Climate influences flowering time and natural selection in a perennial herb

Climate influences both trait expression of flowering time and phenotypic selection among individuals in a perennial herb

ABSTRACT

Climate change is likely to lead to immediate changes in the phenology of individual organisms, as well as to changed selection on traits associated with development and phenology. In this study we used 22 years of data on flowering phenology and fitness in permanently marked individuals of the perennial herb *Lathyrus vernus*, to examine how climatic variation influence timing of flowering, and phenotypic selection on flowering phenology. This species often occurs in the understory of deciduous forests, where availability of light is high during spring, but rapidly decreases when canopy closes in early summer. The results show that plants flowered earlier when springs were warmer and precipitation high. Phenotypic selection favored early flowering in 21 of 22 years but strength of selection differed among years, and was stronger in years when spring temperatures were higher and spring precipitation lower. Our results thus 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 be associated with both co-gradient patterns, as for spring temperature in our study, and with counter-gradient patterns, as for precipitation in our study. An important implication of our results is that to predict the long-term responses of traits, such as phenology, to global climate change, we need to consider both the immediate effects on trait expression and the potential evolutionary responses to climate induced changes in selection.

INTRODUCTION

Climate change is rapidly altering the environmental conditions experienced by organisms (Diffenbaugh & Field 2013; IPCC 2014). This is likely to result 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 seasonal 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 an organism can modify its interactions with the surrounding abiotic environment and with other species. Short-term, plastic trait responses to climatic variation, e.g. earlier development as a consequence of increasing temperatures, are relatively well documented in many species (Charmantier *et al.* 2008; Anderson *et al.* 2012; CaraDonna *et al.* 2014). However, we know much less about how variation in environmental factors and climate influence natural selection on timing (Chevin *et al.* 2010; MacColl 2011; Visser *et al.* 2015; Marrot *et al.* 2018; Siepielski *et al.* 2017). While 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), we still know very little about how among-year variation in climate influence selection on phenological traits in plants.

The phenotypic expression of many phenological traits depends on environmental factors, such as temperature, timing of snowmelt or precipitation (Via 1993; Via *et al.* 1995). For organisms in temperate seasonal environments, we expect higher temperatures in spring and earlier dates of snowmelt to often be associated with plastic responses, in terms of an earlier phenology (Charmantier *et al.* 2008; Wadgymar *et al.* 2018). However, it is less clear how we should expect climate to influence the direction and intensity of natural selection on phenological traits. It has been argued that global warming is expected to influence selection on timing because of documented phenotypic mismatches between adaptive optima and phenotypic means following warming trends (Gienapp *et al.* 2008; Marrot *et al.* 2018). However, the direction of the selection induced by such increases in temperature will depend on how strong the 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 temperature and at earlier mean phenology (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, then we should expect selection for earlier phenology to be weaker at higher temperatures (i.e. selecting for a counter-gradient response).

In order to explore the effects of climatic variation on both the expression of traits associated with timing and natural selection on these traits, we need replicated estimates of the relationships between climate and phenotypic trait expression (i.e. phenotypic plasticity), and between climate and the covariance between traits and fitness (i.e. phenotypic selection). In spite of the recognized importance of temporal variation in selection (Siepielski *et al.* 2009), few long-term assessments on selection on plant traits exist (Siepielski *et al.* 2017), the longest spanning 10-11 years (Campbell & Powers 2015; Thomann et al. 2018). Here, we examined the effects of climatic variation on timing of flowering, as well as on phenotypic selection on flowering phenology over 22 years, using information from permanently marked individuals in a natural population of the long-lived spring-flowering forest herb *Lathyrus vernus*. This species often occurs in the understory of deciduous forests, where availability of light is high during spring, but rapidly decreases when canopy closes in early summer. Timing of flowering has been shown to be correlated with differences in fruit set, and this pattern might be influenced by differences in both resource and pollen availability (Ehrlen 1992). Previous studies have also shown that early-flowering individuals are more damaged by vertebrate grazing than late-flowering (Ehrlén & Münzbergová 2009). 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 climatic conditions during spring?

