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## Butterfly seed predators mediate shifts in selection on flowering phenology in their host plant

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1 TITLE: Butterfly seed predators mediate shifts in selection on flowering phenology in  
2 their host plant

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9 performed the data analysis. AV and JE wrote the manuscript.

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14 *alcon*, *Myrmica*, spatial variation, myrmecophily

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## 21 ABSTRACT

22 Variation in selection among populations and years has important implications for  
23 evolutionary trajectories of populations. Yet, the agents of selection causing this  
24 variation have rarely been identified. We investigated if differences in the direction of  
25 phenotypic selection on flowering phenology among 20 populations of *Gentiana*  
26 *pneumonanthe* were related to the presence of the butterfly seed predator *Phengaris*  
27 *alcon*, and if butterfly incidence was associated with the abundance of a second host,  
28 *Myrmica* ants. In plant populations without the butterfly, phenotypic selection favored  
29 earlier flowering. In populations where the butterfly was present, it preferentially  
30 attacked early-flowering individuals, shifting the direction of selection to favoring later  
31 flowering. Butterfly incidence increased with ant abundance. Our results demonstrate  
32 that antagonistic interactions can shift the direction of selection on flowering  
33 phenology, and suggest that such shifts might be associated with differences in the  
34 community context.

INTRODUCTION

Timing of reproduction within a season is a key trait influencing interactions both with the physical environment and with other organisms. For plants in temperate regions, selection on flowering phenology is mediated by abiotic conditions (Franks et al., 2007, Giménez-Benavides et al., 2011) and by species interactions (Elzinga et al., 2007, Sletvold et al., 2015). Mutualistic pollinators might select for both earlier (e.g. Munguía-Rosas et al., 2011a, Chapurlat et al., 2015), and later flowering (e.g. Sandring & Ågren, 2009). Likewise, antagonistic interactions with herbivores or pre-dispersal seed predators might favor both later (e.g. Parachnowitsch & Caruso, 2008, König et al., 2015) and earlier flowering (e.g. Fukano et al., 2013). Moreover, plants can simultaneously experience selection for earlier and later flowering mediated by different agents. For example, flowering early might be advantageous for plants at northern latitudes because it increases pollinator availability and the time available for seed development (Munguía-Rosas et al., 2011b), but at the same time costly if early flowering increases the exposure to antagonists. In these cases, net selection depends on the relative strengths of these interactions (e.g. Ehrlén and Münzbergová 2009, Sletvold et al. 2015).

Spatial variation in selection mediated by species interactions has been documented in many systems (Thompson 2005, Siepielski et al. 2013), and may result in local adaptation and adaptive population divergence. Variation in selection strength may potentially be driven by differences in interaction intensities or trait preferences of the interactors among populations. For plant-animal interactions, such as pre-dispersal seed predation, studies have demonstrated that differences in selection on plant traits among populations or years can be associated with differences in both interaction intensities (Benkman 2013, Benkman et al., 2013, Vanhoenacker et al., 2013), and trait

