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BRIEF COMMUNICATION

Earlier spring reduces potential for gene flow via reduced flowering synchrony across an elevational gradient

Sébastien Rivest[1,4], Geneviève Lajoie[2], David A. Watts[3], and Mark Vellend[3]

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[1] Department of Biology, University of Ottawa, Ottawa K1N 6N5, Ontario, Canada

[2] Département des Sciences Biologiques, Université du Québec à Montréal, Montréal H2X 1Y4, Quebec, Canada

[3] Département de Biologie, Université de Sherbrooke J1K 2R1, Québec, Canada

[4] Author for correspondence (e-mail: srive028@uottawa.ca)

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PREMISE: One of the best-documented ecological responses to climate warming involves temporal shifts of phenological events. However, we lack an understanding of how phenological responses to climate change vary among populations of the same species. Such variability has the potential to affect flowering synchrony among populations and hence the potential for gene flow.

METHODS: To test whether an earlier start of the growing season affects the potential for gene flow among populations, we quantified the distributions of flowering times of two spring-flowering plants (*Trillium erectum* and *Erythronium americanum*) over 6 years along an elevational gradient. We developed a novel model-based metric of potential gene flow between pairs of populations to quantify the potential for pollen-mediated gene flow based on flowering phenology.

RESULTS: Earlier onset of spring led to greater separation of peak flowering dates across the elevational gradient for both species investigated, but was only associated with a reduction in potential gene flow in *T. erectum*, not *E. americanum*.

CONCLUSIONS: Our study suggests that climate change could decrease gene flow via phenological separation among populations along climatic gradients. We also provide a novel method for quantifying potential pollen-mediated gene flow using data on flowering phenology, based on a quantitative, more biologically interpretable model than other available metrics.

KEY WORDS climate change; elevation gradient; flowering synchrony; gene flow; phenology; populations; spring ephemerals.

Climate warming is causing temporal shifts of phenological events around the globe (Parmesan and Yohe, 2003; Primack et al., 2009), and those shifts represent one of the best-documented responses of organisms to climate change (Forrest and Miller-Rushing, 2010). Phenological shifts in biological events occur because, for many organisms, temperature acts as a cue or a driver of developmental events (Heard et al., 2012). There is, however, substantial spatial and interspecific variability in the phenological responses of organisms to climate warming (Parmesan and Yohe, 2003; Primack et al., 2009; Pau et al., 2011).

Since different populations of a species often differ genetically (Linhart and Grant, 1996; Leimu and Fischer, 2008) and occur in different environments, phenological sensitivity to climate could vary spatially within a species (Heard et al., 2012; Diez et al., 2012). For plants, such variability could potentially affect gene flow by altering flowering synchrony between populations (Fox, 2003), in particular when populations experience different environmental conditions (Fox, 2003; Heard et al., 2012; Matter et al., 2013). Gene flow in plants occurs via both seed and pollen dispersal, but the contribution of pollen—the focus of the present study—is typically much larger (Ennos, 1994; Petit et al., 2005). By affecting local adaptation and genetic variability, gene flow has an important impact on evolutionary trajectories (Garant et al., 2007; Ellstrand,

2014). In the context of cold-range-edge populations under climate change, gene flow is thought to have a positive effect on adaptive capacity by facilitating the spread of adaptive alleles (Aitken and Whitlock, 2013; Matter et al., 2013; Aguilée et al., 2016). Franks and Weis (2009) observed an evolutionary change in reproductive timing of *Brassica rapa* following a drought, resulting in increased potential gene flow between two populations, while Whittet et al. (2017) found the least synchrony in pollen release across populations of *Pinus sylvestris* in the warmest of three study years. However, we still have limited knowledge of how global change impacts phenological synchrony and potential gene flow.

