Received: 13 January 2023 | Accepted: 12 July 2023

DOI: 10.1111/1365-2745.14175

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Warmer springs increase potential for temporal reproductive isolation among habitat patches in subalpine flowering plants

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Funding information
Canadian Network for Research and Innovation in Machining Technology, Natural Sciences and Engineering Research Council of Canada; University of Ottawa; National Science Foundation, Grant/Award Number: DEB-0922080, DEB-1354104, DEB-1912006 and DEB-2016749

Handling Editor: Nicole Rafferty

Abstract

1. Flowering phenology can vary considerably even at fine spatial scales, potentially leading to temporal reproductive isolation among habitat patches. Climate change could alter flowering synchrony, and hence temporal isolation, if plants in different microhabitats vary in their phenological response to climate change. Despite the importance of temporal isolation in determining patterns of gene flow, and hence population genetic structure and local adaptation, little is known about how changes in climate affect temporal isolation within populations.
2. Here, we use flowering phenology and floral abundance data of 50 subalpine plant species over 44 years to test whether temporal isolation between habitat patches is affected by spring temperature. For each species and year, we analysed temporal separation in peak flowering and flowering overlap between habitat patches separated by 5–950 m.
3. Across our study species, warmer springs were associated with more temporal differentiation in flowering peaks among habitat patches, and less flowering overlap, increasing potential for temporal isolation within populations.
4. *Synthesis*. By reducing opportunities for mating among plants in nearby habitat patches, our results suggest that warmer springs may reduce opportunities for gene flow within populations, and, consequently, the capacity of plant populations to adapt to environmental changes.

KEYWORDS
flowering, overlap, phenology, plant–climate interactions, reproductive isolation, subalpine plants, synchrony

1 | INTRODUCTION

Anthropogenic climate warming is causing widespread shifts in phenology—the seasonal timing of biological events (Parmesan & Yohe, 2003; Primack et al., 2009). Flowering times, annual emergences and spring migrations have all tended to advance their dates (Inouye et al., 2000), although at different rates for different species (CaraDonna et al., 2014; Prather et al., 2023). Some of these shifts have been shown to disrupt synchrony with interacting species and optimal environmental conditions, potentially affecting population demography and ecosystem processes (Forrest & Miller-Rushing, 2010; Iler, CaraDonna, et al., 2021; Inouye, 2008; Visser & Gienapp, 2019). Phenological shifts could also disrupt synchrony among individuals of the same species, which could in turn impact demography and evolution, although this possibility has received considerably less empirical attention (but see Prevéy et al., 2017; Rivest et al., 2021).

The seasonal timing of reproductive events not only determines organisms' synchrony with their biotic and abiotic environments, but also synchrony among potential mates (e.g. Augspurger, 1981). Because mating can only occur between individuals with some overlap in timing of reproduction, variation in reproductive phenology can lead to temporal reproductive isolation—hereafter 'temporal isolation' (i.e. phenological assortative mating, or isolation by time; Fox, 2003; Hendry & Day, 2005; Rosser, 2016; Schuster et al., 1989; Weis et al., 2005, 2014). In plants, for example, flowering phenology can vary considerably among habitat patches due to small-scale variation in environmental conditions such as snowpack, sun exposure and temperature (Billings & Bliss, 1959; Denney et al., 2020; Jackson, 1966; Theobald et al., 2017; Yamagishi et al., 2005). Climate change could alter patterns of temporal isolation if phenological response to climate change varies in space (e.g. among habitat patches or populations), leading to altered synchrony (Prevéy et al., 2017; Rivest et al., 2021). This could occur, for example, if individuals located in different microhabitats exhibit genetic variation in responsiveness to climate, or if climate directly increases spatial variability in the environmental drivers of phenology.

Temporal isolation in turn can affect the capacity of populations to respond adaptively to changing environmental conditions, including climate change (Wadgymar et al., 2015). Like spatial isolation, temporal isolation can reduce gene flow across landscapes (Fox, 2003; Heard et al., 2012; Ison et al., 2014; Peters & Weis, 2019) and therefore increase or decrease local adaptation and fine-scale genetic structure (Ellstrand, 2014; Garant et al., 2007; Holt & Gomulkiewicz, 1997). Conversely, synchronized reproduction can allow gene flow even among spatially segregated habitat patches (Kitamoto et al., 2006; Yamagishi et al., 2005), although the extent of gene flow will also depend on the distance between habitat patches and the mode of pollination (e.g. wind vs. animal). Such avenues for the influx of non-resident alleles or genotypes are particularly relevant in the context of anthropogenic climate change, as they could affect the capacity of populations to respond to new environmental conditions. The degree of temporal isolation can therefore influence how quickly plants can adapt to climate change, and how quickly alleles conferring tolerance to new environmental conditions spread within and among populations (Aguilée et al., 2016; Aitken & Whitlock, 2013; Godineau et al., 2021; Matter et al., 2013; Weis et al., 2014; Zettlemoyer & Peterson, 2021). In patchily distributed populations, temporal isolation among habitat patches could also reduce reproductive success or lead to inbreeding depression by reducing the number and diversity of available mates in the landscape (Ison et al., 2014, but see Ison & Wagenius, 2014; Munguía-Rosas et al., 2011).

Considering the importance of flowering synchrony for populations' adaptive responses to environmental change, it is critical to understand the extent to which intraspecific flowering synchrony is affected by climate change. However, while populations have been shown to vary in their phenological sensitivity to temperature (Fisogni et al., 2022; Parmesan & Yohe, 2003; Pau et al., 2011; Prevéy et al., 2017; Primack et al., 2009; Rivest et al., 2021; Zohner et al., 2018), information about how flowering synchrony within populations is affected by climate warming is extremely limited. Indeed, most long-term data on flowering phenology consider population-level trends in mean, median or first flowering dates (e.g. Menzel et al., 2006; Rafferty et al., 2020; Wang et al., 2015, but see Inouye et al., 2019), which does not permit comparison of phenological responses within populations. Moreover, trends in population mean, median or first flowering dates do not allow us to characterize changes in flowering duration (at the scale of individuals, habitat patches or populations), which also contribute to temporal isolation. For example, all else being equal, shorter flowering durations within habitat patches should reduce flowering overlap between patches.

