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Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms[1]

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■ *Premise of the study*: Climate change has resulted in major changes in the phenology of some species but not others. Long-term field observational records provide the best assessment of these changes, but geographic and taxonomic biases limit their utility. Plant specimens in herbaria have been hypothesized to provide a wealth of additional data for studying phenological responses to climatic change. However, no study to our knowledge has comprehensively addressed whether herbarium data are accurate measures of phenological response, and thus applicable to addressing such questions.

■ *Methods*: We compared flowering phenology determined from field observations (years 1852-1858; 1875; 1878-1908; 2003-2006; 2011-2013) and herbarium records (1852-2013) of 20 species from New England, USA.

■ *Key Results*: Earliest flowering date estimated from herbarium records faithfully reflected field observations of first flowering date and substantially increased the sampling range across climatic conditions. Additionally, although most species demonstrated a response to inter-annual temperature variation, long-term temporal changes in phenological response were not detectable.

■ *Conclusions*: Our findings support the use of herbarium records for understanding plant phenological responses to changes in temperature, and also importantly establish a new use of herbarium collections: inferring primary phenological cueing mechanisms of individual species (e.g., temperature, winter chilling, photoperiod). These latter data are lacking from most investigations of phenological change, but are vital for understanding differential responses of individual species to ongoing climate change.

Key words: climate change, climate variability, phenology, herbarium specimens, museum collections

The impacts of global climatic change on organisms have been well documented in recent years (Walther et al., 2002; Root et al., 2003; Parmesan, 2006). Changes in phenological events—i.e., the timing of specific life-history events—are used widely to assess responses of different organisms to climate change. Typically, researchers have focused on relatively common phenological events that are easily measured and have a history of regular observation such as leaf-out, flowering, or fruiting in plants, or eclosion and migration in animals (Sparks and Carey, 1995; Bradley et al., 1999; Fitter and Fitter, 2002; Menzel, 2002; Parmesan and Yohe, 2003; Root et al., 2003; Parmesan, 2007; Bertin, 2008; Miller-Rushing et al., 2008; Miller-Rushing and Primack, 2008; Visser, 2008; Willis et al., 2008; Körner and Basler, 2010; Willis et al., 2010; Panchen et al., 2014). However, the paucity of long-term data sets necessary to identify the influence of climatic change on phenological events remains problematic, even for regions where associated climatic data are available (e.g., long-term temperature trends). Moreover, most data sets of this nature show a strong geographical and taxonomic bias—they are largely from temperate regions, mostly include a small subset of species or functional types within particular assemblages (e.g., dominant woody species), and do not sample the variation in phenological response across the range of a species (Wolkovich, Cook, and Davies, 2014).

The enormous collections of plants housed in herbaria around the world provide a potential, largely untapped, alternative body of data for studying long-term phenological responses to climatic change (Vellend et al., 2013; Kharouba and Vellend, 2015). Herbarium specimens represent snapshots of phenological events (e.g., flowering, fruiting) at a specific place and time. Observations from numerous specimens collected at multiple locations and times may allow us to determine whether a given species has changed its phenology in parallel with climate. Previous efforts have used herbarium specimens in this manner (Primack et al., 2004; Miller-Rushing et al., 2006; Robbirt et al., 2011; Panchen et al., 2012), but only recently has this effort been scaled-up to investigate patterns of phenology across large numbers of species and vast geographical areas. For example, Calinger *et al.* (2013) combined data from herbarium specimens of 141 species with climatic records to determine that peak flowering has advanced 2.4 days/°C of warming over the last century across ~116,000 km^2 in north-central North America. They further identified differences in phenological responses based on whether a species was native, its pollination syndrome, growth form, functional group, and flowering season. Similarly, Everill *et al.* (2014) examined ~1600 herbarium records from 1834-2008 of 27 common New England tree species. They reported that spring leaf-out dates were strongly associated with spring temperatures and that tree species leafed out ~2.0 days/°C earlier now than in the past.

We applaud these efforts to leverage herbarium data to investigate recent effects of climate change. Despite the promise of these studies, however, the efficacy and bias of herbarium records as accurate measures of phenological response have seldom been assessed (Gardner et al., 2006; Moerman and Estabrook, 2006; Loiselle et al., 2008; Sastre and Lobo, 2009). This is relevant because the purpose of herbarium collections, at least historically, has not been to document phenological phenomena *per se*, but rather to sample representative specimens of a species throughout its geographic distribution. Thus, phenological data collected from herbarium records are subject to numerous potential biases. For example, botanists might collect samples at the same time every year out of habit or convenience, with little regard to inter-annual climatic variation, occurrence of date of first flowering, or time of spring leaf-out. Other sources of bias include: misidentification of closely related species that vary in phenological response; temporal gaps in collecting effort that impede efforts to assess long-term change [e.g., decline in collecting efforts since the mid-20th century (Gardner et al., 2006)]; spatial gaps in collecting; and spatial preferences when collecting samples (e.g., easily-accessible urban areas, trails, roadsides).

At the same time, ecologists' emphasis on reconstructing phenology overlooks other important uses of herbarium data. With rare exceptions (e.g., crops and model species like *Arabidopsis thaliana* (L.) Heynh.), we know relatively little about environmental cues that regulate onset and duration of phenological events for most plant species. These cues govern physiological mechanisms that initiate phenological events associated with fitness traits (e.g., initiation of buds or flowering), and timing of these events ultimately may determine how species will respond to future climatic change. For example, species for which flowering is most sensitive to temperature likely will be strongly affected by changing temperatures, especially when this could create temporal mismatches with key pollinators that are more (or less) sensitive to temperature cues (Burkle, Marlin, and Knight, 2013). Fortunately, recent advancements in process-based modeling and data-model fusion have allowed researchers to distinguish among the relative importance of major environmental cues (e.g., temperature, winter chilling, and photoperiod) (Richardson et al., 2006; Morisette et al., 2008; Migliavacca et al., 2012; Archetti et al., 2013; Siniscalco et al., 2014). These models, however, require extensive and temporally dense (>50years) collections of standardized observational data. As a result, the application of process-based models to studies of phenological change has been limited to only a few dozen species, often from fairly restricted phylogenetic and life history groups. To the extent that they prove to be reliable measures of inter-annual phenological response, herbarium data offer the potential for researchers to expand the temporal depth of phenological data for large numbers of species, and simultaneously propose new hypotheses regarding physiological controls on phenological events.

In this paper we address both the validity of herbarium records for investigating phenological change and the use of herbarium records for identifying potential cueing mechanisms of phenological events. Our focal region—New England in the United States—is where researchers have suggested that many plants today flower much earlier than in the past because they are responsive to changes in temperature, which has been rising rapidly in this region (Miller-Rushing and Primack, 2008). We first combine direct observational records and rich herbarium records collected during the last 160 years from Concord, Massachusetts and adjacent counties to assess whether herbarium flowering data correspond with first flowering dates observed at irregular intervals in the field. We then leverage these results to identify likely physiological cueing responses necessary for regulating flowering phenology. We focused on 20 species previously shown to exhibit dynamic responses to climatic change (Willis et al., 2008; Willis et al., 2010).

These species are abundant in New England herbaria because they are frequently collected, conspicuous plants that produce large flowers, which are easy to score from herbarium collections (e.g., lilies, orchids). Ultimately, results of our efforts will help to build a refined regional picture of how climatic change has affected plant phenology given the range of physiological mechanisms by which plants are cued to flower and fruit, and how this is likely to shape plant diversity in the near future.

MATERIALS AND METHODS

Study site–Concord (42°27’38” N; 71°20’54” W) is a ~67 km^2 town in Massachusetts (MA) with a wide range of habitats, including peatlands, deciduous hardwood forests, and prairies. Although Concord has undergone extensive development since the 1850s, ~60% of the town’s land area remains undeveloped or has been permanently protected from future development (Willis et al., 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009). During the mid-19th and early 20th centuries, Henry David Thoreau and Alfred Hosmer, respectively, documented plant species occurrences and first-flowering dates there (Miller-Rushing and Primack, 2008; Willis et al., 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009). These data, combined with contemporary observations, have suggested that, depending on spring temperatures, some species flower as many as 8 days earlier now than they did in the 1850s (Miller-Rushing and Primack, 2008; Willis et al., 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009). Moreover, Willis *et al.* (2008, 2009) identified phylogenetic effects in these data on species diversity: clades whose first flowering time are less sensitive to temperature and have shown little phenological change also have declined significantly in abundance.

