TITLE (provisional): Butterfly seed predation: effects on plant reproductive output and context-dependence

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ABSTRACT

The intensity of antagonistic interactions and their effects on plant reproductive output might depend on the context of the interaction. Abiotic conditions and the abundance of other members of the community might affect interaction intensities and trait preferences of the interactors. Using the interaction between the butterfly seed predator *Phengaris alcon* and its host plant *Gentiana pneumonanthe* as a model system, we investigated the direct and indirect (predator-mediated) effects of plant traits and context on plant reproductive output in three populations. We found higher predation intensities in plants that flowered earlier, in plants that had more flowers and in plants that had higher shoots. Predation intensity was also related to abiotic conditions and community context; predation intensities were higher at low soil temperatures, at low height of surrounding vegetation, and at high abundances of *Myrmica* ants, the second host of the butterfly. Butterfly seed predation strongly reduced fruit production and reproductive output. Despite increased predation, flowering early, producing high shoots and many flowers was still advantageous in *G. pneumonanthe*, due to direct positive effects of these traits on fruit and seed production. Soil temperature increased fruit production both directly and indirectly (by reducing predation intensity), while community context affected reproductive output only indirectly; higher abundances of host ants close to plants were associated with higher predation intensities and a decreased reproductive output. Our results highlight the importance of taking into account the context when studying the outcomes of antagonistic interactions and their consequences for plant reproductive output.

Keywords: predispersal seed predation, context-dependence, plant phenology, plant-animal interactions, *Gentiana pneumonanthe*, *Phengaris alcon*, *Myrmica*, piecewise SEM

INTRODUCTION

(…)

Objectives / questions

1) What are the effects of plant traits (phenology, flower production, shoot height), environmental context (soil temperature, height of surrounding vegetation) and community context (ant abundance at the plant level) on intensity of predispersal seed predation?

H1: Predispersal seed predation is stronger on plants with an early phenology, high number of flowers and higher shoots, when the surrounding vegetation is short and when ants are present in the proximity of the plant. Reasons for an effect of soil temperature?

2) What are the (direct / indirect) effects of plant traits and context on fitness / reproductive output (fruit / seed production) / (fruit / seed set)?

H2: Higher soil temperatures (speeding up fruit maturation?) and higher flower production directly increase fitness. The effects of phenology, height and ants are indirect effects through seed predation (plants flowering early, being higher than the surrounding vegetation and having high ant abundance have higher seed predation and therefore lower fitness).

METHODS

**Study system**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb (10-20 years,Oostermeijer et al. 1992), occurring in open habitats, such as wet heathlands and grasslands (Simmonds 1946). In natural populations, individuals do not flower until their third year (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 brought to the nest by ants. Only ants belonging to the genus *Myrmica* can act as second hosts of *P. alcon* (Mouquet et al. 2005). 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 3 populations of *G. pneumonanthe* where the butterfly *P. alcon* was present, located in the county of Västra Götaland in SW Sweden (…). In each of these populations (Högsjön, Remmene and Tånga Hed, hereafter referred to as H, S and T), data on plant reproductive traits, interaction intensity and reproductive output, as well on the environmental and community contexts, were collected in 100 marked plant individuals during July-October 2015. The number of shoots per individual ranged from 1 to 13 (mean = 2.5). For recordings, we selected the most phenologically advanced shoot in each individual. In these focal shoots, we recorded 3 reproductive traits at the end of July (between the 29th and 31st): shoot height (in cm), number of flowers, and developmental stage of the most advanced bud at the day of recording, assigned to one of six ordinal categories: (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. Each of these stages corresponded to a time interval of flower development, and the average duration of stages 2-5 was about one week during this study (A. Valdés, pers. obs.). Higher values of this variable indicate a more advanced floral development at the day of recording, i.e. an earlier flowering phenology. According to the observed average duration of the stages, a one-unit increase in this measure 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 2-3 visits to each population. Eggs were counted twice in the second half of July, and in one population they were counted again at the end of August because we observed that more eggs had been laid since our last visit.

We counted the number of intact (i.e. not damaged by the butterfly) and predated fruits on 3 visits to each population (from the end of August until the end of October), and collected a sample of fruits. When possible, we collected one fruit of each type (i.e. intact and predated) per plant. Plant reproductive output was estimated by 1) the maximum observed number of intact (i.e. not damaged by the butterfly) fruits on the focal shoot, and 2) the estimated total number of seeds produced on the focal shoot, calculated as (n seeds per intact fruit \* n intact fruits) + (n seeds per predated fruit \* n predated fruits). The total number of seeds was only calculated for plants where we had information on seed number per fruit for all fruit types present on the plant (n = 245 plants).

