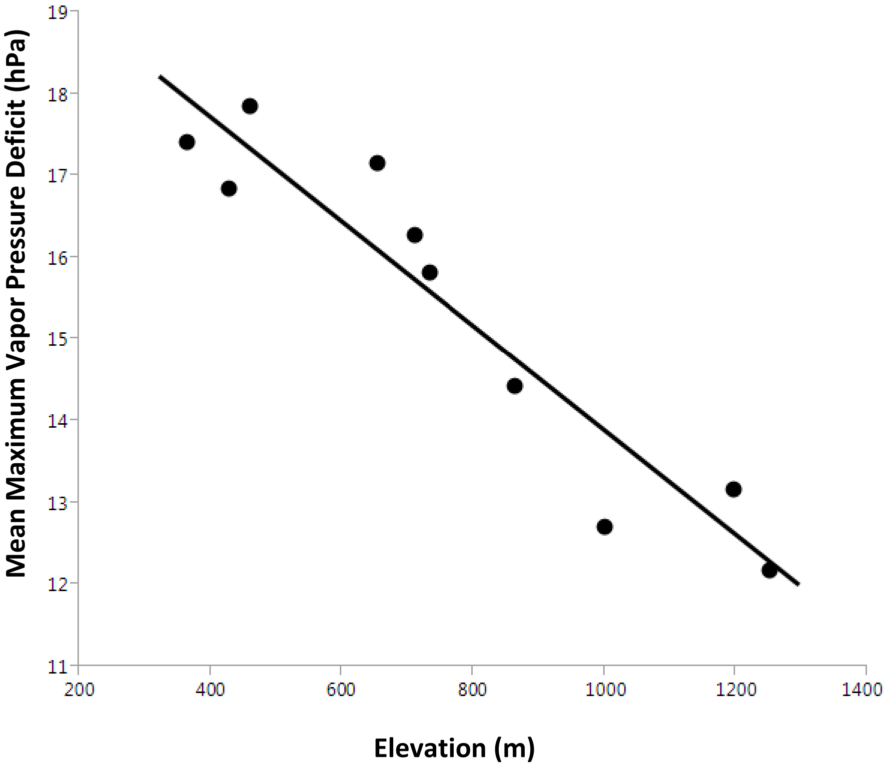
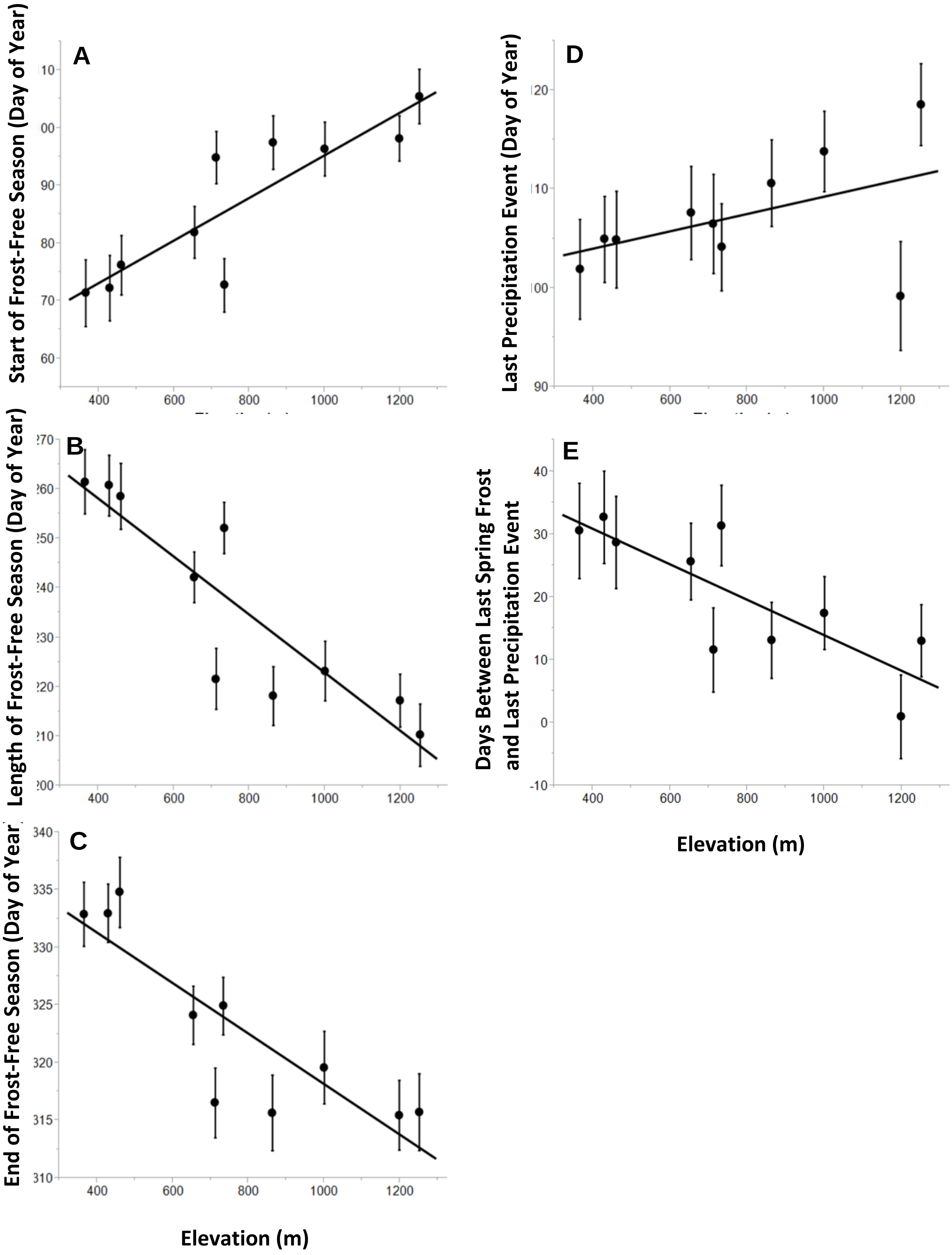


Figure 4. Elevational gradients of the timing of climatic milestones in the southern Sierra Nevada Mountains. Each point is a long-term norm during the January-May period, ±1 SD. See Table 2 for statistical analyses.



Genetically Based Geographic Variation in Flowering Phenology is Associated with Long-Term Climatic Conditions in a Semi-Arid Ecosystem

Abstract

Phenotypic traits that enhance fitness are often associated with local climatic conditions. In such cases, it is a reasonable assumption that natural selection played a role in generating the observed differentiation among individuals and populations. While previous investigations of wild plant species have detected genetically based differentiation among populations in the timing of flowering onset associated with local climatic conditions, none have simultaneously evaluated population differentiation in mean individual lifetime flowering duration or the synchrony of flowering among population members. In addition, none have detected the independent effects of flowering onset and duration on the degree of synchrony among population members. Understanding the quantitative relationships among all three phenological parameters, and their associations with climatic conditions, is required to forecast the effects of climatic change on these ecologically important reproductive attributes.

To investigate whether long-term winter-spring climatic conditions may have influenced the evolution of flowering onset, duration, and synchrony in a semi-arid ecosystem, I conducted a common garden experiment with seeds collected from 29 populations of a California endemic annual wildflower (*Clarkia unguiculata*) representing the entire latitudinal and elevational range of the species. Extensive genetically based differences were detected among populations for each phenological parameter. When grown in a common environment, populations originating from low latitudes and elevations

characterized by relatively warm and dry winter-spring conditions flowered significantly earlier, for a longer duration, and with lower synchrony than populations originating from higher latitudes and elevations characterized by relatively cool and mesic conditions. Overall, latitudinal and elevational clines in flowering phenology mirrored latitudinal and elevational gradients in long-term climatic conditions. However, variation in flowering duration was best explained by days to flowering, and variation in synchrony was best explained by duration. If these geographic patterns reflect the outcome of adaptive evolution on flowering time, then the warm and dry conditions forecasted for California in the coming decades are likely to exert direct selective pressures on flowering time, which may cause the evolution of earlier onset of flowering, longer potential flowering duration, and lower flowering synchrony.

Introduction

Changes in phenology are among the most ubiquitous responses of plants to climate change and have now been reported in hundreds of species worldwide (Menzel et al. 2006; Parmesan 2007; Polaczanska et al. 2013; IPCC 2015). As a result, understanding the links between a species' phenological parameters and changing climatic conditions has become a natural goal in ecology, evolution, and natural resource management (Enquist et al. 2014). Syntheses of long-term records have been instrumental in characterizing phenological sensitivities of a modest number of plant species to changes in average temperatures (Willis et al. 2008; Davis et al. 2010; Mazer et al. 2013). Yet our understanding of climatic drivers of phenological patterns remains limited for two key reasons. First, long-term records do not exist for most species, particularly in water-limited ecosystems (but see Crimmins et al. 2010). Second, the mean date of flowering onset within populations has historically received much more attention than other flowering parameters, namely flowering duration and the degree of flowering synchrony among population members (Munguia-Rosas et al. 2011). This limits our understanding of how the suite of phenological parameters that optimize flowering time in the short term might facilitate or constrain adaptive responses to environmental change over the long term. Expanding our knowledge on the quantitative relationships among flowering onset, duration, and synchrony, and their associations with multiple climatic conditions, is essential to improving our ability to forecast the effects of climatic change on these ecologically important reproductive attributes.

In the absence of long-term records, or to complement ongoing monitoring efforts, predictions of a species' phenological responses to climate change can be informed by studies of geographic co-variation between local climatic conditions and population-level plant phenology (see Etterson et al. 2016 and references therein). This space-for-time approach (Pickett 1989), when applied to wild populations under natural conditions, can generate statistical associations between mean population phenotypes and *recent* local climatic conditions (Mazer et al. 2015). Alternatively, the space-for-time approach can be applied to common garden and growth chamber experiments that are designed to minimize environmental variation and reveal genetically based differences among mean population phenotypes. In these settings, where genetically based differences among populations are strongly and consistently associated with *long-term* climatic conditions in their natal locations, climate-driven natural selection may be inferred to have played a role in generating the observed differentiation (Stinchcombe et al. 2004; Schneider & Mazer 2016).

The drive to understand the adaptive links between intraspecific variation in plant phenology and climatic conditions has long inspired common garden experiments across latitudinal and/or elevational gradients (Claussen et al. 1940; Jonas & Geber 1999; Kooyers et al. 2014; see also reviews by Korner 2007 and DeFrenne et al. 2013). The onset of flowering in wildflowers has been the focus of intensive investigation because it is a key life history transition that is relatively easy to monitor, it can be highly sensitive to environmental conditions (Menzel et al. 2006), and early onset of flowering is often associated with higher reproductive success and therefore under strong selection (Munguia-Rosas et al. 2011, Kingsolver et al. 2012). Clinal variation in flowering onset has been reported in several wildflower species, such that, when grown in a common environment, populations from northern latitudes or higher elevations are genetically determined to begin flowering earlier than conspecifics from southern or lower elevation sites (e.g., *Capsella bursa-pastoris* – Neuffer & Hurka 1986; *Campanulastrum americana* – Kalisz & Wardle 1994; *Solidago* *altissima* and *S. gigantea* – Weber & Schmid 1998; *Arabidopsis thaliana* – Stinchcombe et al. 2004; *Mimulus guttatus* – Kooyers et al. 2014). This pattern is generally interpreted as populations from northern latitudes and higher elevations adapting to a shorter frost-free growing season, however the co-variance between clines in flowering onset and the duration of the frost-free season remains to be demonstrated. Additionally, in two studies in California's semi-arid region that included both latitudinal and elevational gradients in their sampling design, the strength or direction of latitudinal clines in flowering onset differed with elevation (and vice versa such that the strength or direction of elevational clines differed with latitude) (*Clarkia unguiculata* – Jonas & Geber 1999; *Mimulus guttatus* – Kooyers et al. 2014). Both studies pointed to local variation in climatic parameters that disrupted regional climatic gradients, though only the latter study statistically tested for phenology-climate associations and demonstrated that this complex pattern was due to an interaction between temperature and precipitation. Much remains to be learned about the climatic drivers of phenological adaptation in semi-arid ecosystems, particularly in California, which is forecasted to become warmer and have more variable precipitation including drier wet seasons in the coming decades (Cayan et al. 2008; Berg & Hall 2015).

