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Butterfly-mediated shifts in selection on flowering phenology depend on host ant abundance

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TITLE: Butterfly-mediated shifts in selection on flowering phenology depend on host ant abundance

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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 mediated by the butterfly seed predator *Phengaris alcon*, and if incidence of the butterfly 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 increasing ant abundance. Our results demonstrate that antagonistic interactions can shift the direction of selection on timing of reproduction, and suggest that the community context may drive such antagonist-mediated 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., in press), and later flowering (e.g. Sandring & Ågren, 2009). Likewise, antagonistic interactions with herbivores or pre-dispersal seed predators might favor both later (e.g. Parachnowitsch & Caruso, 2008, König et al., 2015) and earlier flowering (e.g. Fukano et al., 2013). Moreover, plants can simultaneously experience selection for earlier and later flowering mediated by different agents. For example, flowering early might be advantageous for plants at northern latitudes because it increases pollinator availability and the time available for seed development (Munguía-Rosas et al., 2011b), but at the same time costly if early flowering increases the exposure to antagonists. In these cases, net selection depends on the relative strengths of these interactions (e.g. Ehrlén and Münzbergová 2009, Sletvold et al. 2015).

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

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3 preferences (Rey et al., 2006; Kolb et al., 2007). Interaction intensities and animal
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5 preferences for plant traits might, in turn, depend on the environmental context in terms
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7 of the abiotic conditions or in terms of other species (community context). For example,
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9 the intensity of plant-seed predator interactions has been shown to be influenced by
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11 light availability (e.g. Arvanitis et al., 2001; Kolb & Ehrlén, 2010) and soil moisture
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13 (von Euler et al., 2014). Community context, in terms of natural enemies, competitors
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15 or alternative hosts, have also been shown to influence the outcome of plant-animal
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17 interactions (Strauss & Irwin, 2004; Siepielski & Benkman, 2007; Chamberlain et al.,
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19 2014). For example, nectar robbers can affect selection by pollinators (Irwin, 2006), and
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21 plant community heterogeneity may determine host plant selection by insect herbivores
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23 (Agrawal et al., 2006). In spite of an increasing awareness of the ubiquitous spatial and
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25 temporal variation in selection, the role of community context as a source of variation in
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27 selection on a focal organism has been little explored. Analyses of selection mediated
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29 by species interactions have mostly focused on pairwise interactions, and analyses of
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31 more complex multispecies interactions rarely have assessed effects on selection
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33 (Strauss and Irwin, 2004). Unravelling the ways in which community context influences
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35 species interactions and the resulting selection is therefore a key step to link analyses of
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37 selection to community structure and species networks.
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44 One way in which community context may influence selection mediated by a
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46 focal interaction is when the outcome of a plant-consumer interaction depends on the
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48 abundance of a second resource for the consumer. For example, large Blue butterflies of
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50 the genus *Phengaris* Doherty, 1891 (the senior synonym of *Maculinea* van Eecke,
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52 1915) are specialist predispersal seed predators during their first larval instars, and often
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54 show strong preferences for plant developmental stages (Thomas & Elmes, 2001; Van
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56 Dyck & Regniers, 2010). This means that plant individuals for which the developmental
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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, in terms of 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 associated with 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, influences the probability of butterfly presence within plant populations. To test these hypotheses, we assessed selection gradients for flowering phenology, seed predation intensities and ant abundances in 11 *G. pneumonanthe* populations where the butterfly was present and 9 where it was absent.

