

**Resource overlap and dilution effects shape host plant use
in a myrmecophilous butterfly**

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Complete List of Authors:	Valdés, Alicia; Stockholm University, Department of Ecology, Environment and Plant Sciences; Stockholm University, Bolin Centre of Climate Research Ehrlén, Johan; Stockholm University, Department of Ecology, Environment and Plant Sciences; Stockholm University, Bolin Centre of Climate Research
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3 AUTHORS: Valdés, Alicia*,^{a, b} and Ehrlén, Johan^{a, b}

4

5 ^a Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91

6 Stockholm, Sweden

7 ^b Bolin Centre of Climate Research, Stockholm University, Stockholm, Sweden

8 *Corresponding author: aliciavaldes1501@gmail.com

9

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15 ABSTRACT

16 The effects of consumers on fitness of resource organisms are a complex function of the spatio-
17 temporal distribution of the resources, consumer functional responses and trait preferences, and
18 availability of other resources. Using the butterfly consumer *Phengaris alcon* and its two
19 sequential resources, the host plant *Gentiana pneumonanthe* and the host ants *Myrmica* spp., we
20 investigated how butterfly egg-laying depended on focal host plant quality, density and quality of
21 neighboring host plants and host ant abundance. Butterflies preferred plants that simultaneously
22 maximized the availability of both larval resources, i.e., they chose higher-quality early-
23 flowering plants where host ants were abundant. Both the probability of oviposition and the
24 number of eggs were lower in plant individuals with a high neighbor density than in more
25 isolated plants, and this dilution effect was stronger when neighbors flowered early and thus
26 were of high quality. Our results show that plant-herbivore interactions simultaneously depend
27 on the spatio-temporal distribution of a focal resource, and on the small-scale spatial variation in
28 the abundance of other herbivore resources. Given that consumers have negative effects on
29 fitness and prefer certain traits of the resource organisms, this implies that processes acting at the
30 levels of individuals, populations and communities simultaneously contribute to variation in
31 consumer-mediated natural selection.

32

33 Keywords: butterflies, flowering phenology, myrmecophily, plant-herbivore interactions,
34 resource use, spatial variation

35

36 INTRODUCTION

37 Resource-consumer interactions often have important effects on the performance of both partners
38 and the distribution and abundance of consumers is expected to match the spatio-temporal
39 distribution of their resources. Resource use by insect herbivores is often conditioned by
40 oviposition site selection by females. As larval stages usually show low mobility, insect
41 oviposition preferences are predicted to match the spatial and temporal distribution of host plants
42 suitable for offspring development (Gripenberg, Mayhew, Parnell, & Roslin, 2010; Jaenike,
43 1978; Scheirs & De Bruyn, 2002). The suitability of host plants, in turn, might depend on factors
44 such as host plant nutritional quality (Chen, Lin, Wang, Yeh, & Hwang, 2004), size (Rabasa,
45 Gutiérrez, & Escudero, 2005), microclimatic conditions (Eilers, Pettersson, & Öckinger, 2013) or
46 enemy-free space (Ballabeni, Włodarczyk, & Rahier, 2001). Most consumers depend on multiple
47 resources throughout their life cycle, e.g. different sources of food, shelter and resting sites
48 (Dennis, Shreeve, & Van Dyck, 2003, 2006). The availability of one resource might thus
49 influence the use of other resources. For example, the availability of nectar resources for adults
50 (Öckinger, 2008) or secondary hosts for larvae (Wynhoff, Bakker, Oteman, Arnaldo, & van
51 Langevelde, 2015) might determine host plant choices in butterflies.

52 The quantitative relationship between the abundance of consumers and the abundance of
53 resource organisms, and thus resource exploitation, will depend both on the birth and death
54 processes and on consumer behavior. Consumer behavior may specifically cause consumer
55 density and resource exploitation to increase (cf. resource concentration hypothesis, e.g.
56 Hambäck & Englund, 2005; Root, 1973; Stephens & Myers, 2012) or decrease (cf. resource
57 dilution, e.g. Otway, Hector, & Lawton, 2005) with increasing patch size and resource

