TITLE (provisional): Selection on flowering time in *Gentiana pneumonanthe* is mediated by seed predators and dependent on the community context

Selection on flowering phenology/time mediated by seed predators and dependent on the community context

Butterflies and ants as selective agents for flowering phenology/time

Butterfly seed predators and ants as selective agents for flowering phenology/time

Butterfly seed predators and their second hosts as selective agents for flowering phenology/time

Butterfly seed predators and their second hosts: selective agents for flowering phenology/time

Selection on flowering phenology/time: the role of (butterfly) seed predators and their second hosts

Selection on flowering phenology/time: mediated/determined by (butterfly) seed predators and their second hosts

Butterfly seed predators mediate selection on flowering phenology/time with help from ants

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INTRODUCTION

Timing of reproduction in plants affects their fitness by influencing interactions both with the physical environment and with other organisms. Both pollinators (mutualists) and predispersal seed predators (antagonists) can exert selection on flowering phenology (Elzinga *et al.*, 2007) and this leads to different and sometimes opposing selective forces (Brody, 1997; Strauss & Irwin, 2004). Phenotypic selection on floral traits by pollinators is positively related to the degree of pollen limitation (Totland, 2001), and pollinator preference for certain floral traits does not always translate into higher fitness. However, preference of a phenotype by seed predators most likely decreases the fitness of that particular phenotype (Parachnowitsch & Caruso, 2008). Nowadays, there is weak empirical support for pollinator-mediated selection on flowering time (Ehrlén, 2015), but growing evidence for predispersal seed predators affecting selection on plant traits (Kolb *et al.*, 2007). The latter have been shown to exert phenotypic selection on flowering time (Ehrlén & Münzbergová, 2009) and in other reproductive traits as flower number (Leimu *et al.*, 2002) or inflorescence height (Kolb & Ehrlén, 2010). Plant phenology may be correlated with other traits also subject to natural selection (Lande & Arnold, 1983). Earlier flowering plants usually have more available resources and thus are able to grow higher and produce more flowers than later-flowering plants (Ison & Wagenius, 2014), causing a fitness decrease over the season due to an indirect effect of phenology. Selection on these correlated traits might act indirectly on flowering phenology (Kingsolver & Diamond, 2011)

This can lead to indirect selection on plant phenology via selection on other traits.

Spatiotemporal variation in species interactions and in selection intensity on a given trait may result in selection mosaics (Thompson, 2005), and lead to different coevolutionary trajectories of plants and their animal interactors. The relationship between plant reproductive traits and predispersal seed predation may vary among populations and years (Kolb *et al.*, 2007), and can contribute to the existence of geographic mosaics of coevolution between plants and their seed predators (Benkman, 1999; Rey *et al.*, 2006). Seed predators might thus vary in their importance as selection agents, due to variations in their abundance, in interaction intensity (predation rates), and in the direction and strength of predator preferences regarding reproductive traits.

Variation in phenotypic selection by seed predators might also be related to the context: the environment where the interaction occurs has been acknowledged to affect coevolutionary dynamics (Kolb & Ehrlén, 2010; König *et al.*, 2014, 2015; von Euler *et al.*, 2014), but the relations with other community members (i.e. the community context, Brandt & Foitzik, 2004; Siepielski & Benkman, 2007) might also influence interactions and thus modify selection. In some cases, the outcome of an interaction between a pair of species (e.g. a plant and its seed predator) may be dependent on the interaction with a third species present in the community (Strauss & Irwin, 2004). Large Blue butterflies (*Maculinea* spp.) are predispersal predators of specific host plants during their first larval instars, but they need a second host to complete their development, and they are parasites of ant nests (*Myrmica* spp.) during later instars (Als *et al.*, 2004). Thus, in this case the interaction between the butterfly and the ants is needed to maintain the interaction between the butterfly and its host plant. Oviposition patterns of *Maculinea* butterflies, However, the community context in terms of the abundance of the second host might also indirectly affect phenotypic selection on host plant traits by modifying interaction intensities, as the distribution and abundance of the butterfly could be related to ant abundance.

In this study, we assess how phenotypic selection on flowering time in the perennial herb *Gentiana pneumonanthe* is mediated by the interaction with its specialist predispersal seed predator, the butterfly *Maculinea alcon*, and how this interaction depends on the community context where it occurs. Specifically, we seek to answer two main questions:

1) Is there phenotypic selection on flowering phenology in *G. pneumonanthe* mediated by the interaction with its seed predator (*M. alcon*)?

