TITLE:

Seed predator-mediated shifts in selection on flowering phenology and depend on a second host

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ABSTRACT

Plants can experience opposed selection on timing of reproduction within a flowering season mediated by abiotic factors and species interactions, the direction of net selection depending on the relative importance of different selective agents. Selection mediated by species interactions, in turn, can be influenced by both abiotic factors and the community context where the interaction occurs. We investigated if differences in phenotypic selection on flowering phenology among 20 populations of the perennial herb *Gentiana pneumonanthe* during two years were mediated by the butterfly seed predator *Maculinea alcon*, and if the intensity of the butterfly-plant interaction was associated with the abundance of *Myrmica* ants, which act as a second host for the butterfly. In *G. pneumonanthe* populations where the predator was absent, phenotypic selection favored earlier flowering in both years. In plant populations where the predator was present, it attacked preferentially early-flowering individuals, and shifted the direction of selection to favoring later flowering. Incidence of butterflies in plant populations, and thus predator-mediated shifts in selection on host plant phenology, were associated with the community context, in terms of the abundance of ants. Our results demonstrate that antagonistic interactions are able to shift the direction of selection on flowering phenology, and that the community context may influence the intensity of interactions, and of phenotypic selection, among populations.

INTRODUCTION

Timing of reproduction within a flowering 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), both mutualistic (e.g. Munguía-Rosas et al., 2011a, Chapurlat et al., in press) and antagonistic (e.g. Ehrlén & Münzbergová, 2009, König et al., 2015). Mutualistic pollinators might select for both earlier (e.g. Munguía-Rosas et al., 2011a), 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) and earlier flowering (e.g. Fukano et al., 2013). Plant species can thus simultaneously experience selection for earlier and later flowering mediated by different agents. In these cases, net selection depends on the relative strengths of these interactions (e.g. Ehrlén and Münzbergová 2009, Sletvold et al. 2015). 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 costly if early flowering increases the exposure to antagonists.

Spatial variation in selection mediated by species interactions has been documented in many systems (Thompson 2005, Siepielski et al. 2013), and may result 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 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 or in terms of other species (community context). For example, the intensity of plant-seed predator interactions has been shown to be influenced by light availability (e.g. Arvanitis et al., 2001; Kolb & Ehrlén, 2010) and soil moisture (von Euler et al*.*, 2014). Other community members, being natural enemies, competitors or alternative hosts, have also 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 in terms of productivity, species and genetic diversity may determine host plant selection by insect herbivores (Agrawal et al*.*, 2006). In spite of the increasing awareness of the ubiquitous spatial and temporal variation in selection, the role of community context as a source of variation in selection on a focal organism has been little explored. Analyses of selection mediated by species interactions have mostly focused on pairwise interactions, and analyses of more complex multispecies interactions rarely have assessed effects on selection (Strauss and Irwin, 2004). Unravelling the ways in which community context influences species interactions and the resulting selection is therefore a key step to link analyses of selection to community structure and species networks.

One interesting way in which community context may influence selection mediated by a focal interaction 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 (*Maculinea* spp.) are specialist predispersal seed predators during their first larval instars, and butterflies often show strong preferences for plant developmental stages (Thomas & Elmes, 2001; Van Dyck & Regniers, 2010; Czekes et al*.*, 2014). 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. *Maculinea* 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, in terms of effects of ant host abundance on butterfly abundance and seed predation intensity. 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 *Maculinea alcon*, and if variation in this interaction is associated with the community context in terms of the abundance of the second host, *Myrmica* ants. Based on the observation of that developmental stages suitable for oviposition in early-flowering plants usually coincide with the oviposition period of the butterfly (A. Valdés and J. Ehrlén, *pers. obs.*), we arrived at two main hypotheses: (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, influences the probability of butterfly presence within plant populations.

MATERIALS AND METHODS

**Study system**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, 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. 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 (*Maculinea 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 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 *Maculinea* species, which prey on ant brood, *M. 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. In our study area, *M. ruginodis* is thought to be the most commonly used ant host 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. *M. alcon* was present in 11 of the study populations 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. For recordings, we selected one shoot of median length in each individual. In these focal shoots, we measured shoot height (in cm), reproductive development stage, and number of flowers from the end of July to the beginning of August. To assess the reproductive development of individuals, we counted the number of buds and flowers in each of six 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 showing signs of wilting. From this data, we calculated two different measures of the reproductive development stage of each individual: (1) the mean development stage of all flowers and buds, and (2) the stage of the most advanced bud. In both cases, higher values indicate earlier flowering. The average duration of stages 2-5 was about one week (A. Valdés, *pers. obs.*); a one-unit increase in these measures thus roughly corresponding to one week earlier development.

