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

1

1   TITLE:

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

3

4   Ehrlén, Johan*, a, b and Valdés, Alicia*, a, b

5   a Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91

6   Stockholm, Sweden

7   b Bolin Centre of Climate Research, Stockholm University, Stockholm, Sweden

8   *These authors contributed equally to the work

9   Short running title: Climate influences selection on phenology

10   Keywords: phenology, climate, phenotypic selection, phenotypic plasticity, timing of

11   reproduction, *Lathyrus vernus*, selection gradients

12   Type of article: Letter

13   Number of words in the abstract: 150

14   Number of words in the main text: 4889

15   Number of references: 63

16   Number of figures: 4

17   Number of tables: 2

18   Corresponding author: johan.ehrlen@su.se

25

2

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.

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-tern 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

5

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.

105

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).

6

113   One or several erect shoots emerge from the over-wintering rhizome in March-April every

114   year (Ehrlen 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 ± SD = 13.45 ± 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 (Ehrlen 1992). Individuals

125   produce a small number of large seeds (mean ± SD = 12.0 ± 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

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

138 and disappeared about two months before flowering. In 1987, all flowering individuals in an

139 area of 825 m^2 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^2 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 mean 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).

159 The number of open flowers produced by an individual was obtained from counts of

160 the number of buds, flowers, fruits and scars from aborted flowers at each recording. The

161 number of flowers in individuals that were grazed before the first recording of flower number

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

163   At the time of fruit maturation, we measured vegetative size and recorded the number

164 of mature fruits, intact seeds and seeds damaged by seed predators. To estimate size of

165 individuals, we measured the height and diameter of all shoots, calculated the volume of each

166 shoot as $\pi \times (\text{diameter}/2)^2 \times \text{height}$, and summed the volumes of all shoots (see Appendix S1

167 for details). Fruits with mature seeds that had not yet opened were examined directly in the

168 field. The number of seeds was counted and pre-dispersal seed predation was identified by the

169 presence of larval entrance holes in a seed. After examination, seeds were dropped to the

170 ground near the parent plant mimicking natural dispersal. Fruits that had opened prior to the

171 recording were brought to the lab where the numbers of intact and preyed seeds were

172 estimated based on the number of placentas and larval entrance holes (see Appendix S1 for

173 details). The number of intact seeds produced by an individual was used as an estimate of

174 fitness.

175   Weather data for March, April and May 1961–2017 was obtained from the Swedish

176 Meteorological and Hydrological Institute (www.smhi.se). Data from the 26-year period

177 (1961–1986) immediately before the onset of the study, and from 1997–2005 (when no

178 recordings of phenology were made), was used to study climatic variation in the study area

179 over a longer period than the one covered by the study. Daily mean, minimum and maximum

180 temperature values were averaged from two meteorological stations: Oxelösund (58.6777 N,

181 17.1223 E, 41 km from the study population) and Södertälje (59.2142 N, 17.6289 E, 29 km

182 from the study population). In cases where values for one station were missing, we imputed

183 values using the relationship between values from the two stations (see Appendix S1 for

184 details). Daily precipitation values were obtained from a third station located in Åda (58.9279

185 N, 17.5358 E, 5 km from the study population).

186 **Statistical analyses**

187 To account for leap years, we converted calendar dates to number of days after the vernal

188 equinox. To describe the yearly position of the flowering season we calculated three

189 measures: start - the date at which 10% of plants had started flowering, mean - the mean first

190 flowering date of all individuals, and end - the date at which 90% of plants had started

191 flowering. We chose the 10 and 90% cutoffs to remove the extremes of the distribution of

192 flowering dates. Other cutoff values (20 and 80%) yielded similar results (not shown). We did

193 not have information about when individuals ceased flowering, and the end of the flowering

194 season thus does not represent the date at which most individuals had ceased flowering. The

195 yearly duration of the flowering season was defined as the number of days between the start

196 and the end of the flowering season.

197 To test our hypotheses regarding the effects of climate on flowering phenology and

198 selection, we calculated 12 variables from weather data: monthly averages of daily minimum,

199 mean and maximum temperatures, and monthly sums of precipitation, for March, April and

200 May in the year of flowering (Table S2). Including also climatic variables from the year

201 preceding flowering did not improve the fit of models (results not shown).

202 To assess how much of variation in FFD of individual plants was explained by year,

203 we calculated the R^2-value from a linear model of FFD with year as a nominal variable. The

204 effects of climate on individual FFD-values were examined using linear mixed models with

205 plant individual as a random effect (because FFD was sometimes recorded in the same

206 individuals in multiple years) and number of flowers as a covariate. All climatic variables

207 were standardized by subtracting the mean and dividing by the standard deviation. We

208 constructed a candidate model set, using the 12 climatic variables as predictors. To avoid

209 overcomplex models, we did not include climate variables from other months or years, or

210 interactions between climatic predictors. We constrained the set of candidate models by

211 including only models with pairs of predictors having a Pearson correlation coefficient below

212 0.5. We ranked the resulting candidate models using the corrected second-order Akaike

213 information criterion (AICc). To account for model uncertainty, we performed model

214 averaging of parameter estimates across all models with $\Delta AICc < 2$. The effects of climate on

215 the start, mean, end and duration of the flowering season were examined using linear models,

216 including climatic predictors that had a significant effect in the averaged model of FFD for

217 individual plants. To explore how long-term trends influenced our estimates of climate

218 effects, we ran the models including also year as a covariate.