58 abundance. Observed differences in the relationships between resource exploitation and patch
59 size or patch density among different consumers have been suggested to be the result of
60 differences in how emigration and immigration rates depend on patch size and patch density, and
61 on the scale of consumer selection (Andersson, Löfstedt, & Hambäck, 2013; Hambäck &
62 Englund, 2005). For example, a strong negative relationship between consumer density and
63 patch area observed in visual searchers like butterflies might be the result of that immigration is
64 diameter-dependent while emigration is perimeter-dependent. Not only should the abundance of
65 the resource organism be important for consumer responses, but also its quality. For example, in
66 herbivores that depend on a specific developmental stage of their host plant, the density of plants
67 in suitable developmental stages rather than the overall density is expected to influence host use.
68 Although the effects of resource density on plant-insect interactions (Kim & Underwood, 2015;
69 Kula, Castillo, Dudash, & Fenster, 2014; Verschut, Becher, Anderson, & Hambäck, 2016;
70 Yamamura, 2002) are well studied, the role of resource quality and the effects of the availability
71 of other resources have rarely been considered in these studies. Moreover, while the ecological
72 and evolutionary effects of variation in consumer-resource interaction intensities have been
73 documented for many systems (e.g. Chamberlain, Bronstein, & Rudgers, 2014; Kolb, Ehrlén, &
74 Eriksson, 2007; Vanhoenacker, Ågren, & Ehrlén, 2013), the simultaneous effects of factors at
75 different levels: individuals (e.g. traits), populations (e.g. resource density), and communities
76 (e.g. availability of other resources) have rarely been explored. This is particularly true for small
77 spatial scales, such as variation in the intensity of herbivory among individuals within plant
78 populations.

79 Myrmecophilous butterflies of the genus *Phengaris* need two sequential hosts to

80 complete their development: the caterpillars are predispersal seed predators during their first
81 instars and parasitize ant nests (*Myrmica* spp.) during later stages. Butterflies are very specific
82 regarding both host plant and host ant species and the developmental stage of host plants (G. W.
83 Elmes et al., 1998; Thomas & Elmes, 2001; Valdés & Ehrlén, 2017). Female butterflies oviposit
84 preferentially on buds in early phenological stages, probably because these constitute higher-
85 quality food; the developing seeds might become tougher, drier and more difficult to digest as
86 buds develop into flowers and fruits (Arnaldo, Gonzalez, Oliveira, Langevelde, & Wynhoff,
87 2014). In some species, the presence of the preferred bud developmental stages has been shown
88 to overlap more with the period of butterfly oviposition in early- than in late-flowering plants,
89 and individuals flowering early in the season receive more eggs (Valdés & Ehrlén, 2018).
90 Moreover, the mobility of caterpillars after leaving their host plants is low (Hayes, 2015), and
91 further development is possible only if the foraging range of suitable host ants is sufficiently
92 close to the host plant individual (usually a few meters). Butterflies are therefore expected to
93 oviposit on host plants in suitable development stages only if plants occur at micro-sites where
94 ant hosts are present (Wynhoff, Grutters, & Langevelde, 2008; Wynhoff & van Langevelde,
95 2017).

96 In this study, we investigated how the quality, in terms of the developmental stage of the
97 reproductive structures, of one resource, the focal host plant *Gentiana pneumonanthe*, the density
98 and quality of neighboring conspecific plants, and the abundance of host ants influence resource
99 use, in terms of egg-laying, by the consumer butterfly *Phengaris alcon*. We used an extensive
100 field data set to test two hypotheses: (1) Effects of plant quality and ant abundance on egg
101 distribution patterns are interdependent: butterflies choose plants for oviposition that

102 simultaneously maximize the availability of both resources, i.e. they have a stronger preference
103 for high-quality early-flowering host plants where host ants are abundant, and (2) The probability
104 of a plant receiving at least one egg, as well as the number of eggs in oviposited plants, decreases
105 with increasing conspecific neighbor density (i. e. a dilution effect), and this decrease is stronger
106 when neighbors are of high quality.

107 MATERIALS AND METHODS

108 Study system

109 The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, occurring in
110 open habitats, such as wet heathlands and grasslands (Simmonds, 1946). Plants can have one to
111 many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by
112 bumblebees. The species is self-compatible and flowers in July and August in the study area (SW
113 Sweden). Fruits are capsules containing a high number of minute, wind-dispersed seeds (mean
114 seed number per fruit \pm SD = 490 ± 243 , mean seed weight = 0.041 mg, authors' unpublished
115 data). *Gentiana pneumonanthe* is the host plant of the Alcon Blue butterfly (*Phengaris alcon*), a
116 specialist predispersal seed predator which oviposits on young flower buds in July and August
117 (Appelqvist & Bengtsson, 2007). During an oviposition event, adult female butterflies usually
118 lay more than one egg on the same plant and even on the same bud, and there is no evidence of
119 avoiding plants that already carry eggs (Van Dyck & Regniers, 2010). The caterpillars feed
120 inside the capsule until they reach the fourth instar, when they drop to the ground to be picked up
121 by *Myrmica* ants. Caterpillars mimic the surface chemistry of the ant brood (Nash, Als, Maile,
122 Jones, & Boomsma, 2008) and the acoustic signals of queen ants (Sala, Casacci, Balletto,
123 Bonelli, & Barbero, 2014), and these features cause ants to carry them to their nest, where they