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 (58.9513 N, 17.6098 E), during the years 1987 – 1996 and 2006 – 2017. *Lathyrus vernus* (L.) Bernh. (Fabaceae) is distributed across Europe and parts of northern Asia. Individuals of this long-lived species grow fairly slow, have high annual survival rates and produce few, large seeds after many years of vegetative growth. One or several erect shoots emerge from the over-wintering rhizome early in spring (Ehrlen 1995). Growth of shoots is determinate and the number and sizes of shoots as well as flower number is determined in the previous year (Ehrlén & Van Groenendael 2001). Racemes with flowers grow from the leaf axes and the phenology of vegetative and reproductive growth are closely correlated (Sola & Ehrlén 2007). Vegetative growth usually starts in April, and flowering starts about 4 weeks after the start of vegetative growth (Sola & Ehrlén 2007). Flowering thus occurs before the canopy of deciduous trees develops in early June, when light availability falls to a small proportion of the levels available in April and May. Late frosts can damage flower buds on developing shoots in some years, but no water deficit has been observed during spring-early summer (J. Ehrlén pers. obs.). Shoots die back in autumn each year. *Lathyrus vernus* lacks organs for vegetative spread. Large individuals do not flower in all years but frequently skip flowering in some years. Some plants do not produce above-ground parts in every year but may stay dormant in one season and reappear only in the following year. Flower number, usually between 5 and 30, is closely correlated with aboveground biomass and therefore strongly related to plant resource state and environmental conditions (Sola & Ehrlén 2007). Flowering is acropetally sequential within shoots, starting with the basal flower in the basal raceme. The flowers are large and pollinated by bumblebees (*Bombus* spp.). *Lathyrus vernus* is self-compatible, but lacks mechanisms for self-pollination (J. Ehrlén, unpubl. data). Individuals produce a small number of relatively large seeds and recruitment from seeds is frequent (Ehrlén & Eriksson 1996). Seeds lack specific dispersal mechanisms and are dispersed up to a few meters when the dry pods dehisce explosively. Developing seeds are often damaged by the pre-dispersal seed predator *Bruchus atomarius*. Grazing by roe deer *(Capreolus capreolus*) often removes entire flowering shoots and prevents seed production. Previous evidence from a common garden study indicates that there is heritable variation in flowering time among populations of *L. vernus* (Widén & Schiemann 2003).

The average conditional life span of flowering individuals has been estimated to 44.3 years (Ehrlén & Lehtilä 2002). The long life span of *L. vernus* and an observed low turnover of individuals in the study population means that, in spite of the long duration of the study, observed variation in trait distributions among years is likely to be mainly the result of phenotypic plasticity, while changes in the genetic structure of the population are likely to have played a minor role.

**Data collection**

Data was collected during 22 reproductive seasons, 1987 – 1996 and 2006 – 2017. A permanent study plot of … m2 was established in 1987. All flowering individuals in this plot were permanently marked with flags and surveyed in each year from 1987 to 1996. No recordings were made from 1997 to 2005, and markings were not maintained. In 2006, a permanent study plot of … m2 adjacent to the initial plot was established. This new plot was surveyed in the same way as the initial plot from 2006 to 2017, i.e. one set of individuals were followed from 1987 to 1996, and a second set of individuals were followed from 2006 to 2017. Within each of the two study periods, the identity of individuals marked as flowering in one year was checked in each of the following years also when not flowering, in order 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. At each visit up to opening of the first flower, we recorded also the size and developmental stage of the most advanced bud in each individual, and assigned it to one of four size categories: small, medium, large and very large. At each visit, we also recorded if one or several shoots had been grazed by roe deer or other vertebrates. 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 attacked by pre-dispersal seed predators. We estimated vegetative size of an individual as their above-ground volume. To estimate above-ground volume, we measured the height and diameter of all shoots, calculated the volume of each shoot as π × (diameter/2)2 × 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 directly examined in the field. The number of seeds was counted and pre-dispersal seed predation was identified by the presence of one or several larval entrance holes in a seed. Fruits that had opened prior to the recording were brought to the lab where the number of intact and preyed seeds was 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.

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 was estimated based on vegetative size (see Appendix S1 for details).

First flowering day (FFD), i.e. the day of year when the first flower was fully unfolded with the banner petal folded upwards, was estimated for each plant. The data on the presence or absence of open flowers from each visit allowed us to with certainty determine during which 5-day interval an individual had started to flower. To estimate also the most likely date within this 5-day interval at which the first flower opened, we used information about the developmental stage of the most developed bud at the last recording before the recording with open flowers and the number of open flowers at the first recording with open flowers (see Appendix S1 for details).