Study species–We investigated 20 biennial or perennial species in nine families (Table 1), each of which met four criteria. [1] The species were represented in historical field observations by Hosmer (1878-1903) from Concord (Miller-Rushing and Primack, 2008). These data are unique in providing a reliable, uninterrupted 15-year period of phenological monitoring. [2] The species had relatively large flowers, which facilitated rapid and accurate assessment of flowering from herbarium specimens. [3] The species were well represented in herbarium collections from Massachusetts, including Middlesex County (which includes Concord) and nearby counties. [4] Species with showy, ephemeral flowers and with relatively short flowering time, such as orchids (Orchidaceae) and irises (Iridaceae), were preferred because they were more likely to have been collected near to their first flowering date (Robbirt et al., 2011). We also included non-native and invasive species (e.g., *Barbarea vulgaris* [Brassicaceae], *Chelidonium majus* [Papaveraceae], respectively) as well as species such as *Vaccinium angustifolium*, which previously have been shown to be phenologically responsive to warming (Ellwood et al., 2013) and thus more likely to exhibit long-term phenological shifts associated the secular trend of rising mean temperatures. Additionally, taxon sampling is representative of the breadth of seasonal flowering (e.g., spring ephemeral vs. summer flowering species).

Flowering time data–Field observations of first flowering date for Concord were recorded by Thoreau (1852–1858), Hosmer (1875, 1878–1903), Miller-Rushing & Primack (2003–2006) (Miller-Rushing and Primack, 2008), and this paper's co-authors Davis & Connolly (2011–2013: field data first reported and used herein). Field observations by Davis & Connolly applied a similar method to the one outlined by Primack et al. (2009): from April to September, multiple sites throughout the Concord area were visited 1-3 times weekly by Davis & Connolly to systematically record flowering dates. Similar to Primack et al., Davis & Connolly also consulted local botanical experts about the location and flowering time of certain species.

Estimates of earliest flowering dates for herbarium records were based on data collected during visits to the Harvard University Herbaria (HUH), New York

Botanical Garden's William and Lynda Steere Herbarium (NY), Yale University Herbarium (YU), and University of Connecticut's George Safford Torrey Herbarium (CONN) by co-author Kelly. These herbaria collectively represent the largest holdings of plants of the Northeastern United States and include both very old collections (HUH, YU) and more recent ones (CONN). We first identified flowering specimens from MA for each of our target species. Following Primack et al. (2004), we recorded locality, collection date, accession number (when provided), and collector for specimens with fully open flowers. When multiple flowers were present on a specimen, it was recorded as flowering if ≥ 75% of them were fully opened. Specimens that had a majority of flower buds or fruit were ignored, as were those with insufficient or illegible collection data. The majority of herbarium specimens were collected between the late 1800s and mid-1900s. When there were multiple specimens for the same species in a given year (which occurred only for <3% of the data collected), we used the earliest record for a species × county combination in a given year as our estimate of earliest flowering date.

Finally, we emphasize that the earliest flowering date estimated from herbarium specimens is different from first flowering date recorded by field observers. Observational records are the gold standard for phenological research, and estimate first flowering date with a high degree of accuracy. In contrast, data from herbarium specimens provides only an approximation of earliest flowering date for those specimens, which may or may not be correlated with first flowering date observed in the field. However, our goal was to assess changes in flowering as a function of both inter-annual temperature variation and long-term changes in climate. Although we expected differences between the observed first day of flowering and estimated date of flowering estimated from herbarium specimens, the aim of this study was to assess whether these two data types estimate similar responses to both short-term and long-term climatic change. We hypothesized that estimated changes in earliest flowering date determined from herbarium specimens would be correlated with observed changes in first flowering date as the climate has changed.

Temperature records–Mean monthly temperatures (1885–present) at Great Blue Hill, ~33 km southeast of Concord were obtained from NOAA's Global Historical Climatology Network (http://ncdc.noaa.gov/ghcnm/). We used the GHCNM v3 quality controlled unadjusted data. These data are highly correlated ($r \geq 0.995$) with available, but sparser, climatological data from Concord (Miller-Rushing and Primack, 2008). Monthly temperature data from 1831 – 1884 collected by the Blue Hill Meteorological Observatory were provided to us by A. Miller-Rushing. Mean annual temperatures (Fig. 1a) were calculated by averaging the mean monthly temperatures for each year. Mean spring temperatures (Fig. 1b) were calculated as the average of each year's February – May mean monthly temperatures. These months were used because they had been found previously to represent the months that are most predictive of flowering time (Miller-Rushing and Primack, 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009).

Statistical analysis–We used linear mixed-effects models in the *nlme* library (Pinheiro et al., 2015) of the R statistical software system (Team', 2014) version 3.1.0 to test for overall and species-specific relationships between spring temperature, calendar year, and earliest flowering date. Earliest flowering date in a given year—either from field or herbarium observations—was the response variable in all models. In the "climate" model, mean spring temperature was treated as a fixed predictor variable, whereas in the "year" model, calendar year was the fixed predictor variable. In both models, data type—field observation or herbarium record—was also treated as a fixed predictor variable. Species identity was included as a random effect. The values of the random effects (i.e., equivalent to the *y* intercept for each species) ordered the species from earliest to latest flowering, so we also regressed (using a linear regression model) the rate of change in earliest flowering date for each species (i.e., the slope of the line relating earliest flowering date to climate) against its random effect term. This latter analysis provided additional insights about potential species-specific sensitivity to spring temperature as a phenological cue.

To test the hypothesis that estimated changes in earliest flowering date determined from herbarium specimens were correlated with observed changes in first flowering date as the climate has changed, we plotted the slopes of the lines fit to either the field observational data or the herbarium data in the "climate" model. We tested the relationship between the slopes generated by these two models in two ways. First, we did a simple paired *t*-test on the slopes (paired by species). Failure to reject the null hypothesis of no difference would suggest that the observed and herbarium data are recording similar responses to climate. We also fit a Model-II regression to the paired slopes (Model-II, or reduced major-axis regression makes no assumption about the "independent" or "dependent" variable (Gotelli and Ellison, 2012). The slope of this regression tests whether the two sets of data vary in parallel, and the intercept is an estimate of how the expected shift in flowering date differs between the two datasets.

In the main text, we report data only for the 600 field observations from Concord combined with 297 herbarium records from Middlesex County (where the town of Concord is located). The results were qualitatively identical when we combined the Concord observations with the 680 herbarium records from four nearby counties (results in Supplementary Online Material). Raw data and model code are publicly accessible from the Harvard Forest Data archive (http://harvardforest.fas.harvard.edu/data-archive), dataset HF-XXX (will provide DOI in galley).

RESULTS AND DISCUSSION

Data density of field observations and herbarium records–Field observations (which we refer to henceforth as "observational data") of early flowering dates have been highly episodic (Fig. 1c). Thoreau recorded dates of first flowering in Concord annually from 1852 to 1858; Hosmer recorded first flowering in 1875, and then annually between 1878 and 1903; Primack and Miller-Rushing's data span 2003–2006, and our own observational data include 2011–2013. In contrast, we have 1108 herbarium records (which we refer to henceforth as "herbarium data") of flowering occurrences in the state of Massachusetts collected between 1852 and 2012 (Fig. 1d) with 297 records from the same county as Concord (Middlesex), and 680 remaining records from four nearby counties (Supplementary Online Material, Fig. S1).

During the combined data interval (1852–2013), mean spring temperatures varied widely, ranging from < 1 to > 8 °C. Similarly, mean annual temperatures ranged from < 6 to > 11 °C. To characterize this variation in temperature, we defined the "climatic space" of Concord since 1852 as the region encompassed by the range of mean spring temperatures and mean annual temperatures (Fig. 1e). Three points are worth emphasizing about the sampling coverage of this climatic space. First, herbarium data covered a much larger percentage of this climatic space than observational data (91% versus 76%, respectively). Second, observational data were notably lacking in years with unusually cool springs (i.e., those below the regression line in Fig. 1e). Third, despite broad inter-annual variability for both mean spring and mean annual temperatures (Figs. 1a, 1b), the historical and contemporary observational data represent extreme endpoints in climatic space. Note that the historical data amassed by Thoreau and Hosmer were collected during a relatively cold period, whereas the more contemporary data were collected during a relatively warm period (i.e., Miller-Rushing & Primack, Davis & Connolly) (Figs. 1a, 1b). This sampling artifact could bias inference about potential long-term secular trends on phenology. However, the statistical bias of observational records caused by this lack of overlap across the climate data is potentially ameliorated by the herbarium data, which is distributed randomly across the climatic space (Fig. 1e).

Herbarium data parallel field observations, but reduce long-term estimates of phenological advancement attributed to climate change–Overall, earliest recorded flowering dates in Middlesex County were negatively associated with mean spring temperatures for all species (i.e., flowering was earlier; Fig. 2; overall slope = –3.8 days/°C; $F_{1,696} = 96.4$, $P < 0.001$); results were similar for the four nearby counties (Supplemental Online Material, Fig. S2). There was no significant interaction between mean spring temperature and observation type on earliest flowering date

($F_{1,696} = 0.5$, $P = 0.47$), suggesting that that the overall relationship (i.e., slope) between mean spring temperature and earliest flowering date did not differ between field observations and herbarium data.