124 spend the rest of their larval period as brood parasites. Females of *Phengaris* detect host ant
125 presence through plant chemicals produced by ants or by plants in response to root tissue damage
126 caused by ants (Patricelli et al., 2015; van Dyck et al., 2000; Wynhoff & van Langevelde, 2017).
127 Contrary to the majority of *Phengaris* species, which prey on ant brood, *P. alcon* is a “cuckoo”
128 species (Als et al., 2004), and caterpillars feed primarily on regurgitants from ant workers,
129 trophic eggs (i.e. nutritious, infertile eggs which are fed to the queens and ant larvae) and prey
130 items brought to the nest by ants. Only ants belonging to *Myrmica* sp. can act as hosts of *P.*
131 *alcon*. In Sweden, *M. rubra* is considered to be the most common host ant species (Graham W.
132 Elmes et al., 1994).

133 **Data collection**

134 The study was carried out in a population of *G. pneumonanthe*, where the butterfly *P.*
135 *alcon* was present, located in Tånga Hed nature reserve in the county of Västra Götaland, SW
136 Sweden (58°01'40.0"N 12°49'47.0"E). Field work was conducted between 9 July and 3
137 September 2016. The plant population occupies a fenced meadow that is grazed by cattle at the
138 end of the summer every year. A study plot measuring 69 m × 45 m was established in the
139 meadow. This plot was further divided into a grid comprising 345 3 m × 3 m subplots with
140 corners permanently marked by wooden poles (Fig. 1A). In 154 of the subplots where *G.*
141 *pneumonanthe* was present, all reproductive shoots of *G. pneumonanthe* were mapped (Fig. 1B),
142 and their phenology and the number of eggs of *P. alcon* were recorded between 29 July and 5
143 August (18 additional occupied subplots were not possible to fully survey due to time
144 constraints). Shoots were mapped by recording their coordinates within subplots. We mapped
145 shoots rather than plant individuals (that may have one or several shoots) because we judged that

146 individual shoots are the relevant unit for the butterfly to assess the food resource quality and
147 because individual shoots act as the unit of attraction for butterfly females. For each shoot,
148 phenology was recorded as the developmental stage of the most advanced bud, according to six
149 ordinal categories: (1) the sepals covering the bud completely, (2) bud becoming visible, (3) bud
150 growing over the sepals, (4) bud turning blue, (5) flower opening, and (6) flower showing signs
151 of wilting (Valdés & Ehrlén, 2017). Each stage corresponds to a time interval of flower
152 development, with higher values indicating a more advanced floral development at the day of
153 recording, i.e. an earlier flowering phenology. The number of eggs was counted on the whole
154 shoot.

155 The ant community was sampled at each corner of the occupied subplots (a total of 254
156 points) at two occasions, 14 July and 3 September (Fig. 1B). Baits consisted of a 50-ml plastic
157 tube in which we placed a sugar cube. The tubes were left open on the ground in the evening, and
158 collected the next morning. Ants collected were transferred to 70° ethanol and the number of ants
159 of different *Myrmica* species were counted in the lab (using Douwes, Abenius, Cederberg, &
160 Wahlstedt, 2012 for identification). Ants belonging to other genera where not identified to
161 species, but counted as “other ants”.

162 **Interpolations of ant abundances**

163 We also incorporated the locations of the 254 points where the ant community was sampled into
164 the GIS. We used inverse distance weighted interpolation (Pebesma, 2004) based on the values at
165 the sampling points to generate values of the abundance of ant species over the surface of all
166 occupied subplots. A maximum distance of 3 m was used because it represents the ground
167 foraging distance of most investigated *Myrmica* species (G. W. Elmes et al., 1998). From these

168 interpolated surfaces (see Figs. S1-S5), we extracted values of abundance of the different ant
169 species for each of the mapped shoots.

