

## RESEARCH ARTICLE

# Beyond the usual climate? Factors determining flowering and fruiting phenology across a genus over 117 years

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## Abstract

**Premise:** Although changes in plant phenology are largely attributed to changes in climate, the roles of other factors such as genetic constraints, competition, and self-compatibility are underexplored.

**Methods:** We compiled >900 herbarium records spanning 117 years for all eight nominal species of the winter-annual genus *Leavenworthia* (Brassicaceae). We used linear regression to determine the rate of phenological change across years and phenological sensitivity to climate. Using a variance partitioning analysis, we assessed the relative influence of climatic and nonclimatic factors (self-compatibility, range overlap, latitude, and year) on *Leavenworthia* reproductive phenology.

**Results:** Flowering advanced by ~2.0 days and fruiting by ~1.3 days per decade. For every 1°C increase in spring temperature, flowering advanced ~2.3 days and fruiting ~3.3 days. For every 100 mm decrease in spring precipitation, each advanced ~6–7 days. The best models explained 35.4% of flowering variance and 33.9% of fruiting. Spring precipitation accounted for 51.3% of explained variance in flowering date and 44.6% in fruiting. Mean spring temperature accounted for 10.6% and 19.3%, respectively. Year accounted for 16.6% of flowering variance and 5.4% of fruiting, and latitude for 2.3% and 15.1%, respectively. Nonclimatic variables combined accounted for <11% of the variance across phenophases.

**Conclusions:** Spring precipitation and other climate-related factors were dominant predictors of phenological variance. Our results emphasize the strong effect of precipitation on phenology, especially in the moisture-limited habitats preferred by *Leavenworthia*. Among the many factors that determine phenology, climate is the dominant influence, indicating that the effects of climate change on phenology are expected to increase.

## KEYWORDS

Brassicaceae, gladdress, global change, *Leavenworthia*, phenological shift, precipitation, relative humidity, temperature, variance partitioning, winter annual

Earlier plant reproduction has been well documented in response to climate change (Menzel et al., 2006; Parmesan, 2006; Miller-Rushing and Primack, 2008; Ganjurjav et al., 2020), with some species advancing phenology by up to 2.5 days per °C over 30 years (Menzel et al., 2006). Climate cues contribute significantly toward

phenological variance. For example, the accumulation of days above or below certain temperature thresholds has a strong effect on the timing of germination, leaf-out, flowering, and fruiting (Pemadasa and Lovell, 1974; Müller and Schmitt, 2018; Meng et al., 2021). While advanced phenology due to warming is the most commonly

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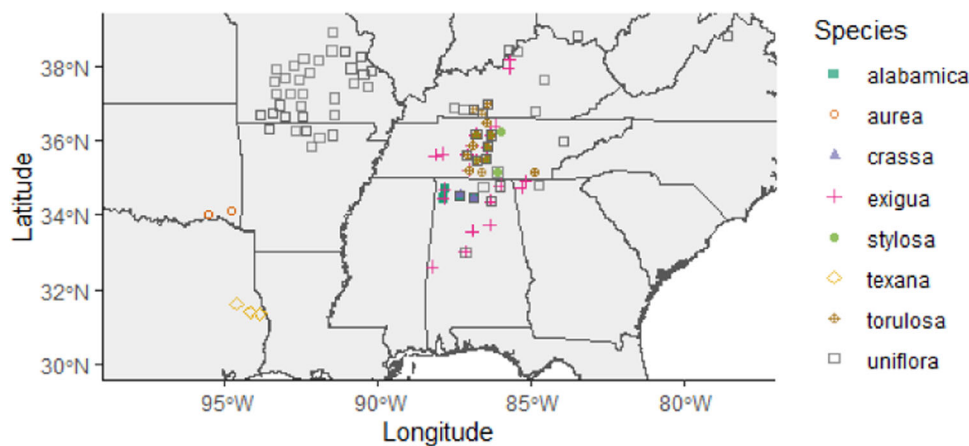
documented response (Menzel et al., 2006; Miller-Rushing and Primack, 2008; Suonan et al., 2017; Piao et al., 2019), other studies found contradictory outcomes, including delayed phenology due to an unmet chilling requirement (Yu et al., 2010; Hart et al., 2014) and advanced phenology following regional cooling (Banaszak et al., 2020). Changes in precipitation also interact with shifting temperature to influence phenology in varied ways (Ganjurjav et al., 2020; Zettlemoyer et al., 2021; Currier and Sala, 2022), especially in moisture-limited environments (Lesica and Kittelson, 2010; Shen et al., 2015). Factors such as latitude and year of observation are highly correlated with climatic variation and are statistically associated with plant phenology (Munguía-Rosas et al., 2011; Yue et al., 2015). Focusing solely on the effect of climate, however, ignores the possible influence of other variables. For example, the timing of leaf-out in the Northern Alps of Europe is best predicted when latitudinal variation in photoperiod is incorporated alongside temperature (Meng et al., 2021).

While climate change is a well-known driver of phenological shifts, we do not yet understand the relative influence of climatic and non-climatic factors on variance in reproductive phenology. Phenological variation among species is well documented (Harrison et al., 2015; Cole and Sheldon, 2017), and this variation may reflect both climatic and species-, population-, and community-level factors. For example, the degree of relatedness among species can shape their reproductive timing (e.g., Rafferty and Nability, 2017; Mazer et al., 2021). Closely related species may flower at similar times due to genetic constraints (Brearley et al., 2007; Davis et al., 2015) or ecological and environmental factors (Gavini et al., 2021). On the other hand, related, co-occurring taxa may avoid coflowering to better access pollinators and avoid heterospecific pollen transfer (e.g., Campbell, 1985a, b; Stone et al., 1998). Finally, differing reproductive traits may also drive phenological variation (Gorman et al., 2020). For example, self-incompatibility can affect reproductive timing because

the flowering time of individuals that rely on outcrossing is constrained by the phenology of conspecifics and their pollinators (Bartomeus et al., 2011).

Understanding phenological responses to different environmental factors requires long-term data on the timing of plant reproductive events. Herbarium (Davis et al., 2015; Willis et al., 2017; Meineke et al., 2018; Austin et al., 2022) and citizen science records (Belitz et al., 2020; Iwanycki Ahlstrand et al., 2022) can provide a valuable source of such long-term data. Many herbaria contain records spanning decades, if not centuries, with each record preserving a specimen's unique phenological phase at a certain time and place. Citizen science records are typically more recent but are the fastest growing in biodiversity databases (Barve et al., 2020). Such herbarium and citizen science records can also contain further information relevant to phenological studies, such as the year, species, or coordinates of the collection.

