



Climate drives among-year variation in natural selection on flowering time

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TITLE:

Climate drives among-year variation in natural selection on flowering time

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ABSTRACT

Climate change may result in both immediate plastic responses of life history traits, and altered selection on these traits. We used data from 22 flowering seasons to examine how climate influences flowering phenology and phenotypic selection on phenology in the perennial herb *Lathyrus vernus*. Plants flowered earlier in springs with higher temperatures and higher precipitation. Selection favored early flowering in nearly all years, but selection strength differed among years. Climatic variables explained most of this among-year variation in selection, early flowering being more advantageous in springs with higher temperatures and lower precipitation. Our results show that climate both induces plastic responses and influences natural selection on phenology, and that effects show both co-gradient patterns, as for spring temperature, and counter-gradient patterns, as for precipitation. To predict long-term responses of phenology to climate change, both immediate effects on trait expression and evolutionary responses to climate-induced changes in selection will be important.

INTRODUCTION

Climate change is rapidly altering the environmental conditions experienced by organisms (Diffenbaugh & Field 2013; IPCC 2014), likely resulting in both immediate plastic responses of individual organisms and altered evolutionary trajectories of populations. Responses to climatic variation often involve changes in phenology, i.e. in the timing of life cycle events (Visser & Both 2005; Cleland *et al.* 2007; Charmantier & Gienapp 2014). In seasonal environments, timing is crucial for survival, and phenological changes constitute the main way in which organisms can modify their interactions with the abiotic and biotic environment. Short-term plastic responses to climatic variation, e.g. earlier development with increasing spring temperatures or earlier snowmelt have been documented for many species (Charmantier *et al.* 2008; Thomson 2010; Anderson *et al.* 2012; CaraDonna *et al.* 2014; Wadgymar *et al.* 2018; Valdés *et al.* 2019).

While phenotypic responses of phenology to climate are well documented, it is less clear how climatic variation influences the direction and intensity of natural selection on the seasonal timing of life history events (Chevin *et al.* 2010; MacColl 2011; Visser *et al.* 2015; Siepielski *et al.* 2017; Marrot *et al.* 2018). A few studies have identified climatic factors as important drivers of temporal variation in selection on timing of reproduction in animals (Réale *et al.* 2003; Visser *et al.* 2015; Marrot *et al.* 2018), but climatic effects on selection on phenology in plants remain largely unexplored. It has been argued that increased temperatures are expected to influence selection on timing through changes in the relationship between adaptive optima and phenotypic means (e.g. Visser *et al.* 1998, 2006; Gienapp *et al.* 2008; Marrot *et al.* 2018; Valdés *et al.* 2019). However, the direction of the selection induced by temperature increases will depend on how strong plastic responses are compared with changes in the optimal phenology. If higher temperatures favor a stronger response of phenology to temperature, then we should expect selection for earlier phenology to be stronger at higher

temperatures, when mean phenology is early (i.e. selecting for a co-gradient response, cf. Conover & Schultz 1995; Conover *et al.* 2009). On the other hand, if higher temperatures favor a weaker response, we should expect selection for earlier phenology to be stronger at lower temperatures when mean phenology is late (i.e. selecting for a counter-gradient response).

In order to explore the effects of climatic variation on the expression of phenological traits and on natural selection on these traits, we need estimates of climatic variables, phenotypic traits and trait-fitness covariances over multiple years. Despite the fundamental importance of temporal variation in selection, few long-term assessments on selection on plant traits exist (Siepielski *et al.* 2017), the longest spanning up to 10-11 years (Campbell & Powers 2015; Thomann *et al.* 2018). Here, we use 22 years of data from permanently marked individuals in a natural population of the long-lived spring-flowering understory herb *Lathyrus vernus* to examine the effects of climatic variation on flowering phenology, and on phenotypic selection on phenology. This species often occurs in the understory of deciduous forests, where availability of light rapidly decreases when canopy closes in late spring. We addressed the following questions: (1) Does flowering time vary among years in response to climatic conditions during spring?, (2) Does the direction and strength of phenotypic selection on flowering time vary among years?, and (3) If so, is variation in phenotypic selection among years related to spring climate?

MATERIALS AND METHODS

Study system

The study was carried out in a population of *Lathyrus vernus* in a deciduous forest in the Tullgarn area, SE Sweden (58.9496 N, 17.6097 E), during 1987 – 1996 and 2006 – 2017. Individuals of this long-lived species grow fairly slow, have high annual survival rates and