Interaction intensity was estimated by the maximum number of *M. 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 number of intact (i.e. not damaged by the butterfly) mature fruits on the focal shoot. 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).

Ant abundance was estimated 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 with 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 errors 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 standardized reproductive traits and population in 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 selection, we also tested for correlational selection by examining the effects of interaction terms in a model also including the linear terms, and for non-linear selection by examining the effect of quadratic terms in a model also including the linear terms. Results for models using mean and most advanced flower developmental stages within shoots were very similar in all cases. Below, we present only results for the stage of the most advanced bud, hereafter referred to as “phenology”.

To test if linear selection on reproductive traits differed among populations with vs. without *M. alcon*, we created a variable “Predation”, coded as 0 in populations without *M. alcon* and as 1 in populations with *M. 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 *M. 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, mediated by seed predation, effects of reproductive traits on fitness (Grace, 2006). 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 ant host of *M. 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 *M. 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 Core Team, 2014).

RESULTS

We found evidence of directional phenotypic selection on flowering phenology in both study years, but selection differed among populations (Table 1A). This among-population variation in selection on flowering phenology was strongly associated with the incidence of the predator in plant populations (Table 2, Appendix S2). 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).

We also found evidence of directional selection on flower number and shoot height in both study years, but again selection differed among populations (except for selection on shoot height in 2010, Table 1A). We also found evidence of correlational selection (Table 1B), and a significant non-linear effect of flower number on fitness in both study years, these effects also differing among populations (Table 1C).

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, Appendix S3). 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 S3). Multigroup path-analyses revealed significant among-population differences in trait-fitness relationships (Appendix S4, Tables S4.1-2). Nevertheless, models fitted for each population and year consistently identified effects of phenology on fitness mediated by seed predator preference for early-flowering plants (Appendix S4, Tables S4.3-5). Direct effects of traits on fitness were less consistent.

The probability of *M. alcon* presence increased significantly with increasing abundance of *Myrmica* ants in the population (Fig. 2A). Although there were populations with high ant abundance where *M. alcon* was absent, ant abundance was rarely low in populations where it was present. In populations where the butterfly was present, neither the proportion of plants with *M. alcon* eggs nor the mean number of eggs per plant was related to ant abundance (Fig. 2B and C).

DISCUSSION

In this study, we have shown that the butterfly pre-dispersal seed predator *M. 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 *M. 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, in turn, did depend on community context, i.e., 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 (Munguía-Rosas et al*.*, 2011b), and the competition with other plants 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). Such patterns could be the result of that early flowering directly increases fitness, or of that other plant traits that are correlated with early flowering have a positive effect. 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.

Our results also clearly show that selection for earlier flowering in the absence of antagonists 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 was the result of that *M. alcon* consistently preferred early-flowering plants for oviposition within populations, thus increasing the relative fitness of late-flowering plants. This strongly suggests that the predator mediates shifts in selection from favoring early to favoring late flowering. Previous studies with this system have shown that females of *Maculinea* sp. prefer to oviposit on buds that are not fully developed, 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 the result of that the presence of suitable floral developmental stages overlap more with the oviposition period of *M. alcon* in early-flowering plants than in late-flowering. Our findings with *G. pneumonanthe* also 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 can shift the direction of selection, and that the distribution of antagonists among plant populations can explain 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 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 show that the presence of a butterfly seed predator mediates a shift in the direction of selection on flowering phenology, the next step in linking variation in selection to environmental variation is to identify the factors influencing butterfly distribution. Our results suggested that predator occurrence did depend on the abundance of its second host. *Maculinea 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. The butterfly is unable to complete its life cycle without ants. Caterpillars need to be fed in the ant nest, to be able to reach their final size, pupate, and eclose as adults (Mouquet et al. 2005). thus among-populationThis is turn would be an example of that the community context influence the likelihood or intensity of plant-animal interactions (Strauss & Irwin, 2004), and 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).

