TITLE:

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

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ABSTRACT

Evolution of timing of reproduction in plants can be determined by different abiotic and biotic factors. Plants can simultaneously experience selection for early or late flowering mediated by different agents, the direction of net selection depending on the relative strengths of selective forces. When selection is mediated by animal interactors, spatiotemporal differences in interaction intensities and in interactor preferences can lead to variations in selection and selection mosaics. Intensity of interactions may in turn be determined by the environment where they occur, including relations of interactors with other community members. Using 20 populations of the perennial herb *Gentiana pneumonanthe* in SW Sweden, we investigated how phenotypic selection on flowering phenology in this species is mediated by the interaction with its butterfly seed predator *Maculinea alcon*, and how this interaction depends on the abundance of *Myrmica* ants, which act as a second host for the butterfly, in two study years. In *G. pneumonanthe* populations where the predator was absent, phenotypic selection favored earlier flowering. In populations where the predator was present, it attacked preferentially early-flowering individuals, which caused a shift in selection towards later flowering. We also observed selection on other plant traits correlated with phenology (shoot height and number of flowers), although it was less consistent among populations and years. Predator-mediated selection on host plant traits depended on the community context, as the probability of presence of the predator increased with ant abundance at the population level. Our results demonstrate that antagonistic interactions are able to shift the direction of selection on flowering phenology, and that the community context where interactions occur is an important source of spatial variation in species interactions, and therefore in phenotypic selection.

INTRODUCTION

Timing of reproduction in plants is a key trait influencing interactions both with the physical environment and with other organisms. For plants in temperate regions, selection on flowering phenology may be mediated by several factors, as abiotic conditions (Franks *et al.*, 2007) and biotic interactions (Elzinga et al., 2007). Both mutualistic (Munguía-Rosas et al., 2011a, Aizen, 2003) and antagonistic interactors (Ehrlén & Münzbergová, 2009, Biere & Antonovics, 1996) have been pointed out as agents of selection for flowering phenology. Some of these factors have been reported to select for earlier flowering. For example, warm temperatures and early snowmelt dates have been suggested to drive an advancement of flowering phenology (Anderson *et al.*, 2012). Pollinators have also been mentioned as agents of selection for early flowering (Elzinga et al., 2007), although empirical evidence of pollinator-mediated selection on phenology is scarce (but see Sandring & Ågren, 2009). However, other factors may simultaneously act to favor later flowering. For example, growing evidence shows that predispersal seed predators can mediate selection on flowering phenology (Elzinga *et al.*, 2007, Ehrlén & Münzbergová, 2009, Ehrlén 2015), and that this sometimes favors later flowering (Parachnowitsch & Caruso, 2008). If the period of maximum predator activity occurs earlier than the peak of flower abundance, late flowering plants would be favored, as they would be able to escape herbivory and thus have a higher fitness (Pilson, 2000).

Therefore, a plant species can simultaneously experience selection for earlier and for later flowering mediated by different agents (e.g. mutualists and antagonists, Ehrlén and Münzbergová 2009, Slevtod et al. 2015), and net selection could shift in direction depending on the relative strengths of selective forces. The relative interaction intensities and the trait preferences of the interactors might differ among populations and years, leading to spatially and temporally varying selection and selection mosaics (Thompson, 2005). For example, variation in relationships between plant reproductive traits and predispersal seed predation in different populations and years (Kolb *et al.*, 2007) may contribute to spatiotemporal variation in selection (Benkman, 1999; Rey *et al.*, 2006). Interaction intensities and preferences of seed predators might, in turn, be related to the environmental context. Species interactions have been shown to be influenced by the physical environment where they occur, in terms of e.g. canopy cover (Arvanitis et al, 2001; Kolb & Ehrlén, 2010) or soil moisture (von Euler *et al.*, 2014). Also other community members, being natural enemies, competitors or alternative hosts, i.e. the community context, might influence interactions (Brandt & Foitzik, 2004; 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 alter selection by insect herbivores (Agrawal *et al.*, 2006). Analyses of selection mediated by species interactions have mostly focused on pairwise interactions, and analyses of more complex multispecies interactions (Strauss and Irwin 2004) rarely have assessed effects on selection. 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 ecology.