219   To assess total and direct phenotypic selection on FFD, we estimated phenotypic

220 selection differentials and gradients. Fitness, in terms of the number of intact seeds, was

221 relativized within years by dividing individual values by yearly means. Traits were

222 standardized within years by subtracting yearly means and dividing by yearly standard

223 deviations. Selection differentials were calculated for each year using univariate regressions

224 of relative fitness on standardized FFD (Lande & Arnold 1983). Selection gradients were

225 calculated using multiple linear regressions of relative fitness on standardized FFD and

226 number of flowers. We included the number of flowers as a measure of resource state in these

227 analyses (Rausher 1992). We estimated non-linear (quadratic and correlational) selection by

228 examining the effect of quadratic and interaction terms in models including also linear terms

229 (Lande & Arnold 1983; Arnold 1986). Quadratic regression coefficients and their standard

230 errors were doubled (Stinchcombe *et al.* 2008).

231   Among-year differences in total and direct phenotypic linear selection on flowering

232 time were evaluated using models including also the interaction between standardized FFD

233 and year. The main effect of year was not included as fitness was relativized within years.

234 Plant individual was included as a random effect. In this analysis, we considered only

235 variation in linear selection because non-linear selection was significant only in very few

236 years (see Results).

237   To examine if variation in total phenotypic selection among years was related to

238 climatic conditions during spring, we ran models including standardized FFD and the

239 interactions between FFD and the 12 climatic variables. The main effects of climatic variables

240 on fitness were not included as fitness was relativized within years. Plant individual was

241 included as a random effect. For the effects of interactions with climatic variables, we

242 performed model selection and model averaging as explained above. Interaction terms that

243 had a significant effect in the averaged model (based on z, the Wald test statistic of the effect,

244 Table S3) were included in an analysis of deviance using the Wald Chi-square test. We used

245 analogous models to examine among-year variation in both total and direct selection, the

246 latter including also the standardized number of flowers.

247   To assess how much of the among-year variation in total and direct selection was

248 explained by climate, we regressed selection coefficients on the climatic variables whose

249 interactions with FFD were significant in the selection models, accounting for uncertainty in

250 the estimates (i.e. standard errors). Using the R package MCMCglmm (Hadfield 2010), we

251 calculated the posterior distribution of the variance in selection associated with the climatic

252 variables, and applied equation 12 in Hunter *et al.* (2018) to estimate the proportion of the

253 total variation in selection attributed to climate (as the mean of the posterior distribution).

254   To examine how effects of climatic variables on selection gradients for FFD

255 depended on changes in trait means, trait variances, fitness means and trait-fitness

256 relationships, we ran two types of models (cf. Hunter *et al.* 2018). First, we ran four linear

257 mixed models testing the effects of climatic variables and number of flowers on individual

258 yearly absolute FFD values, individual absolute distances from the yearly mean FFD and

259 individual absolute fitness, and the effects of number of flowers, FFD and the interactions of

260 FFD with climatic variables on individual absolute fitness. Each model included the set of

261 standardized climatic predictors that had significant effects on selection gradients for FFD,

262 and plant individual as a random effect. Second, to assess the relative importance of these

263 four components for among-year variation in selection, we regressed yearly estimates of

264 selection gradients for FFD on yearly estimates of trait means, trait variances, fitness means

265 and FFD-fitness relationships (estimated by the regression coefficient of absolute fitness on

266 FFD and number of flowers).

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

268

269 RESULTS

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

271 S4). Minimum, mean and maximum daily temperature increased significantly from 1961 to

272 2017 (Table S4, Figs. S1-S3).

273 Yearly mean FFD ranged from 48.0 to 71.6 (mean = 58.1) days after the vernal

274 equinox (Fig. 1B). Differences among years explained 59% of the total variation in individual

275 FFD-values. Differences in climate explained a large part of the variation in FFD of

276 individuals, flowering occurring significantly earlier in years with higher mean temperatures

277 in April and May, and in years with higher precipitation in March and April (Table 1A, Fig.

278 2). Climatic predictors explained most of the among-year variation in the start (72%), mean

279 (76%), end (84%), and duration (57%) of the flowering season (Table 1B and C). The start,

280 mean and end of the flowering season were earlier in years with higher mean temperatures in

281 April and May (Table 1B, Fig. 3A-B). The flowering season was longer in years with higher

282 mean April temperatures (Table 1C, Fig. 3C). There were no effects of precipitation on the

283 position and duration of the flowering season. All effects of climatic variables were

284 independent of potential long-term trends (Table S5).

285 Selection differentials showed that earlier-flowering individuals had significantly

286 higher fitness in 18 of 22 years (mean = 0.44, range = 1.03 - 0.28), while non-linear

287   selection was significant in only 2 years (Table 2A and S6). Selection gradients indicated that

288   earlier flowering was favored in 21 of 22 years (mean = 0.28, range = 0.78 - 0.08), but

289   selection differed in strength and was significant in 8 years (Fig. 1D, Table S7). Non-linear

290   direct selection on FFD was significant in one year, and correlational selection on FFD ×

291   number of flowers in three years (Table S7)

292   Differences in total and direct selection among years were related to climatic

293   conditions (Table 2B and S3, Fig. 4). There was stronger selection for early flowering in years

294   with higher minimum April temperatures (Fig. 4A) and lower March precipitation (Fig. 4B).

295   These two climatic variables explained 70 and 68 % of among-year variation in total and

296   direct selection, respectively.