We evaluated two measures of the environmental context of the plants: soil temperature and height of the surrounding vegetation. An iButton data logger was buried 5 cm deep in the soil as close as possible to each marked plant in mid-July 2015, before data collecting started. Data loggers were programmed to record temperature every third hour and were kept on the field until the end of August. After testing several temperature variables (averages of daily mean, maximum, minimum, standard deviation and range), we decided to use the average of daily minimum temperature because it showed the strongest correlation with predation intensity and reproductive output. Hereafter, we refer to this measure as soil temperature. Height (in cm) of the surrounding vegetation (1 m around each plant) was measured for each marked plant, using the average of 2 measures if height was variable.

At the base of each marked plant, ants were baited 4 times from the end of July until the end of August, avoiding cold and rainy days. In the morning (9-10 am), a sugar cube was placed on a small piece of white paper as close as possible to the plant stem. These baits were checked every 3 hours until around 6 pm, and each time we counted the number of *Myrmica* ants and the number of ants belonging to other genera. We used the maximum number of *Myrmica* ants observed in all baiting events (hereafter, *Myrmica* abundance) as a measure of the community context for each plant.

**Statistical analyses**

The effects of reproductive traits (flowering phenology, number of flowers, shoot height), environmental context (soil temperature, vegetation height), and community context (*Myrmica* abundance) on predispersal seed predation intensity (number of *P. alcon* eggs) were examined using a generalized linear model (GLM) with a negative binomial distribution. The effects of reproductive traits, environmental context (here, only soil temperature) and predation intensity on plant reproductive output estimated as the number of intact fruits were examined using a GLM with a Poisson distribution. We did not fit a GLM with the number of seeds as a response because we expected the effects of reproductive traits and context on this variable to be mostly indirect (i.e. mediated by effects on the number of intact fruits), and therefore we decided to explore them with a structural equation model (see below). As the reproductive traits were correlated amongst each other (Table S1), multicollinearity was an issue in models including the three traits as predictors at the same time (Tables S2-S4), and could lead to inflations in the standard errors of these predictors and difficulties in detecting significance (Zuur et al. 2010). To solve this problem, we performed a principal components analysis (PCA, Figure S1) with the three traits and retained the first axis (explaining 69% of the variance), to be used as a predictor variable in the GLMs. This variable indicated early flowering, high number of flowers and high shoots, as the PCA axis was positively correlated with the three traits. Predictors were standardized before including them in the models, by subtracting the mean and dividing by the standard deviation. As we expected to find among-population differences in reproductive traits, context and predation intensity, we included population and its interactions with all other predictors as fixed factors in our models. We were not specifically interested in differences among populations, but we wanted to account for this variation in our models and it was not advisable to include population as a random factor due to the low number of levels (Bolker et al. 2009). A set of candidate models was constructed for each response variable (number of eggs and number of intact fruits), including all combinations of terms from simple models to a global model including all predictors. Candidate models were ranked using the corrected version of the second-order Akaike information criterion (AICc). The relative importance of each predictor was calculated as the sum of Akaike weights over all models including the predictor (Burnham and Anderson 2002). To account for model uncertainty, we used the set of models with ΔAICc < 2 to calculate model-averaged parameter estimates.