The paired *t*-test comparing the slopes of the species-specific regression lines for observed and herbarium data shown in Fig. 2 found no significant differences ($t_{18} = 0.45$, $P = 0.65$). Although the slopes of observed and herbarium data do not fall on a 1:1 line (Fig. 3), the *y*-intercept of the plot, –2.8 days, suggests that observed first flowering dates are, on average, just under 3 days earlier than estimated earliest flowering date of herbarium specimens. Thus, we were confident (contra CaraDonna, Iler, and Inouye, 2014), that we could fit a common climate model to these data as a whole, combining herbarium data to fill the gaps in the field observational data (black lines in Fig. 2). To minimize effects of outliers, however, we fit this common slope using robust linear models (Venables and Ripley, 2002).

Overall, our results support a single previous study that has looked at the fidelity of herbarium records with respect to field observations, but for a greatly reduced number of species and phylogenetic diversity. Robbirt et al. (2011) compared an abundance of field and herbarium data from across Europe for the single terrestrial orchid *Ophrys sphegodes*, and found no significant difference between the two data types for estimates of peak flowering time as a function of spring temperature.

Although the response of earliest flowering date was similar both for field and herbarium data, the intercepts differed by 2.8 days. This result should not be surprising because the observational data that we used were collected with the explicit purpose of capturing the earliest flowering day. The natural historians and ecologists who collected these data routinely sampled several, often consecutive days before flowering occurred and consulted local residents and experts to increase the likelihood of identifying first flowering events. In contrast, the collections represented by the herbarium data that we used rarely were made expressly to capture first flowering events, but rather to document interesting, frequently abundant plants in an area at multiple developmental stages (e.g., flowering, fruiting), usually meant for systematic and floristic research. These samples often were obtained after the time that the first flowers appeared. Furthermore, our more conservative scoring of flowering time for specimens (≥ 75% open flowers) potentially contributed to the overall later date among herbarium records. Nonetheless, our results provide the first broad validation, for a region in central New England, that herbarium records can be used to address spatial and temporal trends in phenology when and where field observational data are unavailable. These results underscore the enormous promise of leveraging herbarium records for understanding the impacts of climate change in New England, and perhaps more broadly.

We also demonstrated that the inter-annual variability in climate covered by the herbarium data fully encompassed and was substantially larger than the range of climate space encompassed by observational data (Fig. 1e). This was true despite the potential biases observed in sampling temporal variability, including episodic field observations, and herbarium specimens collected predominantly before 1960. This result demonstrates for the first time to our knowledge that herbarium records represent key sources of data for filling those parts of the climatic space for which direct field observations are unavailable, and for determining how species dynamically adjust their flowering time to inter-annual temperature variation. Analysis of our combined field and herbarium data suggested an earlier flowering by 3.5 days/°C (Fig. 2), similar to estimates from larger-scale studies that have used herbarium records to assess phenological effects of climatic change. Calinger et al., (2013), for example, reported an average of change of 2.4 days/°C for flowering in 141 species in the Midwestern United States. Similarly, Everill et al., (2014) reported an advancement of leaf out by 2 days/°C for 27 common deciduous woody species in the northeastern United States.

Importantly, however, concluding that plants flower earlier following warmer springs (or in warmer years) is not the same as saying that these plants flower earlier now than they did in the 1850s or early 1900s (cf. Miller-Rushing and Primack, 2008; Ellwood et al., 2013). When we regressed earliest flowering date on calendar year, no significant effect was observed ($F_{1,696} = 3.28$, $P = 0.07$; Fig. 4), nor was there an interaction between data type (field observation vs. herbarium) and calendar year ($F_{1,696} = 0.01$, $P = 0.75$). Even though both mean spring and mean annual temperatures are clearly rising (Figs. 1a, 1b), inter-annual variation in both spring or annual temperatures (> 7°C) far exceed the long-term trend in temperature (1.5 °C/century): in fact, spring of 2012 was the warmest (8.3 °C) on record, but the spring of 2013 was nearly as cold (5.9 °C) as some of the warmest springs during Hosmer's observations more than a century ago (1898: 5.2 °C; 1903: 6.7 °C; Fig. 1b). Phenological events in recent years illustrate this point remarkably well. For example, in 2012, most species flowered early in the year (mean observed first flowering date of all 20 species was 27 April), but in 2013, most species flowered much later (mean observed first flowering date was 23 May). Thus, our findings indicate that researchers should approach long-term phenological assessments using field or observational data with caution given the high degree of inter-annual variability in temperature.

There are two likely explanations for the discrepancies in long-term phenological trends we observed between our own results and past studies for New England (Primack et al., 2004; Miller-Rushing et al., 2006; Willis et al., 2008; Panchen et al., 2012). First, historical and contemporary observational data were collected in non-overlapping regions of climatic space (Fig. 1e). Despite pronounced inter-annual variability in annual and spring temperatures (Figs. 1a, 1b), historical field observations were made during relatively cool periods with late springs, while more recent observations have been made during a record-setting warm period with early springs (Fig. 1e). Consequently, the use of field observational data alone is biased towards finding strong shifts in flowering over the last century. As we have indicated above, herbarium data greatly help to alleviate this sampling bias in climatic space. Second, because spring flowering species are thought to be on average more responsive to temperature, other studies of phenological advancement have focused on these species with the premise that they would likely exhibit the greatest long-term response, which indeed they do (Miller-Rushing & Primack, 2008). Our analyses, however, demonstrate that the inclusion of later-flowering species (summer and early fall) results in this long-term trend being non-significant, and thus far less dramatic when the seasonal variation of flowering across the flora is considered (Fig. 5). This is not to say that climatic change has not impacted or will not continue to impact spring ephemeral communities. However, we caution against making long-term phenological predictions based only on short-term trends especially where inter-annual variability is high (regression lines in Figs. 1a, 1b).

We obtained qualitatively similar results when we included herbarium data from the four adjacent counties in our analysis (Supplemental Online Material, Fig. S3). However, with the inclusion of additional herbarium data, the interaction term between calendar year and observation type was significant (Supplemental Online Material, Table S2). In other words, not only the intercepts (as in the Middlesex County data alone) but also the slopes of the regression lines relating flowering date to calendar year differed between observational and herbarium data. Although the common slope fitted to each species was essentially flat, the slopes fit to the observational data and the herbarium data were not parallel to one another. This result illustrates that inter-annual temperature variability among locations is substantial and suggests potential limitations in using herbarium data from areas that are not closely co-located with observational data. Specifically, the lack of co-located observational data may lead to potentially spurious interpretation of phenological change across larger areas where only herbarium records are available.

Previous studies of the Concord flora have drawn a clear link between short-term phenological sensitivity to temperature and declining abundance (Willis *et al.*, 2008). Our revised estimates of phenological sensitivity to inter-annual spring temperature raise questions about one recently hypothesized mechanism driving this decline—phenological mismatch. Bartomeous et al. (2011) found that several common New England insect pollinators were sensitive to spring temperature, advancing their flight times by 3.6 days/°C . This is remarkably similar to our own flowering phenology results of 3.5 days/°C. In contrast, Bartomeous et al. (2011) also found that these same pollinators had advanced their phenology over the last century by ~10 days, which is on par with previous studies of plant phenology in New England (Miller-Rushing and Primack 2008). Their interpretation of these results was that ecological mismatches in plant-pollinator mutualisms were unlikely to explain the decline among plants in the region. Our results, however, demonstrated no significant trend in long-term flowering shifts among New England plants (Figs. 4, S4, Table S2). This indicates that pollinators may be emerging significantly earlier than their plant hosts for all but the most temperature-sensitive plant species. This re-interpretation of Bartomeous et al.'s conclusions reopens the question of the importance of pollinator mismatches to the decline of those less-temperature-sensitive species in New England.

Using herbarium records to assess phenological cueing mechanisms–Finally, we suggest that analysis of herbarium data can be used to identify variability in physiological mechanisms that cue phenological events (Fig. 5). It is clear that species vary in their flowering response to spring temperatures (Figs. 2, 4). Including the random effects term (i.e., the species effect) in the model substantially improved model fit (AIC full model = 5584, AIC model without species = 6902). Regression of observed phenological advancement (days/°C) on the random effects term for each species (i.e., the change in *y* intercept relative to a common model) revealed several interesting patterns (Fig. 5). First, spring-blooming species have much less variability in their phenological responses to mean spring temperatures than do summer- or fall-blooming species. This suggests that flowering in spring-blooming species (i.e., those that bloom before early June) is strongly controlled by temperature. In contrast, the large variability in response of summer-, and fall-blooming species suggests that flowering in these species is controlled by a variety of different factors, including photoperiod and winter chilling (Körner and Basler, 2010). When we included herbarium data from nearby counties in this analysis (Supplementary Online Material, Fig. S4), the variability in response of later-blooming species was somewhat reduced and the relationship between the species-specific random effect size and advancement of flowering time was more pronounced. Nonetheless, the variability in response of later-blooming species still exceeded that of spring-blooming species by more than two-fold.