297   Climatic variables influenced trait means, trait variances, mean fitness, and absolute

298   trait-fitness relationships (Tables S8-11). Variation in trait-fitness relationships explained

299   most of the among-year variation in selection gradients for FFD (57%), while variation in trait

300   means, trait variances and mean fitness among years had little effect (Table S12).

301

302   DISCUSSION

303   Twenty-two years of data for the perennial herb *Lathyrus vernus* showed that variation in

304   climatic conditions during spring was related to both variation in flowering time and

305   differences in phenotypic selection. Plants flowered earlier when springs were warmer and

306   precipitation higher, and early flowering was most advantageous in years with high spring

307   temperatures and low precipitation. Although climatic variation influenced also trait

308   distributions and absolute fitness, among-year variation in phenotypic selection gradients was

309   mostly the result of differences in trait-fitness relationships. Our results imply that climatic

310   conditions are related to not only the expression of phenological traits, but also to natural

311   selection on these traits. They also suggest that climate can elicit plastic responses that are

312 both co-gradient with the optimal phenotype in a given year, as for spring temperature in our

313 study, and counter-gradient, as for precipitation.

314 Because survival in *L. vernus* is high, and time from seed germination to flowering is

315 very long, we do not expect plants recruited during the study to have flowered. We thus

316 assume that the observed variation in FFD-values among years was mainly due to plastic

317 responses. Moreover, the fact that the rank order of FFD-values was weakly correlated among

318 years (Pearson r, mean ± SD = 0.16 ± 0.17, N = 22 pairs of adjacent years), indicates that

319 there are no consistently early- or late-flowering individuals.

320 Phenotypic responses to among-year climatic variation were strong, individual plants

321 starting flowering on average 1.8 and 2.5 days earlier with a one-degree increase in mean

322 temperature in April and May, respectively. This is a slightly weaker response to temperatures

323 than the average shifts recorded in other studies (Fitter *et al*. 1995; Miller-Rushing & Primack

324 2008; Ibáñez *et al*. 2010; Iler *et al*. 2013; Tansey *et al*. 2017). Given the effects of spring

325 temperatures on flowering phenology observed in our system, the recorded increase in spring

326 temperatures over the last 57 years is expected to result in an earlier flowering of *L. vernus*.

327 We did indeed observe such an advance in our data, but this trend was stronger than what

328 would be expected based only on changes in temperature (results not shown). This suggests

329 that other factors, such as increases in individual size during the study contributed to the

330 trend.

331 For early-flowering species in seasonal environments, higher temperatures up to

332 flowering start can extend the flowering season, while higher temperatures during flowering

333 might shorten it (Theobald *et al.* 2017). In agreement, we found that for *L. vernus* the duration

334 of the flowering season increased with increasing temperatures in April, the effect on the start

335 of the flowering season being much stronger than on the end. In contrast, May temperature

336   had roughly equal effects on the start and end of the flowering season, and did thus not affect

337   the duration.

338   In temperate environments with little drought stress during spring, the expected

339   phenological responses to precipitation are less clear than responses to temperature.

340   Experimental studies examining responses of phenology to precipitation have either found no

341   effects (Phoenix *et al.* 2001; Cleland *et al.* 2006), or variable responses (Peñuelas *et al.* 2004).

342   In *L. vernus*, higher precipitation during early spring was associated with an earlier flowering.

343   This might be because early-spring precipitation is associated with lower risk of frost damage

344   if falling as snow, and with relatively mild temperatures if falling as rain. However, the

345   effects of precipitation were weaker than those of temperature (plants flowered on average 0.5

346   and 0.2 days earlier with a 10 mm increase in precipitation in March and April, respectively).

347   In our study, the strength of selection on flowering time varied significantly over the

348   22 study years. Still, there was a tendency towards early flowering being favored in almost all

349   years, which agrees with many previous studies (Harder & Johnson 2009; Munguía-Rosas *et*

350   *al.* 2011; Austen *et al.* 2017). Several explanations for such persistent phenotypic selection for

351   earlier flowering have been suggested (Forrest 2014; Ehrlén 2015; Austen *et al.* 2017). For

352   example, selection estimates are often based on a single fitness component, such as seed

353   production in one year, and trade-offs between current and future reproduction can potentially

354   counteract observed effects on seed production. In *L. vernus*, such costs of reproduction are

355   small (Ehrlén & van Groenendael 2001), and therefore unlikely to eliminate the observed

356   selection for earlier flowering, but might potentially weaken it. Another suggested

357   explanation for observed persistent phenotypic selection for earlier flowering is that

358   individuals in a better condition both flower earlier and produce more seeds. In our study, we

359   did not experimentally examine how condition influenced the relationship between phenology

360   and fitness. However, the fact that the advantage of early-flowering differed significantly

361   among years with climatic conditions in spring, suggests that there is a causal relationship

362   between flowering time and fitness, which is influenced by climate.

363   Identifying the environmental drivers of natural selection is key to understand past

364   evolution and to predict the evolutionary implications of ongoing environmental changes.

365   Yet, the factors underlying temporal variation in natural selection on many traits, like plant

366   flowering phenology, remain poorly known (but see Siepielski *et al*. 2017). One suggested

367   driver of variation in selection is climatic fluctuations (Réale *et al*. 2003; Siepielski *et al*.

368   2009, 2017; Visser *et al*. 2015; Siepielski *et al*. 2017; Marrot *et al*. 2018). In our study, which

369   included one of the longest records of natural selection in plants to date, we found that most

370   of observed among-year variation in selection could be explained by climatic variation. The

371   strength of phenotypic selection for early flowering was associated with climatic variables,

372   increasing with minimum temperatures in April and decreasing with precipitation in March.

