Historical changes in flowering phenology are governed by temperature x precipitation interactions in a widespread perennial herb in western North America

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Running head: Historical changes in flowering phenology

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Summary

- For most species, a precise understanding of how climatic parameters determine the timing of seasonal life cycle stages is constrained by limited long-term data. Further, most long-term studies of plant phenology that have examined relationships between phenological timing and climate have been local in scale or have focused on single climatic parameters. Herbarium specimens, however, can expand the temporal and spatial coverage of phenological datasets.
- Using *Trillium ovatum* specimens collected over >100 years across its native range, we analyzed how seasonal climatic conditions (mean minimum temperature [Tmin], mean maximum temperature, and total precipitation [PPT]) affect flowering phenology. We then examined long-term changes in climatic conditions and in the timing of flowering across *T. ovatum*'s range.
- Warmer Tmin advanced flowering, whereas higher PPT delayed flowering. However, Tmin and PPT interact: the advancing effect of warmer Tmin was strongest where PPT was highest, and the delaying effect of higher PPT was strongest where Tmin was coldest. The direction of change in climatic parameters and in the timing of flowering depended on geographic location. Tmin, for example, decreased across the observation period in coastal regions, but increased in inland areas.
- Our results highlight the complex effects of climate and geographic location on phenology.

Key words: climate, elevation, herbarium records, latitude, longitude, minimum temperature, *Trillium ovatum* (Pacific trillium)

Introduction

Phenology is the study of the timing of seasonal life cycle stages (*phenophases*), such as the flowering and fruiting of plants, the migration of birds and mammals, and the emergence of insect pollinators and pests. Shifts in the timing of phenophases are a well-documented response to climate change (Menzel *et al.*, 2006; Parmesan, 2006), and these shifts can have profound and immediate effects on species interactions (Visser & Both, 2005; Both *et al.,* 2006, Ozgul *et al.,* 2010, McKinney *et al.,* 2012), as well as longer term effects on species abundance and distribution (Moller *et al.,* 2008, Chuine, 2010, Miller-Rushing *et al.,* 2010, Willis *et al.,* 2010, Cleland *et al.,* 2012), and on ecosystem function and services (Richardson *et al.,* 2010). For flowering plants, the timing of reproductive phenophases is particularly important, as it can influence the strength of mutualistic or antagonistic interactions between plants and their pollinators, seed dispersers, herbivores, and seed predators (Elzinga *et al.,* 2007; Yang & Rudolf, 2010; Forrest, 2015; Rafferty *et al.,* 2015).

In order to identify the causes and consequences of recent or historical shifts in phenology and to predict future climate-change induced shifts, large-scale efforts to document contemporary plant and animal phenology are underway (Schwartz *et al.,* 2012). These efforts include national-level programs, such as the USA National Phenology Network and Project BudBurst, as well as regional programs, such as the California Phenology Project (Haggerty *et al.,* 2013; Denny *et al.,* 2014; Mazer *et al.,* 2015). Two primary goals of these projects are to maximize the quantity and accessibility of high-quality phenological data with respect to the frequency and duration of monitoring, the numbers of species targeted for monitoring, and the variety of geographic locations monitored, and to link inter-annual and geographic variation in phenology to local climatic conditions.

Despite these efforts, our current understanding of plant phenology and its relationships with climatic parameters is constrained by a dearth of historical data against which contemporary observations can be compared. This gap can be mitigated by accessing phenological information preserved in natural history collections, and this approach has been particularly effective for examining patterns of plant reproductive phenology using herbarium specimens (Primack *et al.,* 2004; Lavoie & Lachance, 2006; Miller-Rushing *et al.,* 2006; Gallagher *et al.,* 2009; Gaira *et al.,* 2011; Robbirt *et al.,* 2011; Park, 2014; Hart *et al.,* 2014). Most of the herbarium-based phenological studies to date examined local patterns of plant phenology and used natural history collections to expand the temporal range of phenological observations at a given location or within a relatively small region. However, herbarium collections can also expand the spatial range of historical datasets (e.g., Park, 2012). Datasets that are geographically widespread and that represent many decades can comprise greater variation in both phenological and climatic data than datasets based on single locations or shorter-term surveys. Further, with datasets representing a broad geographic range — which can be provided by herbarium specimens — larger-scale relationships among geographic, climatic, and temporal variables and plant phenophases can be identified and quantified.

In this study, we examined herbarium records of *Trillium ovatum* to build a dataset representing flowering dates (including both day of year and year) and locations across the entire native range of this species. *Trillium ovatum* is particularly valuable for herbarium-based phenological research because the flowering status of sampled plants is unambiguous: plants typically produce a single stem per year and stems produce only one flower (older plants have been found occasionally to produce more than one stem; Jules & Rathcke, 1999; Ream, 2011). With this dataset, we ask four questions related to how flowering phenology varies across climatic, geographic, and temporal gradients: (1) Which climatic variables (e.g., minimum temperature, maximum temperature, and cumulative precipitation) and which seasonal time periods (three 3-month windows from January – May, prior to flowering) best explain variation in the day of year on which *Trillium ovatum* specimens were collected in flower? (2) Can we detect interactions between temperature and precipitation in their effects on *Trillium ovatum* flowering phenology? For example, where precipitation is not limiting, we expect that temperature will have a stronger effect than where precipitation is limiting. (3) When controlling statistically for geographic location (i.e., latitude, longitude and elevation, which affect seasonal temperatures and precipitation), can we detect long-term temporal change in the climatic variables that affect flowering phenology? (4) Finally, controlling statistically for geographic parameters, can we detect long-term inter-annual change in the onset date of spring flowering over the past 122 years? The application of multivariate models to historical climatic data and herbarium-derived phenological records provided a way to detect a suite of novel interactions between rainfall and temperature that affected the estimated onset of flowering and between geographic variables and collection year that affected local climate conditions.

Materials and methods

Study organism

Trillium ovatum Pursh (Western Trillium; MELIANTHACEAE) is a long-lived perennial herb that is common in mesic coniferous and mixed coniferous-deciduous forests in western North America. Its range extends from Northern California in the United States to Southern British Columbia and Alberta in Canada (USDA 2014; Figure 1). Most plants produce a single stem per year, although older reproductive individuals rarely produce two or three stems per plant (Jules & Rathcke, 1999; Ream, 2011). *Trillium ovatum* flowers in spring, with reproductive individuals producing a single flower per stem. Individual flowers last ~22 days (Jules & Rathcke, 1999) providing a reasonable estimate of its flowering onset date given the wide range in specimen collection dates across the species' geographic range (mean collection day of year= 122; range= 32-239).