2) Are *M. alcon* presence and seed predation intensity related to the community context in terms of abundance of their second host (*Myrmica* ants)?

MATERIALS AND METHODS

**Study system and study area**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb that may reach 45 cm in height, overwintering as a rosette of short shoots with small leaves (Simmonds, 1946). Plants can have one to many shoots and produce deep blue flowers pollinated by bumblebees (although the species is entirely self-compatible). It flowers in July and August in SW Sweden. Fruits are capsules containing a high number of minute, wind-dispersed seeds. The species inhabits open habitats as wet heathlands and grasslands. Although it appears in some other places in Europe, it is red-listed as vulnerable in Sweden. *G. pneumonanthe* is the primary host of the Alcon Blue butterfly (*Maculinea alcon*), a specialist predispersal seed predator which lays its eggs on young gentian buds during its flight period (July and August, Appelqvist *et al.*, 2007). The caterpillars consume a large fraction of developing seeds in some populations. Fourth-instar caterpillars drop to the ground and are then picked up by their second host: *Myrmica* ants (probably *M. ruginodis* in our study area, Appelqvist *et al.*, 2007). 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), whose larvae are fed primarily on regurgitations from ant workers, trophic eggs and prey items.

This 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 and often surrounded by forests, roads or urbanized areas. *M. alcon* was present in 11 of the study populations and absent from 9.

**Data collection**

We collected data on reproductive traits, plant fitness and interaction intensity in 100 marked individuals in each of the study populations during 2010 and 2011. For each individual, we selected one focal shoot as the one having median length. All measures were carried out on this focal shoot. We measured shoot height, phenological state, number of flowers and number of *M. alcon* eggs in late July – early August. Phenology of each individual, in terms of the developmental stages of flowers, was estimated by counting the number of buds and flowers in each of six developmental stages: a) small bud (the sepals covering the bud completely), b) medium-sized bud (from the time the bud begins to protrude outside the sepals until it grows over the sepals) c) large bud (the bud becomes elongated and rather high, *M. alcon* eggs usually start appearing) d) not fully blooming flower (from the time the bud starts changing into blue until it becomes a solid blue, tightly twisted bud) e) blooming flower (from the time the bud starts to open, when the petals start to spin up, to a fully opened flower) and f) wilted flower (merged and started to wither). We then calculated two different measures of phenology. A phenological index (PI) was calculated for each individual as PI = (1a + 2b + 3c + 4d + 5e + 6f) / nfl, with nfl = total number of flowers and buds. Besides, the state of the most advanced bud was recorded based on the abovementioned categories, assigning numbers 1-6 respectively to categories a-f.

Populations were visited up to when all fruits had matured (until mid-October), and the maximum number of eggs and the final number of intact fruits (i.e. not damaged by the butterfly) were assessed, to be used respectively as an estimate of interaction intensity and as an estimate of plant fitness. In 2011, so

Ant abundance was measured in each of the 20 study populations using ant counts on sugar cubes. In 2010, a 20-m transect was established through each population, and a sugar cube was placed every meter (i.e. 20 sugar cubes per population). In 2010, two 20-m parallel transects or one 40-m transect were established, placing thus 40 sugar cubes per population. The number of *Myrmica* sp. ants in each of the cubes was counted after 30 minutes. We then calculated the average number of ants per sugar cube in each population and year, and used the highest of both yearly values as a measure of ant abundance for each population.

**Statistical analyses**

Analyses were performed separately for 2010 and 2011.

To evaluate if there was phenotypic selection on flowering phenology, and whether this selection varied among populations, we performed selection gradient analyses (Lande & Arnold, 1983) by regressing relative fitness on standardized phenological values, together with other reproductive traits (flower number and shoot height). Within each population, absolute fitness (number of intact fruits) was relativized to have a mean of one (by diving it by the mean number of intact fruits), and reproductive traits (phenology, flower number and shoot height) were standardized to have a mean of zero and a standard deviation of one (by subtracting the mean and dividing by the standard deviation). We tested for differences in phenotypic selection gradients among populations by fitting a linear model with Type II sums of squares, using standardized reproductive traits and their interactions with population as predictor variables, and relative fitness as response variable. The main effect of population was not included because fitness was relativized within populations prior to analysis. We tested for directional selection (linear terms), but also for correlational selection (examining the effects of interaction terms in a model also including the linear terms) and non-linear selection (examining the effect of quadratic terms in a model also including the linear terms). As both measures of phenology (PI and state of the most advanced bud) were strongly correlated (r = 0.86), we tested separate models including either one or another. Their performance was very similar in all models, so we only show analyses using the state of the most advanced bud, hereafter referred to as “phenology” variable (with higher values indicating earlier flowering).