Weather data for the months of March, April and May during the period 1961-2017 was obtained from the Swedish Meteorological and Hydrological Institute ([www.smhi.se](http://www.smhi.se/)). Daily mean, minimum and maximum temperature values were averaged from two meteorological stations located in Oxelösund (58.6777 N, 17.1223 E, 41 km from the study plot) and Södertälje (59.2142 N, 17.6289 E, 29 km from the study plot). Missing temperature values were estimated using the relationship between values of both stations in each month over all years (see Appendix S1 for details). Daily precipitation values were obtained from one station located in Åda (58.9279 N, 17.5358 E, 5 km from the study plot).

**Data management**

In order to take into account leap years, we converted all FFD from calendar dates (dd/mm/yy) to the number of days after the vernal equinox. We then calculated three measures of the yearly position of the flowering season: the start, calculated as the date when 10% of the plants had started flowering, the mean, calculated as the mean first flowering date of individuals, and the end, calculated as the date when 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.

Using weather data from the meteorological stations, we calculated in total 12 climatic variables: 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 the shoot development of *L. vernus* in March, April and May should result in an earlier flowering, as development of shoots and flower buds is strongly influenced by temperature (Fogelström et al. unpubl. data). Higher temperatures should also lead to higher mean fitness as both fertilization and seed set are likely to be favored, and there is no shortage of water during spring in these environments. Lastly, higher temperatures should lead to stronger selection for earlier flowering, as individuals flowering early would benefit most from favorable conditions before canopy closure. Lower temperatures in April and May might lead to weaker selection for earlier flowering because early buds might be damaged by frosts and early flowers might experience a shortage of pollinators. We also expected that the duration of flowering should be positively associated with temperatures in April, as higher temperatures early during the season would mean an earlier start of flowering, and negatively associated with higher May temperatures, as this would increase the speed of development and result 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 and lead to an earlier onset of growth and an earlier flowering. Precipitation during early spring falling as rain would often be associated with relatively mild temperatures, and thus also be correlated with an earlier growth and reproduction. Precipitation later in spring is associated with lower than average temperatures and lower availability of light, and thus poorer conditions for growth, fertilization and seed development. This would result in later flowering, lower mean fitness, and possibly weaker selection for early flowering.

**Statistical analyses**

We first checked for trends across years in climate, flowering time and mean fitness by performing univariate linear regressions of all climatic variables, mean first flowering date and number of intact (non-predated) seeds against year. We checked for trends in climate for the 22 study years and for the period 1961-2017. We also performed a visual inspection of the data to identify non-linear trends.

To assess how much of the variation in first flowering date for individual plants was explained by year, we calculated the R2 from a univariate linear regression of first flowering date with year as an ordinal variable. The effects of yearly climate on first flowering date for individual plants 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. In order to avoid collinearity among predictors, we constrained the number of candidate models by avoiding models including pairs of variables with a Pearson correlation coefficient larger than 0.5. We then ranked the resulting candidate models using the corrected version of the second-order Akaike information criterion (AICc). To account for model uncertainty, we performed model averaging of parameter estimates across all models with ΔAICc < 2. The effects of climate on the yearly position (start, mean and end) and duration of the flowering season were examined using linear models with the climatic variables that had a significant effect in the averaged model for first flowering date for individual plants as predictors. In order to explore how long-term trends influenced our estimates of effects of climate, 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 R2 from a univariate linear regression of fitness with year as an ordinal variable. The effects of yearly climate on individual fitness were examined using linear mixed models with number of flowers as a condition trait and plant individual as a random effect. We performed model selection and model averaging using the 12 climatic variables as described above.