In this study, we used herbarium and citizen science records to examine the relative contribution of climatic and nonclimatic factors on shifts in phenology across the entire taxonomic and spatial distribution of a single genus. *Leavenworthia* (Brassicaceae), commonly known as glade grass, is a genus of herbaceous annuals (Rollins, 1963; Al-Shehbaz and Beck, 2010) found across the southern and southeastern United States (Figure 1). While previous studies have explored the causes and consequences of the unique mating systems of *Leavenworthia* (Solbrig and Rollins, 1977; Busch et al., 2010; Busch and Werner, 2012), less attention has been paid to the factors shaping the reproductive phenology within the genus (Banaszak et al., 2020). Given that four of the eight species in the genus are imperiled or critically imperiled (NatureServe ranks G1 or G2; NatureServe, 2022) and one is listed under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, 2020), understanding this taxon's reproductive timing is key to its conservation. Changing phenology affects the biotic and abiotic conditions under which plants



**FIGURE 1** *Leavenworthia* occurrence records mapped by species. Each point corresponds to the centroid of the county of collection. Darker and/or layered points indicate numerous records collected from a single county. Note the highly restricted range of most species in contrast to the broader distribution of *L. uniflora*, the only species to span the Mississippi River.

reproduce, affecting factors ranging from pollination to seed success (Morellato et al., 2016). Discovering what shapes *Leavenworthia* phenology can also help us better understand phenological variation in other winter annuals.

In our study, we aimed to quantify changes over time in *Leavenworthia* flowering and fruiting, determine the climatic variables and periods to which phenology was most sensitive, and examine the relative influence of climatic and nonclimatic factors on *Leavenworthia* reproductive phenology. By analyzing 924 records spanning 117 years, we predicted that *Leavenworthia* flowering and fruiting have advanced in response to warming temperatures. Given the well-documented but non-uniform relationship between climate and phenology, we also predicted that climatic variation will have the strongest influence on flowering and fruiting dates, but with substantial variation explained by nonclimatic factors such as self-compatibility and *Leavenworthia* species richness. To assess these predictions, we partitioned the variance in flowering and fruiting phenology separately using a set of climatic variables (temperature, relative humidity, precipitation, year, and latitude) plus species- (species, self-compatibility) and community-level factors (species richness) that we expected to influence phenology.

## MATERIALS AND METHODS

### Study system

*Leavenworthia* comprises eight nominal species that are largely endemic to glade habitats across the southern and southeastern United States (Figure 1). These habitats are characterized by shallow rocky soil, limestone bedrock, and extreme variation in local temperature and moisture (Rollins, 1963). Individuals of the species are often found in the areas with the shallowest soils in micro-depressions and seeps that retain moisture during the spring reproductive period (A. B. Smith, personal observations). *Leavenworthia* are winter annuals: seeds are dispersed during late spring and early summer, then germinate in the fall, and individuals overwinter as quiescent rosettes before flowering begins in the early spring (Baskin and Baskin, 1971). Among the eight *Leavenworthia* species, there is high variation in the degree of sympatry: Species diversity and co-occurrence are concentrated in the Central Basin of Tennessee, while one species, *L. uniflora*, encompasses nearly the entire range of the genus and four species have very restricted ranges (1–4 counties for each, <2000 km<sup>2</sup>; Koelling and Mauricio, 2010).

A notable characteristic of *Leavenworthia* is the variation in self compatibility among species. While most are self-compatible (SC), *L. stylosa* is self-incompatible, requiring outcrossing to reproduce (Rollins, 1963; Beck et al., 2006). In *L. uniflora*, *L. alabamica*, and *L. crassa*, self-compatibility varies by population (Lloyd, 1965; Busch, 2005; Busch and Werner, 2012).

### Data collection

Our initial data set to assess *Leavenworthia* phenology consisted of 1214 *Leavenworthia* herbarium records collected between 1877 and 2001. This data set comprised all specimens at 10 herbaria, which were either geographically relevant (BRIT, IND, LL, MO, SMU, UNA, VDB) and/or large national collections known to archive large sets of specimens cited in *Leavenworthia* studies (G, NY, US) (Rollins, 1963). At the time of compilation, we estimated that the data set contained ~80% of all *Leavenworthia* herbarium specimens. A subset of this data set was previously used to assess phenology change in *L. stylosa* (Banaszak et al., 2020).

To increase sampling from the 21st century, we supplemented these data with *Leavenworthia* occurrence records downloaded from the Global Biodiversity Information Facility (GBIF.org, 2021; <https://doi.org/10.15468/dl.v6zf9r>), including both herbarium records and iNaturalist observations made between 2001 and 2019 (the most recent year for which climate data were available at the time of analysis). In total, we obtained 212 new records from GBIF and added them to the raw data set ( $N = 1426$ ).

For a sizable portion of records, the precise coordinates of collection could not be determined due to incomplete locality descriptions or endangered species protections. To ensure a uniform analysis, we obtained coordinates for our records by georeferencing each record's coordinates to the centroid of the county in which the record was collected, which was the finest level of spatial resolution we could achieve across all samples. Standardizing our record coordinates to the county level sacrifices a degree of detail, specifically in relating climate data to location. However, counties in this region inhabited by *Leavenworthia* have low topographic heterogeneity, and given the characteristic distance of spatial autocorrelation in temperature and precipitation (several 100 km; Fick and Hijmans, 2017), we expected the spatial autocorrelation of climate conditions within a county to be high enough to not bias our results (Getis, 2010). We excluded any records for which the species, phenophase, date or county of collection, self-compatibility, or monthly climate data could not be determined. After filtering, we had 924 records.

### Phenocoding

We assigned each *Leavenworthia* record a phenophase based on its observed reproductive status: flowering, fruiting, both, or neither. This categorical scoring method has been used widely (Diez et al., 2014; Davis et al., 2015; Banaszak et al., 2020) and accurately and efficiently assesses phenophase for a large number of specimens (Pearson, 2019). Flowering was defined by anthesis, or open flowering. Fruiting included both immature and mature fruits. In cases where multiple individuals were present on one herbarium sheet or photo, phenophase was scored

collectively for all present individuals. Of our 924 complete records, 647 were scored as flowering, 849 were scored as fruiting, and 1 was scored as neither. Flowering and fruiting co-occurred in most specimens.

## Self-compatibility

We assigned self-compatibility based on each record's species and/or population. All records of *L. stylosa* were designated as self-incompatible (SI), as defined by Beck et al. (2006). All *L. exigua*, *L. torulosa*, *L. aurea*, and *L. texana* records were designated as self-compatible (SC), as defined by Rollins (1963) and Beck et al. (2006). Three *Leavenworthia* species are known to have both SI and SC populations: *L. alabamica*, *L. crassa*, and *L. uniflora*. For records of these species, we attempted to assign self-compatibility based on subspecies, locality information, or precise collection coordinates. All records identified as *L. alabamica* var. *brachystyla* or *L. crassa* var. *elongata* were assigned SC status (Lloyd, 1965). For records not identified to subspecies, we determined the population from which the record was collected. Busch (2005) identified selfing and nonselfing populations of *L. alabamica*, listed coordinates for each population, and named them based on nearby localities. Where possible, we matched locality description and/or precise collection coordinates of our *L. alabamica* records to the populations outlined by Busch (2005) to determine self-compatibility. We did the same for *L. crassa* records, according to the population names and coordinates listed by Lloyd (1965). If the locality description or coordinates of a record did not exactly match those provided for a specific population, we did not attempt to assign self-compatibility for that record.