Much of what we know about the adaptive significance of flowering phenology and the capacity of plants to adapt to changing climatic conditions is restricted to the timing of the onset of flowering. This phenological parameter is commonly used as a proxy for the subsequent progression of reproduction, whereas lifetime flowering duration and the synchrony of flowering among conspecific population members are generally overlooked. What little information we have about the progression of the flowering season remains inconclusive. For example, in a meta-analysis of 105 species studied in natural settings, Munguia-Rosas et al. (2011) found no net phenotypic selection on flowering synchrony, even though selection on higher synchrony (e.g., Pettersson 1991) and lower synchrony (e.g., Rathcke 1983; Devaux & Lande 2009) was detected within some of the studies that were reviewed. As a result far less is understood about the adaptive significance of these reproductive attributes of individuals and populations relative to the onset of flowering.

To investigate whether long-term multidimensional climatic conditions may have influenced the independent or integrated evolution of flowering onset, duration, and synchrony in a semi-arid ecosystem, I conducted a common garden experiment to cultivate field-collected seeds representing 29 populations of a California annual wildflower, *Clarkia unguiculata*, collected from across the entire latitudinal and elevational range of the species. By growing populations together in one greenhouse and using initial mean individual seed mass as a covariate in all analyses, environmental sources of variation are reduced, allowing phenotypic differences observed among population means to be interpreted as having a genetic basis. If population differences in mean phenotype correlate with climatic differences among sampled sites, it suggests that selection caused by climatic conditions has caused phenological parameters to evolve. In sum, this chapter addresses the following questions:

1. What is the extent of geographic variation in multiple climatic parameters that may serve as drivers for evolutionary change (by natural selection) in *Clarkia unguiculata's* phenological parameters?
2. When populations sampled from across *unguiculata's* geographic range are raised in a common environment, are genetically based clines detectable in the onset of flowering, the lifetime flowering duration and/or in the synchrony of flowering among population members?

3. What is the extent of geographic co-variation between multiple long-term climatic parameters from populations' natal sites and *unguiculata's* mean population flowering onset, duration, and synchrony?
4. Is there evidence for the joint evolution of flowering onset, duration, and synchrony in response to geographic variation in climatic conditions, or are phenological parameters in *unguiculata* independent of one another?

Methods & Analysis

Seed collections & study populations

In the spring of 2009, I identified candidate regions across the known latitudinal range of *Clarkia unguiculata* where populations were likely to be found across local elevational gradients. This search was informed by personal observations and communications; published records of population locations (Jonas & Geber 1999); and herbarium records maintained and available online (Consortium of California Herbaria and the Jepson Interchange for California Floristics; http://ucjeps.berkeley.edu/). In each of seven identified regions (Table 1), I conducted extensive roadside surveys during May-July 2009 to locate candidate populations and to collect seeds.

To be considered for seed collections, populations had to be comprised of at least several hundred individuals, easily accessible but in a natural habitat, within a large but well-defined area (approximately 0.5-1.0 hectare), and ripe fruits had to be available on nearly all (>95%) individuals. Across each elevational transect, populations were selected from across the available elevational range in order to collect seeds from the lowest, middle, and highest possible elevations with the goal of maintaining intervals between collection sites of approximately 100-300 meters elevation and at least 1 km distance. In one transect (Kern), seed collections occurred over shorter elevation intervals because these populations are the focus of additional field studies; in another transect (Merced), two overlapping transects were later merged, and two pairs of populations (MR1 vs. YS1 and YS1 vs. MR2) were < 100 m apart in elevation (Table 1). Attributes of each population were recorded at the time of seed collection, including location (GPS; Garmin Ltd., Switzerland), slope (clinometer; Suunto Oy, Finland), and aspect (compass; Suunto Oy). Seed collections were conducted in 29 populations distributed among 7 elevational gradients (Table 1) spanning 6.7° latitude (range: 33.25°N – 39.93°N) and 1200m elevation (range: 54m – 1251m), representing the known latitudinal and elevational range of the species. Although the geographic range of *Clarkia unguiculata* includes both the coastal and inland mountain ranges that surround California's Central Valley, populations were collected only from the inland range (Sierra Nevada Mountains) because of the steeper elevational gradients in climate and the higher elevations that occur there.

Within each sampled population, seeds were collected separately from up to 75 haphazardly selected individuals (maternal families), with >1 meter between sampled individuals. On each plant, 5-10 capsules were collected from the main stem, placed in a coin envelope, sealed, and labeled by maternal family, population, and transect. Seed packets were stored in the lab for 2-3 months in cool dry conditions before being processed for the experiment.

Common Garden Study

During July-September 2009, each of up to 75 seed packets for each of 29 populations (N=2175) was processed in the lab by isolating seeds from fruit and stem fragments. Twenty seeds per packet (maternal family) were separated and weighed using a microbalance to estimate the mean individual seed mass of each maternal family. Weighed seeds were stored in the lab in a separate packet until the start of the experiment. Once all seeds had been weighed, ten seeds per maternal family were then selected from the weighed seeds, broadcast on an agar-filled petri dish, and placed in a cold room under constant darkness at 10°C for 10 days for vernalization. Dishes were then brought to the lab September 25 where they were arrayed on lab benches under ambient light and room temperature and checked daily for germination.

During October 7-16, three seedlings per dish were transferred to one labeled cone-tainer (model SC10 UV-stabilized; Stuewe & Sons, Tangent, OR, USA) that was filled with well-mixed potting soil (Sunshine #4 potting mix; Sun Gro Horticulture, Agawam, Massachusetts, USA) and three slow-release fertilizer pellets (14-14-14 NPK; Osmocote Scotts-Sierra horticultural Products, Marysville, Ohio, USA). All seedlings were transferred to cone-tainers within one day of cotyledons opening so that transplanting was achieved at similar developmental stages. The transfer date was recorded for the subsequent calculation of the number of days to the onset of flowering. Additional seedlings were transplanted as needed if all initial transplants died and the new transplant date was recorded.

Cone-tainers were randomly arrayed into 98-hole trays (RL98 Tray; Stuewe & Sons). Each tray was covered with a 6-inch tall clear plastic lid to maintain humidity and moved to three growth chambers where they were hand-misted daily and kept at 20°C under a 14:10 L:D cycle. Trays were re-arranged daily among growth chambers to minimize any environmental heterogeneity among chambers. After 7d in the growth chambers, each cone-tainer was thinned to one seedling, the tallest individual being kept in every case, and all trays were uncovered and brought to a UC Santa Barbara greenhouse under a 14:10 L:D cycle (400W high-pressure sodium bulbs) where seedlings were kept moist by an automated misting system. To reduce any potential influence that microclimates within the greenhouse might cause, trays were re-assigned positions on the greenhouse tables every two days during the seedling stage. After 3 weeks, misting was stopped and hand-watering was initiated every 2 days or as needed throughout the experiment.

Once plants reached 0.5m height they were clipped to and supported by a heavy-gauge metal wire stake that was taped to the outside of each cone-tainer. The primary stem of each plant was clipped to the stake as needed with ½-inch plastic loops that were cut from letter-size paper binding combs (GBC CombBind). From November 19-23, in order to reduce density in the trays to accommodate the growing plants, all conetainers were re-distributed across additional trays by randomized assignment, leaving 12 cone-tainers representing different maternal families and populations per tray. At this point, due to space constraints, all racks were transferred to a different larger greenhouse where the same light and water regimes were maintained. Trays were positioned in the larger greenhouse by random assignment.

The first flower in the experiment appeared on December 12, after which daily monitoring was conducted to record the date of flowering onset for every individual, and then monitoring was continued every 2 days to record the number of open flowers produced by all plants in flower. A flower was considered open if the reproductive structures (anthers and style) were visible. On January 21, 2010, the number of plants for which data were collected was reduced due to logistical constraints, although all plants physically remained in the trays in the greenhouse. Within each population, individuals were selected by randomized assignment so that the sample size was cut to N=30 (each representing a different field-collected maternal family). For populations for which the sample size already was below 30, all individuals were retained. All plants and cone-tainers remained in their trays until all plants had finished flowering, at which point the experiment was concluded. After accounting for plant mortality and missing data, a complete data set was recorded for 755 individuals (maternal families) representing all 29 populations.

Climate information

The life history schedule of *Clarkia unguiculata* in natural populations is to germinate with winter rainfall and complete reproduction by the end of spring or early summer (Lewis & Lewis 1955). To evaluate the potential influence of average winter and spring climatic conditions on flowering phenology observed in the greenhouse, I obtained long-term climatic data from two online databases for the months January-May covering the time period 1981-2010.

Thirty-year monthly norms for most climatic parameters were obtained from PRISM Climate Group (Oregon State University, http://prism.oregonstate.edu, created 4/7/2016) by entering the latitude and longitude of each site into the PRISM Data Explorer. Stability of all obtained PRISM climatic data were designated “stable” in the output, indicating that reporting climate networks had finalized their information and PRISM Climate Group is unlikely to update the data in the future. I obtained norms for mean monthly values in metric units at 800m resolution with interpolated grid cell values for each of the following climatic parameters: minimum daily temperature (Tmin); mean daily temperature (Tmean); maximum daily temperature (Tmax); monthly accumulated precipitation (PPT); and maximum vapor pressure deficit (VPDmax). The long-term mean for each month was then averaged for the January-May period and used in subsequent analyses.

In addition, I obtained daily values of Tmin and Tmax for each site from the PRISM Data Explorer for the time period 1981-2010 in order to calculate monthly mean growing degree day accumulation (GDD) during January-May. Daily values were available only at 4km resolution, and data stability for all data sets was reported in the output as “stable”. GDD is a measure of daily heat accumulation, calculated as the number of degrees that the mean daily temperature is above a certain threshold (10°C in this study). For each site, I calculated the sum of GDD values for each month January-May, for each year 1981-2010. I then calculated the monthly mean GDD sum for each year, and then calculated the long-term mean; this is the value that was used in subsequent analyses.

Long-term monthly norms of drought severity during January-May were obtained from the Western Regional Climate Center’s WestWide Drought Tracker (University of Idaho Desert Research Institute, http://www.wrcc.dri.edu/wwdt/, created 4/8/2016). The Palmer Z-Index is a meteorological drought index that generally ranges from -4 or less (extreme drought) to +4 or more (extremely wet), and is calculated from recent precipitation and temperature as well as local available water content of the soil (Palmer 1965; Alley 1984). This index was selected over other available drought severity indices (e.g., Palmer Drought Severity Index, Self-Calibrated Palmer Drought Severity Index) because its calculation is limited to each month’s meteorological activity without being weighted by conditions in preceding months or years. Thus, the Palmer Z-Index is generally accepted as a more ecologically-relevant measure of short-term drought and is commonly used in short-term agricultural planning. The WestWide Drought Tracker obtains precipitation and temperature data from the PRISM Climate Group at 4km resolution and soils data from the Natural Resources Conservation Service STATSGO soils database to calculate the Palmer Z-Index.

Phenological parameters

Days to flowering (DTF) was calculated for each individual as the number of days from transplant date to the date of flowering onset. Flowering duration (Duration) was calculated for each individual as the number of days from first flowering to the date that the last recorded flower opened. Flowering synchrony (Synchrony) is a context-dependent measure of overlap in flowering between each individual and its population (Primack 1980; Augspurger 1983; Elzinga et al. 2007), and was calculated for each individual using the equation described in Elzinga et al. (2007):

$$\sum_{t=1}^{k}\left[\left(\frac{x_t}{\sum_{t=1}^{k} x_t}\right)(f_t)\right]$$

Here, k is the maximum number of census days for a given population; x_t is the total number of open flowers on an individual plant on census day t; and f_t is the proportion of the total number of open flowers in that individual's population on census day t. This equation generates a value between -1 (asynchronous) and +1 (synchronous) for each individual. Thus, if an individual produces a large floral display over a short period of time that corresponds to its population reaching peak flowering, that individual is highly synchronous. Alternatively, if an individual produces a large floral display over a short period of time that is off its population's peak, or if an individual maintains a small floral display over a long period of time relative to its population, that individual is more asynchronous. Consequently, whereas there is one way for an individual to be synchronous, there may be two ways to be asynchronous. From these data, population-level means and variances can be calculated, allowing for quantitative comparisons of flowering synchrony among populations.