To assess total and direct phenotypic selection on flowering time (FFD) for each year, we estimated phenotypic selection differentials and gradients, respectively, using linear regression models. 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. Phenotypic selection differentials, providing estimates of the combination of direct selection, and indirect selection mediated through correlated characters on a trait, were calculated for each year using univariate regressions of relative fitness on standardized FFD and number of flowers (Lande & Arnold 1983). Phenotypic selection gradients, providing estimates of direct selection on a trait, 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 in order to reduce bias due to environmentally induced covariance between FFD and fitness (Rausher 1992). In addition to directional (linear) selection, we also assessed non-linear (quadratic and correlational) selection (Lande & Arnold 1983; Arnold 1986). Non-linear (quadratic and correlational) selection was estimated by examining the effect of quadratic and interaction terms in a model including also the linear terms. 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. To assess how much of the variation in total selection was explained by year, we subtracted the R2 from a phenotypic selection model including only FFD from the R2 from a phenotypic selection model including also the interaction FFD:year as an ordinal variable. To assess how much of the variation in direct selection was explained by year, we subtracted the R2 from a phenotypic selection model including only FFD and number of flowers from the R2 from a phenotypic selection model including also the interaction FFD:year as an ordinal variable.

Among-year differences in total and direct phenotypic linear selection on flowering time were evaluated using phenotypic selection models including also the interaction between standardized first flowering date 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 examined only variation in linear selection because non-linear selection was only signigicant in very few years (see Results).

To examine if variation in directional phenotypic selection among years was related to climatic conditions during spring, we performed phenotypic selection models, including the interactions between standardized FFD and 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 climatic variables, we performed model selection and model averaging as explained above, using the interactions between standardized first flowering date and each of the 12 climatic variables. Interaction terms that had a significant effect in the averaged model were then used in the phenotypic selection model. We used analogous models to examine among-year variation in both total phenotypic selection and direct selection, but the latter included also the standardized number of flowers.

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

RESULTS

There was considerable variation in climate in the study area during the period 1961-2017 (Fig. 1, Figs. S1-S4). Minimum, mean and maximum daily temperature increased significantly during the period 1961-2017, but trends were not significant over the 22 study years (Table S1, Figs. S1-S3).

Mean FFD during the study years ranged from 48.0 to 71.6 days (mean = 58.1) after the vernal equinox. Differences among years explained 59% of the variation in FFD among plant individuals. There was a significant linear trend of an earlier mean first flowering date over the study period (linear regression coefficient = -0.267, t = -2.42, p = 0.025, adjusted R2 = 0.19, Fig. 1B). Differences in climate explained a large part of the variation in FFD among years, flowering occurring significantly earlier in years with higher mean temperatures in April and May, and higher precipitation in March and April (Table 1A, Fig. 2). Climatic predictors explained most of the variation among years in the start (72%), mean (76%), end (84%) and duration (57%) of the flowering season (Table 1B and C). Both the start, the mean and the 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 temperatures in April (Table 1C, Fig. 4). There were no effects of precipitation on the position and duration of the flowering season. All these effects of climate variables were independent of long-term trends (Table S2).

Mean fitness of individuals ranged from 0.2 to 18.6 (mean = 5.0) intact seeds per flowering individual across years, and there was no significant trend over the study period (Fig. 1C). Differences among years explained 17% of the variation in fitness of individual plants. Fitness increased with precipitation in March, and decreased with maximum temperatures in April, minimum temperatures in May and precipitation in April (Table 2, Fig. 5).

Selection differentials for FFD were negative, i.e. favored earlier flowering, in all years (mean = - 0.xx, range = -0.xx - 0.xx). Linear selection for early flowering was significant in 18 of 22 years, while non-linear selection was significant in only 2 years (Table 3 and S3). Differences among years explained 0.54% of the variation in total selection. 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 of 22 years (Table S4). Non-linear direct selection on flowering time was significant only in one year, and correlational selection on FFD × number of flowers only in three years (Table S4). There was no significant trend in selection estimates over the study period (Table 3, Fig. 1D). Differences among years explained 0.60% of the variation in direct selection.

Interestingly, differences in total and direct phenotypic selection among years were related to climatic conditions, in terms of significant effects of the interactions between climatic variables and first flowering date on fitness (Table 4 and S5, Fig. 6). Specifically, 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 temperatures in April (Fig. 6A) and lower precipitation in March (Fig. 6B).

DISCUSSION

Our results show that among-year variation in flowering time, fitness and phenotypic selection on flowering time, is related to climatic conditions during spring in the perennial herb *Lathyrus vernus*. Plants flowered earlier when springs were warmer and precipitation high. Phenotypic selection favored early flowering in 21 of 22 years but strength of selection differed among years. Phenotypic selection for early flowering was stronger in springs with higher temperatures and lower precipitation. 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 show both co-gradient patterns, as for spring temperature in our study, and counter-gradient patterns, as for precipitation in our study.