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3 61 preferences (Rey et al., 2006; Kolb et al., 2007). Interaction intensities and animal  
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5 62 preferences for plant traits might, in turn, depend on the environmental context in terms  
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7 63 of the abiotic conditions (e.g. light availability, Arvanitis et al., 2007; Kolb & Ehrlén,  
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9 64 2010, soil moisture, von Euler et al., 2014). The community context, in terms of natural  
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11 65 enemies, competitors or alternative hosts, has also been shown to influence the outcome  
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13 66 of plant-animal interactions (Strauss & Irwin, 2004; Siepielski & Benkman, 2007;  
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15 67 Chamberlain et al., 2014). In spite of an increasing awareness of the ubiquitous spatial  
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17 68 and temporal variation in selection, the biotic selective agents causing this variation as  
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19 69 well as how the environmental context influences the impact of a given selective agent  
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21 70 have been little explored.  
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26 71 Large Blue butterflies of the genus *Phengaris* Doherty, 1891 (the senior  
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28 72 synonym of *Maculinea* van Eecke, 1915) are specialist predispersal seed predators  
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30 73 during their first larval instars, and often show strong preferences for particular plant  
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32 74 developmental stages (Thomas & Elmes, 2001; Van Dyck & Regniers, 2010). This  
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34 75 means that plant individuals for which the developmental stage preferred for oviposition  
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36 76 coincides with the peak of butterfly activity suffer most from seed predator attacks.  
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38 77 *Phengaris* larvae need also a second host to complete their development, and most  
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40 78 species are parasites of ant nests (*Myrmica* spp.) during later instars (Als et al., 2004).  
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42 79 Butterfly-mediated selection on plant traits in this system might thus be influenced by  
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44 80 the community context, if the incidence and intensity of predation are related to host ant  
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46 81 abundance. In this study, we examined if among-population variation in phenotypic  
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48 82 selection on flowering phenology in the perennial herb *Gentiana pneumonanthe* is the  
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50 83 result of differences in the intensity of interactions with its specialist predispersal seed  
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52 84 predator, the butterfly *Phengaris alcon*, and if variation in the incidence of the butterfly  
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54 85 in plant populations is associated with the community context in terms of the abundance  
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of the second host, *Myrmica* ants. As developmental stages preferred for oviposition in early-flowering plants usually coincide with the oviposition period of the butterfly (A. Valdés and J. Ehrlén, *pers. obs.*), we hypothesized that: (1) The presence of the butterfly seed predator in plant populations shifts the direction of phenotypic selection on flowering phenology in *G. pneumonanthe*, from favoring early flowering to favoring late flowering, and (2) Community context, in terms of host ant abundance, is related to the probability of butterfly presence within plant populations. To test these hypotheses, we assessed selection gradients for flowering phenology, seed predation intensities and ant abundances in 11 *G. pneumonanthe* populations where the butterfly was present and 9 where it was absent.

MATERIALS AND METHODS

**Study system**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb (10-20 years, Oostermeijer et al. 1992), occurring in open habitats, such as wet heathlands and grasslands (Simmonds, 1946). In natural populations, individuals do not flower until their third year (Simmonds, 1946). Plants can have one to many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by bumblebees. The species is self-compatible and flowers in July and August in SW Sweden. Fruits are capsules containing a high number (usually 300-700, Appelqvist et al., 2007) of minute (mean seed weight = 0.044 mg, Simmonds, 1946), wind-dispersed seeds. *Gentiana pneumonanthe* is the primary host of the Alcon Blue butterfly (*Phengaris alcon*), a specialist predispersal seed predator which oviposits on young buds in July and August (Appelqvist et al., 2007). The caterpillars feed inside the capsule until they reach the fourth-instar, when then they drop to the ground to be picked up by *Myrmica* ants

(Mouquet et al., 2005). Caterpillars mimic the surface chemistry of the ant brood (Nash et al., 2008) and the acoustic signals of queen ants (Sala et al. 2014), and this makes ants carry them to their nest, where they spend the rest of their larval period as parasites (Mouquet et al., 2005). Contrary to the majority of *Phengaris* species, which prey on ant brood, *P. alcon* is a “cuckoo” species (Als et al., 2004), and larvae feed primarily on regurgitations from ant workers, trophic eggs (i.e. nutritious, infertile eggs which are fed to the queens and larvae) and prey items brought to the nest by ants. Only ants belonging to the genus *Myrmica* can act as second hosts of *P. alcon* (Mouquet et al., 2005). In our study area, *M. ruginodis* is thought to be the most commonly used host ant species (Appelqvist et al., 2007).

## Data collection

The study was carried out in 20 populations of *G. pneumonanthe* located in the county of Västra Götaland in SW Sweden (see Appendix S1 in Supporting Information for details). The populations are mainly located in moist heathlands. *P. alcon* was present in 11 of the study populations and absent from 9. Predation intensity varied considerably among the populations where *P. alcon* was present (Appendix S1).

