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# Indirect effects of microclimate on plant reproductive performance via an antagonistic interaction

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Keywords:	micro-climatic variation, plant reproduction, plant-animal interactions, butterflies, <i>Myrmica</i> , environmental context
Abstract:	<p>Climatic variation can influence plant reproduction directly, but also via changes in plant traits, interactions with animals, and the surrounding environment. Such indirect effects can often be complex and involve multiple steps. While the joint effects of climatic variation and indirect effects in terms of plant-animal interactions have sometimes been assessed at larger spatial scales, little is known about how microclimatic variation affects within-population variation in reproductive performance. Here, we studied the direct and indirect effects of microclimate on reproductive performance of the plant <i>Gentiana pneumonanthe</i> in presence of the butterfly seed predator <i>Phengaris alcon</i>. We found that microclimatic effects on plant performance were mainly indirect. The number of seeds per flower of <i>G. pneumonanthe</i> decreased in warm microsites, and this effect was mediated by increased seed predation by <i>P. alcon</i>. The effect of soil temperature was particularly pronounced in sites with high soil moisture. Effects of soil temperature and moisture on the incidence of predation and plant performance were also mediated by effects on plant phenology, density and phenology of neighboring host plants and host ant abundance. Plants that flowered earlier and where host ants were more abundant, and plants surrounded by fewer and later-flowering neighbors, produced fewer seeds per flower because of a higher incidence of predation. Our results demonstrate that effects of microclimatic variation on plant reproductive performance are mostly indirect and largely mediated by species interactions. These findings highlight that among-individual variation in small-scale environmental conditions within populations can cause variation in individual plant performance through multiple pathways.</p>

## 1 INTRODUCTION

2 Climate is a main driver of plant distributions, and differences in climatic conditions over many  
3 different spatial scales might be important for plant performance (Woodward and Williams 1987).  
4 Sexual plant reproduction is particularly sensitive to climatic variations (Hedhly et al. 2009), and  
5 several stages of the reproductive process, from flower development to pollination, fertilization and  
6 seed and fruit maturation, might be affected (Bykova et al. 2012). Experimental evidence also  
7 shows that the production of plant reproductive structures might depend on temperature and water  
8 availability. For example, warming can increase seed biomass (Klady et al. 2011), and drought may  
9 reduce seed set due to resource limitation (del Cacho et al. 2013). Understanding how climatic  
10 variation over different spatial scales influences the reproductive performance of plants is thus  
11 important to understand differences in performance among and within natural plant populations.  
12 While the effects of climatic variability on plant performance are relatively well-studied at  
13 moderate and large spatial scales (e.g. García et al. 2000, De Frenne et al. 2009, 2010, Dainese  
14 2011), they have not been explored at smaller spatial scales, although it is well-known that  
15 organisms do not sample environmental data at such coarse scales (Geiger et al. 2009).

16       The influence of climatic variability on plant reproductive performance might also be  
17 indirect, and mediated by changes in plant traits, such as reproductive phenology (Inouye 2008), or  
18 changes in interactions with other species (Bale et al. 2002, Memmott et al. 2007). Changes in  
19 interactions are likely to play an important role, as they have been shown to strongly influence plant  
20 fitness (Agrawal et al. 2012, Ågren et al. 2013), and animals interacting with plants might be  
21 sensitive to the environmental context of the plant (Chamberlain et al. 2014). Moreover, indirect  
22 effects of climate on plant performance might often involve multiple steps. For example, climatic  
23 variation might induce changes in plant phenology, which in turn influences the intensity of  
24 mutualistic or antagonistic interactions (Elzinga et al. 2007, Valdés and Ehrlén 2017) and plant  
25 reproductive performance. Climate might also affect the context of plant-animal interactions, for

example through effects on vegetation height (Valdés and Ehrlén 2018), canopy cover (Kolb and Ehrlén 2010), community composition (Biere and Tack 2013, Wynhoff and van Langevelde 2017), and density and quality of conspecific or heterospecific plant neighbors (Hambäck et al. 2014, Valdés and Ehrlén 2019). For example, small-scale variation in soil temperature or water availability may condition plant density, and differences in plant densities might in turn lead to differences in intensity of herbivory and in plant reproductive output (Shea et al. 2000). Taken together, this means that identifying the effects of variation in microclimate on reproductive performance and the complex indirect effects of climate is important for understanding within-population variation in plant performance.

In this study, we investigated the effects of microclimate on reproductive performance of the plant *Gentiana pneumonanthe* in presence of an antagonist, the specialized seed-predator butterfly *Phengaris alcon*. The caterpillars of these butterflies live inside the fruits of *G. pneumonanthe* during their first instars, and parasitize the nests of ants (*Myrmica* spp.) during later stages. It has previously been shown that the effects of plant phenology and ant presence on oviposition patterns are interdependent: butterflies oviposit preferentially on early-flowering plants where host ants are abundant (Valdés and Ehrlén 2019). Also the density and phenology of neighboring host plants influences oviposition patterns, with higher oviposition probability in plants with few late-flowering neighbors (Valdés and Ehrlén 2019). Here, we examine how microclimate may affect plant performance of *G. pneumonanthe*, through effects on butterfly oviposition, plant phenology, plant density, and the distribution of ant hosts. We addressed the following questions: 1) What are the direct effects of microclimate on plant reproductive performance?, 2) What are the indirect effects of microclimate on plant reproductive performance, mediated by changes in plant phenology and predation by *P. alcon*?, and 3) What are the indirect effects of microclimate on plant reproductive performance, mediated in two steps by effects via plant phenology or the plant context, on predation by *P. alcon*?