In either case, distinguishing more precisely between different phenological cues, at least at broad scales, might now be possible with the greatly expanded geographic and temporal sampling available from herbarium records. This approach can further guide more focused experiments to establish cues for different species, but even the correlative associations we have identified between climate and phenology are valuable. This is likely to be especially so for species whose cueing mechanisms are simpler and restricted primarily to a single variable. Such data are in great demand, yet are seldom available for a large diversity of species across a region. A recent review of flowering cues by Pau et al., (2011) underscores this demand. Their meta-analysis summarized 115 studies from field observational data. From these studies, they identified sufficient data for only 325 species. While several tropical and boreal species were included, the majority were from temperate regions, primarily in the United States and Western Europe (Pau et al., 2011). Furthermore, all of these studies were restricted to single sites, and thus failed to capture the potential geographic variation within species. These findings greatly emphasize the limited taxonomic and geographic scope of field observational data available for large-scale phenological research (Wolkovich, Cook, and Davies, 2014).

In contrast, herbarium data hold great promise for overcoming this impasse and improving assessments of how species will respond to future climate change. In particular, our results could be used in process-based models to distinguish the relative important of temperature, chilling, and photoperiod across a wide diversity of species (Richardson et al., 2006; Morisette et al., 2008; Migliavacca et al., 2012; Archetti et al., 2013; Siniscalco et al., 2014). Previous studies have been limited to a few dozen species with sufficient inter-annual sampling, typically derived from a small number of well-documented, long-term ecological study sites. The reliability of herbarium data, however, offers the promise of greatly expanding these studies to understand how species will respond to recent climatic change and the potential to untangle the relative importance of multiple cues (e.g., photoperiod, temperature) and how they vary across space. Finally, the ability to study a broader diversity of species could greatly expand our knowledge of deeper phylogenetic patterns involving phenological response mechanisms (Davies et al., 2013).

Future directions–Our results indicate that herbarium data represent a valuable resource for studying both temporal trends *and* mechanisms of phenological change. The next challenge is to scale-up our assessments of phenological responses and mechanisms to include the thousands of species on the landscape that are also represented in herbarium collections. These spatially and temporally explicit records of biodiversity are increasingly becoming available digitally as a result of investment in high-throughput digital imaging, GIS, and rigorous spatial analyses. The Harvard University Herbaria, along with several collaborating institutions, are presently enhancing the digital infrastructure for the flora of New England by capturing specimen-level metadata and images into digital form. Alongside this effort, co-authors Davis and Willis have created a crowdsourcing platform ("Curio") with Edith Law (University of Waterloo) to engage volunteer botanists in detecting flowers, buds, and fruits on herbarium records. We intend to use this platform to capture phenological data from the ~1 million digitized specimens from New England and use these data to understand how plants have responded, and will respond, to climatic change in this region. Future studies focused on species that differ in their flowering season and may respond differently to climatic change (e.g., *Vaccinium angustifolium* versus *Daucus carota*) but have large geographic ranges, are well represented in herbaria, and can be identified easily by amateur botanists, making them especially valuable for these efforts. Moreover, by taking advantage of crowdsourcing, we will be able to assess all of the relevant stages of plant phenology critical to climate change, including leaf-out, transitions from bud to flower, peak flowering time, and transition to fruiting.

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Table 1. Summary of study species. Scientific names, common names, native/introduced status, and growth habit from USDA PLANTS (http://plants.usda.gov/java/).

Species	Common Name	Family	Date range	No. of years of data	Native status
Daucus carota L.	Queen Anne's lace	Apiaceae	1853-2008	35	Introduced
Aralia nudicalis L.	wild sarsaparilla	Araliaceae	1858-2012	47	Native
Barbarea vulgaris W.T. Aiton	garden yellowrocket	Brassicaceae	1877-2005	51	Introduced
Gaultheria procumbens L.	eastern teaberry	Ericaceae	1877-2011	32	Native
Gaylussacia baccata (Wangenh.) K. Koch	black huckleberry	Ericaceae	1858-2011	36	Native
Vaccinium angustifolium Aiton	lowbush blueberry	Ericaceae	1878-2012	46	Native
Vicia cracca L.	bird vetch	Fabaceae	1877-2006	21	Introduced
Iris prismatica Pursh ex Ker Gawl.	slender blue iris	Iridaceae	1877-1934	24	Native
Arethusa bulbosa L.	dragon's mouth	Orchidaceae	1861-1980	30	Native
Calopogon tuberosus (L.) Britton, Sterns & Poggenb.	tuberous grasspink	Orchidaceae	1857-1984	19	Native
Corallorhiza maculata (Raf.) Raf.	summer coralroot	Orchidaceae	1854-1930	22	Native
Cypripedium acaule Aiton	moccasin flower	Orchidaceae	1861-2012	51	Native

Platanthera grandiflora (Bigelow) Lindl.	greater purple fringed orchid	Orchidaceae	1861-1960	25	Native
Platanthera lacera (Michx.) G. Don	green fringed orchid	Orchidaceae	1854-1949	39	Native
Platanthera psycodes (L.) Lindl.	lesser purple fringed orchid	Orchidaceae	1854-1958	21	Native
Pogonia ophioglossoides (L.) Ker Gawl.	snakemouth orchid	Orchidaceae	1852-1962	44	Native
Chelidonium majus L.	celandine	Papaveraceae	1877-2011	21	Introduced
Aquilegia canadensis L.	red columbine	Ranunculaceae	1882-2012	39	Native
Ranunculus acris L.	tall buttercup	Ranunculaceae	1858-2011	34	Native/Introduced

Figure legends

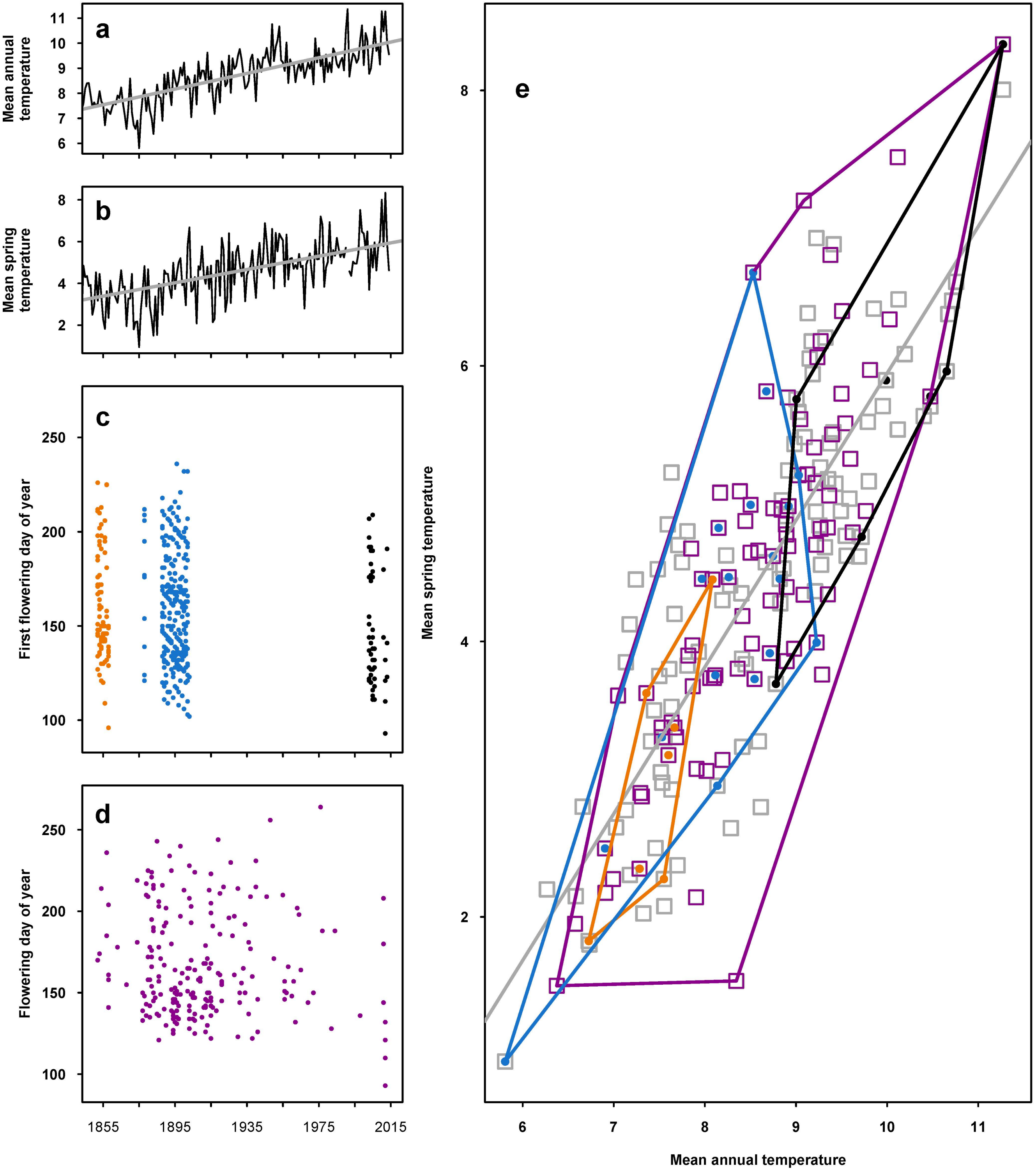
Fig. 1. Climatic and phenological data. **(A)** Mean annual temperatures (°C) and **(B)** mean monthly temperatures recorded at Great Blue Hill, Massachusetts (1885-present) and reconstructed by Miller-Rushing and Primack (1852-1884). **(C)** Observed first flowering dates in Concord, MA and **(D)** earliest flowering dates on herbarium sheets from Middlesex County of the 20 species listed in Table 1. **(E)** Coverage of the climatic space (1852-2013; all boxes) by herbarium data (magenta boxes and magenta convex hull), Thoreau's observations (orange dots and orange convex hull), Hosmer's observations (blue dots and blue convex hull), and contemporary observations (black dots and black convex hull). Unsampled points in the climate space are represented by grey boxes without colored dots. Convex hulls encompass the outer boundaries of the climate space defined by the most extreme observations; they were fit using the "chull" function in R (base graphics). The grey line is the best-fit regression line relating mean spring temperature to mean annual temperature.