170 **Calculation of neighborhood measures**

171 Field maps were digitized using a Geographic Information System (GIS) created with
172 ArcGIS 10.3.1, assigning relative coordinates to each recorded reproductive shoot of *G.
173 pneumonanthe* (Fig. 1B). For each shoot, we defined a buffer zone with a 3-m radius, centered at
174 the focal shoot (see example in Fig. 1B), and calculated the number of reproductive shoots of *G.
175 pneumonanthe* per m² within this buffer zone (hereafter, “neighbor density”) as well as the mean
176 phenology of these shoots (hereafter, “neighbor phenology”), excluding in each case the focal
177 shoot.

178 **Statistical analyses**

179 The effects of shoot phenology, host ant abundance, neighbor density and neighbor phenology on
180 *P. alcon* egg distribution were examined using two Generalized Linear Models (GLMs). Effects
181 on the probability of a shoot having at least one egg were evaluated using a binomial GLM, and
182 effects on the number of eggs in shoots with at least one egg were evaluated using a negative
183 binomial GLM. Both models included also the effects of three interactions. First, we included the
184 interaction between shoot phenology and ant abundance in order to test if the effects of the two
185 resources on egg distribution patterns are interdependent. Second, we included the interaction
186 between neighbor density and neighbor phenology in order to test if the effect of host plant
187 density depends on phenology of neighboring host plants. Third, we included the interaction
188 between shoot phenology and neighbor phenology to account for the fact that the relationship
189 between egg distribution and phenology of the focal plant might depend on the phenology of

190 neighboring host plants. As a measure of host ant abundance, we used the summed abundances
191 of *M. rubra* and *M. schencki* (hereafter, “ant abundance”). *M. rubra* is considered to be the most
192 common host ant species of *P. alcon* in Sweden (Graham W. Elmes et al., 1994), and *M. schencki*
193 has been cited as host in several places in Europe (Vilbas, Esperk, & Teder, 2016). Our data
194 agreed with this previous knowledge, as the combined abundance of *M. rubra* and *M. schencki*
195 had the strongest positive effect on the probability of host plants receiving eggs (Table S1).

196 All predictors were standardized by subtracting the mean and dividing by the standard
197 deviation before the analyses. We checked for spatial autocorrelation in the residuals of models
198 by plotting spatial correlograms and by calculating global Moran's I with a permutation test
199 (1000 random permutations). Moran's I was calculated based on a connectivity matrix of
200 pairwise Euclidean distances among the shoots up to a distance of 30 m. Residual spatial
201 autocorrelation could lead to biased model estimates and invalid statistical inference (Dormann
202 et al., 2007). A significant spatial autocorrelation was found in the residuals of the binomial
203 GLM, but not for the negative binomial GLM (global Moran's I = 0.0150, $p < 0.001$ and global
204 Moran's I = 0.0023, $p = 0.613$, respectively, Figs. S6-S7). We therefore applied Moran's
205 eigenvector mapping (MEM), which translates the spatial arrangement of data points into
206 explanatory variables (eigenvectors) that capture spatial effects, to the binomial model
207 examining effects on the probability of a shoot having at least one egg (Dray, Legendre, & Peres-
208 Neto, 2006; Thayn & Simanis, 2013). The model was refitted including two eigenvectors issued
209 from MEM as spatial predictors, and this strongly reduced the residual spatial autocorrelation
210 (global Moran's I = 0.0003, $p = 0.03297$, Fig. S6). Although residual spatial autocorrelation was
211 still significant, the low value of Moran's I and visual inspection of the spatial correlogram (Fig.

212 S7) indicated that almost all of the residual spatial autocorrelation was removed by the spatial
213 predictors. The results of this model were similar to the results of the binomial GLM not
214 including the spatial predictors (Table S2). Statistical analyses were carried out in R 3.4.0 (R
215 Core Team 2017).

216 RESULTS

217 Eggs of *P. alcon* were found on 731 (8.3%) of 8848 reproductive shoots of *G.*
218 *pneumonanthe* recorded in 154 subplots. On shoots with at least one egg, the mean number of
219 eggs was 5.03 ± 0.20 SE.