Based on the long life-span of *L. vernus* and on the very low turn-over of plants observed during our study, we assume that most observed variation in phenotypic values of FFD among years was due to plastic responses. These plastic responses were strongly influenced by among-year climatic variation, with earlier first flowering dates of individual plants and an earlier position of the flowering season in years with warmer springs. Individual plants started flowering on average 2.2 days earlier with a one-degree increase in mean temperature in April and and 3.8 days earlier with a one-degree increase in May. Plants also started flowering earlier in springs with more precipitation before and during the onset of the flowering season. Higher temperatures in April and May were associated with an earlier start and end of the flowering season. Several previous studies with other plant species 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). Particularly strong effects of warming have been shown in early-spring flowering species (Dunne *et al.* 2003). The duration of the flowering season increased with increasing temperatures in April, probably because this duration was more conditioned by the start than by the end of the flowering season (which is confirmed by the values of the Pearson correlation coefficients; r = -0.56 for start and duration, and r = -0.02 for end and duration). Some previous studies report shortenings of flowering duration with increasing temperatures (Høye *et al.* 2013; Bock *et al.* 2014). It seems likely that early-flowering species in seasonal environments increase the length of the flowering season in response to higher temperatures before flowering while higher temperatures later might lead to an earlier end and shorter duration of the flowering season (Theobald *et al.* 2017). Climatic variation was considerable during our study period and, although we found no warming trends accross 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 such a significant advance in first flowering dates over the study period.

Together with increasing temperatures, current and future climatic changes entail shifts in precipitation (IPCC 2014). However, in temperate environments the expected phenological responses to altered precipitation regimes are less clear than responses to changes in temperatures. Experimental studies have either found no effects (Phoenix *et al.* 2001; Cleland *et al.* 2006), or report 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). 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. Our results show that higher precipitation during early spring, i.e. before and at the beginning of the flowering season of *L. vernus*, is associated with an advanced start of flowering, although the effects of precipitation in FFD of indivduals were weaker than those of temperature, and effects of precipitation on the position and duration of the flowering season were not significant. As there is no shortage of water during spring or early summer in our study area, the observed effect of spring precipitation on the start of flowering might be indirect and mediated by temperature. If precipitation during early spring falls as snow, higher precipitation might protect overwintering shoots and flower buds from very low temperatures and frost damage (Inouye *et al.* 2002), which in turn might lead to an earlier flowering. Precipitation falling as rain, on the other hand, might be associated with relatively mild temperatures during early spring.

FITNESS: Fitness increased significantly with precipitation in March, and decreased significantly with maximum temperatures in April, minimum temperatures in May and precipitation in April.

PICTURE CUT

Temporal variation in selection is important because it determines the overall direction and magnitude of selection, and because it may constrain adaptive evolution. Several studies 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, estimates of phenotypic selection on flowering time varied considerably among years. Both total and direct selection on flowering time varied in strength, from strongly favoring early flowering in some years to no selection in other years. However, we did not observe important changes in the direction of selection (phenotypic selection differentials and gradients were negative in, respectively, 20 and 21 out of 22 study years, and close to zero in the remaining years).

One suggested as an underlying mechanism causing temporal variation in selection is variation in environmental conditions driven by climatic fluctuations (Siepielski *et al.* 2009, 2017; Visser *et al.* 2015; Marrot *et al.* 2018). Yet, the actual causes of temporal variation in selection on many traits, like flowering phenology, are still poorly known. In our study, 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 at higher temperatures because flowering early when spring temperatures are low increases the risk of frost damage (Inouye 2008; Pardee *et al.* 2018). Lastly, lower spring temperatures might also be associated with lower availability of pollinators early during the flowering season (Elzinga *et al.* 2007). Our results with a perennial plant, agrees with the results of previous studies on birds (Visser *et al.* 2015; Marrot *et al.* 2018) and mammals (Réale *et al.* 2003), where selection for earlier reproduction have been found to increase with warmer temperatures. 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 a higher precipitation before the growing season is associated with an earlier onset of flowering it also means that selection for early flowering decreases (see Fig. 6B). A possible mechanism underlying this pattern is that early spring precipitation may result in a lower abundance of pollinators early in the season, thus decreasing the fitness advantage of flowering early in the season. Moreover, early spring precipitation might increase the intensity of antagonistic interactions, such as mollusk herbivory (Crawford-Sidebotham 1972), which have strong negative effects on fitness by damaging emerging shoot buds in *L. vernus* (Ehrlén 2002).