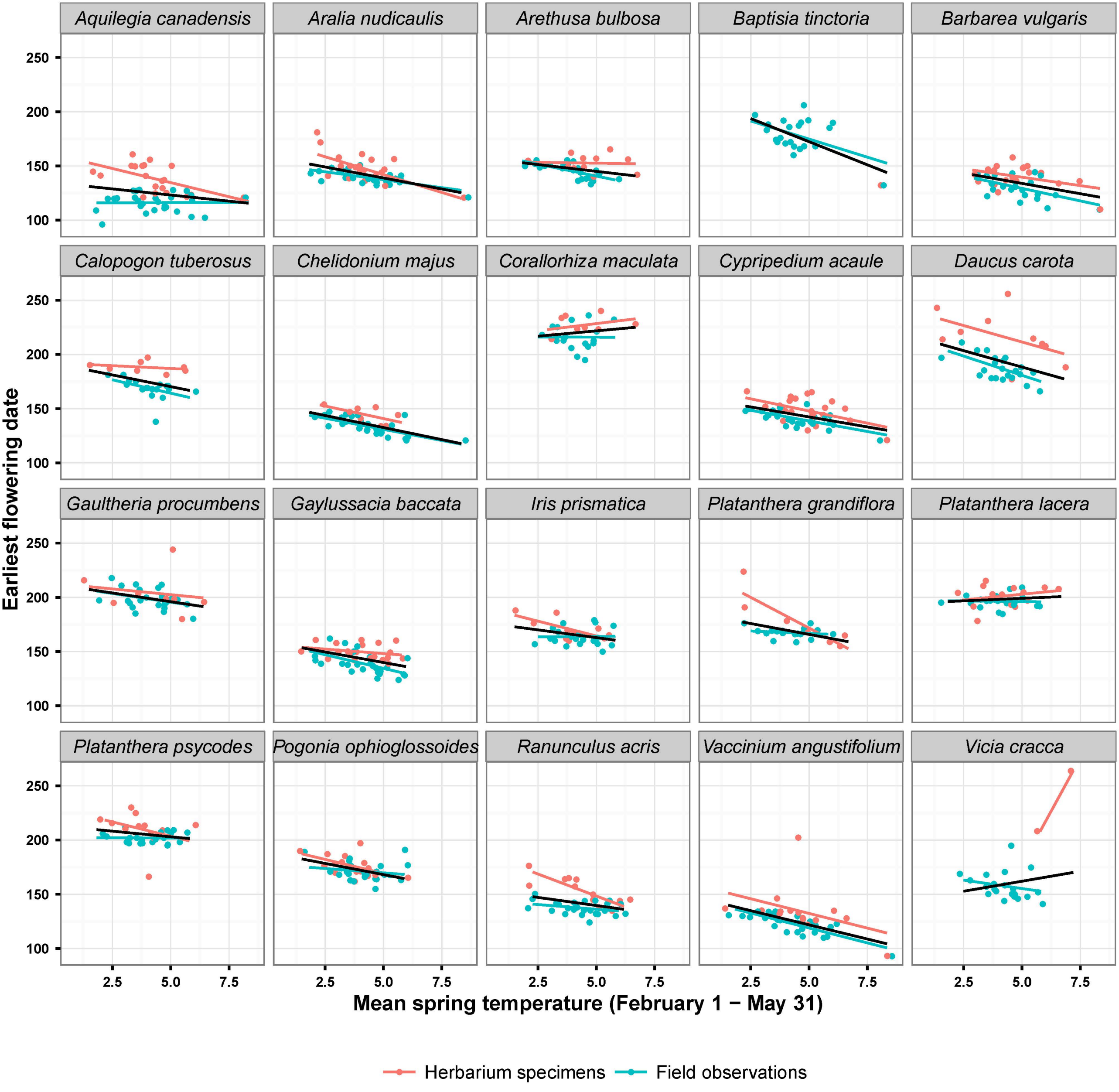
Fig. 2. Relationship between mean spring temperature and earliest flowering date from field observations in Concord, MA or recorded on herbarium specimens from Middlesex County, MA. The blue points and lines are data and associated linear regressions for field observations; the red points and lines are data and associated linear regressions for herbarium specimens (fit using linear mixed effect models); and the black lines are common species-specific robust linear regressions.

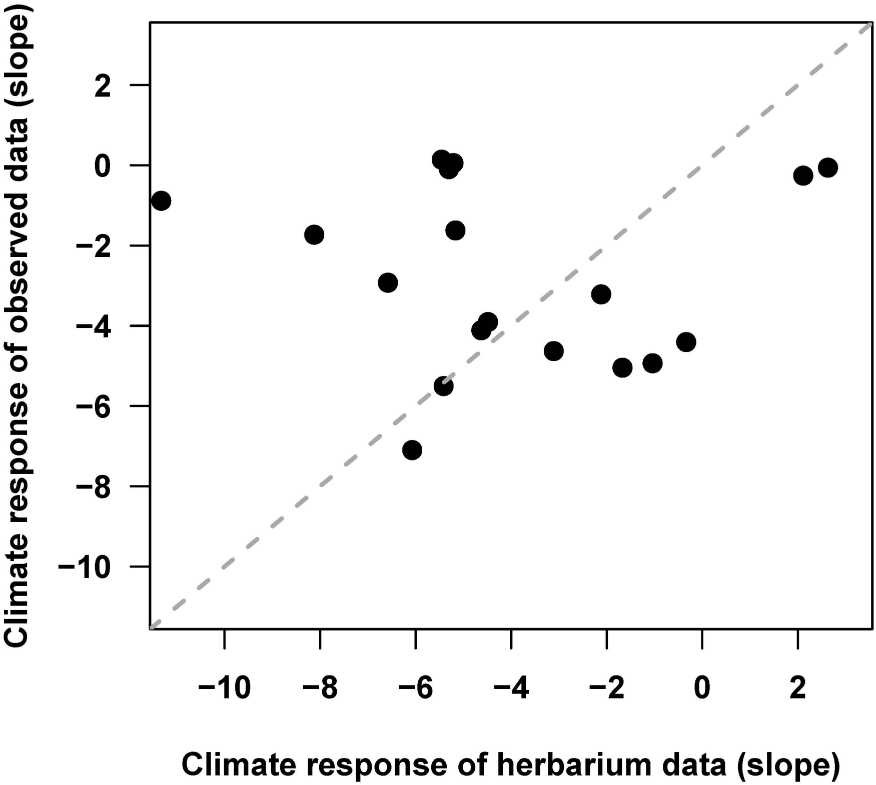
Fig. 3. Relationship between phenological responses to climate estimated from herbarium specimens and observed in the field. The values on the *x*-axis are the slopes estimated for herbarium specimens (red lines in Fig. 2) and the values on the *y*-axis are the slopes estimated for observational data (blue lines in Fig. 2). The dashed grey line is a 1:1 reference line.