220 The effects of shoot phenology and ant abundance on *P. alcon* oviposition were
221 interdependent. The probability of a shoot of *G. pneumonanthe* having at least one egg increased
222 with an earlier phenology (i.e. with a more advanced floral development at the day of recording,
223 Table 1, Fig. 2A). This increase was stronger in shoots where host ants were more abundant
224 (significant effect of the interaction shoot phenology \times ant abundance, Table 1, Fig. 2A). The
225 number of *P. alcon* eggs on individuals with at least one egg increased with an earlier shoot
226 phenology and with a higher host ant abundance, but there was no significant effect of the
227 interaction between phenology and ant abundance (Table 1, Fig. 2B and C).

228 High densities of host plant shoots resulted in a dilution effect, and the strength of this
229 effect depended on the quality of neighboring plants. Both the probability of having at least one
230 egg and the number of eggs on shoots with at least one egg decreased with the overall density of
231 neighboring host plant shoots (Table 1, Fig. 3). In both cases, the decrease was stronger when
232 neighboring host plants were of higher quality, i.e. had an earlier phenology (significant effect of
233 the interaction neighbor density \times neighbor phenology, Table 1, Fig. 3).

234 DISCUSSION

235 The results of this study show that resource use in the myrmecophilous butterfly *P. alcon*
236 is shaped by both the spatial overlap of the two key larval resources and by a dilution effect.
237 Specifically, we found that butterflies prefer to oviposit on early-flowering plants that have a
238 high abundance of host ants, that plants surrounded by many conspecific neighbors receive fewer
239 eggs, and that the dilution effect is stronger when neighbors are of a high quality.

240 We found that both the probability of *P. alcon* butterflies laying at least one egg on a host
241 plant as well as the number of eggs increased with an earlier phenology of its host plant *G.
pneumonanthe*. This agrees with previous studies showing that insects usually prefer plants with
242 a particular phenology (Elzinga et al., 2007), and more specifically, that antagonists
243 preferentially attack early-flowering plants (König, Wiklund, & Ehrlén, 2015; Parachnowitsch &
244 Caruso, 2008; Valdés & Ehrlén, 2017, 2018). Interestingly, in our study the strength of this
245 preference for an early plant phenology did depend on the abundance of the antagonist's second
246 resource, i.e. the two host ant species *Myrmica rubra* and *M. schencki*. The preference of *P.
alcon* for early-flowering host plants was stronger at locations where the host ants were more
247 abundant. For example, the probability of a shoot with the earliest possible phenology receiving
248 at least one egg was 0.07 at sites where host ants were absent, and 0.40 at sites with the
249 maximum ant abundance observed ($n = 34$ ants). This indicates that the butterflies are able to
250 simultaneously track the distribution of the two resources, and to preferentially oviposit on plants
251 where both the availability of high-quality food for the caterpillars and the probability of
252 adoption by the right species of *Myrmica* are higher. When suitable host ants were absent or very
253 scarce, host plant phenology did not seem to affect egg-laying, and the probability that early-

256 flowering plants should receive eggs was very low. Spatial overlap between the distribution of
257 host plants and host ant nests at larger scales has previously been shown to increase the density
258 and population persistence of the congeneric species *P. arion* (Casacci et al., 2011; Thomas,
259 Simcox, & Clarke, 2009). Other studies with *Phengaris* butterflies have demonstrated effects of
260 host plant phenology (Thomas & Elmes, 2001; Valdés & Ehrlén, 2017, 2018), host ant presence
261 (van Dyck et al., 2000; Wynhoff et al., 2008; Wynhoff & van Langevelde, 2017) or both of these
262 factors (Casacci et al., 2011) for oviposition site selection. Our study shows that the spatial
263 overlap between these two resources also at the scale of the host plant individual is associated
264 with butterfly oviposition preference, and that the effects of plant phenology and ant abundance
265 are interactive. An important general implication of these findings is that differences in
266 availability of a resource over very small spatial scales can influence the use of other resource by
267 consumers that depend on multiple resources. The results also provide evidence of that the
268 effects of factors at the levels of the individual (plant phenology) and the community
269 (availability of the ant resource) on small-scale spatial variation in consumer-resource
270 interactions are interactive.

