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Shifts in flowering phenology in response to spring temperatures in eastern Tennessee

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Abstract

Premise: Plant phenological shifts are among the clearest indicators of the effects of climate change. In North America, numerous studies in the northeastern United States have demonstrated earlier spring flowering compared to historical records. However, few studies have examined phenological shifts in the southeastern United States, a highly biodiverse region of North America characterized by dramatic variations in abiotic conditions over small geographic areas.

Methods: We examined 1000+ digitized herbarium records along with location-specific temperature data to analyze phenological shifts of 14 spring-flowering species in two adjacent ecoregions in eastern Tennessee.

Results: Spring-flowering plant communities in the Blue Ridge and the Ridge and Valley ecoregions differed in their sensitivity to temperature; plants in the Ridge and Valley flower 0.73 days earlier/°C on average compared to 1.09 days/°C for plants in the Blue Ridge. Additionally, for the majority of species in both ecoregions, flowering is sensitive to spring temperature; i.e., in warmer years, most species flowered earlier. Despite this sensitivity, we did not find support for community-level shifts in flowering within eastern Tennessee in recent decades, likely because increases in annual temperature in the southeast are driven primarily by warming summer (rather than spring) temperatures.

Conclusions: These results highlight the importance of including ecoregion as a predictor in phenological models for capturing variation in sensitivity among populations and suggest that even small shifts in temperature can have dramatic effects on phenology in response to climate in the southeastern United States.

KEYWORDS

climate change, herbarium, phenological sensitivity, phenology, southeastern United States, spring

Shifts in phenology in response to warming temperatures are among the best-demonstrated effects of recent climate change (Parmesan, 2006; Davis et al., 2015; Jones and Daehler, 2018). Because the timing of developmental events in spring-flowering plant species is highly sensitive to environmental conditions, these species are excellent indicators of climate change (Polgar and Primack, 2011) and have been the focus of several phenological studies (reviewed by Willis et al., 2017). In the understory of temperate forests, spring-flowering species are adapted to a seasonal climate that includes cyclical fluctuations in temperature and light availability after canopy closure. As a consequence, phenological shifts in these species have the potential to affect plant fitness by altering carbon gain (Heberling et al., 2019), synchrony with pollinators

(Forrest, 2015; Kudo and Cooper, 2019), and the length of the growing season (Meineke et al., 2021).

Long-term observational data sets are considered the "gold standard" of phenological data (Davis et al., 2015) and are valuable resources for studying phenological trends. Studies utilizing such data sets have revealed patterns in phenological events that are easy to measure, such as earlier dates for first flowering (Piao et al., 2019) and spring bud break and leaf out of woody plants (Panchen et al., 2014) in response to warming temperatures. However, such studies require long-term monitoring efforts and are often limited in taxonomic scope (Wolkovich et al., 2014; Park et al., 2019). Herbarium collections have become increasingly popular resources for phenological research over the past decade because they contain location-specific historical data that

allow for the exploration of long-term trends predicted with climate change (Jones and Daehler, 2018). Herbarium specimens provide a snapshot of a plant at a given date and place and hold a wealth of information including plant morphology, reproductive status, herbivory, and other traits that cannot be captured with observational data alone. Further, flowering dates estimated from herbarium records reflect field observations, substantially increase sampling range, and alleviate sampling bias in climatic space when comparing historic and contemporary observational data across climatic conditions (Davis et al., 2015).

Much of the phenological research in North America using herbarium specimens has taken place in temperate environments above 38° latitude, primarily in the northeastern United States (Primack et al., 2004; Willis et al., 2010; Calinger et al., 2013; Bertin, 2015; Davis et al., 2015; Gallinat et al., 2018). These studies demonstrate significant advances in flowering phenology over the past century in response to rising temperatures, with the strongest shifts typically in spring-flowering species. In contrast, long-term phenological trends in the southeastern United States, a North American biodiversity hotspot, remain poorly understood. One study in West Virginia showed that two spring ephemerals, *Erythronium americanum* Ker-Gawl and *Dentaria laciniata* Muhl., have advanced their flowering dates by 0.91 days per decade on average over the past century (Petrauski et al., 2019). However, the generality of these results is limited because only two species were included in the study. Another analysis of over 19,000 records of spring-, summer-, and autumn-flowering species in South Carolina revealed that the earliest-flowering species are the most sensitive to increasing March temperatures, but that there have been no long-term advances in spring flowering nor spring temperature over the past century (Park and Schwartz, 2015). Another study found that spring-flowering species in the Asteraceae family in the southeastern coastal plain flowered 1.8–2.3 days earlier per 1°C increase in spring temperature, a level of sensitivity comparable to those estimated for northern temperate species (Pearson, 2019). Thus, more work is clearly needed to understand the importance of spring temperature in driving flowering phenology in plant communities of the southeastern United States.

One factor that likely inflates variations in phenological response within plant species and communities, and that to date has been overlooked in plant phenological studies, is ecoregional variation. Such inflation can occur if collection data are pooled over broad geographical areas that contain high ecoregional diversity such as the southeastern United States. An ecoregion is defined as an area of relative homogeneity in abiotic and biotic factors, including soils, vegetation, climate, geology, and physiography (Griffith et al., 1997). Ecoregions are defined by a hierarchical system that divides North America into increasingly narrower regions based on these shared features. If ecoregions are not accounted for in statistical analyses, phenological studies could miss key information on how phenological sensitivity varies within and among plant communities and individual species across relatively small geographic areas because climatic change is not geographically uniform.

The easternmost portion of Tennessee contains two ecoregions, the Blue Ridge and the Ridge and Valley, that have different geological formations and different climates. The Ridge and Valley (elevation 152–1311 m a.s.l., annual rainfall 1350 mm, average winter and summer temperatures 2° and 25°C, respectively) is characterized by a series of parallel, even-crested ridges and valleys of primarily limestone and a mesothermal climate with short, mild winters and long, hot summers (Hart et al., 2008). In contrast, the Blue Ridge (elevation 600–1600 m a.s.l., annual rainfall 1600 mm, average winter and summer temperatures 0.56° and 22°C, respectively) is characterized by steep slopes and narrow valleys (Surasinghe and Baldwin, 2014). The Blue Ridge ecoregion contains the Great Smoky Mountains, the most biodiverse National Park in the country.

In this study, we focused on these two distinct ecoregions to address the following questions: (1) Are spring-flowering species in eastern Tennessee presently flowering earlier than they did in the last half of the 20th century? (2) How phenologically sensitive are spring-flowering species and communities to spring temperature in eastern Tennessee? (3) To what extent does ecoregion explain variation in community- and species-level responses to spring temperature?