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, often ovipositing only on specific plant developmental states (Thomas & Elmes, 2001; Van Dyck & Regniers, 2010; Czekes *et al.*, 2014). *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). Selection on plant traits might thus be influenced by the community context, in terms of the abundance of the ant host affecting butterfly abundance and seed predation intensity. In this study, we examined how phenotypic selection on flowering phenology 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 in terms of the abundance of the second host, *Myrmica* ants. We addressed two main questions: 1) Does the butterfly seed predator influence the direction and strength of phenotypic selection on flowering phenology in *G. pneumonanthe*?, and 2) Does community context, in terms of the abundance of their second host, influence butterfly occurrence and seed predation intensity?

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 entirely self-compatible and flowers in July and August in SW Sweden. Fruits are capsules containing a high number of minute (mean seed weight = 0.044 mg, Simmonds, 1946), wind-dispersed seeds (usually 300-700 per capsule, Appelqvist *et al.*, 2007). *Gentiana pneumonanthe* is the primary host of the Alcon Blue butterfly (*Maculinea alcon*), a specialist predispersal seed predator which lays its eggs on young buds during its flight period (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 are fed 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 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 reproductive traits, plant fitness and interaction intensity in 100 marked individuals in each of the 20 study populations during 2010 and 2011. For each individual, we selected one shoot of median length. All measurements were carried out on this focal shoot. We measured shoot height, reproductive phenological state, number of flowers, and number of *M. alcon* eggs during late July – early August. Phenology of each individual, in terms of the developmental stages of flowers on the focal shoot at a given date, was estimated by counting the number of buds and flowers in each of six developmental stages: a) the sepals covering the bud completely, b) from the time the bud begins to be visible between the sepals until it grows over the sepals, c) from the time the bud grows over the sepals until it starts to turn blue, d) from the time the bud starts to turn blue until it becomes a solid blue, tightly twisted bud. e) from the time the bud starts to open, until the flower is fully opened and f) from the time the flower shows signs of wilting. Using this data, we derived two different measures of the reproductive developmental stage of each individual: (1) the mean developmental stage of all flowers and buds within a shoot, and (2) the stage of the most advanced bud in each shoot. In both cases, higher values indicate earlier flowering.

Populations were visited once at the end of July-beginning of August 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). The maximum number of eggs observed during these visits was used as an estimate of interaction intensity. The number of intact (i.e. not damaged by the butterfly) mature fruits was used as an estimate plant fitness. In 2011, information on fruit production was not available for 4 of the populations.

Ant abundance was estimated in each of the 20 study populations, using ant counts along transects where a sugar cube was placed every meter. Assessments were only carried out under dry (no precipitation) and warm conditions. The number of *Myrmica* sp. ants at each of the sugar cubes was counted 30 minutes after presenting them to ants. In 2010, 20-m transects were established, and in 2011 we used a 40-m transect in each population. We used the highest yearly value of the average number of ants per sugar cube in each population as a measure of ant abundance. This was done to partly decrease errors associated with remaining variation in ant abundance due to varying weather conditions.

**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 diving 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 also interactions between standardized reproductive traits and population. The main effect of population was not included because 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 fixed effects. 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 in 2010 from these analyses because only one plant individual was attacked.

We also examined the direct effects of reproductive traits on fitness, and the indirect effects mediated by the probability of predator attack or interaction intensity, using path analyses (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* influenced phenotypic selection in the plant host by increasing the probability of predator presence or the intensity of the interaction. First, we performed a logistic regression of butterfly presence on log-transformed ant abundance, using the 20 study populations. Second, we performed linear regressions of 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 for directional selection on flowering phenology, flower number and shoot height in both study years (Table 1A). Linear selection on flowering phenology and flower number differed among populations in both study years and selection on shoot height differed in 2011. We also found evidence of correlational selection, although effects differed among populations (Table 1B). In both study years, we found a significant quadratic effect of flower number on fitness, but this effect also differed among populations (Table 1C).

The direction of phenotypic selection on flowering phenology differed between populations where the predator was absent and populations where it was present (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).

In both study years, early flowering 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, while the effect differed among populations in 2010. The relationship between probability of attack and shoot height was not significant in 2010 and differed among populations in 2011. The effects of phenology and flower number on predation intensity (number of eggs, Table 3, Appendix S3) differed between populations in both years studied.