51

52 MATERIAL AND METHODS

53 **Study system**

54 The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb which occurs in  
55 open habitats as wet heathlands and grasslands (Simmonds 1946). Plants have one to many shoots  
56 measuring up to 45 cm. The species is self-compatible and flowers in July and August in the study  
57 area (SW Sweden), producing deep blue flowers that are pollinated by bumblebees. Fruits are  
58 capsules containing many small, wind-dispersed seeds (mean seed number per fruit  $\pm$  SD =  $490 \pm$   
59  $243$ , mean seed weight =  $0.041$  mg, authors' unpublished data). *G. pneumonanthe* is the host plant  
60 of the Alcon Blue butterfly (*Phengaris alcon*), a specialist predispersal seed predator. Adult female  
61 butterflies oviposit on young flower buds in July and August (Appelqvist et al. 2007), and usually  
62 lay more than one egg on the same plant or even on the same bud. Females have not been shown to  
63 avoid plants that already carry eggs (Van Dyck and Regniers 2010). The caterpillars feed inside the  
64 fruit until their fourth instar, when they drop to the ground and are picked up by *Myrmica* ants. Due  
65 to caterpillars mimicking the surface chemistry of the ant brood (Nash et al. 2008) and the acoustic  
66 signals of queen ants (Sala et al. 2014), ants carry them to their nest, where they live as brood  
67 parasites for the rest of their larval period. *Phengaris* females detect host ants through plant  
68 chemicals produced by ants or by plants in response to root tissue damage caused by ants (Van  
69 Dyck et al. 2000, Patricelli et al. 2015, Wynhoff and van Langevelde 2017). *P. alcon* is a “cuckoo”  
70 species and does not prey on ant brood (Als et al. 2004), but instead caterpillars consume  
71 regurgitations from ant workers, trophic eggs (i.e. nutritious, infertile eggs which are fed to ant  
72 queens and larvae) and prey items brought to the nest by ants. *Myrmica* sp. ants are the only hosts  
73 of *P. alcon*, and *M. rubra* is considered to be the most common host ant species in Sweden (Elmes  
74 et al. 1994).

75 **Data collection**

76 Field work was carried out between 9 July and 3 September 2016 in a population of *G.*  
77 *pneumonanthe*, where the butterfly *P. alcon* was present, located in Tånga Hed nature reserve  
78 (Västra Götaland, SW Sweden, 58°01'40.0"N 12°49'47.0"E). The study population occupies a  
79 fenced meadow grazed by cattle late in the summer every year. A study plot of 69 m × 45 m was  
80 established in the meadow. This plot was subdivided into a grid of 345 3 m × 3 m subplots with  
81 corners marked by wooden poles (see Valdés and Ehrlén 2019, Fig. 1). In 154 of the subplots that  
82 were occupied by *G. pneumonanthe*, all reproductive shoots were mapped by recording their  
83 coordinates within subplots, and their phenology and the number of eggs of *P. alcon* were recorded  
84 between 29 July and 5 August (18 additional occupied subplots were not surveyed due to time  
85 constraints). Shoots were mapped instead of plant individuals (having one or several shoots)  
86 because we judged that individual shoots are the most relevant unit for the butterfly female to assess  
87 the food resource quality, and act as the unit of attraction for butterfly females. Phenology of each  
88 shoot was recorded as the developmental stage of the most advanced bud, according to six ordinal  
89 categories: (1) sepals covering the bud completely, (2) bud becoming visible, (3) bud growing over  
90 the sepals, (4) bud turning blue, (5) flower opening, and (6) flower wilting (Valdés and Ehrlén  
91 2017, 2018, 2019). Higher values of this phenology measure indicate a more advanced floral  
92 development at the day of recording and thus an earlier flowering phenology, with a one-unit  
93 increase roughly corresponding to one week earlier development (Valdés and Ehrlén 2017, 2018,  
94 2019). Eggs were counted on the whole shoot.

95 Up to five *G. pneumonanthe* shoots belonging to five different plants were permanently  
96 marked into each subplot to measure plant reproductive performance. Less shoots (1-4) were  
97 marked if less than five plants were available in the subplot. In the marked shoots, we recorded  
98 number of flowers between 29 July and 5 August, and counted the number of intact (i.e. not  
99 damaged by the butterfly) and preyed fruits between 30 August and 1 September. We also took a  
100 sample of one intact and preyed fruit (when available) per shoot. The number of developed seeds in

these fruits was then counted in the lab. For each of the marked shoots, we calculated the number of seeds per flower as a measure of plant reproductive performance, as:

$$nseedsperflower = \frac{nseedsintactfruit \times nintactfruits + nseedspreyedfruit \times npreyedfruits}{nflowers}$$

The ant community was sampled using sugar baits at each corner of all occupied subplots (a total of 254 points, (Valdés and Ehrlén 2019, Fig. 1) at two occasions, 14 July and 3 September. Baits consisted of a sugar cube placed into a 50-ml plastic tube which was left open on the ground in the evening, and collected the next morning. Ants collected were transferred to 70° ethanol and the number of ants of different *Myrmica* species were then counted in the lab (using Douwes et al. 2012 for identification).

Two microclimatic variables (soil temperature and soil moisture) were recorded at the same 254 points. At each point, an iButton data logger was buried in the soil at 5 cm depth in mid-July 2016. Data loggers recorded temperature every third hour from 14 July to 2 September. We used this information to calculate the average of daily mean temperatures over the recording period (hereafter, soil temperature). At the same points, we measured soil moisture (%) with a Theta Probe sensor connected to a HH2 moisture meter (Delta-T Devices, Cambridge, UK) on one occasion (30 July). We chose a date when the conditions were semi-dry (i.e. 5 days after the last rain), in order to get maximum resolution in moisture measurements. Although moisture values might vary a lot from one day to another, the relative differences should be similar, independently of the date. We took three moisture measurements at each point, and calculated the average of the three values (hereafter, soil moisture).

## **Data preparation**

Field maps were digitized using a Geographic Information System (GIS) in ArcGIS 10.3.1, where we assigned relative coordinates to each recorded shoot of *G. pneumonanthe*. We defined a buffer area with a 3-m radius, centered at each shoot (see Valdés and Ehrlén 2019, Fig. 1), and calculated the number of reproductive shoots of *G. pneumonanthe* per m<sup>2</sup> within this buffer area

(hereafter, “neighbor density”) as well as the mean phenology of these shoots (hereafter, “neighbor phenology”), excluding in each case the focal shoot.

The locations of the 254 points where the ant community and microclimatic variables were sampled were also incorporated into the GIS. We used inverse distance weighted interpolation (Pebesma 2004) based on the values at the sampling points with a maximum distance of 3 m to generate values of *Myrmica rubra* abundance over the surface of all occupied subplots (see Valdés and Ehrlén 2019 for more details on the choice of ant species and maximum distance). From this interpolated surface (see Supporting Information Figure S1 in Valdés and Ehrlén 2019), we extracted values of *M. rubra* abundance for each of the mapped shoots (hereafter, “ant abundance”).

