TITLE:

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

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ABSTRACT

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

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 both abiotic conditions (Franks et al., 2007 …) and species interactions (Elzinga et al., 2007 …). Both mutualistic (e.g. Munguía-Rosas et al., 2011a, Aizen, 2003) and antagonistic interactors (e.g. Ehrlén & Münzbergová, 2009, Biere & Antonovics, 1996) can be important agents of selection for flowering phenology. While mutualist pollinators have been suggested to select for earlier flowering in several systems (e.g. Elzinga et al., 2007, Anderson et al*.*, 2012 …), antagonistic interactions with herbivores or pre-dispersal seed predators have been shown to favor later flowering in several cases (check Kolb et al review for references here, Pilson, 2000, Parachnowitsch & Caruso, 2008 …). In plant species simultaneously experiencing selection for earlier and later flowering mediated by different agents, net selection depend on the relative strengths of these interactions ….

Spatial variation in selection…(Thompson, 2005, Siepielski et al., 2013 …). Such spatial variation in selection may be driven by differences in interaction intensities or trait preferences of the interactors among populations. For example, it has been shown that relationships between plant reproductive traits and intensity of predispersal seed predation differ among populations and years (Benkman, 1999; Rey et al*.*, 2006; Kolb et al*.*, 2007). Interaction intensities and preferences of seed predators might, in turn, depend on the environmental context in terms of the abiotic conditions or in terms of other species (community context). For example, the intensity of plant-seed predator interactions have been shown to be influenced by light availability (e.g. Arvanitis et al., 2001; Kolb & Ehrlén, 2010) and soil moisture (von Euler et al*.*, 2014). Other community members, being natural enemies, competitors or alternative hosts, have also been shown to influence the outcome of plant-animal interactions (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). In spite of the increasing awareness of that selection vary ubiquitously over space and time, the role of community context as a source of spatial variation in selection on a focal organism has been little explored. Analyses of selection mediated by species interactions have mostly focused on pairwise interactions, and analyses of more complex multispecies interactions rarely have assessed effects on selection (Strauss and Irwin, 2004). Unravelling the ways in which community context influences species interactions and the resulting selection is therefore a key step to link analyses of selection to community structure and species networks.

One interesting way in which community context may influence selection mediated by a focal interaction is when the outcome of a plant-consumer interaction depends on the abundance of a second resource for the consumer. For example, large Blue butterflies (*Maculinea* spp.) are specialist predispersal seed predators during their first larval instars, 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 in these systems 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 tested two main hypotheses: 1) The presence of the butterfly seed predator in plant populations shift 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 the abundance of ant hosts, influence the probability of butterfly presence within plant populations.

MATERIALS AND METHODS

**Study system**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, occurring in open habitats, such as wet heathlands and grasslands (Simmonds, 1946). Plants can have one to many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by bumblebees. The species is self-compatible and flowers in July and August in SW Sweden. Fruits are capsules containing a high number (usually 300-700, Appelqvist et al*.*, 2007) of minute (mean seed weight = 0.044 mg, Simmonds, 1946), wind-dispersed seeds. *Gentiana pneumonanthe* is the primary host of the Alcon Blue butterfly (*Maculinea alcon*), a specialist predispersal seed predator which oviposits on young buds in July and August (Appelqvist et al*.*, 2007). The caterpillars feed inside the capsule until they reach the fourth-instar, when then they drop to the ground to be picked up by *Myrmica* ants (Mouquet et al*.*, 2005). Caterpillars mimic the surface chemistry of the ant brood (Nash et al*.*, 2008), and this makes ants carry them to their nest, where they spend the rest of their larval period as parasites (Mouquet et al*.*, 2005). Contrary to the majority of *Maculinea* species, which prey on ant brood, *M. alcon* is a “cuckoo” species (Als et al*.*, 2004), and larvae feed primarily on regurgitations from ant workers, trophic eggs (i.e. nutritious, infertile eggs which are fed to the queens and larvae) and prey items. In our study area, *M. ruginodis* is thought to be the most commonly used ant host species (Appelqvist et al*.*, 2007).