Here we use flowering phenology and floral abundance data collected for the whole flowering period of 50 subalpine plant species over 44 years in the Rocky Mountains of Colorado, USA, to test whether the potential for temporal isolation among habitat patches is affected by spring temperature. We measured, for each plant species, the overlap in flowering distribution between habitat patches. Our 'habitat patches' are 21 individual plant community plots, located 5–950 m from one another, that represent five distinct but interdigitating habitat types that co-occur within our 30-ha study area (Supporting information, Figure S1). Subalpine plant communities are characterized by a short growing season limited by cold temperatures and snowfall. In such communities, snowmelt timing and spring temperature have a strong influence on flowering phenology because they determine the start of the growing season and the availability of soil moisture (Inouye et al., 2002). In this system, the start of the growing season, and consequently flowering phenology, can vary considerably among microhabitats located within a few metres of each other due to topographic and aspect differences (Forrest et al., 2010). Changes in phenological synchrony among habitat patches could therefore influence gene flow within and among distinct habitat types. Moreover, despite considerable interannual variation in spring temperature and snowmelt timing, there has been a trend towards warmer springs and earlier snowmelt in the Rocky Mountains since the 1970s (Funk et al., 2014).

2 | METHODOLOGY

2.1 | Data collection

Data on the flowering phenology of angiosperms were collected at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (~2900 m above sea level). From 1974 to 2020, the number of flowers or inflorescences was recorded approximately every other day in 21 2 × 2 m plots throughout the growing season (from day of year 129 ± 21 to 267 ± 19; mean ± SD) for all the flowering plant species present in the plots. Flowers were counted for species with conspicuous flowers, while inflorescences were counted for species with small, clustered flowers (see Table S1 for details on the reproductive unit counted for each species). Missed years or incomplete censuses in 1976, 1978 and 1990 result in 44 years of observations.

The plots are not randomly distributed in the landscape but were selected to encompass different types of habitats (specifically: dry rocky meadows [$n = 7$], aspen forest [$n = 2$], wet meadow [$n = 5$],

Veratrum-dominated meadows [$n=2$] and the edges of wet meadows with partial willow cover [$n=5$]; see also Figure S1). The plots therefore represent haphazard samples of the broader landscape, with the different habitat types sometimes occurring in closer proximity than is reflected by the spatial distribution of the plots; for example, there are patches of wet meadow habitat within 350 m of dry rocky meadow habitat, although our wet meadow plots are all >500 m away from our dry rocky meadow plots (Figure S1).

The date of snowmelt, defined as the date of first bare ground in spring, has been recorded every year at a fixed station located within 1 km of the plots (Barr, 2022). Temperature data were obtained from the National Oceanic and Atmospheric Administration weather station in Crested Butte, CO, approximately 9 km from the phenology plots (and 221 m lower than their average elevation). Temperature from this station is highly correlated with temperature from a more recently established station located within 1 km of the plots (and 4 m lower than their average elevation) ($r=0.85$, $p<0.0001$, $n=21$ years, for the values of spring temperature measured as described below). Spring temperature was determined as the average maximal daily temperature for the months of April, May and June (selected a priori). In our system, snowmelt at the measurement station typically occurs in late May, but sometimes as early as late April (average day of year of snowmelt = 140, range = 114–170), and the first day of flowering across species and plots is similar to the timing of snowmelt (average day of first flowering = 139, range = 104–160). To describe each plot's microclimate, we estimated the long-term average date of snowmelt, slope (angle from horizontal; range = 2.2–25.0), and aspect (southness; range = −0.99–1.00) of each of the 21 plots using spatial datasets from the RMBL Spatial Data Platform (spatial resolution = 1 m for slope and aspect, and 27 m for date of snowmelt; based on 30 years of data) (Breckheimer & Williams, 2023; Breckheimer et al., 2023).

2.2 | Statistical analysis

2.2.1 | Curve fitting

Flowering distributions for each plot–year–species combination were obtained by fitting curves to the number of flowers recorded on each census day using piecewise cubic Hermite interpolation of polynomials with the pchip function from the pracma package of R (Borchers, 2021). This method forces the curves to pass through each observed value of flower abundance (without oscillating between interpolation points like standard cubic splines). Because statistical estimates of phenology have been shown to be unreliable at high spatial and temporal resolution (Iler, Humphrey, et al., 2021), our conservative method of curve fitting should be more appropriate for our fine-scale phenological data.

2.2.2 | Measures of synchrony

For each species and each year, we estimated the potential for temporal isolation between each pair of plots by measuring the phenological overlap between flowering curves. Flowering overlap (Equation 1) was calculated as the area of overlap between the standardized flowering curves of two plots, '*a*' and '*b*'. This area was calculated by summing the minimum number of flowers open across the two plots at each time *i*, between the first ($i=1$) and last ($i=n$) days of flowering across the two plots. Flowering curves were standardized by dividing the number of flowers open at each time *i* (a_i and b_i) by the total number of flowers summed across the flowering period within that plot (expressed as $\sum a$ and $\sum b$), such that:

$$\text{Overlap}_{ab} = \sum_{i=1}^{n} \min\left(a_i / \sum_{i=1}^{n} a_i, b_i / \sum_{i=1}^{n} b_i \right) \quad (1)$$

The total standardized area of a flowering curve corresponded to 1, thus the measure of overlap gives values of proportion of overlap (ranging from 0 to 1). This metric has been used to measure phenological synchrony (Fox, 2003; Miller-Rushing et al., 2010) and as a proxy for potential gene flow between populations (Carscadden et al., 2022; Franks & Weis, 2009; Matter et al., 2013; Rivest et al., 2021).