Considerable effort has been devoted to understanding how climate affects phenological synchrony or mismatch between species (Visser and Gienapp, 2019). However, few studies have examined phenological synchrony among populations within species, which will be altered if there is among-population variability in phenological sensitivity to temperature (Wang et al., 2015). Menzel et al. (2006) and Wang et al. (2015) observed greater sensitivity to climate warming in populations from warmer environments, which should reduce synchrony among populations, and therefore potential gene flow, with warming. In contrast, Rafferty et al. (2020) observed species-specific decreases or increases in synchrony of flowering between populations at different elevations. However, these long-term studies did not allow direct estimation of overlap in flowering time distributions, but rather relied on first or mean flowering dates to describe population flowering phenology, despite weak correlations of these parameters with other attributes of flowering phenology (CaraDonna et al., 2014). In particular, the shape and temporal spread of flowering in different populations are needed to determine consequences for potential gene flow.

In this study, we test how advanced spring phenology affects potential gene flow via pollen between populations (henceforth simply "gene flow"). To do so, we quantified flowering phenology in populations of two spring-flowering plants (*Trillium erectum* and *Erythronium americanum*) along an elevation gradient for 6 years. Along elevational gradients, climate and phenology vary across short distances (e.g., Lajoie and Vellend, 2015), such that gene flow between populations is expected to be particularly sensitive to phenological synchrony (Kitamoto et al., 2006; Matter et al., 2013). We developed a novel model-based metric of potential interpopulation gene flow; compared to traditional measures of phenological overlap, our metric has a much clearer biological interpretation: the proportion of pollen in a given population that would be received from the other population if reproduction was random between individuals of both populations combined. Our results demonstrate the potential for gene flow to be altered by earlier onset of spring.

MATERIALS AND METHODS

Study species

Erythronium Americanum Ker Gawl. (Liliaceae) and *Trillium erectum* L. (Melanthiaceae) are perennial herbs native to the deciduous forests of eastern North America. Both species flower early in the spring, and their flowering phenology is correlated with spring temperature and snowmelt (Muller, 1978; Routhier and Lapointe, 2002). *Erythronium americanum* flowers are pollinated primarily by Hymenoptera and Coleoptera (Bernhardt, 1977), which at our study site include bumblebees (*Bombus* spp.), solitary bees, and the pollen beetle *Asclera ruficollis* (S. Rivest, personal observations). Although data on pollinator foraging ranges is limited for our region, in general bumblebees have foraging ranges up to 1.5 km or more (Osborne et al., 2008; Wolf and Moritz, 2008), while solitary bees have foraging ranges of 150 m to 1200 m (Gathmann and Tscharntke, 2002). *Trillium erectum* flowers produce a fetid odor that principally attracts dipterans of the families Anthomyiidae, Sciaridae, and Sarcophagidae (Davis, 1981; Irwin, 2000). Sciaridae have been observed transporting pollen up to 200 m (Rader et al., 2011), and Sarcophagidae have been observed traveling up to 2.5 km over 6 days (Braack and De Vos, 1990). As such, with populations of *Trillium* and *Erythronium* all within 1 km of one another (see below), gene flow among populations is possible, contingent on overlap in flowering times (see also Irwin, 2001 for evidence of a lack of genetic isolation of *T. erectum* populations up to 1.5 km apart).

Study site

This study was conducted at Mont-Mégantic National Park (45°26′51″N, 71°06′52″W), located in Québec, Canada, in the northern range of the Appalachian Mountains (Lajoie and Vellend, 2015). The park represents a protected area of 55 km^2, covering an elevational gradient of 600 m with a maximal elevation of 1105 m a.s.l. (Savage and Vellend, 2015). The climate varies from continental humid dominated by sugar maple (*Acer saccharum*) at low elevation (approximately 500 m a.s.l.) to boreal dominated by balsam fir (*Abies balsamea*) at high elevation (Savage and Vellend, 2015). Annual mean temperature decreases by 0.64°C per 100 m increase in elevation, ranging from 3.6°C to 0.4°C along the elevational gradient (Lajoie and Vellend, 2015).

Data collection

Two elevational transects, approximately 800 m apart, were established in 2013 on the east-facing slope of Mont St-Joseph within the park (see Lajoie and Vellend, 2015). Each transect comprises six 26 × 4 m plots (104 m^2), with 55–85 m of elevation between plots. Plots were established only in areas under full tree canopy to avoid disturbed habitats and wetland areas. Our focal species were selected given their high abundance and presence across most of the elevational gradient. The focal species were present in five plots per transect (absent at the highest elevation), covering an elevation gradient from 697 m to 951 m a.s.l. The geographic distance between plots varied from 123 m for adjacent plots up 914 m for the two most distant plots on different transects.