Analysis

Four main sets of least squares regression analysis were conducted to examine 1) geographic variation in climatic norms; 2) geographic variation in population mean phenological parameters; 3) relationships among population mean phenological parameters; and 4) relationships between climatic norms and population mean phenological parameters. All variables met requirements for normality except precipitation, which was normalized with a natural log transformation. For each regression with a phenological parameter as a response variable, mean individual seed mass (mg) was included as a covariate in order to control for the potential influence of maternal or field effects on phenotypes observed in the greenhouse (Roach & Wulff 1987). In each model, each independent effect was checked for multicollinearity by calculating variance inflation factors (VIF); a VIF above 5 (Rogerson 2001) or 10 (Kennedy, 1992; Hair et al. 1995; Neter et al. 1995) is considered to be an indication of correlation among independent variables that can inflate standard errors and model explanatory power. For all regression models, VIFs for each effect remained below 5 with a typical range of 1.0-2.1 and the two highest values were 3.8 and 4.8. All statistical analyses were conducted using JMP v11 Pro.

Geographic associations between climatic norms and mean phenological parameters were evaluated with least squares multivariate regression. The effects of latitude, elevation, their interaction, and their quadratic terms were assessed on each of seven climatic response variables, three phenological parameters, and mean individual seed mass. For each of these regressions where a phenological parameter or mean individual seed mass was the response variable, quadratic terms were not significant so were excluded from the final model.

To create bivariate plots with which to visualize latitudinal gradients of each climatic parameter independent of the effects of elevation, residuals were first obtained from regressions of each climatic parameter against elevation. These residuals were then regressed against latitude (and vice versa for elevational gradients). Surface profiling was conducted to provide additional detail on each geographic gradient by graphing simultaneously the relationships among latitude, elevation, and each response variable. This statistical modeling technique results in a three-dimensional graph that enables visual inspection of, for example, how the steepness of a latitudinal gradient or cline in a response variable can be influenced by elevation (i,e., a latitude x elevation interaction).

Relationships among population mean phenological parameters were evaluated with univariate and multivariate regressions. First, Pearson correlation coefficients were calculated for pair-wise relationships of phenological parameters (Duration vs DTF; Synchrony vs DTF; Synchrony vs Duration). Second, multivariate regression was conducted to test the effects of DTF, Duration, their interaction, and quadratic terms on Synchrony.

Relationships between climatic norms and phenological parameters were evaluated with multivariate regression and stepwise regression. To evaluate the influence of temperature and precipitation on flowering phenology in *Clarkia unguiculata*, two separate models were run for each phenological parameter. First, the effects of Tmax, precipitation, their interaction, and their quadratic terms were assessed on each phenological parameter with mean individual seed mass as a covariate. Due to high collinearity among the three measures of average temperatures (Tmin, Tmean, Tmax), Tmax was chosen for this analysis because it exhibited slightly steeper geographic gradients and consistently had stronger effects on each phenological parameter than the others. Second, to evaluate how heat accumulation rather than average temperatures might influence the evolution of flowering phenology, the effects of growing degree days, precipitation, their interaction, and their quadratic terms were assessed on each phenological parameter with seed size as a covariate. For both sets of regressions where Duration or Synchrony were the response variable, additional phenological parameters were included as covariates to control for the potential influence of trait correlations on detecting climatic effects on Duration and Synchrony. For the Duration models, Days to Flowering was included as a covariate. For Synchrony, three models were evaluated: (a) a model with DTF as a covariate; (b) a model with Duration as a covariate, and; (c) a model with DTF and Duration as covariates.

Finally, forward stepwise regression was conducted to identify the best predictors of each phenological parameter. The initial model included all climatic, geographic, and phenological parameters as well as seed size (10 parameters for DTF; 11 for Duration; 12 for Synchrony). Regressions were run in a forward direction using corrected minimum Akaike information criterion (AICc; Hurvich & Tsai 1989), although backward stepwise regressions resulted in the same outcomes. Each significant effect ($p<0.05$) was then used in the final model for each phenological parameter.

Results

Geographic variation of climatic norms

Mean winter-spring climatic conditions (i.e., the 30-year normals) varied substantially among the 29 sites (Table 1) and regression analysis revealed significant latitudinal and elevational gradients for each climatic parameter (Table 2). Generally, populations originating from lower latitudes and elevations are exposed to warmer and drier winter-spring conditions than populations originating from higher latitudes and elevations.

Precipitation increases significantly with latitude and with elevation, and a positive quadratic effect of latitude indicates non-linear increases in precipitation at northern latitudes (Table 2; Figure 2 A,B). Surface profiling shows that the latitudinal gradient of precipitation is steeper at higher elevation, and that the elevational gradient is steeper at northern latitudes (Figure 3A).

Mean temperatures (Tmin, Tmean, Tmax) decrease significantly with latitude and with elevation (graphed for Tmax only, Figure 2 C,D), with Tmax showing a slightly steeper rate of change across both gradients than Tmin and Tmax (Table 2). A negative quadratic effect of elevation on Tmax and Tmean indicates a non-linear decrease of temperature at higher elevations (Figure 2 C,D). Additionally, a significant latitude*elevation effect for Tmean and Tmax (Table 2) can be seen in the surface profile for Tmax (Figure 3B) which shows steeper elevational decline in temperature at northern latitudes (relative to southern latitudes) and steeper latitudinal decline at high elevations (relative to low elevations).

Heat accumulation (growing degree days) also decreases significantly at higher latitudes (independent of elevation) and elevations (independent of latitude) (Table 2; Figure 2 E,F). A significant latitude*elevation effect can be seen in surface profiling which, similar to mean temperatures, reveals a steeper elevational decline in growing degree days at northern latitudes and a steeper latitudinal decline of GDD at higher elevations (Figure 3C).

Maximum vapor pressure deficit decreases significantly with latitude (independent of elevation), and a significant negative quadratic elevational effect indicates a peak of VPDmax at mid elevations (Table 2; Figure 2 G,H). This indicates that, for the 29 sites in this study, the atmosphere is drier in southern and mid-elevation sites. Surface profiling indicates that the latitudinal gradient for VPDmax also is steeper at high elevations and that the elevational gradient is steeper at high latitudes (Figure 3D).

Finally, the Palmer Z-Index increases significantly with latitude independent of elevation (Table 2), indicating that southern sites have stronger drought conditions than northern sites (Figure 2I). Across elevation, the Palmer Z-Index is similar to VPDmax in exhibiting a significant negative quadratic effect across elevation (Table 2). However, whereas higher values of VPDmax indicate a stronger atmospheric driving force of water loss from leaves, it is lower values of the Palmer Z-Index that indicate stronger drought conditions through a combination of soil properties and temperature and precipitation values. Thus, the negative quadratic effect for the Palmer Z-Index indicates that some high and low elevation sites experience stronger drought conditions than mid-elevation sites (Figure 2J). Surface profiling indicates that the latitudinal gradient for the Palmer Z-Index is steeper at low elevations (Figure 3E).

In sum, populations originating from lower latitudes and elevations are generally exposed to warmer temperatures (Tmin, Tmean, Tmax), greater heat accumulation (GDD), lower precipitation (PPT), and higher potential drought stress indicated by higher VPDmax and lower Palmer Z-Index values. All climatic norms also show non-linear changes over latitude, elevation, or both. Additionally, latitudinal gradients of all climatic parameters are steeper at higher elevations with the exception of the Palmer Z-Index which is steeper at lower elevations, and elevational gradients of all climatic parameters are steeper at higher latitudes except for the Palmer Z-Index which is steeper at lower latitudes.

Geographic variation of population mean phenological parameters

Analysis of population mean phenological parameters measured in the greenhouse revealed extensive differences among populations for mean flowering onset, duration, and synchrony (Table 1; Figure 4). There was no significant change in mean individual seed mass with either latitude or elevation (Table 3; Figure 5 G,H). In the multivariate regressions containing only mean individual seed mass as a covariate, elevational clines were not detected for any of the phenological parameters (Table 4; Figure 5 B,D,F). Significant latitudinal clines, however, were detected for Days to Flowering and Flowering Duration, and a near-significant latitudinal cline ($p=0.07$) was detected for Flowering Synchrony (Table 3). Thus, when grown in the common greenhouse environment provided here, populations originating from lower latitudes started flowering significantly earlier (Figure 5A), flowered for a significantly longer time (Figure 5C), and tended to flower less synchronously than populations from higher latitudes (Figure 5E).

In these models, a significant latitude*elevation interaction was detected for Duration and Synchrony (Table 3). Surface profiling indicates that the latitudinal cline for both traits is stronger at higher elevations than at relatively low elevations (Figure 6 B,C). Although the latitude*elevation effect was not detected for DTF, surface profiling suggests a similar pattern where populations from higher elevations show a steeper latitudinal cline than populations from low elevations (Figure 6A). These surface profiles also show that the effect of elevation on each of the three phenological traits is qualitatively different at low versus high latitudes. At low latitudes, the slope of the elevational gradient is negative for DTF and Synchrony, and positive for Duration. However the opposite is true at high latitudes – the slope of the elevational gradient is positive for DTF and Synchrony, and negative for Duration.

The latitudinal clines in Duration and Synchrony that were detected with these regression models were lost when other phenological parameters were included as covariates. For each of the following models, multicollinearity among independent factors was checked with variance inflation factors; VIFs were always below 5 and never higher than 3.4, indicating no significant multicollinearity among independent factors. When DTF was added to the model designed to detect geographic sources of variation in Duration, the negative effect of latitude on Duration was no longer detectable and DTF was the only variable with a significant (negative) effect on Duration; earlier-flowering individuals flowered for significantly longer periods than late-flowering individuals (Table 3). In this model, DTF explained over twice as much of the variation in Duration than the model that included only latitude and elevation as main effects (R^2 of 0.63 vs. 0.30). Similarly for Synchrony, when either DTF or Duration was added to the model, the near-significant latitudinal cline was lost and each phenological trait tested alone explained over 3 times as much variation in Synchrony as the geographic effects on their own. Finally, when DTF and Duration were both included as covariates in the model predicting Synchrony, only Duration had a statistically significant effect on Synchrony, and the model explained 75% of the variance in Synchrony (Table 3). Thus, geographic variation in Synchrony is influenced more by variation in Flowering Duration than by geographic location, and Flowering Duration is influenced more by clinal variation in DTF than geographic location.

Regression analyses to examine the relationships among phenological parameters (Table 4) revealed that populations that start flowering early also flower significantly longer (Figure 7A) and less synchronously (Figure 7B) than populations that start flowering later. Lower synchrony also is associated with long flowering duration (Figure 7C). While the surface profile for these three parameters also indicates that low synchrony is associated with long duration, it does not reveal any general relationships between DTF and either Duration or Synchrony (Figure 7D).

Climatic sources of variation on phenological parameters and seed size

In contrast to latitude and elevation, differences in flowering phenology among populations were significantly associated with climatic differences. This was determined with three sets of regressions, one set for each phenological parameter (Figure 8; Table 5).

First, with seed size as a covariate, regressions of Days to Flowering on each of the climatic norms (i.e., five separate regressions) indicated that early flowering is significantly associated with warmer temperatures (high Tmax and GDD), lower precipitation, drier conditions (VPDmax), and stronger drought (low Palmer Z-Index) (Figure 8 A-E). Similarly longer Flowering Duration is associated with warmer temperatures (high Tmax & GDD), low precipitation, and drier conditions (VPDmax) (Figure 8 F-J). Flowering Synchrony was significantly associated only with VPDmax whereby low synchrony was associated with drier atmospheric conditions (high VPDmax; Figure 8 K-O). Thus, simple univariate regressions indicate that warm and dry conditions are associated with early, long duration, and low synchrony flowering (analyses below, however, detect the independent effects of each climatic variable on phenological traits.)