We also measured the number of days between flowering peaks and between first flowering dates for each pair of plots (measured in absolute values), as well as the flowering duration of each plot. This allowed us to determine whether changes in flowering overlap were due to changes in flowering synchrony between plots or changes in flowering duration within plots. A species' flowering peak within a plot was defined as the day with the maximal floral abundance within the plot (note that with our method of curve fitting, the estimated flowering peak always corresponded to the observed flowering peak). When the maximal floral abundance was observed for multiple days, we took the average of those dates. Flowering duration was defined as the number of days between the first and last days for which the floral abundance of a given flowering curve was greater than zero.

2.2.3 | Models

To avoid potential biases in the analysis, we removed, for each species, pairs of plots that occurred in fewer than 15 years of observation (the plots from those pairs were also removed from the analysis of flowering duration). We also removed plot–species–year combinations in which only one flower was observed or for which flowers were observed on only one census date, and species–year combinations for which either the beginning or end of flowering was missed by censuses in at least one plot (this occurred mostly with early-flowering species in earlier years of the study). Because our plots encompassed different types of habitats, the investigated plant species occurred only in a subset of those plots (see Table S1).

We used a hierarchical Bayesian model with a zero–one-inflated beta distribution to test for an effect of spring temperature (β_{temp}) on flowering overlap among plots. Beta distributions are well suited to model proportional data such as proportional overlap (Douma &

Weedon, 2019). A zero–one-inflated distribution was used to handle values of overlap of zero and one, which are not possible with standard beta distributions. Spring temperature and snowmelt date were highly correlated in our dataset ($r = -0.83$, $n = 44$ years), so we only incorporated spring temperature in the models, as model comparison using leave-one-out cross-validation (with the function loo from the R package loo; Vehtari et al., 2016) demonstrated that spring temperature offered better predictive accuracy than snowmelt date. In addition to this model, we tested for an effect of spring temperature on the number of days between flowering peaks and first flowering dates among plots using a gamma distribution with hurdle models, and on flowering durations within plots using a gamma distribution. Gamma distributions are well suited for continuous data confined to positive values and with skewed distributions (Bolker, 2008). Hurdle models were used to handle the presence of zeros, which are not accommodated in standard gamma distribution models.

Year was included as a continuous covariate (β_{year}) to prevent spurious relationships between spring temperature and phenological patterns due to both variables potentially changing similarly across years (effectively detrending by year; Iler et al., 2017). The spatial distance (β_{dist}) between plots was used as a covariate to control for the effect of distance between plots on flowering synchrony. We incorporated an interaction term between this variable and spring temperature ($\beta_{temp*dist}$) because the effect of spring temperature on temporal isolation might be stronger for more spatially distinct plots. Species identity was modelled as a random effect with a random intercept (a_{sp}) and a random slope for spring temperature ($\beta_{temp,sp}$) and year ($\beta_{year,sp}$). To account for repeated observations of pairs of plots and habitat types during the study period, plot-pair-by-species combinations (a_p; $n = 169$) nested within habitat-pair-by-species combinations (a_h; $n = 15$, see Section 2.1 above) were included as random intercepts. The models were of the form:

$$\mu_i = a_{sp} + a_h + a_p + \beta_{temp} + \beta_{year} + \beta_{dist} + \beta_{temp*dist} + \beta_{temp,sp} + \beta_{year,sp} \quad (2)$$

where μ_i is the mean value of flowering synchrony or duration across observations i, except for the model of flowering duration. In the latter model, β_{dist} and $\beta_{temp*dist}$ were not included, and the random effects included the plot and habitat type from which duration was measured rather than the plot or habitat pairs. The fixed effects were standardized by subtracting the mean value and dividing by the standard deviation to improve model convergence. Because the flowering period of some species (*Androsace septentrionalis*, *Boechera stricta*, *Collomia linearis*, *Polygonum douglasii* and *Ranunculus inamoenus*) is sometimes characterized by two periods of flowering separated by a period with no flowering, the model of flowering duration was run both with and without these species. Both models gave similar results, so we present only the results from the model including all species.

We used another model to test whether three plot microclimatic variables (date of snowmelt, slope and aspect) and one species trait (average flowering peak, i.e. average of the dates of flowering peaks across plots and years) predicted flowering overlap and changes in flowering overlap with spring temperature (correlations between plot microclimatic variables are not sufficiently strong to cause problems of collinearity; see Table S2). This model was similar to the model of flowering overlap (above) but also incorporated the plot and species characteristics and their interaction with spring temperature as additional explanatory variables. Note that this model could not be used to estimate species-level effects of temperature on flowering overlap; which was done using the model described in (Equation 2) because this requires modelling species as a random effect only.

We used non-informative priors for all parameter estimates. The models were run using Hamiltonian Monte Carlo sampling, with four chains of 10,000 iterations, of which the first 2500 were discarded as burn-in. The thinning intervals were set to 10 to reduce autocorrelation in the Markov chains. Convergence of chains for all parameters was verified both visually with trace plots and with the Gelman–Rubin convergence statistic ($\hat{R} < 1.01$ for all parameters) (Gelman & Rubin, 1992). The models were run using the brm function from the brms package in R (Bürkner, 2017). We checked for temporal autocorrelation in our variables by incorporating a first-order autoregressive term in the models (using the brms package; Bürkner, 2017), but no detectable autocorrelation was observed (the credible interval for the autoregressive term in the overlap model was −0.88–0.88). We also tested for the presence of phylogenetic signal by building a phylogenetic tree with the phylo.maker function from the V.PhyloMaker package (Jin & Qian, 2019), but because incorporating phylogeny did not improve the models (using leave-one-out cross-validation with the loo function from the brms package in R; Bürkner, 2017), phylogeny was not incorporated in the final models.