MATERIALS AND METHODS

Study system

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, 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 (*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. 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 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 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 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). 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. 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 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 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 *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 \times population and trait \times predation interactions were only included in these models if the trait \times 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 (predator-mediated) 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 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 (Arbuckle, 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 \pm 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 \pm 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 *P.alcon* presence increased significantly with increasing abundance of *Myrmica* ants in the population (Fig. 2A). 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, neither the proportion of plants with *P.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 *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, in turn, did

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3 depend on community context, i.e., the incidence of the predator in host plant
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5 populations increased with the abundance of the host ants.
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8 In populations where the seed predator was absent, phenotypic selection favored
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10 early flowering in *G. pneumonanthe*. In late-flowering plant species like *G.*
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12 *pneumonanthe*, earlier flowering might be beneficial at northern latitudes, where the
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14 growing season is short, because it increases the time and resources available for seed
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16 maturation. It is also possible that the availability of pollinators is higher or that the
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18 competition with other plants is less intense earlier during the season. The pattern of
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20 early-flowering plants having higher fitness in the absence of seed predators found in *G.*
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22 *pneumonanthe* is consistent with the general trend suggested by Munguía-Rosas et al.
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24 (2011b). It is important, however, to bear in mind that higher fitness in earlier-flowering
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26 plants could be the result of that early flowering directly increases fitness, or that other
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28 plant traits correlated with early flowering have a positive effect on fitness. Positive
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30 correlations between early flowering and high fitness may also be the result of
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32 environmental covariance, i.e., both early flowering and fitness are correlated with
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34 favorable microsite conditions and high resource availability (Rausher, 1992; Ehrlén,
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36 2015). In our study, we tried to alleviate problems by incorporating traits that we
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38 considered likely to be correlated with flowering phenology, as well as traits likely to be
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40 correlated with plant resource state, as covariates in our models.
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46 Irrespective of the selective agents responsible for the observed selection for
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48 earlier flowering in the absence of antagonists, our results clearly show that this
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50 selection is reversed to selection for later flowering when antagonists are present. In our
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52 study system, the direction of selection on phenology differed markedly between
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54 *Gentiana* populations with vs. without the butterfly pre-dispersal seed predator. This
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56 happened because *P. alcon* consistently preferred early-flowering plants for oviposition
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within populations, thus increasing the relative fitness of late-flowering plants. This means that the predator mediates 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. 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 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 predator occurrence depends on 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). Our results thus strongly suggest that the observed among-population variation in selection on plant flowering phenology mediated by the butterfly seed predator ultimately depends on the factors influencing the abundance of the second host of the butterfly. Taken together, our results and 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), suggest that the community context has important effects on 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 can explain among-population variation in the 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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REFERENCES

1.
Agrawal, A.A., Lau, J.A. & Hambäck, P.A. (2006). Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Q. Rev. Biol.*, 81, 349–376.

2.

Alonso, C. (1997). Choosing a place to grow. Importance of within-plant abiotic microenvironment for *Yponomeuta mahalebella*. *Entomol. Exp. Appl.*, 83, 171–180.

3.

Als, T.D., Vila, R., Kandul, N.P., Nash, D.R., Yen, S.-H., Hsu, Y.-F., *et al.* (2004). The evolution of alternative parasitic life histories in large blue butterflies. *Nature*, 432, 386–390.

4.

Appelqvist, T., Bengtsson, O., Sverige & Naturvårdsverket. (2007). *Åtgärdsprogram för alkonblåvinge och klockgentiana 2007-2011: Maculinea alcon och Gentiana pneumonanthe : hotkategori: sårbara (vu)*. Naturvårdsverket, Stockholm.

5.

Arbuckle, J.L. (2007). *AMOS 16 Users Guide*.

6.

Arceo-Gómez, G. & Ashman, T.-L. (2014). Coflowering community context influences female fitness and alters the adaptive value of flower longevity in *Mimulus guttatus*. *Am. Nat.*, 183, E50–E63.

7.

Arvanitis, L., Wiklund, C. & Ehrlén, J. (2007). Butterfly seed predation: effects of landscape characteristics, plant ploidy level and population structure. *Oecologia*, 152, 275–285.

8.

Benkman, C.W. (2013). Biotic interaction strength and the intensity of selection. *Ecol. Lett.*, 16, 1054–1060.