MATERIALS AND METHODS

Study region and focal species

Because herbarium specimen locality information is organized by county, we selected 16 counties in eastern Tennessee from which to source herbarium specimens within the Blue Ridge and the Ridge and Valley ecoregions (Figure 1). Counties within each ecoregion were selected to maintain an approximately equal sample area per ecoregion. Because four counties are nearly equally split between the Blue Ridge and the Ridge and Valley ecoregions (i.e., Blount, Monroe, Sevier and Cocke; see Figure 1), we obtained specific locality descriptions (e.g., mountain peaks, trail heads, cities) for specimens from those counties when available and then used GeoLocate (www.geo-locate.org) to assign them to an ecoregion. If a specimen from one of the four counties did not have specific locality information, we removed it from the data set.

We focused on spring-flowering plant species because they are known to be particularly sensitive to spring temperature when compared to later-flowering species (Fitter and Fitter, 2002; Park and Schwartz, 2015) and are excellent indicators of climate change (Polgar and Primack, 2011). To select focal species for inclusion in our study, we first performed a preliminary search of the Southeast Regional Network of Expertise and Collections (SERNEC) data portal to acquire locality information for a
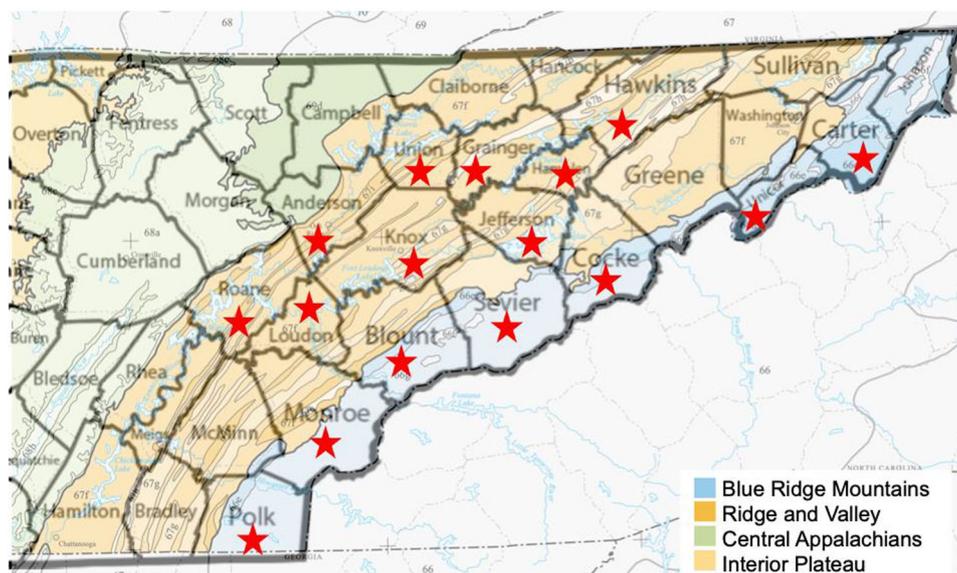
FIGURE 1 Map of ecoregions within eastern Tennessee counties. Red stars indicate counties included in this study. Ecoregion map adapted from U.S. Geological Survey Level IV TN Ecoregions Map (https://store.usgs.gov/assets/MOD/StoreFiles/Ecoregion/21632_tn_front.pdf).

TABLE 1 Number of specimens in each ecoregion and observation time span of focal species. RV = number of specimens in Ridge and Valley ecoregion, BR = number of specimens in Blue Ridge ecoregion.

Code	Species	Common name	Family	Date range	BR	RV
DENDIP	*Dentaria diphylla* Michx.	crinkleroot	Brassicaceae	1923–2014	44	40
DENLAC	*Dentaria laciniata* Muhl. Ex Willd.	cutleaf toothwort	Brassicaceae	1933–2019	26	47
ERYAME	*Erythronium americanum* Ker-Gawl.	dogtooth violet	Liliaceae	1925–2010	19	39
GERMAC	*Geranium maculatum* L.	wild geranium	Geraniaceae	1934–2012	48	38
HEPACU	*Hepatica acutiloba* DC.	sharp lobe hepatica	Ranunculaceae	1934–2019	36	13
MAIRAC	*Maianthemum racemosum* (L.) Link.	false Solomon's seal	Asparagaceae	1931–2019	34	42
PODPEL	*Podophyllum peltatum* L.	mayapple	Berberidaceae	1925–2015	32	36
POLBIF	*Polygonatum biflorum* (Walt.) Ell.	true Solomon's seal	Asparagaceae	1925–2012	40	36
SANCAN	*Sanguinaria canadensis* L.	bloodroot	Papaveraceae	1919–2019	41	30
THATHA	*Thalictrum thalictroides* (L.) Eames & B. Boivin	rue anemone	Ranunculaceae	1925–2003	41	37
TIACOR	*Tiarella cordifolia* L.	Allegheny foamflower	Saxifragaceae	1925–2016	79	54
TRILUT	*Trillium luteum* (Muhl.) Harbison	yellow trillium	Melanthiaceae	1914–2015	40	40
UVUGRA	*Uvularia grandiflora* Sm.	largeflower bellwort	Colchicaceae	1925–2019	9	28
VIOSOR	*Viola sororia* Willd.	common blue violet	Violaceae	1928–2017	79	40

list of over 20 spring-flowering species collected in our study region. SERNEC is a consortium of over 233 southeastern herbaria that offers an extensive digitized collection of specimens spanning >200 years of observations for public download online (www.sernecportal.org). To be included in our analysis, each species had to meet the following criteria: (1) There were at least 50 unique observations backed by vouchered herbarium specimens available with county-level locality information. (2) Date information included the year, month, and day of collection. (3) Reproductive structures were easily identifiable. (4) There were at least 15 observations per species in each ecoregion. These criteria resulted in a group of 14 species that span 11 plant families and flower across the spring growing season (Table 1). After compiling our list of focal species, we obtained phenological data from digitized herbarium

specimens from (1) SERNEC, (2) the University of Tennessee Herbarium (TENN), and (3) the Great Smoky Mountains National Park Collections Preservation Center (GSMNP).

Although studies including elevation as a covariate in phenology models often use the mean elevation of a county (e.g., Park et al., 2019), this metric is not helpful for our study because elevations within counties split between the Blue Ridge and the Ridge and Valley can differ by >1500 m (e.g., in Sevier County, city of Sevierville ≈ 275 m, Clingman's Dome ≈ 2025 m). Additionally, the majority of specimens in this study (especially ones from the earlier half of the 19th century) lack locality information specific enough to determine a latitude and longitude needed to derive elevation data. Thus, we did not include elevation as a covariate.