We collected data on plant reproductive traits, interaction intensity and plant fitness in 100 marked individuals in each of the 20 study populations during 2010 and 2011. The number of shoots per individual ranged from 1 to 44 (mean = 3). For recordings, we selected one shoot of median length in each individual. In these focal shoots, we recorded shoot height (in cm), number of flowers, and developmental stage of each bud and flower. Buds and flowers were assigned to one of six developmental stages: (1) the sepals covering the bud completely, (2) bud becoming visible, (3) bud growing over the sepals, (4) bud turning blue, (5) flower opening, and (6) flower

134 showing signs of wilting. All plant traits were measured once per year, at the end of  
135 July-beginning of August. Phenology of plant individuals was estimated based on the  
136 reproductive development stage of flowers at the day of recording. We calculated two  
137 different phenology measures: (1) the mean development stage of all flowers and buds  
138 within the focal shoot (a continuous variable ranging from 1 to 6), and (2) the stage of  
139 the most advanced bud within the focal shoot (an ordinal variable ranging from 1 to 6).  
140 In both cases, higher values indicate a more advanced floral development at the day of  
141 recording, i.e. an earlier flowering. The average duration of stages 2-5 was about one  
142 week (A. Valdés, *pers. obs.*). A one-unit increase in these measures thus roughly  
143 corresponds to one week earlier development.

144 Interaction intensity was estimated by the maximum number of *P.alcon* eggs  
145 observed on the focal shoot during 2-6 visits to each population. Populations were  
146 visited once at the end of July-beginning of August (when data on reproductive traits  
147 was collected) and from 1 to 5 times from the end of August until all fruits had matured  
148 in mid-October (the number of visits depending on the time needed for fruit  
149 maturation).

150 Plant fitness was estimated by the maximum observed number of intact (i.e. not  
151 damaged by the butterfly) mature fruits on the focal shoot (assessed from counts of  
152 fruits on 1 to 5 visits to each population, see above). Although some seeds might remain  
153 in attacked fruits, the larvae consume a large proportion of seeds in the capsules (A.  
154 Valdés *pers. obs.*), and the total number of mature seeds in intact and attacked fruits is  
155 strongly correlated with the number of intact fruits ( $r = 0.85$ ,  $N = 1136$  individuals in  
156 2010). In 2010, information on fruit production was collected in all 20 populations but  
157 in 2011 we were only able to get information from 16 of the populations.



Ant abundance was estimated once per study year along transects in each of the 20 study populations. In 2010, 20-m transects were established, and in 2011 we used a 40-m transect in each population. Sugar cubes were placed at one-meter intervals along the transects, and the number of *Myrmica* sp. ants at each of the sugar cubes was counted 30 minutes after presenting them to ants. Assessments were only carried out under dry (no precipitation) and warm conditions. To further reduce variation due to varying weather conditions during ant counts, we used the highest yearly value of the average number of ants per sugar cube in each population as a measure of ant abundance.

### Statistical analyses

To assess phenotypic selection on flowering phenology, we performed selection gradient analyses in each of the two years by regressing relative fitness on standardized estimates of phenology, flower number and shoot height (Lande & Arnold, 1983). Fitness, in terms of the number of intact fruits, was relativized within populations by dividing individual values by population mean values. Traits were standardized by subtracting the population mean and dividing by the population standard deviation. To test for differences in phenotypic selection gradients among populations, we included the interaction between standardized reproductive traits and population in the models. The main effect of population was not included as fitness was relativized within populations prior to analysis. We fitted a linear model with Type II sums of squares. In addition to tests of directional (linear) selection, we also tested for non-linear (quadratic and correlational) selection. Quadratic selection is defined as the covariance between relative fitness and the squared deviations of trait values from the mean, while correlational selection is the covariance between relative fitness and the product of trait deviations (Lande & Arnold, 1983; Arnold, 1986). Non-linear selection was assessed by

183 examining the effects of quadratic and interaction terms in a model including also the  
184 linear terms. Results for models using mean and most advanced flower developmental  
185 stages within shoots as estimates of phenology were very similar in all cases. Below, we  
186 present only results for the stage of the most advanced bud (results for selection  
187 gradient analyses using the mean flower developmental stage are shown in Appendix S2  
188 in Supporting Information).

