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

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ABSTRACT

Climatic conditions 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 among-individual variation in reproductive performance of the plant *Gentiana pneumonanthe* in presence of the butterfly seed predator *Phengaris alcon*. We found that microclimatic effects on plant performance were mainly indirect, and that effects of temperature and moisture were interactive. The number of seeds per flower of *G. pneumonanthe* decreased in cold and moist microsites, and these effects were mediated by an increased oviposition by *P. alcon* in these microsites. The effects of soil temperature and moisture on the incidence of oviposition and plant performance were 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 especially plants surrounded by fewer and later-flowering neighbors, produced fewer seeds per flower because of a higher incidence of oviposition. 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.

KEYWORDS: micro-climatic variation, plant reproduction, plant-animal interactions, butterflies, *Myrmica*, environmental variation, environmental context

DECLARATIONS

- Author contributions: A.V. and J.E. designed the study. A.V. collected the data. A.V. analyzed the data with inputs from J.E. A.V. and J.E. wrote the manuscript. Both authors gave final approval for publication.

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INTRODUCTION

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

The influence of climatic variability on plant reproductive performance might not only be direct but also indirect, and mediated by changes in plant traits, such as reproductive phenology (Inouye 2008), or interactions with other species (Bale et al. 2002, Memmott et al. 2007). Changes in interactions are likely to play an important role, as they have been shown to often strongly influence plant fitness (Agrawal et al. 2012, Ågren et al. 2013), and as many animals interacting with plants are sensitive to the environmental context of the plant (Chamberlain et al. 2014). Moreover, such animal-mediated effects of climate on plant performance might often involve multiple steps. For example, climatic variation might induce changes in plant phenology, which in turn influences the intensity of mutualistic or antagonistic interactions (Elzinga et al. 2007, Valdés and Ehrlén 2017). Climate might also affect the context of plant-animal interactions 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 both the direct and complex indirect effects of variation in microclimate is important to understand within-population variation in plant reproductive 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). The density and phenology of neighboring host plants also 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 more specific questions: 1) Does microclimate have direct effects on plant reproductive performance?, 2) Does microclimate influence plant reproductive performance indirectly, through changes in plant phenologyor oviposition by *P. alcon*?, and 3) Can indirect effects of microclimate on plant reproductive performance be mediated in two steps, by effects via plant phenology or the plant context on oviposition by *P. alcon*?

MATERIAL AND METHODS

**Study system**

The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb which occurs in open habitats as wet heathlands and grasslands (Simmonds 1946). Plants have one to many shoots measuring up to 45 cm in height. The species is self-compatible and flowers in July and August in the study area (SW Sweden), producing deep blue flowers that are pollinated by bumblebees. Fruits are capsules containing many small, wind-dispersed seeds (mean seed number per fruit ± SD = 490 ± 243, mean seed weight = 0.041 mg, authors’ unpublished data). *G. pneumonanthe* is the host plant of the Alcon Blue butterfly (*Phengaris alcon*), a specialist predispersal seed predator. Adult female butterflies oviposit on young flower buds in July and August (Appelqvist et al. 2007), and usually lay more than one egg on the same plant or even on the same bud. Females have not been shown to avoid plants that already carry eggs (Van Dyck and Regniers 2010). The caterpillars feed inside the fruit until their fourth instar. Seed predation by *P. alcon* caterpillars strongly reduces seed production, and preyed fruits produce fewer seeds than intact fruits (mean ± SD = 13.8 ± 57.8 vs. 493.1 ± 208.6, calculated from a sample of 459 plants). After the fourth instar, the caterpillars drop to the ground and are picked up by *Myrmica* ants. Due to caterpillars mimicking the surface chemistry of the ant brood (Nash et al. 2008) and the acoustic signals of queen ants (Sala et al. 2014), ants carry them to their nest, where they live as brood parasites for the rest of their larval period. *Phengaris* females detect host ants through chemicals produced by ants or by plants in response to root tissue damage caused by ants (Van Dyck et al. 2000, Patricelli et al. 2015, Wynhoff and van Langevelde 2017). *P. alcon* is a “cuckoo” species and does not prey on ant brood (Als et al. 2004), but instead caterpillars consume regurgitations from ant workers, trophic eggs (i.e. nutritious, infertile eggs which are fed to ant queens and larvae) and prey items brought to the nest by ants. *Myrmica* sp. ants are the only hosts of *P. alcon*, and *M. rubra* is considered to be the most common host ant species in Sweden (Elmes et al. 1994).