Herbarium data

Trillium ovatum is well-represented in herbaria throughout its range and produces solitary, showy flowers, making it a good candidate for study via preserved herbarium specimens. We obtained loans from five California herbaria, including: Rancho Santa Ana Botanic Garden Herbarium (RSA), University of California, Riverside (UCR), Santa Barbara Botanic Garden (SBBG), and the Jepson Herbarium (JEPS) and the University Herbarium (UC) at University of California, Berkeley. Because *Trillium ovatum* produces a single, relatively large flower per stem, its phenological status is also simple to observe via photographs; consequently, we were able to expand the size and geographic coverage of our dataset by downloading specimen images through the Consortium of Pacific Northwest Herbaria website (www.pnwherbaria.org). These specimens are housed in the following herbaria: H.J. Andrews Experimental Forest (HJAEF), Stillinger Herbarium at University of Idaho (ID), Montana State (MONT), Pacific Luthern University (PLU), Reed College (REED), Rocky Mountain Herbarium at University of Wyoming (RM), and Western Washington University (WWB).

We examined each specimen and recorded its collection date (day, month, and year), collection location (latitude, longitude, and elevation), and phenological status (flowering or not). Specimens that were missing detailed label information (e.g., the exact day, month, and year of collection) were excluded. Many specimen labels did not include geographic coordinates, but provided a detailed description of the collection location (e.g., a county and road name).

These specimens were geo-referenced using online tools (e.g., GEOLocate: http://www.museum.tulane.edu/geolocate/) and United States Geological Survey topographic maps. We estimated elevation for each collection location using georeferenced coordinates. Specimens for which the labels provided insufficient location information to enable the assignment of GPS coordinates or elevations were rejected. Finally, if there was more than one stem preserved on an herbarium sheet, only one datum was recorded. Our final dataset included 289 flowering specimens that met these criteria.

Climate data

The link between temperature and plant phenology is well-documented (Menzel *et al.*, 2006; Parmesan, 2006; Gallagher *et al.,* 2009; and references therein), but fewer studies have examined the degree to which precipitation drives phenological variation and how temperature and precipitation may interact to influence phenology (but see Crimmins *et al.,* 2011 and Mazer *et al.,* 2015). Because our study area covers a large geographic range and climate stations are available at few of our sample locations, we accessed climate data for our study area from the PRISM dataset (PRISM Climate Group). The PRISM dataset includes 4km gridded data for the conterminous U.S., interpolated from point station data; PRISM data are readily-available online and have been used frequently in phenological research (Crimmins *et al.,* 2011; Park 2014; Mazer *et al.*, 2015). For the georeferenced location of each specimen, we downloaded monthly climate data for the year of the collection. For each collection event (a combination of the collection location and date), we obtained monthly mean maximum temperature, mean minimum temperature, and total precipitation (the three climate variables provided by the PRISM dataset). We then generated composite seasonal climate parameters representing the mean maximum temperature, mean minimum temperature, and total precipitation during three three-month windows preceding the collection date of *Trillium ovatum* specimens: JFM (January, February, and March), FMA (February, March, and April), and MAM (March, April, and May).

Statistical Analysis

Effects of temperature and rainfall on flowering date

We constructed multiple linear regression models to detect the effect of each site- and year-specific climate variable on flowering day of year (DOY). For each specimen, we calculated flowering DOY as the number of days after January 1st (e.g., April 1 is day 90) on which it was collected. We first constructed saturated models, which included (for each specimen's georeferenced location) the three seasonal climate parameters (mean minimum temperature [Tmin], mean maximum temperature [Tmax], and total precipitation) and their interactions during each of the three-month windows (JFM, FMA, or MAM); in these models, DOY was the response variable, and Tmin, Tmax, total precipitation, and the interactions among them were the independent variables. Each seasonal window (JFM, FMA, and MAM) was analyzed separately. Because the first year represented in the PRISM dataset is 1895, collection events prior to 1895 were not used in any analysis that included climate data (N = 282). Precipitation values were log transformed to achieve normality. We identified a minimal adequate model through backward elimination, where non-significant predictors ($p > 0.05$) were removed in successive steps (Crawley, 2007**)**. A stepwise approach to multiple regression analysis is frequently used in phenological research studies (Keatley *et al.,* 2002; Moller *et al.,* 2008; Hulme, 2011; Mazer *et al.,* 2015) and has the benefit of identifying the independent variables that have the strongest influence on phenology (Roberts, 2009). The statistically significant regression coefficients associated with the independent variables were examined to determine whether DOY was advanced or delayed in response to higher temperatures and/or precipitation. The relative sensitivity of DOY to each of the three seasonal windows was also examined to determine whether flowering DOY is more sensitive to winter or to spring conditions.

Statistically significant two-way interaction terms were examined graphically to reveal how the effect of one factor (e.g., Tmin) on DOY depended on the value of a second (i.e., interacting) factor (e.g., PPT). We used the equation estimated by the linear model to generate three lines, each of which plotted the predicted DOY against a range of values for the first climate variable in the interaction term while using one of three values of the second climate variable in the interaction term: the minimum value, mean value, and maximum value. All other significant predictors were included in the equation at their mean value. For example, we used the equation of the linear model to illustrate the effects of Tmin on DOY using the minimum, mean, and maximum values of PPT (see Figure 2a). We similarly created three lines in which predicted DOY was plotted against a range of values for the second climate variable in the significant interaction term, where each line used one of three values of the first climate variable in the interaction (again, the minimum, mean, and maximum value; see Figure 2b).