Each plot was divided into 26 subplots each 2 × 2 m in which the number of flowers of *Trillium erectum* and *Erythronium americanum* was recorded approximately every 4 days throughout the flowering season for 6 years, 2013–2018 inclusive. Flowers were included in the count from the start of flower opening to the start of flower senescence (identified by signs of drying, wrinkling, or discoloration of petals or sepals). Since flower abundance decreased with elevation, for high-elevation plots (850 m and higher), we also recorded individuals within less than 2 m from the plots to obtain enough data to determine the flowering start, peak, and end; the number of individuals was standardized to correspond to the density of individuals in an area of 104 m^2 (the area of one plot). The term "population" is used to describe all individuals of a species in a plot.

Statistical analyses

Distributions of flowering times were quantified for each year by fitting curves to observations of the number of flowers per 104-m^2 plot over time using a locally weighted scatterplot smoother with the loess function from the stats package of R (R Core Team, 2020). The smoothing factor and the curve degree were fixed at 0.75 and 2, respectively, based on visual examination of all the fits. When the predicted number of flowers was <0, values were adjusted to zero. These curves permitted estimation of the number of open flowers in each population over time. To facilitate efficient, discrete-time quantitative comparisons of effectively continuous distributions, we used these curves to calculate the number of open flowers in 0.1-day intervals; these numbers were used in the overlap and potential gene flow calculations described below.

For each pair of populations separated by more than 100 m of elevation, we measured the time between flowering peaks and a common metric of overlap between curves, as well as our new metric of potential gene flow (see below). The 100-m separation criterion meant excluding pairs of populations with the same elevation on different transects; our model accounts for differences in phenology, not geographic position; excluded pairs represent replicates of the same "phenological population". Given a particular interest in range-edge populations and gene flow from contrasting environments, we also conducted analyses contrasting only the two highest vs. the two lowest elevation populations on each transect.

The time between flowering peaks was measured as the number of days separating the mode of the flowering distributions of the two curves. For a measure of phenological overlap (Eq. 1), we calculated the area of overlap between the standardized flowering curves of two given populations, "a" and "b". This area was calculated by summing the minimum number of flowers open across the two populations at each time i (0.1 day increments), between the first ($i = 1$) and last ($i = n$) time increments of flowering across the two populations. 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 time increments (expressed as $\sum a$ and $\sum b$). This step effectively standardized the total area under each flowering curve to equal 1.0. Therefore, overlap values range between 0 (no overlap) and 1 (complete overlap). This metric has been used in the past to measure phenological synchrony (Fox, 2003; Miller-Rushing et al., 2010) and as a proxy for the potential for gene flow between populations (Franks and Weis, 2009; Matter et al., 2013).

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

As a more biologically relevant metric of potential gene flow between populations, we developed an ecological model to predict the potential for gene flow based on phenological data (Eq. 2). The model estimates for a given population "b" the expected proportion of pollen received originating from a second population "a", assuming that reproduction is random among individuals of both populations combined. The model focuses entirely on phenology rather than any dependence of gene flow on geographic distance (Levin, 1981), and thus gives an upper-bound estimate of potential gene flow. Since the model estimates the potential proportion of exchanged pollen, values range between 0 and 1. For each time i, we first calculate the proportion of total flowers from the two populations that are in the donor population a: $a_i / \left(a_{i+} b_i\right)$. We then calculate a weighted sum of these values across time (0.1 day increments), with weights equal to the number of flowers open in the recipient population b at time i as a proportion of the total number of flower-days in that population during the entire flowering period ($b_i / \sum_{i=1}^{n} b_i$).

$$\text{PGF}_{a \to b} = \sum_{i=1}^{n} \frac{a_i}{a_{i+} b_i} \cdot \frac{b_i}{\sum_{i=1}^{n} b_i} \quad (2)$$

This metric of potential gene flow (PGF) offers two main advantages over the standardized measure of overlap described above. First, the value of PGF has a clear, quantitative, biological meaning, instead of being a rough statistical proxy. Indeed, the standardized overlap metric only takes into account the minimum number of flowers across two populations of interest at a given time (see Eq. 1), while gene flow is determined by the number of flowers in both populations. Second, our model allows for asymmetry in gene flow (i.e., $\text{PFG}_{a \to b} \neq \text{PFG}_{b \to a}$), which is expected to be widespread in nature (Servedio and Kirkpatrick, 1997; Fedorka et al., 2012), and therefore a key part of range-edge evolution models (García-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997). In particular, gene flow from central to range-edge populations is expected to be much greater than the opposite (García-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997), with important implications for adaptation to climate change (see introduction). In our analyses, we measured potential for gene flow in both directions for each pair of populations.