## *L. uniflora*

*Leavenworthia uniflora* is the most widespread *Leavenworthia* species, featuring notable geographic variation in its reproductive habits. Populations of *L. uniflora* west of the Mississippi River self-fertilize almost exclusively, while eastern populations have a mixed mating system (Busch and Werner, 2012). We conducted a *t*-test using base R (version 4.2.0, R Core Team, 2022) to determine whether the mean day of year of *L. uniflora* reproduction differed based on population (east or west of the Mississippi River; Appendix S1, Figure S1). However, given that both eastern and western populations are self-compatible and do self-fertilize (Beck et al., 2006; Busch and Werner, 2012), we designated all *L. uniflora* records as SC.

## Climatic data

Using the year and coordinates of each *Leavenworthia* record, we obtained monthly climatic data for our records

using ClimateNA v6.40a (Wang et al., 2016), which provides monthly-resolution estimates of climate interpolated across North America from 1901 to the present. Using the coordinates for every county centroid in our data set, we extracted monthly (1) mean temperatures, (2) total precipitation, and (3) mean annual relative humidity (RH) across all available and sampled years (1902–2019). We chose these climatic variables due to their hypothesized or empirical influence on germination and/or reproductive phenology of *Leavenworthia* (Rollins, 1963; Baskin and Baskin, 1971; Solbrig and Rollins, 1977). Keeping the winter-annual habit of this genus in mind, we defined fixed seasonal periods in which we calculated relevant climatic variables: the summer seed dormancy period (June–August, in the year before collection), the fall germination period (September–November, before collection), the winter quiescent period (December of the year prior, plus January–February of the year of collection), and the spring reproductive period (March–May of the year of collection; Baskin and Baskin, 1971; Banaszak et al., 2020). Mean monthly temperature and RH were each averaged across each climate period. Monthly precipitation was summed across each period. Our final data set included 647 flowering and 849 fruiting records between 1902 and 2019, each matched with climate data corresponding to stages in the plant's life history.

## Climatic analysis

To identify the most predictive climatic variables for inclusion in the final variance partitioning model, we ran two separate sets of models—one for flowering and one for fruiting with all species combined—using the *lm* linear regression function in R to model the day of year (DOY) of flowering and fruiting against every possible pairwise combination of average temperature with total precipitation or RH, each across all four of the fixed climatic periods. Climatic variables were scaled and centered before analysis. We compared the  $R^2$  values across the resulting 32 models in each set. The flowering and fruiting models with the highest  $R^2$  in each set were used in the final variance partitioning analyses.

The climate variables from the most predictive models were plotted against year to determine the change in relevant climate over time. The most-predictive climatic variables were also plotted against the day of year of both flowering and fruiting to assess phenological sensitivity or against the change in phenophase timing per unit change in each climate variable (Davis et al., 2015). We used the *sma* function in the R package *smatr* (version 3.4-8; Warton et al., 2012) to conduct one-sample tests for differences in slopes with all species combined between flowering and fruiting, determining whether the phenophases differed in terms of phenological sensitivity to climate (DOY vs. unit climate) or rate of phenological change across years (DOY vs year). We also used the *sma* function to test for

differences in phenological sensitivity or rate of change among *Leavenworthia* species.

To test for collinearity between climatic and the continuous non-climatic variables, we used the `cor` function in base R, generating a matrix displaying the Pearson's correlation coefficient between every possible climate variable pair, plus year and latitude.

## Variance partitioning

We conducted separate variance partitioning analyses for flowering and fruiting dates. Our final models consisted of a linear regression comparing the DOY of flowering or fruiting against the two climatic variables found to be most explanatory above (mean temperature and total precipitation during the reproductive period) and our selection of relevant species- (species and self-compatibility), population- (year and latitude), and community-level variables (the number of *Leavenworthia* species recorded in the county of collection: species richness), year, and latitude. All continuous variables were scaled and centered before analysis, but species and self-compatibility were categorical and were thus not transformed. To account for variation in species responses to climate, we also included interaction terms between species and mean reproductive temperature and between species and total reproductive precipitation. Preliminary models also included identity of the phenology scorer (KBB or JBB) as a covariable to test for bias between individuals responsible for phenophase scoring. Because scorer had no significant effect, we dropped it from the final models.

We ran a variance partitioning analysis on the flowering and fruiting models in R (version 4.2.0, R Core Team, 2022) using the `calc.relimp` function in the `relaimpo` package (version 2.2-6; Ulrike 2006). In this procedure, all possible subsets of models are run, then differences in  $R^2$  between models with and without a focal variable are calculated, resulting in the total amount of  $R^2$  attributable to that

variable (Chevan and Sutherland, 1991). We divided the  $R^2$  attributable to each variable by the model's total  $R^2$  to determine the percentage of total variance explained attributable to each variable. Finally, we used the `boot.relimp` function to calculate 95% confidence intervals and test for significant differences between variables via 1000 bootstrapping replicates (Fox and Monette, 2002; Ulrike, 2006).

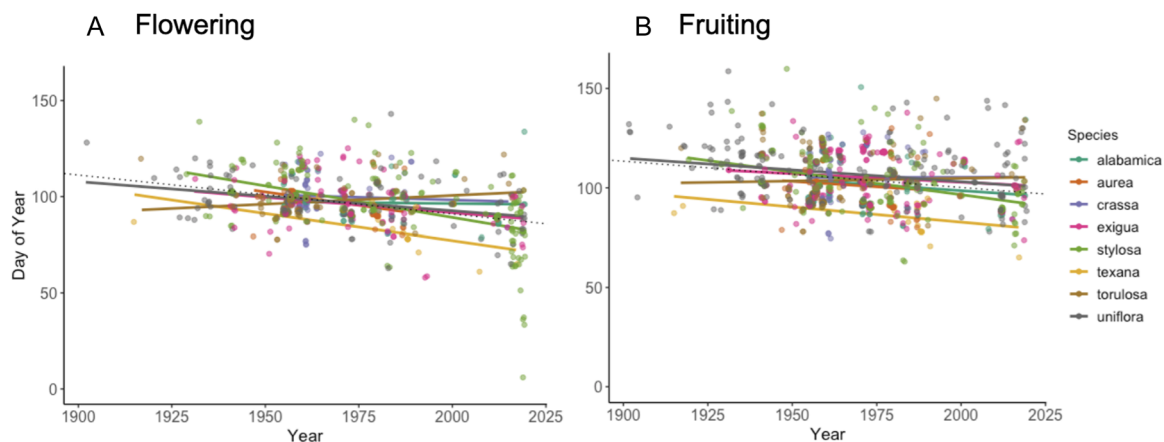
## RESULTS

*Leavenworthia* flowering and fruiting dates each advanced significantly over 117 years (Figure 2). Flowering advanced by approximately 2.0 days per decade (slope  $-0.20 \pm 0.02$  d/yr,  $r^2 = 0.12$ ,  $df = 645$ ,  $P < 0.005$ ), while fruiting advanced approximately 1.3 days per decade (slope of  $-0.13 \pm 0.02$  d/yr,  $r^2 = 0.04$ ,  $df = 847$ ,  $P < 0.005$ ). Flowering date advanced significantly faster than fruiting (likelihood ratio = 6.46,  $df = 2$ ,  $P < 0.05$ ). Species' rates of phenological change were also significantly different (likelihood ratio = 54.57,  $df = 7$ ,  $P < 0.005$ ).