We used an ordinary kriging interpolation (autoKrige function of the R package automap, Hiemstra et al. 2009) based on the values of soil temperature and soil moisture at the sampling points to generate values of these two microclimatic variables over the surface of all occupied subplots. This method first produces a variogram to describe the spatial autocorrelation between every pair of points. Then, an interpolation model is fitted to this variogram to assign values (with standard errors) over the interpolated surface based on the distance between sampling points and the location to be predicted. From these interpolated surfaces, we extracted values of soil temperature and soil moisture for each of the mapped shoots.

### Statistical analyses

First, in order to evaluate the overall relationship between reproductive performance of *G. pneumonanthe* (measured as the number of seeds per flower) and microclimate, we performed univariate linear regressions of number of seeds per flower against soil temperature and soil moisture.

Second, in order to decompose the overall relationship into different pathways, we applied piecewise structural equation modeling (piecewise SEM) to assess the relationships between microclimate, plant phenology and context (in terms of host ant presence, density and phenology of

151 neighboring host plants), occurrence of seed predation by *P. alcon*, and the number of seeds per  
 152 flower. We chose a piecewise approach, rather than the traditional variance-covariance based SEM,  
 153 because it allows to fit multiple separate linear models with non-normal distributions. In our  
 154 models, we considered both direct effects of microclimate on number of seeds per flower, indirect  
 155 effects mediated by seed predation or plant phenology, and indirect effects mediated by effects of  
 156 changes in plant phenology and plant context, on seed predation. Before performing the SEMs, we  
 157 checked for a possible correlation between soil temperature and soil moisture. As the correlation  
 158 was relatively high (Pearson's  $r = -0.40$ ,  $p < 0.001$ ), we performed a principal component analysis  
 159 (PCA, Table A1) on these two variables. The first and second axes of this PCA explained,  
 160 respectively, 68.5% and 31.5% of the total variance. The first axis (hereafter, "PC1") was positively  
 161 correlated with soil temperature and negatively correlated with soil moisture, while the second axis  
 162 (hereafter, "PC2") was positively correlated with both soil temperature and soil moisture. PC1 and  
 163 PC2 were then used as explanatory variables in the SEM.

164         The piecewise SEM consisted of: 1) a linear model (LM) with plant phenology as the  
 165 response variable and PC1 and PC2 as predictor variables; 2) a generalized linear model (GLM)  
 166 with a negative binomial error distribution with ant abundance as the response variable and PC1 and  
 167 PC2 as predictor variables; 3) a LM with neighbor density as the response variable and PC1 and  
 168 PC2 as predictor variables; 4) a LM with neighbor phenology as the response variable and PC1 and  
 169 PC2 as predictor variables; 5) a GLM with a binomial error distribution with a binomial variable  
 170 "egg occurrence" (being 1 when the plant had at least one egg, and 0 otherwise) as the response  
 171 variable and plant phenology, ant abundance, the interaction plant phenology  $\times$  ant abundance, and  
 172 the interaction neighbor density  $\times$  neighbor phenology as predictor variables; and 6) a LM with the  
 173 number of seeds per flower as the response variable and plant phenology, egg occurrence, PC1 and  
 174 PC2 as predictor variables. We first constructed a global model containing all possible paths (Fig.  
 175 A1). We then simplified this model by backward stepwise removing paths based on Akaike



information criterion corrected for small sample sizes (AICc). For this, we constructed alternative models by removing one path at a time, and considered that alternative models improved the model fit to the data if the AICc was more than two units lower than the AICc of the original model (models with  $\Delta\text{AICc} < 2$  are considered to fit the data equally well, Burnham and Anderson 2002). All alternative models showed a  $\Delta\text{AICc} < 2$ , and we therefore retained the global model. Overall fit of the model was assessed using Shipley's test of direct separation (d-separation, Shipley 2009), which evaluates the probability that none of the paths missing from the hypothesized causal network contain useful information. This test yields the Fisher's C statistic that is  $\chi^2$ -distributed. A  $\chi^2$ -value of Fisher's C below the significance level ( $p < 0.05$ ) indicates that the model is inconsistent with the data, and should be rejected. The test of direct separation suggested several missing paths between variables. We modeled these paths as correlated errors, as no causal relationships between them appeared meaningful (see Table A2). Finally, obtaining standardized coefficients for model 2) was not possible because of transformation via the link function of the GLM. Therefore, we repeated the piecewise SEM using a LM for model 2) in order to obtain standardized coefficients for this model (although their significance was assessed using the previously fitted GLM).

Statistical analyses were carried out in R ver. 3.6.2 (R Core Team 2020) using (apart from the above mentioned) the packages MASS (Venables and Ripley 2002) and piecewiseSEM (Lefcheck 2016).

194

## 195 RESULTS

There was considerable variation in microclimate among *G. pneumonanthe* plants: soil temperature ranged from 14.7 to 17.2 °C with a mean of 16.2 °C, and soil moisture ranged from 35.4 to 80.4 % with a mean of 66.1 %. Plant reproductive performance, measured as the number of seeds per flower, decreased with soil temperature (Fig. 1A,  $\beta = -66.41$ ,  $p = 0.002$ , adjusted  $R^2 = 0.02$ ) and showed a tendency to increase with soil moisture (Fig. 1B,  $\beta = 1.53$ ,  $p = 0.059$ , adjusted  $R^2 = 0.01$ ).

201           The piecewise SEM model had a reasonable fit to the data (Fisher's  $C = 13,61$ ,  $p = 0.093$ ).  
 202 Reproductive performance decreased with increasing soil temperature, especially in combination  
 203 with increasing soil moisture (negative effects of PC1 and strong negative effects of PC2 on number  
 204 of seeds per flower). However, there was no evidence of significant direct effects of microclimate  
 205 on plant reproductive performance. Instead, microclimate affected reproductive performance  
 206 indirectly through multiple pathways (Fig. 2, Table 1). Predation by *P. alcon* was more likely to  
 207 occur in plants located at microsites with a high temperature (significant positive effects of PC1 and  
 208 PC2 on egg occurrence), and this increased occurrence of predation in warmer microsites led to a  
 209 reduced number of seeds per flower. There were no significant indirect effects of microclimate on  
 210 reproductive performance mediated only by changes in plant phenology, as plant phenology did not  
 211 have a significant direct effect on reproductive performance.

