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

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ABSTRACT

Spatial variation in selection might be driven by differences in the relative importance of selective agents acting in opposed directions. One potentially important source of variation is that the effects of a given selective agent depend on the community context. We investigated if differences in phenotypic selection on flowering phenology among 20 populations of *Gentiana pneumonanthe* were related to the presence of the butterfly seed predator *Phengaris alcon*, and if butterfly incidence was associated with the abundance of a second host, *Myrmica* ants. In plant populations without the butterfly, phenotypic selection favored earlier flowering. In populations where the butterfly was present, it preferentially attacked early-flowering individuals, shifting the direction of selection to favoring later flowering. Butterfly incidence increased with ant abundance. Our results demonstrate that antagonistic interactions can shift the direction of selection on flowering phenology, and suggest the community context as a potential driver of such shifts in selection.

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 preferences (Rey et al*.*, 2006; Kolb et al*.*, 2007). Interaction intensities and animal preferences for plant traits might, in turn, depend on the environmental context in terms of the abiotic conditions (e.g. light availability, Arvanitis et al., 2007; Kolb & Ehrlén, 2010, soil moisture, von Euler et al*.*, 2014) or in terms of other species (community context). The community context, in terms of natural enemies, competitors or alternative hosts, havs been shown to influence the outcome of plant-animal interactions (Strauss & Irwin, 2004; Siepielski & Benkman, 2007; Chamberlain et al*.*, 2014). For example, nectar robbers can affect selection by pollinators (Irwin, 2006), and plant community heterogeneity may determine host plant selection by insect herbivores (Agrawal et al*.*, 2006). In spite of an increasing awareness of the ubiquitous spatial and temporal variation in selection, the role of community context as a source of variation in species interactions, and potentially in selection on a focal organism has been little explored. Unravelling the ways in which community context is related to species interactions is a key first step to link analyses of selection to community structure and species networks.

One way in which community context may be related to a focal interaction which can potentially mediate selection is when the outcome of a plant-consumer interaction depends on the abundance of a second resource for the consumer. For example, large Blue butterflies of the genus *Phengaris* Doherty, 1891 (the senior synonym of *Maculinea* van Eecke, 1915) are specialist predispersal seed predators during their first larval instars, and often show strong preferences for particular plant developmental stages (Thomas & Elmes, 2001; Van Dyck & Regniers, 2010). This means that plant individuals for which the developmental stage preferred for oviposition coincides with the peak of butterfly activity suffer most from seed predator attacks. *Phengaris* larvae need also a second host to complete their development, and most species are parasites of ant nests (*Myrmica* spp.) during later instars (Als et al*.*, 2004). Butterfly-mediated selection on plant traits in this system might thus be influenced by the community context, if the incidence and intensity of predation are related to host ant abundance. In this study, we examined if among-population variation in phenotypic selection on flowering phenology in the perennial herb *Gentiana pneumonanthe* is the result of differences in the intensity of interactions with its specialist predispersal seed predator, the butterfly *Phengaris alcon*, and if variation in this interaction is related to the community context in terms of the abundance 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 and to predation intensity 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). Plants can have one to many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by bumblebees. It does not flower before its third year in natural populations (Simmonds, 1946). 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. 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 for details). The populations are mainly located in moist heathlands. *P. alcon* was present in 11 of the study populations (where different interaction intensities were observed, Appendix S1) and absent from 9.

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. 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 measured shoot height (in cm), phenology, and number of flowers. These traits were measured once per plant in each of the two years, at the end of July-beginning of August. Phenology was estimated as the reproductive development stage of individuals at the given measuring date (i.e. one single measure per study year). For this, we counted the number of buds and flowers that were in each of six developmental stages at the measuring date: (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 showing signs of wilting. From this data, we calculated two different measures of the reproductive development stage of each individual, which were used as estimates of phenology: (1) the mean development stage of all flowers and buds (continuous variable ranging from 1 to 6), and (2) the stage of the most advanced bud (ordinal variable ranging from 1 to 6). In both cases, higher values indicate an earlier phenology, i.e. plants flowering earlier in the season. The average duration of stages 2-5 was about one week (A. Valdés, *pers. obs.*). A one-unit increase in these measures thus roughly corresponds to one week earlier development.