271 Our results show both that there is a dilution effect of local host plant density on butterfly
272 resource use, and that the strength of this effect depends on the quality of neighboring plants.
273 Higher densities of neighboring host plants were associated with both a decreasing probability of
274 a focal plant receiving at least one egg and a lower number of eggs in plants with eggs, i.e. a
275 lower consumer pressure. The results of previous studies with this species (Van Dyck &
276 Regniers, 2010) suggest that females usually lay more than one egg on the same plant, and that
277 they do not avoid oviposition on plants that already carry eggs. If this is true also in our study,

278 then the eggs observed on an individual plant correspond to the offspring of either a single or
279 several females (but this is something we cannot determine because oviposition was not directly
280 observed in this study). Because we cannot be sure whether the lower number of eggs was the
281 result of fewer females visiting the plant, or of each visiting female laying fewer eggs, it is more
282 appropriate here to talk about “consumer pressure” than about “consumer abundance”. In any
283 case, the observed negative relationship between consumer pressure and overall resource
284 abundance was stronger when the neighbors represented high-quality resources during the period
285 of butterfly flight, i.e. when surrounding plants flowered early in the season. In other words, at a
286 given conspecific density more eggs were found in *G. pneumonanthe* plants surrounded by non-
287 attractive, low-quality neighbors (i.e. late-flowering plants). In our study, plant phenology thus
288 influences butterfly oviposition not only because the phenology of a focal plant interacts with
289 host ant availability to determine plant suitability for oviposition, but also because the phenology
290 of neighbors modulates the strength of host plant density effects on oviposition. Therefore, not
291 only factors at the levels of individuals and communities, but also at the level of the plant
292 population, drive variation in resource use.

293 Our results suggest that oviposition decisions in myrmecophilous butterflies are taken at
294 two levels. When flying over an area populated with host plants, females might primarily use
295 visual cues (Van Dyck & Regniers, 2010) to evaluate host plant availability (i.e. density) and
296 identify high-quality (i.e. early-flowering) host plants. When butterflies approach host plants, or
297 after alighting on a plant, females may use both visual cues to evaluate plant quality and
298 chemical cues to detect ant presence (Patricelli et al., 2015; Wynhoff et al., 2015; Wynhoff & van
299 Langevelde, 2017), thereby evaluating the spatial overlap of the two resources needed by their

300 offspring. If females use plant developmental stage as a cue indicating plant quality, host plants
301 surrounded by low-quality neighbors would be more attractive to butterflies than host plants
302 surrounded by high-quality neighbors. Negative density-dependence in antagonists has been
303 documented also in other systems, where egg clumping on plants with few conspecific neighbors
304 was explained by a disproportionate attraction of insect herbivores to more isolated host plants
305 (Hasenbank & Hartley, 2015; Shea, Smyth, Sheppard, Morton, & Chalimbaud, 2000). For
306 herbivores feeding on several plant species, associational effects linked to the surrounding plant
307 community have been documented more frequently (e.g. Bergvall, Rautio, Kesti, Tuomi, &
308 Leimar, 2006; Hjältén, Danell, Lundberg, & Hjalten, 1993). Our study shows that associational
309 effects are present also for a specialized herbivore using a single host species, and that the
310 strength of these density-dependent effects depends on the quality of conspecific neighboring
311 host plants.

312 Our results are important to understand the factors causing variation in the intensity of
313 interactions between consumers and their resources. Given that consumers often have important
314 effects on fitness in the consumed organisms and that they show preferences for traits of the
315 resource organisms, identifying the factors that influence the intensity of interactions is also
316 important to understand the mechanisms underlying variation in natural selection. In our study
317 system, the butterfly seed predator has strong negative effects on plant fitness and shows a
318 preference for plants with an early flowering phenology, leading to butterfly-mediated selection
319 for later flowering (Valdés & Ehrlén, 2017). The results of the current study show that the
320 strength of butterfly preferences for early-flowering plants is influenced by both population
321 context, in terms of population density and the trait distribution with the population, and by

322 community context in terms of the abundance of the ant species that function as the second host
323 of butterfly caterpillars. Taken together, these findings illustrate that observed spatial variation in
324 intensities of interactions and consumer-mediated selection on traits of the resource organisms
325 can be the result of processes acting at the levels of individuals, populations and communities.

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Table 1: Results of the GLMs of the effects on the probability of a flowering shoot of *Gentiana pneumonanthe*

333 receiving at least one egg from the butterfly *Phengaris alcon* (binomial GLM)
 334 and the number of eggs in shoots with at least one egg (negative binomial GLM), of shoot
 335 phenology, ant abundance, neighbor density and neighbor phenology, as well as three two-way
 336 interactions of these predictors. In the binomial GLM, two eigenvectors issued from Moran's
 337 eigenvector mapping (MEM) are also included as predictors, in order to reduce residual spatial
 338 autocorrelation (see details in the text).