212           Indirect effects of microclimate on plant reproductive performance also acted through  
 213 pathways involving multiple steps (Fig. 2, Table 1). Microclimate was related to plant phenology  
 214 and to the plant context, and these factors influenced predation and reproductive performance.  
 215 Plants flowered earlier in microsites with higher temperature in combination with lower moisture  
 216 (high values of PC1), and later in microsites with higher temperature in combination with higher  
 217 moisture (high values of PC2). Early-flowering plants, in turn, had a higher occurrence of predation  
 218 than late-flowering plants and therefore produced less seeds per flower. The increase in egg  
 219 occurrence with an earlier phenology was stronger in plants where host ants were abundant, as  
 220 evidenced by the significant effect of the interaction plant phenology  $\times$  ant abundance. Ant  
 221 abundance, in turn, was higher in microsites with higher temperature in combination with lower  
 222 moisture (high values of PC1). Moreover, pathways involving conspecific neighbors also mediated  
 223 effects of microclimate on plant performance. Neighbor density was lower and neighbors flowered  
 224 earlier in microsites with higher temperature in combination with lower moisture (high values of  
 225 PC1), while the opposite was true in microsites with higher temperature in combination with higher

226 moisture (high values of PC2). Plants surrounded by few late-flowering neighbors showed a higher  
227 egg occurrence, as evidenced by the significant effect of the interaction neighbor density  $\times$  neighbor  
228 phenology, and therefore produced less seeds per flower.

229

## 230 DISCUSSION

231 Our results show that the effects of microclimate on reproductive performance of the plant  
232 *G. pneumonanthe* were mainly indirect. Microclimate, in terms of soil temperature and moisture,  
233 was related to seed predation by the butterfly *P. alcon*, which had a strong negative effect on plant  
234 performance. Microclimate also influenced plant phenology, host ant abundance and density and  
235 phenology of neighboring host plants, which in turn affected seed predation and plant reproductive  
236 output.

237 We found no direct effects of microclimate on reproductive performance of *G.*  
238 *pneumonanthe*. Direct effects of climatic variation might be more important at larger spatial scales  
239 than the microclimatic differences among plants within a population that we studied. Still, the role  
240 of direct effects in previous larger-scale studies reporting climatic influences on plant reproduction  
241 is difficult to assess because these studies do not separate direct and indirect effects (García et al.  
242 2000, De Frenne et al. 2009, 2010, Dainese 2011). Experimental studies with other species have  
243 found effects of small-scale warming and drought on seed production (Klady et al. 2011, del Cacho  
244 et al. 2013). These studies also did not discriminate between direct and indirect effects on  
245 reproductive performance, but suggested that the observed responses to experimental treatments  
246 might partly be due to effects of warming on plant phenology (Klady et al. 2011), or due to effects  
247 of drought on plant size (del Cacho et al. 2013). Although the range of variation, at least in soil  
248 temperature, was larger in our study than in these previous studies, we did not detect direct effects  
249 of microclimate on plant performance.

250 In contrast to the lack of direct effects, we detected several indirect effects of microclimate  
 251 on reproductive performance of *G. pneumonanthe*. The overall impact of these indirect effects was  
 252 that the number of seeds per flower was lower in warm microsites, particularly when soil moisture  
 253 was high. The incidence of predation by *P.alcon* was higher in warm microsites, irrespective of soil  
 254 moisture. Microclimatic effects on the intensity of plant-animal interactions, such as seed predation,  
 255 might be due to effects on larval growth and survival (Bonebrake et al. 2010). Our results agree  
 256 with previous studies showing an increased incidence of butterfly oviposition in warmer  
 257 microclimates, and that this effect is particularly pronounced near the margins of their geographical  
 258 range (Eilers et al. 2013, Fourcade and Öckinger 2016). The increased incidence of eggs in *G.*  
 259 *pneumonanthe* individuals in warmer microsites was associated with a reduced reproductive output.  
 260 Our results therefore show that antagonistic interactions play an important role in mediating effects  
 261 of microclimate on local-scale environmental variation on plant performance.

262 Microclimate was also related to incidence of predation and performance of *G.*  
 263 *pneumonanthe* individuals through effects on plant phenology, host ant abundance, and neighboring  
 264 conspecific plants. Plants flowered earlier in warm microsites if the soil moisture content was low,  
 265 but later if moisture was high. The few studies that have documented variations in plant phenology  
 266 at small scales in relation to soil temperature (Dahlgren et al. 2007, Toftegaard 2016), found that  
 267 plants flowered earlier in warmer microsites. Earlier flowering has also been related to higher  
 268 nutrient levels (Dahlgren et al. 2007), and one possibility is that the later flowering of *G.*  
 269 *pneumonanthe* observed in warm and moist microsites is due to that moister microsites are also  
 270 poorer in nutrients. In accordance with previous findings (Valdés and Ehrlén 2018), variation in  
 271 plant phenology only affected reproductive performance indirectly, through its effects on the  
 272 antagonistic interaction with *P.alcon*.

273 There was also a higher abundance of host ants (i.e. *Myrmica rubra*) in warm and dry  
 274 microsites. *Myrmica* ants occupy different ecological niches with respect to vegetation and soil

275 characteristics (Elmes et al. 1998). Specifically, *M. rubra* shows preferences for intermediate soil  
276 temperature and moisture (Elmes et al. 1998), and might thus avoid moist microsites. Lastly, the  
277 fact that neighbor density was higher in warm and moist microsites, might be the result of that a  
278 higher water availability in the soil can support higher plant densities.

279         As a consequence of the earlier phenology, higher abundance of host ants and lower  
280 neighbor density, the incidence of *P. alcon* attacks increased, and reproductive performance of *G.*  
281 *pneumonanthe* decreased, in warm and dry microsites. However, these effects of microclimate were  
282 partly counteracted by that individuals in warm and dry microsites also had neighbors with an  
283 earlier phenology, and this acted to decrease the incidence of attacks and increase plant  
284 reproductive performance. In a previous study with this species it was shown that incidence of *P.*  
285 *alcon* eggs increased with an earlier plant phenology, and the preference for early-flowering host  
286 plants was stronger where host ants were abundant (Valdés and Ehrlén 2019). Moreover, plants  
287 with neighbors that were less preferred by *P. alcon* for oviposition had a higher probability of  
288 receiving eggs. As a result of these interactive effects, plants that flowered earlier and where host  
289 ants were more abundant, and plants surrounded by fewer and later-flowering neighbors produced  
290 fewer seeds per flower because they had a high occurrence of predation. The fact that both plant  
291 phenology, host ant abundance and density and phenology of neighboring conspecific plants were  
292 related to microclimate, implies that microclimate indirectly affects plant reproduction not only by  
293 directly influencing the intensity of plant-animal interactions, but also by modifying plant  
294 phenology and the plant context, which in turn influence interactions. Although indirect effects of  
295 climate on plant reproduction through altered species interactions have been investigated before  
296 (e.g. Lemoine et al. 2017, Richman et al. 2020), the type of complex indirect effects of  
297 microclimate on within-population differences in plant reproductive performance found in this  
298 study have not previously been explored.