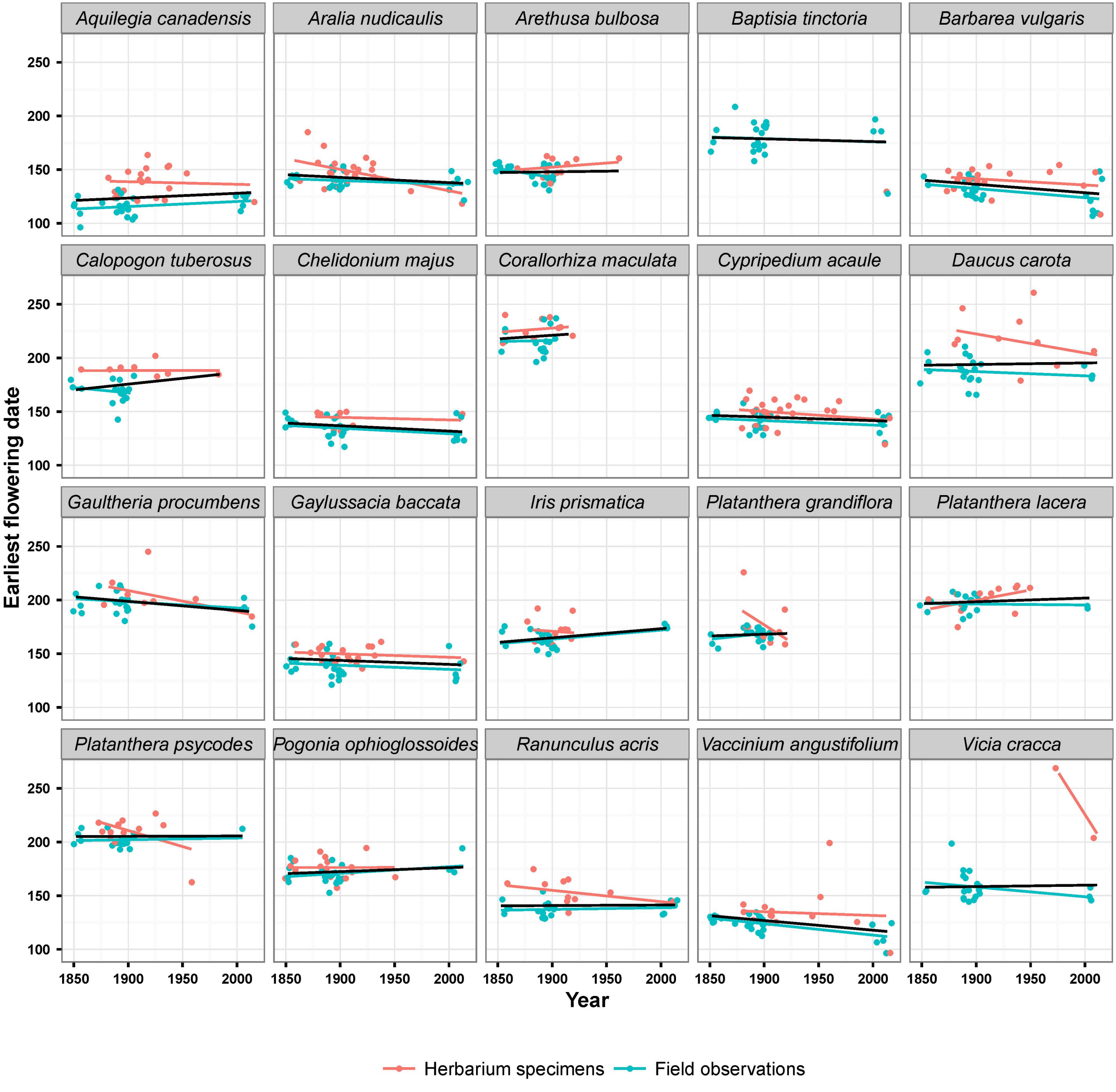
Fig. 4. Relationship between calendar year and earliest flowering date observed in the field or recorded on herbarium specimens from Middlesex County. The blue points and lines are data and associated linear regressions for field observations; the red points and lines are data and associated linear regressions for herbarium specimens; and the black lines are common species-specific robust linear regressions.

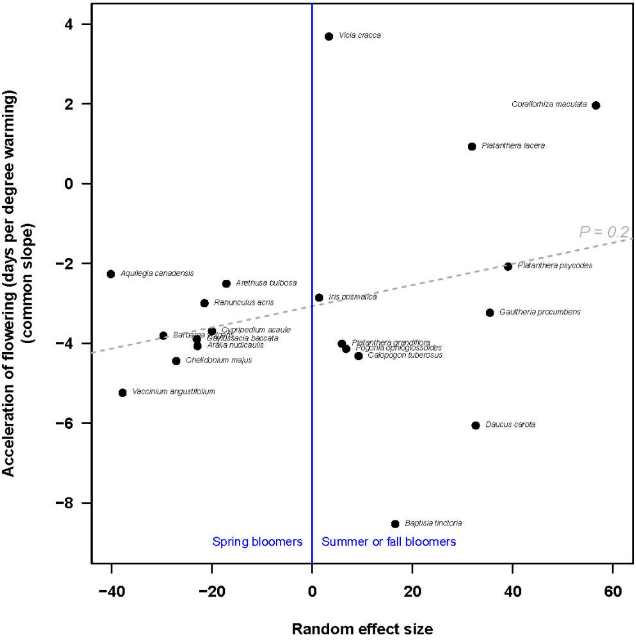
Fig. 5. Relationship between species-specific random effect size and the acceleration of flowering (i.e., the values of the common slopes fit in Fig. 2).


a
Mean annual temperature
11
10
9
8
7
6
b
Mean spring temperature
8
6
4
2
c
First flowering day of year
250
200
150
100
d
Flowering day of year
250
200
150
100
1855
1895
1935
1975
2015
e
Mean spring temperature
8
6
4
2
6
7
8
9
10
11
Mean annual temperature


Aquilegia canadensis
Aralia nudicaulis
Arethusa bulbosa
Baptisia tinctoria
Barbarea vulgaris
Calopogon tuberosus
Chelidonium majus
Corallorhiza maculata
Cypripedium acaule
Daucus carota
Gaultheria procumbens
Gaylussacia baccata
Iris prismatica
Platanthera grandiflora
Platanthera lacera
Platanthera psycodes
Pogonia ophioglossoides
Ranunculus acris
Vaccinium angustifolium
Vicia cracca
Earliest flowering date
250
200
150
100
2.5
5.0
7.5
Mean spring temperature (February 1 − May 31)
Herbarium specimens
Field observations


Climate response of observed data (slope)
2
0
−2
−4
−6
−8
−10
−10
−8
−6
−4
−2
0
2
Climate response of herbarium data (slope)


Aquilegia canadensis
Aralia nudicaulis
Arethusa bulbosa
Baptisia tinctoria
Barbarea vulgaris
Calopogon tuberosus
Chelidonium majus
Corallorhiza maculata
Cypripedium acaule
Daucus carota
Gaultheria procumbens
Gaylussacia baccata
Iris prismatica
Platanthera grandiflora
Platanthera lacera
Platanthera psycodes
Pogonia ophioglossoides
Ranunculus acris
Vaccinium angustifolium
Vicia cracca
Earliest flowering date
250
200
150
100
Year
1850
1900
1950
2000
Herbarium specimens
Field observations


Acceleration of flowering (days per degree warming)
(common slope)
Random effect size
4
2
0
−2
−4
−6
−8
−40
−20
0
20
40
60
P = 0.2
Vicia cracca
Corallorhiza maculata
Platanthera lacera
Platanthera psycodes
Gaultheria procumbens
Aquilegia canadensis
Arethusa bulbosa
Ranunculus acris
Iris prismatica
Cypripedium acaule
Barbarea vulgaris
Gaylussacia baccata
Aralia nudicaulis
Platanthera grandiflora
Pogonia ophioglossoides
Calopogon tuberosus
Chelidonium majus
Vaccinium angustifolium
Daucus carota
Baptisia tinctoria
Spring bloomers
Summer or fall bloomers

Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms

Charles C. Davis, Charles G. Willis, Bryan Connolly, Courtland Kelly, and Aaron M. Ellison

Supplementary Online Information

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1. Geographic distribution of herbarium data

Most of the 1103 herbarium specimens of our 20 target species from Massachusetts (1852-2012) were collected from Worcester County (317) and Middlesex County (297) (Fig. S1). Among the 14 Massachusetts counties, only three other counties, Bristol, Essex, and Norfolk, had > 100 herbarium specimens.

