



**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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## 26 ABSTRACT

27 To predict long-term responses to climate change we need to understand how changes in  
28 temperature and precipitation elicit both immediate phenotypic responses and changes in  
29 natural selection. We used 22 years of data for the perennial herb *Lathyrus vernus* to examine  
30 how climate influences flowering phenology and phenotypic selection on phenology. Plants  
31 flowered earlier in springs with higher temperatures and higher precipitation. Early flowering  
32 was associated with a higher fitness in nearly all years, but selection for early flowering was  
33 significantly stronger in springs with higher temperatures and lower precipitation. Climate  
34 influenced selection through trait distributions, mean fitness and trait-fitness relationships, the  
35 latter accounting for most of the among-year variation in selection. Our results show that  
36 climate both induces phenotypic responses and alters natural selection, and that the change in  
37 the optimal phenotype might be either weaker, as for spring temperature, or stronger, as for  
38 precipitation, than the optimal response.

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INTRODUCTION

Climate change is rapidly altering the environmental conditions experienced by organisms (Diffenbaugh & Field 2013; IPCC 2014). To predict the long-term effects of climate change, we need to assess not only immediate phenotypic responses of individuals, but also how changes in climate affect the optimal phenotypes, natural selection, and evolutionary trajectories of populations. 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). The direction of selection induced by increased temperature will depend on how strong plastic responses are compared with changes in the optimal phenotype. If the optimal response is stronger than the response elicited by increased temperatures, then we expect selection for stronger responses at higher temperatures (cf. Conover & Schultz 1995; Conover *et al.* 2009). If the optimal response is weaker, then we expect selection for weaker responses at higher temperatures. Many recent studies have documented phenotypic trait responses to climate, but it is less clear how climate influences the optimal phenotypes, and the direction and intensity of natural selection (Chevin *et al.* 2010; MacColl 2011; Siepielski *et al.* 2017). Moreover, the extent to which variation in climate or other environmental factors influence selection gradients through changes in trait-fitness relationships vs. changes in fitness and trait distributions is still largely unknown (Hunter *et al.* 2018). Long-term studies examining the relationships between climatic variables, phenotypic trait distributions, absolute fitness and trait-fitness relationships, are therefore essential to understand how climate change influences the evolutionary trajectories of plant and animal populations.

Responses to climatic variation often involve changes in the timing of different 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

64 main way in which organisms can modify their interactions with the abiotic and biotic  
65 environment. Short-term plastic responses, such as earlier development with higher spring  
66 temperatures or earlier snowmelt, have been documented for many species (Visser *et al.* 1998,  
67 2006; Charmantier *et al.* 2008; Anderson *et al.* 2012; CaraDonna *et al.* 2014; Wadgymar *et al.*  
68 2018).

69 However, we still know relatively little about how climate influences selection on  
70 phenology, or to what extent observed phenotypic changes have a genetic basis (Gienapp *et*  
71 *al.* 2008, Franks *et al.* 2014, Merilä & Hendry 2014). A few studies have identified climatic  
72 factors as important drivers of temporal variation in selection on timing of reproduction in  
73 animals (Réale *et al.* 2003; Visser *et al.* 2015; Marrot *et al.* 2018). Although climate is  
74 expected to also influence selection on phenology in plants (Munguía-Rosas *et al.* 2011;  
75 Anderson *et al.* 2012; Franks *et al.* 2014), such effects remain largely unexplored.

76 Despite the fundamental importance of temporal variation in selection, few long-term  
77 assessments on selection on plant traits exist (Siepielski *et al.* 2017), the longest spanning up  
78 to 10-11 years (Campbell & Powers 2015; Thomann *et al.* 2018). In this study, we use 22  
79 years of data from permanently marked individuals in a natural population of the spring-  
80 flowering understory herb *Lathyrus vernus* to examine the effects of climatic variation on  
81 flowering phenology, and on phenotypic selection on phenology. In this species, the  
82 development time from onset of shoot growth to flowering strongly depends on temperature.  
83 Spring frosts might damage developing shoots or flower buds in some years, and snow might  
84 protect shoot buds from extreme low temperatures in early spring. We asked: (1) Does  
85 flowering time vary among years in response to climatic conditions during spring? (2) Does  
86 the direction and strength of phenotypic selection on flowering time vary among years in  
87 response to differences in climatic conditions during spring? and (3) If so, what is the relative