Our results illustrate the complexity of the relationship between local-scale environmental variation and plant performance, and highlight that species interactions can play an important role in mediating effects of climate. We simultaneously assessed the effects of microclimatic variation via multiple pathways, including direct effects of microclimate on plant performance, effects via plant-animal interactions, as well as effects of microclimate on plant traits and the environmental context that influence these interactions. This enabled us to provide a more comprehensive understanding of the many and complex ways in which among-individual variation in small-scale climatic conditions within populations can affect individual plant performance. Approaches considering multiple pathways, such as the one used in this study, provide a powerful way to identify the environmental causes of differences in performance of individuals over small spatial scales, which is necessary to understand the ecological and evolutionary dynamics of plant populations.

#### DATA ACCESSIBILITY STATEMENT

Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository and the data DOI will be included at the end of the article.

#### REFERENCES

- Agrawal, A. A. et al. 2012. Insect herbivores drive real-time ecological and evolutionary change in plant populations. - *Science* 338: 113–116.
- Ågren, J. et al. 2013. Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. - *Proc. Natl. Acad. Sci.* 110: 18202–18207.
- Als, T. D. et al. 2004. The evolution of alternative parasitic life histories in large blue butterflies. - *Nature* 432: 386–390.
- Appelqvist, T. et al. 2007. Åtgärdsprogram för alkonblåvinge och klockgentiana 2007-2011: *Maculinea alcon* och *Gentiana pneumonanthe*. - Naturvårdsverket.
- Bale, J. S. et al. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. - *Global Change Biology* 8: 1–16.
- Biere, A. and Tack, A. J. M. 2013. Evolutionary adaptation in three-way interactions between plants, microbes and arthropods. - *Funct. Ecol.* 27: 646–660.
- Bonebrake, T. C. et al. 2010. Oviposition behavior and offspring performance in herbivorous insects: consequences of climatic and habitat heterogeneity. - *Oikos* 119: 927–934.

- Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical information-theoretic approach. - Springer.
- Bykova, O. et al. 2012. Temperature dependence of the reproduction niche and its relevance for plant species distributions. - J. Biogeogr. 39: 2191–2200.
- Chamberlain, S. A. et al. 2014. How context dependent are species interactions? - Ecol. Lett. 17: 881–890.
- Dahlgren, J. P. et al. 2007. Variation in vegetative and flowering phenology in a forest herb caused by environmental heterogeneity. - Am. J. Bot. 94: 1570–1576.
- Dainese, M. 2011. Impact of land use intensity and temperature on the reproductive performance of *Dactylis glomerata* populations in the southeastern Alps. - Plant Ecol. 212: 651–661.
- De Frenne, P. et al. 2009. Unravelling the effects of temperature, latitude and local environment on the reproduction of forest herbs. - Glob. Ecol. Biogeogr. 18: 641–651.
- De Frenne, P. et al. 2010. Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. - For. Ecol. Manag. 259: 809–817.
- del Cacho, M. et al. 2013. Reproductive output in Mediterranean shrubs under climate change experimentally induced by drought and warming. - Perspect. Plant Ecol. Evol. Syst. 15: 319–327.
- Douwes, P. et al. 2012. Steklar: Myror-getingar: Hymenoptera: Formicidae-Vespidae. - ArtDatabanken, Sveriges lantbruksuniversitet.
- Eilers, S. et al. 2013. Micro-climate determines oviposition site selection and abundance in the butterfly *Pyrgus armoricanus* at its northern range margin. - Ecol. Entomol. 38: 183–192.
- Elmes, G. W. et al. 1994. Differences in host-ant specificity between Spanish, Dutch and Swedish populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.)(Lepidoptera). - Memorab. Zool. 48: 55–68.
- Elmes, G. W. et al. 1998. The ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. - J. Insect Conserv. 2: 67–78.
- Elzinga, J. A. et al. 2007. Time after time: flowering phenology and biotic interactions. - Trends Ecol. Evol. 22: 432–439.
- Fourcade, Y. and Öckinger, E. 2016. Host plant density and patch isolation drive occupancy and abundance at a butterfly's northern range margin. - Ecol. Evol. 7: 331–345.
- García, D. et al. 2000. Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. - J. Ecol. 88: 435–446.
- Geiger, R. et al. 2009. The climate near the ground. - Rowman & Littlefield Publishers.
- Hambäck, P. A. et al. 2014. Effects of plant neighborhoods on plant–herbivore interactions: resource dilution and associational effects. - Ecology 95: 1370–1383.
- Hedhly, A. et al. 2009. Global warming and sexual plant reproduction. - Trends Plant Sci. 14: 30–36.
- Hiemstra, P. H. et al. 2009. Real-time automatic interpolation of ambient gamma dose rates from the Dutch radioactivity monitoring network. - Comput. Geosci. 35: 1711–1721.
- Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. - Ecology 89: 353–362.
- Klady, R. A. et al. 2011. Changes in high arctic tundra plant reproduction in response to long-term experimental warming. - Glob. Change Biol. 17: 1611–1624.
- Kolb, A. and Ehrlén, J. 2010. Environmental context drives seed predator-mediated selection on a floral display trait. - Evol. Ecol. 24: 433–445.
- Lefcheck, J. S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. - Methods Ecol. Evol. 7: 573–579.
- Lemoine, N. P. et al. 2017. Responses of plant phenology, growth, defense, and reproduction to interactive effects of warming and insect herbivory. - Ecology 98: 1817–1828.