**Data collection**

The study was carried out in 20 populations of *G. pneumonanthe* located in the county of Västra Götaland in SW Sweden (see Appendix S1 for details). The populations are mainly located in moist heathlands. *M. alcon* was present in 11 of the study populations and absent from 9.

We collected data on plant reproductive traits, interaction intensity and plant fitness in 100 marked individuals in each of the 20 study populations during 2010 and 2011. For each individual, we selected one shoot of median length. In these focal shoots, we measured shoot height (in cm), reproductive development stage, and number of flowers during late July – early August. To assess reproductive development stage of individuals, we counted the number of buds and flowers in each of six developmental 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 within a shoot, and (2) the stage of the most advanced bud in each shoot. In both cases, higher values indicate earlier flowering. The average duration of stages 2-5 was about one week (NN *pers. obs.*), and a one-unit increase in these measures roughly correspond to one week earlier development.

Interaction intensity was estimated by…. 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.

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 NN , and the number of mature seeds in intact and preyed fruits is strongly correlated with the number of intact fruits reveal a very (r = 0.85, N = xxx individuals in 2010).

Ant abundance was estimated along transects in each of the 20 study populations. Sugar cubes were placed with one-meter intervals along the transects, and t. Assessments were only carried out under dry (no precipitation) and warm conditions. o further reduce thevarying 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 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 (Appendix S1) 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 simultaneously, 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* 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 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 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 if the predator was present or not in plant populations (Table 2, Appendix S2). In populations where the predator was absent, there was selection for earlier flowering (mean ± 95% CI of selection gradients, 2010: 0.22 ± 0.15, 2011: 0.30 ± 0.17), while in populations where the predator was present there was selection for later flowering (mean ± 95% CI of selection gradients, 2010: -0.19 ± 0.15, 2011: -0.10 ± 0.11, Fig. 1).

For traits other than plant reproductive phenology, we found evidence for directional selection on flower number and shoot height in both study years, but also in these cases 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, effects differing among populations (Table 1C).

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, but the effect of flower number differed among populations in 2010. Shoot height did not influence probability of attack in 2010 and in 2011 the effect differed among populations. Also the number of eggs per plant was correlated with phenology and flower number within populations but relationships varied among populations in both study years (Table 3, Appendix S3). Multigroup path-analyses revealed significant among-population differences in trait-fitness relationships (Appendix S4, Tables S4.1-2). Nevertheless, models fitted for each population and year consistently identified effects of phenology on fitness mediated by seed predator preference for early-flowering plants (Appendix S4, Tables S4.3-5). Direct effects of traits on fitness were less consistent.

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

DISCUSSION

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

In populations where the seed predator was absent, phenotypic selection favored early flowering in *G. pneumonanthe*. This is consistent with the general trend suggested by Munguía-Rosas et al*.* (2011b). Such patterns could be the result of early flowering directly increasing fitness, or of that other plant traits that are correlated with early flowering have a positive effect. 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. In late-flowering plant species at northern latitudes where the growing season is short, like *G. pneumonanthe*, earlier flowering might be beneficial because it increases the time and resources available for seed maturation. It is also possible that the availability of pollinators is higher (Munguía-Rosas et al*.*, 2011b), and the competition from other plants less intense earlier during the season.

Our results also clearly show that selection for earlier flowering in the absence of antagonists, is reversed to selection for later flowering when antagonists are present. In our study system, the direction of selection on phenology differed markedly between *Gentiana* populations with vs. without the butterfly pre-dispersal seed predator. This strongly suggests that the predator mediates shifts in selection from favoring early to favoring late flowering. 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 is most likely the result of a higher temporal overlap between the presence of floral developmental stages suitable for oviposition and the oviposition period of *M. alcon* in early-flowering plants. Our findings with *G. pneumonanthe* also agree with studies demonstrating predator-mediated selection for late flowering in other systems (Pilson, 2000; Kolb et al*.*, 2007; Parachnowitsch & Caruso, 2008), although some studies have reported on pre-dispersal seed predators selecting for early flowering (Kolb et al*.*, 2007). Taken together, our results with *G. pneumonanthe* shows that the presence of the butterfly seed predator shifts selection from favoring early to favoring late flowering plants, and that the occurrence of butterflies within plant populations explain the observed spatial variation in the direction of selection among plant populations.