We also used piecewise structural equation modelling (piecewise SEM, Lefcheck 2015) to infer the direct and indirect effects (mediated by predation intensity) of reproductive traits and context on plant reproductive output. This analysis allows detecting the independent effects of each reproductive trait, as correlations among variables can be incorporated into the model. We chose a piecewise approach (instead of the traditional variance-covariance based SEM) because of its ability to fit multiple separate linear models with non-normal distributions, which was well-suited for our data. As reproductive output could only be estimated as number of seeds in a subset of plants due to missing data (see above), we constructed two different SEMs using, respectively, the number of intact fruits (n = 303 plants) and the number of seeds (n = 245 plants) as estimates of reproductive output. We based the a priori structure of these SEMs on the significances and relative importances obtained in the previous analyses, as well as on ecological knowledge about the study system. We also included population as a fixed factor in our component models, as well as the interactions among population and other variables that were significant in previous analyses (see Results). The first SEM consisted of 3 component models: 1) a Poisson GLM with the number of intact fruits as response, and reproductive traits, soil temperature, number of eggs and population as predictors, 2) a negative binomial GLM with the number of eggs as response, and reproductive traits, soil temperature, vegetation height, ant abundance and population as predictors, and 3) a negative binomial GLM with ant abundance as response, and vegetation height and population as predictors. We also tested including an effect of soil temperature on ant abundance, as well as an effect of vegetation height on soil temperature, but these paths were not significant and therefore not included in the final SEM. The interaction population x vegetation height was significant in the previous analyses and was therefore initially included in model 2), although we finally decide to remove it because it did not show significance in the piecewise SEM. The second SEM consisted of 4 component models: the 3 included in the first model and 4) a quasipoisson GLM with the number of seeds as response, and reproductive traits, soil temperature, number of eggs, number of intact fruits and population as predictors. We included all possible correlations among the 3 traits in both SEMs. In order to compare the importance of paths among variables measured on different units, standardized path coefficients need to be calculated (Grace 2006). However, this is not possible (REF??) in a piecewise SEM where the component models do not follow a normal distribution. Therefore, we refitted our two SEMs using standardized variables and normal distributions in all the component models in order to obtain standardized path coefficients. However, the significance of path coefficients, the R2s for the component models and the overall fit of the piecewise SEMs (evaluated using Shipley’s test of d-separation and Fisher’s C statistic, Shipley 2009) were assessed using the SEMs with non-normal distributions, as they showed a better fit to our data.

Statistical analyses were carried out in R 3.2.4 (R Core Team 2016) using the packages MASS (Venables and Ripley 2002), MuMIn (Bartoń 2016) and piecewiseSEM (Lefcheck 2015).

RESULTS

Interaction intensity ranged from 0 to 37 *P. alcon* eggs per plant (mean 4.5 ± 0.38 SE). Reproductive output ranged from 0 to 15 intact fruits (mean 1.19 ± 0.11 SE) and from 0 to 6386 seeds per plant (mean 604.73 ± 76.04 SE).

Interaction intensity (Table 1) responded positively to the first axis of the PCA of reproductive traits, i.e. higher numbers of eggs were observed in early flowering plants with high number of flowers and high shoots. The significant positive effect of the three traits on the number of eggs per plant was confirmed by the piecewise SEM (Fig. 1). The environmental and community contexts also showed significant effects on interaction intensity. Higher numbers of eggs were observed in plants where soil temperature was lower and where *Myrmica* ants were more abundant. Plants surrounded by low vegetation received also more eggs, although this effect differed among populations according to the GLM. *Myrmica* ants were more abundant near plants surrounded by high vegetation. Interaction intensity differed among populations.

The number of intact fruits also responded positively to the first axis of the PCA (Table 2), although the piecewise SEM revealed that only flower number and shoot height increased the number of intact fruits per plant, while the effect of flowering phenology was not significant (Fig. 1). Number of intact fruits increased with soil temperature, decreased with predation intensity, and differed among populations. The effects of reproductive traits, soil temperature and interaction intensity were similar among populations, as none of the interaction effects was included in the best candidate GLM. The number of seeds per plant increased, as expected, with the number of intact fruits, leading to indirect effects of traits and context on seed production. Reproductive traits affected number of seeds mostly through indirect effects mediated by intensity of predation by *P. alcon*, although there were also small direct effects, as number of seeds increased with early flowering and with shoot height. There were no direct effects of the environmental context and predation intensity on the number of seeds, and it also differed among populations.

DISCUSSION

In this study, we have shown that seed predation by the butterfly *P. alcon*, and as a consequence, the reproductive output of its host plant *G. pneumonanthe*, are influenced both by plant reproductive traits and by the context where this interaction occurs. (More summary here).