	Probability of having at least one egg (n = 8848, R ² = 0.45)		Number of eggs (n = 731, R ² = 0.22)	
	Estimate	SE	Estimate	SE
Shoot phenology	1.462 ***	0.079	0.257 ***	0.036
Ant abundance	-0.017	0.052	0.089 **	0.031
Neighbor density	-1.691 ***	0.102	-0.368 ***	0.067
Neighbor phenology	-1.150 ***	0.124	-0.121 **	0.041
Shoot phenology × Ant abundance	0.132 *	0.062	0.004	0.031
Shoot phenology × Neighbor phenology	0.068	0.074	0.014	0.035
Neighbor density × Neighbor phenology	-0.500 ***	0.087	-0.100 *	0.042
MEM Vector 1	0.289 ***	0.030		
MEM Vector 2	0.324 ***	0.052		

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

FIGURE LEGENDS

Figure 1: Sampling design. A) Study plot ($69 \times 45 \text{ m}^2$), comprising 345 3 m^2 subplots. B) Detail of several occupied subplots with reproductive shoots of *Gentiana pneumonanthe* and sampling points for ants. Shoots are represented by points, and colored according to shoot phenology. Numbers indicate the number of *Phengaris alcon* eggs found on the shoot. An example of a 3-m buffer used for neighbor measures on a focal shoot (represented by a star) is shown.

Figure 2: Effects of shoot phenology and host ant abundance on the distribution of *Phengaris alcon* eggs on its host plant *Gentiana pneumonanthe*. A) Effects on the probability of a shoot having at least one egg, the relationship between the probability of having at least one egg and shoot phenology shown for different ant abundances. B) Effects of shoot phenology on the number of eggs in shoots with at least one egg. Higher values of shoot phenology indicate earlier flowering. C) Effects of ant abundance on the number of eggs in shoots with at least one egg. In A), binomial GLM fit lines are represented with a color gradient for a continuous increment in ant abundance within the range of observed values. In B) and C), negative binomial GLM fit lines and confidence intervals are shown.

Figure 3: Effects of the interaction between neighbor density and neighbor phenology on the probability of a flowering shoot of *Gentiana pneumonanthe* having at least one egg of the butterfly *Phengaris alcon* (A) and the number of eggs in shoots with at least one egg (B). The relationships with neighbor density are shown for different values of neighbor phenology. Binomial (A) or negative binomial (B) GLM fit lines are represented with a color gradient for a continuous increment in neighbor phenology within the range of observed values. Higher values of neighbor phenology indicate earlier flowering.