## Climatic analysis

The climatic variables included in each model had absolute values  $< 0.7$  for Pearson's  $r$  product-moment pairwise correlations (Appendix S1, Figure S2;  $|r| \leq 0.10$  for reproductive temperature and precipitation across flowering [ $df = 645$ ] and fruiting [ $df = 847$ ],  $P < 0.005$  for both), and so were not expected to confound one another due to collinearity (Murray and Conner, 2009; Dormann et al., 2013). Pairwise Pearson's  $r$  correlations between the climatic variables, year, and latitude also fell below 0.7 (Dormann et al., 2013).

Comparing the phenological responses to 32 combinations of climatic variables across four periods of *Leavenworthia*'s



**FIGURE 2** Flowering and fruiting day of year over time. (A) Flowering and (B) fruiting date plotted against year of collection. Each point represents a *Leavenworthia* record, color-coded by species. Colored regression lines show phenological shifts by species. Dotted black line is the best fit across all records within the genus, illustrating overall phenological change over time (flowering =  $-0.20 \pm SE 0.02$  (SE) days/year; fruiting =  $-0.13 \pm 0.02$  days/year).

**TABLE 1** Flowering and fruiting models ranked by  $R^2$ . Flowering and fruiting dates were modeled against every two-variable combination of mean temperature, mean relative humidity, and total precipitation across four seasons (germination, September–November; quiescent, December–February; reproductive, Mar–May; and dormancy, June–August). The five best-predictive flowering models and the five best-predictive fruiting models are listed above, ranked from highest  $R^2$  to lowest  $R^2$ . The climate variables from the best-predictive models were used with nonclimatic variables in the variance partitioning analyses.

Model	Temperature slope ( $\pm$ SE)	Moisture slope ( $\pm$ SE)	$R^2$
<b>Flowering</b>			
Reproductive temperature + Reproductive precipitation	-3.36 $\pm$ 0.46	6.49 $\pm$ 0.46	0.287
Quiescent temperature + Reproductive precipitation	-2.48 $\pm$ 0.48	5.91 $\pm$ 0.48	0.258
Germination temperature + Reproductive precipitation	-2.16 $\pm$ 0.47	6.55 $\pm$ 0.47	0.252
Dormancy temperature + Reproductive precipitation	-1.42 $\pm$ 0.47	6.54 $\pm$ 0.47	0.238
Quiescent temperature + Germination relative humidity (RH)	-4.27 $\pm$ 0.50	-3.22 $\pm$ 0.50	0.139
<b>Fruiting</b>			
Reproductive temperature + Reproductive precipitation	-4.62 $\pm$ 0.42	5.96 $\pm$ 0.42	0.294
Quiescent temperature + Reproductive precipitation	-3.87 $\pm$ 0.45	5.25 $\pm$ 0.45	0.259
Germination temperature + Reproductive precipitation	-3.53 $\pm$ 0.43	6.32 $\pm$ 0.43	0.252
Dormancy temperature + Reproductive precipitation	-2.67 $\pm$ 0.44	6.31 $\pm$ 0.44	0.227
Quiescent temperature + Germination precipitation	-5.41 $\pm$ 0.45	-2.46 $\pm$ 0.45	0.166

annual life history (Table 1; all 32 models ranked in Appendix S1, Table S3), we found that average temperature and total precipitation during the spring reproductive season best predicted both flowering date ( $R^2 = 0.287$ ,  $df = 645$ ,  $P < 0.005$ ) and fruiting date ( $R^2 = 0.294$ ,  $df = 847$ ,  $P < 0.005$ ). Based on these findings, reproductive temperature and precipitation were included as climatic variables in our final variance partitioning models.

