Changes in flowering phenology of *Cardamine concatenata* and *Erythronium americanum* over 111 years in the Central Appalachians

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Abstract Spring ephemerals in the Central Appalachians are a key component of deciduous forest communities and can be indicators of shifting phenology due to climate changes in this ecosystem. The objectives of this study were to (1) determine if there have been any changes in date of flowering for the Cutleaf Toothwort (*Cardamine concatenata*) and Yellow Trout Lily (*Erythronium americanum*) in West Virginia over the last 111 years; (2) determine which climatic factors affect the blooming date of these perennial, spring ephemeral wildflowers; and (3) evaluate the effect of elevation on changing blooming dates using herbarium specimens and photographs from 1904 to 2015. Both species are widespread throughout the woodlands of eastern North America. Both species have significantly advanced their spring flowering over the last century ($\bar{x} = 0.91$ days/decade). Spring temperature was the strongest predictor of

blooming date (2.91 and 3.44 days earlier/1 °C increase in spring temperature, respectively). Flowers at < 500 m elevation bloomed earlier and demonstrated a stronger shift in flowering date over time than flowers at > 1000 m elevations. Lower elevations, higher spring and winter temperatures, and low amounts of precipitation were associated with earlier spring flowering. This research demonstrates the plasticity of phenological response to a variety of climatic variables, the usefulness of using herbarium specimens to reconstruct flowering dates over a topographically variable area, and the contrasting effects of climate change on high elevation regions of West Virginia.

Keywords Blooming phenology · Herbarium specimens · Climate change · Historical phenology

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Introduction

Climate affects the phenology of plants and animals, and changes in climate pose possible risks for phenological mismatches and shifts in vegetative communities (Memmott et al. 2007; Crimmins et al. 2008) if species cannot shift their distributions in synchrony with the changing climate (Ash et al. 2016). Flowering of herbaceous plants has been heavily studied due to the sensitivity of this phenophase to

changes in climate (Gezon et al. 2016) and the fitness insights gained by studying the reproductive phase of plant life (Inouye 2008). Flowering phenology can be influenced by a number of abiotic factors, including increasing spring temperatures (Primack et al. 2004) [especially in the months before flowering (Miller-Rushing and Primack 2008)], spring precipitation (Matthews and Mazer 2016), elevation (Crimmins et al. 2011; Čufar et al. 2012), and snowmelt (Inouye 2008; Livensperger et al. 2016).

Geographic location, including elevation, is a strong contributing factor in the sensitivity of plant phenology (Matthews and Mazer 2016), due to its association with microclimates, which can influence temperature or the risk of frost damage (Inouye 2008). Elevation has an effect on the strength of phenology shifts in horse-chestnut trees *Aesculus hippocastanum* L. (Defila and Clot 2001), and changes in climate affect phenology to a greater degree in higher elevations than lower elevations in Slovakia (Čufar et al. 2012). In the Himalayas, flowers were found to bloom an average of 26 days earlier per 1000 m decrease in elevation (Gaira et al. 2011). Due to a warming climate, plants are able to expand their range and grow at higher elevations (Lenoir et al. 2008), which can affect the way these species respond to changes in climate. However, there can be confounding factors involved with shifting ranges such as precipitation or moisture availability. For example, Crimmins et al. (2011) found that plants in California shifted to lower elevations despite increases in temperature, due to decreased water availability in higher elevations. Geographic location also includes latitude, which can give insight into larger patterns of phenology (McKinney et al. 2012). For example, the phenology of species in higher latitudes in the northern hemisphere and lower latitudes in the southern hemisphere is more strongly affected by changes in climate than latitudes closer to the equator (Guyon et al. 2011). Therefore, it is important to examine latitude in conjunction with elevation as factors influencing phenological shifts.

Studies in the eastern United States have shown shifts of flowering times, ranging from an average of 7 days earlier since the 1850s in Concord, Massachusetts (Miller-Rushing and Primack 2008) to an average of 2.4 days earlier since the 1970s in Washington, DC (Abu-Asab et al. 2001). The strongest predictor of spring flowering phenology has been

temperature (Jackson 1966; Beaubien and Freeland 2000; Primack et al. 2004; Miller-Rushing and Primack 2008), which covaries with other environmental factors such as snowmelt (Inouye 2008) and elevation (Gaira et al. 2011). Warmer winter temperatures have been linked to earlier flowering dates for a diversity of herbaceous and woody plant species (Beaubien and Freeland, 2000; Miller-Rushing and Primack 2008) and increased precipitation has been associated with later flowering of spring ephemerals such as western trilliums *Trillium ovatum* Pursh (Matthews and Mazer 2016).