We created a dummy variable “Predation”, coded as 0 (in populations without *M. alcon*) and 1 (in populations with *M. alcon*). We constructed a linear hierarchical mixed model (Type II sums of squares) for each year, with relative fitness as response variable. Predictors included standardized reproductive traits, their interactions with population (as random effects) and with predation (as fixed effects). Trait x population and trait x predation interactions were only included if the trait x population interaction had shown significant in the previous selection gradient analyses. We did not include an individual intercept by population because fitness was relativized within populations prior to analysis. We consider only linear effects in these models, as they showed to be the most important (see Results).

Within each population, we estimated linear selection gradients (’s) from multiple regressions of relative fitness on standardized reproductive traits, and we compared linear selection gradients for phenology between populations with and without predator using one-way ANOVAs.

To investigate among-population variation on the relationship between the occurrence of predation or its intensity and reproductive traits (i.e. differences in predator preferences), we regressed both the probability of attack by *M. alcon* (using a dummy variable “attack”, coded as 0 when number of eggs = 0, and 1 when number of eggs > 0) and the number of eggs on reproductive traits, population and their interactions. We excluded data from population D in 2010 from these analyses because predation intensity was extremely low (only 1 plant with 2 eggs).

We also examined the causal effects of phenology and other reproductive traits on fitness using path analysis (Grace, 2006) in the subset of populations with the predator (excluding also data from population D in 2010). We included both direct and indirect effects of traits on fitness, the latter mediated by the probability of predator attack or interaction intensity. To test for spatial variation in selective scenarios among populations, we used multigroup analysis (Grace, 2006). Afterwards, we fitted the path models in each population separately because they differed statistically. Details and results of this additional analysis are given in Appendix S2.

Finally, to see if the second host of *M. alcon* can condition phenotypic selection by increasing: a) the probability of presence of the predator or b) the intensity of the interaction, we performed a) a logistic regression of butterfly presence on log-transformed ant abundance, using the 20 study populations, and b) per-year linear regressions of the mean number of eggs per plant and the proportion of plants with *M. alcon* eggs on log-transformed ant-abundance, using the 11 populations where the butterfly was present.

Analyses were conducted in R 3.1.2 (R Core Team, 2014), with the exception of the path analyses shown on Appendix S2, which were conducted in Amos 16.0 (Arbukle, 2007).

RESULTS

We found evidence for linear (directional) selection (model A in Table 1) on flowering phenology, flower number and shoot height in both study years. Linear selection on flowering phenology and flower number varied among populations in both study years. Linear selection on shoot height was constant among populations in 2010, with relative fitness increasing in plants with higher shoots, but varied among populations in 2011. We also found some evidence of correlational and non-linear selection. Some trait interactions showed significant effects (model B in Table 1), although they were dependent on the population. In both study years, we found a significant quadratic effect of flower number on fitness that was dependent on the population (model C in Table 1).

Phenotypic selection on flowering phenology was mediated by the interaction with *M. alcon*, as it differed between populations where the predator was absent and present in both study years (and phenotypic selection on shoot height did so in 2011, Table 2). This was also shown by the significant differences in selection gradients for phenology between both population groups (Fig. 1). 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). Values of selection gradients for each population and year are detailed in Appendix S3.

In both study years, early-flowering increased the probability of being attacked by the predator, and this effect was constant between populations (i.e. the predator consistently preferred early-flowering plants in all populations, Table 3, Appendix S4). In 2010, there were differences between populations in the relationship between probability of attack and flower number, while in 2011 the predator consistently preferred plants with higher number of flowers in all populations. In 2011, there were differences between populations in the relationship between probability of attack and shoot height. There were also differences between populations in the effects of phenology and flower number on predation intensity (number of eggs, Table 3, Appendix S4) in both years studied.

Multigroup analyses revealed significant among-population variation in selective scenarios (Appendix S2). Although there was a high variation between populations and years, models fit for each population (Appendix S2) show that indirect effects of phenology on fitness (i.e. mediated by the seed predator preference for early-flowering plants, which in general reduced their fruit production) were more commonly significant than direct effects.