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

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

Ant abundance was estimated along transects in each of the 20 study populations, once per study year. 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 the variation associated with 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 examining the effects of quadratic and interaction terms in a model also including the linear terms. Results for models using mean and most advanced flower developmental stages within shoots as estimates of phenology were very similar in all cases. Below, we present only results for the stage of the most advanced bud, hereafter referred to as “phenology”. Results for selection gradient analyses using the mean flower developmental stage are shown in Appendix S2 for comparison.

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

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

We also used path analyses to examine the relative importance of direct and indirect effects of reproductive traits on fitness (Grace, 2006), the latter mediated by the probability of predator attack or by interaction intensity (two different saturated models were considered, see Appendix S5 for details). To test for differences among populations, we used multigroup analysis (Grace, 2006). Because populations differed significantly, we fitted separate models for each population and year. We excluded population D in 2010 also from these analyses.

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

Path analyses were carried out in Amos 16.0 (Arbukle, 2007). All other analyses were conducted in R 3.1.2 (R Development Core Team, 2014).

RESULTS

We found evidence of directional phenotypic selection on flowering phenology in both study years, and selection varied among populations (Table 1A). Statistically significant selection on phenology was found in 5 out of 20 populations in 2010, and in 3 out of 16 populations in 2011 (Appendix S3). This among-population variation in selection on flowering phenology was strongly associated with the incidence of the predator in plant populations (Table 2, Appendix S3). In populations where the predator was absent, there was selection for earlier flowering (mean ± 95% CI of selection gradients, 2010: 0.22 ± 0.15, 2011: 0.30 ± 0.17), while in populations where the predator was present there was selection for later flowering (mean ± 95% CI of selection gradients, 2010: - 0.19 ± 0.15, 2011: - 0.10 ± 0.11, Fig. 1). However, some variation in phenotypic selection intensity still remained among populations with the predator and among populations without the predator (Appendix S3). In populations with the predator, this among-population variation in selection on flowering phenology was not related to interaction intensity, as relationships between selection gradients for phenology and the mean number of eggs per population were not significant in any of the two study years (2010:  = -0.01, R2 = 0.01, p = 0.808; 2011:  = -0.03, R2 = 0.30, p = 0.080).

We also found evidence of directional selection on flower number and shoot height in both study years, and again selection varied among populations (except for selection on shoot height in 2010, Table 1A). We also found some evidence of non-linear selection (Table 1B). There was a quadratic effect of flower number on fitness which varied among populations in both study years, and some interaction effects were significant, although they also varied among populations.

In both study years, early development of floral structures increased the probability of being attacked by the predator within all populations where the predator was present (Table 3), although the strength of this relationship was different among populations (Appendix S2). The probability of being attacked was higher also in plants with higher number of flowers in 2011, but the effect of flower number differed among populations in 2010. Shoot height did not influence probability of attack in 2010, and in 2011 the effect differed among populations. Also the number of eggs per plant was correlated with phenology and flower number within populations, but relationships varied among populations in both study years (Table 3, Appendix S4). Multigroup path-analyses revealed significant among-population differences in trait-fitness relationships (Appendix S5, Tables S5.1-2). Nevertheless, models fitted for each population and year consistently identified effects of phenology on fitness mediated by seed predator preference for early-flowering plants. Considering together models with the probability of predator attack and with interaction intensity, the direct effect of phenology on fitness was significant or marginally significant in 30% of the models in 2010 and in 0% in 2011, while the indirect effect mediated by the seed predator was significant or marginally significant in 35% of the models in 2010 and in 50% in 2011 (for detailed results, see Appendix S5, Tables S5.3-5).

The probability of *P. alcon* presence increased significantly with increasing abundance of *Myrmica* ants in the population (Fig. 2). Although there were populations with high ant abundance where *P. alcon* was absent, ant abundance was rarely low in populations where it was present. In populations where the butterfly was present and in both study years, neither the proportion of plants with *P. alcon* eggs nor the mean number of eggs per plant was significantly related to ant abundance in linear regressions (results not shown).