Temporal changes in temperature and rainfall

We analyzed data comprised of each specimen's latitude, longitude, elevation, year of collection, and climatic parameters to quantify the relationship between the seasonal climate parameters that were identified as significant predictors of flowering phenology in the previous analysis (as the dependent variables) and the collection year, controlling for variation in climate that is associated with latitude, longitude, and elevation. We used an analytical approach similar to the previous analysis of flowering dates and climate variables. We built multiple linear regression models, using a seasonal climate parameter (e.g., mean Tmin in JFM) as the response variable and collection year (treated as a continuous variable), geographic parameters (latitude, longitude, and elevation), and their interactions as independent variables. In this model, significant effects of collection year on the response variable were interpreted as a significant long-term temporal trend, and the values of the statistically significant regression coefficients associated with year, latitude, longitude, and elevation were examined to determine whether each of the climatic variables increased (or decreased) over time (independent of geographic location) or in association with geographic location (independent of temporal effects).

Where significant interactions between two variables were detected, we again used a graphical approach to visualize how the effects of one factor depended on the value of a second factor. We graphed the predicted values of the seasonal climate parameters against a range of values for the first variable contributing to the interaction term and, for each of three separate lines, one of three levels of the second variable contributing to the interaction term (the minimum value, mean value, and maximum value of the second variable). For example, the interacting effects on FMA Tmin of collection year and longitude were examined by graphing predicted FMA Tmin against collection year using each of three longitude values (the westernmost, mean, and easternmost longitude values represented by the specimens; see Figure 3a).

Long-term temporal changes in flowering date

We used multiple linear regression to quantify the relationship between flowering phenology (DOY) and collection year. To control for environmental effects on DOY associated with geographic location rather than temporal changes in climate, we created a regression model with flowering DOY as the response variable and collection year, geographic variables (latitude, longitude, and elevation), and their interactions as independent variables. The sign of the regression coefficient associated with collection year was examined to determine whether the DOY has become significantly delayed or advanced (earlier) over time, controlling for environmental variation (climatic or biotic) associated with geographic location that may have also influenced DOY. In addition, the regression coefficients associated with latitude, longitude, and elevation were examined to corroborate the prediction that DOY would be delayed at higher latitudes and elevations and to detect, if present, an association between flowering DOY and longitude.

All statistical analyses were performed in R (R Development Core Team, 2013).

Results

Our dataset spanned a 122-year period, from 1888-2009. The mean collection day of year was 122 (May 3rd) + SD = 40.29 (range= 32-239; SE = ± 2.37; Figure 4).

Effects of seasonal temperature and rainfall on flowering date

Temperature and precipitation in both winter and spring influenced DOY. For each of the three seasonal windows, there were significant effects of mean Tmin, total precipitation, or their interaction on flowering DOY (Table 1). In none of the models did mean Tmax have a significant effect on DOY. The climate models account for 34-36% of the variation in flowering DOY.

Flowering DOY is advanced (earlier) where January – March mean Tmin is warmer and delayed (later) where January – March total precipitation is higher. For the February – April and March – May climate windows, the main effects of Tmin and precipitation were similar, but there was also a significant interaction between mean Tmin and total precipitation. The advancing effect of warmer mean Tmin was stronger where total precipitation was higher (Figure 2a shows this interaction for the FMA window), and the delaying effect of increased precipitation was stronger where mean Tmin values were lower (Figure 2b shows this interaction for the FMA window).

Temporal changes in temperature and rainfall

We detected long-term temporal change in mean Tmin and total precipitation, independent of variation associated with geographic location. Across all three seasonal windows, there were significant independent effects of year, elevation, latitude, longitude and their interactions on mean Tmin and total precipitation (Table 2; Table 3). The models account for 74-81% of the variation in Tmin and 41-52% of the variation in precipitation.

The model of January – March mean Tmin as influenced by the geographic variables detected significant interactions between each pair of geographic parameters (e.g., elevation*latitude; elevation*longitude; latitude*longitude; Table 2a), indicating complex effects of geography on winter minimum temperatures. The effects of elevation on mean Tmin, for example, depend on latitude and longitude. In contrast, the effect of geographic parameters on mean Tmin in the FMA and MAM windows was primarily attributed to the main effects, with lower Tmin values associated with higher latitudes (more northern sites), higher elevations, and more easterly (inland) sites (Table 2b and 2c). In all three seasonal windows, there was a significant interaction between year and longitude: Tmin increased over time at inland (eastern) sites, whereas Tmin decreased at coastal (western) sites (Figure 3a illustrates this relationship for the FMA seasonal window).

The sign and statistical significance of the regression coefficients in the models of precipitation as influenced by geographic parameters differed among the three-month focal windows (Table 3). The negative coefficients associated with longitude, however, indicate that precipitation consistently declined from western to eastern collection localities. The models detected at least one interaction among geographic variables in each seasonal window, although total precipitation generally decreased with higher elevation and higher latitudes. Temporal trends in precipitation were complex. In the JFM window, there was a significant interaction between year and latitude; at northern latitudes, precipitation increased over time, while at southern latitudes, precipitation decreased over time (Figure 3b). In the MAM window, there was a significant interaction between year and longitude during the MAM window: total precipitation increased across the observed period at eastern (inland) sites, whereas total precipitation decreased at western (coastal) sites (Figure 3c). The FMA window was the only season in which there was no temporal trend in precipitation (Table 3b).

Long-term, temporal changes in flowering date

Collection year and geographic variables explained 48% of the variation in flowering DOY. The effect of year on flowering date, however, depended upon geographic location. The model detected two significant three-way interaction terms that included year and geographic parameters (year*elevation*latitude and year*elevation*longitude) and several two-way interaction terms between year and the geographic parameters (Table 4). For example, a significant two-way interaction between year and elevation indicates that the long-term direction of change in flowering DOY depends on elevation. The only significant main effect detected was that of elevation on DOY, with advanced flowering dates associated with high elevations.

Discussion

Effects of temperature and rainfall on flowering date

Flowering DOY is associated with winter and spring mean Tmin and total precipitation. Higher spring Tmin is associated with earlier flowering phenology, and more spring precipitation is associated with delayed flowering. Advanced flowering phenology as a response to increased spring temperatures has been reported for many species in temperate regions (Menzel *et al.,* 2006; Miller-Rushing *et al.,* 2007; Beaubien & Hamann, 2011; Panchen *et al.,* 2012). Although phenological responses to precipitation have been less well studied, it appears that the phenological response to precipitation may be less consistent than that with temperature. Some authors have found no effect of precipitation on flowering phenology (Abu-Asab *et al.,* 2001), whereas others have found that increased precipitation resulted in delayed flowering (Von Holle *et al.,* 2010; Mazer *et al.,* 2013) or earlier phenophase onset dates (Crimmins *et al.,* 2010; Lambert *et al.,* 2010).