373   High temperatures early during the flowering period are likely to be associated with beneficial

374   conditions, in terms of more rapid development, higher light availability (Kudo *et al*. 2008),

375   decreased frost damage risk (Inouye 2008; Pardee *et al*. 2018), or higher pollinator

376   availability (Kudo & Ida 2013), and early-flowering individuals might benefit most from this.

377   Our results agree with the results of previous studies on birds (Visser *et al*. 2015; Marrot *et al*.

378   2018) and mammals (Réale *et al*. 2003), suggesting that stronger selection for earlier

379   reproduction in warmer springs might occur in many groups of organisms in temperate

380   seasonal environments.

381   Although precipitation has recently been suggested to be an important driver of

382   selection (Siepielski *et al*. 2017), we still lack studies relating long-term variation in

383   precipitation to variation in phenotypic selection. Our results show that higher March

384   precipitation was associated with a weaker selection for early flowering. A possible

385   explanation for this pattern is that early-spring precipitation is associated with smaller fitness

386   advantages of earlier-flowering individuals because growth conditions and light availability

387   are poorer, and pollinator abundance lower, early in the season. Higher precipitation in early

388   spring might also increase the intensity of antagonistic interactions, such as mollusk herbivory

389   (Crawford-Sidebotham 1972), which have strong negative effects on fitness in *L. vernus*

390   (Ehrlén 2002).

391   To fully understand the causes of variation in natural selection, we need not only to

392   establish relationships between environmental factors and selection estimates, but also to

393   examine the ecological mechanisms underlying such relationships (Chevin *et al.* 2010, Hunter

394   *et al.* 2018). The pathways through which climate influences selection are still largely

395   unexplored, and studies examining how effects act through changes in trait distributions,

396   mean fitness and trait-fitness relationships are important to further our understanding (Hunter

397   *et al.* 2018). Our results show that even if climatic variation influenced both trait distributions

398   and mean fitness, most of the among-year variation in selection gradients for flowering time

399   was explained by effects of climatic factors on trait-fitness relationships. This means that

400   differences in selection among years were mostly a consequence of differences in trait-

401   absolute fitness relationships, and that climatic conditions during spring influenced how much

402   fitness increased with an earlier flowering.

403   Our 22-year assessment of among-year variation in selection on timing of flowering

404   shows that climatic variation not only influences the expression of phenological traits, but

405   also natural selection on these traits. They also show that effects of climate on selection are

406   largely the result of changes in trait-fitness relationships. Lastly, our results illustrate that

407   climatic variables can elicit plastic responses that are either co- or counter-gradient with the

408   optimal response (cf. Conover & Schultz 1995; Conover *et al.* 2009). In our study, higher

409   spring temperatures were associated with both an earlier flowering and an increased fitness

410   advantage for plants flowering early, suggesting that the plastic response was weaker than the

411 optimal response. In contrast, higher precipitation before the flowering season was associated

412 with an earlier phenology, but also with a decreased fitness advantage for plants flowering

413 early, suggesting that the observed response was stronger than the optimal response. An

414 important broader implication of this study is thus that in order to predict long-term responses

415 of life-history traits to global climate change, we need to consider both the immediate effects

416 on trait expression and the potential evolutionary responses to climate-induced changes in

417 selection.

418

423

424 REFERENCES

Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 3843–3852.

Arnold, S.J. (1986). Limits on stabilizing, disruptive, and correlational selection set by the opportunity for selection. *The American Naturalist*, 128, 143–146.

Austen, E.J., Rowe, L., Stinchcombe, J.R. & Forrest, J.R.K. (2017). Explaining the apparent paradox of persistent selection for early flowering. *New Phytologist*, 215, 929–934.

Campbell, D.R. & Powers, J.M. (2015). Natural selection on floral morphology can be influenced by climate. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20150178.

CaraDonna, P.J., Iler, A.M. & Inouye, D.W. (2014). Shifts in flowering phenology reshape a
subalpine plant community. *PNAS*, 111, 4916–4921.

Charmantier, A. & Gienapp, P. (2014). Climate change and timing of avian breeding and
migration: evolutionary versus plastic changes. *Evolutionary Applications*, 7, 15–28.

Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C.
(2008). Adaptive phenotypic plasticity in response to climate change in a wild bird
population. *Science*, 320, 800–803.

Chevin, L.-M., Lande, R. & Mace, G.M. (2010). Adaptation, plasticity, and extinction in a
changing environment: towards a predictive theory. *PLOS Biology*, 8, e1000357.

Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A. & Field, C.B. (2006). Diverse
responses of phenology to global changes in a grassland ecosystem. *PNAS*, 103, 13740–
13744.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. (2007). Shifting plant
phenology in response to global change. *Trends in Ecology & Evolution*, 22, 357–365.

Conover, D.O., Duffy, T.A. & Hice, L.A. (2009). The covariance between genetic and
environmental influences across ecological gradients: reassessing the evolutionary
significance of countergradient and cogradient variation. *Annals of the New York
Academy of Sciences*, 1168, 100–129.

Conover, D.O. & Schultz, E.T. (1995). Phenotypic similarity and the evolutionary
significance of countergradient variation. *Trends in Ecology & Evolution*, 10, 248–252.

Crawford-Sidebotham, T.J. (1972). The influence of weather upon the activity of slugs.
Oecologia, 9, 141–154.