Multigroup 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 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 seldom low in populations where it was present. In populations where the butterfly was present, neither the mean number of eggs per plant (Fig. 2B) nor the proportion of plants with *M. alcon* eggs (Fig. 2C) was related to ant abundance.

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.

Our study shows that in the absence of the predator, phenotypic selection favored early flowering. This is consistent with the general trend suggested by Munguía-Rosas *et al.* (2011b). Such patterns could potentially be the result both of early flowering directly increasing fitness, and of selection on other plant traits that are correlated with flowering phenology. Moreover, positive correlations between early flowering and high fitness may also occur because both early flowering and fitness are correlated with favorable microsite conditions and high resource availability, i.e., they are the result of environmental covariance (Rausher, 1992; Ehrlén, 2015). In our study, we tried to alleviate these problems by incorporating traits that we considered likely to be correlated with flowering phenology and traits likely to be correlated with plant resource state as covariates in our models. This suggests that the positive effects of early flowering in the absence of the predator may indeed be direct and causal in our system. Flowering early in *Gentiana pneumonanthe* can have several advantages, e.g. avoiding competition for pollinators and favoring outcrossing (Munguía-Rosas *et al.*, 2011b). Plants flowering during the summer in northern latitudes, where the growing season is short, might also benefit from flowering earlier in order to increase the time available for seed maturation in suitable weather conditions, as temperatures are expected to decrease along the summer.

Our results also show that such selection for earlier flowering in the absence of antagonists may shift to selection for later flowering if 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 strongly suggests that the predator mediates shifts in selection from favoring early to favoring late flowering. Moreover, *M. alcon* consistently preferred attacking early-flowering plants within populations, thus increasing the relative fitness of late-flowering plants. Previous studies with this study system have focused on the relationship between oviposition and bud phenological state, and showed that females of *Maculinea* sp. prefer laying eggs on not fully developed buds, thereby increasing time available for brood feeding and development (Thomas & Elmes, 2001; Patricelli *et al.*, 2011). In contrast, our study 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 phenological stage, plants starting bud development early in the season are more prone to be attacked by the butterfly than plants flowering late. This seems to result from a temporal match between early-flowering plants and the peak of fecundity of *M. alcon*, as butterflies flying early in the season have been shown to deposit higher number of eggs than those flying later (Arnaldo *et al.*, 2014). The reason behind this is probably the occurrence of warmer temperatures at the beginning of the flight period, which could increase egg maturation and oviposition rates (Berger et al. 2008), as well as favor larval development (Audusseau et al. 2013). On the other hand, higher temperatures could also increase abundance of ants, the second host of *M. alcon*, and this could have partly driven the evolution of a higher fecundity in early-flying butterflies. Our findings with *G. pneumonanthe* agree with previous studies demonstrating predator-mediated selection for late flowering (Pilson, 2000; Parachnowitsch & Caruso, 2008), although other studies (see review by Kolb *et al.*, 2007) have reported predators selecting for both early and late flowering. In this system, the butterfly seed predator is responsible for shifting selection from early to late flowering phenology in its host plant.

We also found evidence for phenotypic selection on other plant traits. Butterflies preferred to oviposit on taller shoots, which are more conspicuous and represent a visually attractive target (Nowicki et al. 2005). Taller shoots are probably also a predator-safe oviposition site for the female butterflies and, as they receive more solar radiation, buds on these shoots might show a warmer, more suitable microclimate for larval growth (Van Dyck & Regniers, 2010). The preference for taller shoots was only observed in some populations and years (Fig. S4.3). This could possibly be explained by variation in height of the surrounding vegetation. High shoots may protrude out of the vegetation and be preferred for oviposition (Küer & Fartmann, 2005). This effect is likely to vary with the height of the surrounding vegetation. *M. alcon* attacked plants with higher numbers of flowers in one of the study years, but not in the other.

Our study not only shows that the presence of a butterfly seed predator mediates a shift in the direction of selection on flowering phenology, but also suggests that predator presence depends 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, their obligate second host. Caterpillars need to be fed into the ant nest, where they will gain most of their final biomass, pupate, and eclose as adults (Als et al. 2001; Mouquet et al. 2005). Previous studies with other systems have shown that the community context can affect the likelihood or intensity of plant-animal interactions (Strauss & Irwin, 2004), as selection on plant traits by either mutualists or antagonists can be altered by interactions with other community members. In this case, the interaction between the plant and the butterfly is conditioned by the interaction between the butterfly and its ant host; in populations where ants are more abundant, there is a higher probability for the predation interaction to happen. Therefore, we have shown that the community context, represented in this case by the abundance of a second host, can be an important determinant of spatial variation in selection on flowering phenology mediated by seed predators.