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2
3 lack organs for vegetative spread. They flower only after several years of vegetative growth
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5 and frequently skip flowering in some years. Growth of shoots is determinate and the number
6
7 of flowers is determined in the previous year (Ehrlén & Van Groenendael 2001). One or
8
9 several erect shoots emerge from the over-wintering rhizome in April every year (Ehrlén
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11 1995). Flowering starts about 4 weeks after shoot emergence, before canopy development in
12
13 early June (Sola & Ehrlén 2007). Late frosts can damage flower buds on developing shoots in
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15 some years, but there is no water deficit during spring-early summer (J. Ehrlén pers. obs.).
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17 Flower number (usually 5 – 30) is closely correlated with aboveground biomass and plant
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19 resource state (Sola & Ehrlén 2007). Flowering is acropetally sequential within shoots,
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21 starting with the basal flower in the basal raceme. The large flowers are pollinated by
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23 bumblebees (*Bombus* spp.). *Lathyrus vernus* is self-compatible, but lacks mechanisms for
24
25 self-pollination (J. Ehrlén, unpubl. data). Timing of flowering is correlated with differences in
26
27 fruit set, and this pattern might be influenced by differences in both resource and pollen
28
29 availability (Ehrlén 1992). Individuals produce a small number of relatively large seeds and
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31 recruitment from seeds is frequent (Ehrlén & Eriksson 1996). Developing seeds are often
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33 damaged by the pre-dispersal seed predator *Bruchus atomarius*. Grazing by roe deer
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35 (*Capreolus capreolus*) sometimes removes entire flowering shoots, and early-flowering
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37 individuals are more often grazed than late-flowering (Ehrlén & Münzbergová 2009). There is
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39 heritable variation in flowering time among populations of *L. vernus* (Widén & Schiemann
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41 2003).
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49 The average conditional life span of flowering individuals has been estimated to 44.3
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51 years (Ehrlén & Lehtilä 2002). The long life span and the observed low turnover of
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53 individuals in the study population means that, despite the long duration of this study, among-
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55 year observed variation in trait distributions mainly results from phenotypic plasticity, while
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57 changes in the genetic structure of the population are of minor importance.
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Data collection

Data was collected in 22 years, 1987 - 1996 and 2006 - 2017. In 1987, all flowering individuals in an area of 825 m² were permanently marked and surveyed in each year to 1996. New flowering individuals in the plot were marked and included in the study in each year. No recordings were made from 1997 to 2005, and markings were not maintained. In 2006, a new set of individuals in an area of 162 m² within the same population were marked, and surveyed in the same way as the initially marked individuals to 2017. One set of 606 individuals was thus followed during 1987 - 1996, and a second set of 228 individuals was followed 2006 - 2017. In total, our data set included 2411 flowering events. The identity of individuals marked as flowering in one year was checked in each of the following years, to keep track of individuals flowering only in some study years. Field recordings in each season started when shoots initiated growth in April and were continued every fifth day until the last plant had finished flowering. At all visits, we recorded the number of buds, open flowers, scars from aborted flowers and fruits. We also recorded if one or several shoots had been grazed. At each visit up to opening of the first flower, we recorded the size and developmental stage of the most advanced bud in all individuals, and assigned it to one of four size categories: small, medium, large or very large. At the time of fruit maturation, we measured vegetative size and recorded the number of mature fruits, the number of intact seeds and the number of seeds damaged by pre-dispersal seed predators in each individual. To estimate vegetative size, we measured the height and diameter of all shoots, calculated the volume of each shoot as $\pi \times (\text{diameter} / 2)^2 \times \text{height}$, and summed the volumes of all shoots of an individual (see Appendix S1 for details). Fruits that had not yet opened at the time of recording were examined directly in the field. The number of seeds was counted and pre-dispersal seed predation was identified by the presence of larval entrance holes in a seed. Fruits that had opened prior to the recording were brought to the lab where the numbers of intact and preyed

seeds were estimated based on the number of placentas and larval entrance holes (see Appendix S1 for details). The total number of intact seeds produced by an individual was used as an estimate of fitness.

We used first flowering day (FFD), i.e. the day of year when the first flower was fully unfolded with the banner petal folded upwards, as an estimate of flowering phenology of individuals. Information about the presence or absence of open flowers from each visit allowed us to determine during which 5-day interval an individual had started flowering. To estimate the most likely date within this interval at which the first flower opened, we used information about the developmental stage of the most developed bud at the beginning of this 5-day interval, and the number of open flowers at the end of the interval (see Appendix S1 for details).

The total number of open flowers produced by an individual during the flowering season was obtained from counts of the total number of buds, flowers, fruits and scars from aborted flowers at each recording. The total number of flowers in individuals that were grazed before the first recording of flower number was estimated based on vegetative size (see Appendix S1 for details).

Weather data for March, April and May each year during 1961-2017 was obtained from the Swedish Meteorological and Hydrological Institute (www.smhi.se). Daily mean, minimum and maximum temperature values were averaged from two meteorological stations: Oxelösund (58.6777 N, 17.1223 E, 41 km from the study population) and Södertälje (59.2142 N, 17.6289 E, 29 km from the study population). In cases where values for one station were missing, we imputed values using the relationship between values from the two stations (see Appendix S1 for details). Daily precipitation values were obtained from a third station located in Åda (58.9279 N, 17.5358 E, 5 km from the study population).