Table 5 provides summaries of eight multivariate regression models that include different combinations of covariates. For Days to Flowering, lower winter-spring precipitation is associated with earlier onset of flowering. No effect of temperature was detected on DTF. For Flowering Duration, the base model with mean individual seed mass included as a covariate indicates that higher Tmax, as well as high values of the Tmax*Precipitation interaction, are associated with longer flowering duration. However, when DTF is included as a covariate to the Flowering Duration model, the effect of Tmax is no longer significant, while the effect of Precipitation is nearly statistically significant (p=0.07), as is the effect of DTF. Thus, longer flowering duration is strongly associated with earlier flowering and is mildly associated with higher winter-spring precipitation.

For Flowering Synchrony, the base model with seed mass included as a covariate indicates that high values of the Tmax*Precipitation interaction term result in lower flowering synchrony (Table 5). Again, however, this effect is lost when either DTF or Duration are included as a covariate; flowering synchrony is not affected by temperature or precipitation independent of DTF or Duration. These models indicate that higher flowering synchrony is associated either with later onset of flowering (higher DTF) or with shorter flowering duration. However, when both DTF and Duration are included as covariates in the model, Duration is the only factor that has a significant association with Synchrony – higher synchrony is associated with shorter duration. Thus, precipitation is strongly positively associated with DTF, and DTF is negatively associated with the duration of flowering (duration also is mildly influenced by precipitation); in addition, the duration of flowering is negatively associated with the synchrony of flowering.

To examine the potential influence of winter-spring heat accumulation (GDD) instead of mean maximum temperatures on population differentiation in flowering phenology, the same set of multivariate regressions was conducted including GDD and Precipitation as main effects (Table 6). The results are qualitatively similar to the model with Tmax (Table 5), with the exception that higher seed mass is associated with higher values of the GDD*Precipitation interaction term.

Finally, the fourth set of regressions that were conducted to evaluate the relationships between climatic and phenological parameters was a set of stepwise regressions to identify the best geographic, climatic, and phenological predictors for each phenological parameter. The three phenological parameters differed in their significant predictors (Table 7), and the final models informed by these predictors are presented in Table 8 and visualized with surface profiling in Figure 9.

Days to Flowering was predicted best by precipitation and maximum vapor pressure deficit – earlier flowering in the greenhouse is associated with drier long-term conditions (low precipitation; high VPDmax) (Table 8). Although a significant Precipitation*VPDmax interaction was not detected in the model, the surface profile suggests that the effect of precipitation on DTF is diminished when VPDmax is high, and the effect of VPDmax on DTF is similarly lost when Precipitation is low (Figure 9A). Thus, populations from historically dry sites flower just as early regardless of whether the dry conditions are due only to low precipitation, only to dry air, or to both.

Flowering Duration was predicted best by DTF and Palmer Z-Index – longer flowering duration is associated primarily with earlier flowering but also with reduced drought conditions (i.e., higher Palmer Z-Index) (Table 8). While a significant interaction effect was not detected, the surface profile suggests that, for populations that flower early, alleviating drought conditions has little effect on flowering duration (Figure 9B).

Flowering synchrony was predicted best by Duration and Seed Mass – low flowering synchrony is associated with longer flowering duration, higher seed mass, and their interaction (Table 8). The surface profile indicates that the effect of flowering duration on synchrony is diminished when seed mass is high (Figure 9C). It also indicates that the effect of seed mass on synchrony is diminished when flowering duration is long.

Discussion

I examined the extent to which geographic variation and long-term climatic conditions may have influenced the evolution of flowering phenology of a California endemic annual wildflower, *Clarkia unguiculata*. In this common garden study, I evaluated the geographic covariance of average winter-spring climatic conditions and the onset, duration, and synchrony of flowering for 29 populations sampled from across the species' entire latitudinal and elevational range and raised in a common environment.

Climatic differences among the sampled sites in turn are associated with latitudinal and elevational differences. Early flowering onset, long flowering duration, and low flowering synchrony are associated with warm and dry conditions that are characteristic of the low latitude and low elevation sites in this study. Overall, the latitudinal cline in flowering phenology is stronger than that of the elevational clines, mirroring climatic gradients that also are generally stronger across latitude than elevation.

Geographic variation in multidimensional climatic conditions may drive evolutionary change in phenological parameters

Among the 29 sampled sites, multiple dimensions of long-term winter-spring climatic conditions were found to exhibit strong latitudinal and elevational gradients. Whereas mean temperatures, heat accumulation, and drought indicators strongly decrease with increased elevation and latitude, accumulated precipitation strongly increases. Moreover, six of seven climatic parameters exhibited steeper elevational gradients at higher latitudes and steeper latitudinal gradients at higher elevations. The remaining climatic parameter (Palmer Z-Index) exhibited similarly strong gradients but in the opposite direction – steeper elevational gradients at lower latitudes and steeper latitudinal gradients at lower elevations.

The nature of these opposing and non-linear gradients provides a foundation for an ecological setting in which a complex combination of temperature, precipitation, and annual drought are very likely to have both regional and local influences on plant productivity. This study also provides quantitative evidence that climatic gradients do not necessarily change linearly with latitude or elevation, an assumption often made when climatic gradients are inferred – and not statistically evaluated – from geographic gradients (Korner 2007; DeFrenne et al. 2013). The recent availability of extensive gridded climatic data through central databases (e.g., PRISM Climate Group) poises ecologists, evolutionary biologists, and natural resource managers to integrate multiple climatic parameters into their research programs and help improve our understanding of how multidimensional climatic conditions may act as selective agents on plant phenology.

Genetically based clines in flowering onset, duration, and synchrony

The detection of significant differences in flowering onset, duration, and synchrony among populations in a common environment, controlling statistically for variation in the mean individual seed mass of sown seeds, supports the hypothesis that these differences are genetically based. The extent of genetically based differences among populations is easily seen when population means are rank-ordered by trait values (Figure 4), which shows a difference of >50 days in mean population DTF, >40 days difference in mean population Duration, and a two-fold difference in Synchrony.

The phenological clines detected in this study – though comparable to those described in other wildflower species (*Prunellus vulgaris* – Bocher 1949; *Verbascum thapsus* – Reinartz 1984a, 1984b; *Datura stramonium* – Weaver et al. 1985; *Campanulastrum americanum* – Kalisz & Wardle 1994) – extend previous knowledge on the genetic basis to geographic variation in flowering phenology. Conventional wisdom holds that natural populations will evolve delayed, rapid, and synchronous flowering at higher latitudes and elevations where the growing season is abbreviated by shorter frost-free seasons (Rathcke & Lacey 1989; Korner 2007; deFrenne et al. 2013). In semi-arid ecosystems, however, where water and vapor pressure deficit may strongly limit plant growth and reproduction, the realized growing season for many annual and perennial wildflowers occurs during the cool rainy season when mean temperatures may be at their annual lowest.

In California, for example, opposing temperature and precipitation gradients (Figure 2) suggest that both temperature and precipitation, as well as the onset of annual summer drought indicated by VPDmax and Palmer Z-Index, regulate plant productivity and flowering phenology. Indeed, in the case of *Clarkia unguiculata*, when phenological parameters are regressed separately onto temperature and precipitation, higher precipitation appears to delay DTF, lower temperatures are associated with shorter Flowering Duration, and lower values of the temperature*precipitation interaction term are associated with higher Synchrony (Tables 5-6). Thus, flowering phenology in this study is inferred to be genetically differentiated for delayed, rapid, and synchronous flowering at higher latitudes and elevations, a pattern likely due to complex interactions with multiple climatic parameters.

Geographic co-variation between long-term climatic conditions and correlated phenological parameters

The associations between long-term climatic conditions and flowering onset, duration, and synchrony are consistent with the view that the geographic variation observed here is the outcome of selection caused by climatic factors. Among the phenological traits observed here, Days to Flowering has the strongest relationship with long-term climatic conditions. Although simple bivariate regressions showed strong associations between earlier DTF and higher Tmax and growing degree days (Figure 8), results from the stepwise regression indicate that early flowering onset in *unguiculata* is associated more strongly with dry soil and atmospheric conditions than with winter-spring temperatures (Table 7). This is seen in the models that detected that best predictors for variation in DTF were precipitation and maximum vapor pressure deficit. By comparison, the strongest predictor of variation in

Duration was DTF, and for Synchrony it was Duration (Tables 7-8). Interestingly, stepwise regression also indicated that, in addition to low synchrony being associated with longer flowering duration, it also is associated with higher seed mass (Tables 7-8). To my knowledge this is the first example of seed mass being associated with flowering synchrony, though how exactly this relationship may manifest warrants further experimental investigation.

Given their close phenotypic associations with each other and with DTF, flowering duration and synchrony are more likely to evolve in response to changes in DTF than to climatic factors directly. Thus, it is not only plausible that DTF is the primary target of selection by dry winter-spring conditions but also that flowering duration and synchrony evolve in response to phenological traits that precede them in seasonal sequence.

In the case of *Clarkia unguiculata*, which flowers during the transition into annual drought, selection on early flowering may be due to the requirement to complete flowering and seed maturation before the lack of rain brings an end to the growing season. This is supported in the data in Tables 5-8, which are consistent with the inference that precipitation and maximum vapor pressure deficit have had a strong influence on the evolution of flowering onset over recent decades. Thus, if there is little to no survival cost associated with flowering extremely early in natural settings (e.g., spring killing frosts), earlier flowering may be able to evolve within populations where there is both sufficient adaptive variation in flowering time and pollinators also shift their phenology in coordinated time.

Whether or not these phenological parameters continue to evolve and differentiate among *unguiculata* populations will depend on their associations with plant performance as well as the climatic agents of selection. The results of this study suggest that the warmer and drier conditions projected in California for the forthcoming decades (Cayan et al. 2008; Berg & Hall 2015) will exert phenotypic selection for earlier flowering, and if populations evolve earlier flowering in response, then they also will evolve the capacity to flower for a longer duration, the result of which will be the evolution of lower flowering synchrony.

Conclusion

The broad goal of this common garden study was to improve our understanding of the response of flowering phenology to climatic conditions in semi-arid ecosystems. Genetically based differences in flowering onset, duration, and synchrony that were found among 29 populations of *Clarkia unguiculata* are inferred to be the outcome of selection caused by long-term climatic conditions. However, due to their close phenotypic associations with each other and with flowering onset, flowering duration and synchrony are more likely to evolve in response to changes in flowering onset than to climatic factors directly. Consequently, warm and dry conditions forecasted for the coming decades in California are forecasted to promote the evolution of earlier flowering onset, a change that will also result in longer potential flowering duration and lower flowering synchrony.

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Table 1. Geographic attributes, sample sizes, mean climatic norms, mean phenological parameters and mean individual seed mass of the 29 *Clarkia unguiculata* populations cultivated in this common garden experiment. Transect locations are mapped in Figure 1 (Elev: elevation; Tmax: maximum temperature; GDD: monthly growing degree day; PPT: precipitation; VPDmax: maximum vapor pressure deficit). Table continues next page.

Transect	Pop	N	Latitude	Longitude	Elev (m)	
San Diego	DG1	30	33.255756	-117.241081	54	⋯
	DG2	23	33.252371	-117.165142	199	⋯
San Gabriel	GB1	30	34.232965	-118.311836	410	⋯
	GB2	27	34.619982	-118.561252	622	⋯
Santa Ynez	SY1	26	34.567275	-119.950531	252	⋯
	SY3	30	34.769887	-119.936879	566	⋯
	SY4	30	34.736205	-119.924992	884	⋯
Kern	PH	31	35.466794	-118.754680	365	⋯
	LO	31	35.480184	-118.747994	429	⋯
	SCr	31	35.474821	-118.726986	460	⋯
	CG	30	35.533671	-118.646827	654	⋯
	CP	30	35.566675	-118.567572	712	⋯
	MC	31	35.537767	-118.613511	733	⋯
	GR	31	35.690679	-118.731678	863	⋯
	JS	31	35.795832	-118.702449	1000	⋯
	GH	31	35.721339	-118.500858	1198	⋯
	SL	31	35.805945	-118.665221	1251	⋯
Sequoia	SQ1	29	36.466376	-118.836995	610	⋯
	SQ2	20	36.449952	-118.793208	766	⋯
Merced	WLMS	22	37.799512	-120.640639	102	⋯
	MR1	19	37.607848	-120.135641	277	⋯
	YS1	18	37.603833	-119.965602	371	⋯
	MR2	17	37.597143	-120.130210	399	⋯
	MR3	6	37.583667	-120.117309	508	⋯
	YS2	27	37.672566	-119.791202	570	⋯
	YS3	8	37.696286	-119.725430	1012	⋯
Chico	CH1	30	39.460681	-121.598125	41	⋯
	CH2	31	39.647498	-121.548539	315	⋯
	CH3	29	39.934255	-121.315626	598	⋯

Table 1 continued. Geographic attributes, sample sizes, mean climatic norms, mean phenological parameters and mean individual seed mass of the 29 *Clarkia unguiculata* populations cultivated in this common garden experiment. Transect locations are mapped in Figure 1 (Elev: elevation; Tmax: maximum temperature; GDD: monthly growing degree day; PPT: precipitation; VPDmax: maximum vapor pressure deficit). Table continues next page.