For each of these three measures of phenological separation or overlap (the mean across pairs), we then estimated its slope in relation to the timing of the start of the flowering season (with years as replicates) using general linear models (stats package, R Core Team, 2020). Statistical significance of slopes was tested using a null model (described below). The date of the start of the flowering season was quantified as the mean of the two mean flowering dates of the lowest-elevation (i.e., earliest-flowering) populations of each transect. Given a fairly small number of years of data ($N = 6$), we also calculated interannual variance of the three separation/overlap measures. Interannual variance is a prerequisite for climate-driven variation, and the data permit a stronger test for the presence of significant interannual variation than for a correlation of those variables with the start of the flowering season.

The null model for significance testing randomized the flowering curves for a given site among years, after first standardizing the overall mean flowering date across years by setting it to zero for all years. This standardization prevents variation in mean flowering date among *years* from artificially reducing flowering overlap among populations within years in the null model (e.g., comparing a population in an early year with a population in a late year). Effectively, we are isolating the precise phenomenon of interest: phenological overlap among populations and how this overlap varies across years. After standardization, we randomly shuffled (without replacement) flowering curves across years for each population 10,000 times ("sample" function in R). Annual values of the separation/overlap metrics were recorded in each simulation. We then compared the observed values of the slopes (vs. the start of the flowering season) and interannual variance to the distribution of values from the null model. For the values of the slopes, we used a two-sided test where the observed slopes were considered

significantly different from the null model if they were lower or higher than 97.5% of the simulated values. For the interannual variance, we used a one-sided test where the variance was considered significantly different from the null model if the value was higher than 95% of simulated variance values.

For each population of each species in each year, we measured the duration of the flowering period by calculating the number of days between the first and the last day of flowering, using the curves fitted to the phenological data. We tested for an effect of the start of the flowering season on the duration of the flowering period with plot identity as a random effect, using linear mixed models (stats package, R Core Team, 2020). Separate tests were performed for *Erythronium americanum* and *Trillium erectum*.

RESULTS

The date of the start of the flowering period varied substantially among the years encompassed by this study. For *Trillium erectum*, the earliest start of the flowering period (2013) was 9.5 days earlier than the latest start of the flowering period (2014), while the difference between those 2 years was 10.2 days for *Erythronium americanum*. The comparison of our data to a null model demonstrated that, for both *T. erectum* and *E. americanum*, years with earlier starts of the flowering period were associated with an increase in the mean time between flowering peaks of populations along the elevation gradient, although the earliest year (2013) had a major influence for both species (Figs. 1, 2; Table 1). For *T. erectum*, we also found a significant decrease in the overlap between populations' phenological curves and potential for gene flow with earlier starts to the flowering season (i.e., toward the left on the *x*-axis in Fig. 2). However, for *E. americanum*, there was no significant difference between our data and the null model regarding the effect of the start of the flowering period on the overlap and the potential for gene flow. For *T. erectum*, interannual variance in the time between flowering peaks and the phenological overlap were also significantly higher than those obtained from the null model (Appendix S1; see Supplemental Data with this article). For *E. americanum*, only the number of days between flowering peaks had significantly higher interannual variance than in the null model. Results were

