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

ABSTRACT

….

INTRODUCTION

Climate change is rapidly altering the environmental conditions experienced by organisms (Diffenbaugh & Field 2013; IPCC 2014). 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. Climatic variation can influence not only the phenotypic expression of traits associated with timing in a given genotype (Charmantier *et al.* 2008; Anderson *et al.* 2012; CaraDonna *et al.* 2014), but also natural selection on these traits (Visser *et al.* 2015; Marrot *et al.* 2018). Short-term, plastic responses to climatic variation, e.g. earlier development as a consequence of increasing temperatures, are relatively well documented in many species (REFS). We know much less about how variation in environmental factors and climate influences natural selection on timing (Chevin *et al.* 2010, MacColl 2011, but see REF; reviewer + Soay sheep, Siepielski *et al.* 2017). Some studies have identified climatic factors as important drivers of temporal variation in selection on phenological traits in animals (Réale *et al.* 2003; Visser *et al.* 2015; Marrot *et al.* 2018), but we know little about how climatic variation influence selection on timing of reproduction in plants.

The phenotypic expression of many phenological traits depends on environmental factors, such as temperature, timing of snowmelt or precipitation (Via *et al.* 1995; … REFS). For most organisms in temperate seasonal environments, higher temperatures and earlier dates of snowmelt are associated with an earlier phenology (Charmantier *et al.* 2008; Wadgymar *et al.* 2018). It is less clear how we should expect climatic variation 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 a change in temperature will depend on whether plastic responses are unable to bring phenotypic means fully to the optima or whether they bring means beyond the optima. Selection on timing might thus act to change the sensitivity of phenology to temperature (i.e. the thermal reaction norm) in the same or in opposite direction as the effect of temperature on phenotypic plasticty (cf. Conover & Schultz 1995; Conover *et al.* 2009). In other words, if higher temperatures favor a stronger response of phenology to temperature, we would expect selection for earlier phenology to be stronger at higher temperatures (i.e. selecting for a co-gradient response). On the other hand, if higher temperatures favor a weaker response, we would 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 this study, we examined the effects of climatic variation on timing of flowering, as well as on phenotypic selection on flowering phenology, using information from permanently marked individuals over 22 years in a natural population of the long-lived spring-flowering forest herb *Lathyrus vernus*. This species often occurs in the understories of deciduous forests, where availability of light is high during spring but 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 (Ehrlén 1992). Previous studies have also shown that early-flowering individuals are more damaged by vertebrate grazing than late-flowering (Ehrlén and Munzbergova 2009). In this study, we addressed the following questions: (1) Do flowering time and fitness vary among years in response to weather 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 weather 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 (58.951289 N, 17.609774 E), during the years 1987 – 1996 and 2006 – 2017.

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. Ehrlén & Van Groenendael 2001 ShootsSome 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). is acropetally sequential within shoots, starting with the basal flower in the basal raceme. The flowers are large and pollinated by bumble-bees. *L. 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 and Eriksson 1996). 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 and 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 mostly 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 in 22 years, 1987 – 1996 and 2006 – 2017. A permanent study plot measuring … was established in 1987. All flowering individuals in this plots 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 adjacent to the initial one was re-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 all individuals, 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 more details). The final number of fruits was recorded for all individuals in the field. 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. For estimation of the number of intact and preyed seeds in fruits that had opened prior to the recording, see Appendix S1. The total number of intact seeds produced by an individual was used as an estimate of fitness.

We used total number of flowers to mean the cumulative number of open flowers produced by an individual during the flowering season. The total number of flowers 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 …… (see Appendix S1 for details).

For each plant, we estimated first flowering day (FFD), i.e. the day of year when the first flower was fully unfolded with the banner petal folded upwards. The data on the presence or absence of open flowers from each visit also allowed us to with certainty determine during which 5-day interval an individual had started to flower. To increase the resolution, we also assigned the most likely FFD to each individual based on ….. (see Appendix S1 for details).