**Data collection**

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

Up to five *G. pneumonanthe* shoots belonging to five different plants were permanently marked into each subplot to measure plant reproductive performance. Fewer shoots (1-4) were marked if few than five plants were available in the subplot. In the marked shoots, we recorded number of flowers once per shoot between 29 July and 5 August, and counted the number of intact (i.e. not damaged by the butterfly) and preyed fruits once per shoot between 30 August and 1 September. We also took a 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:

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 70º 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). While it is true that daily mean temperature in the soil might not accurately represent the microclimate experienced by the flowers, this measure integrates temperature conditions over a long time period. Relative differences among plants in soil temperature are thus likely to be similar to relative differences in air temperature. Moreover, soil temperature might be more related to some of the variables representing the plant context (e.g. host ant abundance, plant density, see below) than air 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, we expect relative differences to remain similar over the flowering season. 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 m2 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 at which soil temperature and soil moisture were 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. As residual spatial autocorrelation could lead to biased model estimates and invalid statistical inference (Dormann et al., 2007), we checked for spatial autocorrelation in the residuals of the univariate linear regressions by plotting spatial correlograms and by calculating global Moran's I with a permutation test (1000 random permutations), based on a connectivity matrix of pairwise Euclidean distances among the shoots up to a distance of 30 m. We found significant autocorrelation in the residuals of both univariate linear regressions (global Moran's I = 0.011, p = 0.005 for soil temperature and global Moran's I = 0.013, p = 0.006 for soil moisture, Figure A1). We thus applied Moran's eigenvector mapping (MEM, Dray et al. 2006, Thayn and Simanis 2013) to both regressions. MEM translates the spatial arrangement of data points into eigenvectors that capture spatial effects. We then refitted the regressions including, in each case, one eigenvector issued from MEM as spatial predictor, and this removed the residual spatial autocorrelation (global Moran's I = -0.001, p = 0.270 for soil temperature and global Moran's I = -0.002, p = 0.323 for soil moisture, Figure A1).

Second, in order to decompose the overall relationship into different pathways, we applied piecewise structural equation modelling (piecewise SEM) to assess the relationships between microclimate, plant phenology and context (in terms of host ant presence, density and phenology of neighboring host plants), occurrence of oviposition by *P. alcon*, and the number of seeds per flower. We chose a piecewise approach, rather than the traditional variance-covariance based SEM, because it allows to fit multiple separate linear models with non-normal distributions. In our models, we considered both direct effects of microclimate on number of seeds per flower, indirect effects mediated by occurrence of oviposition or plant phenology, and indirect effects mediated by effects of changes in plant phenology and plant context, on occurrence of oviposition.

The piecewise SEM consisted of six component models: 1) a linear model (LM) with plant phenology as the response variable and soil temperature, soil moisture and their interaction as predictor variables; 2) a generalized linear model (GLM) with a negative binomial error distribution with ant abundance as the response variable and soil temperature, soil moisture and their interaction as predictor variables; 3) a LM with neighbor density as the response variable and soil temperature, soil moisture and their interaction as predictor variables; 4) a LM with neighbor phenology as the response variable and soil temperature, soil moisture and their interaction as predictor variables; 5) a GLM with a binomial error distribution with a binomial variable “egg occurrence” (being 1 when the shoot had at least one egg, and 0 otherwise) as the response variable and plant phenology, ant abundance, the interaction plant phenology  ant abundance, neighbor density, neighbor phenology, the interaction neighbor density  neighbor phenology, soil temperature, soil moisture and their interaction as predictor variables; and 6) a LM with the number of seeds per flower as the response variable and plant phenology, egg occurrence, soil temperature, soil moisture and their interaction as predictor variables. We used a LM for plant phenology, and thus treated phenology as a numeric variable rather than an ordinal variable, because a one-unit increase in phenology roughly corresponds to one week earlier development. We also used a LM for neighbor phenology because it could take any values between 1 and 6. We use egg occurrence as a variable accounting for the existence of an interaction between the butterfly and the plant, but not as a measure of presence or absence of predation, as some shoots with eggs might finally not suffer from seed predation (e.g. if eggs do not hatch). One might thus argue that the presence of eggs is a better estimate of the intensity of the butterfly-plant interaction, while seed predation is more of an estimate of effects on plant fitness. For the model of egg occurrence, we included only interactions that were biologically plausible based on our knowledge of the study system and on previous evidence (Valdés and Ehrlén 2019). In all component models, we considered the possibility of interactive effects of both microclimatic variables by testing for effects of the interaction soil temperature  soil moisture, but removed the interaction term when it was not significant to allow for simpler models.