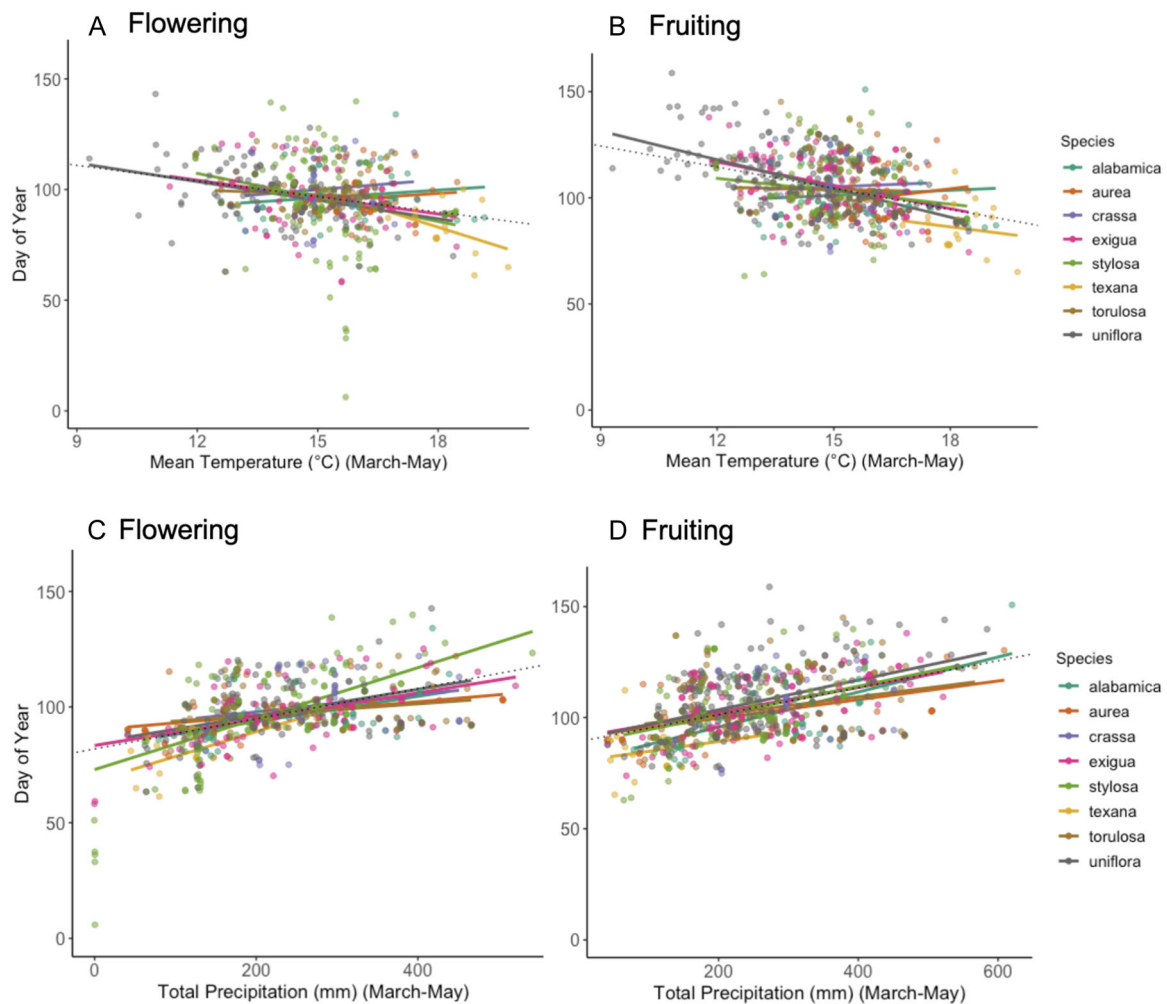
We found that 1°C warming during the spring reproductive period led to a  $2.34 \pm 0.35$  (mean  $\pm$  SE) day advance in flowering ( $r^2 = 0.06$ ,  $df = 645$ ,  $P < 0.005$ ) and a  $3.33 \pm 0.30$  day advance in fruiting ( $r^2 = 0.12$ ,  $df = 847$ ,  $P < 0.005$ ) across the genus (Figure 3). Average reproductive season temperature in counties inhabited by *Leavenworthia* increased by  $0.02^\circ\text{C} \pm 0.002$  per year ( $r^2 = 0.08$ ,  $df = 922$ ,  $P = 0.005$ ), or approximately  $2.09^\circ\text{C}$  over 117 years (Figure 4). For a 1-mm increase in total precipitation during the reproductive period, flowering was delayed by  $0.070 \pm 0.005$  days ( $r^2 = 0.23$ ,  $df = 645$ ,  $P < 0.005$ ) and fruiting by  $0.060 \pm 0.004$  days ( $r^2 = 0.19$ ,  $df = 847$ ,  $P < 0.005$ ) (Figure 3). However, total precipitation during the reproductive period decreased by  $\sim 83$  mm over 117 years (Figure 4;  $0.71 \text{ mm} \pm 0.14$  per year,  $r^2 = 0.03$ ,  $df = 922$ ,  $P = 0.005$ ).

Flowering date was significantly more sensitive to changes in both reproductive temperature (likelihood ratio = 11.05,  $df = 2$ ,  $P < 0.005$ ) and precipitation (likelihood ratio = 30.59,  $df = 2$ ,  $P < 0.005$ ) than fruiting. Species also varied in both the direction and magnitude of their phenological sensitivity to reproductive warming (likelihood ratio = 82.71,  $df = 7$ ,  $P < 0.005$ ) for both flowering and fruiting (Figure 3 A, B). Three species significantly advanced phenology: *L. uniflora* (likelihood ratio = -8.45,  $P < 0.005$ ),

*L. stylosa* (-16.57,  $P < 0.005$ ), *L. exigua* (-9.83,  $P < 0.005$ ). The phenology for five species had no significant response to spring warming—*L. torulosa* (-14.17,  $P = 0.73$ ), *L. texana* (-17.94,  $P = 0.32$ ), *L. alabamica* (10.32,  $P = 0.58$ ), *L. aurea* (18.65,  $P = 0.51$ ), *L. crassa* (16.03,  $P = 0.55$ ). Species' responses to reproductive period precipitation varied in magnitude (likelihood ratio = 47.52,  $df = 7$ ,  $P < 0.005$ ), but all advanced in response to decreasing precipitation (Figure 3C, D).

## Variance partitioning

The full models with the best climatic covariates and with all nonclimatic covariates predicted 35.4% of total variance in the day of year of flowering ( $df = 619$ ,  $P < 0.005$ ), and 33.9% of total variance in fruiting ( $df = 821$ ,  $P < 0.005$ ). Reproductive season precipitation was the best predictor of both flowering date and fruiting date (Figure 5). Precipitation accounted for 51.3% of the total explained variance in flowering date ( $R^2$  attributable to reproductive precipitation = 0.196, bootstrap lower and upper 95% confidence intervals: 0.147, 0.246), and 44.6% of the total variance in fruiting date ( $R^2 = 0.161$ , lower = 0.115 upper = 0.203). Year was the second-best predictor of flowering date, accounting for 16.6% of explained variance ( $R^2 = 0.063$ , lower = 0.037, upper = 0.097). In contrast, year only explained 5.4% of fruiting date variance ( $R^2 = 0.019$ , lower = 0.007, upper = 0.036). Reproductive season temperature was the second-best predictor of fruiting date ( $R^2 = 0.070$ , lower = 0.046, upper = 0.098; 19.3% of explained variance) and the third-best predictor of flowering date ( $R^2 = 0.041$ , lower = 0.021, upper = 0.070; 10.6% of explained variance). Latitude was the third-best



**FIGURE 3** Sensitivity of *Leavenworthia* flowering and fruiting date to reproductive period climate. (A, C) Flowering and (B, D) fruiting date plotted against mean temperature (A, B) and total precipitation (C, D) during the spring reproductive period (March–May). Each point represents a *Leavenworthia* record, color-coded by species. Colored regression lines demonstrate species phenological responses to changes in temperature and precipitation. Dotted black line is the best fit across all *Leavenworthia* records, illustrating mean phenological sensitivity across the genus to spring warming ( $-2.3 \pm 0.35$  days/°C for flowering;  $-3.3 \pm 0.30$  days/°C for fruiting) or drying ( $-0.07 \pm 0.004$  days/mm for flowering;  $-0.06 \pm 0.004$  days/mm for fruiting).

predictor of fruiting date ( $R = 0.054$ , lower = 0.036, upper = 0.076; 15.1% of explained variance) but had nominal influence on flowering. Interactions between species identity and climate affected flowering more than fruiting (total  $R^2$  from interactions = 0.05 or 13.8% of total explained flowering variance vs.  $R^2 = 0.02$  or 5.1% of fruiting).

According to our bootstrap analysis, reproductive precipitation explained significantly more flowering and fruiting variance than any other variable. Nonclimatic factors (species, richness, and self-compatibility) combined were comparatively weak predictors, accounting for only 5.3% of explained flowering variance and 10.5% of fruiting (Figure 5).