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88 importance of changes in trait means, trait variances, fitness means and trait-fitness  
89 relationships for effects of climatic variables on selection gradients?  
90 We predicted that higher temperatures during shoot development in March, April and  
91 May result in faster development and earlier flowering. We also expected earlier-flowering  
92 individuals to benefit more from their earlier start in years when temperatures are high during  
93 the early phase of the flowering season. High temperatures might be beneficial because they  
94 are associated with increased assimilation, or with earlier emergence and higher activity of  
95 pollinators (cf. Kudo & Cooper 2019). On the other hand, low spring temperatures might lead  
96 to frost damage of early buds and flowers (J. Ehrlén pers. obs.). We also hypothesized that  
97 precipitation during early spring falling as snow might protect overwintering shoot buds from  
98 extreme low temperatures (Inouye *et al.* 2002), and lead to an earlier flowering. Because no  
99 negative effects of drought during spring have been observed in the study species, we also  
100 hypothesized that high precipitation later in spring should be associated with reduced  
101 photosynthetic active radiation, later flowering, and weaker selection for early flowering.  
102 Taken together, this means that we expected temperature and precipitation to influence both  
103 the timing of flowering and mean fitness, but that changes in selection gradients largely  
104 would correspond to changes in absolute trait-fitness relationships.

106 MATERIALS AND METHODS

107 **Study species**

108 Individuals of *Lathyrus vernus* grow fairly slow, lack organs for vegetative spread, usually  
109 flower only after 10-15 years of vegetative growth and frequently skip flowering in some  
110 years (J. Ehrlén pers. obs.). The average conditional life span of flowering individuals has  
111 been estimated to 44.3 years (Ehrlén & Lehtilä 2002). Growth of shoots is determinate and  
112 the number of flowers is determined in the previous year (Ehrlén & Van Groenendael 2001).

113 One or several erect shoots emerge from the over-wintering rhizome in March-April every  
114 year (Ehrlén 1995). At the study site, flowering starts about 4 weeks after shoot emergence,  
115 before canopy develops and light availability decreases in early June. Bud development and  
116 the onset of flowering therefore depend on climatic conditions during March, April and May.  
117 The time from shoot emergence to flowering varies considerably among years (J. Ehrlén  
118 unpublished data). Flower number (mean  $\pm$  SD =  $13.45 \pm 17.60$ ) is closely correlated with  
119 aboveground biomass and plant resource state (Sola & Ehrlén 2007). Flowering is acropetally  
120 sequential within shoots, starting with the basal flower in the basal raceme. The only  
121 pollinators of the large flowers are bumblebees (*Bombus* spp.). *Lathyrus vernus* is self-  
122 compatible, but lacks mechanisms for autogamy (J. Ehrlén, unpublished data). Timing of  
123 flowering is correlated with differences in seed production, and this pattern might be  
124 influenced by differences in both resource and pollen availability (Ehrlén 1992). Individuals  
125 produce a small number of large seeds (mean  $\pm$  SD =  $12.0 \pm 3.5$  mg, N = 200, Ehrlén 2002),  
126 and recruitment from seeds is frequent (Ehrlén & Eriksson 1996). Developing seeds are often  
127 damaged by the pre-dispersal seed predator *Bruchus atomarius*. Roe deers (*Capreolus*  
128 *capreolus*) sometimes consume flowering shoots, and early-flowering individuals are more  
129 often grazed than late-flowering (Ehrlén & Münzbergová 2009). There is heritable variation  
130 in flowering time among populations of *L. vernus* (Widén & Schiemann 2003), but the  
131 amount of genetic variation for flowering time within the study population is not known.

132

### 133 **Data collection**

134 The study was carried out in a population of *L. vernus* in a deciduous forest in the Tullgarn  
135 area, SE Sweden (58.9496 N, 17.6097 E), during 1987–1996 and 2006–2017. The study area  
136 is quite homogeneous with regard to altitude, slope and canopy cover. A permanent snow  
137 cover was lacking in most years, and when present, snow cover was usually less than 20 cm

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138 and disappeared about two months before flowering. In 1987, all flowering individuals in an  
139 area of 825 m<sup>2</sup> were permanently marked and surveyed in each year to 1996. New flowering  
140 individuals in the plot were included in the study in each year. No recordings were made  
141 1997–2005. In 2006, a new set of individuals in an area of 162 m<sup>2</sup> within the same population  
142 were marked, and surveyed in the same way as the initially marked individuals to 2017. In  
143 total, we recorded 2411 flowering events, and followed 606 individuals 1987–1996, and 228  
144 individuals 2006–2017.

145       Recordings in each season started when shoots initiated growth in April and continued  
146 every fifth day until all plants had finished flowering. At all visits, we recorded the number of  
147 buds, open flowers, scars from aborted flowers and fruits. We also recorded if shoots had  
148 been grazed. At each visit up to opening of the first flower, we assigned the size of the most  
149 advanced bud in all individuals to one of four categories: small, medium, large or very large.