- Memmott, J. et al. 2007. Global warming and the disruption of plant–pollinator interactions. - Ecology Letters 10: 710–717.
- Nash, D. R. et al. 2008. A mosaic of chemical coevolution in a Large Blue butterfly. - Science 319: 88–90.
- Patricelli, D. et al. 2015. Plant defences against ants provide a pathway to social parasitism in butterflies. - Proc R Soc B 282: 20151111.
- Pebesma, E. J. 2004. Multivariable geostatistics in S: the gstat package. - Comput. Geosci. 30: 683–691.
- R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Richman, S. K. et al. 2020. Asynchronous range shifts drive alpine plant–pollinator interactions and reduce plant fitness. - Glob. Change Biol. 26: 3052–3064.
- Sala, M. et al. 2014. Variation in butterfly larval acoustics as a strategy to infiltrate and exploit host ant colony resources. - PLoS ONE 9: e94341.
- Shea, K. et al. 2000. Effect of patch size and plant density of Paterson’s curse (*Echium plantagineum*) on the oviposition of a specialist weevil, *Mogulones larvatus*. - Oecologia 124: 615–621.
- Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. - Ecology 90: 363–368.
- Simmonds, N. W. 1946. *Gentiana pneumonanthe* L. - J. Ecol. 33: 295–307.
- Toftegaard, T. 2016. Temperature and the synchrony of plant-insect interactions.
- Valdés, A. and Ehrlén, J. 2017. Caterpillar seed predators mediate shifts in selection on flowering phenology in their host plant. - Ecology 98: 228–238.
- Valdés, A. and Ehrlén, J. 2018. Direct and plant trait-mediated effects of the local environmental context on butterfly oviposition patterns. - Oikos 127: 825–833.
- Valdés, A. and Ehrlén, J. 2019. Resource overlap and dilution effects shape host plant use in a myrmecophilous butterfly. - J. Anim. Ecol. 88: 649–658.
- Van Dyck, H. et al. 2000. Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? - Proc. R. Soc. Lond. B Biol. Sci. 267: 861–866.
- Van Dyck, H. and Regniers, S. 2010. Egg spreading in the ant-parasitic butterfly, *Maculinea alcon*: from individual behaviour to egg distribution pattern. - Anim. Behav. 80: 621–627.
- Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S. - Springer.
- Woodward, F. I. and Williams, B. G. 1987. Climate and plant distribution at global and local scales. - Vegetatio 69: 189–197.
- Wynhoff, I. and van Langevelde, F. 2017. *Phengaris (Maculinea) teleius* butterflies select host plants close to *Myrmica* ants for oviposition, but *P. nausithous* do not. - Entomol. Exp. Appl. 165: 9–18.



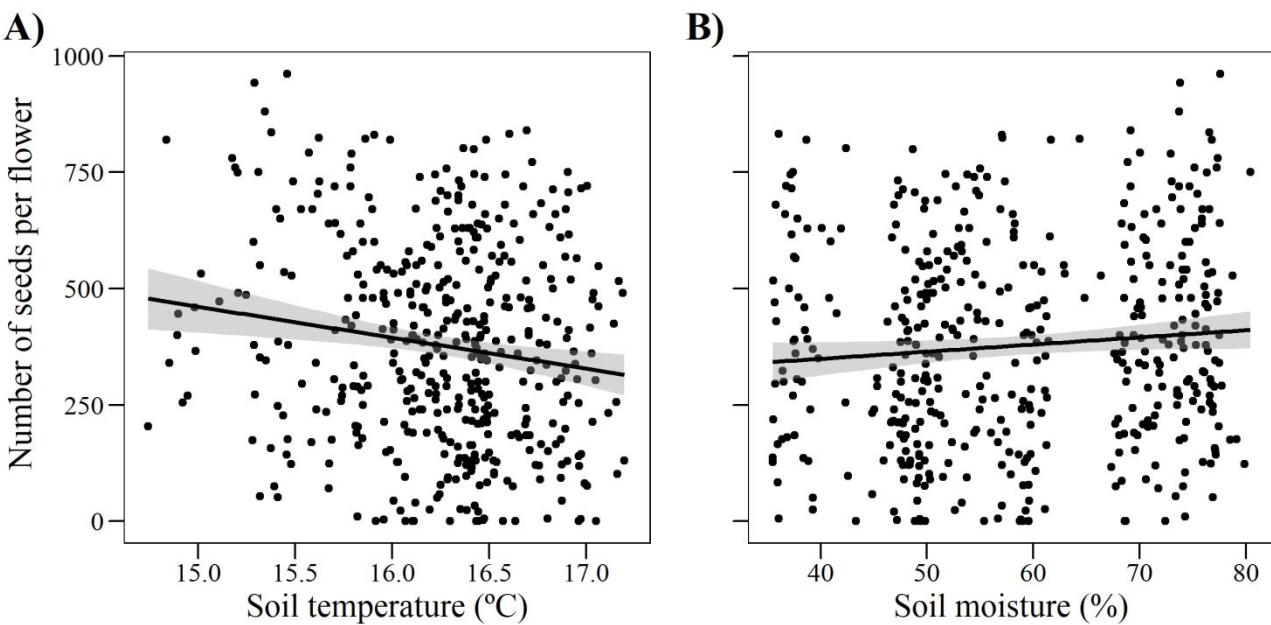
Table 1: Effects of microclimate (first and second axes of a PCA performed on soil temperature and soil moisture) on plant reproductive performance (number of seeds per flower) calculated from the piecewise SEM (Fig. 1). Total, direct and indirect effects are shown, and indirect effects are divided into different pathways.

Type of effect on number of seeds per flower	Microclimatic variable	
	PC1 (high temperature, low moisture)	PC2 (high temperature, high moisture)
Total effect	-0.033	-0.169
Direct effect	-0.032	-0.040
Indirect effect	-0.001	-0.129
Through changes in predation	-0.052	-0.073
Through changes in phenology	0.025	-0.026
Through effects of changes in plant phenology and ant abundance on predation	-0.050	0.055
Through effects of changes in neighbour density and phenology on predation	0.076	-0.084