In the current study, multiple linear regression models also detected a significant interaction between mean Tmin and total precipitation during late winter and spring (the February – April and March – May windows) affecting flowering day of year (DOY). In these windows, the advancing effect of warmer mean Tmin was stronger where total precipitation was higher (Figure 2a). One proximal explanation for this pattern is that flowering phenology more closely tracks minimum temperatures where precipitation is not limiting. Another interpretation is that, where total precipitation is relatively high, DOY is delayed (cf. the effects of precipitation as a main effect) and, accordingly, there is greater potential for higher temperatures to advance DOY towards earlier values without risking reproductive failure. Advancing DOY in response to increasing temperature may not be possible where DOY is already relatively early without risking floral failure due to late winter or early spring frost events. These interpretations are not mutually exclusive and may both contribute to the interaction. In any case, the ultimate evolutionary or physiological mechanisms underlying these interactions cannot be deduced from these patterns alone; to our knowledge this is the first report of such a pattern in any wild species.

The temperature*precipitation interaction is also a result of the delaying effect of precipitation being stronger where Tmin values were colder, suggesting that future changes in precipitation in the western U.S. will have greater effects on the flowering time of *T. ovatum* in cooler locations (in Figure 2b, the positive slope of the line representing the minimum value of mean Tmin [solid, red] is steeper than slope of the lines representing the mean and maximum values of mean Tmin [dashed, green and dotted, blue]); based on the patterns detected here, any reductions in precipitation will advance flowering, particularly where the climate is relatively cool.

This result was unexpected; where Tmin values are low, flowering is relatively late. The delaying effect of high precipitation, then, is strongest where flowering is *already* delayed. In contrast, we might expect that variation in precipitation would have the strongest effect on the onset of flowering in *T. ovatum* where Tmin is highest and flowering is relatively early, i.e., precipitation would have a delaying effect where plants are flowering early and there is greater potential for phenology to be delayed. One possible explanation for the observed pattern is that under cooler conditions, precipitation may freeze and be deposited as snow, requiring additional time for snow to melt and for soils to warm before plants are able to initiate growth and reproduction. Under warmer climate conditions, by contrast, the effect of precipitation on flowering time is not as strong. Given that very few studies have documented interactions between Tmin and precipitation (but see Fu *et al.,* 2014), a better understanding of phenological responses to precipitation is needed if we are to model and forecast phenological changes more effectively, particularly in water-limited ecosystems.

Finally, climatic conditions during the later seasonal windows (FMA and MAM) explained slightly more variation in the flowering phenology of *Trillium ovatum* than the earlier window. Previous studies have found that flowering phenology of some taxa is more sensitive to climatic conditions in certain months or seasons than in others (Hart *et al.,* 2014; Mazer *et al.,* 2015), but the mechanism driving this pattern is unclear. In our study, sensitivity to the later seasonal windows may be due to the individuals in our study that flowered relatively late (e.g., a flowering DOY > 150, or May 30th; Figure 2); these plants may be more sensitive than earlier-flowering individuals to the more recent climate conditions (e.g., those in observed in FMA and MAM).

One limitation of the current study is that the models included only contemporaneous temperature and rainfall (i.e., climatic parameters experienced in the same set of months). Mazer *et al.* (2015) found that, for some California native woody species, the effects on a phenophase's DOY of Tmin during one month depended on the level of rainfall in another month. For example, precipitation in one winter month influenced an individual plant's sensitivity to the Tmin experienced in a subsequent month. Examining the effects of non-synchronous combinations of temperature and rainfall was beyond the scope of the current study, but the variance in DOY explained by multivariate models might be increased by including such interactions.

Temporal changes in temperature and rainfall

Seasonal Tmin values varied across the >100 years of observation (1895-2009) in our climate dataset, but the direction of change depended upon the location of observation. Observations from the western, coastal portion of *Trillium ovatum*'s range revealed that minimum temperatures have decreased across the observation period, whereas in the eastern, inland portion of the range, minimum temperatures have increased. Lebassi *et al.* (2009) reported similar patterns for summer temperature over a 50-year observation period (from 1948 to 2004) in California: summer temperatures have become cooler at low-elevation, coastal sites, which are open to marine air penetration, whereas summer temperatures at inland sites increased in recent years. Likewise, the temporal changes in precipitation were complex, with the direction of change depending upon location. In the January – March window, long-term temporal changes in total precipitation depended on latitude, whereas in March – May, temporal change in precipitation depended on longitude. To our knowledge, the fact that temporal trends in temperature and/or precipitation vary regionally has not previously been accounted for in studies of species' responses to climate change and is an important consideration for any widespread species, in which long-term phenological patterns in one part of its range may differ from those in the another part of its range due to regional variation in the direction or magnitude of climate change.

Climate models for the Pacific Northwest generally predict warmer and similar to slightly wetter conditions in the future; the climate models available in The Nature Conservancy's online climate wizard tool (http://www.climatewizard.org/; accessed November 9, 2014), for example, predict warmer springs (March –May) and relatively little change in precipitation in the Pacific Northwest by the 2080's (Girvetz *et al.,* 2009). We found that warmer spring Tmin values were associated with advanced flowering and the delaying effect of precipitation was more pronounced when Tmin values were lower. If the climate predictions hold true for this region, we expect the inter-annual trend in flowering phenology of *Trillium ovatum* to shift towards earlier flowering in the upcoming decades.

Long-term, temporal changes in flowering date

Given the complexity of long-term, temporal changes in the climate variables that affect flowering phenology, it is not surprising that the long-term, temporal trend in flowering date was similarly complex and location-dependent. Surprisingly, few studies have emphasized the importance of considering location- or region-specific trends in phenology (but see Cocu *et al.,* 2005, which found location-specific trends in aphid phenology across Europe), perhaps because most studies have been limited to local or regional scales.