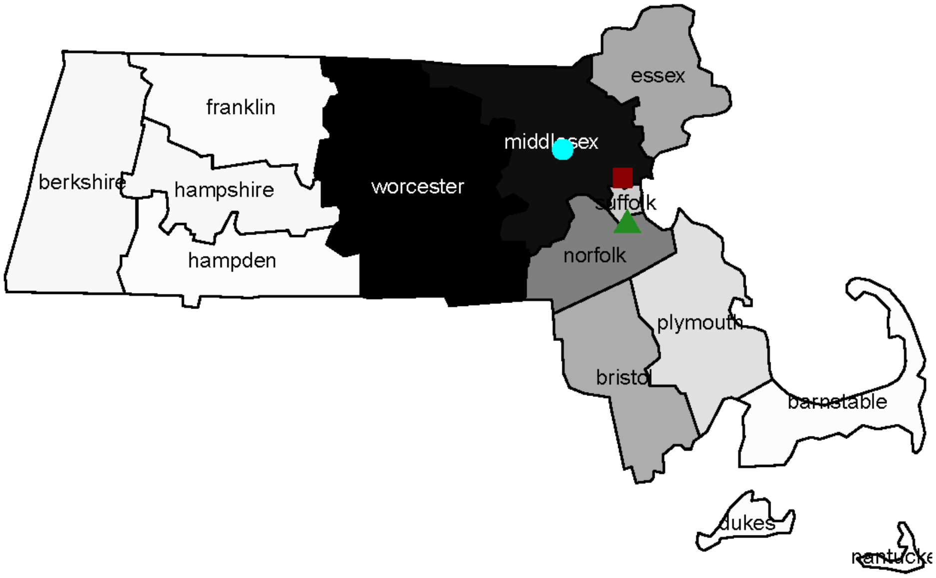

Figure S1. Map of the number of herbarium specimens (black = 317, white = 1) in the 14 Massachusetts counties. Also shown are the locations of Concord (light blue circle), the Great Blue Hill meterological station (green triangle), and the Harvard University Herbaria (red square).

2. Climatic effects on phenology from the five counties

In the main text, we present analyses of the effects of mean spring temperatures on plants observed in Concord and collected from Concord and other towns in Middlesex County. Inclusion of data from the other four counties (Worcester, Norfolk, Bristol, and Essex) for which we have reasonable sample sizes yielded similar patterns (Fig. S2) to what we observed for Middlesex County alone (main text, Fig. 2).

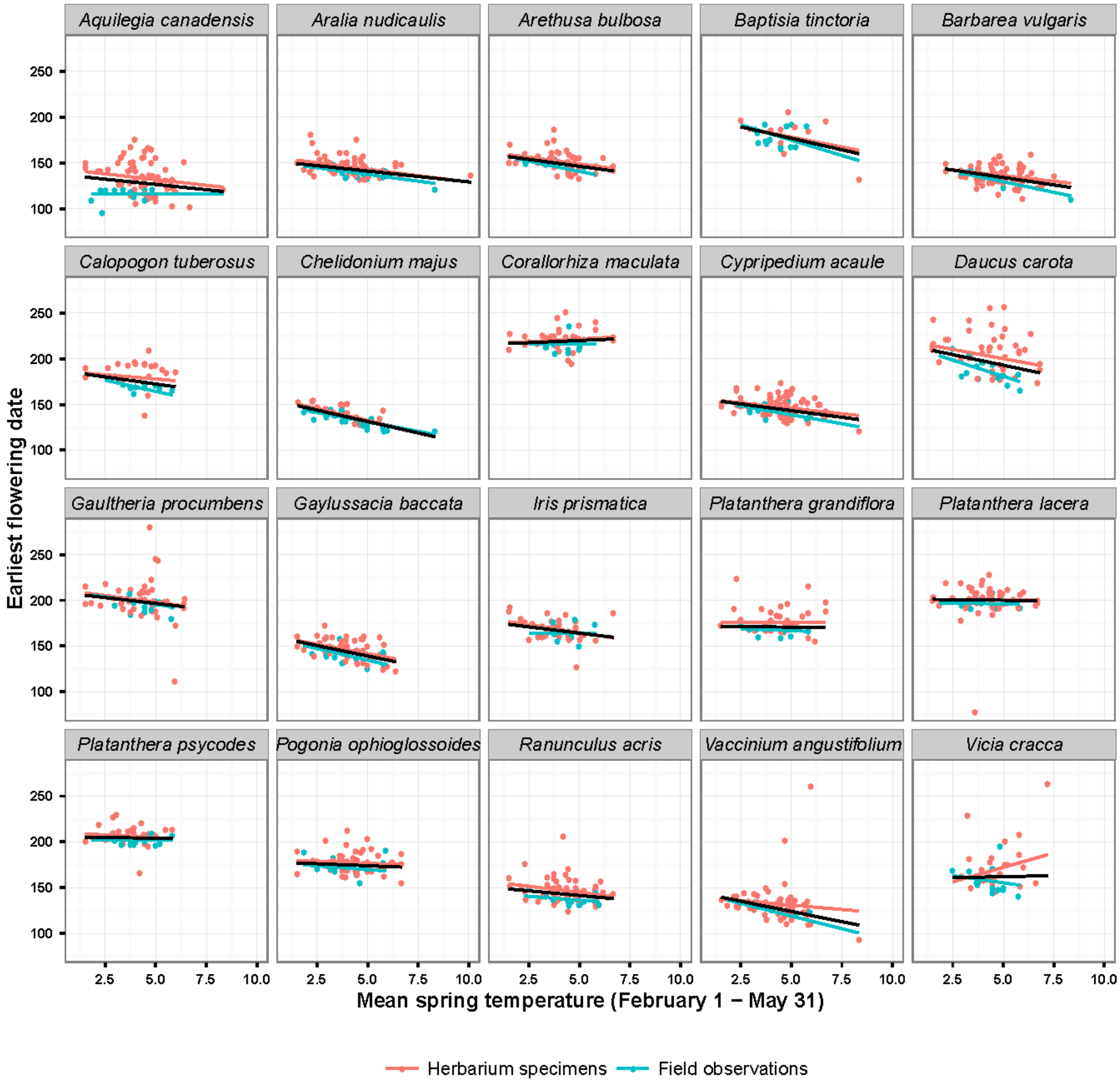

Figure S2. Relationship between mean spring temperature and earliest flowering date observed in the field or recorded on herbarium specimens. The blue points and lines are data and associated linear regressions for field observations; the red points and lines are data and associated linear regressions for herbarium specimens; and the black lines are common species-specific robust linear regressions.

As with the Middlesex County data alone, the main effects (mean spring temperature) and datum type (observed or herbarium) were significant, but their interaction was not (Table S1). Inclusion of the species-specific random effect also improved the fit of the model (AIC full model = 12,761; AIC of reduced model = 15,248).

Table S1. ANOVA table for effects of fixed variables (mean spring temperature) and datum type (herbarium or field observation) and their interaction on earliest flowering date (as day of year) for five Massachusetts counties. The model specification in R was:

```
library(nlme)
model.temps <- lme(Date ~spring.mean.temp*DatumType, random= –1|Species)
```

	Estimate	df	F	*P*
Intercept	177.2	1, 1560	712.3	< 0.001
Mean spring temperature	–2.2	1, 1560	78.0	< 0.001
Datum type	–2.8	1, 1560	101.7	< 0.001
Temp × Datum type	–1.6	1, 1560	2.3	0.09

3. Climatic effects are not the same as annual changes

As we pointed out in the main text, concluding that plants flower earlier following warmer springs or in warmer years is not the same as saying that these plants flower earlier now than they did 100 years ago. This can be observed when we regress earliest flowering date on calendar year for either Middlesex County data alone (main text Fig. 4), or for data from all five counties with adequate herbarium specimens (Fig. S3; Table S2).