189 To test if linear selection on reproductive traits differed among populations with  
190 vs. without *P. alcon*, and to avoid basing contrasts on estimated selection coefficients,  
191 we created a variable “Predation”, coded as 0 in populations without *P. alcon* and as 1  
192 in populations with *P. alcon*, and constructed a linear hierarchical mixed model (Type II  
193 sums of squares) for each year. Predictors included standardized reproductive traits and  
194 their interactions with population as random effects, and predation as a fixed effect.  
195 Effects of trait  $\times$  population and trait  $\times$  predation interactions were only included in  
196 these models if the trait  $\times$  population interaction was significant. We did not include an  
197 individual intercept by population because fitness was relativized within populations  
198 prior to analysis.

199 In populations where the butterfly was present, we investigated predator  
200 preferences, by regressing the probability (0 or 1) and intensity (number of eggs in all  
201 individuals) of attack by *P. alcon* on reproductive traits, population and their  
202 interactions in each of the two years. We excluded population D (Appendix S1) in 2010  
203 from these analyses because only one plant individual was attacked.

204 We also used path analyses to examine the relative importance of direct and  
205 indirect effects of reproductive traits on fitness (Grace, 2006), indirect effects being  
206 mediated by either the probability or the intensity of predator attack (i.e., two different

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3 207 saturated models were considered, see Appendix S3 in Supporting Information for  
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5 208 details). To test for differences among populations, we used multigroup analysis (Grace,  
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7 209 2006). Because populations differed significantly, we fitted separate models for each  
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10 210 population and year. We excluded population D in 2010 also from these analyses.

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12 Finally, we examined if the abundance of the host ant of *P. alcon* was associated  
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14 with an increased probability of predator presence or an increased interaction intensity.  
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17 213 First, we performed a logistic regression of butterfly presence on log-transformed ant  
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19 214 abundance, using the 20 study populations. Second, we regressed the mean number of  
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21 215 eggs per plant and the proportion of plants with *P. alcon* eggs in each of the two years  
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23 216 on log-transformed ant-abundance, using the 11 populations where the butterfly was  
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26 217 present.

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29 218 Path analyses were carried out in Amos 16.0 (Arbuckle, 2007). All other analyses  
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31 219 were conducted in R 3.1.2 (R Development Core Team, 2014).  
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## 34 220 RESULTS

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37 221 Directional phenotypic selection on flowering phenology varied among populations,  
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39 222 and was statistically significant in 5 populations in 2010, and in 3 populations in 2011  
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41 223 (Table 1A, Appendix S4 in Supporting Information). Among-population variation in  
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43 224 selection on flowering phenology was strongly associated with the incidence of the  
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46 225 predator in plant populations (Table 2, Appendix S4). In populations where the predator  
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48 226 was absent, there was selection for earlier flowering (mean  $\pm$  95% CI of selection  
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50 227 gradients, 2010:  $0.22 \pm 0.15$ , 2011:  $0.30 \pm 0.17$ ), while in populations where the  
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52 228 predator was present there was selection for later flowering (mean  $\pm$  95% CI of  
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55 229 selection gradients, 2010:  $-0.19 \pm 0.15$ , 2011:  $-0.10 \pm 0.11$ , Fig. 1). The intensity of  
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57 230 selection varied also among populations with the butterfly (Appendix S4). Differences  
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231 in selection gradients for phenology among populations with the seed predator were not  
232 significantly related to predation intensity, in terms of the mean number of eggs per  
233 individual (2010:  $b = -0.01$ ,  $p = 0.808$ ; 2011:  $b = -0.03$ ,  $p = 0.080$ ).

234 We found evidence of directional selection also on flower number and shoot  
235 height in both study years, and again selection varied among populations (except for  
236 selection on shoot height in 2010, Table 1A). We found evidence of non-linear  
237 selection, in terms of quadratic effects of flower number and correlational effects of  
238 flower number  $\times$  phenology, and these effects varied among populations (Table 1B).