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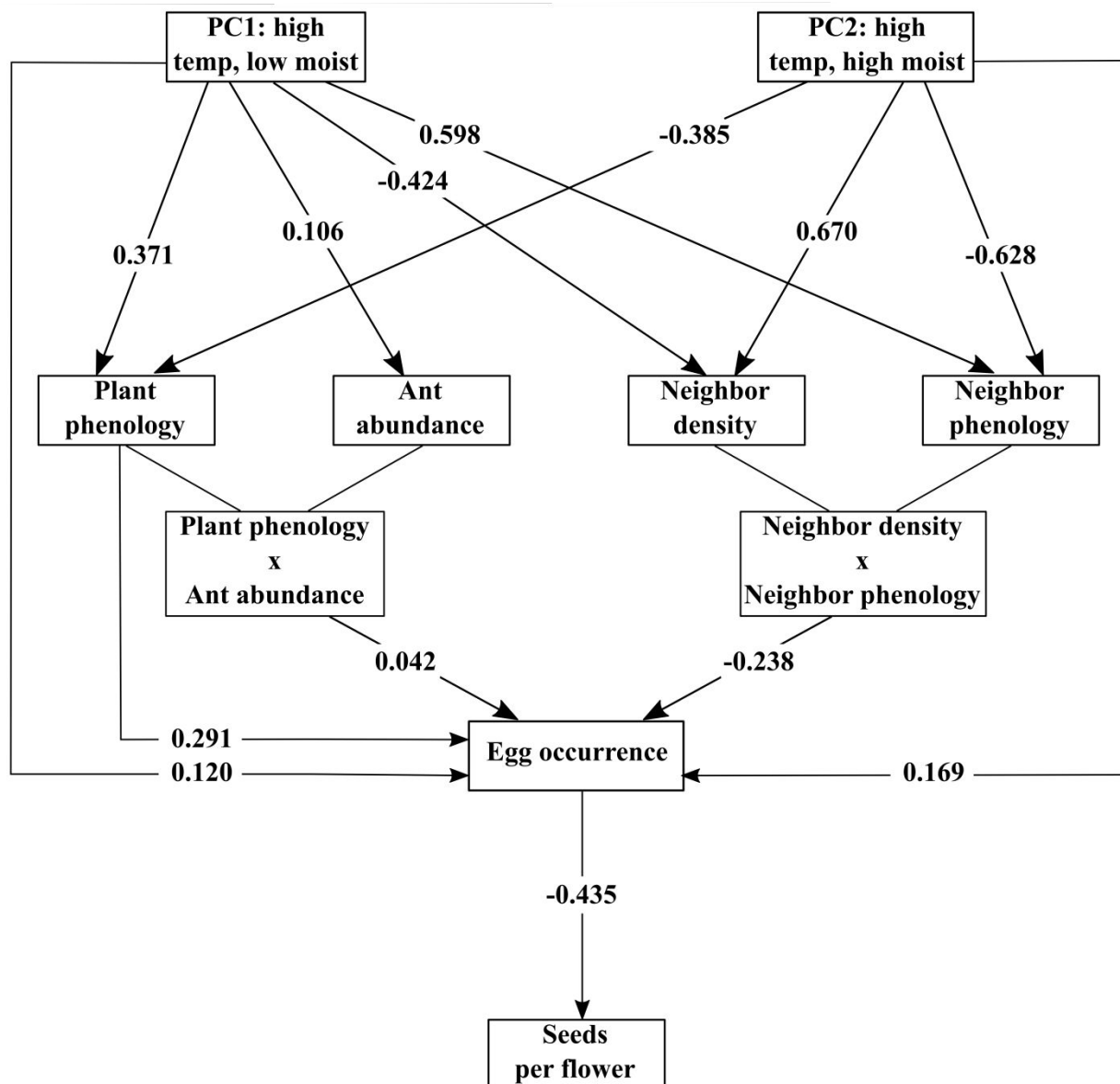
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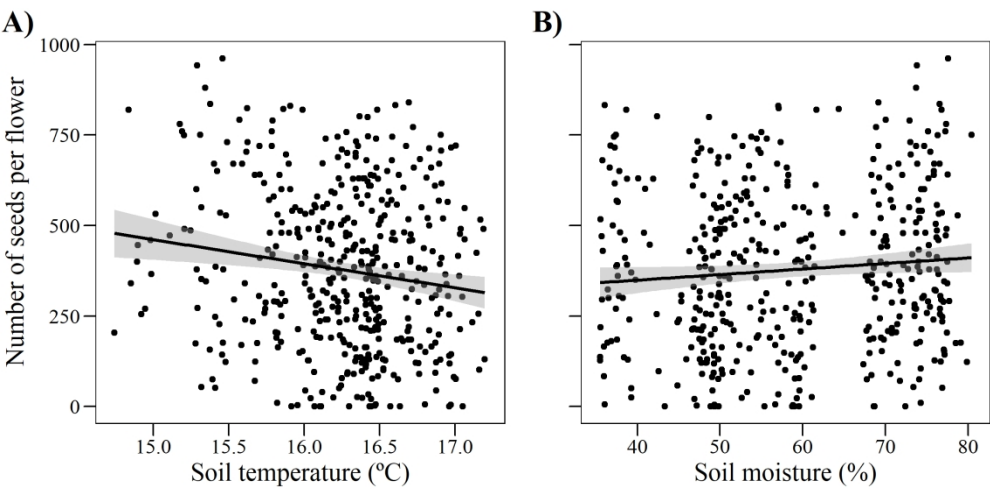
319 Figure 1: Relationships among the number of seeds per flower in *Gentiana pneumonanthe* and A)  
320 soil temperature and B) soil moisture. Fit lines and confidence intervals for linear models (n = 454  
321 plants where seed data was available) are shown in each case.