3 | RESULTS

Across subalpine plant species, we found a positive association between spring temperature and temporal isolation. Specifically, warmer springs were associated with more temporal separation in flowering peaks (Figure 1a), and less flowering overlap, between habitat patches (Figures 1b and 2). In both cases, the Bayesian credible interval (BCI) of the estimated effect of spring temperature did not overlap zero (95% BCI [0.013, 0.081] for separation in flowering peaks, and [−0.121, −0.050] for overlap). However, the effect of spring temperature on temporal separation in first flowering dates was weaker than that for flowering peaks, and the estimated slope overlapped zero (95% BCI [−0.004, 0.047]). We found no effect of spring temperature on flowering duration across the subalpine species in our dataset (95% BCI [−0.028, 0.004]). This indicates that changes in temporal isolation were more likely due to changes in flowering synchrony between plots than changes in flowering duration within plots.

While we detected an overall negative association between spring temperature and flowering overlap between habitat patches, this effect varied among species (Figure 2). Most species

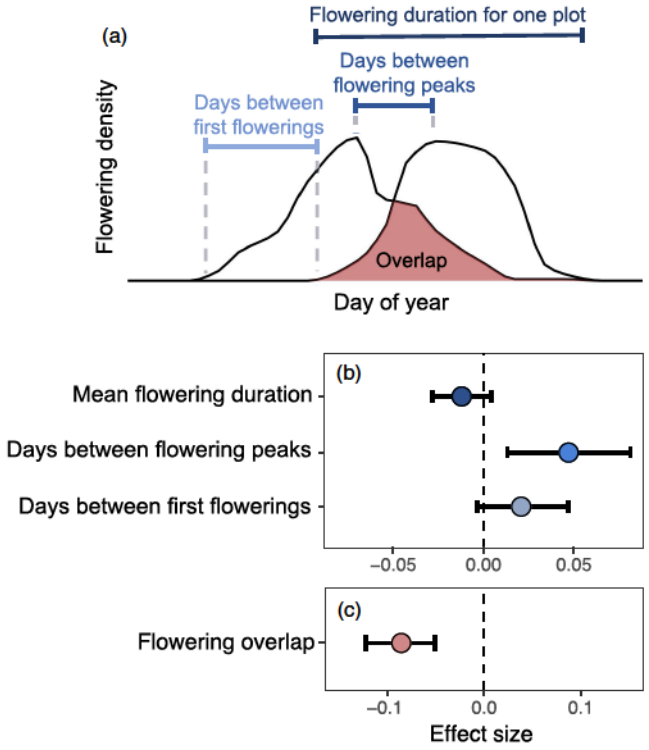

FIGURE 1 Effect of spring temperature on phenological synchrony. (a) Schematic representation of the different indices of synchrony between pairs of plots used in the study. (b, c) Effect of spring temperature on different measures of phenological synchrony with 95% credible intervals. Effect sizes are comparable only within panels because different distributions were used to model the effect of spring temperature in different panels: gamma distributions for (b), and zero-inflated beta distributions for (c). In (b), the back-transformed, unstandardized, marginal effect sizes correspond to −0.20 days per °C for flowering duration, 0.21 days per °C for days between flowering peaks and 0.08 days per °C for days between first flowerings. In (c), the back-transformed marginal effect size corresponds to a reduction in proportional overlap of 0.013 per °C for flowering overlap. Note that because the relationships between spring temperature and the response variables are nonlinear (in the back-transformed space), the marginal effects hold only when all the explanatory variables are at their mean values, and are therefore provided only to aid interpretation.

experienced a decrease in flowering overlap with warmer springs. The BCI of this relationship did not overlap zero in 20 of the 50 investigated species (Figure 2a). However, many species exhibited similarly strong patterns but with lower confidence in the strength of the effect due to smaller sample sizes. Some species exhibited no decrease in flowering overlap among habitat patches with increasing spring temperature. Finally, a few species exhibited a trend towards increased flowering overlap with warmer springs, but the BCIs of these relationships always overlapped zero (Figure 2a,b). Across all investigated species, we found a reduction in proportional flowering overlap of 0.013 (i.e. −1.3%) per °C (Figure 2b). However, many species showed considerably higher decreases in flowering overlap with increasing temperatures (Figure 2b).

Microclimate was an important driver of flowering synchrony. Flowering overlap was lower between plots with larger differences in estimated snowmelt dates and slopes (95% BCI [−0.231, −0.140] for snowmelt date and [−0.255, −0.173] for slope), but not between plots with more distinct aspects (95% BCI [−0.022, 0.054]) (Figure 3b–d; illustrated as the difference in intercepts). More spatially separated plots also exhibited less flowering overlap, even when controlling for microclimatic variables (95% BCI [−0.255, −0.103]; Figure 3a). The effect of temperature on flowering overlap was partially mediated by microclimatic differences between plots: plots with more distinct slopes exhibited greater reductions in flowering overlap with increasing temperature (95% BCI [−0.040, −0.008]; Figure 3). However, differences in snowmelt date, aspect and spatial location did not explain variation in the flowering overlap–temperature relationship (95% BCI [−0.0270, 0.009] for snowmelt date, [−0.024, 0.003] for aspect, and [−0.030, 0.007] for spatial distance; i.e., there is no evidence that regression slopes differ in Figure 3a,c,d). Flowering phenology did not explain interspecific differences in the effect of spring temperature on temporal isolation; that is, species that flowered earlier did not exhibit more or less change in overlap with temperature (95% BCI [−0.044, 0.017]).