150       We used first flowering day (FFD), i.e. the day of year when the first flower was fully  
151 unfolded with the banner petal folded upwards, as an estimate of flowering phenology of  
152 individuals. For some of the study years we had data also on median flowering date, and in  
153 these years FFD was strongly correlated with median flowering day (Pearson's  $r = 0.89$ ).  
154 Information about the presence of open flowers from each visit allowed us to determine  
155 during which 5-day interval an individual had started flowering. To estimate the most likely  
156 date within this interval at which the first flower opened, we used information about the size  
157 of the most developed bud at the beginning of this 5-day interval, and the number of open  
158 flowers at the end of the interval (see Appendix S1 for details). To account for leap years, we  
159 converted calendar dates to number of days after the vernal equinox.

160       The number of open flowers produced by an individual was obtained from counts of  
161 the number of buds, flowers, fruits and scars from aborted flowers at each recording. The



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).

At the time of fruit maturation, we measured vegetative size and recorded the number of mature fruits, intact seeds and seeds damaged by seed predators. To estimate size of individuals, 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 (see Appendix S1 for details). Fruits with mature seeds that had not yet opened 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. After examination, seeds were dropped to the ground near the parent plant mimicking natural dispersal. 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 number of intact seeds produced by an individual was used as an estimate of fitness.

Weather data for March, April and May 1961–2017 was obtained from the Swedish Meteorological and Hydrological Institute ([www.smhi.se](http://www.smhi.se)). Data from the 26-year period (1961–1986) immediately before the onset of the study, and from 1997–2005 (when no recordings of phenology were made), was used to study climatic variation in the study area over a longer period than the one covered by the study. 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). To test our hypotheses regarding the effects

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of climate on flowering phenology and selection, we calculated 12 variables from weather data: monthly averages of daily minimum, mean and maximum temperatures, and monthly sums of precipitation, for March, April and May in the year of flowering (Table S2).

**Statistical analyses**

*Variation in flowering time in response to spring climate*

To assess how much of variation in FFD of individual plants was explained by year, we calculated the R<sup>2</sup>-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 (because FFD was sometimes recorded in the same individuals in multiple years) and number of flowers as a covariate. Flower number was included in models as a measure of plant resource state to account for the fact that FFD might be related to plant condition. While climate also in the previous year might have an effect on phenology through effects on the resource state of individuals, such effects should to a large extent be accounted for by including the number of flowers as a predictor. All climatic variables were standardized by subtracting the mean and dividing by the standard deviation. We constructed a candidate model set, using the 12 climatic variables as predictors. To avoid overcomplex models, we did not include climate variables from other months or years, or interactions between climatic predictors. We constrained the set of candidate models by including only models with pairs of predictors having a Pearson correlation coefficient below 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  $\Delta AICc < 2$ . To explore how long-term trends influenced our estimates of climate effects, we ran the models including also year as a covariate.

212 *Variation in phenotypic selection on flowering time in response to spring climate*

213 To assess total and direct phenotypic selection on FFD, we estimated phenotypic selection  
214 differentials and gradients. Fitness, in terms of the number of intact seeds, was relativized  
215 within years by dividing individual values by yearly means. Traits were standardized within  
216 years by subtracting yearly means and dividing by yearly standard deviations. Selection  
217 differentials were calculated for each year using univariate regressions of relative fitness on  
218 standardized FFD (Lande & Arnold 1983). Selection gradients were calculated using multiple  
219 linear regressions of relative fitness on standardized FFD and number of flowers. We included  
220 the number of flowers as a measure of resource state also in these analyses (Rausher 1992).  
221 We estimated non-linear (quadratic and correlational) selection by examining the effect of  
222 quadratic and interaction terms in models including also linear terms (Lande & Arnold 1983;  
223 Arnold 1986). Quadratic regression coefficients and their standard errors were doubled  
224 (Stinchcombe *et al.* 2008).

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

231       To examine if variation in total phenotypic selection among years was related to  
232 climatic conditions during spring, we ran models including standardized FFD and the  
233 interactions between FFD and the 12 climatic variables. The main effects of climatic variables  
234 on fitness were not included as fitness was relativized within years. Plant individual was  
235 included as a random effect. For the effects of interactions with climatic variables, we  
236 performed model selection and model averaging as explained above. Interaction terms that

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237 had a significant effect in the averaged model (based on  $z$ , the Wald test statistic of the effect,  
238 Table S3) were included in an analysis of deviance using the Wald Chi-square test. We used  
239 analogous models to examine among-year variation in both total and direct selection, the  
240 latter including also the standardized number of flowers.

241 To assess how much of the among-year variation in total and direct selection was  
242 explained by climate, we regressed selection coefficients on the climatic variables whose  
243 interactions with FFD were significant in the selection models, accounting for uncertainty in  
244 the estimates (i.e. standard errors). Using the R package MCMCglmm (Hadfield 2010), we  
245 calculated the posterior distribution of the variance in selection associated with the climatic  
246 variables, and applied equation 12 in Hunter *et al.* (2018) to estimate the proportion of the  
247 total variation in selection attributed to climate (as the mean of the posterior distribution).