239 In both study years, early development of floral structures increased the  
240 probability of being attacked by the predator within all populations where the predator  
241 was present (Table 3). The probability of being attacked was higher in plants with  
242 higher number of flowers in 2011, while the effect of flower number differed among  
243 populations in 2010. Shoot height did not influence probability of attack in 2010, and in  
244 2011 the effect differed among populations. Also the number of eggs per plant was  
245 correlated with phenology and flower number within populations, but relationships  
246 varied among populations in both study years (Table 3, Appendix S5 in Supporting  
247 Information). Multigroup path-analyses revealed significant among-population  
248 differences in trait-fitness relationships (Appendix S6 in Supporting Information).  
249 Nevertheless, models fitted for each population and year consistently identified effects  
250 of phenology on fitness mediated by seed predator preference for early-flowering plants,  
251 effects being significant or marginally significant in 40% of the cases (considering  
252 models with probability and intensity of predator attack for two years; Appendices S7-9  
253 in Supporting Information). Direct effects of traits on fitness were less consistent,  
254 effects being significant or marginally significant in 14% of the cases.

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3 255 The probability of *P. alcon* presence increased significantly with increasing  
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5 256 abundance of *Myrmica* ants in the population (Fig. 2). Although there were populations  
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7 257 with high ant abundance where *P. alcon* was absent, ant abundance was rarely low in  
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9 258 populations where it was present. In populations where the butterfly was present,  
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11 259 neither the proportion of plants with *P. alcon* eggs (2010:  $b = -3.74$ ,  $p = 0.571$ ; 2011:  $b$   
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13 260  $= -5.31$ ,  $p = 0.246$ ) nor the mean number of eggs per plant (2010:  $b = -0.30$ ,  $p = 0.552$ ;  
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15 261 2011:  $b = -0.61$ ,  $p = 0.575$ ) were significantly related to ant abundance.  
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## 19 262 DISCUSSION

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22 263 In this study, we have shown that the butterfly pre-dispersal seed predator *P. alcon*  
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24 264 shifts the direction of phenotypic selection on flowering phenology in its host plant *G.*  
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26 265 *pneumonanthe*. In the absence of the seed predator, phenotypic selection favored earlier  
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28 266 flowering. Because *P. alcon* preferentially attacked earlier-flowering individuals within  
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30 267 populations, selection favored later flowering in populations where the predator was  
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32 268 present. Moreover, the incidence of the predator in host plant populations was higher in  
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34 269 populations with a high abundance of host ants, suggesting that community context in  
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36 270 terms of the second host of the seed predator influences selection on flowering  
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38 271 phenology in the host plant.  
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43 272 In populations where the seed predator was absent, phenotypic selection favored  
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45 273 early flowering in *G. pneumonanthe*. In late-flowering plant species like *G.*  
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47 274 *pneumonanthe*, earlier flowering might be beneficial at northern latitudes, where the  
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49 275 growing season is short, because it increases the time and resources available for seed  
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51 276 maturation. It is also possible that the availability of pollinators is higher or that the  
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53 277 competition with other plants is less intense earlier during the season. The pattern of  
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55 278 early-flowering plants having higher fitness in the absence of seed predators found in *G.*  
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3 279 *pneumonanthe* is consistent with the general trend suggested by Munguía-Rosas et al.  
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5 280 (2011b). It is important, however, to bear in mind that higher fitness in earlier-flowering  
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7 281 plants could result from early flowering directly increasing fitness, or from other plant  
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9 282 traits correlated with early flowering having a positive effect on fitness. Positive  
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11 283 correlations between early flowering and high fitness may also be the result of  
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13 284 environmental covariance, i.e., both early flowering and fitness are correlated with  
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15 285 favorable microsite conditions and high resource availability (Rausher, 1992; Ehrlén,  
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17 286 2015). In our study, we tried to alleviate problems by incorporating traits that we  
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19 287 considered likely to be correlated with flowering phenology, as well as traits likely to be  
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21 288 correlated with plant resource state, as covariates in our models.  
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26 289 Irrespective of the selective agents responsible for the observed selection for  
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28 290 earlier flowering in the absence of antagonists, our results clearly show that this  
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30 291 selection is reversed to selection for later flowering when antagonists are present. In our  
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32 292 study system, the direction of selection on phenology differed markedly between  
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34 293 *Gentiana* populations with vs. without the butterfly pre-dispersal seed predator. This  
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36 294 happened because *P. alcon* consistently preferred early-flowering plants for oviposition  
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38 295 within populations, thus increasing the relative fitness of late-flowering plants. This  
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40 296 means that the predator mediates shifts in selection from favoring early to favoring late  
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42 297 flowering. The relationship between selection on flowering time and butterfly presence  
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44 298 in this study was based on observational data, meaning that we did not prove causation  
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46 299 in a strict sense. However, the facts that we know from direct observations that butterfly  
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48 300 attack reduces fitness by larval feeding on seeds, and that butterflies preferentially  
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50 301 attack early-flowering individuals, strongly suggest that the observed relationship is  
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52 302 indeed a casual one. Previous studies with this system have examined butterfly  
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54 303 preferences and shown that females of *Phengaris* sp. prefer to oviposit on buds that are  
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not fully developed (corresponding to stages 1-3 in our classification), thereby increasing time available for brood feeding and development (Thomas & Elmes, 2001; Patricelli et al., 2011). Our study instead focused on the response of the predator to the timing of bud development of individuals, and showed that given the butterfly preference for a given developmental stage, plants starting bud development early in the season are more prone to be attacked by the butterfly than plants flowering late. This is most likely because the presence of suitable floral developmental stages overlaps more with the oviposition period of *P. alcon* in early-flowering plants than in late-flowering. Our findings with *G. pneumonanthe* agree with studies in other systems demonstrating predator-mediated selection for late flowering (Pilson, 2000; Kolb et al., 2007; Parachnowitsch & Caruso, 2008). However, selection for early flowering mediated by pre-dispersal seed predators has also been reported (Kolb et al., 2007). Although most plant populations where the seed predator was present experienced selection for later flowering in our study, the intensity of this selection varied. The fact that these differences in intensity of selection were not related to predation intensity within populations suggests that also other, unidentified, selective agents contributed to net selection on flowering phenology. In conclusion, our results with *G. pneumonanthe* demonstrate that the presence of antagonists in plant populations can shift the direction of selection, and that the distribution of antagonists among plant populations is strongly related to the observed patterns of among-population variation in the direction of selection.