**Effects of plant traits and context on predispersal seed predation**

Predispersal seed predation was more intense (i. e. the butterflies laid more eggs per plant) on plants flowering early in the season and producing many flowers. Previous studies have found different responses of predispersal seed predators to plant phenology (Kolb et al. 2007a): they either might prefer to attack plants with an earlier (Pilson 2000, Parachnowitsch and Caruso 2008) or later phenology (Kawagoe and Kudoh 2010, Sletvold et al. 2015), or show no specific preference (Dieringer 1991, Ehrlén 1996). *Phengaris* females have been shown to prefer ovipositing on buds that are not fully developed (Thomas and Elmes 2001, Patricelli et al. 2011), in order to increase time available for brood feeding and development. In *G. pneumonanthe*, the presence of these preferred bud developmental stages overlaps more with the oviposition period of *P. alcon* in early- than in late flowering plants, explaining why *P. alcon* might prefer plants flowering early in the season (Valdés and Ehrlén, under review). On the other hand, the presence of a high number of flowers per stem represents an attractive display both for pollinators and for floral antagonists (Ashman et al. 2004), and higher numbers of flowers have been previously shown to lead to an increased predispersal seed predation (Leimu et al. 2002). Indeed, flower number is the most important trait determining predation intensity in our study system (the path from flower number to number of eggs has a standardized coefficient of 0.48, while that from flowering phenology is 0.29 and that from shoot height is not significant, Fig. 1). On the contrary, the preference of butterflies for plants with higher shoots is not very strong, in accordance with previous results with this system on the same study area (Valdés and Ehrlén, under review), which showed that effects of shoot height on butterfly oviposition differed among plant populations and years. Several other studies with *P. alcon* (Czekes et al. 2014, Wynhoff et al. 2015) have found stronger positive effects of shoot height on butterfly oviposition. Tall shoots are more conspicuous (Nowicki et al. 2005), and probably also safer for oviposition (Van Dyck and Regniers 2010), but this trait seems to be less important than phenology and flower production for defining butterfly preferences in our study area. This might be due to differences in the environmental context between our study and previous studies, as for example in overall vegetation height. It has been shown that pollinators select tall plants when they are surrounded by tall vegetation, but not when the vegetation is short (Sletvold et al. 2013), and the same might occur with seed predators if the vegetation height in our study sites is lower than in the sites used in other studies.

In accordance with recent evidence on the widespread context-dependence of species interactions (Chamberlain et al. 2014, Maron et al. 2014), we have found that predation intensity is not only determined by plant reproductive traits, but also by the context where the interaction occurs. Both abiotic and biotic conditions (i.e. the environmental and community contexts) affect the intensity of seed predation by *P. alcon* on *G. pneumonanthe*. On the one hand, we have found higher predation intensity, i.e. higher amounts of eggs, in plants located in colder microsites. In general, butterfly mobility and egg-laying rates have been shown to increase with temperature (Davies et al. 2006, Dennis and Sparks 2006, Cormont et al. 2010, Radchuk et al. 2013). Therefore, we would expect butterfly predation intensity to increase with temperature as well, contrary to what we found. However, some studies (Davies et al. 2006, Lawson et al. 2014) have also found a temperature-dependent variation in the choice of oviposition microsite by butterflies, with females choosing warmer microsites at low ambient temperatures and cooler microsites at high ambient temperatures. A possible explanation for the negative effect of soil temperature on *P. alcon* predation intensity is that butterflies have mostly been active when the ambient temperature was high, and therefore relatively colder microsites (i.e. host plants) have been more frequently chosen for oviposition. This could potentially lead to higher accumulations of eggs in plants located in these colder microsites. Additionally, the negative effect of soil temperature on predation intensity could also be due to a correlation with unmeasured factors related to microclimate, as soil moisture or microtopography. On the other hand, we have found lower predation intensity, i.e. lower amounts of eggs, in plants surrounded by higher vegetation. These plants might have lower chances of being oviposited, as seed predators have been shown to prefer shoots that stand out of the vegetation, which are both more visually attractive (Küer and Fartmann 2005) and predator-free (Van Dyck and Regniers 2010).