The probability of presence of *M. alcon* was higher in populations with higher abundance of *Myrmica* ants (Fig. 2A). Although there were populations with high ant abundance where *M. alcon* was absent, in populations where it was present ant abundance was generally high. Conversely, neither the mean number of eggs per plant (Fig. 2B) nor the proportion of plants with *M. alcon* eggs (Fig. 2C) showed any relationship with ant abundance in any of the two years.

DISCUSSION

In this study, we have shown that the interaction between *G. pneumonanthe* and its seed predator *M. alcon* is responsible for phenotypic selection on flowering phenology, and dependent on the community context, represented by ant abundance. *M. alcon* selects for later flowering in its host plant, as earlier flowering increases the probability of predator attack and the intensity of predation, although the selection intensity varies among plant populations and years. Phenotypic selection on flowering phenology is dependent on the community context, as presence of the predator (although not predation intensity) is related to ant abundance.

**Phenotypic selection on flowering phenology is mediated by seed predation**

We have shown that there is phenotypic selection for flowering phenology in the perennial herb *G. pneumonanthe*, and that among-population differences in selection are mediated by the interaction with its seed predator, the butterfly *M. alcon*. In populations where the predator is present, phenotypic selection acts to promote late flowering. This agrees with some previous evidence on predator-mediated selection on phenology (Pilson, 2000; Parachnowitsch & Caruso, 2008), although other studies (see review by Kolb *et al.*, 2007) have reported predators selecting for early flowering.

On the other hand, in populations without the predator there is also evidence for phenotypic selection in flowering time, selecting in this case for early flowering. This is consistent with the trend shown by Munguía-Rosas *et al.* (2011), who suggested that selection generally favors early flowering plants. This could be due to early flowering directly increasing fitness (e.g. by favoring outcrossing, Munguía-Rosas *et al.*, 2011), but also to indirect selection on other correlated reproductive traits (e.g. early-flowering plants produce in general higher shoots and more flowers), or to environmental covariance (i.e. phenology and fitness being both influenced by the environment, Ehrlén, 2015). Our results show some evidence of correlational selection between the studied traits (see model B in Table 1). In any case, the interaction with the seed predator modifies this general trend of selection for early flowering, as plants flowering late have a higher fitness in presence of the predator because they are less prone to be attacked (see section below).

We also found evidence for phenotypic selection on other plant traits. In 2010, plants with higher shoots were positively selected in all populations, while in 2011, there were differences between populations in selection for shoot height that were mediated by seed predation. Butterflies might prefer to lay eggs on tall shoots, as this indicates better plant quality and hence more resources available for larval development (Czekes *et al.*, 2014; Wynhoff *et al.*, 2014). However, plants with higher shoots will also be able to produce more flowers, and this explains why they are positively selected in 2010 and in populations without predator in 2011, as the effect of flower number on fitness might overcome that of shoot height. Phenotypic selection on flower number varied among populations, but these differences were not mediated by predation, and could be explained by variation in the strength of the relationship between flower and fruit production (due to e.g. variation in pollination success or in resources available for fruit set, Zimmerman & Aide, 1989).

**Predators select for late flowering, but selection strength varies among populations**

*M. alcon* consistently preferred attacking early-flowering plants in all populations and in both study years, leading thus to phenotypic selection for late flowering in populations where the predator was present, as early-flowering plants generally decrease their fitness as a result of predation. Although predation intensity responded to phenology with very different strengths among populations (see Appendix S4), egg load always increased with early flowering. Several studies have shown that females of *Maculinea* sp. choose plants for oviposition on the basis of their bud phenology, and prefer laying eggs on slightly immature buds, increasing time available for brood feeding and development (Thomas & Elmes, 2001; Patricelli *et al.*, 2011). However, we show that apart from the developmental state of the bud, the phenology of the plant (i.e. if it flowers early or late in the season) also matters for butterfly oviposition. It has been stated that *M. alcon* butterflies flying early in the season (during the first third of the flight period) deposit higher number of eggs than those flying later (Arnaldo *et al.*, 2014). Therefore, the preference for early-flowering plants might be due to an overlapping between the “optimal” bud developmental state for oviposition in these plants and the moment of higher oviposition activity of *M. alcon* during the season.

Plant phenology seems to be the main trait determining predator preferences, although *M. alcon* also preferred attacking plants with higher number of flowers in one of the study years, but not in the other. However, we found no clear preferences for shoot height. The visibility of food plants for butterflies may be determined by both its height and the size of its floral display (Nowicki *et al.*, 2005), although in our study system, the first factor is not very important, and the importance of the second varies between years. Therefore, the phenotypic selection for lower shoots mediated by the seed predator in 2011 is probably due to the correlated lower number of flowers in plants with low shoots, which reduces the visibility of plants for the butterfly.