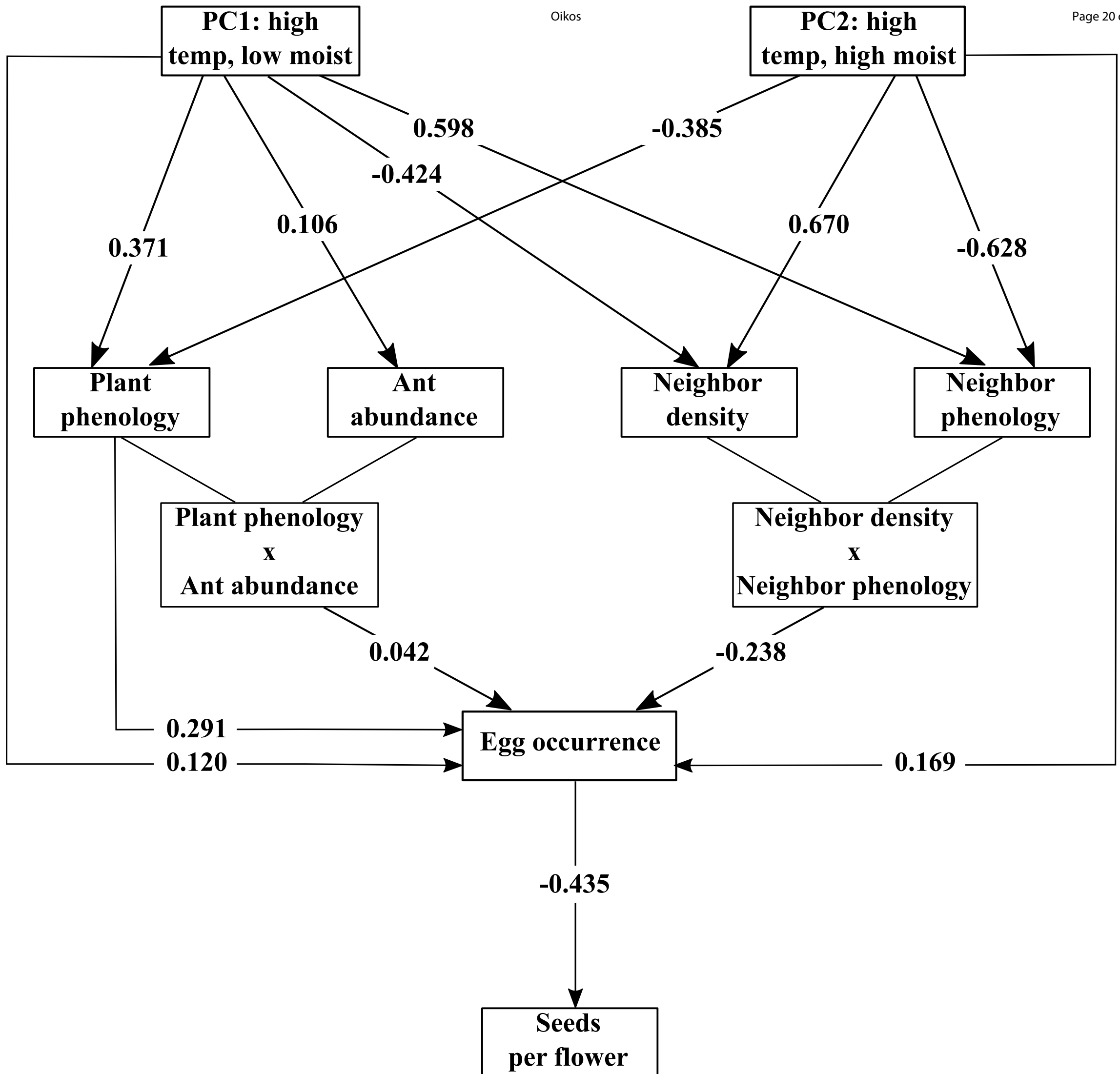
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Figure 2: Results of a piecewise structural equation model (piecewise SEM) examining the relationships among microclimate (first and second axes of a PCA performed on soil temperature and soil moisture), *G. pneumonanthe* phenology and context (host ant abundance, density and phenology of neighboring host plants), occurrence of seed predation by *P. alcon*, and *G. pneumonanthe* reproductive performance (number of seeds per flower). Values shown are standardized coefficients. Non-significant relationships and correlated errors have been omitted from the diagram for clarity (see Table A2 for the full model output).





219x109mm (300 x 300 DPI)



Supplementary material

Table A1: Variable loadings and correlations between each variable and the two axes of a principal component analysis (PCA) performed on soil temperature and soil moisture.

Variables	PC1		PC2	
	Eigenvalue = 1.369		Eigenvalue = 0.631	
	Loading	Correlation	Loading	Correlation
Soil temperature	0.707	0.827	0.707	0.562
Soil moisture	-0.707	-0.827	0.707	0.562

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Table A2: Results of a piecewise structural equation model (piecewise SEM) examining the relationships among microclimate (first and second axes of a PCA performed on soil temperature and soil moisture), *G. pneumonanthe* phenology and context (host ant abundance, density and phenology of neighboring host plants), occurrence of seed predation by *P. alcon*, and *G. pneumonanthe* reproductive performance (number of seeds per flower). All significant and non-significant predictors are shown. Pairs of variables with correlated errors (i.e. those not considered causal but which had a significant correlation) are denoted by  $\sim$ .

Response	Predictor	Unstandardized path coefficient	Standard error	Standardized path coefficient	P	R <sup>2</sup>
Plant phenology	PC1	0.266	0.013	0.378	<0.001	0.11
	PC2	-0.534	0.020	-0.385	<0.001	
Ant abundance	PC1	0.525	0.026	0.106	<0.001	0.04
	PC2	-0.007	0.037	-0.026	0.847	
Neighbor density	PC1	-2.858	0.083	-0.424	<0.001	0.42
	PC2	8.720	0.122	0.670	<0.001	
Neighbor phenology	PC1	0.264	0.003	0.598	<0.001	0.67
	PC2	-0.535	0.005	-0.628	<0.001	
Egg occurrence	Plant phenology	0.865	0.051	0.291	<0.001	0.45
	Ant abundance	-0.184	0.095	-0.130	0.053	
	Neighbor density	0.090	0.045	0.283	0.050	
	Neighbor phenology	-0.105	0.120	-0.023	0.383	
	PC1	0.255	0.043	0.120	<0.001	
	PC2	0.695	0.078	0.169	<0.001	
	Plant phenology $\times$ Ant abundance	0.045	0.019	0.042	0.012	
	Neighbor density $\times$ Neighbor phenology	-0.070	0.012	-0.238	<0.001	
Seeds per flower	Plant phenology	14.770	9.603	0.068	0.125	0.19
	Egg occurrence	-197.745	20.692	-0.435	<0.001	
	PC1	-5.039	6.938	-0.032	0.468	

	PC2	-12.127	12.996	-0.040	0.351
~~ Neighbor phenology	~~ Neighbor density	-0.143		-0.143	<0.001
~~ Plant phenology	~~ Neighbor phenology	0.236		0.236	<0.001
~~ Neighbor phenology	~~ Ant abundance	-0.048		-0.048	<0.001
~~ Neighbor density	~~ Ant abundance	0.081		0.081	<0.001
~~ Plant phenology	~~ Neighbor density	-0.041		-0.041	<0.001

For Review Only



Figure A1: Global piecewise structural equation model (piecewise SEM) examining the relationships among microclimate (first and second axes of a PCA performed on soil temperature and soil moisture), *G. pneumonanthe* phenology and context (host ant abundance, density and phenology of neighboring host plants), occurrence of seed predation by *P. alcon*, and *G. pneumonanthe* reproductive performance (number of seeds per flower). Alternative models were obtained by simplification of the global model through backwards stepwise removal of paths.