Additionally, there is also an indirect positive effect of height of the surrounding vegetation on *P. alcon* predation intensity (Fig. 1), mediated by biotic conditions (i.e. the community context). Plants surrounded by taller vegetation show higher abundances of *Myrmica* ants (the second host of *P. alcon*) and this in turn leads to higher predation intensities. Vegetation structure has been shown to be closely related to the distribution of *Myrmica* ants, although effects are species-dependent. For example, occurrence of *M. sabuleti*, the host ant of *Phengaris arion* in the UK, rapidly decreased with increases in vegetation height resulting from abandonment of grazing, while the non-host *M. scabrinodis* prevailed (Thomas 1995, Thomas et al. 2009). On the contrary, densities of *M. ruginodis* and *M. scabrinodis*, both hosts of *P. alcon* in the Netherlands, were negatively affected by sod cutting (WallisDeVries 2004). Elmes et al. (1998) have shown that European *Myrmica* species occupy different ecological niches regarding soil moisture and soil temperature. Although we found that *Myrmica* abundance (as well as vegetation height) was unrelated to soil temperature within populations, we did not record soil moisture, which might also be an important factor related to changes in vegetation structure, and determining ant distributions (Elmes et al. 1998). Furthermore, we did not distinguish among *Myrmica* species in our baits, and although *M. ruginodis* is thought to be the host of *P. alcon* in our study area (Västra Götaland county, Appelqvist et al. 2007), other potential host ants have been observed in our study populations (i.e. *M. rubra* and *M. scabrinodis*, authors’ unpublished data). Therefore, our abundance data might comprise a mixture of *Myrmica* species that could respond differently to soil temperature and vegetation structure. Accordingly, we currently cannot assure which *Myrmica* species is the host for *P. alcon* in our study populations, and if the abundance of this particular species is affected by the environmental factors studied. However, we can conclude that predation intensity is directly related to the community context, as higher numbers of eggs were found on *G. pneumonanthe* plants growing where many *Myrmica* ants were present. This is an example of how a plant-animal interaction can be altered through changes in population density of another community member (i.e. density-mediated effects, Strauss and Irwin 2004).

Other studies have advocated for the existence of ant-mediated oviposition in *P. alcon* and other species of *Phengaris* (van Dyck et al. 2000, Wynhoff et al. 2008, 2015, Patricelli et al. 2011, but see Thomas and Elmes 2001, Fürst and Nash 2010). The mechanism by which the presence of host ants in the vicinity of the host plant could lead to an increase of the egg load is still unclear, and should involve a direct or indirect detection of the host ants by the female butterflies. An indirect detection mechanism has recently been demonstrated by Patricelli et al. (2015), by showing that *Phengaris arion* females respond to volatile compounds induced by its host plant as a defense reaction to root damage by its host ants. Although there is still no evidence that this holds true for all *Phengaris* species, a similar mechanism might help *P. alcon* to detect its host ants.

**Effects of plant traits, context and seed predation on plant reproductive output**

Predation by *P. alcon* contributes to decrease reproductive output of *G. pneumonanthe*, but the direct positive effects of the three studied traits on reproductive output outweigh their negative indirect effects mediated by increased seed predation. This means that flowering early, producing high shoots and many flowers per shoot is still advantageous in terms of fruit and seed production, in spite of the increased predation in plants with these traits. Plants may experience trade-offs when expressing traits that could increase reproductive output (by e.g. increasing pollinator visitation or time available for seed maturation, Aizen 2003, Munguía-Rosas et al. 2011), because those traits could also attract antagonistic interactors (Ehrlén et al. 2002, Strauss and Whittall 2006, Sletvold et al. 2015), and therefore decrease reproductive output at the same time. The relative importance of direct effects of traits on reproductive output vs indirect effects through seed predation will depend on the intensity of the interaction, which in turn could show important spatiotemporal variation (Kolb et al. 2007a). In this study, the negative effect of seed predation on reproductive output was not strong enough to counteract the positive effects of early phenology, flower production and plant height. Cariveau et al. (2004) also found that flower number had net positive effects on seed set after accounting for the negative effects mediated by seed predation. We have found similar patterns for flowering phenology and shoot height. Flowering phenology in *G. pneumonanthe* directly affected seed production, but not fruit production. This means that that plants that flower early produce more seeds per fruit than plants flowering late, and agrees with previous evidence showing that early-flowering plants often have higher seed set (i.e. the proportion of ovules that develop into seeds, Forrest and Thomson 2010). On the other hand, plants with higher shoots produced both more fruits and more seeds per fruit, and this could relate to higher pollinator attractiveness of tall plants (Ehrlén et al. 2002).