It is well-known that species interactions and selection on plant traits varies both spatially and temporally (Thompson, 2005). However, selection has been reported to vary mainly in strength, but less in direction among populations, and the environmental factors causing this variation, although crucial to understanding the spatial structure of selection, have been rarely identified (Siepielski et al., 2013). In this study, we have not only demonstrated that an antagonistic interaction can mediate selection on plant phenology and shift its direction, but also that the community context can be an important determinant of spatial variation in species interactions, and thus one of the factors determining the existence of selection mosaics. Studies of selection on plant traits mediated by species interactions should not only address how interaction intensity affects selection, but also focus on how interaction intensity is affected by the environmental context where it occurs, as this can indirectly determine the outcome of selection.

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REFERENCES

Agrawal, A.A., Lau, J.A. & Hambäck, P.A. (2006) Community heterogeneity and the evolution of interactions between plants and insect herbivores. *The Quarterly Review of Biology*, **81**, 349–376.

Aizen, M.A. (2003) Influences of animal pollination and seed dispersal on winter flowering in a temperate mistletoe. *Ecology*, **84**, 2613–2627.

Als, T.D., Nash, D.R. & Boomsma, J.J. (2001) Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae) by three *Myrmica* ant species. *Animal Behaviour*, **62**, 99–106.

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

Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, **279**, 3843–3852.

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

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

Arnaldo, P.S., Gonzalez, D., Oliveira, I., Langevelde, F. van & Wynhoff, I. (2014) Influence of host plant phenology and oviposition date on the oviposition pattern and offspring performance of the butterfly *Phengaris alcon*. *Journal of Insect Conservation*, **18**, 1115–1122.

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

Audusseau, H., Nylin, S. & Janz, N. (2013) Implications of a temperature increase for host plant range: predictions for a butterfly. *Ecology and Evolution*, **3**, 3021–3029.

Benkman, C.W. (1999) The selection mosaic and diversifying coevolution between crossbills and Lodgepole pine. *The American Naturalist*, **153**, S75–S91.

Berger, D., Walters, R. & Gotthard, K. (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology*, **22**, 523–529.

Biere, A. & Antonovics, J. (1996) Sex-specific costs of resistance to the fungal pathogen Ustilago violacea (Microbotryum violaceum) in Silene alba. *Evolution*, **50**, 1098–1110.

Brandt, M. & Foitzik, S. (2004) Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology*, **85**, 2997–3009.

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

Czekes, Z., Markó, B., Nash, D.R., Ferencz, M., Lázár, B. & Rákosy, L. (2014) Differences in oviposition strategies between two ecotypes of the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae) under unique syntopic conditions. *Insect Conservation and Diversity*, **7**, 122–131.

Van Dyck, H. & Regniers, S. (2010) Egg spreading in the ant-parasitic butterfly, *Maculinea alcon*: from individual behaviour to egg distribution pattern. *Animal Behaviour*, **80**, 621–627.

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

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

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

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

Franks, S.J., Sim, S. & Weis, A.E. (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, **104**, 1278.

Grace, J.B. (2006) *Structural equation modeling and natural systems*, Cambridge University Press, Cambridge, UK ; New York.

Irwin, R.E. (2006) The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *The American Naturalist*, **167**, 315–328.

Ison, J.L. & Wagenius, S. (2014) Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial. *Journal of Ecology*, **102**, 920–929.

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

Kolb, A., Ehrlén, J. & Eriksson, O. (2007) Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution and Systematics*, **9**, 79–100.

Küer, A. & Fartmann, T. (2005) Prominent shoots are preferred: microhabitat preferences of *Maculinea alcon* (Denis & Schiffermüller, 1775) in Northern Germany (Lycaenidae). *Nota Lepidoptera*, **27**, 309–319.