Interestingly, the model including geographic variables and collection year explained a larger proportion of the variation in flowering date than any of the models with seasonal climate variables (48% [Table 4] vs. 34-36% [Table 1], respectively). While geographic parameters are a good proxy for (and probably capture most) variation in climate, other abiotic factors that affect phenology also are likely to vary geographically and may account for the additional explained variance (e.g., day length, duration of the warmest part of the day, soil nutrients or temperatures, or the intensity of herbivory). Moreover, each season may explain some portion of the variance in flowering DOY, independent of other seasons, a possibility not explored here (since each season was modeled independently). Finally, biotic factors such as the timing of pollinator availability and abundance could determine the optimum flowering time in different regions. If so, natural selection could result in local adaptation and differentiation among populations in flowering time that is somewhat independent of local climatic conditions.

Using natural history collections as a data source

The geographic distribution of *Trillium ovatum* is well represented by the specimens included in our dataset (Figure 1). While herbarium specimens have been used to extend the temporal coverage of phenological datasets (Primack *et al.,* 2004; Robbirt *et al.,* 2011; Panchen *et al.,* 2012), here we show that herbarium specimens can also expand geographic coverage, which allowed us to describe relationships with geographic variables and to capture a wider range of climatic conditions. Many natural history collections are now being digitized, making information contained within them more accessible and allowing researchers to document phenological information without physically visiting herbaria or requesting loan specimens (Park 2012; Park 2014).

As shown here, data derived from natural history collections can illustrate phenological relationships with climate and provide a reference point for comparison with future phenological research. *Trillium ovatum* is a focal species for two national-scale phenological monitoring programs in the U.S., the USA National Phenology Network (www.usanpn.org) and Project Budburst (http://budburst.org/), and we expect that contemporary phenological data across its native range will be increasingly accessible via these online platforms. These herbarium-derived data and results represent a 122-year time series that will provide a baseline upon which to interpret phenological data that are reported to these programs in the future.

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Table 1. Summary of multiple linear regressions conducted to detect significant effects of seasonal climatic variables (mean maximum temperature [Tmax], mean minimum temperature [Tmin], total precipitation [PPT]) and their interaction on the day of year (DOY) of the collection of flowering *Trillium ovatum* specimens. We report the minimal adequate model, identified through backward elimination of predictor variables (see text for details of model selection procedure). Each model tests for the effects on DOY of climatic variables during a different three-month window preceding specimen collection; independent variables represent conditions in: a) January - March; b) February - April; and c) March - May. Parameter estimates are the regression intercepts ad coefficients; values significantly < 0 indicate that flowering times occur earlier with increasing temperature or precipitation, while values that are significantly > 0 indicate that flowering times are delayed with increasing temperature or precipitation. Interaction terms are discussed in the text.

a. Independent Variables: Seasonal Climatic Conditions in January - March

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Tmin	1	134223	126.81	**< 0.001**
Log (PPT + 1)	1	56951	53.81	**< 0.001**
Error	279	295309		
R^2				**0.34**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	24.56	12.88	1.81	.06
Tmin	-4.76	0.42	-11.26	**< 0.001**
Log (PPT + 1)	57.34	7.82	7.34	**< 0.001**

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b. Independent Variables: Seasonal Climatic Conditions in February – April

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Tmin	1	139875	136.52	**< 0.001**
Log (PPT + 1)	1	40368	39.40	**< 0.001**
Tmin * Log (PPT + 1)	1	6592	6.43	**0.01**
Error	279	284832		
R^2				**0.36**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	53.05	11.85	4.48	**< 0.001**
Tmin	1.95	3.05	0.64	.52
Log (PPT + 1)	48.38	7.86	6.16	**< 0.001**

Tmin * Log (PPT + 1)	-5.23	2.06	-2.54	**0.01**

c. Independent Variables: Seasonal Climatic Conditions in March – May

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Tmin	1	107169	104.08	**< 0.001**
Log (PPT + 1)	1	28438	27.62	**< 0.001**
Tmin * Log (PPT + 1)	1	7587	7.37	**< 0.001**
Error	279	286245		
R^2				**0.36**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	25.08	13.61	4.34	**< 0.001**
Tmin	2.39	3.19	0.75	0.45
Log (PPT + 1)	55.31	9.50	5.82	**< 0.001**
Tmin * Log (PPT + 1)	-6.14	2.26	-2.71	**< 0.001**

Table 2. Summary of multiple linear models conducted to detect effects of year, elevation, latitude, longitude, and their interactions on mean minimum temperature (Tmin) during three three-month windows (January – March, February - April, and March - May) preceding the collection date of each sampled specimen. Table 2a reports the independent effects of each dependent variable and their interactions on Tmin from January - March; Table 2b reports the effect of these variables on Tmin from February – April; Table 2c reports the effect of these variables on Tmin from March - May. Parameter estimates are the regression intercept and coefficients of each independent variable.

a) Response variable: mean Tmin (January - March)

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	94.38	20.92	**< 0.001**
Elevation	1	805.30	178.54	**< 0.001**
Latitude	1	272.23	60.35	**< 0.001**
Longitude	1	349.78	77.55	**< 0.001**
Elevation*Latitude	1	1.03	0.22	0.63
Year*Longitude	1	60.46	13.40	**< 0.001**
Elevation*Longitude	1	0.08	0.01	0.89
Latitude*Longitude	1	8.01	1.88	0.18
Elevation*Latitude*Longitude	1	19.94	4.42	**0.04**
Error	272	1226.87		
R^2				**0.81**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-1206.00	405.00	-2.859	**0.004**
Year	0.70	0.19	3.634	**< 0.001**
Elevation	-0.56	0.26	-2.179	**0.03**
Latitude	-5.35	5.65	-0.96	0.34
Longitude	-9.93	3.36	-2.826	**0.005**
Elevation*Latitude	0.01	0.01	2.165	**0.03**
Year*Longitude	0.006	0.001	3.508	**< 0.001**
Elevation*Longitude	-0.005	0.002	-2.162	**0.03**
Latitude*Longitude	-0.04	0.05	-0.894	0.37
Elevation*Latitude*Longitude	0.0001	0.00004	2.16	**0.03**

b) Response variable: mean Tmin (February - April)