Benefits of earlier spring blooming could include high levels of light and moisture and less interspecific competition for pollinators (Forrest and Thomson 2010). However, there may also be consequences associated with earlier flowering, such as frost damage leading to decreased reproductive fitness (Gezon et al. 2016). Past studies found that flowering times respond to increases in spring temperatures by advancing their date of flowering by 3.07 days (Miller-Rushing and Primack 2008), 3.39 days (Park and Schwartz 2015), 3.6 days (Primack et al. 2004), and 6 days (Robbirt et al. 2011) per 1 °C increase in spring temperature.

Spring ephemeral wildflowers, which are especially sensitive to changes in climate, have served as useful indicators of phenological advancement (Fitter et al. 1995). For example, spring ephemerals emerge shortly after snowmelt (Lapointe 2001), and earlier snowmelt has been linked to earlier flowering (Lambert et al. 2010). Winter temperatures and precipitation (Matthews and Mazer 2016) have also been used to analyze flowering phenology of spring perennials. Most studies of flowering phenology are constrained by a lack of long-term historical data (Primack et al. 2004; Robbirt et al. 2011). This is why herbarium specimens and photographs have been explored as a way to reconstruct historical phenology of spring ephemerals and other flowering plants (Lavoie and Lachance 2006; Miller-Rushing et al. 2006; Gaira et al. 2011; Calinger et al. 2013; Everill et al. 2014). Miller-Rushing et al. (2006) and Robbirt et al. (2011) determined that herbarium specimens alone can successfully show flowering response of a variety of flowering plants to changing climate.

The three objectives of this study were to (1) determine if there have been changes in date of flowering for two species of spring ephemerals in West Virginia over the last 111 years, (2) evaluate

which climatic and non-climatic factors affect their blooming dates, and (3) evaluate the effect of elevation on changing blooming dates. It is important to study the factors that shift phenology because any phenophases that are altered can disrupt species interactions. We predicted that spring ephemerals have shifted their flowering dates over the last century in response to changes in climate, especially increased spring temperature, and that flowers at higher elevations would flower later than flowers at low elevations.

Methods

Study area

West Virginia is a heavily forested, mountainous state located in the Appalachian region of the United States. Temperature, elevation, and precipitation differ considerably throughout the state. The average statewide yearly temperature is 6–17 (°C), where the lower southern regions are warmer than the mountainous regions. The average elevation is 457 m with the highest point (Spruce Knob) at 1482 m and the lowest point (Harper's Ferry) at 149 m above sea level. Annual precipitation ranges between 81 and 132 cm statewide. West Virginia is 79% forested and the forests are 94% deciduous hardwoods. Oak-hickory forest type covers 74% of West Virginia forestlands followed by northern hardwood forest type (18%) (Morin et al. 2013). West Virginia is partially composed of hemlock forests, and boreal and red spruce forests in high elevations.

Study species

Cutleaf Toothwort *Cardamine concatenata* (Michx.) Sw. (Brassicaceae) and Yellow Trout Lily *Erythronium americanum* Ker Gawl. (Liliaceae) are both common spring wildflowers in eastern North America (Strausbaugh and Core 1978). Their short blooming-window (1–2 weeks) makes them good candidates for phenology research (Shetler and Wiser 1987; Lapointe 2001). Both species are native perennial herbs that grow in moist bottomland and deciduous forests. Spring ephemerals are among the first plants to flower in the spring. The specific phenophase used for this study was “open flowers” (USA-NPN 2012). For this study, plants with $\geq 50\%$ of all fresh flowers in bloom were

used, which is easily observed in herbarium specimens and pictures.

Observations

We used herbarium specimens and dated photographs to identify locations and flowering dates for *C. concatenata* and *E. americanum* in West Virginia. Herbarium specimens or photographs that showed at least half of the flowers open on a plant were scored as peak-flowering (Primack et al. 2004; Primack and Miller-Rushing 2012). Specimens were processed at six public herbariums, including five in West Virginia and one in Pennsylvania, and the personal herbarium of a West Virginian citizen (Table 1). To obtain elevation data and accurate climatic data, locations of observations were mapped using ArcGIS Version 10.3 using the location descriptions on the herbarium specimens (Fig. 1). A West Virginia citizen contributed 11 dated photographs with known locations (2005–2015), which were added to the herbarium dataset to supplement the recent observations. Each usable herbarium specimen and photograph included date and location. Specimens without locality data or with overly broad localities (i.e., county only) were excluded from the study. Overall, the observations gained from these specimens reflect an average flowering date because each specimen is not necessarily a first or last flowering observation. By using average flowering dates of species with short blooming windows, our conclusions will more accurately represent the shifts and changing phenology of these species as compared to first flowering dates (Caradonna et al. 2014).