9.
Benkman, C.W., Smith, J.W., Maier, M., Hansen, L. & Talluto, M.V. (2013).
Consistency and variation in phenotypic selection exerted by a community of seed
predators. *Evolution*, 67, 157–169.

10.
Biere, A. & Tack, A.J.M. (2013). Evolutionary adaptation in three-way interactions
between plants, microbes and arthropods. *Funct. Ecol.*, 27, 646–660.

11.
Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. (2014). How context dependent are
species interactions? *Ecol. Lett.*, 17, 881–890.

12.
Chapurlat, E., Ågren, J. & Sletvold, N. (in press). Spatial variation in pollinator-
mediated selection on phenology, floral display and spur length in the orchid
Gymnadenia conopsea. *New Phytol.*

13.
Ehrlén, J. (2015). Selection on flowering time in a life-cycle context. *Oikos*, 124, 92–
101.

14.
Ehrlén, J. & Münzbergová, Z. (2009). Timing of flowering: opposed selection on
different fitness components and trait covariation. *Am. Nat.*, 173, 819–830.

15.
Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E. & Bernasconi, G. (2007).
Time after time: flowering phenology and biotic interactions. *Trends Ecol. Evol.*, 22,
432–439.

16.
Fedriani, J.M. & Delibes, M. (2013). Pulp feeders alter plant interactions with subsequent animal associates. *J. Ecol.*, 101, 1581–1588.
17.
Franks, S.J., Sim, S. & Weis, A.E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc. Natl. Acad. Sci.*, 104, 1278.
18.
Fukano, Y., Tanaka, K. & Yahara, T. (2013). Directional selection for early flowering is imposed by a re-associated herbivore - but no evidence of directional evolution. *Basic Appl. Ecol.*, 14, 387–395.
19.
Giménez-Benavides, L., García-Camacho, R., Iriondo, J.M. & Escudero, A. (2011). Selection on flowering time in Mediterranean high-mountain plants under global warming. *Evol. Ecol.*, 25, 777–794.
20.
Grace, J.B. (2006). *Structural equation modeling and natural systems*. Cambridge University Press, Cambridge, UK ; New York.
21.
Irwin, R.E. (2006). The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *Am. Nat.*, 167, 315–328.
22.
Ison, J.L. & Wagenius, S. (2014). Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial. *J. Ecol.*, 102, 920–929.

23.
Kéry, M., Matthies, D. & Fischer, M. (2001). The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. *J. Ecol.*, 89, 418–427.

24.
Kolb, A. & Ehrlén, J. (2010). Environmental context drives seed predator-mediated selection on a floral display trait. *Evol. Ecol.*, 24, 433–445.

25.
Kolb, A., Ehrlén, J. & Eriksson, O. (2007). Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect. Plant Ecol. Evol. Syst.*, 9, 79–100.

26.
König, M.A.E., Wiklund, C. & Ehrlén, J. (2015). Timing of flowering and intensity of attack by a butterfly herbivore in a polyploid herb. *Ecol. Evol.*, 5, 1863–1872.

27.
Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210.

28.
Mouquet, N., Belrose, V., Thomas, J.A., Elmes, G.W. & Clarke, R.T. (2005). Conserving community modules: a case study of the endangered lycaenid butterfly *Maculinea alcon*. *Ecology*, 86, 3160–3173.

29.
Munguia-Rosas, M.A., Ollerton, J. & Parra-Tabla, V. (2011). Phenotypic selection on flowering phenology and size in two dioecious plant species with different pollen vectors. *Plant Species Biol.*, 26, 205–212.

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3 30.

4
5 Munguía-Rosas, M.A., Ollerton, J., Parra-Tabla, V. & De-Nova, J.A. (2011). Meta-
6
7 analysis of phenotypic selection on flowering phenology suggests that early flowering
8
9 plants are favoured. *Ecol. Lett.*, 14, 511–521.
10

11 31.

12
13 Nash, D.R., Als, T.D., Maile, R., Jones, G.R. & Boomsma, J.J. (2008). A mosaic of
14
15 chemical coevolution in a Large Blue butterfly. *Science*, 319, 88–90.
16
17

18 32.