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	9.37	2.81	**0.09**
Elevation	1	748.85	224.70	**< 0.001**
Latitude	1	149.14	44.75	**< 0.001**
Longitude	1	196.23	58.88	**< 0.001**
Year*Longitude	1	32.47	9.74	**0.002**
Error	276	919.84		
R^2				**.80**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-986.60	302.10	-3.26	**0.001**
Year	0.49	0.15	3.17	**0.002**
Elevation	-0.004	0.0002	-14.99	**< 0.001**
Latitude	-0.26	0.04	-6.69	**< 0.001**
Longitude	-8.27	2.53	-3.27	**0.001**
Year*Longitude	0.004	0.001	3.12	**0.002**

c) Response variable: mean Tmin (March - May)

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	0.27	0.19	0.75
Elevation	1	642.44	234.53	**< 0.001**
Latitude	1	72.43	25.93	**< 0.001**
Longitude	1	55.85	20.32	**< 0.001**
Year*Longitude	1	16.51	5.12	**0.01**
Error	276	757.40		
R^2				**.74**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-685.40	274.60	-2.798	**0.01**
Year	0.34	0.14	2.461	**0.01**
Elevation	-0.003	0.0002	-15.301	**< 0.001**
Latitude	-0.18	0.04	-5.138	**< 0.001**
Longitude	-5.83	2.30	-2.539	**0.01**
Year*Longitude	0.003	0.001	2.453	**0.01**

Table 3. Summary of multiple linear models conducted to detect effects of year, elevation, latitude, longitude on mean total precipitation during three three-month windows (January – March, February - April, and March - May) preceding the collection date of each sampled specimen. Table 3 reports the independent effects of year, elevation, latitude, longitude on Tmin from a) January – March; b) February – April; and c) March - May. Parameter estimates are the regression intercepts and coefficients for each independent variable.

a) Response variable: Log (Precipitation + 1) (January-March)

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	0.16	4.99	**0.03**
Elevation	1	3.46	104.24	**< 0.001**
Latitude	1	0.09	2.65	0.11
Longitude	1	8.04	241.90	**< 0.001**
Year*Latitude	1	0.16	4.94	**0.03**
Latitude*Longitude	1	0.31	9.59	**0.002**
Error	275	9.14		
R^2				**.52**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-24.77	15.89	-1.575	0.12
Year	-0.01	0.004	-2.021	**0.04**
Elevation	0.0002	0.00002	10.210	**< 0.001**
Latitude	0.32	0.35	0.900	0.37
Longitude	-0.37	0.10	-3.880	**< 0.001**
Year*Latitude	0.0002	0.0001	2.224	**0.03**
Latitude*Longitude	0.007	0.002	3.097	**0.002**

b) Response variable: Log (Precipitation + 1) (February-Ap

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	0.07	2.20	0.14
Elevation	1	3.02	93.63	**< 0.001**

Latitude	1	0.35	10.91	**0.001**
Longitude	1	6.21	192.53	**< 0.001**
Elevation*Latitude	1	0.21	6.40	**0.01**
Error	276	8.90		
R^2				**0.49**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-9.88	1.15	-8.61	**< 0.001**
Year	0.0006	0.0004	1.48	0.14
Elevation	-0.0006	0.0003	-1.86	0.06
Latitude	0.006	0.004	1.37	0.17
Longitude	-0.08	0.006	-13.88	**< 0.001**
Elevation*Latitude	0.00002	0.000008	2.53	**0.01**

c) Response variable: Log (Sum Precip + 1) (March-May)

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	0.22	5.62	**0.02**
Elevation	1	3.75	93.93	**< 0.001**
Latitude	1	1.52	38.05	**< 0.001**
Longitude	1	5.29	132.49	**< 0.001**
Elevation*Latitude	1	0.24	6.07	**0.01**
Year*Longitude	1	0.17	4.25	**0.04**
Error	275	10.99		
R^2				**0.40**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-78.98	33.13	-2.38	**0.01**
Year	0.04	0.02	2.13	**0.03**
Elevation	-0.0007	0.0004	-1.79	**0.07**
Latitude	0.02	0.005	3.83	**< 0.001**
Longitude	-0.64	0.28	-2.33	**0.02**
Elevation*Latitude	0.00002	0.00001	2.46	**0.01**
Year*Longitude	0.0003	0.0001	2.06	**0.04**

Table 4. Summary of multiple linear regression model conducted to detect effects of year, elevation, latitude, longitude, and their interactions on the day of year (DOY) of the collection of flowering specimens of *Trillium ovatum*. Parameter estimates are the regression coefficients for each variable.

Analysis of Variance

Source	DF	Sum of Squares	F Ratio	p-value
Year	1	4621	5.24	**0.02**
Elevation	1	153943	58.28	**<.0001**
Latitude	1	11422	6.49	**0.001**
Longitude	1	25570	29.04	**<.0001**
Year*Elevation	1	505	0.57	0.45
Year*Latitude	1	2120	2.41	0.12
Elevation*Latitude	1	2157	2.45	0.12
Year*Longitude	1	397	0.45	0.50
Elevation*Longitude	1	130	0.15	0.70
Latitude*Longitude	1	1313	1.49	0.22
Year*Elevation*Latitude	1	6115	6.94	**0.009**
Year*Elevation*Longitude	1	5244	5.96	**0.02**
Error	276	243016		
R^2				**0.48**

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	9305	12460	0.75	0.46
Year	-6.36	6.52	-0.98	0.33
Elevation	-49.37	18.26	-2.70	**0.007**
Latitude	19.53	71.62	0.27	0.79
Longitude	64.32	93.41	0.69	0.49
Year*Elevation	0.025	0.009	2.70	**0.007**
Year*Latitude	0.02	0.03	0.64	0.52
Elevation*Latitude	0.31	0.12	2.66	**0.008**
Year*Longitude	-0.046	0.05	-0.95	0.34
Elevation*Longitude	-0.297	0.12	-2.45	**0.02**
Latitude*Longitude	0.484	0.40	1.22	0.22
Year*Elevation*Latitude	-0.0002	0.00006	-2.64	**0.009**
Year*Elevation*Longitude	0.0002	0.00006	2.44	**0.02**