Butterflies did not only prefer early-flowering individuals but also oviposited more often on taller shoots, which are more conspicuous and represent a visually attractive target (Nowicki et al. 2005). Taller shoots possibly also constitute safer oviposition sites for the female butterflies, allowing them to escape from predators

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329 dwelling in the vegetation, such as lizards or spiders (Van Dyck & Regniers, 2010).  
330 Moreover, eggs laid on taller shoots might experience higher temperatures and a more  
331 suitable microclimate for larval growth (Alonso, 1997). Still, the preference for taller  
332 shoots was only observed in some populations and years, suggesting that the positive  
333 effect of higher shoots depends on environmental context, e.g. in terms of the height of  
334 the surrounding vegetation.

335       Given that our results link among-population differences in the direction of  
336 selection on flowering phenology to the incidence of the butterfly seed predator, the  
337 next step in understanding how environmental variation influences selection is to  
338 identify the factors influencing butterfly distribution and abundance. In our study  
339 system, *P. alcon* was more probable to be present in host plant populations with a high  
340 abundance of *Myrmica* ants. This finding agrees with what is known about the biology  
341 of the butterfly, which is unable to complete its life cycle without ants. In order to be  
342 able to reach their final size, pupate, and eclose as adults, caterpillars need to be fed in  
343 the ant nest (Mouquet et al. 2005). Although we did not demonstrate a causal  
344 relationship between ant abundance and butterfly presence, our correlative results  
345 together with the dependence of the butterfly on ant presence suggest that the observed  
346 among-population variation in selection on plant flowering phenology mediated by the  
347 butterfly seed predator is influenced by the factors influencing the abundance of its  
348 second host. Several other studies showing that selection on plant traits by mutualists  
349 and antagonists is altered by interactions with other community members (Biere &  
350 Tack, 2013, Fedriani & Delibes, 2013, Arceo-Gómez & Ashman, 2014), also suggest  
351 that the community context has important effects on the outcome of plant-animal  
352 interactions.