19
20 Nowicki, P., Witek, M., Skorka, P. & Woyciechowski, M. (2005). Oviposition patterns
21
22 in the myrmecophilous butterfly *Maculinea alcon* Denis & Schiffermueller
23
24 (Lepidoptera: Lycaenidae) in relation to characteristics of foodplants and presence of
25
26 ant hosts. *Pol. J. Ecol.*, 53, 409–417.
27
28

29 33.

30
31 Parachnowitsch, A.L. & Caruso, C.M. (2008). Predispersal seed herbivores, not
32
33 pollinators, exert selection on floral traits via female fitness. *Ecology*, 89, 1802–1810.
34
35

36 34.

37
38 Patricelli, D., Barbero, F., La Morgia, V., Casacci, L.P., Witek, M., Balletto, E., *et al.*
39
40 (2011). To lay or not to lay: oviposition of *Maculinea arion* in relation to *Myrmica* ant
41
42 presence and host plant phenology. *Anim. Behav.*, 82, 791–799.
43
44

45 35.

46
47 Pilson, D. (2000). Herbivory and natural selection on flowering phenology in wild
48
49 sunflower, *Helianthus annuus*. *Oecologia*, 122, 72–82.
50
51

52 36.

53
54 Rausher, M.D. (1992). The measurement of selection on quantitative traits: biases due
55
56 to environmental covariances between traits and fitness. *Evolution*, 46, 616–626.
57
58
59
60

37.

R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

38.

Rey, P.J., Herrera, C.M., Guitián, J., Cerdá, X., Sanchez-Lafuente, A.M., Medrano, M., *et al.* (2006). The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *J. Evol. Biol.*, 19, 21–34.

39.

Sala, M., Casacci, L.P., Balletto, E., Bonelli, S. & Barbero, F. (2014). Variation in butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. *PLoS ONE*, 9, e94341.

40.

Sandring, S. & Ågren, J. (2009). Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution*, 63, 1292–1300.

41.

Siepielski, A.M. & Benkman, C.W. (2007). Selection by a predispersal seed predator constrains the evolution of avian seed dispersal in pines. *Funct. Ecol.*, 21, 611–618.

42.

Siepielski, A.M., Gotanda, K.M., Morrissey, M.B., Diamond, S.E., DiBattista, J.D. & Carlson, S.M. (2013). The spatial patterns of directional phenotypic selection. *Ecol. Lett.*, 16, 1382–1392.

43.

Simmonds, N.W. (1946). *Gentiana pneumonanthe* L. *J. Ecol.*, 33, 295–307.

1
2
3 44.

4
5 Sletvold, N., Moritz, K.K. & Ågren, J. (2015). Additive effects of pollinators and
6
7 herbivores result in both conflicting and reinforcing selection on floral traits. *Ecology*,
8
9 96, 214–221.

10
11 45.

12
13 Strauss, S.Y. & Irwin, R.E. (2004). Ecological and evolutionary consequences of
14
15 multispecies plant-animal interactions. *Annu. Rev. Ecol. Evol. Syst.*, 35, 435–466.

16
17 46.

18
19 Thomas, J.A. & Elmes, G.W. (2001). Food–plant niche selection rather than the
20
21 presence of ant nests explains oviposition patterns in the myrmecophilous butterfly
22
23 genus *Maculinea*. *Proc. R. Soc. Lond. B Biol. Sci.*, 268, 471–477.

24
25 47.

26
27 Thompson, J.N. (2005). *The geographic mosaic of coevolution*. 1 edition. University Of
28
29 Chicago Press, Chicago.

30
31 48.

32
33 Van Dyck, H. & Regniers, S. (2010). Egg spreading in the ant-parasitic butterfly,
34
35 *Maculinea alcon*: from individual behaviour to egg distribution pattern. *Anim. Behav.*,
36
37 80, 621–627.