Statistical analyses

To account for leap years, we converted calendar dates to number of days after the vernal equinox. We then calculated three measures of the yearly position of the flowering season: start - the date at which 10% of the plants had started flowering, mean - the mean first flowering date of all individuals, and end - the date at which 90% of the plants had started flowering. The yearly duration of the flowering season was defined as the number of days between the start and the end of the flowering season. We calculated 12 climatic variables from weather data: monthly averages of daily minimum, mean and maximum temperatures, and monthly sums of precipitation, for March, April and May.

We expected that higher temperatures during shoot development in March, April and May result in faster development and earlier flowering. We also predicted that higher temperatures are associated with higher availability of pollinators and resources, and lead to higher mean fitness. Lastly, we expected higher temperatures to lead to stronger selection for earlier flowering, as individuals flowering early benefit most from favorable conditions before canopy closure. Lower temperatures in April and May might also lead to that early buds and flowers are damaged by frosts, or experience a low availability of pollen. We expected that the duration of flowering is positively associated with temperatures in April, as higher temperatures early during the season mean an earlier start of flowering, and negatively associated with higher May temperatures, as this increases the speed of development, resulting in an earlier end of the flowering season. The expected effects of precipitation during spring on trait expression and natural selection are less obvious. Precipitation during early spring, if falling as snow, might protect overwintering shoot buds from extreme low temperatures (Inouye *et al.* 2002) and lead to an earlier onset of growth and earlier flowering. Precipitation during early spring falling as rain, on the other hand, is often associated with relatively mild temperatures, and is thus correlated with an earlier onset of growth and reproduction. Precipitation later in spring is associated with lower than average temperatures

and lower availability of light, possibly resulting in later flowering and weaker selection for early flowering.

To examine these hypotheses, we first performed univariate linear regressions of all climatic variables, mean FFD and number of intact seeds on year to check for trends across years. We checked for trends in climate for both the 22 study years and for 1961 - 2017.

To assess how much of the variation in FFD of individual plants was explained by year, we calculated the R^2 -value from a linear model of FFD with year as a nominal variable. The effects of climate on individual FFD-values were examined using linear mixed models with plant individual as a random effect and number of flowers as a covariate. All climatic variables were standardized prior to analyses by subtracting the mean and dividing by the standard deviation. We constructed a candidate model set using the 12 climatic variables as predictors. We constrained the set of candidate models by including only models with pairs of predictors having a Pearson correlation coefficient lower than 0.5. We ranked the resulting candidate models using the corrected second-order Akaike information criterion (AICc). To account for model uncertainty, we performed model averaging of parameter estimates across all models with $\Delta AICc < 2$. The effects of climate on the yearly position (start, mean and end) and duration of the flowering season were examined using linear models, including climatic predictors that had a significant effect in the averaged model of FFD for individual plants. In order to explore how long-term trends influenced our estimates of climate effects, we ran the models including also year as a covariate.

To assess how much of the variation in individual fitness was explained by year, we calculated the R^2 -value from a linear model of fitness with year as a nominal variable. The effects of the 12 climatic variables on individual fitness were examined using linear mixed models with number of flowers as a covariate and plant individual as a random effect. We performed model selection and model averaging, as described above.

To assess total and direct phenotypic selection on FFD in each year, we estimated phenotypic selection differentials and gradients, respectively. Fitness, in terms of the number of intact seeds, was relativized within years by dividing individual values by yearly mean values. Traits were standardized within years by subtracting the yearly mean and dividing by the yearly standard deviation. Selection differentials were calculated for each year using univariate regressions of relative fitness on standardized FFD (Lande & Arnold 1983). Selection gradients were calculated for each year using multiple linear regressions of relative fitness on standardized FFD and number of flowers. We included the number of flowers as a measure of resource state in these analyses (Rausher 1992). We also estimated non-linear (quadratic and correlational) selection by examining the effect of quadratic and interaction terms in a model including also the linear terms (Lande & Arnold 1983; Arnold 1986). Quadratic regression coefficients and their standard errors were doubled (Stinchcombe *et al.* 2008). We checked for a trend across years in direct selection on flowering time by performing a univariate linear regression of selection gradients for FFD against year.

Among-year differences in total and direct phenotypic linear selection on flowering time were evaluated using models including also the interaction between standardized FFD and year. The main effect of year was not included as fitness was relativized within years prior to analysis. Plant individual was included as a random effect. In this analysis, we considered only variation in linear selection because non-linear selection was significant only in very few years (see Results).

To examine if variation in total phenotypic selection among years was related to climatic conditions during spring, we ran models including standardized FFD and the interactions between FFD and the 12 climatic variables. The main effects of climatic variables on fitness were not included as fitness was relativized within years prior to analysis. Plant individual was included as a random effect. For the effects of interactions with climatic

variables, we performed model selection and model averaging as explained above. Interaction terms that had a significant effect in the averaged model (based on z , the Wald test statistic of the effect, see Table S5) were then included in an analysis of deviance using the Wald Chi-square test. We used analogous models to examine among-year variation in both total and direct selection, the latter including also the standardized number of flowers.