After removing duplicate, mislabeled, nonreproductive, or damaged specimens that we could not score, the final data set from SERNEC comprised 1249 specimens. To increase our sample size, we also accessed historic specimens from the Great Smoky Mountains National Park that were deposited at the TENN herbarium and digitized for this project as well as specimens from the GSMNP. These added 186 and 48 additional data points to our study, respectively. Before incorporating climate data, the data set contained 1483 unique observations spanning 141 years.

Phenological data collection

The majority of phenological studies using digitized herbarium specimens apply one of two approaches to categorizing reproductive phenology: (1) a binary approach, where a score of 1 or 0 indicates either the presence or absence of flowers on a specimen (e.g., Bertin, 2015; Park and Schwartz, 2015) or (2) a "relative" approach, where a specimen is considered flowering if a given proportion of flowers are open (typically 50% but up to 75%; e.g., Primack et al., 2004; Davis et al., 2015; Park et al., 2019). While these approaches are sufficient for capturing general phenological trends across broad spatial scales, finer-scale scoring methods that assign a phenophase based on the relative number of buds, flowers, and fruits present on a specimen enable more precise estimates of phenological trends (Pearson, 2019). Thus, we defined five "scores" that categorized specimens into phenophases based on the relative proportion of reproductive structures present on a given specimen: 0: no reproductive structures present; 1: early flowering (<50% of reproductive structures are open flowers, fruits absent); 2: peak flowering (>50% of reproductive structures are open flowers, fruits absent or present); 3: late flowering (<50% of reproductive structures are open flowers, fruits present); 4: fruiting (only fruits present).

To assign phenophases to digitized specimen images, we trained 10 observers (undergraduate students at the TENN Herbarium) to recognize the reproductive structures of the 14 individual species included in our study. Observers determined the phenophase of individual specimens by counting the total number of reproductive structures (buds, flowers, and fruits) present on a specimen, then assigning a score from 0 to 4 based on the relative number of each reproductive structure. To ensure consistency in scoring among observers, we created reference sheets that included photographs of reproductive structures from digitized herbarium specimens for each species (Appendix S1) and assessed observers on their data collection until consistency and accuracy were achieved. Willis et al. (2017) used crowdsourcing (Amazon's Mechanical Turk service) to hire anonymous workers with no previous botanical experience to score the phenological stage of herbarium specimens. Their study showed that, with proper training, non-experts produce the same data quality as expert botanists. Thus, we are confident that the phenological scores assigned by our trained observers reflect the true phenophases of the specimens.

Climate data collection

While spring flowering phenology in temperate regions can be influenced by several abiotic factors, including spring temperature (Primack et al., 2004; Miller-Rushing and Primack, 2008), snowmelt (Inouye, 2008), and precipitation (Matthews and Mazer, 2016), short-term records of flowering phenology in the southeastern United States imply that flowering phenology in this region is more closely related to temperature than to precipitation (Funderburk and Skeen, 1976; Abu-Asab et al., 2001). Thus, we chose to use spring temperature in the 2 months preceding flowering as the primary environmental predictor of spring phenology (see Data analyses section in Materials and Methods for a detailed description of how spring temperature was calculated).

Mean monthly temperatures for each county across the 141-year range of the data set were obtained from NOAA's Global Historical Climatology Network (https://ncdc.noaa.gov/ghcnm/). To calculate mean spring temperature within ecoregions in counties split between the Ridge and Valley and Blue Ridge, we obtained data from separate county-level weather stations located within each ecoregion, then assigned the appropriate annual mean spring temperature to individual specimens according to their ecoregion. Because data were not available for all years in all counties, specimens that were collected in a year for which there were no weather data available were removed from the data set. In total, the final data set contained 1088 unique observations.

Data analyses

To analyze changes in flowering and fruiting phenophases over time, we regressed the year of observation against the day of the year that it was observed for each individual

phenophase. We then used Welch's *t*-test, which is robust to unequal variances and sample sizes (Ruxton, 2006), to determine whether there was a difference between historical (pre-1970) and recent (1970 and later) flowering or fruiting dates for each species. The year 1970 was chosen as the dividing year for historical and recent observations because climate data suggest that global surface temperatures began to steadily increase around 1970 (Pachauri et al., 2014). In addition, this year has been used as the cut-off between historical and recent phenophase observations in other herbarium-based phenological studies (e.g., Abu-Asab et al., 2001; Bertin, 2015; Petrauski et al., 2019). Thus, using 1970 to divide historical and recent observations allows us to compare a time frame with a cooler climate and low interannual variation to a more recent time frame where average temperatures were increasing and higher on average (Bertin, 2015).

To characterize phenological sensitivity to spring temperature, we regressed the average temperature of the 2 months before the average month of flowering against the day of the year of a given phenophase for each species. For example, for an April-flowering specimen collected in the Blue Ridge ecoregion, we associated the average monthly temperature of February and March in the Blue Ridge during the year of collection with that particular specimen (see Calinger et al., 2013 for an example of a similar analysis). The temperature-averaging period was determined by calculating Pearson's correlation coefficients for the relationship between the day of the year of flowering (phenophases 1–3) and the average monthly temperature for each month preceding flowering in a given year and ecoregion. This analysis showed that significant correlations occurred for 1, 2, or both 1 and 2 months preceding flowering for most species (Appendix S2). Ideally, to obtain species-specific estimates of phenological sensitivity, we would allow the random slope of each species to vary in response to spring temperature. However, we did not have enough statistical power to do so. Instead, we ran separate models to obtain slope estimates for the linear relationships between spring temperature and the day of year of a phenophase for each individual species in each ecoregion.

To analyze phenological sensitivity at the community level, we applied the same analysis described above to all species binned together with ecoregion as a fixed effect and the year of observation and species as random effects for each phenophase. The late flowering phenophase (stage 3) was excluded due to limited sample size.