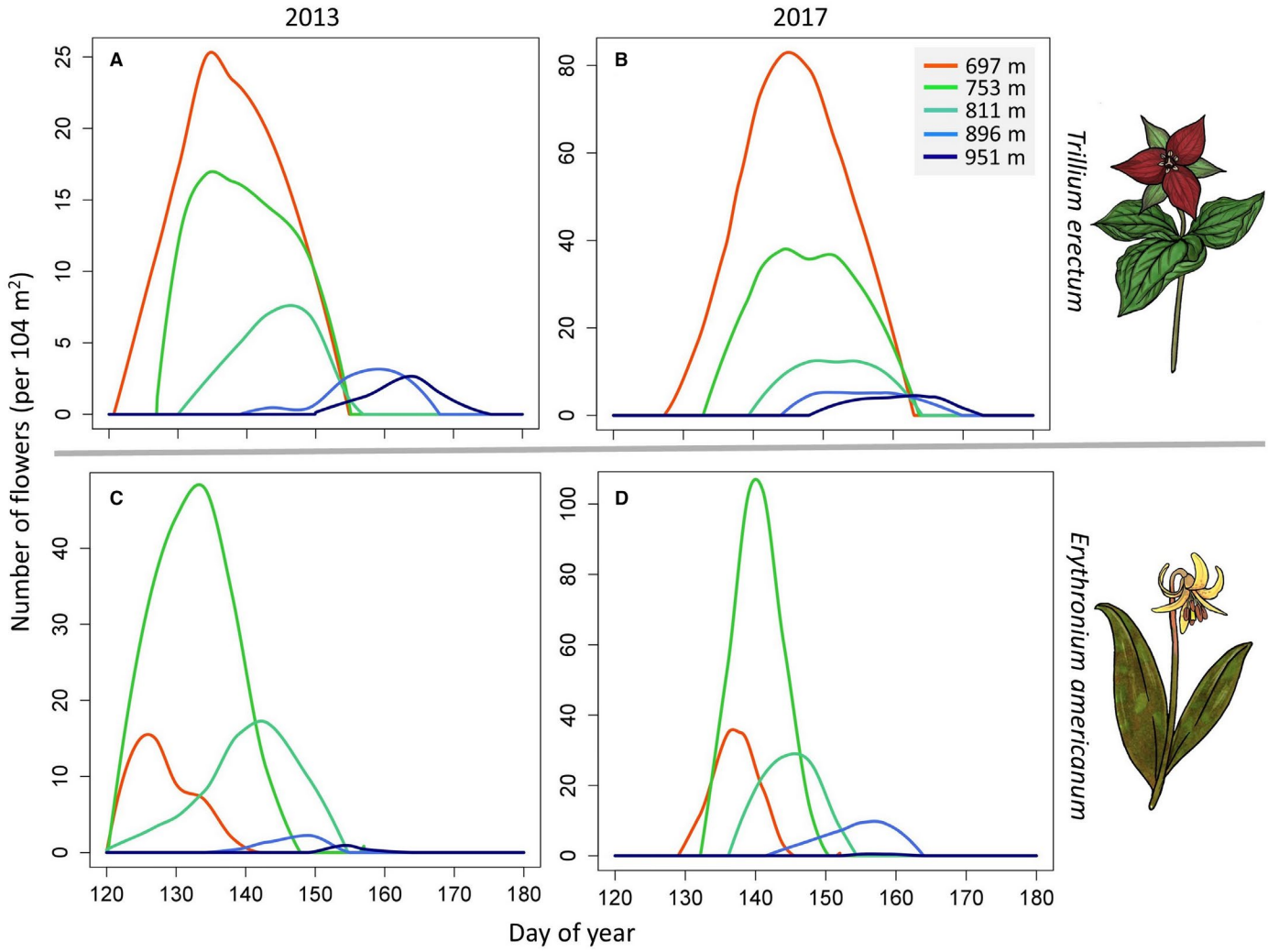

FIGURE 1. Number of open flowers in five populations along an elevational transect (results are similar for both transects) for (A, B) *Trillium erectum* and (C, D) *Erythronium americanum* during a year with an early flowering-season initiation (2013: A, C) and a year with a late flowering-season initiation (2017: B, D). Illustrations by Florence Jean.