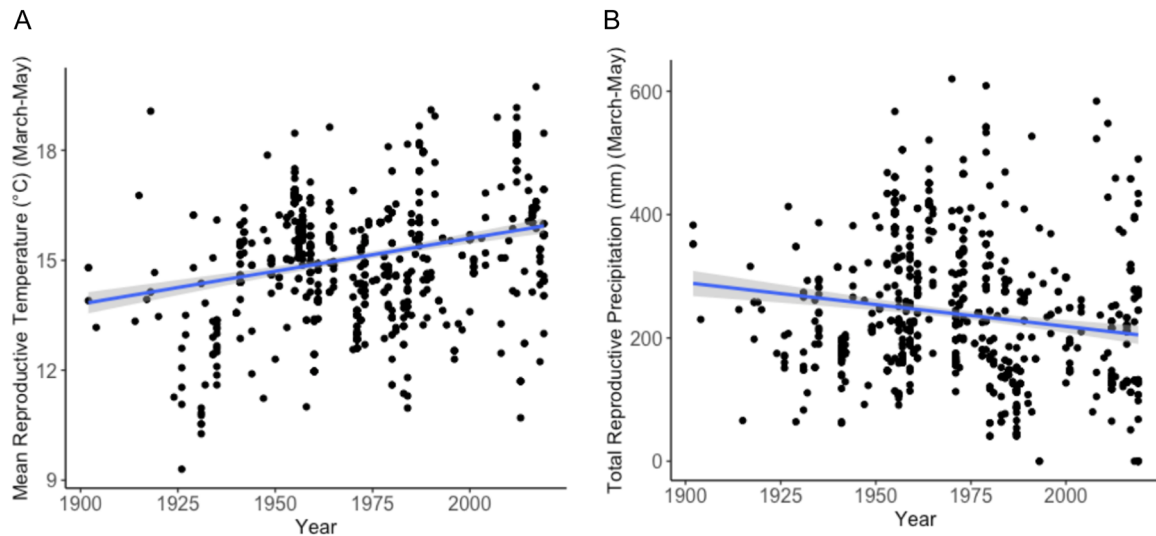
### *L. uniflora*

We found significant phenological differences between eastern and western populations of *L. uniflora* ( $t = -4.26$ ,

$df = 155.46$ ,  $P < 0.005$ ; Appendix S1, Figure S1). The eastern population of *L. uniflora* had a mean reproductive day of year of 102, while the mean day of year in the western population was 111. However, given that both species identity collectively explained comparatively little phenological variance, we chose not to incorporate eastern vs western population as an additional variable in our variance partitioning models.

## DISCUSSION

In this study of phenological changes within the genus *Leavenworthia* and the relative influence of climatic and nonclimatic factors on flowering and fruiting dates, total spring precipitation best predicted both flowering and fruiting dates. Variables of secondary importance included year, reproductive temperature, and interactions between



**FIGURE 4** Change in reproductive season climate over 117 years. Mean temperature (A) and total precipitation (B) during the spring reproductive season (March–May) plotted against year. Each dot represents the calculated spring climate for a single *Leavenworthia* record. Blue lines are best fit across all records, demonstrating change in temperature ( $0.02 \pm 0.35^\circ\text{C}/\text{year}$ ,  $P < 0.005$ ) and precipitation ( $-0.71 \pm 0.14 \text{ mm}/\text{year}$ ,  $P < 0.005$ ) over time. Gray shading illustrates standard error.

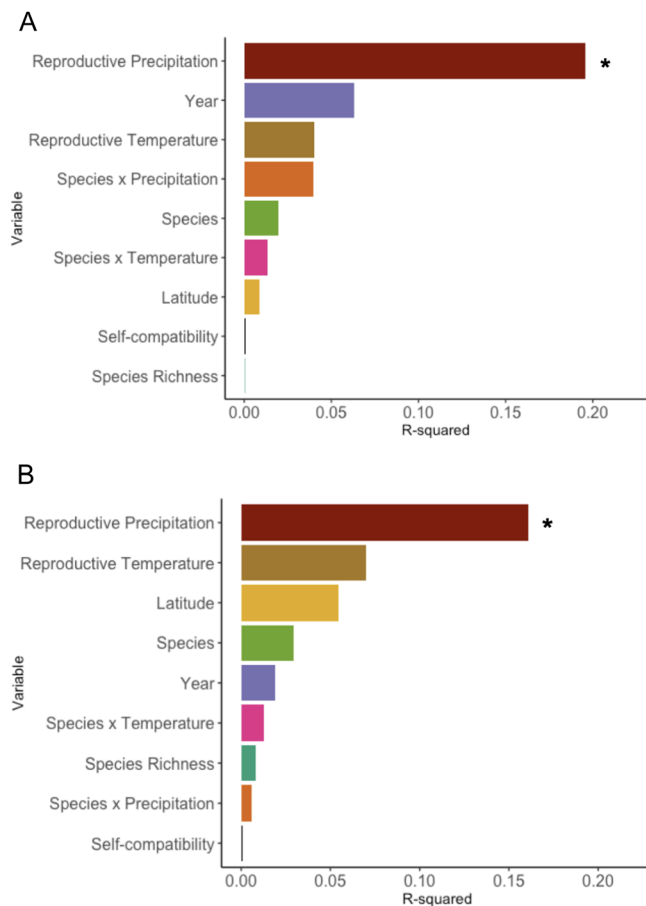
species and precipitation for flowering and between reproductive temperature and latitude for fruiting. These results align with numerous studies that found that shifts in climatic cues prompt changes in plant phenology (e.g., Miller-Rushing and Primack, 2008; Wilczek et al., 2010; Banaszak et al., 2020; Love and Mazer, 2021). We found that a 100-mm decrease in spring precipitation was correlated with a 7-day advance in flowering and 6-day advance in fruiting. For every  $1^\circ\text{C}$  of spring warming, *Leavenworthia* flowering and fruiting advanced by more than 2 days, although there was significant variation between species. Over our 117-year study period, springtime temperature in areas inhabited by *Leavenworthia* increased by  $2.09^\circ\text{C}$ , while spring precipitation declined by  $\sim 83 \text{ mm}$ . As a result, *Leavenworthia* reproduction has advanced by approximately 2 weeks. Nonclimatic factors (species, self-compatibility, and species richness) explained the lowest proportion of the total phenological variance.