Climate

Climate data at 13 stations in West Virginia were recorded by the US Historical Climatology Network (Williams et al. 2007) since 1890. A series of estimation maps was made for each decade (12 decades 1890–2014) using the Radial Basis Function interpolation method in ArcGIS Version 10.3. These decadal interpolations were used to estimate average decadal yearly temperature, spring and winter temperatures, and spring precipitation for each flowering observation. Radial basis functions create surfaces of estimated measurements based on the degree of smoothing from measured points, resulting in an exact

Table 1 Sources of flowering data for *Cardamine concatenata* and *Erythronium americanum* in West Virginia, USA over the last 111 years

Source	Number of records		City	Dates
	<i>Cardamine concatenata</i>	<i>Erythronium americanum</i>		
Fairmont State University Herbarium	1	10	Fairmont, WV	1963–1981
Marshall University Herbarium	72	61	Huntington, WV	1928–2001
University of Charleston Herbarium	0	1	Charleston, WV	1968
West Virginia University Herbarium	63	58	Morgantown, WV	1904–2013
George B. Rossbach Herbarium	17	20	Buckhannon, WV	1929–1999
Carnegie Museum of Natural History	11	6	Pittsburgh, PA	1930–1988
Personal Herbarium	1	1	Old Fields, WV	2013
Personal Photograph Collection	5	6	Morgantown, WV	2005–2015

Fig. 1 Collection locations of *Cardamine concatenata* and *Erythronium americanum* herbarium specimens collected from 1904–2015 in West Virginia, USA

interpolation, which qualifies it as a suitable method for determining unknown atmospheric values based on

known neighboring measurements (Apaydin et al. 2004; Ashiq et al. 2010).

Data analyses

The wildflowers dataset included day of the year (Julian calendar), year, elevation, average decadal spring temperature, average decadal spring precipitation, average decadal winter temperature, average decadal yearly temperature, and species. All variables were normally distributed except elevation, which was log transformed. Correlation and regression were used to test for collinearity of the variables. Yearly temperature was highly correlated with spring temperature ($r = 0.86$) and with winter temperature ($r = 0.85$), and therefore was not used in analyses (Miller-Rushing and Inouye 2009).

To test for changes in flowering date, the dates of flowering for each species were regressed against year. A Welch's t test, which is robust to unequal variances and sample sizes (Ruxton 2006), was used to determine if there was a difference between historical and current flowering dates. Historical flowering dates were considered to be before 1970 and current dates were considered the years 1970 and after, when global surface temperatures began to steadily increase (IPCC 2014).

Observations were divided into several elevation categories (low: under 500 m, mid: 500–1000 m, high: above 1000 m) and the day of flowering was regressed against year for each category to compare the difference in magnitude of phenology shifts as a function of elevation. A one-way Analysis of Variance (ANOVA) was used to test for differences in flowering date among the elevation categories.

Results

Changes in climate

Between 1970 and 2014, average spring temperatures (March–April) in West Virginia increased 10 times as much as they did between 1890 and 1969, and spring temperatures between 1970 and 2014 ($\bar{x} = 8.52$ °C, SE = 0.18) were significantly higher than between 1890 and 1969 ($\bar{x} = 8.08$ °C, SE = 0.16) ($t_{103} = -1.77$, $P = 0.03$). Over the 125 years of weather data, the average climate variables have mostly remained constant when analyzed over the entire state (Fig. 2). Spring precipitation, spring temperature, and winter temperature have only increased slightly

(precipitation increasing 0.030 cm/decade; spring temperature increasing 0.055 °C/decade; winter temperature increasing 0.003 °C/decade) since 1890. However, variation in temperature and precipitation exists within West Virginia and change over time is evident in some parts of the state more than others for spring temperature (Fig. 3a), winter temperature (Fig. 3b), and spring precipitation (Fig. 3c). For example, spring and winter temperatures have especially increased in the Eastern Panhandle region of West Virginia as compared to the rest of the state, and precipitation has increased throughout the state.