To assess how much of the among-year variation in total and direct selection was explained by climate, we carried out regressions of the selection coefficients (i.e. differentials and gradients) on the climatic variables whose interactions with standardized first flowering date were significant in the selection models, accounting for uncertainty in the estimates (i.e. standard errors). Using the R package MCMCglmm (Hadfield 2010) and the method described by Hunter *et al.* (2018), we calculated the posterior distribution of the variance in selection associated with the climatic variables and applied equation 12 in Hunter *et al.* (2018) to calculate the proportion of the total variation in selection attributed to the climatic component of the model (as the mean of the posterior distribution).

All statistical analyses were carried out in R version 3.5.1 (R Core Team 2018).

RESULTS

Climate in the study area varied considerably during the period 1961-2017 (Fig. 1, Figs. S1-S4). Minimum, mean and maximum daily temperature increased significantly from 1961 to 2017, but trends were not significant over the 22 study years (Table S1, Figs. S1-S3).

Yearly mean FFD ranged from 48.0 to 71.6 days (mean = 58.1) after the vernal equinox. Differences among years explained 59% of the total variation in individual FFD-values. There was a significant trend of an earlier mean first flowering date over the study period (Fig. 1B). Differences in climate explained a large part of the variation in FFD of individuals, flowering occurring significantly earlier in years with higher mean temperatures in April and May, and in years with higher precipitation in March and April (Table 1A, Fig.

2). Climatic predictors explained most of the among-year variation in the start (72%), mean (76%), end (84%), and duration (57%) of the flowering season (Table 1B and C). The start, mean and end of the flowering season were earlier in years with higher mean temperatures in April and May (Table 1B, Fig. 3A-B). The duration of the flowering season was longer in years with higher mean April temperatures (Table 1C, Fig. 3C). There were no effects of precipitation on the position and duration of the flowering season. All effects of climatic variables were independent of potential long-term trends (Table S2).

Mean fitness of individual plants ranged from 0.2 to 18.6 (mean = 5.0) intact seeds per flowering individual across years, but there was no significant trend over the study period (Fig. 1C). Differences among years explained 17% of the total variation in fitness among individuals. Fitness increased with March precipitation, and decreased with maximum April temperatures, minimum May temperatures and April precipitation (Table S3, Fig. S5).

Selection differentials for FFD were negative, i.e. favored early flowering, in 20 of 22 years (mean = -0.44, range = -1.03 - -0.28). Directional selection for early flowering was significant in 18 years, while non-linear selection was significant in only 2 years (Table 2A and S4). Selection gradients for FFD differed in strength but favored earlier flowering in 21 of 22 years (mean = -0.28, range = -0.78 - -0.08), and this pattern was significant in 8 years (Table S5). Non-linear direct selection on flowering time was significant in only one year, and correlational selection on FFD \times number of flowers in three years (Table S5). There was no significant trend in selection estimates over the study period (Fig. 1D).

Differences in total and direct selection among years were related to climatic conditions (Table 2B and S6, Fig. 4). There was increased selection for early flowering, i.e. the slope of the relationship between relative fitness and standardized FFD was more negative, in years with higher minimum April temperatures (Fig. 4A) and lower March precipitation (Fig. 4B). These two climatic variables explained 70 and 68 % of the among-

year variation in total and direct selection, respectively (values calculated as the mean of the posterior distribution).

DISCUSSION

Our results show that among-year variation in both flowering time and phenotypic selection on flowering time are related to climatic conditions during spring in the perennial herb *Lathyrus vernus*. Plants flowered earlier when springs were warmer and precipitation higher. Selection favored early flowering in almost every year, but strength of selection varied among years, and was stronger in springs with higher temperatures and lower precipitation, climatic variables explaining most of the among-year variation. Our results imply that climatic variation affects both the expression of traits associated with timing, and natural selection on these traits. They also suggest that effects of climate may favor both co-gradient patterns of phenotypic and genotypic responses, as for spring temperature in our study, and counter-gradient patterns, as for precipitation.

Based on the very low turnover of plants observed during our study, we assume that most observed variation in phenotypic values of FFD among years was due to plastic responses. This assumption is further supported by the fact that adding years as a fixed effect to models of FFD did not alter the effects of climatic variables. Phenotypic responses were strongly influenced by among-year climatic variation, with earlier FFD of individual plants and an earlier position of the flowering season in years with warmer springs. Individual plants started flowering on average 1.8 and 2.5 days earlier with a one-degree increase in mean temperature in April and May, respectively. Several previous studies have attributed advances in flowering phenology to increasing temperatures (Cleland *et al.* 2007; Ibáñez *et al.* 2010; Anderson *et al.* 2012; CaraDonna *et al.* 2014), with particularly strong effects in early-spring flowering species (Dunne *et al.* 2003). Responses to climate change and fine-tuning of the

flowering season to the environmental conditions might be particularly important in temperate early-spring forest plants where the benefits provided by high light availability are constrained by the timing of canopy closure. Although we found no warming trends across the 22 study years, spring temperatures have increased significantly in our study area over the last 57 years. In combination with the observed effects of spring temperatures on flowering phenology, we should thus expect that recent warming leads to an earlier flowering of *L. vernus*. In our study, we did indeed find a significant advance in first flowering dates over the study period.