248  
249 *Relative importance of changes in trait means, trait variances, fitness means and trait-fitness*  
250 *relationships*

251 To examine how effects of climatic variables on selection gradients for FFD depended on  
252 changes in trait means, trait variances, fitness means and trait-fitness relationships, we ran two  
253 types of models (cf. Hunter *et al.* 2018). First, we ran four linear mixed models testing the  
254 effects of climatic variables on individual yearly absolute FFD values, individual absolute  
255 distances from the yearly mean FFD and individual absolute fitness, and on the relationships  
256 between trait values and absolute fitness. The three first models included the set of  
257 standardized climatic predictors that had significant effects on selection gradients for FFD,  
258 flower number, and plant individual as a random effect. The fourth model examined effects of  
259 FFD, the interactions of FFD with the climatic variables, and number of flowers, on  
260 individual absolute fitness, including plant individual as a random effect. Second, to assess  
261 the relative importance of these four components for among-year variation in selection, we

regressed yearly estimates of selection gradients for FFD on yearly estimates of trait means, trait variances, fitness means and FFD-fitness relationships. The relationships between FFD and fitness were estimated by the partial regression coefficient of absolute fitness on FFD, and obtained from yearly multiple regression models of absolute fitness on FFD and number of flowers of each individual.

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

## RESULTS

### *Variation in flowering time in response to spring climate*

Climate in the study area varied considerably during the period 1961-2017 (Fig. 1A, Figs. S1-S4, Table S4).

Yearly mean FFD ranged from 48.0 to 71.6 (mean = 58.1) days after the vernal equinox (Fig. 1B). Differences among years explained 59% of the total variation in individual FFD-values. 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). These effects of climatic variables were independent of potential long-term trends (Table S5).

### *Variation in phenotypic selection on flowering time in response to spring climate*

Selection differentials showed that earlier-flowering individuals had significantly higher fitness in 18 of 22 years (mean = 0.44, range = -1.03 - 0.28), while non-linear selection was significant in only 2 years (Table 2A and S6). Selection gradients indicated that earlier flowering was favored in 21 of 22 years (mean = 0.28, range = -0.78 - 0.08), but selection differed in strength and was significant in 8 years (Fig. 1D, Table S7). Non-linear direct

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selection on FFD was significant in one year, and correlational selection on FFD  $\times$  number of flowers in three years (Table S7)

Differences in total and direct selection among years were related to spring temperature and precipitation (Table 2B and S3, Fig. 3). There was stronger selection for early flowering in years with higher minimum April temperatures (Fig. 3A). Interestingly, selection favored earlier flowering at low levels of March precipitation but later flowering at high levels (Fig. 3B). These two climatic variables explained 70 and 68 % of among-year variation in total and direct selection, respectively. There was also a marginally significant trend towards increased benefits of early flowering with increased April precipitation (Table 2B and S3).

*Relative importance of changes in trait means, trait variances, fitness means and trait-fitness relationships*

Climatic variables influenced trait means, trait variances, mean fitness, and absolute trait-fitness relationships (Tables S8-11). Variation in trait-fitness relationships explained most of the among-year variation in selection gradients for FFD (27%), while variation in trait means, trait variances and mean fitness among years had little effect (Fig. 4, Table S12).

DISCUSSION

Twenty-two years of data for the perennial herb *Lathyrus vernus* showed that variation in climatic conditions during spring was related to both variation in flowering time and differences in phenotypic selection. Plants flowered earlier when springs were warmer and in years when precipitation was higher, and early flowering was most advantageous in years with high spring temperatures and in years with low precipitation. Although climatic variation influenced also trait distributions and absolute fitness, among-year variation in phenotypic

selection gradients was mostly the result of differences in trait-fitness relationships. Our results imply that climatic conditions are related to not only the expression of phenological traits, but also to natural selection on these traits. They also suggest that climate can elicit plastic responses that are both co-gradient with the optimal phenotype in a given year, as for spring temperature in our study, and counter-gradient, as for precipitation.

Because survival in *L. vernus* is high, and time from seed germination to flowering is very long, we do not expect plants recruited during the study to have flowered. We thus assume that the observed variation in FFD-values among years was mainly due to plastic responses. Moreover, the fact that the rank order of FFD-values was weakly correlated among years (Pearson  $r$ , mean  $\pm$  SD =  $0.16 \pm 0.17$ ,  $N = 22$  pairs of adjacent years), indicates that there are no consistently early- or late-flowering individuals. This, in turn, suggests that only a relatively small fraction of the observed variation in flowering phenology can be explained by genotype, and that evolutionary responses to observed selection will be slow.