Weather data for 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 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 mainly from one station located in Åda (58.9279 N, 17.5358 E, 5 km from the study plot). Precipitation data from this station was missing for 254 dates. In those cases, values were obtained from another station located in Sjögärde (58.9752 N, 17.8411 E, 14 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, and the end, calculated as the date when 90% of the plants had started flowering. As measures of the start and the end of the flowering season, we used 10 and 90% FFD instead of the date when the first and last plant started flowering in order to disregard extreme values of flowering dates. 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 meterological 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 mean and maximum 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 is likely to be favored by higher temperatures, 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 beneficial conditions before canopy closure. Lower minimum 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 are slightly less clear. 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 March falling as rain would often be associated with relatively mild temperatures, and thus also be correlated with an earlier growth and reproduction. The effects of early spring precipitation on selection on flowering time is more difficult to predict. Precipitation later in spring, during April and May, is expected to be 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 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 detect any 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 a factor. 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 a factor. The effects of yearly climate, yearly position (mean FFD) and duration of the flowering season on individual fitness were examined using linear mixed models with number of flowers as a condition trait and plant individual as a random effect. For the effects of yearly climate, 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 a factor. 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 a factor.

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.

To examine if variation in total phenotypic selection among years was related to climatic conditions during spring, we performed three phenotypic selection models, including the interactions between standardized FFD and (1) climatic variables, (2) mean FFD, or (3) duration of the flowering season. The main effects of these variables were not included as fitness was relativized within years prior to analysis. Plant individual was included as a random effect. For the effects of climate, we performed model selection and model averaging as explained above, using the interactions between standardized first flowering date and each of the 17 climatic variables. Interaction terms that had a significant effect in the averaged model were then used in the phenotypic selection model. To examine if variation in direct phenotypic selection among years was related to conditions during spring, we repeated the same procedure with models where standardized number of flowers was included as a measure of resource state.

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 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. 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 among years explained a large part of the variation in FFD, 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). Climate 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, but there was no significant trend across years (Fig. 1C). Differences among years explained 17% of the variation in fitness for 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. 5A-D). Fitness was also higher in years with a longer duration of the flowering season (Table 2, Fig. 5E), while the position of the flowering season had no effect on fitness.

Selection differentials for FFD varied among years but there was significant selection for early flowering in 18 of 22 years, but 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 also varied among years, selection estimates being negative (i.e. favoring earlier flowering in xx of 22 years (mean = - 0.28, range = -0.78 - 0.08). Swas significant There was no significant trend in selection estimates over the study period (Table 3, Fig. 1D). Non-linear direct selection on flowering time was significant only in one year, and correlational selection on FFD × number of flowers in three years (Table S4). Differences among years explained 0.60% of the variation in direct selection.

Importantly, differences in total and direct phenotypic selection among years was related to climatic conditions, in terms of significant effects of the interactions between climate 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). Variation in phenotypic selection among years was not related to the position or duration of the flowering season (Table 4).

DISCUSSION

Our results show that flowering phenology in terms of first flowering date in this forest herb is strongly correlated with spring temperature, flowering being xx days earlier with a one-degree increase in mean temperature during April-May (or min vs. max). Phenotypic selection gradients varied significantly among years, from strongly favoring (?) early flowering in some years to … in other years. This variation in selection was, however, not linked to spring temperatures or mean flowering time within the population. Our results thus show that while spring temperature had strong effects on trait expression, it had no detectable effect on phenotypic selection on flowering time. … Provide no support for that we should expect evolutionary responses to changes in temperatures … other factors causing variation in selection on flowering time than those influencing phenotypic plasticity of the trait …

… flowering phenology in terms of first flowering date was strongly correlated with spring temperature, flowering being xx days earlier with a one-degree increase in mean temperature during April-May. … Other potential drivers examined … Explanatory power … Causal mechanisms … Other studies … Concluding remark

… Phenotypic selection gradients varied significantly among years, from strongly favoring (?) early flowering in some years to … in other years. … Other results for selection on flowering time … direction … among-year variation … Among-year variation in selection gradients in general … methodological concerns … implications … [Temporal variation in selection is important because it determines the overall direction and magnitude of selection, and because it may constrain adaptive evolution. Both the direction and strength of selection has been shown to vary geographically and among years, and in some systems temporal variation can be linked to climatic variation (e.g. Thompson 2005; (Siepielski *et al.* 2009).] … Empirical evidence suggest that selection estimates vary both spatially and over time within populations (cf. reviews)… Yet, the prevalence of temporal variation in selection have been questioned based on … Hadfield et al 2xxx). We also know very little about the causes of temporal variation in natural selection (but see (Charmantier *et al.* 2008).]