Transect	Pop	Tmax (°C)	GDD (°C)	PPT (mm)	VPD max (hPa)	Palmer Z-Index	
San Diego	DG1	20.50	130.25	43.25	13.72	-0.52	⋯
	DG2	21.44	137.87	51.13	15.57	-0.55	⋯
San Gabriel	GB1	21.54	124.58	74.07	17.76	-0.14	⋯
	GB2	19.36	75.49	82.76	16.71	-0.13	⋯
Santa Ynez	SY1	21.5	97.58	90.25	17.29	0.02	⋯
	SY3	19.52	69.40	91.46	16.77	0.04	⋯
	SY4	17.5	69.40	104.41	13.97	0.05	⋯
Kern	PH	19.98	77.41	37.27	17.40	-0.18	⋯
	LO	19.5	75.46	37.60	16.84	-0.14	⋯
	SCr	20.32	72.57	37.36	17.85	-0.14	⋯
	CG	19.52	63.14	46.77	17.15	-0.08	⋯
	CP	18.76	45.34	48.78	16.27	-0.01	⋯
	MC	18.38	74.17	50.08	15.81	-0.10	⋯
	GR	17.46	47.31	66.26	14.42	-0.07	⋯
	JS	15.98	49.65	74.85	12.70	-0.29	⋯
	GH	15.36	43.18	58.85	13.16	-0.16	⋯
	SL	14.94	36.07	83.72	12.17	-0.35	⋯
Sequoia	SQ1	18.72	77.63	92.42	13.74	-0.04	⋯
	SQ2	17.72	50.76	102.06	14.04	-0.08	⋯
Merced	WLMS	19.32	93.73	66.24	13.64	-0.04	⋯
	MR1	18.38	59.59	78.20	12.71	0.01	⋯
	YS1	17.82	48.22	96.28	12.53	0.01	⋯
	MR2	18.12	53.26	84.12	12.58	0.01	⋯
	MR3	16.56	53.26	98.72	11.90	0.01	⋯
	YS2	17.32	45.04	112.85	13.42	0.00	⋯
	YS3	15.32	13.84	131.82	12.18	0.01	⋯
Chico	CH1	19.12	98.63	84.89	13.77	0.20	⋯
	CH2	18.84	83.11	133.59	14.13	0.10	⋯
	CH3	16.74	31.52	229.28	13.41	0.14	⋯

Table 1 continued. Geographic attributes, sample sizes, mean climatic norms, mean phenological parameters and mean individual seed mass of the 29 *Clarkia unguiculata* populations cultivated in this common garden experiment. Transect locations are mapped in Figure 1 (Elev: elevation; Tmax: maximum temperature; GDD: monthly growing degree day; PPT: precipitation; VPDmax: maximum vapor pressure deficit).

Transect	Pop	DTF	Duration	Synchrony	Seed Mass (mg)
San Diego	DG1	72.27	43.30	0.0474	0.2229
	DG2	79.00	30.96	0.0638	0.2179
San Gabriel	GB1	95.10	43.27	0.0511	0.2510
	GB2	87.59	45.52	0.0484	0.2588
Santa Ynez	SY1	83.54	59.81	0.0349	0.3988
	SY3	83.00	41.13	0.0462	0.2911
	SY4	90.33	40.87	0.0474	0.2570
Kern	PH	72.65	40.13	0.0444	0.3537
	LO	80.97	34.32	0.0428	0.3479
	SCr	75.13	38.77	0.0439	0.3051
	CG	68.07	55.17	0.0300	0.2197
	CP	92.00	35.23	0.0514	0.2382
	MC	67.23	44.16	0.0430	0.2618
	GR	77.13	61.42	0.0316	0.2419
	JS	83.52	41.84	0.0427	0.3691
	GH	83.84	43.42	0.0373	0.2745
	SL	116.07	13.04	0.0586	0.3001
Sequoia	SQ1	80.03	40.97	0.0501	0.2975
	SQ2	126.50	29.60	0.0470	0.3165
Merced	WLMS	80.36	67.23	0.0367	0.2763
	MR1	119.74	19.69	0.0559	0.3108
	YS1	116.22	27.17	0.0461	0.2884
	MR2	124.12	23.47	0.0711	0.2903
	MR3	124.33	18.83	0.0567	0.3156
	YS2	99.70	30.04	0.0505	0.3094
	YS3	116.38	19.38	0.0592	0.2684
Chico	CH1	100.63	33.37	0.0428	0.3151
	CH2	96.29	38.58	0.0313	0.3404
	CH3	123.83	20.83	0.0612	0.2302

Table 2. Geographic variation of long-term climatic conditions. The effects of Latitude, Elevation, their interaction, and quadratic terms on each of seven climatic parameters were evaluated with multivariate regression for the 29 sites where seeds were collected for this study. Each climatic parameter is long-term mean (1981-2010) of monthly climatic conditions during January-May. See Figure 2 for the corresponding bivariate plots and Figure 3 for three dimensional surface profiles. Each model was statistically significant at the $p \leq 0.0005$ level. Significant model effects are bold with the designated level of significance (*$p \leq 0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$).

		Model		**Latitude**	**Elevation**	**Latitude * Elevation**	**Latitude^2**	**Elevation^2**
Climatic parameter		**R^2**	**F Ratio**	estimate	estimate	estimate	estimate	estimate
Precipitation	Ln(PPT) (mm)	**0.59**	**6.74**	**0.1415****	**0.0007****	9.19e-5	**0.051***	-6.83e-7
Temperature	Tmin (°C)	**0.76**	**14.33**	**-0.5969*****	**-0.0035******	-0.0008	**0.1433***	-5.08e-7
	Tmean (°C)	**0.89**	**37.22**	**-0.6500******	**-0.0037******	**-0.0007***	**0.1040***	**-2.12e-6***
	Tmax (°C)	**0.89**	**38.73**	**-0.7045******	**-0.0039******	**-0.0006***	0.0658	**-3.70e-6***
	GDD (°C)	**0.88**	**35.47**	**-13.19******	**-0.0597******	**-0.0131***	2.5611	2.15e-5
Drought Indicators	VPDmax (hPa)	**0.61**	**7.19**	**-0.9529******	-0.0006	**-0.0001***	0.9022	**-7.6e-6***
	Palmer Z-Index	**0.72**	**11.98**	**0.0431****	5.07e-5	**-0.0001***	0.0007	**-5.82e-7***

Table 3. Geographic sources of variation for phenological parameters and mean individual seed mass. The effects of Latitude, Elevation, and their interaction on each of three phenological parameters and seed mass were evaluated with multivariate regression. Quadratic terms were not significant, so they were excluded from the final models. Seed mass, Days to Flowering and Flowering Duration were included as covariates where noted. Estimates of regression coefficients are reported for each effect and covariate. See Figure 5 for the corresponding bivariate plots and Figure 6 for three dimensional surface profiles. Significant models and model effects are indicated in boldface, with the designated level of significance (‡ $p \leq 0.07$; * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Parameter	Covariate	Model		Latitude	Elevation	Latitude* Elevation	SeedSize	Days to Flowering	Flowering Duration
		R^2	F Ratio	estimate	estimate	estimate	estimate	estimate	estimate
Days to Flowering	----	0.44	**4.68***	**8.6956*****	0.0122	0.0155	16.8826	----	----
Flowering Duration	----	0.30	**2.57‡**	**-4.9695****	-0.0108	**-0.0105***	-14.2043	----	----
	DTF	0.63	**7.80****	-0.3629	-0.0043	-0.0049	-5.2501	**-0.5304****	----
Flowering Synchrony	----	0.22	1.70	**0.0025‡**	2.51e-6	**9.00e-6***	-0.0359	----	----
	DTF	0.59	**6.53****	-0.0012	-2.65e-6	4.56e-6	-0.0430	**0.0004****	----
	Duration	0.72	**12.01*****	-0.0007	-4.40e-6	2.30e-6	-0.0450	----	**-0.0006*****
	DTF Duration	0.75	**10.99*****	-0.0014	-4.81e-6	2.10e-6	-0.0456‡	0.0002	**-0.0005****
Seed Mass	----	0.09	0.78	0.0015	-1.37e-5	-1.95e-5	----	----	----

Table 4. Relationships among phenological parameters. Three separate regressions were conducted for Flowering Synchrony in order to examine the effects of adding different combinations of phenological parameters as covariates. Each row presents the statistical parameters and regression coefficients from a separate regression. See Figure 7 for corresponding bivariate plots and three dimensional surface profile plots. Significant models and model effects are shown in boldface with the designated level of significance (‡ $p \le 0.07$; *$p \le 0.05$; **$p < 0.01$; ***$p < 0.001$).

	Model			**Main effects**			**Covariate**
Response Variable		**R^2**	**F Ratio**	**DTF**	**Duration**	**DTF* Duration**	**Seed Mass**
Flowering Duration vs. …	**DTF**	**0.59**	**18.74*** **	**-0.5343*** **	----	----	10.8662
Flowering Synchrony vs. …	**DTF**	**0.45**	**10.46*** **	**0.0003*** **	----	----	**-0.0620‡**
	Duration	**0.66**	**25.21*** **	----	**-0.0006*** **	----	**-0.0549***
	DTF + Duration	**0.66**	**11.82*** **	5.84e-5	**-0.0005*** **	2.22e-6	**-0.0578***

Table 5. Climatic sources of variation of phenological parameters. The effects of Tmax, PPT, and their interaction on each of three phenological parameters. Each row presents the statistical parameters and regression coefficients from a separate regression model; two or more regressions were conducted for Flowering Duration and Flowering Synchrony in order to examine the effects of adding different phenological parameters as covariates. See Figure 8 for bivariate plots of each phenological parameter on each climatic parameter. Significant model effects are bold with the designated level of significance (‡p≤0.07; *p≤0.05; **p<0.01; ***p<0.001).

	Model			**Main effects**			**Covariates**		
Response Variable	**Phenological Covariate**	**R^2**	**F Ratio**	**Tmax**	**Ln(PPT)**	**Tmax* Ln(PPT)**	**SeedSize**	**Days to Flowering**	**Flowering Duration**
Days to Flowering	----	**0.54**	**7.11*****	-2.53	**25.378*****	-4.418	45.165	----	----
Flowering Duration	----	**0.42**	**4.44****	**2.664***	-5.870	**9.043***	-43.606	----	----
	DTF	**0.74**	**13.03*****	1.207	**8.727**‡	**6.502****	-17.629	**-0.575*****	----
Flowering Synchrony	----	**0.29**	**2.41**‡	4e-4	0.006	**-0.006***	-0.026	----	----
	DTF	**0.56**	**5.89*****	6e-4	-0.005	-0.004	-0.044	**0.0004*****	----
	Duration	**0.70**	**10.65*****	0.001	0.002	4e-4	-0.053	----	**-0.0006*****
	DTF & Duration	0.70	**8.71*****	0.001	3e-4	8e-4	-0.054	8.5e-5	**-0.0006****
Seed Mass	----	0.09	0.84	2e-4	0.007	0.021	----	----	----

Table 6. Climatic sources of variation of phenological parameters. The effects of GDD, PPT, and their interaction on each of three phenological parameters were evaluated with least squares multivariate regression. Seed mass was included as a covariate in each analysis. See Figure 8 for bivariate plots of each phenological parameter on each climatic parameter. Significant models and model effects are bold with the designated level of significance (‡ p≤0.07; *p≤0.05; **p<0.01; ***p<0.001). VIFs lower than 4.8 for all models.