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

Leimu, R., Syrjänen, K., Ehrlén, J. & Lehtilä, K. (2002) Pre-dispersal seed predation in *Primula veris*: among-population variation in damage intensity and selection on flower number. *Oecologia*, **133**, 510–516.

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.

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

Munguía-Rosas, M.A., Ollerton, J., Parra-Tabla, V. & De-Nova, J.A. (2011b) Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology Letters*, **14**, 511–521.

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

Nowicki, P., Witek, M., Skorka, P. & Woyciechowski, M. (2005) Oviposition patterns in the myrmecophilous butterfly *Maculinea alcon* Denis & Schiffermueller (Lepidoptera: Lycaenidae) in relation to characteristics of foodplants and presence of ant hosts. *Polish Journal of Ecology*, **53**, 409–417.

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

Patricelli, D., Barbero, F., La Morgia, V., Casacci, L.P., Witek, M., Balletto, E. & Bonelli, S. (2011) To lay or not to lay: oviposition of *Maculinea arion* in relation to Myrmica ant presence and host plant phenology. *Animal Behaviour*, **82**, 791–799.

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

Rausher, M.D. (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*, **46**, 616–626.

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/.*,.

Rey, P.J., Herrera, C.M., Guitián, J., Cerdá, X., Sanchez-Lafuente, A.M., Medrano, M. & Garrido, J.L. (2006) The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology*, **19**, 21–34.

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

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

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. *Ecology Letters*, **16**, 1382–1392.

Simmonds, N.W. (1946) Gentiana pneumonanthe L. *Journal of Ecology*, **33**, 295–307.

Sletvold, N. & Grindeland, J.M. (2008) Floral herbivory increases with inflorescence size and local plant density in *Digitalis purpurea*. *Acta Oecologica*, **34**, 21–25.

Strauss, S.Y. & Irwin, R.E. (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 435–466.

Thomas, J.A. & Elmes, G.W. (2001) Food–plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proceedings of the Royal Society of London B: Biological Sciences*, **268**, 471–477.

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

TABLES

Table 1: Effects of three 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 are given for significant main effects where the Population × trait interaction is not significant.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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. Means±SE are represented (one-way ANOVAs indicated significant differences, with P<0.001 in both years).

Figure 2: Effects of abundance of the second host (log-transformed maximum *Myrmica* abundance) on A) *M. alcon* presence (fitted curve is a logistic regression, P-value 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. Black symbols in B and C are estimates for 2010 and grey are for 2011.

Figure 1

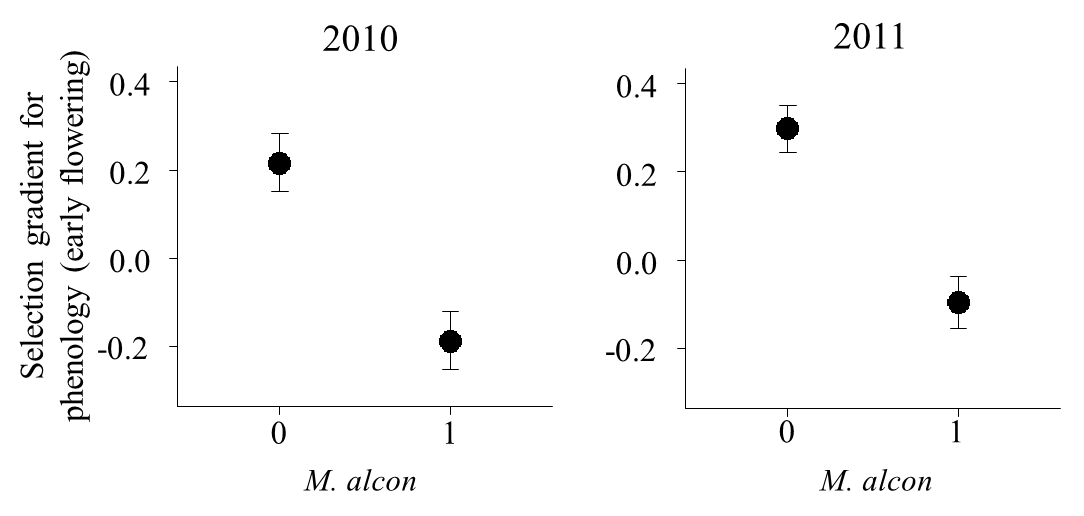


Figure 2