RESULTS

Shifts in phenology over time

Focal species showed varied phenological patterns and sensitivity to climate both at the community level and within species between the Ridge and the Valley and Blue
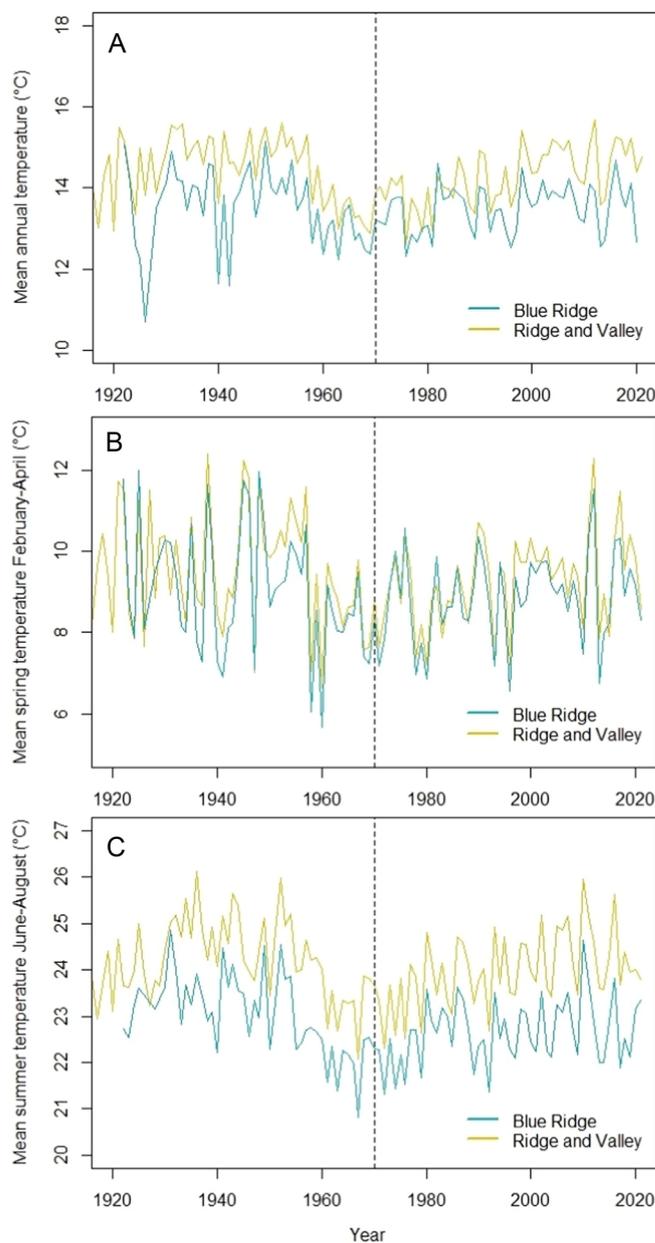
FIGURE 2 Changes in (A) mean annual temperature, (B) mean spring temperature, and (C) mean summer temperature in the Blue Ridge and Ridge and Valley ecoregions over the past century (1920–2020). Dotted lines indicate the division between historical and recent time periods (year 1970) used in analyses of phenological shifts over the past century.

Ridge ecoregions. Across all years of data, annual temperatures in the Blue Ridge were 0.8°C lower on average than those in the Ridge and Valley (Figure 2A). Spring temperatures were highly variable among years but not between ecoregions (Figure 2B), and temperature differences were greatest between the two regions in the summer (Figure 2C). Mean flowering dates of individual species calculated across all years of observation and all flowering phenophases (1–3) ranged from late March (e.g., *Hepatica acutiloba* DC. [sharp lobed hepatica; Ranunculaceae],
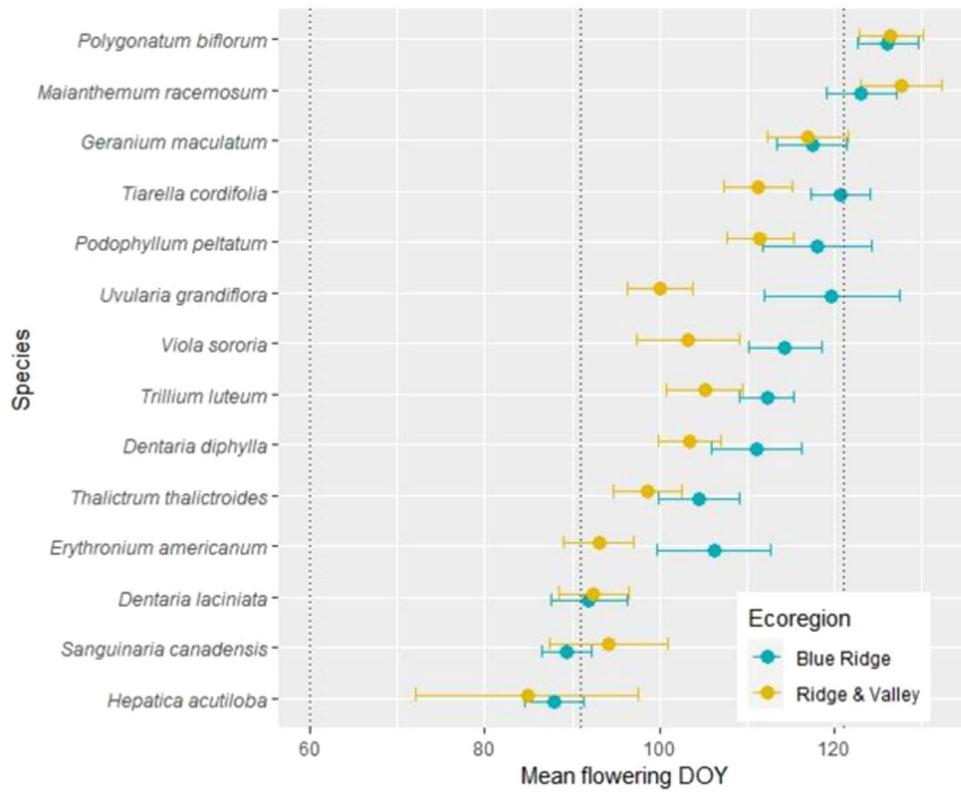
FIGURE 3 Mean flowering dates for focal species in the Blue Ridge and Ridge and Valley ecoregions. Flowering dates were calculated across all years of observation for each species. Points represent means and 95% confidence intervals. Dotted lines indicate the first day of each month in March, April, and May.
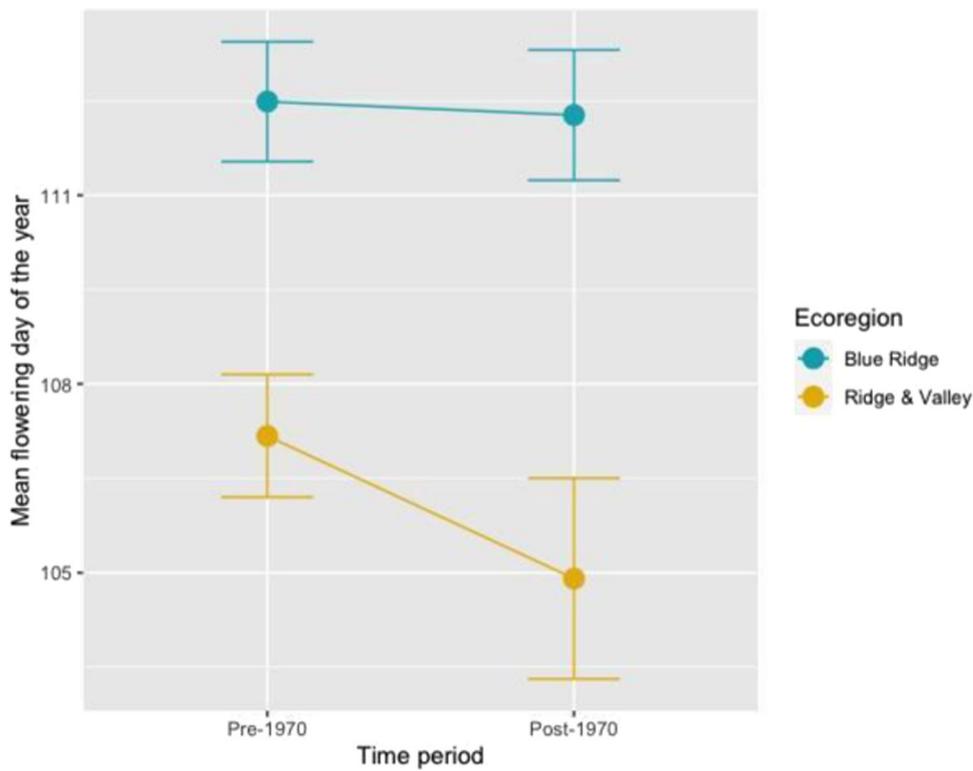
FIGURE 4 Changes in mean flowering times between historical and recent time periods in the Blue Ridge and Ridge and Valley ecoregions. Each point represents the mean and 95% confidence interval for flowering time in a given region and time period.