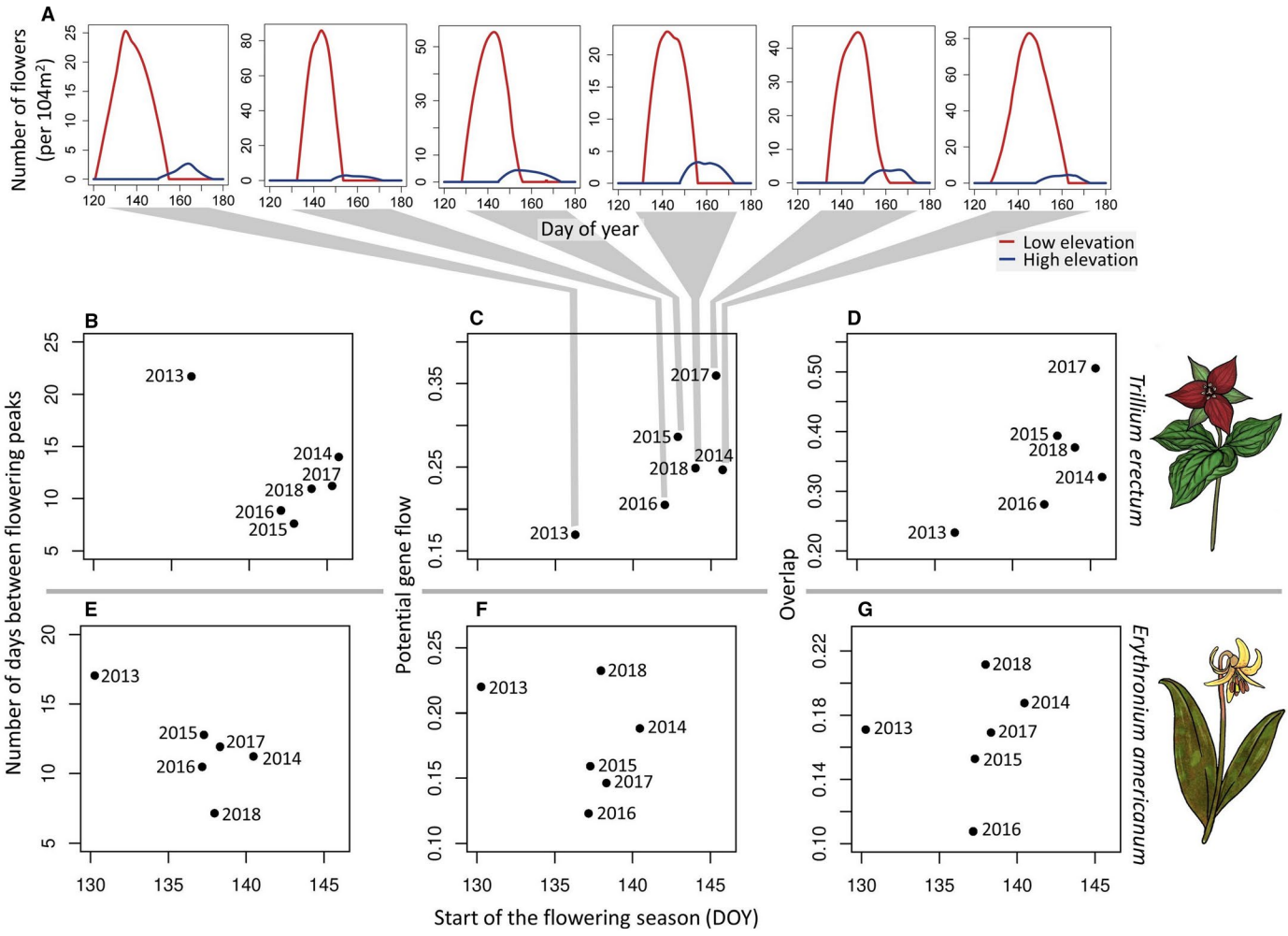

FIGURE 2. Comparison of the interpopulational synchrony and the potential for gene flow among populations for 6 years varying in the timing of the start of the flowering season. (A) Flowering phenology of the lowest- and highest-elevation populations across the different years for *Trillium erectum*. (B–G) Effects of the timing of the start of the flowering season on annual mean number of days between flowering peaks (B, E), potential gene flow (C, F), and overlap (D, G) between all pairs of populations separated by more than 100 m of elevation, distributed along an elevation gradient. Results are shown for *Trillium erectum* (B–D) and *Erythronium americanum* (E–G). Dates for the start of the flowering season correspond to the average of the mean flowering dates of the lowest-elevation populations of each transect. Illustrations by Florence Jean.