The context where the interaction takes place affects reproductive output mostly indirectly through its effects on predation intensity. On the one hand, higher abundances of *Myrmica* ants close to the plants indirectly decrease their reproductive output due to higher predation intensities. Thus, we have demonstrated that the abundance of a community member not directly involved in an antagonistic interaction (i.e. seed predation) can affect not only the intensity of that interaction, but also its outcome for one of the interactors (i.e. the host plant). On the other hand, the height of the surrounding vegetation has both positive and negative indirect effects on *G. pneumonanthe* reproductive output: it contributes to increase reproductive output because plants surrounded by high vegetation show lower predation intensities; however, through its positive effect on ant abundance, which in turn increases predation, it also contributes to decrease reproductive output. This is an example of how two factors related to the context can influence each other to modify the outcome of the interaction. Finally, soil temperature increases reproductive output both directly (by increasing fruit production) and indirectly (by reducing predation intensity). Microclimate might affect pollinator behavior (Herrera 1995) and low temperatures have also been shown to reduce fertilization success and the speed of seed maturation (Totland 2001)

**Concluding remarks**

Our results contribute to gain a better understanding of species interactions by simultaneously assessing the effects of trait variation and of the context on the outcome of the interaction.

Context-dependence of plant-animal interactions implies not only direct, but also indirect effects of the context on the outcome of the interaction. As we have demonstrated, environmental factors can modify the abundance of community members that in turn determine the intensity of plant-animal interactions, as well as their outcome.

Predator preferences on traits can lead to selection…

Few studies have simultaneously assessed the effects of trait variation and the effects of environmental context.

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TABLES

Table 1: Results of model selection and model averaging for the best candidate models (negative binomial GLMs, n = 303) relating intensity of predation by *P. alcon* to plant reproductive traits (PCA1 traits = first axis of the PCA performed with the 3 traits, see Figure S1), environmental and community contexts and population. Averaged coefficients () across all models with ΔAICc < 2 and their adjusted standard errors (Adj. SE) are shown for variables included in this set of models. The relative importance (W) of each variable was calculated as the sum of Akaike weights across all models in which the respective variable occurred. R2 = 0.35 for the best model.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | |  | | Adj. SE | | W |
| Traits | | PCA1 traits | 1.152\*\*\* | | 0.264 | | 1.00 |
| Env. context | | Soil temperature | -0.848\*\*\* | | 0.204 | | 1.00 |
| Vegetation height | -0.940\*\*\* | | 0.224 | | 1.00 |
| Comm. context | | *Myrmica* abundance | 0.491\* | | 0.198 | | 0.99 |
|  |  | | R | T | R | T |  |
| Population |  | | -0.753\* | -1.126\*\*\* | 0.368 | 0.342 | 1.00 |
| Population x | | PCA1 traits | -0.133 | -0.197 | 0.267 | 0.325 | 0.33 |
| Soil temperature | 0.453 | 0.349 | 0.368 | 0.345 | 0.63 |
| Vegetation height | 0.406 | 0.833\*\* | 0.282 | 0.310 | 0.86 |
| *Myrmica* abundance | -0.172 | -0.391 | 0.383 | 0.244 | 0.69 |

\*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.05

Table 2: Results of model selection showing the best candidate model (poisson GLMs, n = 303) relating number of intact fruits of *G. pneumonanthe* to plant reproductive traits (PCA1 traits = first axis of the PCA performed with the 3 traits, see Figure S1), environmental context, intensity of predation by *P. alcon* and population. This was the only model with ΔAICc < 2. Coefficients () and their standard errors (SE) are shown for variables included in this model. The relative importance (W) of each variable was calculated as the sum of Akaike weights across all models in which the respective variable occurred. R2 = 0.83.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | |  | | SE | | W |
| Traits | | PCA1 traits | 0.651\*\*\* | | 0.060 | | 1.00 |
| Env. context | | Soil temperature | 0.211\* | | 0.102 | | 0.78 |
| Pred. intensity | | Number of eggs | -0.424\*\*\* | | 0.060 | | 1.00 |
|  |  | | R | T | R | T |  |
| Population |  | | -0.306 | 1.499\*\*\* | 0.338 | 0.200 | 1.00 |
| Population x | | PCA1 traits |  |  |  |  | 0.24 |
| Soil temperature |  |  |  |  | 0.18 |
| Number of eggs |  |  |  |  | 0.22 |