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 possibly also 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, buds on taller shoots also receive more solar radiation, and might represent a warmer, more suitable microclimate for larval growth (Alonso, 1997). The preference for taller shoots was only observed in some populations and years (Fig. S4.3). This could possibly be explained by that the positive effect of higher shoots depend on the height of the surrounding vegetation and that the height of variation vary among populations.

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). Studies with other systems have shown that the community context can affect the likelihood or intensity of plant-animal interactions (Strauss & Irwin, 2004), and that selection on plant traits by mutualists and antagonists can be altered by interactions with other community members. Our results strongly suggest the observed spatial variation in selection on plant flowering phenology mediated by the butterfly seed predator ultimately depend on the factors influencing the abundance of the second host of the butterfly.

It is well-known that species interactions and selection on plant traits varies both spatially and temporally (Thompson, 2005). Yet, 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 demonstrated that an antagonistic interaction with a butterfly pre-dispersal seed predator can mediate selection on plant phenology and shift the direction of selection, and that among-population variation in selection on flowering time largely can be explained by the presence of butterflies. Moreover, we have shown that the community context can be an important driver of spatial variation in species interactions and selection. These results illustrates that to link variation in the environment to variation in natural selection, we need not only to assess the effects of species interactions on fitness of different phenotypes, but also examine how the physical environment and the community context influence the incidence and abundance of the interacting species.

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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.

Alonso, C. (1997) Choosing a place to grow. Importance of within-plant abiotic microenvironment for *Yponomeuta mahalebella*. *Entomologia Experimentalis et Applicata*, **83**, 171–180.

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.

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*. *Journal of Ecology*, **89**, 418–427.

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.

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

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

Figure 1

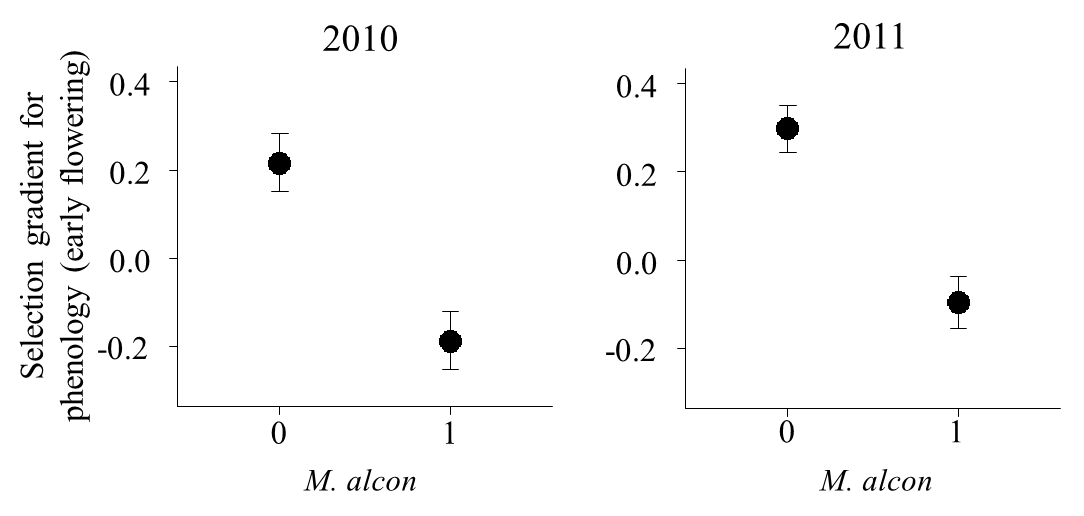


Figure 2