	Model			Main effects			Covariates		
Parameter	**Phenological Covariate**	**R^2**	**F Ratio**	**GDD**	**Ln(PPT)**	**GDD* Ln(PPT)**	**Seed Mass**	**Days to Flowering**	**Flowering Duration**
Days to Flowering	----	**0.54**	**7.20*** **	-0.1808	**24.0736** **	-0.1999	41.8509	----	----
Flowering Duration	----	**0.35**	**3.28***	**0.1638**‡	-3.7786	**0.4377***	-47.7021	----	----
	DTF	**0.71**	**11.10*** **	0.0534	**10.9275***	**0.3156***	-22.1362	**-0.6109*** **	----
Flowering Synchrony	----	**0.30**	**2.55**‡	-3.78e-3	0.0032	**-0.0004***	-0.0133	----	----
	DTF	**0.59**	**6.74*** **	3.84e-5	-0.0069	**-0.0003***	-0.0310	**0.0004*** **	----
	Duration	**0.70**	**10.85*** **	5.98e-5	0.0010	-0.0001	-0.0418	----	**-0.0006*** **
	DTF & Duration	**0.71**	**9.17*** **	6.41e-5	-0.0016	-0.0001	-0.0417	0.0001	**-0.0005** **
Seed Mass	----	0.17	1.73	-1.72e-5	0.0164	**0.0015***	----	----	----

Table 7. Significant predictors for each phenological parameter identified with stepwise regression. Each initial model included all effects listed in the left column. Estimate column is the statistically significant regression coefficient, according to the AIC criteria. Significant predictors were retained for the final model for each phenological parameter (detailed in Table 8).

	Days to Flowering		Duration		Synchrony	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Ln(PPT)	**21.72**	**0.004**				
Tmin						
Tmean						
Tmax						
GDD						
VPDmax	**-3.68**	**0.021**				
Palmer Z-Index			**22.70**	**0.026**		
Latitude						
Elevation						
Seed Mass					**-0.05**	**0.036**
DTF			**-0.61**	**<0.001**		
Duration					**-5.9e4**	**<0.001**

Table 8. Multivariate regressions for each phenological parameter with main effects identified with a stepwise regression process (see Table 7 for initial model). See Figure 9 for three dimensional surface profile for each regression model. (*p≤0.05; **p<0.01; ***p<0.001).

	Model		Main effects			Covariate
	R^2	F Ratio	Ln(PPT)	VPDmax	Ln(PPT) * VPDmax	Seed Mass
Days to Flowering	**0.62**	**9.77***	**22.7103**	**-4.0963**	-5.8379	26.3405

			DTF	Palmer Z-Index	DTF * Palmer Z-Index	Seed Mass
Flowering Duration	**0.67**	**12.14**	**-0.5981***	**21.0373***	-0.4442	-11.3379

			Flowering Duration	Seed Mass	Flowering Duration * Seed Mass	
Flowering Synchrony	**0.72**	**21.44***	**-5.87e-4***	**-0.0810**	**0.0043***	----

Figure 1. Locations of 7 elevation transects (white stars) where 29 populations of *Clarkia unguiculata* were sampled for the common garden study. See Table 1 for geographic attributes of each transect and population. Digital elevation model and background image from PRISM Climate Group (Oregon State University, http://prism.oregonstate.edu).

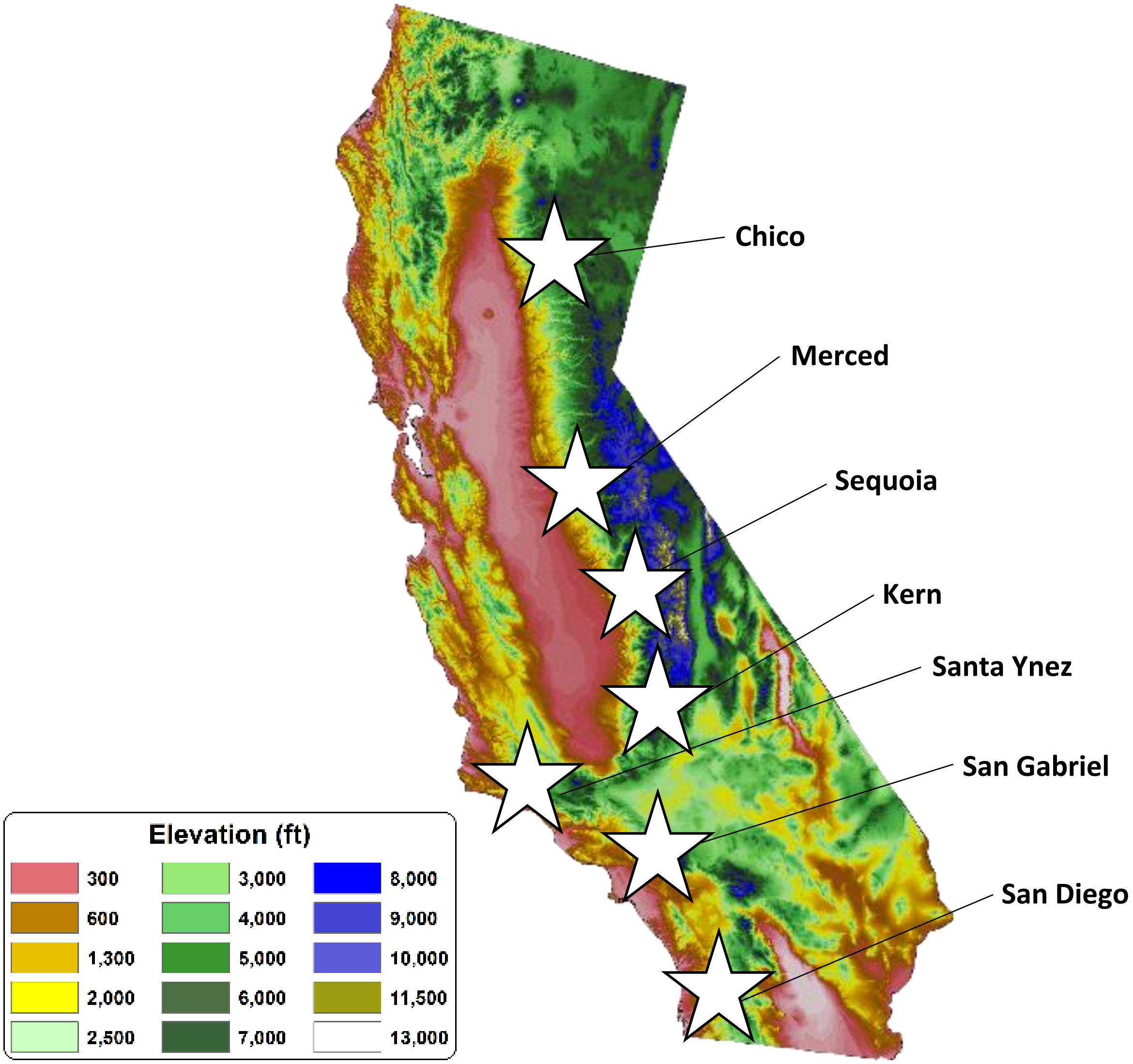

Figure 2. Geographic variation of climatic norms. Each point represents the residual of the long-term mean (1981-2010) of monthly climatic conditions during January-May for one of the sites from which populations were sampled for the common garden study. The x-axis for A, C, E, and G is latitude, and the x-axis for B, D, F, and H is elevation. See Table 2 for summary of statistical tests of the effects of latitude, elevation, and their interaction on each climatic variable. Lines represent the best fit regression model.

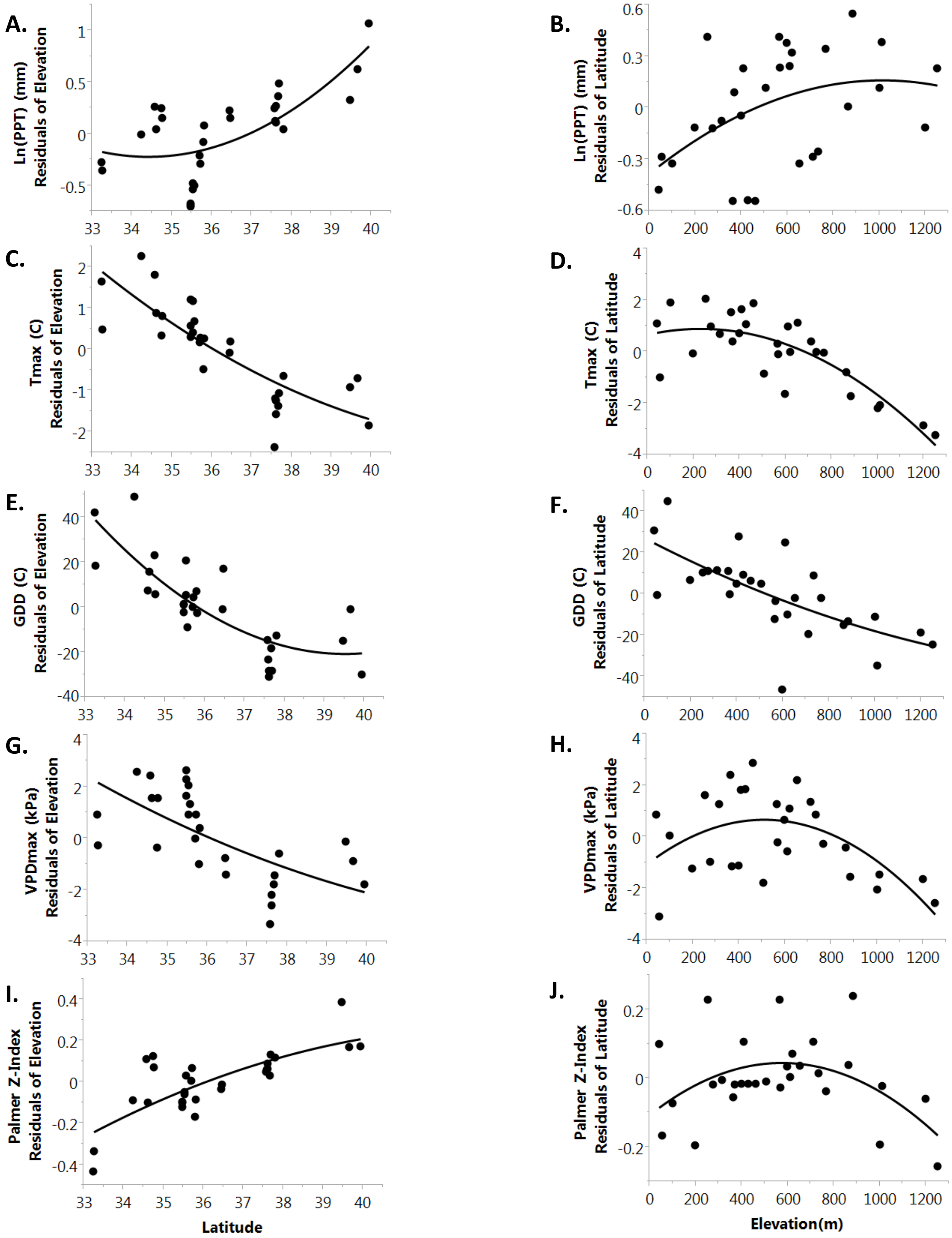

Figure 3. Surface profiles depicting geographic variation of climatic norms. Surface profiles were generated from multivariate regressions among sampled size of each climatic parameter on latitude and elevation. See Table 2 for the corresponding statistical effects of latitude and elevation on each climatic variable. Colors in each profile are used to help visualize the shape of the surface; they do not represent the same parameters or unit scales across graphs.