Phenotypic responses to among-year climatic variation were strong, individual plants starting flowering on average 1.8 and 2.5 days earlier with a one-degree increase in mean temperature in April and May, respectively (based on the coefficients of a model including the predictors identified by model selection in Table 1, but using raw, unstandardized climatic variables). This is a slightly weaker response to temperatures than the average shifts recorded in other studies (Fitter *et al.* 1995; Miller-Rushing & Primack 2008; Ibáñez *et al.* 2010; Iler *et al.* 2013; Tansey *et al.* 2017). In temperate environments with little drought stress during spring, the expected phenological responses to precipitation are less clear than responses to temperature. Experimental studies examining responses of phenology to precipitation have either found no effects (Phoenix *et al.* 2001; Cleland *et al.* 2006), or variable responses (Peñuelas *et al.* 2004). In *L. vernus*, higher precipitation during early spring was associated with an earlier flowering. This might be because early-spring precipitation is associated with

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337 lower risk of frost damage if falling as snow, and with relatively mild temperatures if falling  
338 as rain. However, the effects of precipitation were weaker than those of temperature (plants  
339 flowered on average 0.5 and 0.2 days earlier with a 10 mm increase in precipitation in March  
340 and April, respectively).

341         Some recent studies have argued that measures of heat accumulation, such as growing  
342 degree days (GDD), are more ecologically relevant than calendar dates to describe the period  
343 from wintering conditions to flowering, as plant development during spring often depends on  
344 temperature (Wadgyamar et al. 2018; Ensing & Eckert 2019). Such measures might be  
345 particularly useful in situations where a good knowledge about the relationship between  
346 development rate and temperature is already available. In this study we used calendar date as  
347 a measure of differences in phenology, and explored the effects of multiple climatic factors on  
348 flowering phenology and selection. Indeed, we found that not only temperature variables,  
349 which are likely to have captured most of the variation in GDD, but also spring precipitation  
350 influenced first flowering dates, and that spring temperature and spring precipitation together  
351 explained most of the variation in flowering phenology and phenotypic selection.

352         In our study, the strength of selection on flowering time varied significantly over the  
353 22 study years. Still, there was a tendency towards early flowering being favored in almost all  
354 years, which agrees with many previous studies (Harder & Johnson 2009; Munguía-Rosas *et*  
355 *al.* 2011; Austen *et al.* 2017). Several explanations for such persistent phenotypic selection for  
356 earlier flowering have been suggested (Forrest 2014; Ehrlén 2015; Austen *et al.* 2017). For  
357 example, selection estimates are often based on a single fitness component, such as seed  
358 production in one year, and trade-offs between current and future reproduction can potentially  
359 counteract observed effects on seed production. In *L. vernus*, such costs of reproduction are  
360 small (Ehrlén & van Groenendael 2001), and therefore unlikely to eliminate the observed  
361 selection for earlier flowering, but might potentially weaken it. Another suggested



362 explanation for observed persistent phenotypic selection for earlier flowering is  
363 environmental covariance, i.e. that individuals in a better condition both flower earlier and  
364 produce more seeds (Rauscher 1992). In our study, we did not experimentally examine how  
365 condition influenced the relationship between phenology and fitness. However, including  
366 flower number in our analyses of selection gradients is likely to have reduced problems  
367 associated with environmental covariance.

368         Identifying the environmental drivers of natural selection is key to understand past  
369 evolution and to predict the evolutionary implications of ongoing environmental changes.  
370 Yet, the factors underlying temporal variation in natural selection on many traits, like plant  
371 flowering phenology, remain poorly known (but see Siepielski *et al.* 2017). One suggested  
372 driver of variation in selection is climatic fluctuations (Réale *et al.* 2003; Siepielski *et al.*  
373 2009, 2017; Visser *et al.* 2015; Siepielski *et al.* 2017; Marrot *et al.* 2018). In our study, which  
374 included one of the longest records of natural selection in plants to date, we found that most  
375 of observed among-year variation in selection could be explained by climatic variation. The  
376 strength of phenotypic selection for early flowering was associated with climatic variables,  
377 increasing with minimum temperatures in April and decreasing with precipitation in March.  
378 High temperatures early during the flowering period are likely to be associated with beneficial  
379 conditions, in terms of more rapid development, higher light availability (Kudo *et al.* 2008),  
380 decreased frost damage risk (Inouye 2008; Pardee *et al.* 2018), or higher pollinator  
381 availability (Kudo & Ida 2013), and early-flowering individuals might benefit most from this.  
382 Our results agree with the results of previous studies on birds (Visser *et al.* 2015; Marrot *et al.*  
383 2018) and mammals (Réale *et al.* 2003), suggesting that stronger selection for earlier  
384 reproduction in warmer springs might occur in many groups of organisms in temperate  
385 seasonal environments.