**Concluding remarks**

Our 22-year assessment of selection on plant flowering phenology demonstrated that climatic variation can influence both the expression of phenological traits, and the strength of natural selection acting on these traits. An important implication of these findings is that plant phenology is likely to show both plastic and, to the extent the additive genetic variation for flowering time exists, evolutionary responses to climatic variation and to anthropogenic climate change. Moreover, our results show that the effects of climatic variation on the expression of a trait and on the strength of phenotypic selection on the same trait might be in the same or in opposite directions. In our study, higher spring temperature were associated with both a plastic response in terms of an earlier flowering and an increased fitness advantage for plants flowering early, i.e. suggesting a co-gradient response (Conover & Schultz 1995; Conover *et al.* 2009). In contrast, higher precipitation before the flowering season was associated with an earlier phenology, but also with a decreased fitness advantage for plants flowering early, i.e. suggesting a counter-gradient response. To predict the long-term responses of traits, such as phenology, to global climate change, we therefore need to consider both the immediate effects on trait expression and the potential evolutionary responses to climate induced changes in selection.

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Tables

Table 1: Results of models testing for effects of yearly climate on (A) first flowering date (FFD) for individual plants, and on three measures of the yearly position (B) and the yearly duration (C) of the flowering season of *Lathyrus vernus* in 22 study 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, averaged estimates and z-values across all candidate models with ΔAICc < 2 are shown, and values of marginal and conditional pseudo-R2 (representing, respectively, the variance explained by the fixed effects and by the entire model, (Nakagawa & Schielzeth 2013) are shown for the best model resulting from model selection. ∑wi = Relative variable importance (sum of Akaike weights over all models including each variable).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Response variable | Predictor variable | Estimate | | Std. error | z | ∑wi |
| A) FFD  N = 2411  R2 marg = 0.577  R2 cond = 0.628 | Precipitation March | -0.716 | \*\*\* | 0.105 | 6.85 | 1.00 |
| Precipitation April | -0.345 | \*\* | 0.125 | 2.77 | 0.95 |
| Mean March | -0.074 |  | 0.136 | 0.54 | 0.23 |
| Max March | -0.236 |  | 0.185 | 1.28 | 0.63 |
| Mean April | -2.188 | \*\*\* | 0.134 | 16.29 | 1.00 |
| Mean May | -3.754 | \*\*\* | 0.113 | 33.17 | 1.00 |
| Number of flowers | -2.407 | \*\*\* | 0.101 | 23.86 | 1.00 |
|  |  | Estimate | | Std. error | t | |
| B) Position of the fl. season |  |  |  |  |  | |
| Start  N = 22  R2 = 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 | |
| Mean  N = 22  R2 = 0.764 | Precipitation March | -0.803 |  | 0.724 | -1.11 | |
| Precipitation April | -0.627 |  | 0.765 | -0.82 | |
| Mean April | -3.429 | \*\*\* | 0.816 | -4.20 | |
| Mean May | -4.004 | \*\*\* | 0.671 | -5.97 | |
| End  N = 22  R2 = 0.844 | Precipitation March | -0.461 |  | 0.549 | -0.84 | |
| Precipitation April | -0.810 |  | 0.580 | -1.40 | |
| Mean April | -2.324 | \*\* | 0.618 | -3.76 | |
| Mean May | -4.555 | \*\*\* | 0.508 | -8.96 | |
| C) Duration or the fl. season |  |  |  |  |  |  |
| N = 22  R2 = 0.567 | Precipitation March | 0.532 |  | 0.604 | 0.88 | |
| Precipitation April | -0.458 |  | 0.638 | -0.72 | |
| 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: Results of linear mixed models testing for effects of yearly climate on fitness of *Lathyrus vernus* in 22 study years. All candidate models included number of flowers as a condition trait and plant individual as a random effect. Averaged estimates and z-values across all candidate models with ΔAICc < 2 are shown, and values of marginal and conditional pseudo-R2 (representing, respectively, the variance explained by the fixed effects and by the entire model, (Nakagawa & Schielzeth 2013) were 0.177 and 0.213 for the best model resulting from model selection. ∑wi = Relative variable importance (sum of Akaike weights over all models including each variable). N = 2411 for all models.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Predictor variable | Estimate | | Std. error | z | ∑wi |
| Precipitation March | 0.551 | \*\* | 0.195 | 2.83 | 0.96 |
| Precipitation April | -0.920 | \*\*\* | 0.222 | 4.15 | 1.00 |
| Precipitation May | -0.040 |  | 0.124 | 0.32 | 0.28 |
| Min March | 0.042 |  | 0.128 | 0.33 | 0.20 |
| Max March | -0.109 |  | 0.208 | 0.52 | 0.35 |
| Max April | -0.935 | \*\*\* | 0.236 | 3.97 | 0.99 |
| Min May | -0.545 | \* | 0.214 | 2.55 | 0.82 |
| Number of flowers | 3.731 | \*\*\* | 0.176 | 21.15 | 1.00 |