Sanguinaria canadensis L. [bloodroot; Papaveraceae]) to early May [e.g., *Maianthemum racemosum* (L.) Link (false Solomon's seal; Asparagaceae), *Polygonatum biflorum* Walt. Ell. (Solomon's seal; Asparagaceae)] (Figure 3). The three earliest- and latest-flowering species showed the least variation in mean flowering date between ecoregions, with April-blooming plants flowering earlier on average in the Ridge and Valley compared to the Blue Ridge (Figure 3). Across the full temporal range of observations, plants in the Ridge and Valley flowered 6.03 days earlier on average than plants in the Blue Ridge region (Welch's $t_{1040.7} = 5.5$, $P < 0.001$). Plants in the Ridge and Valley flowered 2.16 days earlier post-1970 than they did pre-1970, although this difference was not significant (Welch's $t_{264.2} = 1.21$ $P = 0.25$, 95% CI: –1.53, 5.85). Plants in the Blue Ridge region showed little change in flowering phenology between historical and recent time frames (Figure 4).

Phenological sensitivity to temperature

At the community level, spring temperature and ecoregion had significant impacts on flowering phenology (Table 2). For every degree-Celsius increase in temperature, spring flowering advanced 1.26 days on average across both ecoregions (95% CI: –1.78, –0.70) when analyzing all stages with flowers present together (stages 1–3). Results of models run separately for each ecoregion revealed that plants in the Blue Ridge ecoregion were more sensitive to spring temperature, with flowering dates advancing by

TABLE 2 Results of mixed-effects models analyzing the relationship between average spring temperature, ecoregion, and day of year of flowering. Full models were analyzed using community-level data (all species binned together) from both ecoregions, and Ridge and Valley and Blue Ridge models were analyzed using community-level data from each respective ecoregion. N.S. = not significant.

Phenological stage	Ecoregion	χ^2	df	P
All flowering stages (flowers present)	**Both ecoregions**			
	Avg. spring temperature	20.6	1	**5.7×10^{-6}**
	Ecoregion	14.3	1	**1.6×10^{-4}**
	Avg. spring temperature × ecoregion			N.S.
	Ridge and Valley			
	Avg. spring temperature	2.5	1	0.11
	Blue Ridge			
	Avg. spring temperature	10.3	1	**0.001**
Early flowering (>50% of reproductive structures are open flowers, fruits absent)	**Both ecoregions**			
	Avg. spring temperature	13.2	1	**2.7×10^{-4}**
	Ecoregion	0.04	1	0.85
	Avg. spring temperature × ecoregion		1	N.S.
	Ridge and Valley			
	Avg. spring temperature	16.5	1	**4.9×10^{-5}**
	Blue Ridge			
	Avg. spring temperature	0.09	1	0.76
Peak flowering (>50% of reproductive structures are open flowers, fruits absent or present	**Both ecoregions**			
	Avg. spring temperature	10.5	1	**0.001**
	Ecoregion	14.6	1	**1.3×10^{-4}**
	Avg. spring temperature × ecoregion			N.S.
	Ridge and Valley			
	Avg. spring temperature	0.11	1	0.74
	Blue Ridge			
	Avg. spring temperature	10.0	1	**0.002**
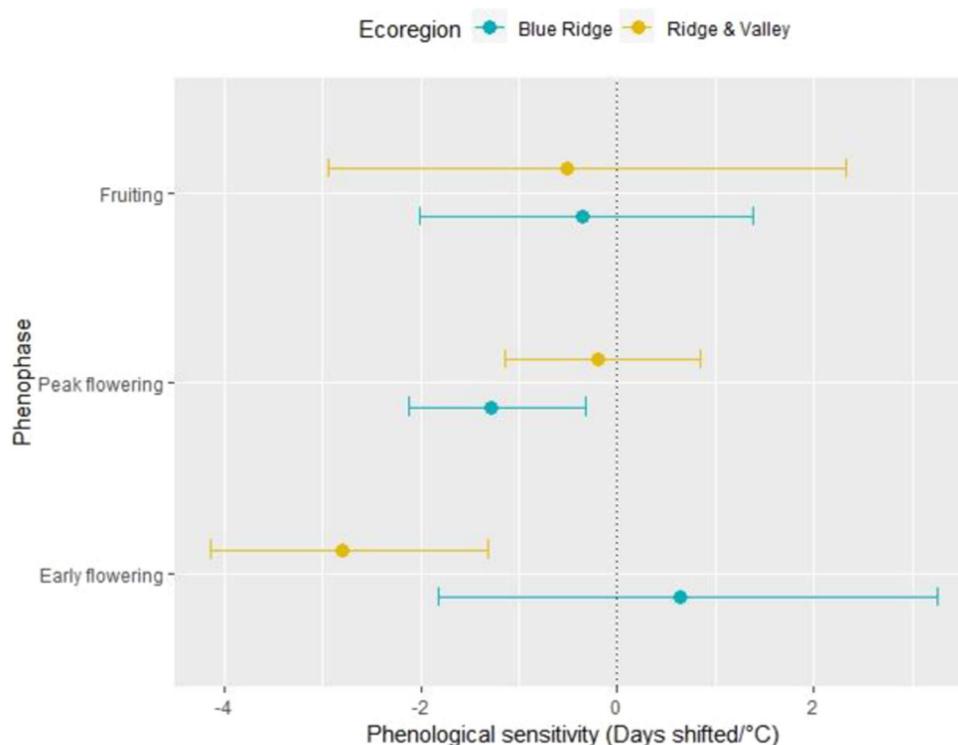
FIGURE 5 Variation in sensitivity of different phenophases to spring temperature in the Blue Ridge and Ridge and Valley ecoregions. Points represent slope estimates and 95% confidence intervals.