DISCUSSION

In this study, we have shown that the butterfly pre-dispersal seed predator *P. alcon* shifts the direction of phenotypic selection on flowering phenology in its host plant *G. pneumonanthe*. In the absence of the seed predator, phenotypic selection favored earlier flowering. Because *P. alcon* preferentially attacked earlier-flowering individuals within populations, selection favored later flowering in populations where the predator was present. Butterfly-mediated selection on host plant flowering phenology might, in turn, be dependent on the community context, as the incidence of the predator in host plant populations increased with the abundance of the host ants.

In populations where the seed predator was absent, phenotypic selection favored early flowering in *G. pneumonanthe*. In late-flowering plant species like *G. pneumonanthe*, earlier flowering might be beneficial at northern latitudes, where the growing season is short, because it increases the time and resources available for seed maturation. It is also possible that the availability of pollinators is higher or that the competition with other plants is less intense earlier during the season. The pattern of early-flowering plants having higher fitness in the absence of seed predators found in *G. pneumonanthe* is consistent with the general trend suggested by Munguía-Rosas et al*.* (2011b). It is important, however, to bear in mind that higher fitness in earlier-flowering plants could result from early flowering directly increasing fitness, or from other plant traits correlated with early flowering having a positive effect on fitness. Positive correlations between early flowering and high fitness may also be the result of environmental covariance, i.e., both early flowering and fitness are correlated with favorable microsite conditions and high resource availability (Rausher, 1992; Ehrlén, 2015). In our study, we tried to alleviate problems by incorporating traits that we considered likely to be correlated with flowering phenology, as well as traits likely to be correlated with plant resource state, as covariates in our models.

Irrespective of the selective agents responsible for the observed selection for earlier flowering in the absence of antagonists, our results clearly show that this selection is reversed to selection for later flowering when antagonists are present. In our study system, the direction of selection on phenology differed markedly between *Gentiana* populations with vs. without the butterfly pre-dispersal seed predator. This happened because, although there are differences between populations regarding the strength of predator preferences (Appendix S4), *P. alcon* consistently preferred early-flowering plants for oviposition within populations, thus increasing the relative fitness of late-flowering plants. This means that the predator is very likely to mediate shifts in selection from favoring early to favoring late flowering. Previous studies with this system have examined butterfly preferences and shown that females of *Phengaris* sp. prefer to oviposit on buds that are 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. The observed among-population variation in the strength of predator preference for early-flowering plants (Appendix S4), and the subsequent variation in phenotypic selection strength (Appendix S3) might be due to different degrees of overlap between the presence of the preferred developmental stages for oviposition and the oviposition period or butterflies, or to differences in butterfly abundances among populations.

We have assessed phenotypic selection using the number of intact fruits as a measure of fitness, but we have to acknowledge that one of the limitations of our study is that fruit (and seed) production are only components of fitness, and that a more integrative measure of fitness would be preferable to evaluate net selection on flowering phenology. For example, population growth rate calculated using a demographic approach would be a better surrogate of lifetime fitness (Ehrlén, 2015). Unfortunately, we are lacking demographic data to develop this approach, but we believe that assessing phenotypic selection based on fruit production is still very interesting in the case of *G. pneumonanthe*, given the high impact of seed predators in this fitness component.

In any case, 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), although selection for early flowering mediated by pre-dispersal seed predators has also been reported (Kolb et al*.*, 2007). In conclusion, our results with *G. pneumonanthe* demonstrate that the presence of antagonists in plant populations is very likely to 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. Providing that this variation is maintained in time, and that there is sufficient genetic variance in flowering phenology, this variation in phenotypic selection could drive evolutionary changes through local adaptation (Thompson, 2005), resulting in genetic differences in flowering phenology among populations.

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 dwelling in the vegetation, such as lizards or spiders (Van Dyck & Regniers, 2010). Moreover, eggs laid on taller shoots might experience higher temperatures and a more suitable microclimate for larval growth (Alonso, 1997). Still, the preference for taller shoots was only observed in some populations and years (Fig. S4.3), suggesting that the positive effect of higher shoots depends on environmental context, e.g. in terms of the height of the surrounding vegetation.