Dunne, J.A., Harte, J. & Taylor, K.J. (2003). Subalpine meadow flowering phenology
responses to climate change: integrating experimental and gradient methods. *Ecological
Monographs*, 73, 69–86.

Ehrlen, J. (1992). Proximate lmits to seed production in a herbaceous perennial legume,
Lathyrus vernus. *Ecology*, 73, 1820–1831.

Ehrlen, J. (1995). Demography of the Perennial Herb Lathyrus Vernus. I. Herbivory and
Individual Performance. *Journal of Ecology*, 83, 287–295.

20

Ehrlén, J. (2002). Assessing the lifetime consequences of plant-animal interactions for the

perennial herb Lathyrus vernus (Fabaceae). *Perspectives in Plant Ecology, Evolution*

and Systematics, 5, 145–163.

Ehrlén, J. (2015). Selection on flowering time in a life-cycle context. *Oikos*, 124, 92–101.

Ehrlén, J. & Eriksson, O. (1996). Seedling recruitment in the perennial herb Lathyrus vernus.

Flora, 191, 377–383.

Ehrlén, J. & Lehtilä, K. (2002). How perennial are perennial plants? *Oikos*, 98, 308–322.

Ehrlén, J. & Münzbergová, Z. (2009). Timing of flowering: opposed selection on different

fitness components and trait covariation. *The American Naturalist*, 173, 819–830.

Ehrlén, J. & Van Groenendael, J. (2001). Storage and the delayed costs of reproduction in the

understorey perennial Lathyrus vernus. *Journal of Ecology*, 89, 237–246.

Fitter, A.H., Fitter, R.S.R., Harris, I.T.B. & Williamson, M.H. 1995. Relationships between

frist flowering date and temperature in the flora of a locality in central England.

Functional Ecology, 9, 55-60.

Forrest, J.R.K. 2014. Plant size, sexual selection, and the evolution of protandry in dioecious

plants. *American Naturalist* 184:338-351.

Franks, S.J., Weber, J.J. & Aitken, S.N. (2014). Evolutionary and plastic responses to climate

change in terrestrial plant populations. *Evolutionary Applications*, 7, 123–139.

Gienapp, P., Teplitsky, C., Alho, J.S., Mills, J.A. & Merilä, J. (2008). Climate change and

evolution: disentangling environmental and genetic responses. *Molecular Ecology*, 17,

167–178.

Hadfield, J.D. (2010). MCMC methods for multi-response generalized linear mixed models:

the MCMCglmm R package. *Journal of Statistical Software*, 33, 1–22.

Harder, L.D. & Johnson, S.D. 2009. Darwin's beautiful contrivances: evolutionary and

functional evidence for floral adaptation. *New Phytologist* 183, 530-545.

Hunter, D.C., Pemberton, J.M., Pilkington, J.G. & Morrissey, M.B. (2018). Quantification

and decomposition of environment-selection relationships. *Evolution*, 72, 851–866.

Ibáñez, I., Primack, R.B., Miller-Rushing, A.J., Ellwood, E., Higuchi, H., Lee, S.D., *et al.* (2010). Forecasting phenology under global warming. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 365, 3247–3260.

Iler, A.M., Hoye, T.T., Inouye, D.W. & Schmidt, N.M. 2013. Nonlinear flowering responses to climate: are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B-Biological Sciences*, 368, 20120489.

Inouye, D., Morales, M. & Dodge, G. (2002). Variation in timing and abundance of flowering by *Delphinium barbeyi* Huth (Ranunculaceae): the roles of snowpack, frost, and La Niña, in the context of climate change. *Oecologia*, 130, 543–550.

Inouye, D.W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, 89, 353–362.

Kudo, G. & Cooper, E. J. 2019. When spring ephemerals fail to meet pollinators: mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society B-Biological Sciences*, 286, 20190573.

Kudo, G. & Ida, T.Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, 94, 2311–2320.

Kudo, G., Ida, T.Y. & Tani, T. (2008). Linkages between phenology, pollination, photosynthesis, and reproduction in deciduous forest understory plants. *Ecology*, 89, 321–331.

Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210–1226.

MacColl, A.D.C. (2011). The ecological causes of evolution. *Trends in Ecology & Evolution*, 26, 514–522.

Marrot, P., Charmantier, A., Blondel, J. & Garant, D. (2018). Current spring warming as a driver of selection on reproductive timing in a wild passerine. *Journal of Animal Ecology*, 87, 754–764.

Merilä, J. & Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, 7, 1–14.

22

Miller-Rushing, A.J. & Primack, R.B. 2008. Global warming and flowering times in Thoreau's concord: A community perspective. *Ecology*, 89, 332–341.

Munguía-Rosas, M.A., Ollerton, J., Parra-Tabla, V. & De-Nova, J.A. (2011). Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology Letters*, 14, 511–521.

Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142.

Pardee, G.L., Inouye, D.W. & Irwin, R.E. (2018). Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers. *Global Change Biology*, 24, 848–857.

Peñuelas, J., Filella, I., Zhang, X., Llorens, L., Ogaya, R., Lloret, F., *et al.* (2004). Complex spatiotemporal phenological shifts as a response to rainfall changes. *New Phytologist*, 161, 837–846.

Phoenix, G.K., Gwynn-Jones, D., Callaghan, T.V., Sleep, D. & Lee, J.A. (2001). Effects of global change on a sub-Arctic heath: effects of enhanced UV-B radiation and increased summer precipitation. *Journal of Ecology*, 89, 256–267.