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 χ2-distributed. A χ2-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 modelled these paths as correlated errors, as we were uncertain regarding the direction of any potential underlying causal relationships between them (see Table A1). Finally, obtaining standardized coefficients for component 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 component model 2) in order to obtain standardized coefficients for this model.

We checked for spatial autocorrelation in the residuals of the six component models of the SEM as explained previously. We found significant autocorrelation in the residuals of component models 1-5 (Figure A2), and thus applied MEM to these models. Each of the models was then refitted including, in each case, one or several (up to five) eigenvectors issued from MEM as spatial predictors, and this removed the residual spatial autocorrelation (global Moran's I was non-significant for all models, Figure A2).

Statistical analyses were carried out in R version 4.1.0 (R Core Team 2021) using (apart from the above mentioned) the packages MASS (Venables and Ripley 2002), spdep (Bivand et al. 2013), adespatial (Dray et al. 2021) and piecewiseSEM (Lefcheck 2016).

RESULTS

There was considerable variation among *G. pneumonanthe* plants regarding the microclimate conditions experienced; soil temperature ranged from 14.7 to 17.2 ºC (mean ± SD = 16.2 ± 0.5 ºC), and soil moisture ranged from 35.4 to 80.4 % (mean ± SD = 66.1 ± 10.8 %). Plant reproductive performance, measured as the number of seeds per flower, did not significantly change with soil temperature after correcting for residual spatial autocorrelation (Table 1A) but decreased with increasing soil moisture (Table 1B).

The piecewise SEM model had a reasonable fit to the data after including the missing paths as correlated errors (Fisher’s C = 10.86, p = 0.093). There was no evidence of significant direct effects of microclimate on plant reproductive performance. Instead, microclimate affected reproductive performance indirectly through multiple pathways (Fig. 1, Table 2, Table A1). Oviposition by *P. alcon* occurred more often in plants located at microsites with a high temperature, resulting in a reduced number of seeds per flower. There were no significant indirect effects of microclimate on reproductive performance mediated only by changes in plant phenology, as plant phenology did not have a significant direct effect on reproductive performance.

Indirect effects of microclimate on plant reproductive performance also acted through pathways involving multiple steps (Fig. 1, Table 2, Table A1). Microclimate was related to plant phenology and to the plant context, and these two parameters influenced oviposition and reproductive performance. Soil temperature and soil moisture showed significant interactive effects on plant phenology, ant abundance, neighbor density and neighbor phenology (Fig. 1, Table A1, Fig. 2). Plants flowered earlier in colder and drier microsites (Fig. 2A). Ant abundance, in turn, decreased with temperature in dry microsites, but increased with temperature in wet microsites (Fig. 2B). Early-flowering plants where host ants were abundant showed a higher occurrence of oviposition, as evidenced by the significant effect of the interaction plant phenology  ant abundance on egg occurrence (Fig. 3A), and therefore produced less seeds per flower (Fig. 1, Table A1). Also pathways involving conspecific neighbors mediated effects of microclimate on plant performance, and their effects were the most important, especially for soil temperature (Table 2). Neighbor density decreased with temperature in dry microsites, but increased with temperature in wet microsites (Fig. 2C), and neighbors flowered earlier in colder and drier microsites (Fig. 2D). Plants surrounded by few, late-flowering neighbors showed a higher egg occurrence, as evidenced by the significant effect of the interaction neighbor density  neighbor phenology on egg occurrence (Fig. 3B), and therefore produced less seeds per flower (Fig. 1, Table A1).

DISCUSSION

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

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

In contrast to the lack of direct effects, we detected several indirect effects of microclimate on reproductive performance of *G. pneumonanthe*. The overall impact of these indirect effects was that the number of seeds per flower increased with soil temperature and decreased with soil moisture, the effect of temperature being more important that the effect of moisture (total effects, Table 2). The occurrence of oviposition by *P. alcon* was higher in warm microsites, but was not related to soil moisture after correcting for residual spatial autocorrelation. Microclimatic effects on the intensity of plant-animal interactions, such as seed predation, might be due to effects on larval growth and survival, as females select oviposition sites that maximize development and survival of larvae (Bonebrake et al. 2010). Our results agree with previous studies showing an increased incidence of butterfly oviposition in warmer microclimates, and that this effect is particularly pronounced near the margins of their geographical range (Eilers et al. 2013, Fourcade and Öckinger 2016). The increased incidence of eggs in *G. pneumonanthe* individuals in warmer microsites was associated with a reduced reproductive output. Our results therefore show that antagonistic interactions play an important role in mediating effects of microclimate on local-scale environmental variation on plant performance.