Given that our results link among-population differences in the direction of selection on flowering phenology to the incidence of the butterfly seed predator, the next step in linking variation in selection to environmental context is to identify the factors influencing butterfly distribution and abundance. Our results suggest that the community context might be one of these factors, as predator occurrence is related to the abundance of its second host. *Phengaris alcon* was more probable to be present in host plant populations with a high abundance of *Myrmica* ants. This finding agrees with what is known about the biology of the butterfly, which is unable to complete its life cycle without ants. In order to be able to reach their final size, pupate, and eclose as adults, caterpillars need to be fed in the ant nest (Mouquet et al. 2005). If the presence of *Myrmica* is a prerequisite for *P. alcon* to be present in a population, this would suggest that the observed among-population variation in selection on plant flowering phenology mediated by the butterfly seed predator could depend to some extent on the factors influencing the abundance of its second host. This hypothesis cannot be confirmed with our current data, but it is in line with other studies showing that selection on plant traits by mutualists and antagonists is altered by interactions with other community members (Biere & Tack, 2013, Fedriani & Delibes, 2013, Arceo-Gómez & Ashman, 2014), and suggesting that the community context has the potential to affect the outcome of plant-animal interactions.

In recent years, the ubiquitous variation in selection among populations and years and its important implications for evolutionary trajectories of populations have been increasingly acknowledged (e.g. Thompson, 2005; Siepielski et al. 2013). Yet, the environmental factors causing this variation have been rarely identified (Siepielski et al., 2013). In this study, we have shown how an antagonistic interactor mediates selection on timing of reproduction in its host plant, and how the presence of this interaction is related to among-population variation in the direction of selection. We have also shown that the presence of the antagonist is associated with the abundance of another community member, suggesting the community context as a potential driver of among-population variation in selection. These results illustrate that in order to link variation in the environment to variation in natural selection, we need to assess the effects of species interactions on fitness of different phenotypes, and to 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, of the plant *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 | Estim |  | 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 2 | 1 | 3.51 |  |  | 1 | 0.32 |
|  | Flower number 2 | 1 | 0.95 |  |  | 1 | 0.02 |
|  | Shoot height 2 | 1 | 0.19 |  |  | 1 | 0.00 |
|  | Population × Phenology 2 | 19 | 0.64 |  |  | 15 | 1.31 |
|  | Population × Flower number 2 | 19 | 1.88\* |  |  | 15 | 2.82 \*\*\* |
|  | Population × Shoot height 2 | 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, of *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 2 values are shown). Effects of population × trait (random effects, not shown) and predation × trait interactions were only included in the models if the population × 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 | 2 |  | df | 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 × Phenology | 1 | 14.72\*\*\* |  | 1 | 14.95\*\*\* |
| Predation × Flower number | 1 | 0.01 |  | 1 | 3.69 |
| Predation × 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 of *G. pneumonanthe* on the probability (0 or 1) and intensity (number of eggs in all individuals) of attack by *P. alcon* in 10 plant populations in 2010 (N = 1000 plants) and 11 populations in 2011 (N = 1099 plants) where the predator was present. 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 | 2 | Estim |  | df | 2 | Estim |  | 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 × Phenology | 9 | 8.62 |  |  | 10 | 16.65 |  |  | 9 | 9.91\*\*\* |  | 10 | 5.55\*\*\* |
| Population × Flower number | 9 | 29.67\*\*\* |  |  | 10 | 14.11 |  |  | 9 | 8.77\*\*\* |  | 10 | 33.61\*\*\* |
| Population × 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 (black) and 2011 (grey). Filed 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

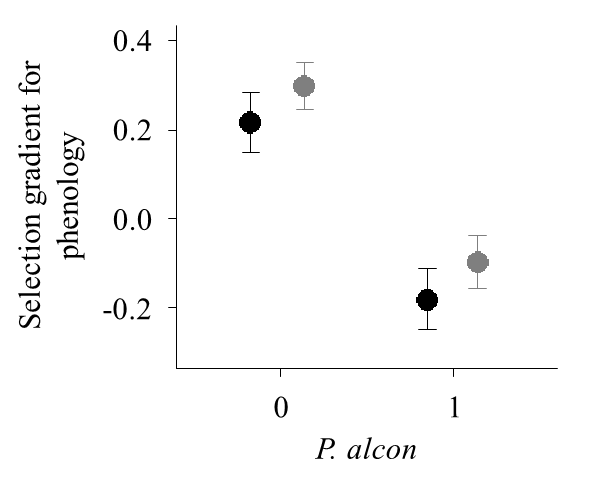


Figure 2