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3 353 In this study we examined how seed predators influenced selection on flowering  
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5 354 time by estimating effects on one fitness component, fruit production. To assess the  
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7 355 likelihood of that observed differences in the direction of selection on flowering time  
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9 356 translate into population divergence, we need to know also how well our fitness  
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11 357 component correlates with lifetime fitness, how consistent differences in selection (i.e.,  
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13 358 persistence of butterfly populations) are over time, and to what extent there is genetic  
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15 359 variation in flowering time. While it is true that selective agents acting on other  
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17 360 components of fitness might influence net selection on flowering time, our study should  
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19 361 still provide a reliable picture of the part of selection on flowering time that is mediated  
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21 362 by pre-dispersal seed-predators. Regarding genetic variation, we know through common  
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23 363 garden experiments that there are significant, genetically based differences among  
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25 364 populations in flowering phenology (A. Valdés and J. Ehrlén, unpublished data).  
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27 365 However, we still lack data from a sufficient number of populations to confirm if these  
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29 366 differences are related to the presence of the predator in the population of origin.  
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35 367 In recent years, the ubiquitous variation in selection among populations and  
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37 368 years and its important implications for evolutionary trajectories of populations have  
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39 369 been increasingly acknowledged (e.g. Thompson, 2005; Siepielski et al. 2013). Yet, the  
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41 370 environmental factors causing this variation have been rarely identified (Siepielski et  
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43 371 al., 2013). In this study, we have shown how an antagonistic interactor mediates  
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45 372 selection on timing of reproduction in its host plant, and how the presence of this  
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47 373 interaction is related to among-population variation in the direction of selection. We  
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49 374 have also shown that the presence of the antagonist is associated with the abundance of  
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51 375 a second host, suggesting that the community context might contribute to among-  
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53 376 population variation in selection. These results illustrate that in order to link variation in  
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55 377 the environment to variation in natural selection, we need to both assess the effects of  
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species interactions on fitness of different phenotypes, and examine how the physical environment and the community context influence the incidence and abundance of the interacting species.

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TABLES

Table 1: Selection gradient analyses for three traits, flowering phenology, flower number, and shoot height, in *G. pneumonanthe* in 2010 (N = 2000 plants in 20 populations) and 2011 (N = 1598 plants in 16 populations). Results are from linear models with Type II sums of squares, including: A) only linear effects, B) linear, quadratic and interaction effects. All models included effects of population × trait interactions. Estimates from a model without interaction terms are given for significant main effects where the population × trait interaction is not significant. Fitness was estimated by the number of intact fruits. Traits were standardized and fitness relativized before analyses.

Source of variation	2010			2011	
	df	F	Estimate	df	F
A) Linear terms					
Phenology (early flowering)	1	0.02		1	0.50
Flower number	1	183.73***		1	50.81***
Shoot height	1	7.18 **	0.128	1	0.47
Population × Phenology	19	3.61***		15	1.98*
Population × Flower number	19	3.88***		15	2.90***
Population × Shoot height	19	1.19		15	2.35**
B) Non-linear terms					
Phenology <sup>2</sup>	1	3.51		1	0.32
Flower number <sup>2</sup>	1	0.95		1	0.02
Shoot height <sup>2</sup>	1	0.19		1	0.00
Population × Phenology <sup>2</sup>	19	0.64		15	1.31
Population × Flower number <sup>2</sup>	19	1.88*		15	2.82 ***
Population × Shoot height <sup>2</sup>	19	1.00		15	0.73
Phenology × Flower number	1	2.09		1	0.06
Phenology × Shoot height	1	0.45		1	2.16
Flower number × Shoot height	1	1.33		1	0.84
Population × Phenology × Flower number	19	2.39 ***		15	2.42**
Population × Phenology × Shoot height	19	0.63		15	0.76
Population × Flower number × Shoot height	19	1.06		15	2.14 **

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001

Table 2: Selection gradient analyses for three traits, flowering phenology, flower number, and shoot height, in *G. pneumonanthe* in 2010 (N = 2000 plants in 20 populations) and 2011 (N = 1598 plants in 16 populations). The presence of butterfly seed predators (Predation), coded as 0 in populations without the predator and as 1 in populations with the predator, was included in all models. Results are from linear hierarchical mixed models with Type II sums of squares (Wald  $\chi^2$  values are shown). Effects of population  $\times$  trait (random effects, not shown) and predation  $\times$  trait interactions were only included in the models if the population  $\times$  trait interaction was significant. Fitness was estimated by the number of intact fruits. Traits were standardized and fitness relativized before analyses.