### Phenological predictors and trends

This study is one of the few to assess differences in phenological shifts across an entire genus (e.g., Debussche et al., 2004). While phenological advances in a single *Leavenworthia* species—*L. stylosa*—have been previously reported (Banaszak et al., 2020), ours is the first to quantify climatic sensitivity and the rate of phenological change for all *Leavenworthia*. Our results somewhat contrast with previous work on *L. stylosa*, which found advanced phenology in response to local cooling and increased precipitation using a subset of the same herbarium data used here (Banaszak et al., 2020). We and Banaszak et al. (2020) found a comparable rate of advancement (1–2 days

per decade); in our study, however, this phenological advance is linked to spring warming and drying across the range of the genus, rather than to year-round cooling within the restricted region examined by Banaszak et al. (2020). We did find, however, that *Leavenworthia* species varied significantly in their rates of phenological advance and climate sensitivities, with three species (*L. aurea*, *L. alabamica*, and *L. crassa*) not responsive to warming. Additionally, differences in the spatial and temporal resolution of climatic data may account for the differing results between our study and Banaszak et al. (2020). We found significant differences between flowering and fruiting in terms of the rate of phenological shifts (days per year) and phenological sensitivity to climate (days per unit climate). Additionally, our variance partitioning revealed notable differences in the factors best explaining variance in flowering versus fruiting dates. Flowering and fruiting are distinct phenophases, and shifts in their timing can create distinct evolutionary and ecological consequences. While shifts in flowering time may affect coflowering and pollination dynamics (Elzinga et al., 2007; Sherry et al., 2007; Kehrberger and Holzschuh, 2019; Rudolf, 2019), a change in fruiting time affects the conditions to which seeds are exposed, ultimately shaping dispersal, dormancy length, and germination times (Lacey et al., 2003; Vergara-Tabares et al., 2016; Du et al., 2020). We found that temperature and latitude explained a greater portion of fruiting variance than flowering. Baskin and Baskin (1971) documented a temperature-sensitive seed dormancy that varies with seed age in *L. torulosa*, *L. stylosa*, and *L. uniflora*. The sensitivity of fruiting time to temperature could be a mechanism to control the conditions to which seed is exposed, which in turn shapes seed dormancy and affects the likelihood of germination. Determining the specific factors that shape





**FIGURE 5** Variance partitioned in *Leavenworthia* (A) flowering and (B) fruiting dates. The total variance explained by our flowering (A) and fruiting (B) models, partitioned by variable. Bars indicate the proportion of model variance explained by each variable. Asterisks indicate variables that explain significant portions of variance. The flowering model explained 35.4% of phenological variance ( $df = 619$ ,  $P < 0.005$ ) and the fruiting model explained 33.9% of variance ( $df = 821$ ,  $P < 0.005$ ). Variables include the temperature and moisture variables of the life stage with greatest explanatory power, species identity, county-level *Leavenworthia* species richness, and latitude and year of each record.

different reproductive events helps to better understand the implications of phenology shifts going forward.

## Influence of climate

Total precipitation during the spring reproductive period (March–May) was the strongest predictor of both flowering and fruiting times. This window of sensitivity coincides with flowering and fruiting dates of the majority of specimens in our data set (Appendix S1, Figure S4) and aligns with a multitude of studies suggesting that spring climatic conditions have an impact on the reproductive phenology of plants (Miller-Rushing and Primack, 2008; Lesica and Kittelson, 2010; Cook et al., 2012a, b) including winter annuals (Banaszak et al., 2020) and range-limited species (Zettlemoyer et al., 2021).

The outsized influence of spring precipitation compared to temperature is of note. While flowering and fruiting dates were positively correlated with reproductive season precipitation, spring precipitation has actually decreased over time. Accordingly, *Leavenworthia* phenology has advanced. This sensitivity to spring moisture could be at least partially attributed to the preference of *Leavenworthia* for limestone prairies and barrens, or glades. Moisture varies widely in the shallow glade soils, ranging from extremely dry in the summers to fully saturated by the early spring (Kucera and Martin, 1957; Rollins, 1963). Given this high intra-annual variability, *Leavenworthia* is likely highly sensitive to moisture changes during the reproductive period. The strong influence of spring precipitation aligns with previous *Leavenworthia* studies, which found that phenological response to temperature and moisture throughout the year depends specifically on reproductive season precipitation (Banaszak et al., 2020). Banaszak et al. (2020) noted that in years when fall and winter were wetter and spring was warmer and drier, flowering of *L. stylosa* advanced. We detected these same climatic trends—increased fall and winter precipitation (Appendix S1, Figure S5), decreased spring precipitation, and increased spring temperature—across the range of the genus since 1902. Our results add to the growing body of literature highlighting the strong but complex effect of precipitation on phenology, where changes in moisture interact with temperature to produce varied and unexpected phenological changes (Ganjurjav et al., 2020; Zettlemoyer et al., 2021; Currier and Sala, 2022).

Spring temperature was also an important predictor of *Leavenworthia* phenology, accounting for the second highest proportion of fruiting variance and third highest proportion of flowering variance. Rising spring temperatures have been widely associated with advanced phenology (Menzel et al., 2006; Miller-Rushing and Primack, 2008; Lesica and Kittelson, 2010; Yu et al., 2010; Cook et al., 2012b), especially in spring-flowering species. However, *Leavenworthia* species varied significantly in their responses to warming spring temperature, with three species significantly advancing phenology (*L. uniflora*, *L. stylosa*, *L. exigua*, *L. torulosa*, *L. texana*) and five species phenologically nonresponsive (*L. aurea*, *L. alabamica*, *L. crassa*, *L. torulosa*, and *L. texana*). Earlier studies on the genus (Baskin and Baskin, 1971) revealed that *L. aurea*, endemic to southeastern Oklahoma, varied from more northern and eastern species in their seed dormancy and temperature requirements for germination. The species least responsive to warming are experiencing the same climatic trends across seasons as those with advanced phenology—spring warming and drying and winter warming and wetting. However, with the exception of *L. torulosa*, these species are some of the southernmost in the genus. The warmer baseline temperatures may shape the responses of these species to warming. Additionally, these are some of the more range-limited species in the

genus. When compared to widespread relatives, extirpated plant species have been shown to have more variable responses to spring warming than their extant counterparts (Zettlemoyer et al., 2021). Given the variation detected between species' flowering and fruiting times, future work could also explore whether seasonality (e.g., early-vs. late-flowering) affects phenological sensitivity to changes in climate. Overall, our results illustrate significant intrageneric variation in phenological sensitivity to climate and that phenological uniformity in related species should not be assumed.