4 | DISCUSSION

Across the subalpine plant species in our dataset, warmer springs increase the potential for temporal isolation among habitat patches within populations. More precisely, warmer springs are associated with more temporal separation in flowering peaks (Figure 1a), and less flowering overlap among habitat patches (Figures 1b and 2). In other words, flowering phenology becomes less synchronized across our studied subalpine landscape in warmer springs, as the earlier-flowering plots advance phenology more than later-flowering plots, which may reduce opportunities for mating among plants in different habitat patches. This suggests that spring temperature could influence pollen dispersal within populations, and hence potentially gene flow and reproductive success (Aguilée et al., 2016; Aitken & Whitlock, 2013; Godineau et al., 2021; Matter et al., 2013; Weis et al., 2014). In the context of climate change, increased reproductive isolation could reduce the spread of alleles conferring tolerance to new environmental conditions and affect the rate at which populations can adapt to those new conditions (Aguilée et al., 2016; Aitken & Whitlock, 2013; Godineau et al., 2021; Matter et al., 2013; Weis et al., 2014; Zettlemoyer & Peterson, 2021).

Notwithstanding the strong overall patterns (Figure 1), the effect of spring temperature on temporal isolation varied considerably across the 50 plant species we investigated. Although many species experienced a decrease in flowering overlap among habitat patches with increasing spring temperature, some species were unaffected or weakly affected, while a few species had a weak tendency towards increased synchrony (Figure 2). For example, in *Salix monticola*—the most responsive species—the proportion of overlap decreased by 0.062 per °C, corresponding to a

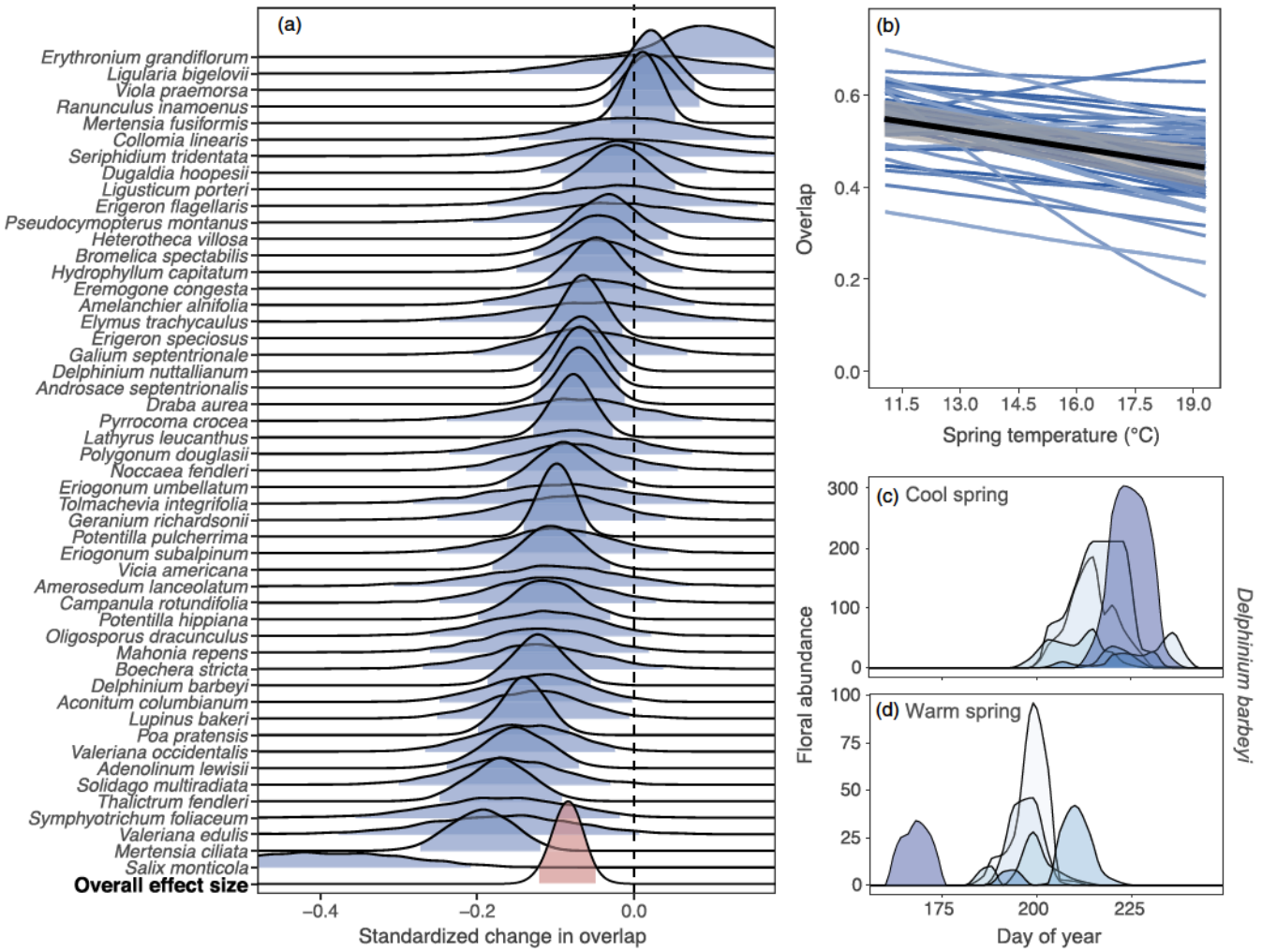

FIGURE 2 Effect of spring temperature on the degree of overlap in flowering phenology between plots for 50 subalpine flowering plant species. (a) Probability density functions of the standardized effect sizes showing the 95% credible intervals (in blue) for the 50 plant species and the overall effect size. (b) Conditional effect of spring mean daily maximum temperature on the degree of flowering overlap for the 50 species (blue) and the overall effect (black, with 95% credible interval in grey). (c, d) Comparison of the flowering phenology of different plots (shown in different colours) between a year with a cold spring (c, 1975; 11.1°C) and a year with a warm spring (d, 1977; 17.2°C) for *Delphinium barbeyi*.