In short, seed predators generally prefer early-flowering plants with high number of flowers, although there are differences between populations regarding the strength of this preference. These differences lead to variations in phenotypic selection intensity. For example, in populations where early-flowering plants receive many more eggs than late-flowering plants (e.g. population I in Fig. S4.1B), phenotypic selection on flowering time will be very strong, as late-flowering plants will have much higher fitness. However, in populations where the difference in egg load between early- and late-flowering plants is not very high (e.g. population C in Fig. S4.1B), phenotypic selection on flowering time will be much weaker. Among-population variation in phenotypic selection intensity is the basis for the occurrence of selection mosaics and local adaptation (Thompson, 2005) and could promote different evolutionary trajectories in different populations. However, this would need differences among populations in phenotypic selection for phenology to be constant among years. Although we found great spatiotemporal variation in selective scenarios for flowering phenology, some populations showed consistently strong phenotypic selection in both study years (e.g. population I). Although more study years would be needed to confirm this trend, these populations could potentially act as “coevolutionary hot spots” (Thompson, 1999). If this strong phenotypic selection is maintained in time, it could drive evolutionary change towards a later flowering phenology.

**Phenotypic selection is dependent on the community context**

Our results also show that phenotypic selection on flowering phenology in *G. pneumonanthe* is indirectly dependent on the community context in terms of abundance of the second host of its butterfly seed predator. The presence of *M. alcon* is more probable in sites with a high abundance of *Myrmica* ants, as the butterfly needs them to complete its life cycle. Therefore, the plant-seed predator interaction can only take place and mediate phenotypic selection in these sites. However, in populations where the butterfly is present, the intensity of seed predation on *G. pneumonanthe* is not related to ant abundance. This suggests that the second host is crucial for the antagonistic interaction to happen, but does not determine its magnitude, at least at the population scale studied. The butterfly’s dependence on ants for choosing an oviposition site at a local scale (i.e. a plant within the population) is somehow controversial, as some studies have found an ant-related oviposition behavior in these species (van Dyck *et al.*, 2000; Wynhoff *et al.*, 2014), while others have not (Nowicki *et al.*, 2005; Fürst & Nash, 2010). Relating interaction intensity to ant abundance at the scale of individual plants could help to clarify to what extent the community context can determine the intensity of antagonistic-mediated selection on flowering phenology in this species.

**Concluding remarks and future directions**

We have demonstrated that selection on flowering time can be determined by the outcome of pairwise interactions between three species: a plant, its predispersal seed predator and another member of the community. Ant abundance conditions presence of the seed predator *M. alcon*, which in in turn mediates phenotypic selection on flowering time in its host plant *G. pneumonanthe*. Thus, both butterflies and ants may act respectively as direct and indirect selective agents that can determine evolution on flowering time in this plant. However, evolutionary change will only occur if 1) the observed phenotypic selection is maintained in time and 2) there is sufficient genetic variance in flowering time to allow a selection response (Putterill *et al.*, 2004). Therefore, longer studies that also investigate the heritability of phenological traits are needed to broaden our knowledge about antagonist-driven evolution of flowering time.

Studies on flowering time are specially timely nowadays, as this plant trait plays a key role in the current scenario of global warming, where many plants are known to be advancing their phenologies towards an earlier flowering (Cleland *et al.*, 2007). However, in our study system, predator-mediated selection favors later flowering. This means that the selection exerted by antagonistic interactors goes in this case on the opposite direction than the phenological shifts expected as a response to changing environmental conditions. Further studies that also focus on climatic conditions would help to find out how evolution of flowering time will respond to these two opposed selection forces.