<u>Figure captions</u>

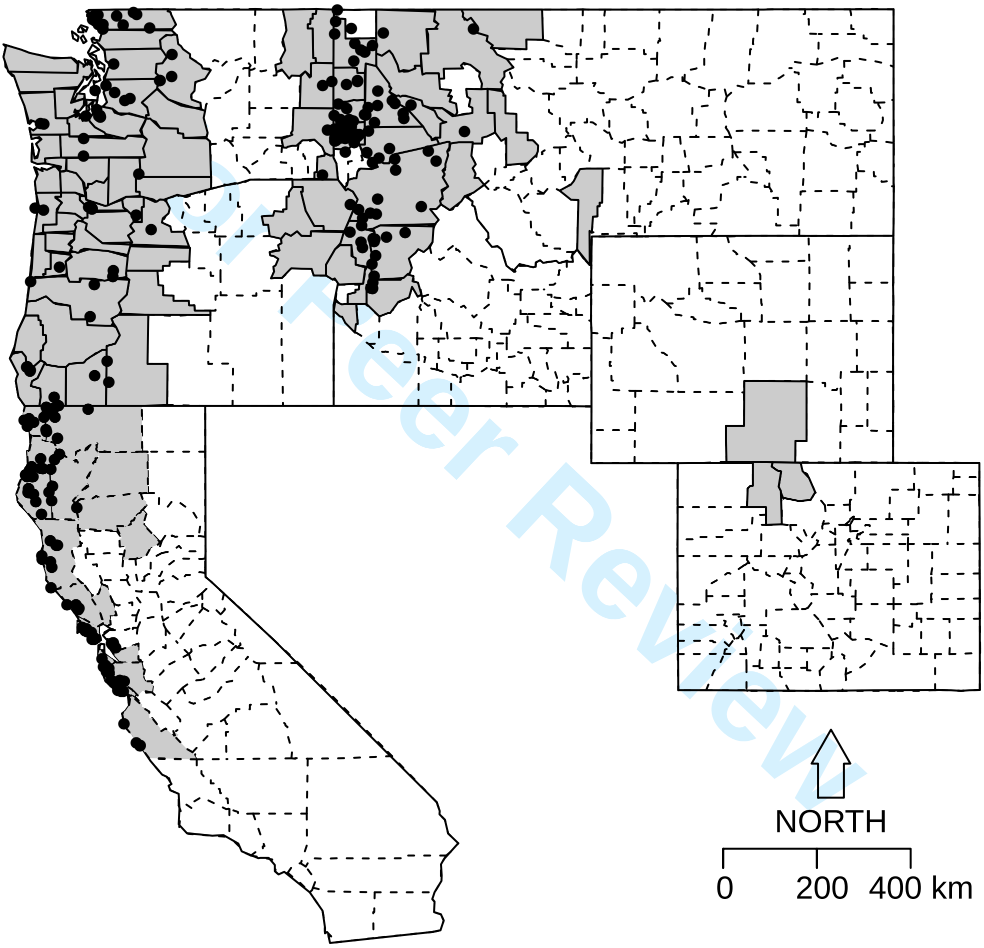
Figure 1. Collection locations of flowering *Trillium ovatum* specimens (n= 289). Black dots are collection locations, which are overlaid on the county-level geographic range information obtained from the USDA PLANTS database (USDA 2014; *Trillium ovatum* occurs in the counties that are shaded grey).

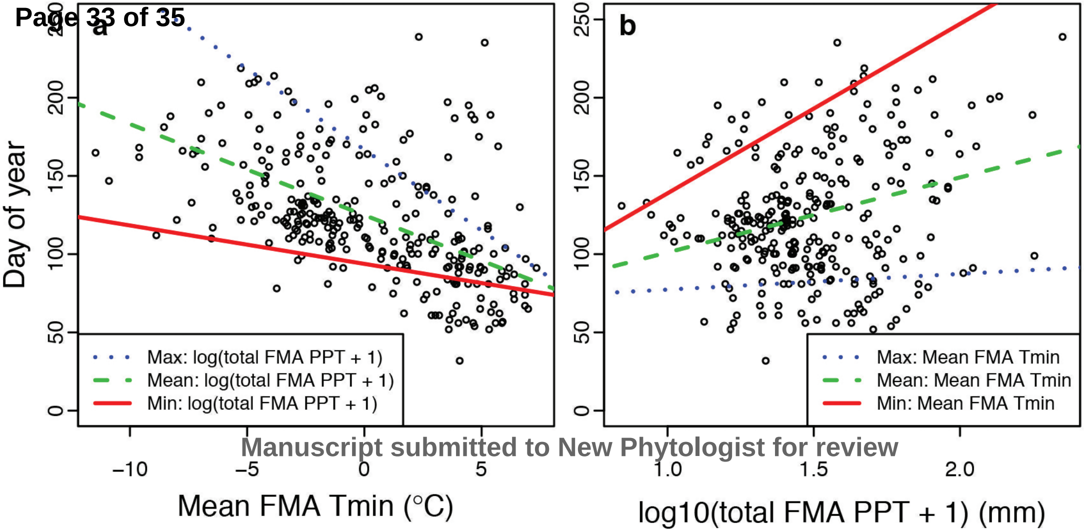
Figure 2. Effect of the interactions between February-March-April (FMA) climate variables (mean Tmin (°C) and total precipitation (mm)) on Day of Year (DOY). Predicted DOY values were plotted as a function of FMA climate variables, based on the equation estimated from the linear model (Table 1b). Panel a shows predicted DOY values as a function of mean Tmin. The dotted blue line represents the predicted DOY at the maximum value of log (Total FMA Precipitation +1); the dashed green line represents the predicted DOY at the mean value of log(Total FMA Precipitation +1); and the solid red line represents the predicted DOY at the minimum value of log(Total FMA Precipitation +1). Panel b shows predicted DOY values as a function of total precipitation. The dotted blue line is the predicted DOY when at the maximum value of Mean FMA Tmin; dashed the green line is the predicted DOY at the mean value of Mean FMA Tmin; and the solid red line is the predicted DOY at the minimum value of Mean FMA Tmin. The lines are superimposed on the actual data to illustrate the bounds of the data.