Changes in flowering phenology

Overall, there were 337 observations of flowering, including 170 for *C. concatenata* and 167 for *E. americanum* between 1904 and 2015 (Table 2). Across historic and contemporary dates, the flowering dates were significantly different ($t_{277.75} = 4.52$, $P < 0.001$) between *C. concatenata* ($\bar{x} \pm \text{SD} = 102$ (April 12) ± 12.3 days, SE = 0.946) and *E. americanum* ($\bar{x} \pm \text{SD} = 101$ (April 11) ± 7.5 days, SE = 0.643), and therefore analyzed separately. Normality was confirmed for flowering dates of *C. concatenata* ($W = 0.987$, $P = 0.135$) and *E. americanum* ($W = 0.994$, $P = 0.784$) using Shapiro–Wilk tests. *C. concatenata* and *E. americanum* have both significantly advanced their spring flowering over the last century (Fig. 4). *C. concatenata* ($F_{1, 168} = 5.76$, $P = 0.017$) has flowered 0.87 days earlier each decade, and *E. americanum* ($F_{1, 165} = 9.78$, $P = 0.002$) has flowered 0.94 days earlier every decade. For both species, there was a significant difference between historical ($\bar{x} \pm \text{SD} = 108$ (April 18) ± 11.8 days, SE = 0.244) flowering dates and current ($\bar{x} \pm \text{SD} = 102$ (April 12) ± 10.0 days, SE = 0.722) flowering dates based on Welch's t tests. *C. concatenata* flowered significantly earlier ($t_{155} = 3.07$, $P = 0.002$) in recent years ($\bar{x} \pm \text{SD} = 99$ (April 9) ± 11.7 days, SE = 1.20) as compared to pre-1970 ($\bar{x} \pm \text{SD} = 105$ (April 15) ± 12.4 days, SE = 1.43), and *E. americanum* also significantly advanced ($t_{111} = 3.66$, $P < 0.001$) its current flowering ($\bar{x} \pm \text{SD} = 105$ (April 15) ± 7.5 days, SE = 0.75) as compared to historical records ($\bar{x} \pm \text{SD} = 110$ (April 20) ± 10.9 days, SE = 1.34). Normality was confirmed using a Shapiro–Wilk test (*C. concatenata*: historical: $W = 0.98$, $P = 0.41$, current:

Fig. 2 Average climate in West Virginia, USA over 125 years (1890–2014). **a** Spring temperature is average March–April temperatures, **b** spring precipitation is average March–April

rainfall, and **c** winter temperature is average January–February temperature based on 13 United States Historical Climatology Network recording stations located in West Virginia

Fig. 3 **a** Spring temperature (March–April), **b** Winter temperature (January–February), **c** Spring precipitation (March–April) interpolations in West Virginia, USA from the 1890 s

(1890–1899) and 2000s (2000–2014). Interpolations are based on climate data from 13 United States Historical Climatology Network recording stations located in West Virginia

Table 2 Observations of *Cardamine concatenata* and *Erythronium americanum* categorized by year from West Virginia, USA

Year of observation	Species	
	<i>Erythronium americanum</i>	<i>Cardamine concatenata</i>
1904–1921	0	3
1922–1929	6	6
1930–1959	42	57
1960–1989	82	58
1990–2015	37	46
Total	167	170

Fig. 4 Flowering dates of **a** *C. concatenata* ($y = -0.088x + 275.53$, $R^2 = 0.02$, $P = 0.017$) and **b** *E. americanum* ($y = -0.094x + 292.76$, $R^2 = 0.05$, $P = 0.002$) in West Virginia, USA from 1904 to 2015

$W = 0.99$, $P = 0.74$; *E. americanum*: historical: $W = 0.98$, $P = 0.48$, current: $W = 0.98$, $P = 0.26$).

Elevation effects

Climate variables and elevation had differing effects on the date of flowering for each species, as determined using simple linear regressions (Table 3). Given that elevation was log transformed, for every 277.5 m (10% of total elevation range) increase in elevation, *C. concatenata* flowered 0.5 days later (SE = 1.81) ($F_{1,168} = 8.55$, $P = 0.004$) and *E. americanum* flowered 0.72 days later (SE = 1.34) ($F_{1,165} = 9.72$, $P = 0.002$) for every 124.1 m increase in elevation. In other terms, for every 1000 m increase in elevation, *C. concatenata* flowered 1.80 days later and *E. americanum* flowered 5.8 days later.