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386           Although precipitation has recently been suggested to be an important driver of  
387 selection (Siepielski *et al.* 2017), we still lack studies relating long-term variation in  
388 precipitation to variation in phenotypic selection. Our results indicated that increases in  
389 March precipitation might shift selection from favoring earlier flowering to favoring later  
390 flowering. A possible explanation for this pattern is that early-spring precipitation is  
391 associated with smaller fitness advantages of earlier-flowering individuals because growth  
392 conditions and light availability are poorer, and pollinator abundance lower, early in the  
393 season. Higher precipitation in early spring might also increase the intensity of antagonistic  
394 interactions, such as mollusk herbivory (Crawford-Sidebotham 1972), which have strong  
395 negative effects on fitness in *L. vernus* (Ehrlén 2002).

396           To fully understand the causes of variation in natural selection, we need not only to  
397 establish relationships between environmental factors and selection estimates, but also to  
398 examine the ecological mechanisms underlying such relationships (Chevin *et al.* 2010, Hunter  
399 *et al.* 2018). The pathways through which climate influences selection are still largely  
400 unexplored, and studies examining how effects act through changes in trait distributions,  
401 mean fitness and trait-fitness relationships are important to further our understanding (Hunter  
402 *et al.* 2018). Our results show that even if climatic variation influenced both trait distributions  
403 and mean fitness, most of the among-year variation in selection gradients for flowering time  
404 was explained by effects of climatic factors on trait-fitness relationships. This means that  
405 differences in selection among years were mostly a consequence of differences in trait-  
406 absolute fitness relationships, and that climatic conditions during spring influenced how much  
407 fitness increased with an earlier flowering. To better understand the ecological mechanisms  
408 underlying variation in selection, an important step would be to extend the analytical  
409 approach for survival developed by Hunter *et al.* (2018), to also enable examinations of  
410 fitness measures with other types of distributions, such as the number of offsprings.

Our 22-year assessment of among-year variation in selection on timing of flowering shows that climatic variation not only influences the expression of phenological traits, but also natural selection on these traits. They also show that effects of climate on selection are largely the result of changes in trait-fitness relationships. Lastly, our results illustrate that climatic variables can elicit plastic responses that are either co- or counter-gradient with the optimal response (cf. Conover & Schultz 1995; Conover *et al.* 2009). In our study, higher spring temperatures were associated with both an earlier flowering and an increased fitness advantage for plants flowering early, suggesting that the plastic response was weaker than the optimal response. 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, suggesting that the observed response was stronger than the optimal response. An important broader implication of this study is thus that in order to predict long-term responses of life-history traits 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.

#### ACKNOWLEDGEMENTS

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Table 1: Results of models testing for effects of climatic factors on first flowering date (FFD) of individual plants of *Lathyrus vernus* over 22 years (number of observations = 2411 flowering events, number of groups = 834 plant individuals). 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  $\Delta\text{AICc} < 2$  are shown. Variance and standard deviation of the random effect are shown for the best model resulting from model selection. The 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 were  $R^2_{\text{marg}} = 0.577$  and  $R^2_{\text{cond}} = 0.628$ . Estimates are on standardized scales.

Predictor variable	Estimate	Std. error	z	P
Precipitation March	-0.716	0.105	6.85	<0.001
Precipitation April	-0.345	0.125	2.77	0.006
Mean temperature March	-0.074	0.136	0.54	0.587
Max temperature March	-0.236	0.185	1.28	0.201
Mean temperature April	-2.188	0.134	16.29	<0.001
Mean temperature May	-3.754	0.113	33.17	<0.001
Number of flowers	-2.407	0.101	23.86	<0.001
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Random effects	Variance		Std. deviation	
Plant individual	2.708		1.65	
Residual	19.550		4.42	

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3	443	Table 2: Phenotypic selection models testing for (A) among-year differences and (B) effects					
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5	444	of climatic factors on total (direct and indirect) and direct phenotypic selection on flowering					
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7	445	time in <i>Lathyrus vernus</i> in 22 study years. Results are from (A) linear mixed models including					
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10	446	the interaction first flowering date × year, testing for among-year differences in selection					
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12	447	differentials and selection gradients for first flowering date, and (B) linear mixed models					
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14	448	including the interactions of first flowering date with climatic variables that were significant					
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16	449	according to model selection (see Table S3). For direct phenotypic selection, number of					
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18	450	flowers is included as a condition trait. Fitness was estimated by the number of intact seeds.					
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20	451	Traits were standardized and fitness relativized within years before analyses. Plant individual					
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22	452	was included as a random effect.					
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## Figures