Table S2 - ANOVA table for effects of fixed variables (year) and datum type (herbarium or field observation) and their interaction on earliest flowering date (as day of year) of plants observed in Concord and collected from Middlesex and the four adjacent counties (Figure S4). The model specification in R was:

```
library(nlme)
model.temps <- lme(Date ~Year*DatumType, random= –1|Species)
```

	Estimate	df	F	*P*
Intercept	146.2	1, 1560	706.5	< 0.001
Year	0.01	1, 1560	0.004	0.95
Datum type	68.9	1, 1560	97.4	< 0.001
Year × Datum type	–0.04	1, 1560	5.23	0.02

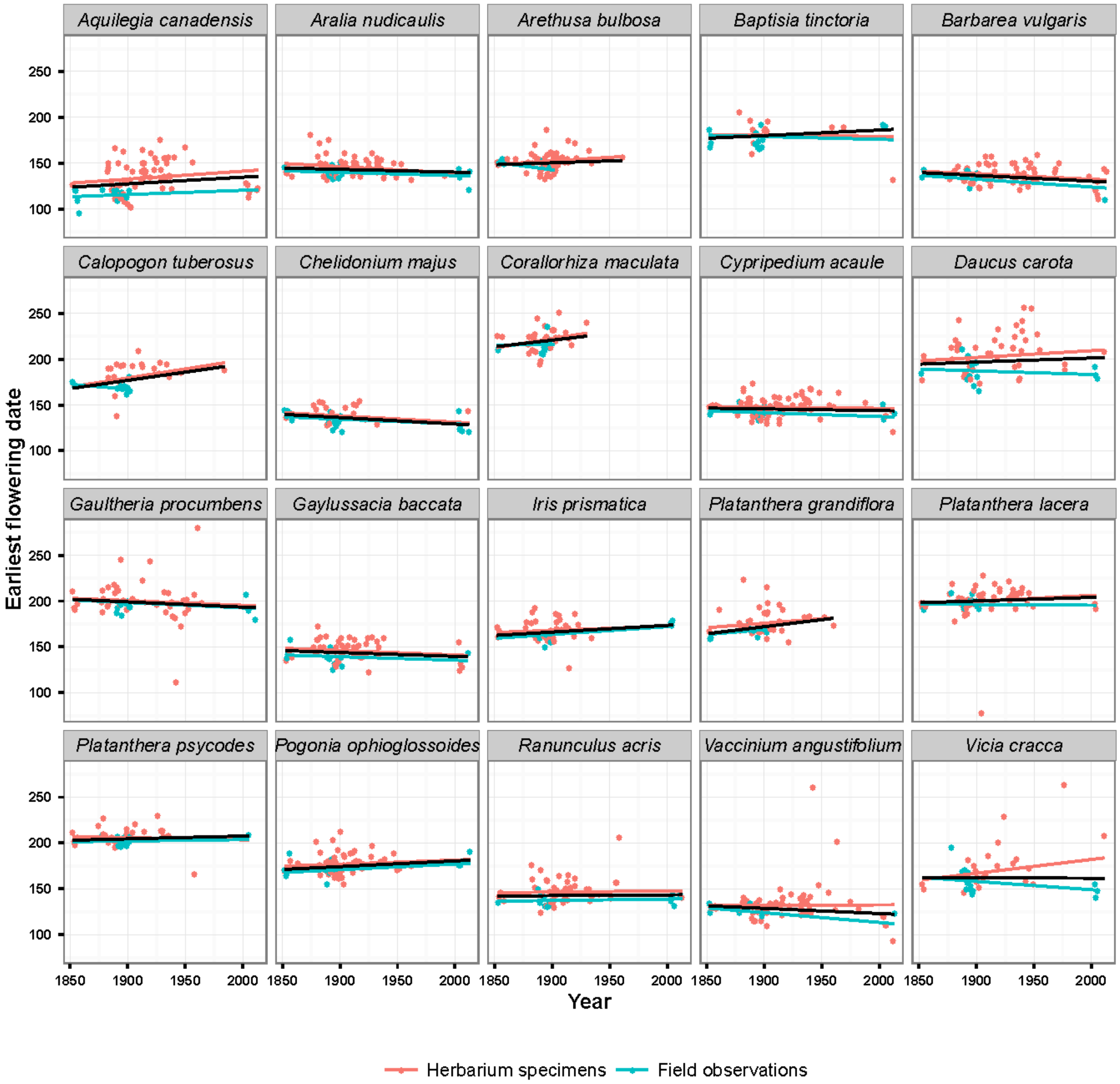

Figure S3. Relationship between calendar year and earliest flowering date observed in the field or recorded on herbarium specimens from Middlesex and the four adjacent counties. The blue points and lines are data and associated ordinary linear regressions for field observations; the red points and lines are data and associated ordinary linear regressions for herbarium specimens; and the black lines are common species-specific robust linear regressions.

4. Using herbarium data to identify physiological mechanisms for cueing phenological events

Including the herbarium data from all five counties in analyzing the species-specific relationship of phenological advancement (days/°C) substantially improved the fit of the model (main text Fig. 5). In particular, we observed less variability in estimates of phenological advance of late flowering species (compare main text Fig. 5 with Fig. S4), and the overall relationship between the species-specific random effect size and advancement of flowering was statistically significant ($P = 0.03$). Nonetheless, the amount of variation explained by this regression remained small ($r^2 = 0.23$ for the regression using data from all five counties; $r^2 = 0.07$ for the regression using data only from Middlesex County).

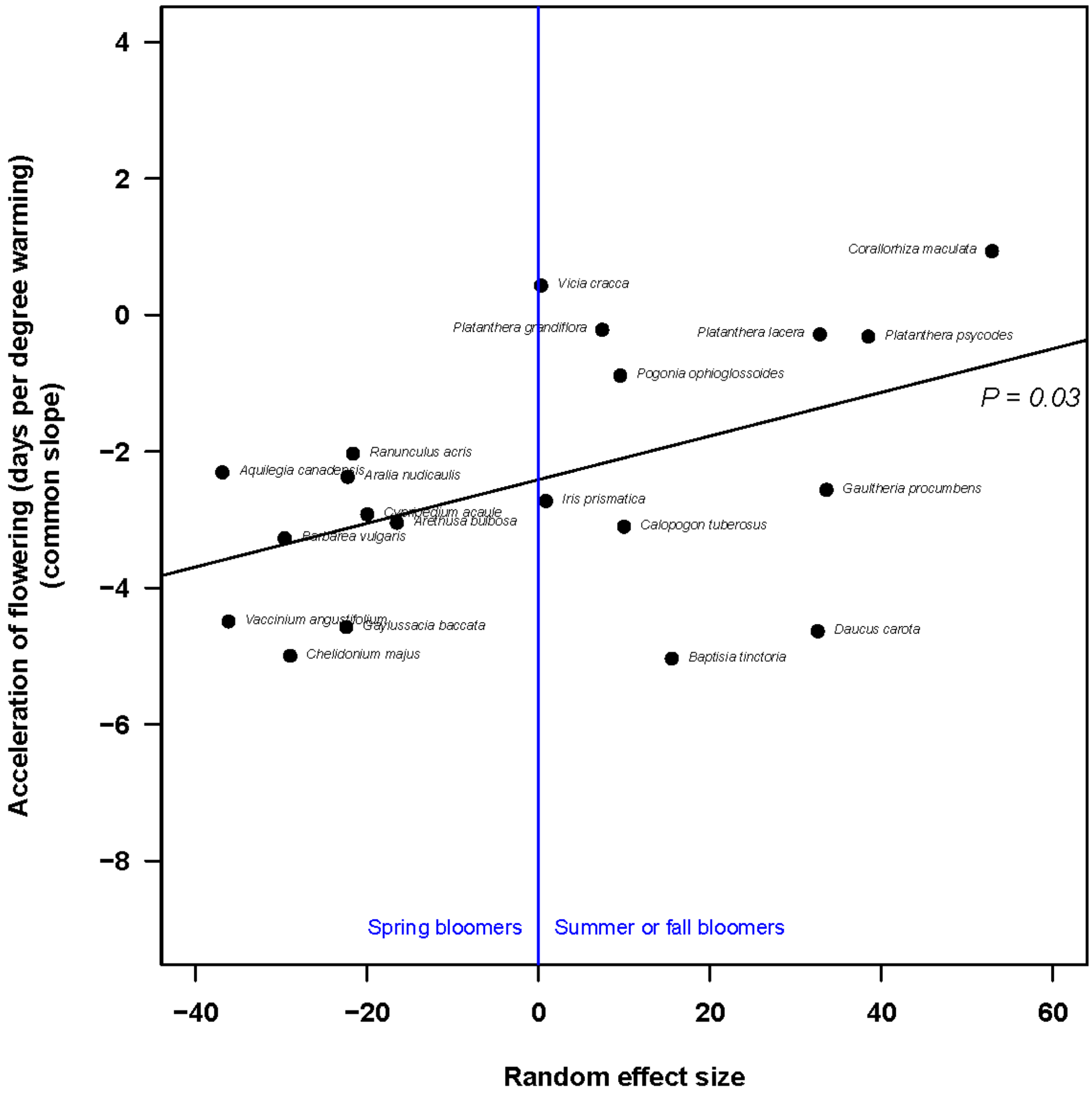

Figure S4. Relationship between species-specific random effect size and the advancement of flowering (common slopes fit in Fig. S2).