R Core Team. (2018). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/*.

Rausher, M.D. (1992). The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*, 46, 616–626.

Réale, D., McAdam, A.G., Boutin, S. & Berteaux, D. (2003). Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, 270, 591–596.

Siepielski, A.M., DiBattista, J.D. & Carlson, S.M. (2009). It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters*, 12, 1261–1276.

Siepielski, A.M., Morrissey, M.B., Buoro, M., Carlson, S.M., Caruso, C.M., Clegg, S.M., *et al.* (2017). Precipitation drives global variation in natural selection. *Science*, 355, 959–962.

Sola, A.J. & Ehrlén, J. (2007). Vegetative phenology constrains the onset of flowering in the perennial herb Lathyrus vernus. *Journal of Ecology*, 95, 208–216.

Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. (2008). Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution*, 62, 2435–2440.

Tansey, C.J., Hadfield, J.D. & Phillimore, A.B. 2017. Estimating the ability of plants to plastically track temperature-mediated shifts in the spring phenological optimum. *Global Change Biology*, 23, 3321-3334.

Theobald, E.J., Breckheimer, I. & HilleRisLambers, J. (2017). Climate drives phenological reassembly of a mountain wildflower meadow community. *Ecology*, 98, 2799–2812.

Thomann, M., Ehrlén, J. & Ågren, J. (2018). Grazers affect selection on inflorescence height both directly and indirectly and effects change over time. *Ecology*, 99, 2167–2175.

Valdés, A., Marteinsdóttir, B. & Ehrlén, J. (2019). A natural heating experiment: Phenotypic and genotypic responses of plant phenology to geothermal soil warming. *Global Change Biology*, 25, 954–962.

Visser, M.E., Noordwijk, A.J. van, Tinbergen, J.M. & Lessells, C.M. (1998). Warmer springs lead to mistimed reproduction in great tits (Parus major). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 1867–1870.

Visser, M.E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, 272, 2561–2569.

Visser, M.E., Holleman, L.J.M. & Gienapp, P. (2006). Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia*, 147, 164–172.

24

Visser, M.E., Gienapp, P., Husby, A., Morrisey, M., Hera, I. de la, Pulido, F., *et al.* (2015).

Effects of spring temperatures on the strength of selection on timing of reproduction in

a long-distance migratory bird. *PLOS Biology*, 13, e1002120.

Wadgymar, S.M., Ogilvie, J.E., Inouye, D.W., Weis, A.E. & Anderson, J.T. (2018).

Phenological responses to multiple environmental drivers under climate change:

insights from a long-term observational study and a manipulative field experiment. *New*

Phytologist, 218, 517–529.

Widén, B. & Schiemann, K. (2003). The pattern of variation in leaflet shape and reproductive

characters in Lathyrus vernus (L.) Bernh. (Fabaceae). *Plant Syst. Evol.*, 240, 41–54.

425 Tables

426 Table 1: Results of models testing for effects of climatic factors on (A) first flowering date

427 (FFD) of individual plants, and on three measures of the yearly position (B) and duration (C)

428 of the flowering season of *Lathyrus vernus* over 22 years. The position of the flowering

429 season was defined by the start, i.e. the date when 10% of the plants had started flowering, the

430 mean, i.e. the mean first flowering date, and the end, i.e. the date when 90% of the plants had

431 started flowering. The duration of the flowering season was defined by the number of days

432 between the start and the end. In (A), linear mixed models with plant individual as a random

433 effect and number of flowers as a covariate were used. Also shown are averaged estimates

434 and z-values across all candidate models with ΔAICc < 2, and values of marginal and

435 conditional pseudo-R^2 (representing, respectively, the variance explained by the fixed effects

436 and by the entire model, Nakagawa & Schielzeth 2013) and variance and standard deviation

437 of the random effect for the best model resulting from model selection. Estimates are on

438 standardized scales.

Response variable	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
A) FFD	Mean temperature March	-0.074	0.136	0.54	0.587
N = 2411	Max temperature March	-0.236	0.185	1.28	0.201
R^2_{marg} = 0.577	Mean temperature April	-2.188	0.134	16.29	<0.001
R^2_{cond} = 0.628	Mean temperature May	-3.754	0.113	33.17	<0.001
	Number of flowers	-2.407	0.101	23.86	<0.001
		Variance		Std. deviation	
	Plant individual (random)	2.708		1.65	
		Estimate	Std. error	t	P
B) Position of the fl. season					
Start	Precipitation March	-0.993	0.888	-1.12	0.279
N = 22 R^2 = 0.719	Precipitation April	-0.353	0.938	-0.38	0.712

26

	Mean temperature April	-4.465	1.001	-4.46	<0.001
	Mean temperature May	-3.531	0.823	-4.29	<0.001
Mean N = 22 $R^2 = 0.764$	Precipitation March	-0.803	0.724	-1.11	0.283
	Precipitation April	-0.627	0.765	-0.82	0.423
	Mean temperature April	-3.429	0.816	-4.20	<0.001
	Mean temperature May	-4.004	0.671	-5.97	<0.001
End N = 22 $R^2 = 0.844$	Precipitation March	-0.461	0.549	-0.84	0.412
	Precipitation April	-0.810	0.580	-1.40	0.180
	Mean temperature April	-2.324	0.618	-3.76	0.002
	Mean temperature May	-4.555	0.508	-8.96	<0.001