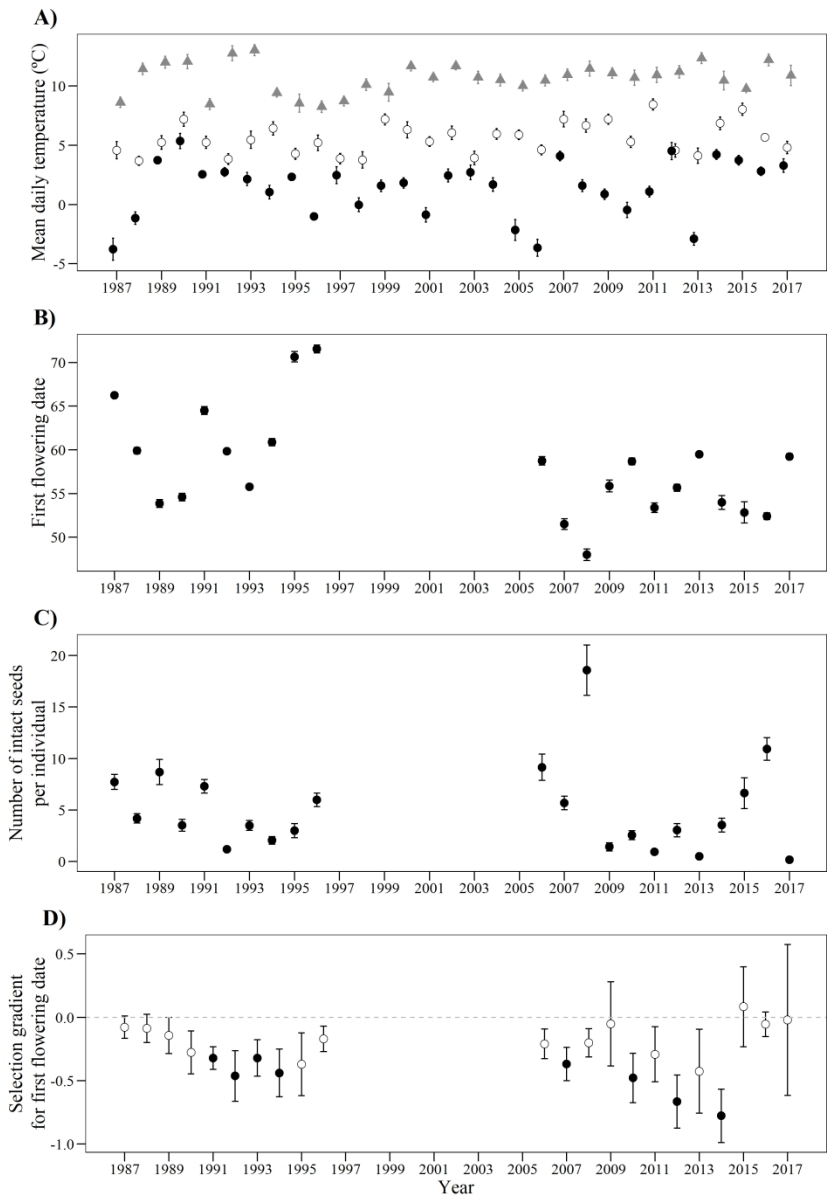
Figure 1: Variation in temperature, flowering time, fitness and selection on flowering time during the study period. Symbols represent means (dots) and standard errors (whiskers) for yearly values of (A) mean daily temperature during March (black circles), April (white circles) and May (grey triangles), (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 (A), data points are slightly jittered along the x-axis in order to prevent overplotting. In (D), filled symbols indicate selection gradients significant at  $\alpha = 0.05$ , and open symbols indicate selection gradients 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 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, in different years. Lines represent linear model fits.

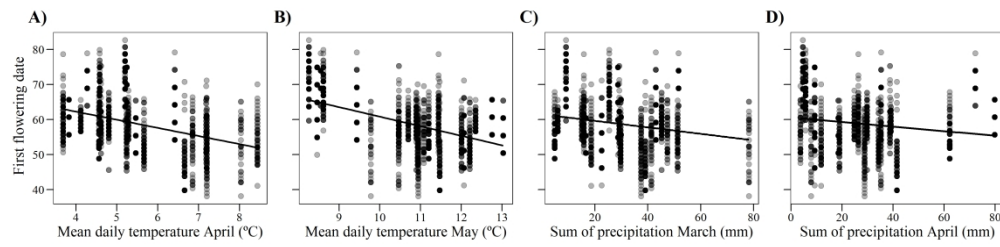
Figure 3: Partial regression plots (i.e. added variable plots) showing the independent effects of climatic variables on selection gradients for first flowering date (FFD) in *Lathyrus vernus* in 22 study years. The effect of standardized first flowering date on relative fitness, measured as the relative 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.

Figure 4: Relationship between selection gradients for first flowering dates (FFD) and partial regression coefficients of absolute fitness on FFD obtained from yearly multiple regression models of absolute fitness on FFD and number of flowers of each individual, in *Lathyrus vernus* in 22 study years. Linear model fit line and 95% confidence intervals are shown.