\*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.05

FIGURE LEGENDS

Figure 1: Path diagram showing the results of the piecewise SEMs of reproductive traits, context and intensity of predation by *P. alcon* as predictors of reproductive output of *G. pneumonanthe*. The diagram combines results from two different SEMs with either number of intact fruits (n = 303; Fisher′s C = 14.99; P = 0.242) or number of seeds (n = 245; Fisher′s C = 14.38; P = 0.570) as estimates of reproductive output. All path coefficients except those for the effects on number of seeds were obtained from the first model. Arrows represent unidirectional relationships among variables. Numbers on the arrows indicate path coefficients, both unstandardized (in italics) and standardized (see text for details on calculation of both types of coefficients). Solid black arrows represent significant paths (P < 0.05 for unstandardized coefficients), solid grey arrows represent non-significant paths, and dotted arrows represent paths that were tested in previous models but not included in the final model. When only the standardized coefficient was not significant, “ns” is noted). R2s for component models are shown in the boxes of endogenous variables. Population effects on all endogenous variables and correlations among traits were significant but are not included in the figure for simplicity (see Table S1 for coefficients of correlation among traits).

Figure 1

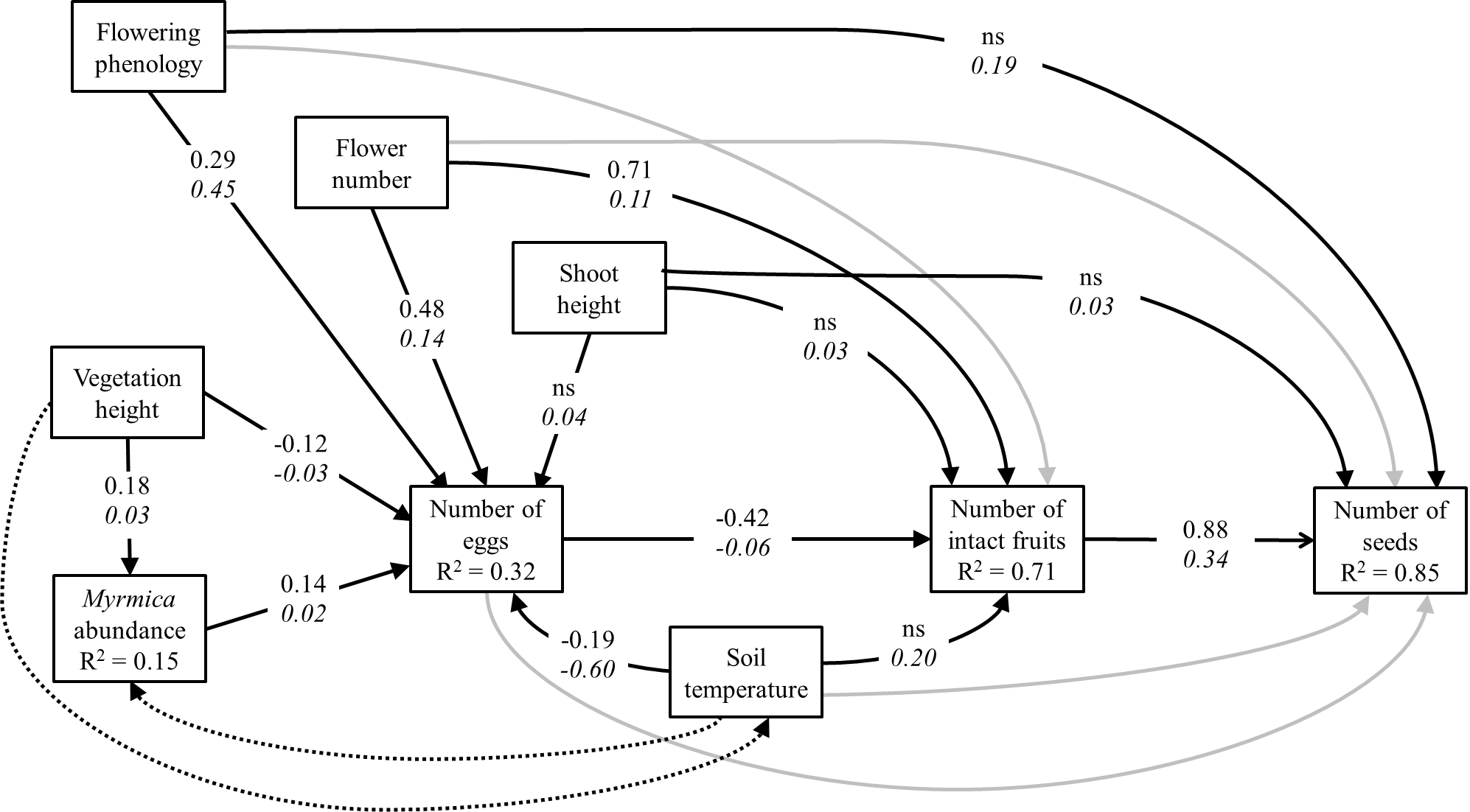


Table S1: Correlation matrix for the 3 reproductive traits measured. Values are Pearson correlation coefficients and were significant in all cases.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Flowering phenology | Flower number | Shoot height |
| Flowering phenology | 1 |  |  |
| Flower number | 0.69 | 1 |  |
| Shoot height | 0.33 | 0.57 | 1 |