qualitatively similar when calculating pairwise metrics for only the two lowest and two highest populations of each transect (Appendix S2), and indeed the overall effect was due in large part to pairwise contrasts involving the highest-elevation (range-edge) populations (Appendix S3d). We found a significant effect of the start of the flowering season on the duration of the flowering period for *Erythronium americanum* (df = 39, $t = -3.02$, $p = 0.005$), but not for *Trillium erectum* (df = 39, $t = -1.86$, $p = 0.07$) (Fig. 3).

DISCUSSION

Climate change affects organisms in multiple ways. Using a novel method to measure potential gene flow based on phenological data, we demonstrate that climate change could decrease gene flow via phenological separation among populations. Specifically, earlier onset of spring was associated with a reduction in potential gene flow between populations of *Trillium erectum*. Gene flow plays an important role in the capacity of species to adapt to changing environments and therefore is expected to have a major impact on responses to anthropogenic climate change (Aitken and Whitlock, 2013; Matter et al., 2013; Aguilée et al., 2016). For high-elevation populations, where genes from lower elevation might be pre-adapted to warmer conditions, the observed decrease in potential gene flow with earlier springs might compromise the capacity of *T. erectum* populations to adapt to the climate change. Gene flow from high to low elevation is, at present, much lower given smaller populations at high elevation.

The timing of the start of the flowering season was quite variable among the 6 years of our study, with approximately 10 days between the earliest and the latest starts of the growing season. Considering the reported paces of advances in flowering phenology, ranging from 1.2 to 3.1 days per decade (Walther et al., 2002), the interannual phenological variation encompassed by this study should correspond to several decades of climate warming. We observed that, for *T. erectum*, the potential for gene flow was roughly twice as high in 2017, a late-spring year, as it was in 2013, an early-spring year (Figs. 1, 2), suggesting not just statistically significant

TABLE 1. Comparison between observed and simulated values from a null model of the number of days between flowering peaks, potential gene flow, and overlap, between all pairs of populations separated by more than 100 m of elevation, distributed along an elevation gradient. Comparisons were made between the observed and simulated slopes of the annual mean values of the variables in relation to the flowering-season initiation dates. Flowering-season initiation dates correspond to the mean of the mean flowering dates of the lowest-elevation populations of each transect.

Species	Variable	Observed slope	Percentile 2.5 from null model distribution	Percentile 97.5 from null model distribution	*P*
T. erectum	Days between flowering peaks	–0.978	–0.810	0.727	**0.007**
	Overlap	0.0183	–0.0150	0.0167	**0.030**
	Potential gene flow	0.0140	–0.0106	0.0137	**0.045**
E. americanum	Days between flowering peaks	–0.692	–0.531	0.491	**0.004**
	Overlap	0.00194	–0.00870	0.00835	0.663
	Potential gene flow	–0.00419	–0.0104	0.0101	0.428

Values in bold indicate statistically significant difference from the null model ($P < 0.05$).

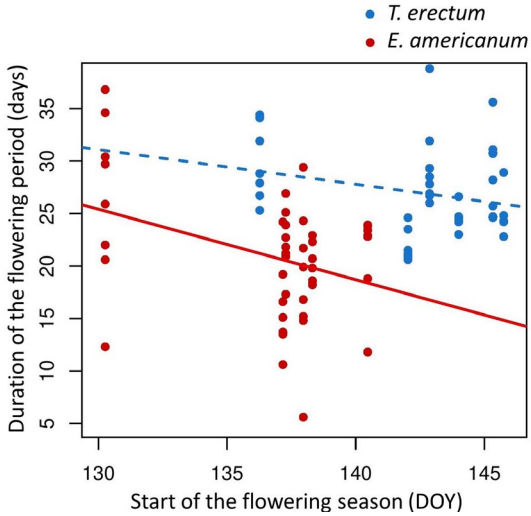

FIGURE 3. Relationship between the start of the flowering period and the length of the flowering period across years for different populations of *Trillium erectum* (df = 39, $t = -1.86$, $p = 0.07$, dotted line indicating trend not statistically significant) and *Erythronium americanum* (df = 39, $t = -3.02$, $p = 0.005$, solid line indicating significant trend) along an elevational gradient.

but biologically important impacts on gene flow. However, offering precise predictions regarding the impact of phenological advance on the potential for gene flow will require longer-term phenological data sets providing information on the entire flowering season of different populations along latitudinal or elevational gradients—data that are not available at present.