The duration of the flowering season increased with increasing temperatures in April, but was not affected by temperatures later during the season. In contrast, previous studies have reported shorter flowering seasons with higher temperatures (Høye *et al.* 2013; Bock *et al.* 2014). For early-flowering species in seasonal environments, higher temperatures up to flowering start might increase the length of the flowering season, while higher temperatures during flowering might lead to a shorter duration of the flowering season (Theobald *et al.* 2017).

Together with increasing temperatures, current and future climatic changes entail shifts in precipitation, although predictions regarding the direction of changes in precipitation are less clear than for temperature (IPCC 2014). In temperate environments with little drought stress during spring or early summer, the expected phenological responses to altered precipitation regimes are also less clear than responses to changes in temperatures. Experimental studies examining responses of phenology to precipitation changes have either found no effects (Phoenix *et al.* 2001; Cleland *et al.* 2006), or reported species-specific responses (Peñuelas *et al.* 2004). Drier conditions have been shown to delay the onset of flowering in the Mediterranean region (Llorens & Peñuelas 2005) and higher summer precipitation in the previous year led to earlier flowering in an alpine environment (Lambert

et al. 2010). We show that higher precipitation during early spring, i.e. before and at the beginning of the flowering season of *L. vernus*, was associated with an earlier flowering. This is consistent with that precipitation during early spring, falling as snow or rain, is associated with lower risks of frost damage and relatively mild temperatures, respectively, and therefore with an earlier flowering. The effects of precipitation were weaker than those of temperature (plants flowered on average 0.5 and 0.2 days earlier with a 10 mm increase in precipitation in March and April, respectively), and there were no significant effects of precipitation on the position and duration of the flowering season.

Reviews of selection estimates from natural populations have shown that both the direction and the strength of selection vary geographically (Siepielski *et al.* 2013) and temporally (Siepielski *et al.* 2009, but see Morrissey & Hadfield 2012). In our study, the strength of both total and direct phenotypic selection on flowering time varied significantly among years, from strongly favoring early flowering in some years to no detectable selection in other years. Still, the direction of selection was the same in almost all years. The persistent selection for early flowering shown in our 22-year study is in agreement with many previous studies (Harder & Johnson 2009; Munguía-Rosas *et al.* 2011; Austen *et al.* 2017). Several explanations for observed persistent phenotypic selection for earlier flowering have been suggested (Forrest 2014; Ehrlén 2015; Austen *et al.* 2017). For example, it has been proposed that selection acting on other fitness components, or later during the season, may counter observed patterns of seed production, or that individuals that are in a better condition are able to both flower earlier and to produce more seeds. We did not specifically examine the validity of these explanations. However, the fact that strength of selection varied among years and that most of this variation was explained by differences in spring climatic conditions, suggest that explanations based solely on selection through other fitness components or condition-dependence might not sufficiently explain the prevalence of selection for earlier flowering.

Identifying the environmental drivers of natural selection is key to understand past evolution and to predict the evolutionary implications of ongoing changes in climate and environment. Yet, the factors underlying temporal variation in natural selection on many traits, like plant flowering phenology, remain poorly known (e.g. MacColl, 2011, but see Siepielski *et al.* 2017). One mechanism suggested to underlie temporal variation in selection are climatic fluctuations (Réale *et al.* 2003; Siepielski *et al.* 2009, 2017; Visser *et al.* 2015; Siepielski *et al.* 2017; Marrot *et al.* 2018). In our study, which included one of the longest records of natural selection in plants recorded, we were able to explain most of the observed among-year variation in selection by climatic variation. The strength of phenotypic selection for early flowering increased with minimum temperatures in April and decreased with precipitation in March. The association among high spring temperatures and strong selection for early flowering in *L. vernus* might be due to earlier-flowering individuals maximizing the benefits of high light availability prior to canopy closure under warm conditions (Kudo *et al.* 2008). Selection for early flowering might also increase with higher spring temperatures because the frost damage risk decreases in early-flowering individuals (Inouye 2008; Pardee *et al.* 2018). Lastly, higher spring temperatures might be associated with higher pollinator availability early during the flowering season (Kudo & Ida 2013). Our results with a perennial plant agree with the results of previous studies on birds (Visser *et al.* 2015; Marrot *et al.* 2018) and mammals (Réale *et al.* 2003), suggesting that increased selection for earlier reproduction with warmer temperatures might be a widespread phenomenon among organisms in temperate seasonal environments.