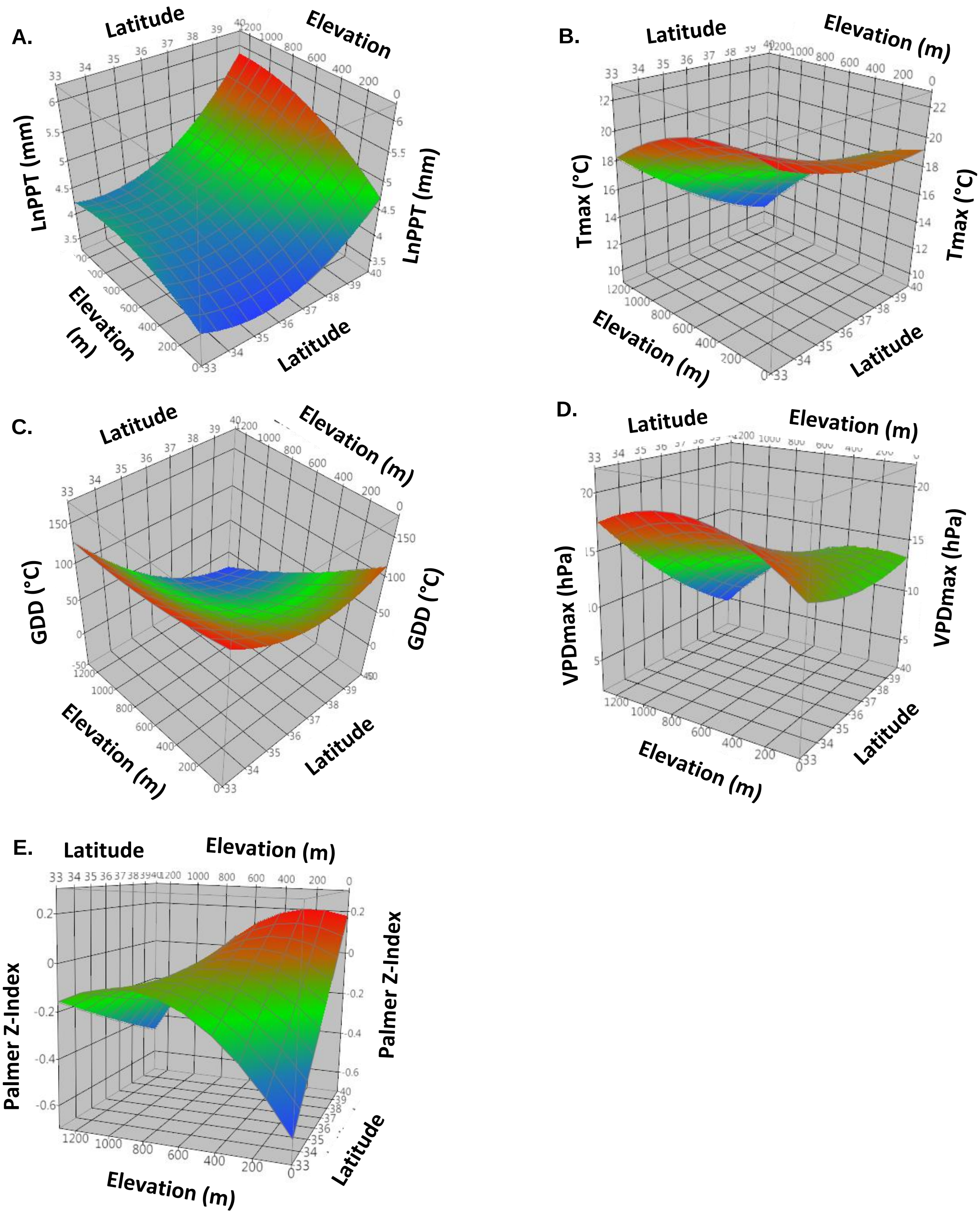

Figure 4. Phenological parameters across the sampled populations. Within each panel, populations are ranked from the highest to the lowest mean phenotypic value of each trait (±SE). The sequence of the populations differs between panels. Synchrony is measured as an index that expresses the degree of flowering overlap among individuals within populations (see text).

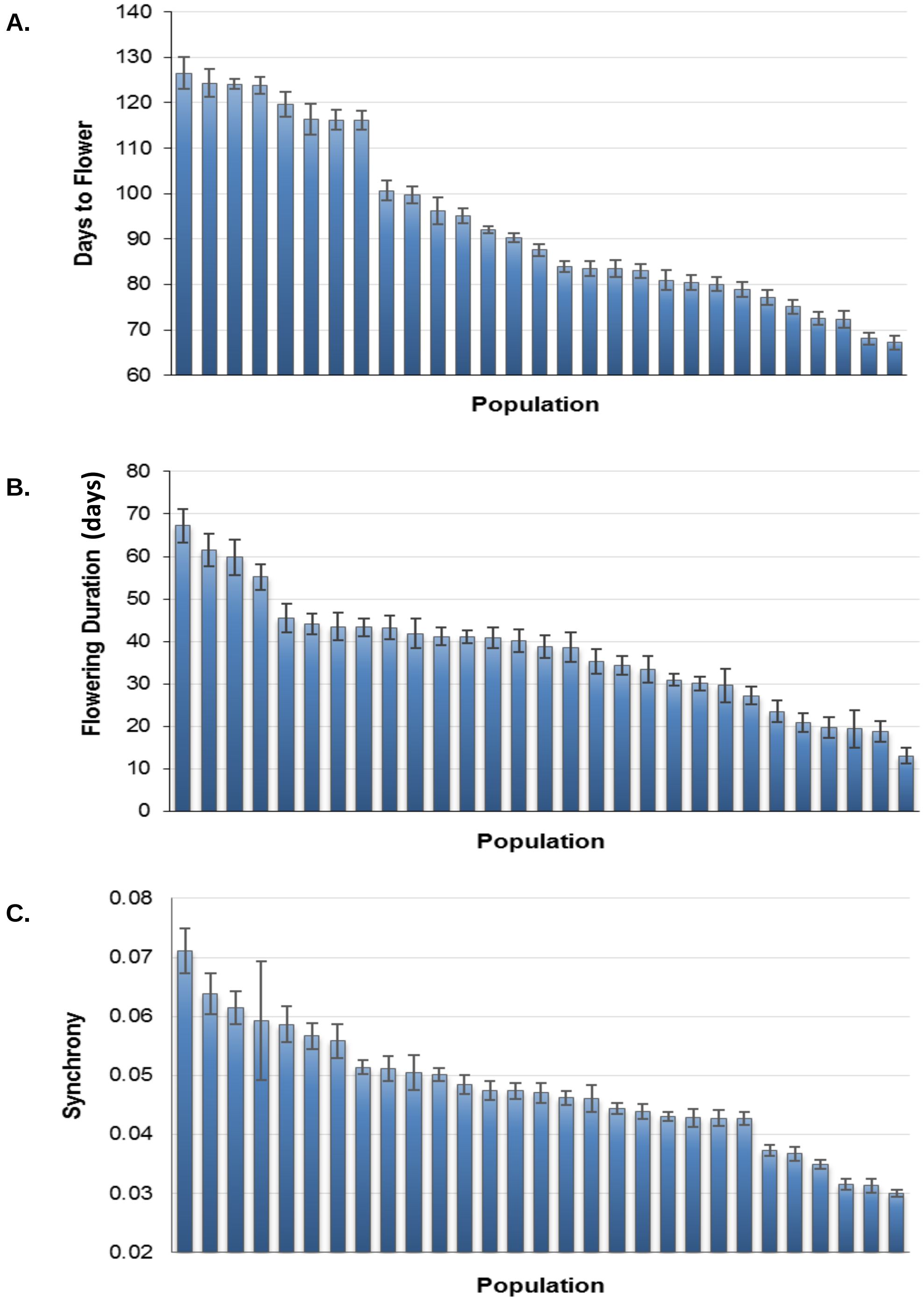

Figure 5. Geographic variation of flowering phenology and seed size for 29 populations in the common garden study. *p<0.05; **p<0.001; ***p<0.0001; ^{NS} not significant.

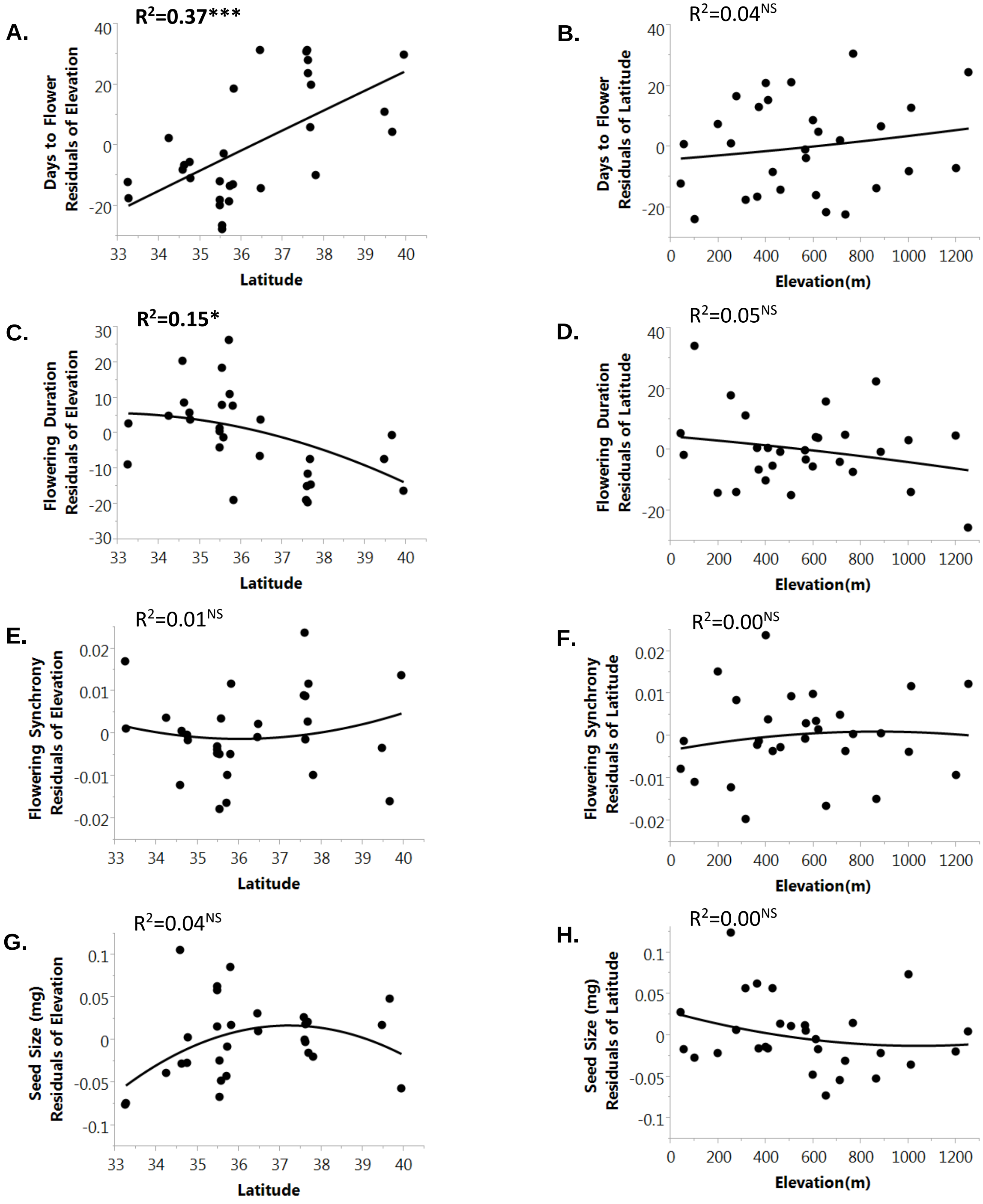

Figure 6. Surface profiles depicting geographic variation of phenological parameters. Each plot was generated from a multivariate regression of each phenological parameter on elevation, latitude, their interaction, and their quadratic terms. Although the statistical parameters reported in Table 3 do not include quadratic terms because they were non-significant, they were included in these surface profiles to improve the visualization of geographic patterns for each phenological parameter.