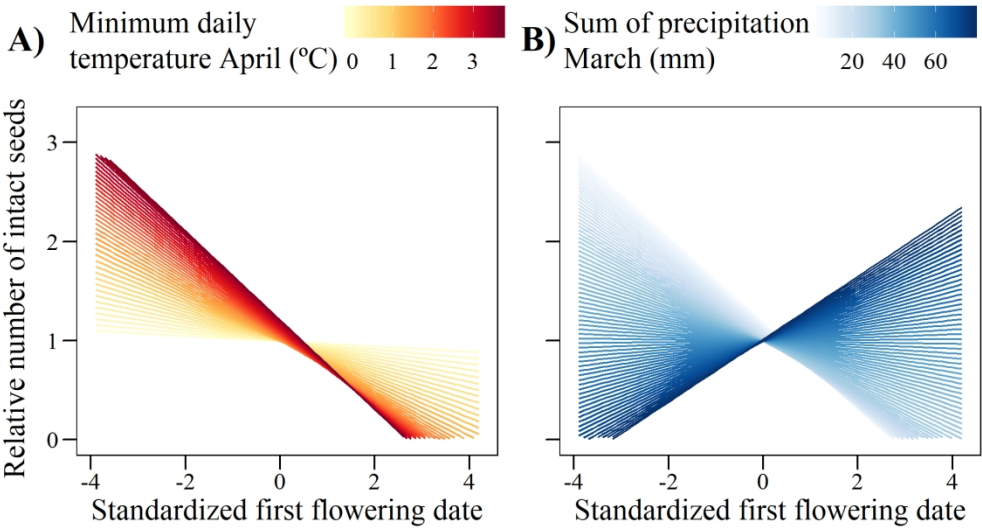
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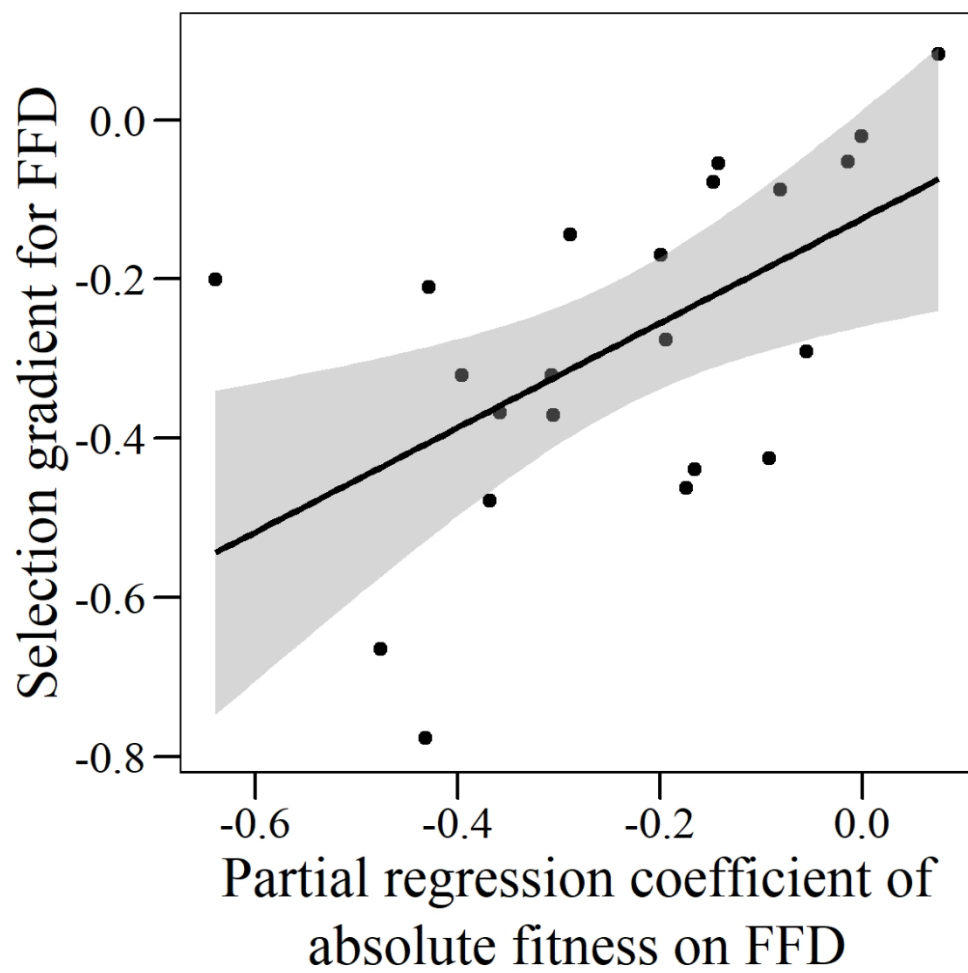


379x89mm (300 x 300 DPI)



189x109mm (300 x 300 DPI)





99x99mm (300 x 300 DPI)