Although precipitation has been recently reported as an important driver of variation in selection worldwide (Siepielski *et al.* 2017), no studies to date have directly related long-term variation in precipitation to changes in phenotypic selection. Our results suggest that while higher precipitation before the growing season is associated with an earlier onset of flowering,

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3 it also means that selection for early flowering decreases (Fig. 4B). A possible mechanism
4 underlying this seemingly contradictory pattern is that early spring precipitation may result in
5 smaller fitness advantages of earlier-flowering individuals because pollinator abundance is
6 lower early in the season. Higher precipitation in early spring might also increase the intensity
7 of antagonistic interactions, such as mollusk herbivory (Crawford-Sidebotham 1972) which
8 have strong negative effects on fitness in *L. vernus* by damaging emerging shoots (Ehrlén
9 2002).

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12 Our 22-year assessment of among-year variation in selection on timing of flowering
13 demonstrates that climatic variation can influence both the expression of phenological traits,
14 and the strength of natural selection on these traits. An important implication of these findings
15 is that plant phenology is likely to show both plastic and, providing there is additive genetic
16 variation for flowering time, evolutionary responses to climatic changes. Moreover, our
17 results show that the effects of climatic variation on the expression of a trait and on the
18 strength of phenotypic selection on the same trait, might act in the same or in opposite
19 directions. In our study, higher spring temperatures were associated with both an earlier
20 flowering and an increased fitness advantage for plants flowering early, suggesting that
21 plastic and genetic responses might follow a co-gradient pattern (Conover & Schultz 1995;
22 Conover *et al.* 2009). In contrast, higher precipitation before the flowering season was
23 associated with an earlier phenology, but also with a decreased fitness advantage for plants
24 flowering early, suggesting a counter-gradient pattern. To predict long-term responses of life-
25 history traits to global climate change, we therefore need to consider both the immediate
26 effects on trait expression and the potential evolutionary responses to climate-induced
27 changes in selection.

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Tables

Table 1: Results of models testing for effects of climatic factors on (A) first flowering date (FFD) of individual plants, and on three measures of the yearly position (B) and duration (C) of the flowering season of *Lathyrus vernus* over 22 years. The position of the flowering season was defined by the start, i.e. the date when 10% of the plants had started flowering, the mean, i.e. the mean first flowering date, and the end, i.e. the date when 90% of the plants had started flowering. The duration of the flowering season was defined by the number of days between the start and the end. In (A), linear mixed models with plant individual as a random effect and number of flowers as a covariate were used. Also shown are averaged estimates and z-values across all candidate models with $\Delta AICc < 2$, and values of marginal and conditional pseudo- R^2 (representing, respectively, the variance explained by the fixed effects and by the entire model, Nakagawa & Schielzeth 2013) for the best model resulting from model selection. $\sum w_i$ = Relative variable importance (sum of Akaike weights over all models including each variable).

Response variable	Predictor variable	Estimate	Std. error	z
A) FFD N = 2411 $R^2_{\text{marg}} = 0.577$ $R^2_{\text{cond}} = 0.628$	Precipitation March	-0.716***	0.105	6.85
	Precipitation April	-0.345**	0.125	2.77
	Mean March	-0.074	0.136	0.54
	Max March	-0.236	0.185	1.28
	Mean April	-2.188***	0.134	16.29
	Mean May	-3.754***	0.113	33.17
	Number of flowers	-2.407***	0.101	23.86
		Estimate	Std. error	t
B) Position of the fl. season				
Start N = 22 $R^2 = 0.719$	Precipitation March	-0.993	0.888	-1.12
	Precipitation April	-0.353	0.938	-0.38
	Mean April	-4.465 ***	1.001	-4.46
	Mean May	-3.531 ***	0.823	-4.29

		Precipitation March	-0.803	0.724	-1.11
Mean		Precipitation April	-0.627	0.765	-0.82
N = 22		Mean April	-3.429 ***	0.816	-4.20
R ² = 0.764		Mean May	-4.004 ***	0.671	-5.97
		Precipitation March	-0.461	0.549	-0.84
End		Precipitation April	-0.810	0.580	-1.40
N = 22		Mean April	-2.324 **	0.618	-3.76
R ² = 0.844		Mean May	-4.555 ***	0.508	-8.96
C) Duration or the fl. season					
		Precipitation March	0.532	0.604	0.88
N = 22		Precipitation April	-0.458	0.638	-0.72
R ² = 0.567		Mean April	2.141 **	0.680	3.15
		Mean May	-1.024	0.560	-1.83

***P < 0.001; **P < 0.01; *P < 0.05

Table 2: Phenotypic selection models testing for (A) among-year differences and (B) effects of climatic factors on total (direct and indirect) and direct phenotypic selection on flowering time in *Lathyrus vernus* in 22 study years. Results are from (A) linear mixed models including the interaction first flowering date \times year, testing for among-year differences in selection differentials and selection gradients for first flowering date, and (B) linear mixed models including the interactions of first flowering date with climatic variables that were significant according to model selection (see Table S5). For direct phenotypic selection, number of flowers is included as a condition trait. Fitness was estimated by the number of intact seeds. Traits were standardized and fitness relativized within years before analyses. Plant individual was included as a random effect.