fl1.09 days/°C (95% CI: –1.81, –0.33) compared to 0.73 days/°C on average (95% CI: –1.57, 0.17) in the Ridge and Valley. When analyzing early and peak owering phenophases separately in each region, early spring flowering had the highest level of sensitivity of any phenological stage for plants in the Ridge and Valley (2.78 day advance/°C, 95% CI: –4.13, –1.31; Figure 5). While sensitivity of peak flowering was lower, it was still significant for plants in the Blue Ridge (0.52 day advance/°C, 95% CI: –2.12, –0.32). Fruiting was not significantly impacted by spring temperature in either ecoregion.

At the species level, sensitivity to temperature varied within species between ecoregions as well. In the Ridge and Valley, confidence limits for the slope of the line relating spring temperature to flowering date were negative (indicating advances in flowering phenology) and did not overlap zero for one of 14 species (*Geranium maculatum* L. [wild geranium; Geraniaceae]) (Figure 6). In contrast, in the Blue Ridge, the confidence limits for three species were negative and did not overlap zero: *Maianthemum racemosum*, *Trillium luteum* (Muhl.) Harbison (yellow trillium; Melanthiaceae), and *Sanguinaria canadensis* L. (bloodroot; Papaveraceae) (Figure 6).

DISCUSSION

Our results demonstrate that, although spring-flowering species in eastern Tennessee vary in the magnitudes of their phenological sensitivity to spring temperature (i.e.,

they flower earlier in warmer springs), mean flowering dates have not consistently advanced over the past century for most species tested. These results are concordant with another study that pooled data from >1700 species in South Carolina across the entire flowering season (Park and Schwartz, 2015). This pattern is likely explained by the fact that, although spring temperature is highly variable among years, it does not appear to have increased appreciably since the latter half of the 20th century (Figure 2B). Further, spring temperature has not changed substantially in the southeastern United States over the 20th century as a whole (Costanza et al., 2016). Interestingly, the most appreciable changes in temperature in the southeastern United States have occurred during the summer (+2°C since 1980, Costanza et al., 2016), a time of year that should not affect spring flowering. Although we did not detect greater increases in summer temperature than in spring temperature in our study region, the average temperature difference between the two ecoregions is greater in the summer than in the spring (Figure 2C). Further studies investigating warming patterns and their effects on spring phenology in this region of the United States are warranted.

Although we did not find evidence of significant advances in average flowering date during the latter half of the 20th century, our result that the average flowering date of spring-flowering species advanced by around 1.26 days/°C in response to warming spring temperatures supports the findings of other studies in the southeast
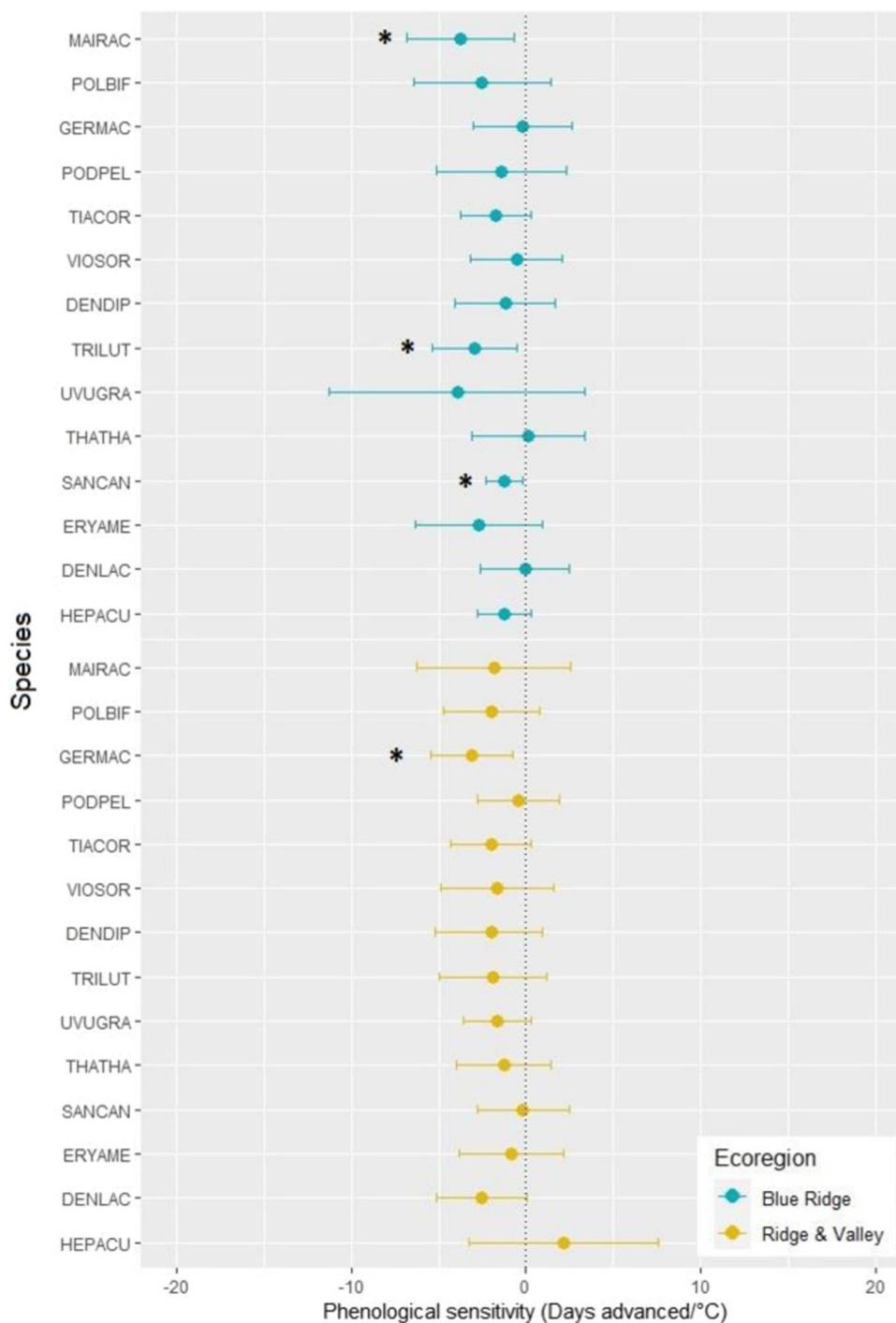
FIGURE 6 Variation in sensitivity of flowering to spring temperature (days advanced/°C) within species between the Blue Ridge and Ridge and Valley ecoregions. Points represent slope estimates and 95% confidence intervals. Asterisks indicate species for which confidence intervals for their estimate of phenological sensitivity do not overlap zero. See Table 1 for species codes.