Source of variation	2010		2011	
	df	$\chi^2$	df	$\chi^2$
Phenology (early flowering)	1	0.00	1	0.86
Flower number	1	53.85***	1	27.82***
Shoot height	1	7.82**	1	0.21
Predation	1	0.11	1	0.00
Predation $\times$ Phenology	1	14.72***	1	14.95***
Predation $\times$ Flower number	1	0.01	1	3.69
Predation $\times$ Shoot height	1	-	1	5.69*

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

Table 3: Effects of population, flowering phenology, flower number and shoot height on the probability (0 or 1) and intensity (number of eggs in all individuals) of attack by *P. alcon* in 10 populations of the plant *G. pneumonanthe* in 2010 (N = 1000 plants) and 11 populations in 2011 (N = 1099 plants) where the predator was present.  $\chi^2$ -values are shown for logistic regressions, and F values for linear regressions. Estimates from a model without interaction terms are given for significant main effects where the interaction with population is not significant

Source of variation	Response: probability of attack						Response: intensity of attack			
	2010			2011			2010		2011	
	df	$\chi^2$	Estimate	df	$\chi^2$	Estimate	df	F	df	F
Phenology (early flowering)	1	39.32***	0.420	1	39.00***	0.545	1	79.57***	1	46.87***
Flower number	1	27.69***		1	81.15***	0.794	1	75.43***	1	240.98***
Shoot height	1	1.93		1	0.04		1	0.01	1	0.03
Population	9	181.74***		10	138.77***		9	29.09***	10	46.00***
Population $\times$ Phenology	9	8.62		10	16.65		9	9.91***	10	5.55***
Population $\times$ Flower number	9	29.67***		10	14.11		9	8.77***	10	33.61***
Population $\times$ Shoot height	9	9.98		10	18.38*		9	0.52	10	0.72

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

## FIGURE LEGENDS

Figure 1: Differences in linear selection gradients for flowering phenology between populations where the predator was present (*P. alcon* = 1, N = 11) and absent (*P. alcon* = 0, N = 9) in 2010 and 2011. Filled circles represent means, and bars standard errors ( $P < 0.001$  in both years, one-way ANOVAs).

Figure 2: Relationship between abundance of *Myrmica* host ants (log mean number of observed individuals), and presence/absence of *P. alcon* in populations of *G. pneumonanthe* (fitted curve is a logistic regression, N = 20 populations,  $p = 0.045$ ).

Figure 1

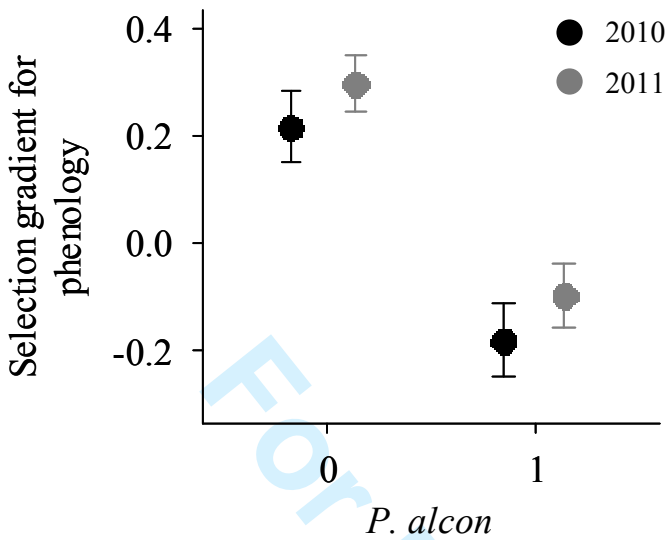


Figure 2