There was a significant difference in flowering dates depending on elevation ($F_{1,335} = 28.74$, $P < 0.001$). Flowers at low elevations ($n = 267$) bloomed earlier ($\bar{x} \pm \text{SD} = 104$ (April 14) ± 10.6 days, SE = 0.651) as compared to mid elevations ($n = 52$, $\bar{x} \pm \text{SD} = 108$ (April 18) ± 12.2 days, SE = 1.953) and high elevations ($n = 18$, $\bar{x} \pm \text{SD} = 117$ (April 27) ± 9.1 days, SE = 2.143). Flowers at low elevations demonstrated a stronger shift toward earlier flowering dates over time (0.95 days earlier each decade) than flowers at mid (0.68 days earlier each decade) or high elevations (1.32 days later each decade). Normality was confirmed for low ($W = 0.994$, $P = 0.311$), mid ($W = 0.978$, $P = 0.647$), and high ($W = 0.980$, $P = 0.950$) elevations using a Shapiro–Wilk test and equal variances were confirmed using a Kolmogorov–Smirnov test ($D = 0.156$, $P = 0.373$).

Climatic variables

Precipitation, spring temperature, and winter temperature had varying effects on the flowering of *C. concatenata* and *E. americanum* (Fig. 5). *C. concatenata* flowered 2.91 days earlier (SE = 0.82) ($F_{1,168} = 12.51$, $P < 0.001$) and *E. americanum* flowered 3.44 days earlier (SE = 0.67) ($F_{1,165} = 31.40$, $P < 0.001$) for each 1 °C increase in spring temperature. *C. concatenata* flowered 1.57 days earlier (SE = 0.76) ($F_{1,168} = 4.28$, $P = 0.040$) and *E. americanum* flowered 1.11 days earlier (SE = 0.51) ($F_{1,165} = 4.82$, $P = 0.029$) for each 1 °C increase in winter temperature. Precipitation had no significant effect on *C. concatenata*'s flowering dates, but *E. americanum* flowered 2.96 days later (SE = 0.69) ($F_{1,165} = 18.45$, $P < 0.001$) for every 1 cm increase in precipitation. Interactions among variables were investigated with no significant effects ($P > 0.05$).

Discussion

Overall, the flowering of *E. americanum* and *C. concatenata* advanced over the last 111 years and the flowers are blooming earlier than they did historically. *Erythronium americanum* advanced its spring flowering by an average of 6 days and was more sensitive to elevation, spring temperature, and spring precipitation than *C. concatenata*, which has also advanced an average of 6 days. *Cardamine concatenata* was slightly more affected by warmer winter temperatures. Lower elevations, higher spring temperatures, higher winter temperatures, and low amounts of precipitation were associated with earlier spring flowering overall.

Table 3 Statistically significant flowering responses of *Cardamine concatenata* and *Erythronium americanum* to increases in climatic variables and elevation from 1904–2015 in West Virginia, USA

Climatic variable	Species	
	<i>Erythronium americanum</i>	<i>Cardamine concatenata</i>
Spring Temperature (+ 1°)	3.44 days earlier (SE = 0.67)	2.91 days earlier (SE = 0.82)
Winter Temperature (+ 1°)	1.11 days earlier (SE = 0.51)	1.57 days earlier (SE = 0.76)
Precipitation (+ 1 cm)	2.96 days later (SE = 0.69)	No sig. effect
Elevation (+ 1000 m)	5.8 days later (SE = 11.90)	1.80 days later (SE = 5.61)

Fig. 5 Flowering responses of *Cardamine concatenata* and *Erythronium americanum* to increases in spring temperature from 1922 to 2015 in West Virginia, USA. Flowering of *E. americanum* in response to **a** spring temperature ($y = -3.44x + 135.83$, $R^2 = 0.15$, $P < 0.001$) **c** winter temperature ($y = -1.11x + 107.3$, $R^2 = 0.02$, $P = 0.03$), and **e** precipitation

($y = 2.96x + 77.50$, $R^2 = 0.10$, $P < 0.001$). Flowering of *C. concatenata* in response to **b** spring temperature ($y = -2.91x + 127.87$, $R^2 = 0.06$, $P < 0.001$) **d** winter temperature ($y = -1.57x + 104.05$, $R^2 = 0.02$, $P = 0.04$), and **f** precipitation ($y = 1.20x + 91.24$, $R^2 = 0.003$, $P = 0.22$)

Spring precipitation only affected the flowering of *E. americanum*, an effect supported by previous research (Matthews and Mazer 2016). Years with higher spring precipitation and lower spring temperatures were associated with later flowering dates.