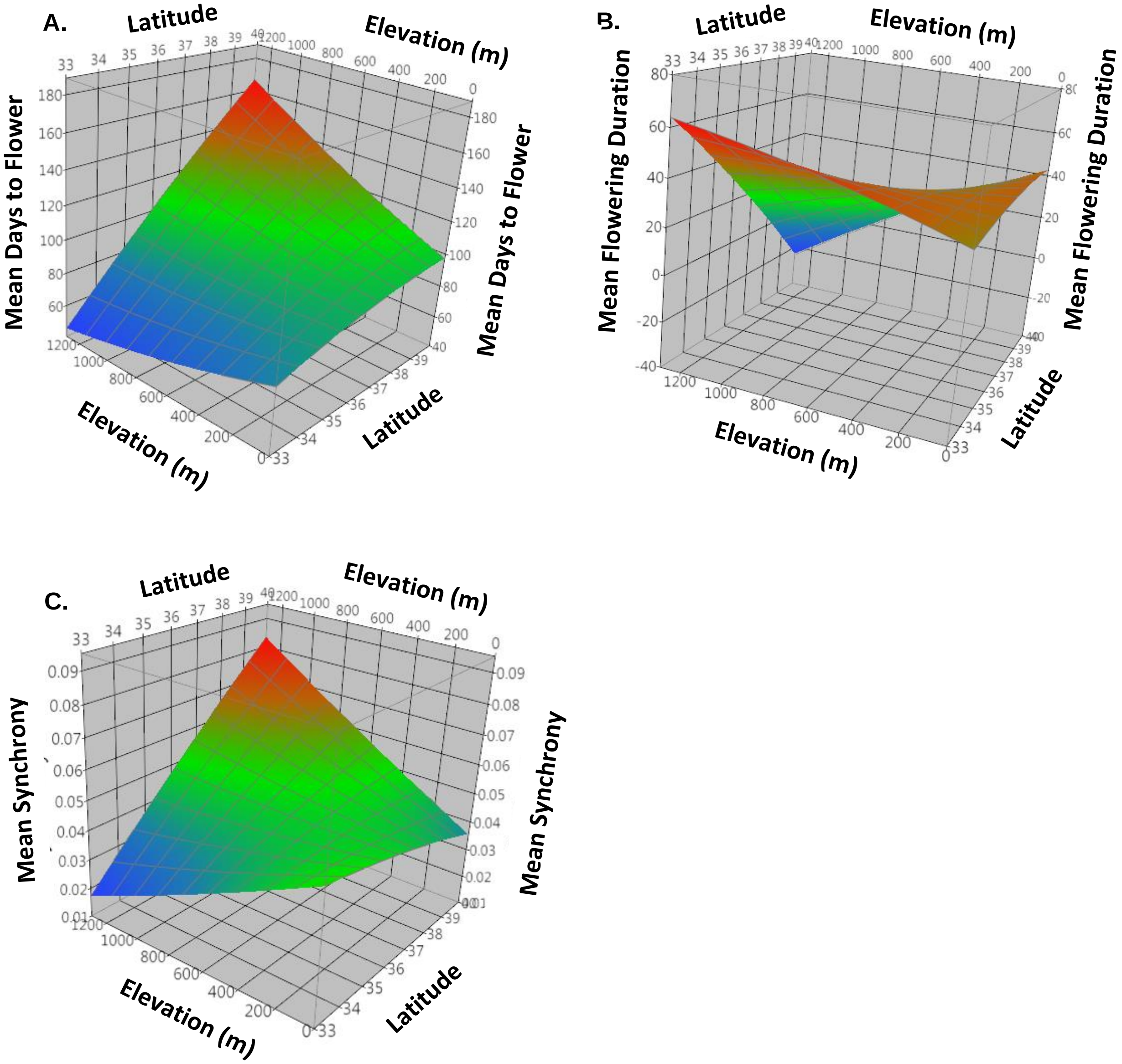

Figure 7. Relationships among phenological parameters. Bivariate plots indicate that early onset of flowering is associated with (A) longer flowering duration and (B) lower flowering synchrony, and that (C) longer flowering duration is associated with lower flowering synchrony. A surface profile incorporating all three phenological parameters (D) indicates that flowering duration has a strong effect on synchrony independent of DTF. See Table 4 for regression outputs. ***p<0.0001

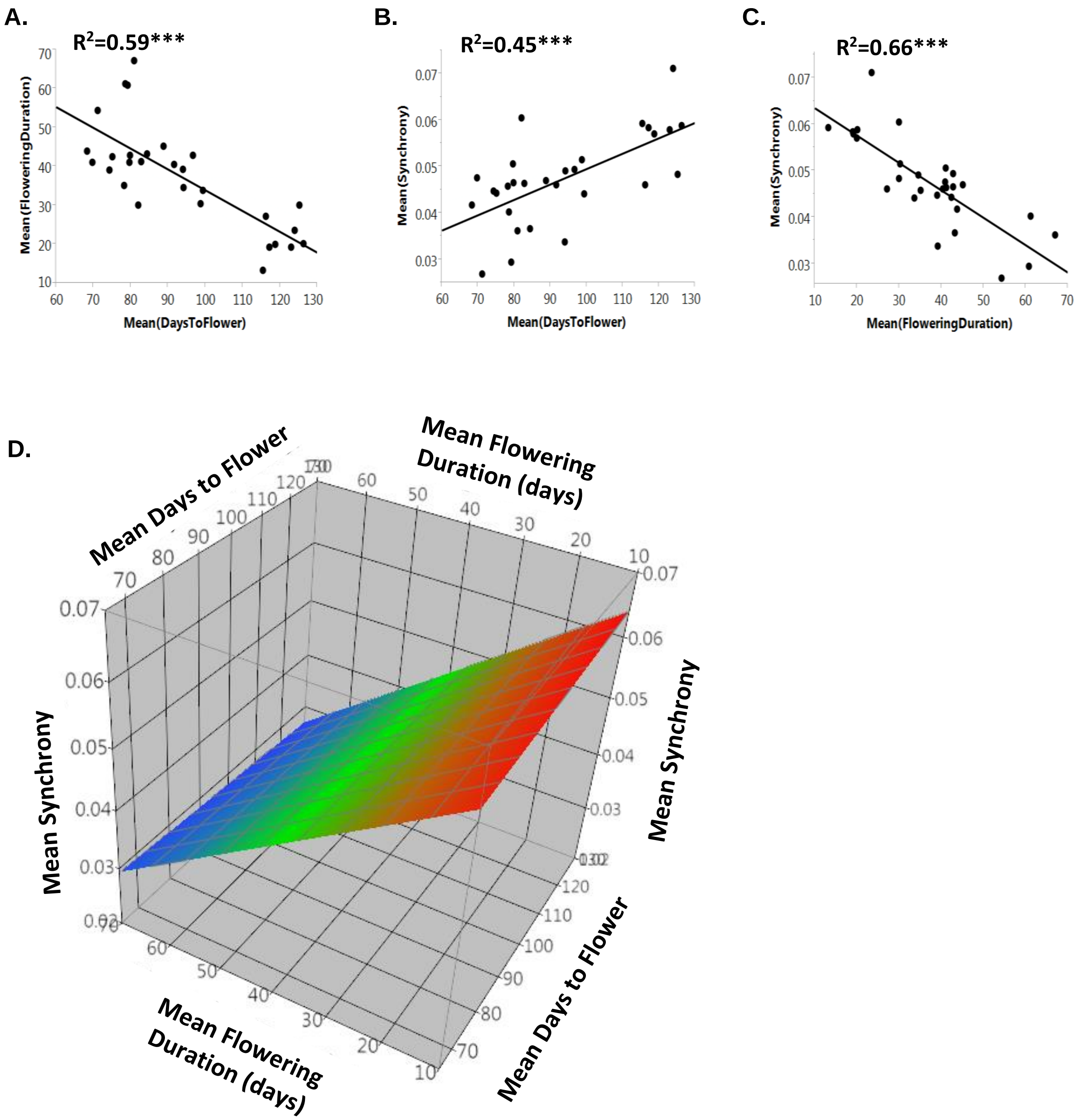

Figure 8. Bivariate relationships between phenological parameters and climatic sources of variation.

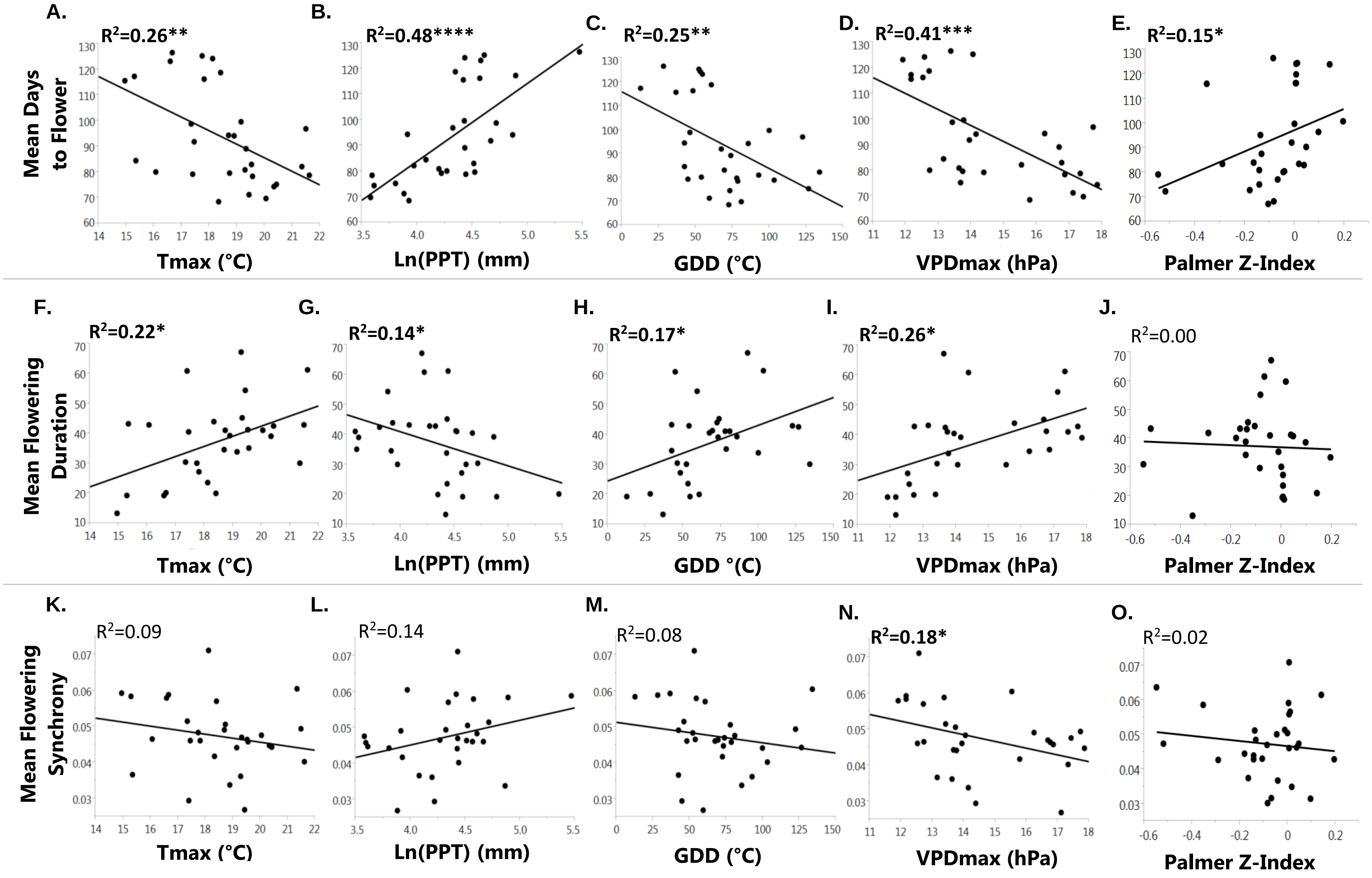

Figure 9. Surface profiles from multivariate regressions of each phenological parameter on the climatic sources of variation identified in a stepwise regression analysis.