C) Duration or the fl. season

N = 22 $R^2 = 0.567$	Precipitation March	0.532	0.604	0.88	0.390
	Precipitation April	-0.458	0.638	-0.72	0.483
	Mean temperature April	2.141	0.680	3.15	0.006
	Mean temperature May	-1.024	0.560	-1.83	0.085

439

440　Table 2: Phenotypic selection models testing for (A) among-year differences and (B) effects

441　of climatic factors on total (direct and indirect) and direct phenotypic selection on flowering

442　time in *Lathyrus vernus* in 22 study years. Results are from (A) linear mixed models including

443　the interaction first flowering date × year, testing for among-year differences in selection

444　differentials and selection gradients for first flowering date, and (B) linear mixed models

445　including the interactions of first flowering date with climatic variables that were significant

446　according to model selection (see Table S2). For direct phenotypic selection, number of

447　flowers is included as a condition trait. Fitness was estimated by the number of intact seeds.

448　Traits were standardized and fitness relativized within years before analyses. Plant individual

449　was included as a random effect.

Predictor variable	Total selection			Direct selection		
	χ^2	Degrees of freedom	P	χ^2	Degrees of freedom	P
A) First flowering date	110.18	1	<0.001	33.89	1	<0.001
Number of flowers	-	-		64.79	1	<0.001
First flowering date : year	36.46	21	0.019	37.87	21	0.013
B) First flowering date	110.44	1	<0.001	33.93	1	<0.001
Number of flowers	-	-		65.55	1	<0.001
First flowering date : Precipitation March	12.48	1	<0.001	14.80	1	<0.001
First flowering date : Precipitation April	3.48	1	0.062	3.83	1	0.050
First flowering date : Min temperature April	10.73	1	0.001	9.90	1	0.002

450

451 Figures

452 Figure 1: Variation in temperature, flowering time, fitness and selection on flowering time

453 during the study period. Symbols represent means (dots) and standard errors (whiskers) for

454 yearly values of (A) mean daily temperature during March (black circles), April (white

455 circles) and May (grey triangles), (B) first flowering date, calculated as the number of days

456 from the vernal equinox, (C) mean fitness, calculated as the number of intact, non-predated

457 seeds, and (D) linear selection gradient for first flowering date. In (A), data points are slightly

458 jittered along the x-axis in order to prevent overplotting. In (D), black circles indicate

459 significant selection gradients at $\alpha = 0.05$ and the grey dashed line indicates a selection

460 gradient not significantly different from 0. Variation in other climatic variables is presented in

461 Figures S1-S4.

462 Figure 2: Relationship between first flowering date of individual plants in different years and

463 (A) mean daily temperature in April, (B) mean daily temperature in May, (C) sum of

464 precipitation in March, and (D) sum of precipitation in April. Lines represent linear model

465 fits.

466 Figure 3: Relationship between the start, mean and end of the flowering season, and (A) mean

467 daily temperature in April and (B) mean daily temperature in May, and (C) between the

468 duration of the flowering season and mean daily temperature in April. The symbols in (A) and

469 (B) denote the start, i.e. the date when 10% of the plants had started flowering (triangles and

470 dashed line), the mean, i.e. the mean first flowering date (points and solid line) and the end,

471 i.e. the date when 90% of the plants had started flowering (stars and dotted line), of the

472 flowering season. The duration of the flowering season was measured as the number of days

473 between the start and the end of the season. Lines represent linear model fits.

474 Figure 4: Partial regression plots (i.e. added variable plots) showing the independent effects of

475 climatic variables on selection gradients for first flowering date (FFD) in *Lathyrus vernus* in
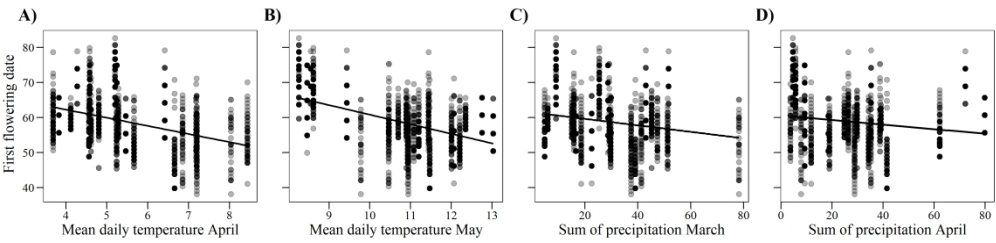
476   22 study years. The effect of standardized first flowering date on relative fitness, measured as

477   the relative number of intact seeds, is shown for (A) different minimum daily temperatures in