… This variation in selection was, however, not linked to spring temperatures or mean flowering time within the population. … Implications … Consequences … Causes … Other selective agents – Not strongly linked to spring temperatures … Other studies … General about effects of (climatic) drivers on trait expression, selection on thermal reaction norms (can not be excluded – further studies needed), counter- and co-gradient patterns, other types of potential relationships .. Concluding remark

We conclude … CC vs. plasticity and selection …

Things we can add from (Siepielski *et al.* 2009):

The strength of selection often varies considerably from year to year. Changes in the direction of selection are frequent. Changes in the form of selection are likely common, but harder to quantify. Variation in environmental conditions driven by climatic fluctuations appears to be a common and important causal mechanism underlying temporal variation in the strength, direction and form of selection. Many studies measuring the strength of selection in Kingsolver et al. 2001 were not temporally replicated. Consideration of long-term selection on traits increases the chance of detecting infrequent bouts of strong selection. Overall, changes in the direction of selection are relatively common (not in our study! - almost always negative selection gradients). Most populations may experience infrequent bouts of strong selection tempered with other bouts of weaker selection (selection may be particularly strong at times, however, it is rarely consistent in strength) and there is a tendency for traits experiencing stronger selection to be accompanied by greater temporal variation in the strength of such selection. Several studies identified fluctuating climate as an important factor causing selection to vary. Patterns of rainfall, in particular, emerged as a principal cause of temporal variation in selection although its effect was more often indirect. In general, determining the causes of selection is more difficult than quantifying selection or testing for its statistical significance. Consequently, our understanding of the causes of selection has greatly lagged behind our ability to detect selection, which clearly hinders our ability to predict how fitness landscapes (and thus the strength, direction and form of selection) will shift in response to climate change or other perturbations.

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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 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).

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| --- | --- | --- | --- | --- | --- | --- |
| 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 (A) climate, (B) position and (C) duration of the flowering season on fitness of *Lathyrus vernus* in 22 study years. Models include the effects of (A) climatic variables, (B) mean first flowering date (Mean FFD), and (C) duration of the flowering season (N days 90-10% FFD). All models include number of flowers as a condition trait and plant individual as a random effect. The marginal and conditional pseudo-R2 (representing, respectively, the variance explained by the fixed effects and by the entire model, (Nakagawa & Schielzeth 2013) are shown. In (A), averaged estimates and z-values across all models with ΔAICc < 2 are shown, and values of R2 are shown 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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Model | Predictor variable | Estimate | | Std. error | z | ∑wi |
| A) Climate  R2 marg = 0.177  R2 cond = 0.213 | 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 |
|  | Estimate | | Std. error | t | |
| B) Position of the fl. season  R2 marg= 0.159  R2 cond = 0.201 |  |  |  |  |  | |
| Mean FFD | 0.002 |  | 0.031 | 0.07 | |
| Number of flowers | 0.208 | \*\*\* | 0.010 | 20.40 | |
| C) Duration of the fl. season  R2 marg= 0.174  R2 cond = 0.219 |  |  |  |  |  | |
| N days 90-10% FFD | 0.384 | \*\*\* | 0.057 | 6.73 | |
| Number of flowers | 0.201 | \*\*\* | 0.010 | 19.98 | |

\*\*\*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 (A) climate, (B) position and (C) duration of the flowering season on 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 (A) climatic variables , (B) mean first flowering date (Mean FFD), and (C) duration of the flowering season (N days 90-10% FFD). Interactions of first flowering date with climatic variables (precipitation in March and minimum daily temperature in April) included in (A) were selected because they had a significant effect in the averaged model resulting from model selection (see Table S5). All models include 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 | Total selection | | |  | Direct selection | | |
| 2 | | Degrees  of freedom |  | 2 | | Degrees  f freedom |
| A) Climate |  |  |  |  |  |  |  |
| 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 |
| B) Position of the flowering season |  |  |  |  |  |  |  |
| First flowering date | 109.59 | \*\*\* | 1 |  | 33.53 | \*\*\* | 1 |
| Number of flowers | - |  | - |  | 63.61 | \*\*\* | 1 |
| First flowering date : Mean FFD | 0.39 |  | 1 |  | 0.17 |  | 1 |
| C) Duration of the flowering season |  |  |  |  |  |  |  |
| First flowering date | 109.62 | \*\*\* | 1 |  | 33.53 | \*\*\* | 1 |
| Number of flowers | - |  | - |  | 63.31 | \*\*\* | 1 |
| First flowering date : N days 90-10% FFD | 0.78 |  | 1 |  | 0.30 |  | 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) and duration of the flowering season (E) 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.