Table S2: Results of model selection and model averaging for the best candidate models (negative binomial GLMs, n = 303) relating intensity of predation by *P. alcon* to 3 plant reproductive traits, environmental and community contexts and population. Averaged coefficients () across all models with ΔAICc < 2 and their adjusted standard errors (Adj. SE) are shown for variables included in this set of models. The relative importance (W) of each variable was calculated as the sum of Akaike weights across all models in which the respective variable occurred. Generalized variance inflation factors (GVIF) were calculated for the global model including all predictors without interactions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | |  | | Adj. SE | | W | GVIF |
| Traits | | Flowering phenology | 0.524 \*\*\* | | 0.121 | | 1 | 2.50 |
| Flower number | 0.509 | | 0.329 | | 0.97 | 3.00 |
| Shoot height | 0.462 \* | | 0.217 | | 0.90 | 2.02 |
| Env. context | | Soil temperature | -0.846 \*\*\* | | 0.209 | | 1 | 2.26 |
| Vegetation height | -1.001 \*\*\* | | 0.262 | | 1 | 1.78 |
| Comm. context | | *Myrmica* abundance | 0.532 \*\* | | 0.187 | | 0.99 | 1.22 |
|  |  | | R | T | R | T |  |  |
| Population |  | | -0.774 \* | -1.231 \*\*\* | 0.362 | 0.359 | 1 | 6.49 |
| Population x | | Flowering phenology |  |  |  |  | 0.16 |  |
| Flower number | 0.040 | -0.204 | 0.366 | 0.354 | 0.48 |  |
| Shoot height | -0.323 | -0.104 | 0.355 | 0.231 | 0.49 |  |
| Soil temperature | 0.421 | 0.331 | 0.372 | 0.349 | 0.58 |  |
| Vegetation height | 0.546 | 0.878\*\* | 0.359 | 0.326 | 0.78 |  |
| *Myrmica* abundance | -0.087 | 0.427 | 0.391 | 0.224 | 0.67 |  |

\*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.05

Table S3: Results of model selection and model averaging for the best candidate models (poisson GLMs, n = 303) relating number of intact fruits of *G. pneumonanthe* to 3 plant reproductive traits, environmental context, intensity of predation by *P. alcon* and population. Averaged coefficients () across all models with ΔAICc < 2 and their adjusted standard errors (Adj. SE) are shown for variables included in this set of models. The relative importance (W) of each variable was calculated as the sum of Akaike weights across all models in which the respective variable occurred. Generalized variance inflation factors (GVIF) were calculated for the global model including all predictors without interactions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | |  | | Adj. SE | | W | GVIF |
| Traits | | Flowering phenology | 0.125 | | 0.095 | | 0.72 | 2.08 |
| Flower number | 0.298 | | 0.209 | | 1 | 2.48 |
| Shoot height | 0.166 | | 0.125 | | 0.92 | 1.97 |
| Env. context | | Soil temperature | 0.227\* | | 0.110 | | 0.78 | 1.67 |
| Pred. intensity | | Number of eggs | -0.404\*\*\* | | 0.061 | | 1 | 1.27 |
|  |  | | R | T | R | T |  |  |
| Population |  | | -0.229 | 1.640\*\*\* | 0.371 | 0.245 | 1 | 2.22 |
| Population x | | Flowering phenology |  |  |  |  | 0.09 |  |
| Flower number | 0.122 | 0.054 | 0.339 | 0.198 | 0.25 |  |
| Shoot height | 0.100 | 0.037 | 0.226 | 0.111 | 0.24 |  |
| Soil temperature |  |  |  |  | 0.17 |  |
| Number of eggs |  |  |  |  | 0.21 |  |

\*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.05

Figure S1: PCA diagram of the reproductive traits, showing their correlation with the two first PCA axes. The black circle represents correlation = 1. Points correspond to individual plants.