(Park and Schwartz, 2015; Petrauski et al., 2019) and elsewhere (Primack et al., 2004; Panchen et al., 2012; Bertin, 2015; Park et al., 2019). Further, species varied in their sensitivities (Figure 6), suggesting that future phenological responses to continued climatic change will be heterogeneous within communities. Although our ability to estimate the sensitivity of individual species was limited by

our sample sizes, our results demonstrate that the flowering phenology of spring-flowering communities in both the Blue Ridge and the Ridge and Valley was sensitive to spring temperature. However, individuals in the Blue Ridge appear to be trending toward greater sensitivity to spring temperature, which could reflect the fact that low phenological sensitivity is "riskier" in the Blue Ridge due to greater

interannual climatic variability (Park et al., 2019). In the higher-elevation Blue Ridge ecoregion, temperatures tend to be lower (Figure 2), and the growing season tends to be shorter, so low temperature sensitivity in this ecoregion may present greater risk for mismatching with mutualists and other negative fitness consequences than it does to individuals in the lower-elevation Ridge and Valley. These differential responses of plant communities in the two ecoregions included in this study agree with the results of a recent study that found local environmental conditions to be an important explanatory factor in predicting the onset and duration of phenological stages of species in five botanical gardens across Germany (Sporbert et al., 2022), highlighting the importance of including microclimatic conditions in future studies of plant phenology.

Differential responses to climate change across relatively small geographic areas (i.e., within a single county located in two ecoregions) could result in changes to the structure and composition of plant communities, potentially altering gene flow and interactions between plants and their mutualistic partners such as pollinators or seed dispersers (i.e., phenological mismatch; Miller-Rushing et al., 2010). For example, species with greater sensitivities to temperature have been found to be at greater risk of herbivory than less-sensitive species (Meineke et al., 2021). In another study, flowering onset of *Corydalis ambigua* Chem. Et Schlecht (Papaveraceae) plants demonstrated greater sensitivity to snowmelt date than pollinator emergence, resulting in reduced seed set in years with earlier snowmelt (Kudo and Cooper, 2019). Thus, the result that the same communities in the Ridge and Valley and the Blue Ridge differ in their sensitivity to spring temperature and the magnitude of their phenological shifts in response to climate over the past century implies that the fitness consequences of continued climatic change on plant species will be heterogeneous, not only within communities, but across the landscape as well.

CONCLUSIONS

The fitness consequences of phenological sensitivity are complex (Prather et al., 2023). In the short term, dynamic phenological tracking of climate via high sensitivity to temperature could be potentially risky in cooler and less-predictable environments because it puts species at risk of freezing temperatures if they flower too early (Park et al., 2019). However, in the long-term, the ability of species' phenologies to track changes in temperature may be necessary for their persistence in light of continued climate warming. Willis et al. (2008) used historical records of the phenology and abundance of 473 spring wildflowers in Massachusetts, United States to assess the relationship between phenological sensitivity to temperature and change in abundance. The authors found that the species whose flowering times did not effectively track seasonal temperature have greatly declined in abundance

over the past 100 years. In the southeastern United States, further work is needed to understand whether other variables related to climate (e.g., snowmelt) or summer warming may be more important for phenological sensitivity and whether being sensitive to temperature is advantageous or detrimental depending on the ecological context. Given that the Blue Ridge ecoregion is a center of biodiversity in the eastern United States and contains the greatest floristic diversity in the entire state of Tennessee (U.S. Environmental Protection Agency, 1997), further work toward understanding the phenological sensitivity of plants is of particular importance in this region. While our study showed that overall plants did not demonstrate high levels of sensitivity to spring temperature, our work highlights the importance of considering ecoregion as a predictor in phenological studies because it allows researchers to account for differences in local abiotic conditions across even relatively small geographical areas that may explain inter- and intraspecific variation in phenological trends across time.

AUTHOR CONTRIBUTIONS
A.S.F. and J.M.B. conceived and planned the study. A.S.F. and M.G.O. acquired the data with the assistance of the undergraduate students listed in the Acknowledgements. A.S.F. created the species reference sheets (Appendix S1). A.S.F. analyzed the data with input from J.M.B., M.G.O., and S.K. Data were interpreted by all authors. A.S.F. wrote the initial draft manuscript and J.M.B, M.G.O., and S.K. edited the manuscript. A.S.F completed revisions on the manuscript after submission.

DATA AVAILABILITY STATEMENT

Data are available in Zenodo at https://doi.org/10.5281/zenodo.7539145.

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REFERENCES

Abu-Asab M., P. Peterson, S. Shetler, and S. Orli. 2001. Earlier plant flowering in spring as a response to global warming in the Washington, DC, area. *Biodiversity Conservation* 10: 597–612.

Bertin, R. I. 2015. Climate change and flowering phenology in Worcester County, Massachusetts. *International Journal of Plant Sciences* 176: 107–119.

Calinger, K. M., S. Queenborough, and P. S. Curtis. 2013. Herbarium specimens reveal the footprint of climate change on flowering trends across north-central North America. *Ecology Letters* 16: 1037–1044.

Costanza, J., S. Beck, M. Pyne, A. Terando, M. J. Rubino, R. White, and J. Collazo. 2016. Assessing climate-sensitive ecosystems in the southeastern United States. Open-file report 2016–1073. U.S. Geological Survey, Reston, VA, USA.

Davis, C. C., C. G. Willis, B. Connolly, C. Kelly, and A. M. Ellison. 2015. Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. *American Journal of Botany* 102: 1599–1609.

Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689–1691.

Forrest, J. R. 2015. Plant–pollinator interactions and phenological change: What can we learn about climate impacts from experiments and observations? *Oikos* 124: 4–13.