15% reduction per °C (relative to the average overlap value of 0.42 for this species)—a value five times higher than the across-species average of 0.013 (corresponding to a 2.5% decrease relative to the average overlap value of 0.52). This interspecific variability in response to climate is consistent with high variability among species in studies of other phenological trends, such as shifts in flowering dates and duration (Bock et al., 2014; CaraDonna et al., 2014; Cook et al., 2012; Fitter & Fitter, 2002; Prather et al., 2023). Therefore, not only the ecological impacts of climate warming, but also its potential evolutionary impacts (e.g. via altered gene flow), could differ considerably among co-occurring species. Specifically, impacts of climate change on pollen exchange and gene flow are more likely for those species, like *S. monticola*, that exhibit strong decreases in overlap with warming, while we would expect little impact on gene flow in species that exhibit little to no change in synchrony with temperature. More studies are needed that investigate how and why species vary in their response to climate, both in their overall phenology and in their intraspecific synchrony (see Chmura et al., 2019; Zohner et al., 2018).

4.1 | Drivers of changes in temporal isolation with temperature

We found that microclimate was an important driver of temporal isolation in our subalpine landscape. Differences in dates of snowmelt and slopes between plots predicted their phenological synchrony: pairs of plots with less synchronized snowmelt and more different slopes also exhibited less synchronized flowering (Figure 3). In turn, these more microclimatically distinct plots (or at least those with different slopes) also experienced a stronger impact of temperature on their phenological synchrony. This suggests that plants growing in different microhabitats become more temporally isolated with warmer springs. It is important to note,

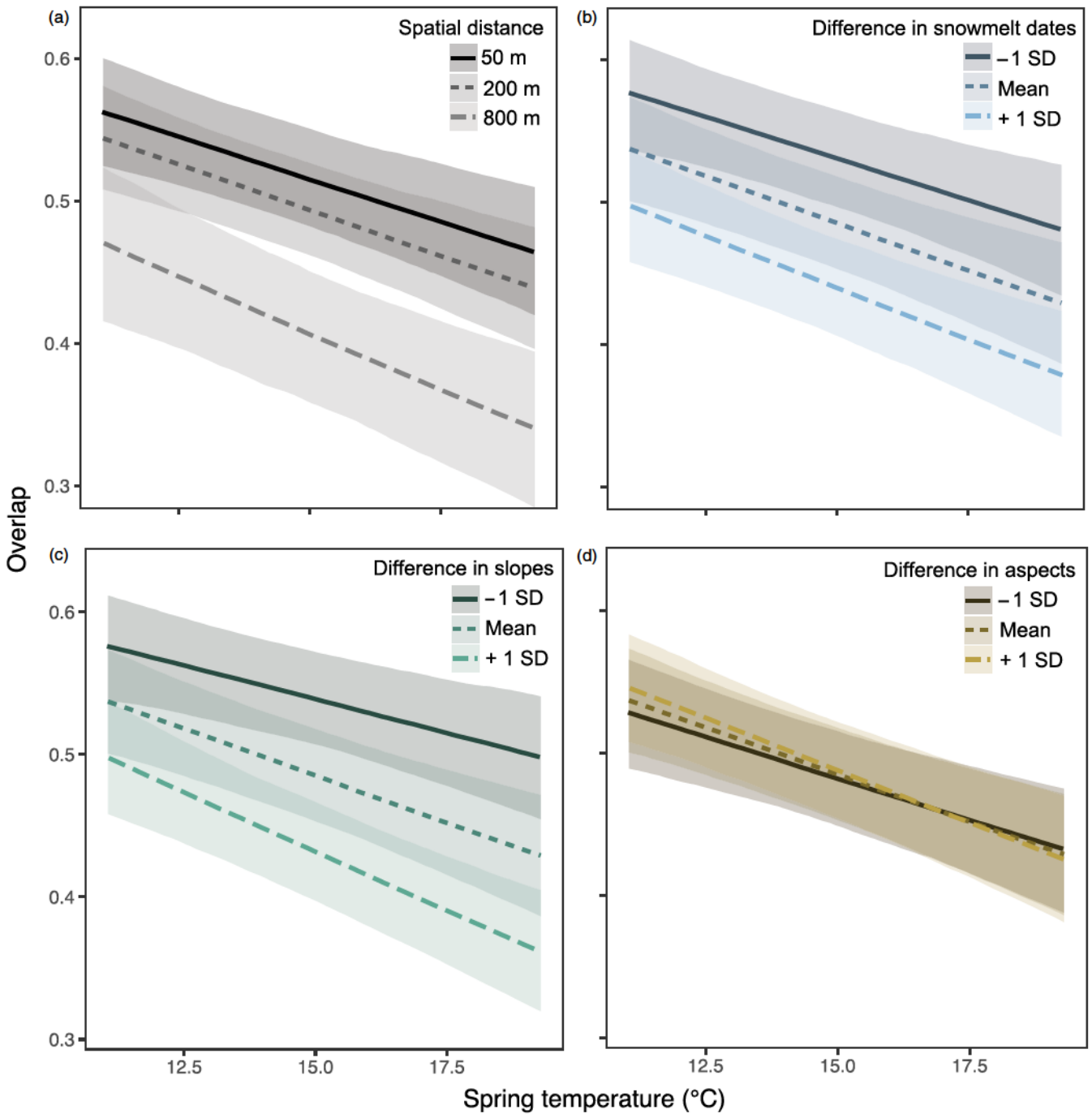

FIGURE 3 Effects of plot differences on the relationship between spring temperature and flowering overlap between plots. Proportional flowering overlap is shown as a function of spring temperature and spatial distance between plots (a), difference between plots in snowmelt dates (b), difference in slopes between plots (c) and difference in aspects between plots (d). The estimate for the interaction between plot differences and spring temperature did not overlap zero (indicating evidence of an interaction) for difference in plot slopes (c), but overlapped zero (indicating lack of evidence of an interaction) for spatial distance (a) and differences in snowmelt dates (b) and aspect (d). The values of spatial distance in (a) were selected to encompass most of the range in spatial distance between plots in our study area (range = 5–950 m).

however, that our plots were not randomly distributed in the landscape but were selected to encompass different habitat types (Figure S1), which limits our ability to disentangle the effects of these variables (as plots of a given habitat type were often clustered in space, although problems of collinearity are unlikely; see Table S2).