Predictor variable	Total selection		Direct selection	
	χ^2	Degrees of freedom	χ^2	Degrees of freedom
A) First flowering date	110.18 ***	1	33.89***	1
Number of flowers	-	-	64.79***	1
First flowering date : year	36.46 *	21	37.87*	21
B) First flowering date	110.44***	1	33.93***	1
Number of flowers	-	-	65.55***	1
First flowering date : Precipitation March	12.48***	1	14.80***	1
First flowering date : Precipitation April	3.48	1	3.83	1
First flowering date : Min April	10.732**	1	9.90**	1

***P < 0.001; **P < 0.01; *P < 0.05

Figures

Figure 1: Variation in temperature, flowering time, fitness and selection on flowering time during the study period. Symbols represent means (dots) and standard errors (whiskers) for yearly values of (A) mean daily temperature during March (light grey), April (medium grey) and May (black), (B) first flowering date, calculated as the number of days from the vernal equinox, (C) mean fitness, calculated as the number of intact, non-predated seeds, and (D) linear selection gradient for first flowering date. In (B), the solid line indicates a significant linear trend of an earlier flowering date across years ($\beta = -0.267$, $t = -2.42$, $p = 0.025$, adjusted $R^2 = 0.19$). In (D), filled circles indicate significant selection gradients at $\alpha = 0.05$ and the grey dashed line indicates a selection gradient not significantly different from 0.

Variation in other climatic variables is presented in Figures S1-S4.

Figure 2: Relationship between first flowering date of individual plants in different years and (A) mean daily temperature in April, (B) mean daily temperature in May, (C) sum of precipitation in March, and (D) sum of precipitation in April. Lines represent linear model fits.

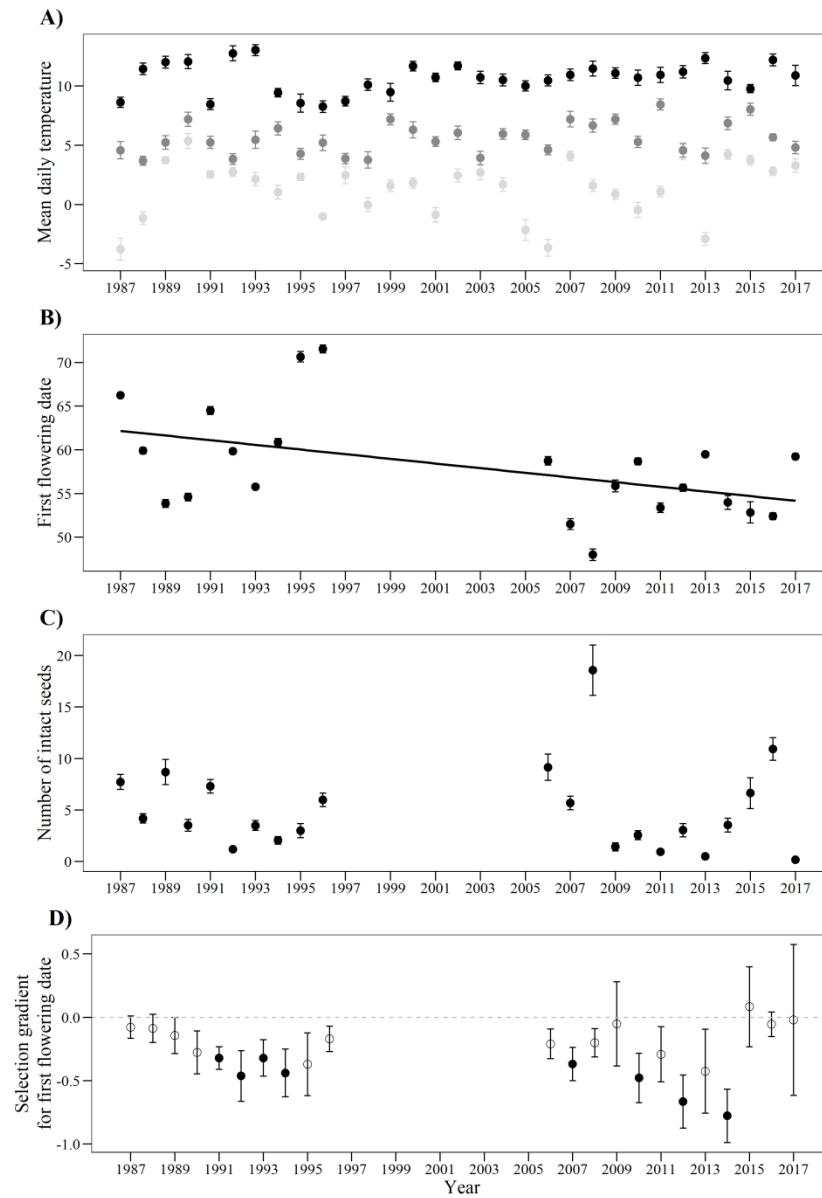
Figure 3: Relationship between the start, mean and end of the flowering season, and (A) mean daily temperature in April and (B) mean daily temperature in May, and (C) between the duration of the flowering season and mean daily temperature in April. The symbols in (A) and (B) denote the start, i.e. the date when 10% of the plants had started flowering (triangles and dashed line), the mean, i.e. the mean first flowering date (points and solid line) and the end, i.e. the date when 90% of the plants had started flowering (stars and dotted line), of the flowering season. The duration of the flowering season was measured as the number of days between the start and the end of the season. Lines represent linear model fits.