One of the major drivers of phenological studies is the risk of phenological mismatch (Root et al. 2003), which can occur when one species advances (Kudo and Ida 2013) or delays (Gezon et al. 2016) its phenology in relation to interacting species. For example, early-blooming spring ephemerals may

incur lower reproductive success due to a difference in phenological response by their pollinators (Rafferty and Ives 2011; Kudo and Ida 2013). However, for *Claytonia lanceolata* Pall. ex Pursh, another spring ephemeral, the rate of pollinator visits was higher for plants that flowered early, but not so early that they were threatened by frost (Gezon et al. 2016). In this case, the spring ephemeral balanced environmental and species interaction constraints for optimal flowering date.

In general, it is thought that earlier blooming due to increased temperatures is beneficial for the plant's reproductive success (Elzinga et al. 2007) partly due to increased pollinator visits (Rafferty and Ives 2011); however, there is some disparity in the literature depending on the pollinator. For example, in Japan, spring ephemerals that bloomed earlier due to increasing temperatures differed in their reproductive success (seed production) based on their pollinators (Kudo et al. 2004)—the seed-set of bee-pollinated ephemerals drastically decreased, whereas fly-pollinated ephemerals experienced no change in their seed production due to the earlier blooming.

Earlier blooming caused by earlier spring onset has been linked to fewer pollinator visits (Petanidou et al. 2014) and a phenological mismatch in the emergence of pollinator bees, which resulted in decreased reproductive success for the spring ephemerals that relied on bees for pollination (Kudo and Ida 2013). Although generalist pollinators do occur in early spring within temperate deciduous forest communities (Motten 1986), *C. concatenata* is primarily pollinated by bees and *E. americanum* is pollinated by ants, so perhaps *C. concatenata* receives more severe consequences and less reproductive benefits for earlier blooming than *E. americanum*, which could help explain why *E. americanum* is advancing spring flowering more than *C. concatenata*. However, data to support this hypothesis are lacking, but this premise could serve as a starting point for a future research project on pollinator response to phenology change for spring ephemerals in the region.

Elevation had a strong effect on the flowering of these species. Flowers at < 500 m elevation bloomed earlier in the spring and advanced their flowering dates over time more than flowers at mid (500–1000 m) and high (> 1000 m) elevations. In addition, the flowers found at > 1000 m in elevation have flowered later over time, which could be attributed to varying effects of climate change on high elevation areas as compared to low elevations. This suggests that wildflower communities at different elevations will be affected differently by changing climate. The high elevation areas of West Virginia also were the areas that experienced the least amount of climatic changes over the last century (Fig. 3), which would explain the lack of earlier flowering in high elevation species.

Our large statewide study area could harbor possible bias in imprecise geographic locations for

flowering observations. Given that the location information on the herbarium specimens was not always exact, factors such as hillshade and aspect, which have been shown to explain variation in flowering phenology (Jackson 1966), could serve as confounding variables. However, we did exclude specimens that had broad localities such as county only to reduce some of this bias. Moreover, the high number of flowering observations can overcome potential sampling bias (Primack et al. 2004).

There are several issues associated with using herbarium specimens to understand phenology. Because the timing of phenophases is highly related to climate, and climatic conditions vary over sampling areas, it is difficult to compare the phenophases of specimens collected over large geographic areas (Lavoie and Lachance 2006). In addition to climatic differences, there is also uncertainty in determining exact full-flowering dates. Depending on the species, the time when an individual plant could be considered “full flowering” could range over several weeks, which could decrease the reliability of the data (Miller-Rushing et al. 2006). By choosing spring ephemeral species with short blooming windows, this risk was mitigated.

Another bias to consider is that of the original collectors of the herbarium specimens. Factors such as proximity to trails and roadways could have affected the geographic distribution of the observations (Miller-Rushing et al. 2006). However, based on the map of observations (Fig. 1), the distribution of the herbarium specimens used in this study is fairly uniform throughout the state.

Based on their advancing flowering dates, *C. concatenata* and *E. americanum* are at risk for phenological mismatches, including pollinator interactions and other species that may rely on the phenophases of these flowers, such as *Pieris virginensis* (the West Virginia white butterfly). This research demonstrates the plasticity of phenological response to a variety of climatic variables, the usefulness of using herbarium specimens to reconstruct flowering dates over a topographically variable area, and the contrasting effects of climate change on high elevation regions of West Virginia. As a key component in deciduous forest communities, spring ephemerals in West Virginia are valuable indicators of shifting phenology in this ecosystem.

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