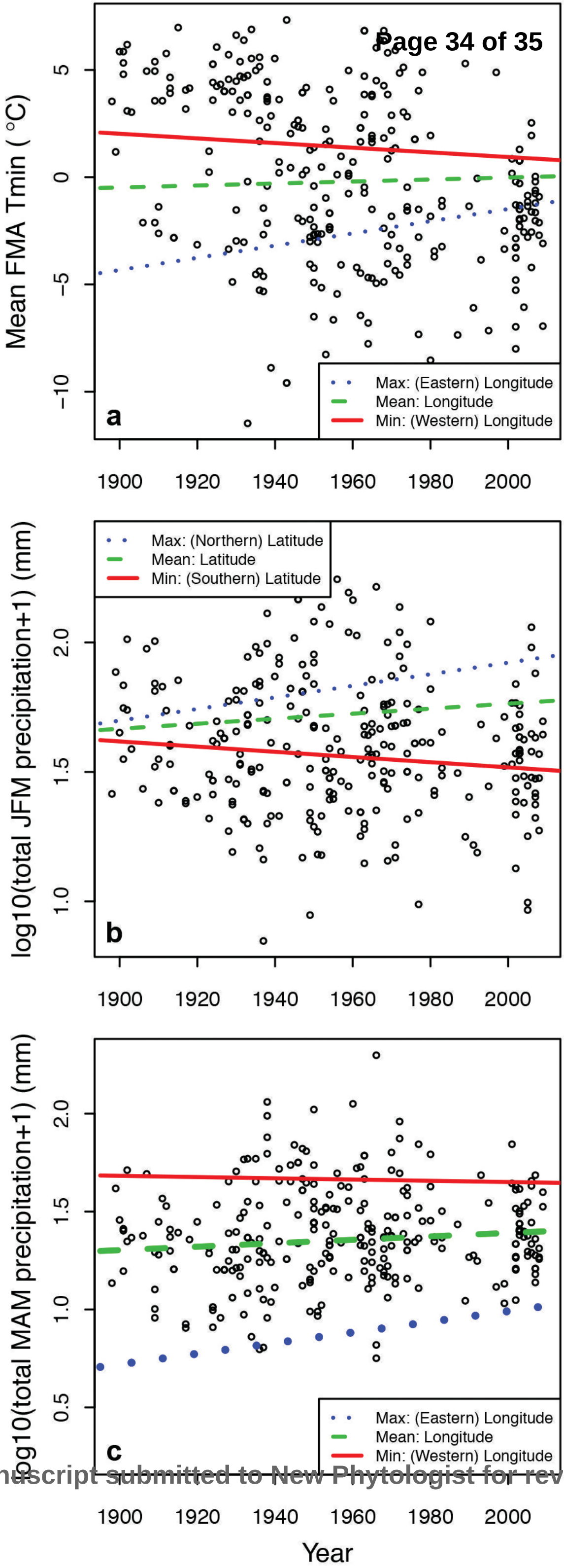
Figure 3. Effect of the interactions between Year and geographic variables on seasonal climate variables. Predicted values of the climate variables were plotted as a function of Year based on the equations estimated from the linear models (Table 2). The lines are superimposed on the actual data to illustrate the bounds of the data. Panel a shows the effect of the interaction between Year and Longitude on Mean FMA Tmin (°C). The dotted blue line is the predicted mean Tmin at the maximum value of longitude (in decimal degrees); these are the Eastern-most collection locations. The dashed green line is the predicted mean Tmin at the mean value of longitude, and the solid red line is the predicted mean Tmin at the minimum (Western-most) longitude value. Panel b shows the effect of the interaction between Year and Latitude on total JFM precipitation (mm). The dotted blue line is the predicted total precipitation at maximum values of latitude (i.e., Northern sites); the dashed green line is the predicted total precipitation at the mean value of latitude; and the solid red line is the predicted total precipitation at the minimum value of latitude

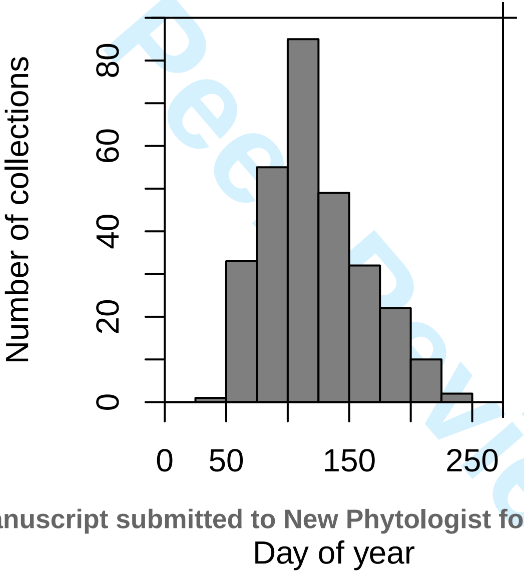
(i.e. Southern sites). Panel c shows effect of the interaction between Year and Longitude on total MAM precipitation (mm). The dotted blue line is the predicted total precipitation at the maximum value of longitude (Eastern locations); the dashed green line is the predicted total precipitation at the mean value of longitude; and the solid red line is the predicted total precipitation at the minimum value of longitude (Western locations).

Figure 4. Frequency distribution of Day of Year (DOY) on which flowering *Trillium ovatum* herbarium specimens were collected (n=289). The mean collection DOY is 122 (May 3rd).


NORTH
0
200
400 km


a
Day of year
0
50
100
150
200
250
Max: log(total FMA PPT + 1)
Mean: log(total FMA PPT + 1)
Min: log(total FMA PPT + 1)
−10
−5
0
5
Mean FMA Tmin (°C)
b
0
50
100
150
200
250
Max: Mean FMA Tmin
Mean: Mean FMA Tmin
Min: Mean FMA Tmin
1.0
1.5
2.0
log10(total FMA PPT + 1) (mm)


Mean FMA Tmin (°C)
5
0
−5
−10
a
Max: (Eastern) Longitude
Mean: Longitude
Min: (Western) Longitude
1900
1920
1940
1960
1980
2000
log10(total JFM precipitation+1) (mm)
Max: (Northern) Latitude
Mean: Latitude
Min: (Southern) Latitude
2.0
1.5
1.0
b
1900
1920
1940
1960
1980
2000
log10(total MAM precipitation+1) (mm)
2.0
1.5
1.0
0.5
c
Max: (Eastern) Longitude
Mean: Longitude
Min: (Western) Longitude
1900
1920
1940
1960
1980
2000
Year


Number of collections
0
20
40
60
80
0
50
150
250
Day of year