Figure 4: Partial regression plots (i.e. added variable plots) showing the independent effects of climatic variables on selection gradients for first flowering date (FFD) in *Lathyrus vernus* in

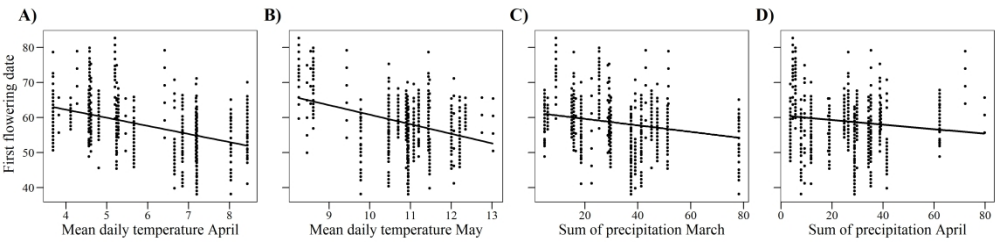
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22 study years. The effect of standardized first flowering date on relative fitness, measured as the relative number of intact seeds, is shown for (A) different minimum daily temperatures in April and (B) different sums of precipitation in March. Lines represent linear model fits. Values were calculated from models in Table 2.

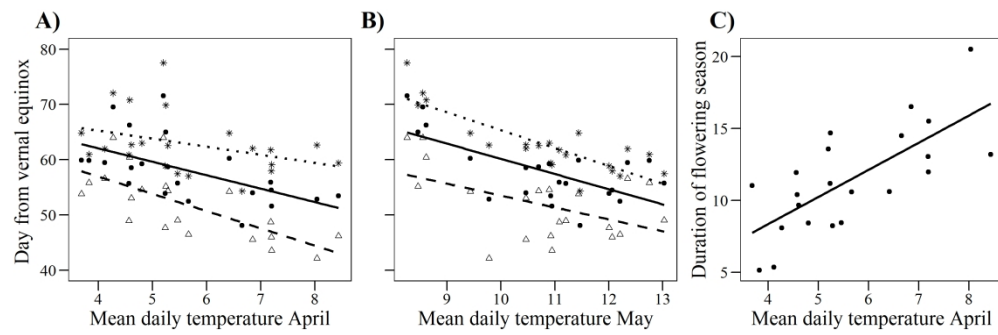
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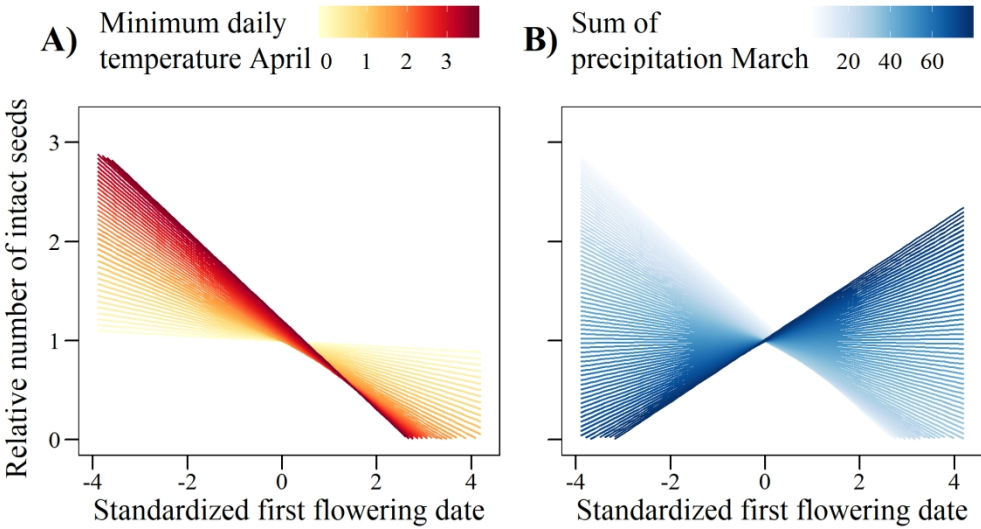
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