In recent years, the fact that selection varies ubiquitously among populations and among years and that this has very important implications for evolutionary trajectories of populations has 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 interaction mediates selection on timing of reproduction in a plant, and how this the presence of this interaction can explain among-population variation in direction of selection. We have also shown that the presence of the antagonist is associated with differences in the community context, suggesting that community context is an important driver of among-population variation in interaction intensity and selection. These results illustrate that in order to link variation in the environment to variation in natural selection, we not only need to assess the effects of species interactions on fitness of different phenotypes, but also 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 showing the effects of three traits, flowering phenology, flower number, and shoot height, and population on fitness (number of intact fruits) of *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 effects and interactions and C) linear and quadratic effects. Estimates are given for significant main effects where the Population × trait interaction is not significant. 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) Interaction terms | |  |  |  |  |  |  |
|  | Phenology × Flower number | 1 | 1.78 |  |  | 1 | 0.08 |
|  | Phenology × Shoot height | 1 | 3.21 |  |  | 1 | 2.16 |
|  | Flower number × Shoot height | 1 | 9.59\*\* |  |  | 1 | 4.57\* |
|  | Population × Phenology × Flower number | 19 | 2.66\*\*\* |  |  | 15 | 1.61 |
|  | Population × Phenology × Shoot height | 19 | 1.02 |  |  | 15 | 1.31 |
|  | Population × Flower number × Shoot height | 19 | 1.82\* |  |  | 15 | 2.37\*\* |
|  |  |  |  |  |  |  |  |
| C) Quadratic terms | |  |  |  |  |  |  |
|  | Phenology 2 | 1 | 2.47 |  |  | 1 | 0.13 |
|  | Flower number 2 | 1 | 4.38\* |  |  | 1 | 2.02 |
|  | Shoot height 2 | 1 | 0.03 |  |  | 1 | 1.50 |
|  | Population × Phenology 2 | 19 | 1.40 |  |  | 15 | 0.94 |
|  | Population × Flower number 2 | 19 | 2.40\*\*\* |  |  | 15 | 3.38\*\*\* |
|  | Population × Shoot height 2 | 19 | 1.22 |  |  | 15 | 1.80\* |

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

Table 2: Effects of three standardized traits, flowering phenology, flower number, and shoot height, predation and their interactions on relative fitness (number of intact fruits) of *G. pneumonanthe* in 2010 (N = 2000 plants in N =20 populations) and 2011 (N = 1598 plants in N = 16 populations). Results are from linear hierarchical mixed models with Type II sums of squares (Wald 2 values are shown). Effects of trait × population (random effects, not shown) and trait × predation interactions were only included in the models if the trait × population interaction was significant.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 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, phenology, flower number and shoot height of *G. pneumonanthe* on probability (0 or 1) and intensity (number of eggs) of attack by *M. alcon* in 10 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 phenology between populations where the predator was present (*M. alcon* =1, N = 11) and absent (*M. alcon* = 0, N = 9) in 2010 and 2011. Filed circles represent means, and bars standard errors (P < 0.001 in both years, one-way ANOVAs).

Figure 2: Relationships between of abundance of *Myrmica* ant-hosts (log mean number of observed individuals), and (A) Presence/absence of *M. alcon* in populations of *G. pneumonanthe* (fitted curve is a logistic regression, N = 20 populations), (B) Mean number of eggs per plant in populations where *M. alcon* was present (N = 11 populations in two years), and (C) The proportion of plants that had at least one *M. alcon* egg in populations where *M. alcon* was present (N = 11 populations in two years). Black circles in B and C are estimates from 2010 and grey are estimates from 2011.

Figure 1

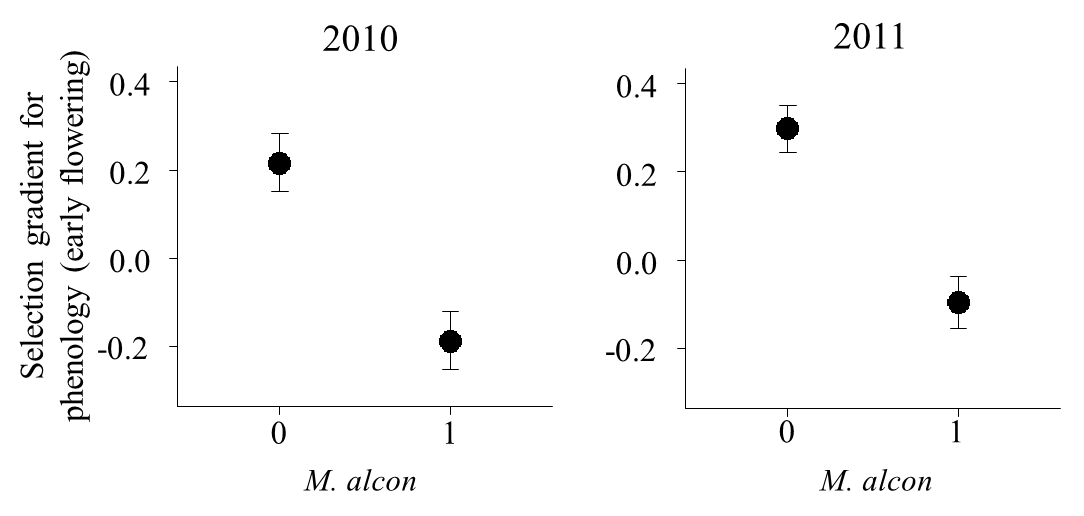


Figure 2