We detected an association between spring temperature and proximity in flowering peaks between habitat patches, but not between temperature and flowering duration within habitat patches (Figure 1a). Therefore, although some studies have observed an effect of climate on the flowering durations of individuals or populations (Bock et al., 2014; CaraDonna et al., 2014; Rivest et al., 2021), changes

in relative timing of flowering peaks, rather than duration, are probably the main drivers of the variation in temporal isolation that we see in this study (see also Figure 2c,d). The skewness of phenological distributions can also affect overlap (Stemkovski et al., 2022), but we did not test for an effect of spring temperatures on skewness; we assume shifts in peak dates and durations will have larger effects on overlap.

What mechanism is responsible for reduced phenological synchrony with warmer springs? First, warmer springs could directly increase spatial variability in the environmental drivers of phenology. For example, in warm springs with low snowpack, exposed microhabitats can lose their reflective snow cover early in spring, leading to snow from subsequent snowfall melting rapidly, while snow can continue to accumulate in less exposed microhabitats. This could increase phenological differences among microhabitats relative to cold years, in which snow can continue to accumulate in both types of microhabitats until melting relatively synchronously during the longer, warmer days of late spring. In support of this hypothesis, the mean snowmelt date of our plots is highly correlated with their variability in snowmelt dates across years (measured as the standard deviation; $r = -0.96$, $n = 30$ years and 21 plots), indicating that plots with earlier snowmelt experience more interannual variability in timing of snowmelt.

Alternatively, genetic differences among plants occupying different habitat patches (i.e. heritable phenotypic plasticity, see Anderson et al., 2012; Nussey et al., 2005), perhaps due to adaptation to different microhabitats, could generate spatial variation in phenological responsiveness to temperature. It is not clear whether our habitat patches were sufficiently isolated to exhibit much genetic differentiation. However, Waser and Price (1991) found evidence of outbreeding depression in individuals of *Delphinium nuttallianum*—one of our study species—separated by 30 m from one another, suggesting that at least in some species, even nearby habitat patches could exhibit considerable genetic differences. On the other hand, in our analysis, microclimate was a stronger driver of changes in temporal isolation with temperature than spatial distance, suggesting that changes in temporal isolation are not necessarily stronger in more spatially—and hence genetically—isolated plants.

Finally, reduced phenological synchrony with rising temperature could originate from complex microgeographical variation in environmental conditions. If habitat patches vary in multiple environmental variables, such as temperature and soil moisture, plants whose flowering phenologies are governed by interactions among these variables might react differently in different patches to changes in a given driver. If this hypothesis is correct, we should expect late-flowering species to experience more change in phenological synchrony with temperature. This is because the flowering phenology of early-flowering plants is often strongly correlated with the timing of snowmelt, while the flowering of later-blooming species is frequently associated with additional variables such as soil moisture (Cook et al., 2012; Dunne et al., 2003; Fitter & Fitter, 2002; Miller-Rushing et al., 2007). However, our data did not support this prediction; across our species, we found no relationship between plant mean phenology (across plots and years) and changes in synchrony with temperature.

To our knowledge, our study is unique in combining data on whole flowering distributions and long-term phenological monitoring to assess the effect of temperature on flowering synchrony within plant populations. Studies investigating the role of temperature or climate change on synchrony at larger spatial scales, along latitudinal or altitudinal gradients, have found contrasting trends: a decrease (Menzel et al., 2008; Rivest et al., 2021; Wang et al., 2015; Zohner et al., 2018), an increase (Fisogni et al., 2022; Prevéy et al., 2017; Rafferty et al., 2020) or no trend (Park et al., 2019) in synchrony with warmer years or over time. The effect of temperature on synchrony that we observed (a decrease in overlap of 2.5% per °C, if expressed relative to our average overlap value of 0.52) is comparable in magnitude to that reported among populations—although comparisons are made challenging by the different metrics of change used in different studies. For example, Fisogni et al. (2022) studied a large elevational gradient over 33 years, and found an increase in overlap over the study period in 3 of 10 elevation pairs, with a change between adjacent pairs of 6.8%. Prevéy et al. (2017) investigated variation in temperature sensitivity of flowering phenology among sites across the Canadian and European Arctic and found that cooler plots were more sensitive to warming—exhibiting a 0.35 day per °C greater sensitivity, per degree difference in summer temperature. This value is comparable in magnitude to our global average of a 0.21 day divergence in peak dates per °C. In these latter studies, the observed variability in synchrony with climate could sometimes be attributed to spatial variability in the intensity of climate warming—for example, colder sites warming faster. It is unlikely that this mechanism plays a role in explaining our results because our study system encompassed a limited elevational range (<110 m). It therefore seems likely that flowering synchrony within populations is governed at least in part by different mechanisms than synchrony among populations, which could explain the discrepancy between our results and those of some studies conducted at larger spatial scales.

Our results, together with other studies, suggest that changes in flowering synchrony might be a widespread consequence of climate change, not only along large climatic gradients, but also within populations that span microclimates. Patterns of genetic structure and local adaptation might therefore be restructuring in complex ways in response to climate changes. Small-scale changes in phenological synchrony could also affect processes such as mast seeding, for which within-population synchronization is critical (Koenig et al., 2015). However, given the strong influence of temperature on phenology in the subalpine plant community that we studied (Forrest et al., 2010; Inouye, 2008), it remains to be determined the extent to which climate affects small-scale temporal isolation in other systems, where temperature may play a less central role.