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

Table 3: Phenotypic selection models testing for among-year differences in (A) indirect and (B) direct phenotypic selection on flowering time in *Lathyrus vernus* in 22 study years. Results are from linear mixed models including the interaction first flowering date : year in order to assess among-year differences in (A) selection differentials and (B) selection gradients for first flowering date. In (B), 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.

|  |  |  |  |
| --- | --- | --- | --- |
|  | 2 | | Degrees of freedom |
| A) Indirect selection | 110.18 | \*\*\* | 1 |
| First flowering date |
| First flowering date : year | 36.46 | \* | 21 |
| B) Direct selection | 33.89 | \*\*\* | 1 |
| First flowering date |
| Number of flowers | 64.79 | \*\*\* | 1 |
| First flowering date : year | 37.87 | \* | 21 |

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

Table 4: Phenotypic selection models testing for effects of yearly climate on (A) total and (B) direct selection on flowering time in *Lathyrus vernus* in 22 study years. Results are from linear mixed models including the interactions of first flowering date with climatic variables. The included interactions were selected because they had a significant effect in the averaged models resulting from model selection (see Table S5). In (B), all candidate models included number of flowers 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 | (A) Total selection | | |  | (B) Direct selection | | |
| 2 | | Degrees  of freedom |  | 2 | | Degrees  f freedom |
| 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 climate, flowering time, fitness and selection on flowering time during the study period. Means (dots) and standard errors (whiskers) of mean daily temperature during March (light grey), April (medium grey) and May (black) (A), first flowering date, calculated as the number of days from the vernal equinox (B), mean fitness, calculated as the number of intact, non-predated seeds (C), and linear selection gradient for first flowering date (D) are shown for each study year. In (B), the solid line indicates a significant linear trend of an earlier flowering date across years (adjusted R2 = 0.19). In (D), filled circles indicate significant selection gradients at α = 0.05 and the grey dashed line indicates a selection gradient of 0. See Figures S1-S4 for variation in other climatic variables.

Figure 2: Relationship among first flowering date for every plant and climatic variables, including mean daily temperature in April (A) and May (B), and sum of precipitation in March (C) and April (D). Lines represent linear model fits.

Figure 3: Relationship among the position of the flowering season and climatic variables, including mean daily temperature in April (A) and May (B). The position of the flowering season was measured as 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). Lines represent linear model fits.

Figure 4: Relationship among the duration of the flowering season, measured as the number of days between the start and the end of the season, and mean daily temperature in April. The line represents a linear model fit.

Figure 5: Partial regression plots (i.e. added variable plots) calculated from models in Table 2 showing the independent effects of maximum daily temperature in April (A), minimum daily temperature in May (B), sum of precipitation in March (C) and April (D) on fitness, measured as the number of intact seeds. Linear model fit lines and confidence intervals are shown.

Figure 6: Partial regression plots (i.e. added variable plots) calculated from models in Table 4 showing the independent effects of climatic variables on direct phenotypic selection on flowering time in *Lathyrus vernus* in 22 study years. The effect of standardized first flowering date on relative fitness, measured as the relative number of intact seeds, is shown for different minimum daily temperatures in April (A) and different sums of precipitation in March (B). Lines represent linear model fits.