Funderburk, D. O., and J. N. Skeen. 1976. Spring phenology in a mature piedmont forest. *Castanea* 41: 20–30.

Gallinat, A. S., L. Russo, E. K. Melaas, C. G. Willis, and R. B. Primack. 2018. Herbarium specimens show patterns of fruiting phenology in native and invasive plant species across New England. *American Journal of Botany* 105: 31–41.

Griffith, G. E., J. M. Omernik, and S. H. Azevedo. 1997. Ecoregions of Tennessee. EPA/600/R-97/022. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, OR, USA.

Hart, J. L., S. L. van de Gevel, and H. D. Grissino-Mayer. 2008. Forest dynamics in a natural area of the southern Ridge and Valley, Tennessee. *Natural Areas Journal* 28: 275–289.

Heberling, J. M., C. McDonough MacKenzie, J. D. Fridley, S. Kalisz, and R. B. Primack. 2019. Phenological mismatch with trees reduces wildflower carbon budgets. *Ecology Letters* 22: 616–623.

Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89: 353–362.

Jones, C. A., and C. C. Daehler. 2018. Herbarium specimens can reveal impacts of climate change on plant phenology; a review of methods and applications. *PeerJ* 6: e4576.

Kudo, G., and E. J. Cooper. 2019. When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society, B, Biological Sciences* 286: 20190573.

Matthews, E. R., and S. J. Mazer. 2016. Historical changes in flowering phenology are governed by temperature × precipitation interactions in a widespread perennial herb in western North America. *New Phytologist* 210: 157–167.

Meineke, E. K., C. C. Davis, and T. J. Davies. 2021. Phenological sensitivity to temperature mediates herbivory. *Global Change Biology* 27: 2315–2327.

Miller-Rushing, A. J., T. T. Høye, D. W. Inouye, and E. Post. 2010. The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 365: 3177–3186.

Miller-Rushing, A. J., and R. B. Primack. 2008. Global warming and flowering times in Thoreau's Concord: a community perspective. *Ecology* 89: 332–341.

Pachauri, R. K., M. R. Allen, V. R. Barros, J. Broome, W. Cramer, R. Christ, J. A. Church, et al. 2014. Climate change 2014: synthesis report, R. K. Pachauri and L. Meyer [eds.], contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Geneva, Switzerland.

Panchen, Z. A., R. B. Primack, T. Aniśko, and R. E. Lyons. 2012. Herbarium specimens, photographs, and field observations show Philadelphia area plants are responding to climate change. *American Journal of Botany* 99: 751–756.

Panchen, Z. A., R. B. Primack, B. Nordt, E. R. Ellwood, A. D. Stevens, S. S. Renner, C. G. Willis, et al. 2014. Leaf out times of temperate woody plants are related to phylogeny, deciduousness, growth habit and wood anatomy. *New Phytologist* 203: 1208–1219.

Park, I. W., and M. D. Schwartz. 2015. Long-term herbarium records reveal temperature-dependent changes in flowering phenology in the southeastern USA. *International Journal of Biometeorology* 59: 347–355.

Park, D. S., I. Breckheimer, A. C. Williams, E. Law, A. M. Ellison, and C. C. Davis. 2019. Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 374: 20170394.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637–669.

Pearson, K. D. 2019. A new method and insights for estimating phenological events from herbarium specimens. *Applications in Plant Sciences* 7: e01224.

Pearson, K. D. 2019. Spring-and fall-flowering species show diverging phenological responses to climate in the Southeast USA. *International Journal of Biometeorology* 63: 481–492.

Petrauski, L., S. F. Owen, G. D. Constantz, and J. T. Anderson. 2019. Changes in flowering phenology of *Cardamine concatenata* and *Erythronium americanum* over 111 years in the Central Appalachians. *Plant Ecology* 220: 817–828.

Piao, S., Q. Liu, A. Chen, I. A. Janssens, Y. Fu, J. Dai, L. Liu, et al. 2019. Plant phenology and global climate change: current progresses and challenges. *Global Change Biology* 25: 1922–1940.

Polgar, C. A., and R. B. Primack. 2011. Leaf-out phenology of temperate woody plants: from trees to ecosystems. *New Phytologist* 191: 926–941.

Prather, R. M., R. M. Dalton, B. Barr, D. T. Blumstein, C. L. Boggs, A. K. Brody, D. W. Inouye, et al. 2023. Current and lagged climate affects phenology across diverse taxonomic groups. *Proceedings of the Royal Society, B, Biological Sciences* 290: 20222181.

Primack, D., C. Imbres, R. B. Primack, A. J. Miller-Rushing, and P. Del Tredici. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *American Journal of Botany* 91: 1260–1264.

Ruxton, G. D. 2006. The unequal variance *t*-test is an underused alternative to Student's t-test and the Mann–Whitney *U* test. *Behavioral Ecology* 17: 688–690.

Sporbert, M., D. Jakubka, S. F. Bucher, I. Hensen, M. Freiberg, A. König, et al. 2022. Functional traits influence patterns in vegetative and reproductive plant phenology – a multi-botanical garden study. *New Phytologist* 235: 2199–2210.

Surasinghe, T., and R. F. Baldwin. 2014. Ghost of land-use past in the context of current land cover: evidence from salamander communities in streams of Blue Ridge and Piedmont ecoregions. *Canadian Journal of Zoology* 92: 527–536.

U.S. Environmental Protection Agency. 1997. Level III ecoregions of the continental United States (revision of Omernik, 1987), Map M-1, various scales. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, OR, USA. Website: https://store.usgs.gov/assets/MOD/StoreFiles/Ecoregion/21632_tn_front.pdf

Willis, C. G., E. R. Ellwood, R. B. Primack, C. C. Davis, K. D. Pearson, A. S. Gallinat, J. M. Yost, et al. 2017. Old plants, new tricks: Phenological research using herbarium specimens. *Trends in Ecology and Evolution* 32: 531–546.

Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences, USA* 105: 17029–17033.

Willis, C. G., B. R. Ruhfel, R. B. Primack, A. J. Miller-Rushing, J. B. Losos, and C. C. Davis. 2010. Favorable climate change response explains non-native species' success in Thoreau's woods. *PLoS One* 5: e8878.

Wolkovich, E. M., B. I. Cook, and T. J. Davies. 2014. Progress towards an interdisciplinary science of plant phenology: building predictions across space, time and species diversity. *New Phytologist* 201: 1156–1162.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Species reference sheets used for scoring phenological stages of herbarium specimens.

Appendix S2. Pearson's correlation coefficients for the relationship between the day of year of flowering and mean monthly temperatures preceding flowering for species flowering on average in March, April, and May.

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