38
39 49.

40
41 Vanhoenacker, D., Ågren, J. & Ehrlén, J. (2013). Non-linear relationship between
42
43 intensity of plant–animal interactions and selection strength. *Ecol. Lett.*, 16, 198–205.

44
45 50.

46
47 von Euler, T., Ågren, J. & Ehrlén, J. (2014). Environmental context influences both the
48
49 intensity of seed predation and plant demographic sensitivity to attack. *Ecology*, 95,
50
51 495–504.

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 effects and interactions and C) linear and quadratic effects. All models included effects of population \times trait interactions. Estimates are given for significant main effects where the population \times 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 \times Phenology	19	3.61***		15	1.98*
Population \times Flower number	19	3.88***		15	2.90***
Population \times Shoot height	19	1.19		15	2.35**
B) Interaction terms					
Phenology \times Flower number	1	1.78		1	0.08
Phenology \times Shoot height	1	3.21		1	2.16
Flower number \times Shoot height	1	9.59**		1	4.57*
Population \times Phenology \times Flower number	19	2.66***		15	1.61
Population \times Phenology \times Shoot height	19	1.02		15	1.31
Population \times Flower number \times 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 \times Phenology ²	19	1.40		15	0.94
Population \times Flower number ²	19	2.40***		15	3.38***

Population × Shoot height ²	19	1.22	15	1.80*
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* 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 χ^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	χ^2	df	χ^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. χ^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	χ^2	Estim	df	χ^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 and 2011. Filled circles represent means, and bars standard errors ($P < 0.001$ in both years, one-way ANOVAs).

Figure 2: Relationships between of abundance of *Myrmica* host ants (log mean number of observed individuals), and (A) Presence/absence of *P. alcon* in populations of *G. pneumonanthe* (fitted curve is a logistic regression, N = 20 populations, $p = 0.045$), (B) Mean number of eggs per plant in populations where *P. alcon* was present (N = 11 populations in two years, $p > 0.05$ in both years), and (C) The proportion of plants that had at least one *P. alcon* egg in populations where *P. alcon* was present (N = 11 populations in two years, $p > 0.05$ in both years). Black circles in B and C are estimates from 2010 and grey are estimates from 2011.

Figure 1

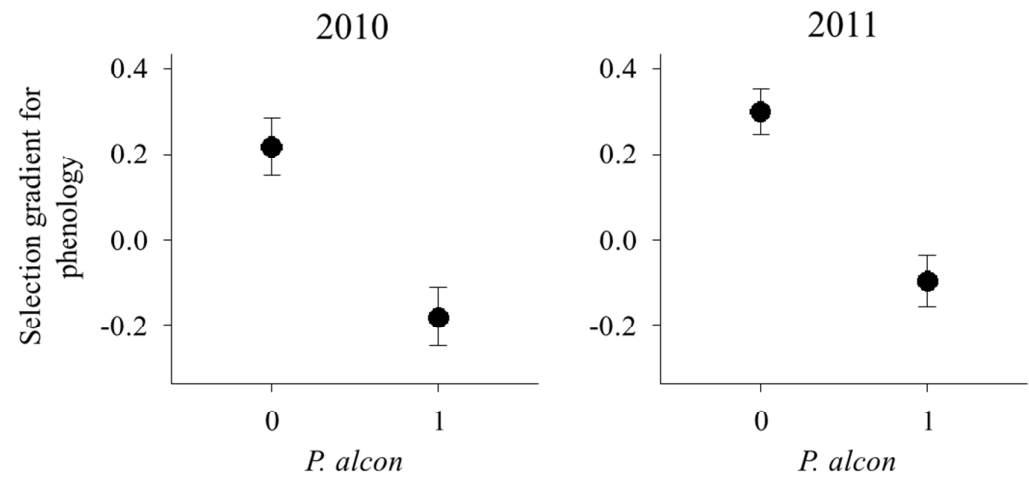


Figure 2