Microclimate also affected oviposition by *P. alcon* and the reproductive performance of *G. pneumonanthe* individuals indirectly via effects on plant phenology, host ant abundance, and neighboring conspecific plants, and the effects of soil temperature and moisture were interactive. Plants flowered latest in warm and wet microsites. In contrast, the few other studies that have documented variations in plant phenology at small scales in relation to soil temperature have found that plants flowered earlier in warmer microsites (Dahlgren et al. 2007, Toftegaard 2016). However, the distribution of soil temperature and soil moisture values shows that there are actually no plants with very low values of temperature and moisture (Fig. A3). Our analyses also suggested that the effects of moisture on plant phenology were more important than those temperature, and this was an effect of the larger range of variation in soil moisture in the study population (Fig. A3). Earlier flowering has also been related to higher nutrient levels (Dahlgren et al. 2007), and one possibility is that the later flowering of *G. pneumonanthe* observed in warm and moist microsites is due to that moister microsites are poorer in nutrients. In accordance with previous findings (Valdés and Ehrlén 2018), variation in plant phenology only affected reproductive performance indirectly, through its effects on the antagonistic interaction with *P. alcon*. Soil temperature and moisture were also correlated with host ant abundance. When soils were dry *M. rubra* were more abundant in cold microsites, but when moisture was high it was more abundant in warm microsites. *Myrmica* ants occupy different ecological niches with respect to vegetation and soil characteristics (Elmes et al. 1998). Specifically, *M. rubra* shows preferences for intermediate soil temperature and moisture (Elmes et al. 1998), and might thus avoid very warm and dry microsites, where there might be a risk of dessication, as well as very cold and wet microsites, which might be too damp. Lastly, neighbor density was the highest in warm and moist microsites. This might be the result of that these microsites can support higher plant densities due to a higher water availability in the soil and a low cover of grasses resulting from low nutrient concentrations (Oostermeijer et al. 1998).

As a consequence of the effects of microclimate on phenology, host ant abundance and neighbor density and phenology, the incidence of *P. alcon* oviposition increased, and reproductive performance of *G. pneumonanthe* decreased in cold and moist microsites (in spite of the occurrence of oviposition being higher in warm microsites). This was mainly due to effects of changes in density and phenology of neighbour on oviposition, which was the pathway with the stronger indirect effects, especially regarding temperature. Effects of changes in plant phenology and ant abundance on oviposition occurrence were less important. A previous study with this species found that the incidence of *P. alcon* eggs increased with an earlier plant phenology, and that the preference for early-flowering host plants was stronger where host ants were abundant (Valdés and Ehrlén 2019). Moreover, plants with neighbors that were less preferred by *P. alcon* for oviposition had a higher probability of receiving eggs. As a result of these interactive effects, 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 they had a high occurrence of oviposition. The fact that both plant phenology, host ant abundance and density and phenology of neighboring conspecific plants were related to microclimate in this study thus implies that microclimate indirectly affects plant reproduction by modifying plant phenology and the plant context, which in turn influence interactions. Although indirect effects of climate on plant reproduction through altered species interactions have been investigated before (e.g. Lemoine et al. 2017, Richman et al. 2020), the type of complex indirect effects of microclimate on within-population differences in plant reproductive performance found in this 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. We also showed that the effects of temperature and moisture are interactive. The approach used enables 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 plausible 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.

Bivand, R. S. et al. 2013. Applied Spatial Data Analysis with R. - Springer New York.

Bonebrake, T. C. et al. 2010. Oviposition behavior and offspring performance in herbivorous insects: consequences of climatic and habitat heterogeneity. - Oikos 119: 927–934.

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.

Dormann, C. F. 2007. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. - Global Ecology and Biogeography 16: 129–138.

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.

Dray, S. et al. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). - ecological modelling 196: 483–493.

Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N. & Wagner, H. H. (2021). adespatial: Multivariate multiscale spatial analysis. R package version 0.3-14. Retrieved from: https://CRAN.R-project.org/package=adespatial

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.

Oostermeijer, J. G. B. et al. 1998. Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. - Conservation Biology 12: 1042–1053.

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 2021. 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.

Thayn, J. B. and Simanis, J. M. 2013. Accounting for spatial autocorrelation in linear regression models using spatial filtering with eigenvectors. - Annals of the Association of American Geographers 103: 47–66.

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.