## Factors correlated with climate

Year of collection was the second-most explanatory variable predicting flowering date, explaining marginally more variation than spring temperature. The unique influence of year on flowering date, as opposed to a specific climatic variable, could be attributed to a variety of factors. Year was not highly correlated with the climatic variables used in our models (Appendix S1, Figure S2,  $|r| < 0.26$ ); yet, year could explain a high proportion of phenological variance because it encompasses climatic conditions across all seasons, rather than a single period. Various studies indicate that climate during the fall and/or winter period can also exert a strong influence on spring flowering time (Miller-Rushing and Primack, 2008; Cook et al., 2012a; Zettlemoyer et al., 2021) including in winter annuals (Wilczek et al., 2010) and *Leavenworthia* in particular (Banaszak et al., 2020). In our analysis, the combination of winter quiescent temperature and spring reproductive precipitation was the second-best model for predicting flowering and fruiting date (Table 1). The inclusion of winter climatic changes over time within “year” could explain the variable's relatively high predictive power. Other climatic cues, that were not included here but which are correlated with year, could also shape flowering time. In the preliminary analyses, we assessed multiple climatic periods and variables beyond basic measures of temperature and precipitation, specifically relative humidity. While humidity did not outperform total precipitation as a phenological predictor, other environmental cues that act more directly yet are not traditionally tested, such as soil temperature or moisture, could have an impact on phenology. Year could also be confounded with herbarium biases such as collection effort (Daru et al., 2017), or other phenologically relevant factors, such as community composition, competition, or rates of disease and parasitism that may vary temporally.

Latitude of collection was the third-most predictive variable in our fruiting model, explaining significantly more variance in fruiting date than year and nonclimatic factors. Latitude was not highly correlated with the climatic variables included here ( $|r| < 0.7$ ; Appendix S1, Figure S2). The stronger influence of latitude over year of collection could indicate that, compared to flowering, changing climate over

time has had a smaller impact on *Leavenworthia* fruiting. Instead, differences in temperature or photoperiod across a latitudinal gradient may be stronger determinants of fruiting date. Complex interactions between temperature, precipitation, and photoperiod shape both vegetative and reproductive phenology (Legros et al., 2009; Müller and Schmidt, 2018; Du et al., 2020). For example, photoperiod can moderate leaf-out date in temperate trees to prevent excessively early or late leaf-out due to temperature fluctuations (Meng et al., 2021). Photoperiod could exert a similar effect on *Leavenworthia* fruiting.

## Nonclimatic factors

We expected to find that nonclimatic factors—self-compatibility, species, and species richness in the county of collection—would predict a significant amount of the phenological variance in *Leavenworthia*. However, we found that nonclimatic variables accounted for very little of the total variance explained by our models. These results suggest that climatic changes are eliciting a similar phenological response across *Leavenworthia*, regardless of species, self-compatibility, or intrageneric competition—a finding that suggests strong phylogenetic niche conservatism in *Leavenworthia* phenology (Wiens, 2007; Wiens et al., 2010). Evolutionary history may influence phenological variance, but due to the number of species in the genus we were unable to test for this here.

## Conservation

While only one *Leavenworthia* species—*L. crassa*—is federally listed under the U.S. Endangered Species Act (U.S. Fish and Wildlife Service, 2020), four of the eight species in the genus—*L. crassa*, *L. alabamica*, *L. aurea*, and *L. texana*—meet the NatureServe criteria for “imperiled” or “critically imperiled” (NatureServe, 2022). These species are habitat specialists on spatially restricted glades, which are threatened by diverse factors such as agricultural development, road maintenance, mining and oil extraction, and invasive species (NatureServe, 2022).

Even barring complete habitat destruction, changes in climate likely will create an uncertain future for the genus. Because *Leavenworthia* and other glade specialists already possess adaptations to extreme heat and aridity, it is possible that the impact of climatic changes is negligible (Miller-Struttman, 2011; Brandt et al., 2014). However, our study demonstrates that *Leavenworthia* phenology is, in fact, sensitive to changes in temperature—a finding in line with other glade specialists (Miller-Struttman, 2011) and range-limited species (Zettlemoyer et al., 2021) being more phenologically responsive to climate than their generalist relatives. The limited seed dispersal ability and specific habitat preferences of species in the genus (Rollins, 1963) restrict their capacity for migration,

meaning that *Leavenworthia* species will most likely have to adapt in place to changing climate. The advanced phenology could have an impact on the success of *Leavenworthia* reproduction. For example, Baskin and Baskin (1971) found that young seeds (0–1 month old) will only germinate at or below 15°C; with age, however (3–5 months old), seeds will germinate at temperatures as high as 25°C. This dynamic dormancy works well when seed sets in May: optimal germination temperatures generally do not match ambient temperatures until the early fall, when seedlings are most likely to successfully establish. We found, however, that the majority of *Leavenworthia* fruiting now occurs in April. If fruiting continues to advance, seeds could be 3–5 months old as early as June or July, causing higher maximum germination temperatures to coincide with high ambient temperatures, which could increase the number of *Leavenworthia* germinating in the summer and decrease seedling establishment (Baskin and Baskin, 1971). Ultimately, if advanced phenology of *Leavenworthia* in response to spring climate creates such barriers to reproduction, this advancement, in combination with habitat destruction, could have serious conservation implications for this imperiled genus (Cartwright, 2019). However, the fitness and viability impacts of changing phenology vary widely (Miller-Rushing et al., 2010; Willis et al., 2010; Iler et al., 2019), and more research is required to determine the specific implications of *Leavenworthia* phenology shifts within glade habitats. More broadly, glade habitats are home to a disproportionate number of the region's endemic species (Ware, 2002; Zollner et al., 2005). Since these other species experience the same general climate and threats as *Leavenworthia*, our results suggest that they may also be experiencing changes in reproductive phenology, with similar consequences for their long-term viability.

## CONCLUSIONS

We demonstrated that *Leavenworthia* flowering and fruiting dates advanced significantly across 117 years, largely due to climatic factors including spring precipitation and temperature. A notable portion of variance in flowering was also explained by year and in fruiting by latitude. By assessing phenological change at the genus level, we utilized a unique approach for determining the factors affecting plant reproductive phenology and demonstrated significant interspecific phenological variance. This study contributes to a narrow body of literature on phenological variation with genera (e.g., Debussche et al., 2004) and supports climate as the dominant factor influencing reproductive timing. Our study also demonstrates the importance of separating flowering and fruiting phenophases when testing for factors influencing phenology. We found that flowering and fruiting times changed at different rates, were primarily determined by different climate-associated factors, and varied in their phenological sensitivity to spring warming.

We presented long-term data on the reproductive habits and sensitivities of a highly imperiled genus, with broad implications for future phenological research under continuing climatic changes.

## AUTHOR CONTRIBUTIONS

K.B.: Conceptualization (equal); investigation (lead); formal analysis (equal); original draft preparation (lead). M.A.: conceptualization (equal); investigation (supporting); review and editing (equal). J.B.: investigation (supporting); review and editing (equal). A.Z.: formal analysis (equal); supervision (equal); review and editing (equal). A.S.: conceptualization (equal); methodology (lead); supervision (equal); review and editing (equal).

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## DATA AVAILABILITY STATEMENT


All data and code used in our analysis are available at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.70rxwdc3f>. The specific GBIF search used to acquire *Leavenworthia* records is available at <https://doi.org/10.15468/dl.v6zf9r>.

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### SUPPORTING INFORMATION

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

**APPENDIX S1.** Ancillary analyses and results for *Leavenworthia* phenology.

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