478   April and (B) different sums of precipitation in March. Lines represent linear model fits.

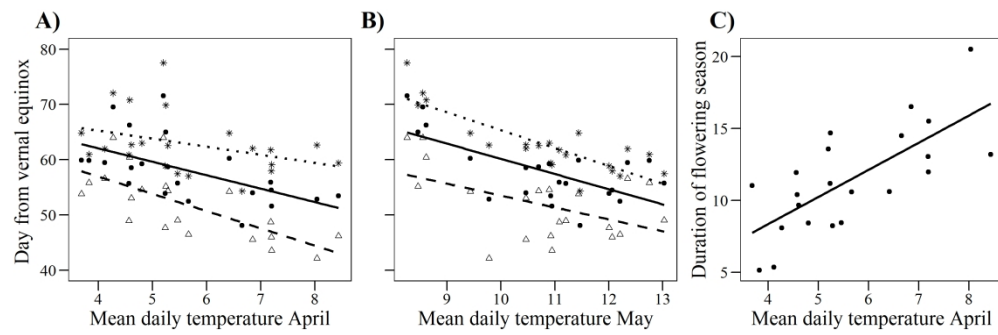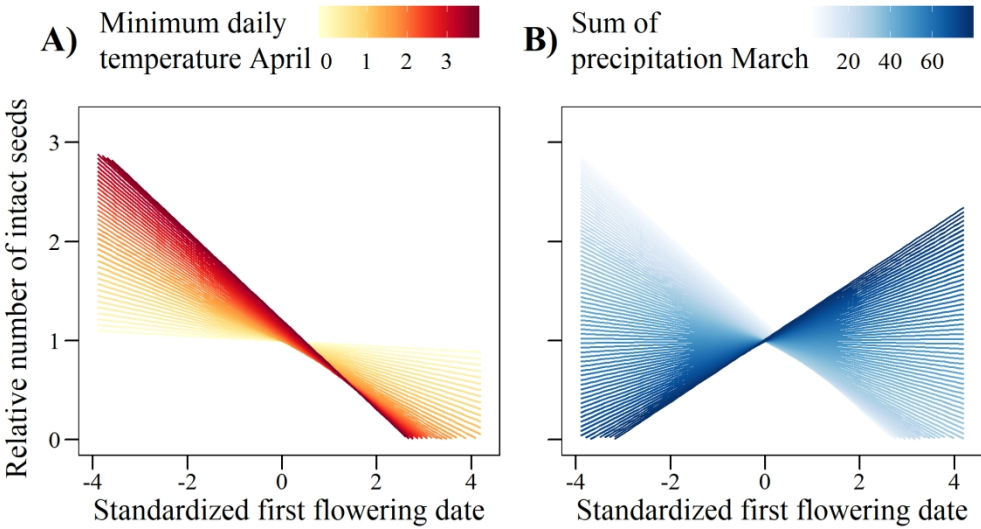479   Values were calculated from models in Table 2.

480
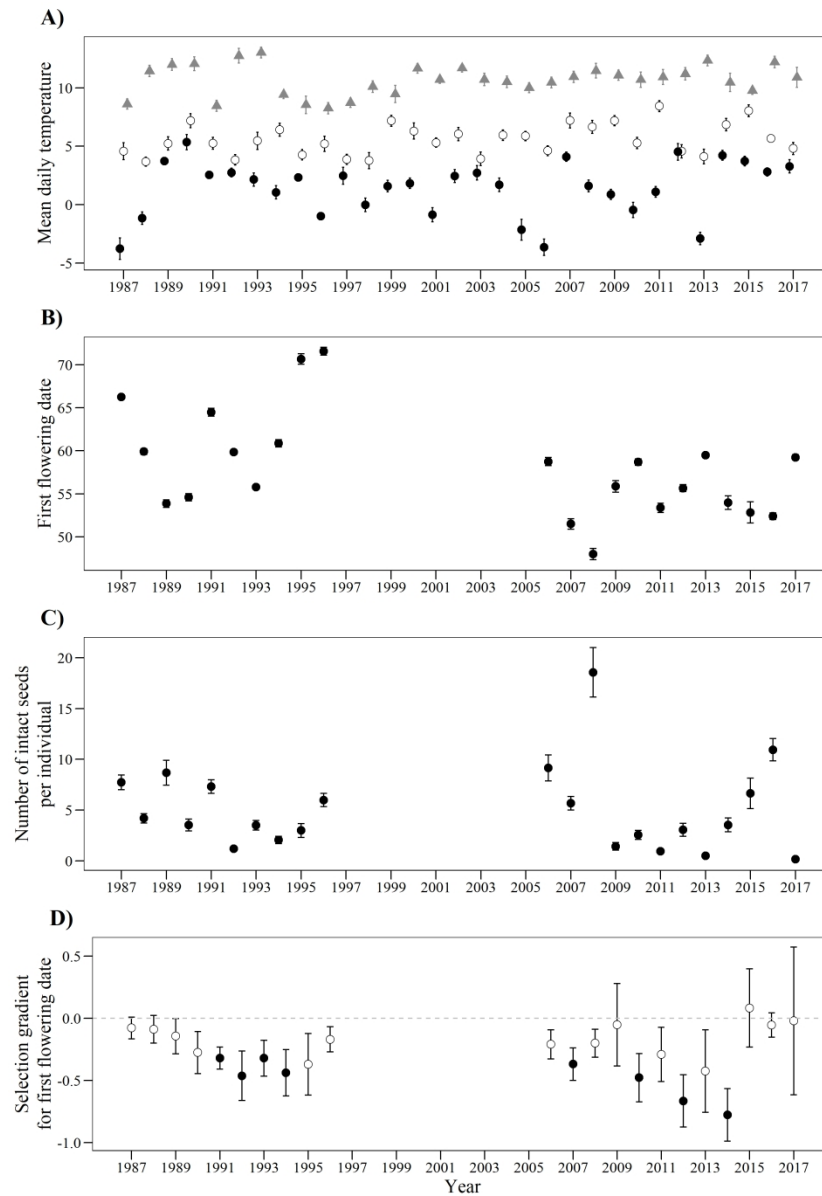
249x359mm (300 x 300 DPI)

379x89mm (300 x 300 DPI)

279x89mm (300 x 300 DPI)

A) Minimum daily temperature April

B) Sum of precipitation March

189x109mm (300 x 300 DPI)