Earlier onset of spring was associated with a reduction in flowering synchrony among populations along the elevation gradient for *T. erectum* and possibly (but less obviously) for *E. americanum*. These results are consistent with long-term studies across latitudinal gradients that have observed greater phenological responsiveness to temperature in populations from warmer environments (Menzel et al., 2006; Wang et al., 2015). Our results, together with these studies, suggest that a reduction in gene flow among populations along spatial temperature gradients might be a widespread consequence of climate warming. However, the fact that the reduction in synchrony in earlier springs did not result in a decrease in the potential for gene flow for *E. americanum* demonstrates that this response to climate variation is species-specific (see also Rafferty et al., 2020).

The lack of consistency between the time between flowering peaks and potential gene flow for *E. americanum* indicates that the use of a single flowering date to characterize flowering phenology, as used in many long-term phenological studies (e.g., Menzel et al., 2006; Bock et al., 2014; Wang et al., 2015), is not a suitable proxy for estimating potential gene flow. Thus, understanding how gene flow will be affected by climate warming will require estimation of the shapes of full flowering-time distributions in populations along climatic gradients over the long term. In addition to affecting first or mean flowering dates, temperature during and before the growing season is likely to affect other components of phenology that influence potential gene flow. Indeed for *E. americanum* (but not *T. erectum*), we found that in years with earlier flowering, the duration of flowering period was longer (Fig. 3). With respect to potential gene flow, increased separation of peak flowering times (negative effect) was thus countered by broader distributions of flowering times (positive effect). Interestingly, while increased duration of the flowering period might counter phenological divergence between populations, it might reduce gene flow within populations by reducing intrapopulation flowering synchrony (Zohner et al., 2018). Several other studies have observed an increase in the length of the flowering season in earlier springs or warmer years at the individual (Miller-Rushing et al., 2007; Arroyo et al., 2013) or population level (Bock et al., 2014; Zohner et al., 2018). Furthermore, CaraDonna et al. (2014) observed, for 60 plant species studied over 39 years, that shifts in the start, peak, and end of flowering were not consistent. Also, the quantity of flowers and the length of the flowering period are potentially affected by numerous abiotic and biotic factors (O'Neill, 1997). Interannual variation in the quantity of flowers and the length of the flowering period and independent of shifts in first or peak flowering dates could reduce the strength of the link between phenological cues and the potential for gene flow.

Our data suggest a potential negative impact of climate warming on the amount of gene flow, at least for one of our study species. Considering the important role that gene flow is assumed to play in the capacity of species to adapt to climate change, our study points to an urgent need for researchers to gather complete populational phenological data across many years. Developing such databases will allow more precise predictions of the effect of climate warming on gene flow and could therefore considerably increase our understanding of how climate change will affect biodiversity.

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AUTHOR CONTRIBUTIONS

S.R. and M.V. developed the idea. G.L. and M.V. designed the sampling protocol. S.R., G.L., M.V., and D.W. collected the data. S.R. and M.V. developed the null model. S.R. performed the analysis and wrote the first draft of the manuscript. All authors read and contributed feedback to the manuscript.

DATA AVAILABILITY

Data and codes for this manuscript are available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.sqv9s4n2j (Rivest et al., 2021).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Comparison between observed inter-annual variance and simulated inter-annual variance from a null model for time between flowering peaks, potential gene flow, and overlap between pairs of populations.

APPENDIX S2. Comparison between observed and simulated values from a null model of the number of days between flowering peaks, potential gene flow from low to high elevation, and overlap, using data only for pairs of populations from the two lowest and two highest elevations of each transect.

APPENDIX S3. Supplementary figures showing some of the raw data used for the curve fitting, the length of flowering duration between years for each population of both species, and the potential for gene flow between each pair of populations for *Trillium erectum*.

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