4.2 | Caveats

By detecting an effect of spring temperature on temporal isolation within plant populations, our study suggests that changes in temporal isolation occur at a scale similar to that of pollen dispersal in many

plant species—and, therefore, that these phenological changes could realistically influence within-population pollen dispersal. Indeed, the distance between our most distant plots (less than 950 m) is similar to the maximal foraging ranges of some of the main pollinator species in our study area (more than 800 m; Elliott, 2009), and most of our study species occurred in only a portion of the plots that were closer to one another than this (e.g. 190 m between the most distant of the 10 plots in which *Delphinium barbeyi* was present). Moreover, the effect of spring temperature on temporal isolation was only slightly more pronounced between highly distant plots relative to the most nearby ones (Figure 3), indicating that the effect we detected was not driven mainly by the highly spatially isolated plots. Therefore, it seems likely that our plots are sufficiently close to one another for pollen transport to be possible, provided flowering times align.

Nevertheless, the potential impacts of increased temporal isolation might vary considerably among plant species. Plants experiencing frequent long-distant pollen dispersal, such as those pollinated by wind, birds or large-bodied bees, might be more affected than plants pollinated primarily by short-distance dispersers (e.g. some flies) and primarily selfing plants, because pollen dispersal might occur more frequently at the scale at which temporal isolation manifests (Gathmann & Tscharntke, 2002; Rader et al., 2011; Wessinger, 2021). Our studied species encompass diverse pollination systems: 7 of the 50 investigated species are wind-pollinated (Table S1); birds and large-bodied bees are common pollinators of many of our studied species (e.g. those in the genera *Aconitum*, *Delphinium*, *Lupinus*, *Mertensia*, *Erythronium*, *Potentilla*, *Hydrophyllum*, *Lathyrus*, *Vicia*, *Amelanchier*); many species are generalists (e.g. Asteraceae species, *Ligusticum*, *Pseudocymopterus*); and some are pollinated mostly by flies and small bees (e.g. *Valeriana edulis*). We should therefore expect the consequences of increased temporal isolation to differ considerably among the 50 species we studied. Moreover, population density and spatial distribution might also influence the impact of temporal isolation. For example, temporal isolation could reduce the availability of potential mates in fragmented or small populations, while plants in dense and well-connected populations could have access to a large pool of mates regardless of the degree of temporal isolation.

In wind-pollinated plants, temporal isolation and pollen dispersal should be directly affected by flowering overlap, as their pollen is carried passively through the landscape (Schuster & Mitton, 2000; Schuster et al., 1989). However, in animal-pollinated plants—a group that includes most of our study species—patterns of pollen dispersal also depend on pollinator behaviour (e.g. Ogilvie & Thomson, 2016). The relationships among flowering overlap, temporal isolation and pollen dispersal need to be interpreted with more caution in these species because pollinator behaviour and the floral characteristics that affect it (e.g. flower abundance and reward content) could themselves be affected by climate (Descamps et al., 2020, 2021; Inouye et al., 2002; Miller-Rushing & Inouye, 2009; Russell & McFrederick, 2022). Therefore, although reductions in flowering overlap among habitat patches should influence pollen movement among animal-pollinated plants—as reproduction can only occur among individuals with overlapping flowering periods—the precise effects of climate change on pollen dispersal will also depend on other factors.

5 | CONCLUSIONS

Phenological synchrony mediates patterns of pollen dispersal and gene flow within and among populations, but as we demonstrate here, climate change could disrupt this synchrony in flowering plants. Our study points to a need to gather more data on the effect of intraspecific synchrony on patterns of pollen dispersal and how climate affects such patterns, for example by directly monitoring pollen movements or using genetic markers (e.g. Kitamoto et al., 2006; Yamagishi et al., 2005). Moreover, most current studies on intraspecific synchrony rely on limited phenological information, such as first flowering dates (e.g. Menzel et al., 2008; Wang et al., 2015; Zohner et al., 2018). Here we detected a stronger effect of temperature on flowering overlap and synchrony in peak flowering dates than on synchrony in first flowering (Figure 1a), highlighting the need for future studies to collect data on whole flowering distributions to accurately describe intraspecific synchrony (Inouye et al., 2019, see also Rivest et al., 2021). Given the key role of pollen dispersal and gene flow in the capacity of species to adapt to climate change, such studies promise to improve our understanding of how climate change will affect biodiversity.

AUTHOR CONTRIBUTIONS

Design, analyses and initial writing were done by Sébastien Rivest. Sébastien Rivest, Brian D. Inouye and Jessica R. K. Forrest contributed to writing and revisions.

ACKNOWLEDGEMENTS

We thank the researchers who collected the long-term phenology data funded by multiple National Science Foundation grants including DEB-2016749, DEB-1912006, DEB-1354104 and DEB-0922080, and Billy Barr for providing the snowmelt data. We also thank Julien Martin and members of the Forrest lab for valuable advice, and D. Inouye for comments. Funding for this project was provided by the University of Ottawa and the Natural Sciences and Engineering Research Council of Canada (NSERC).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14175.

DATA AVAILABILITY STATEMENT

Data and code used for this manuscript are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.dz08kps38 (Rivest et al., 2023).

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SUPPORTING INFORMATION

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

Figure S1. Map of the study site with the 21 monitored plots representing five different habitat types (represented by symbol colours).

Table S1. Plant species list and details of the 50 species present in the study. 'Plot occurrence' represents the number of plots in which the species occurred and that were used for the analysis after data filtering.

Table S2. Correlation between the measures of microclimatic differences between plots used in the model of the effect of plot microclimate and species phenology on flowering overlap.

How to cite this article: Rivest, S., Inouye, B. D., & Forrest, J. R. K. (2023). Warmer springs increase potential for temporal reproductive isolation among habitat patches in subalpine flowering plants. *Journal of Ecology*, *111*, 2257–2268. https://doi.org/10.1111/1365-2745.14175