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TABLES

Table 1: Effects of standardized traits (flowering phenology, flower number, and shoot height), population 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 models with Type II sums of squares, including: A) only linear effects, B) linear effects and interactions and C) linear and quadratic effects. Estimates (from a model without interaction terms in each case) are given for significant main effects where the Population x trait interaction is not significant. Quadratic regression coefficients were doubled to properly estimate stabilizing/disruptive selection (Stinchcombe et al., 2008).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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 x Phenology | 19 | 3.61\*\*\* |  |  | 15 | 1.98\* |
|  | Population x Flower number | 19 | 3.88\*\*\* |  |  | 15 | 2.90\*\*\* |
|  | Population x Shoot height | 19 | 1.19 |  |  | 15 | 2.35\*\* |
|  |  |  |  |  |  |  |  |
| B) Interaction terms | |  |  |  |  |  |  |
|  | Phenology x Flower number | 1 | 1.78 |  |  | 1 | 0.08 |
|  | Phenology x Shoot height | 1 | 3.21 |  |  | 1 | 2.16 |
|  | Flower number x Shoot height | 1 | 9.59\*\* |  |  | 1 | 4.57\* |
|  | Population x Phenology x Flower number | 19 | 2.66\*\*\* |  |  | 15 | 1.61 |
|  | Population x Phenology x Shoot height | 19 | 1.02 |  |  | 15 | 1.31 |
|  | Population x Flower number x 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 x Phenology 2 | 19 | 1.40 |  |  | 15 | 0.94 |
|  | Population x Flower number 2 | 19 | 2.40\*\*\* |  |  | 15 | 3.38\*\*\* |
|  | Population x Shoot height 2 | 19 | 1.22 |  |  | 15 | 1.80\* |

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

Table 2: Effects of 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). Trait x population interactions that were significant in Model A in Table 1 are included here as random effects. Boldface indicates significance.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 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 x Phenology | 1 | 14.72\*\*\* |  | 1 | 14.95\*\*\* |
| Predation x Flower number | 1 | 0.01 |  | 1 | 3.69 |
| Predation x 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 attack by *M. alcon* and number of eggs in 10 populations in 2010 (N = 1000 plants) and 11 populations in 2011 (N = 1099 plants). 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 Boldface indicates significance.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of variation | Response: attack | | | | | | |  | Response: number of eggs | | | | |
| 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 x Phenology | 9 | 8.62 |  |  | 10 | 16.65 |  |  | 9 | 9.91\*\*\* |  | 10 | 5.55\*\*\* |
| Population x Flower number | 9 | 29.67\*\*\* |  |  | 10 | 14.11 |  |  | 9 | 8.77\*\*\* |  | 10 | 33.61\*\*\* |
| Population x 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. P-values from one-way ANOVAs are shown.

Figure 2: Effects of abundance of the second host (log-transformed maximum *Myrmica* abundance for both years) on A) *M. alcon* presence (p from logistic regression fit is shown, N = 20 populations), B) Mean number of eggs per plant, and C) Proportion of plants with *M. alcon* eggs. In B) and C), N = 11 populations where the predator was present, symbol color indicates the study year for interaction intensity measures (black = 2010 and grey = 2011).

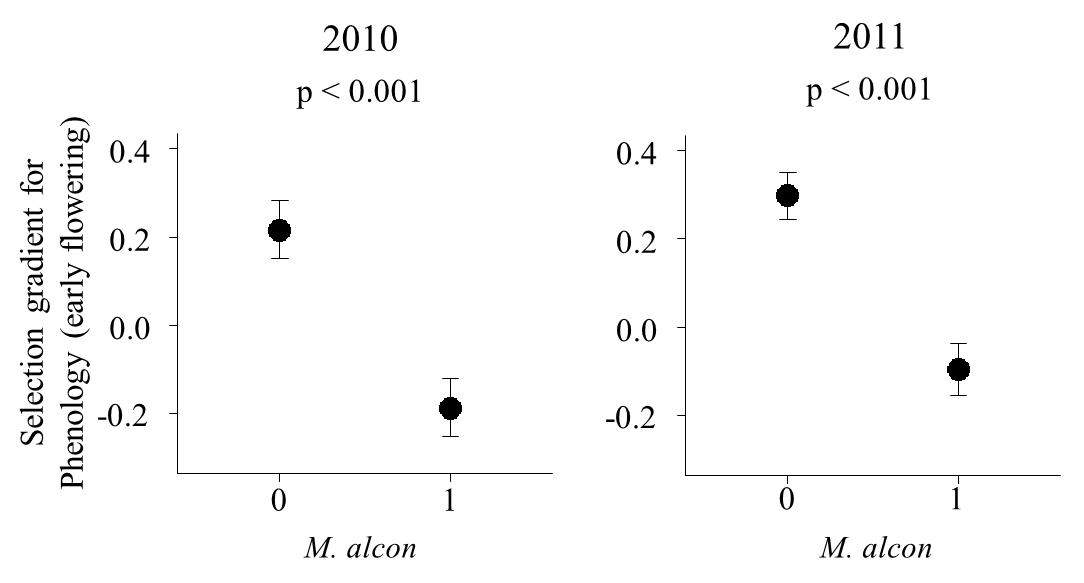
Figure 1

Figure 2