Figure 1

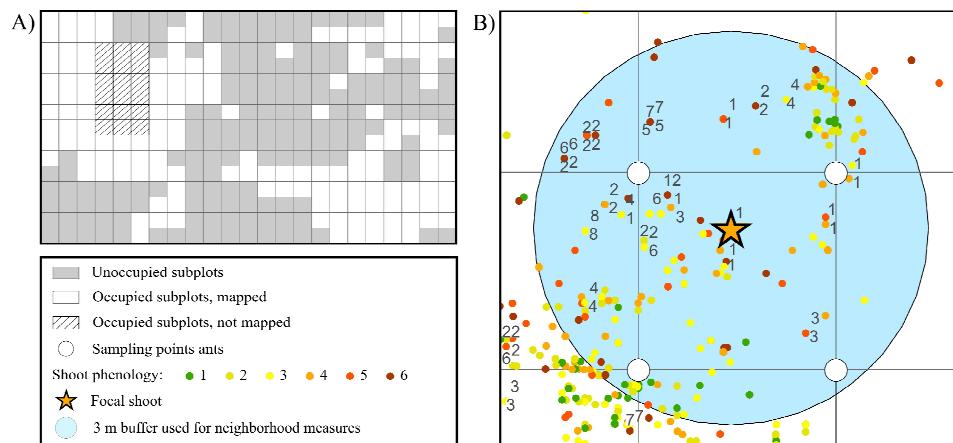


Figure 2

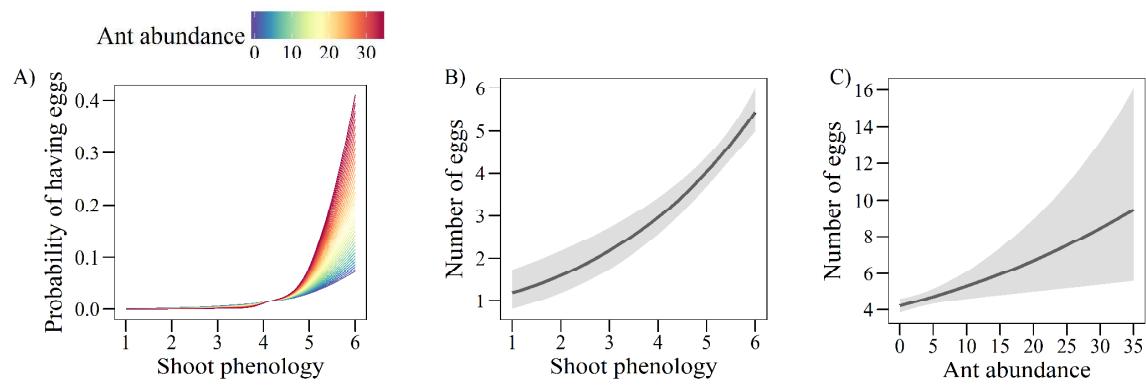
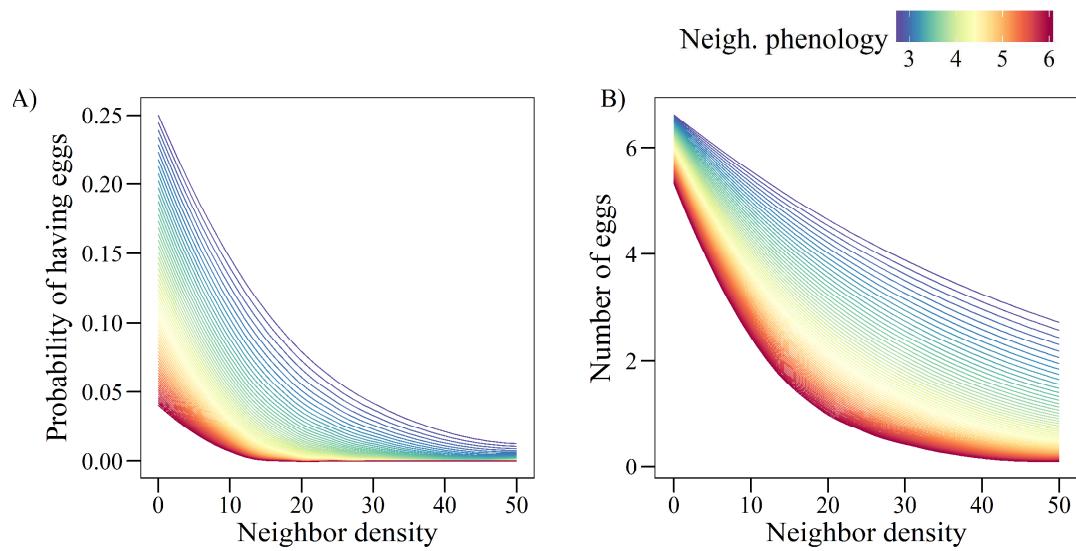


Figure 3



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Supporting Information

Table S1: Coefficients from univariate GLMs relating the probability of having eggs (A, binomial model) and the number of eggs in plants with at least one egg (B, negative binomial model) to the abundance of different ant species.

	A) Probability of having eggs (n = 8848)	B) Number of eggs (n = 731)
All <i>Myrmica</i>	-0.522 ***	-0.006
<i>M. rubra</i>	0.174 ***	0.137 ***
<i>M. scabrinodis</i>	-1.364 ***	-0.120 ***
<i>M. ruginodis</i>	-0.215 ***	-0.016
<i>M. schencki</i>	0.358 ***	0.006
Other ants	-0.403 ***	-0.061
<i>M. rubra + M. schencki</i>	0.360 ***	0.115 ***

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

Table S2: Results of the binomial GLM ($n = 8848$, $R^2 = 0.42$) relating the probability of a shoot of *G. pneumonanthe* having eggs of *P. alcon* to shoot phenology, ant abundance, neighbor density and neighbor phenology, and to three different two-way interactions of these predictors.

	Estimate	SE
Shoot phenology	1.384 ***	0.077
Ant abundance	0.061	0.051
Neighbor density	-1.943 ***	0.092
Neighbor phenology	-1.014 ***	0.120
Shoot phenology x Ant abundance	0.120 *	0.058
Shoot phenology x Neighbor phenology	-0.022	0.071
Neighbor density x Neighbor phenology	-0.650 *	0.084

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

Figure S1: Result of the inverse distance weighted interpolation for abundance of *Myrmica rubra* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

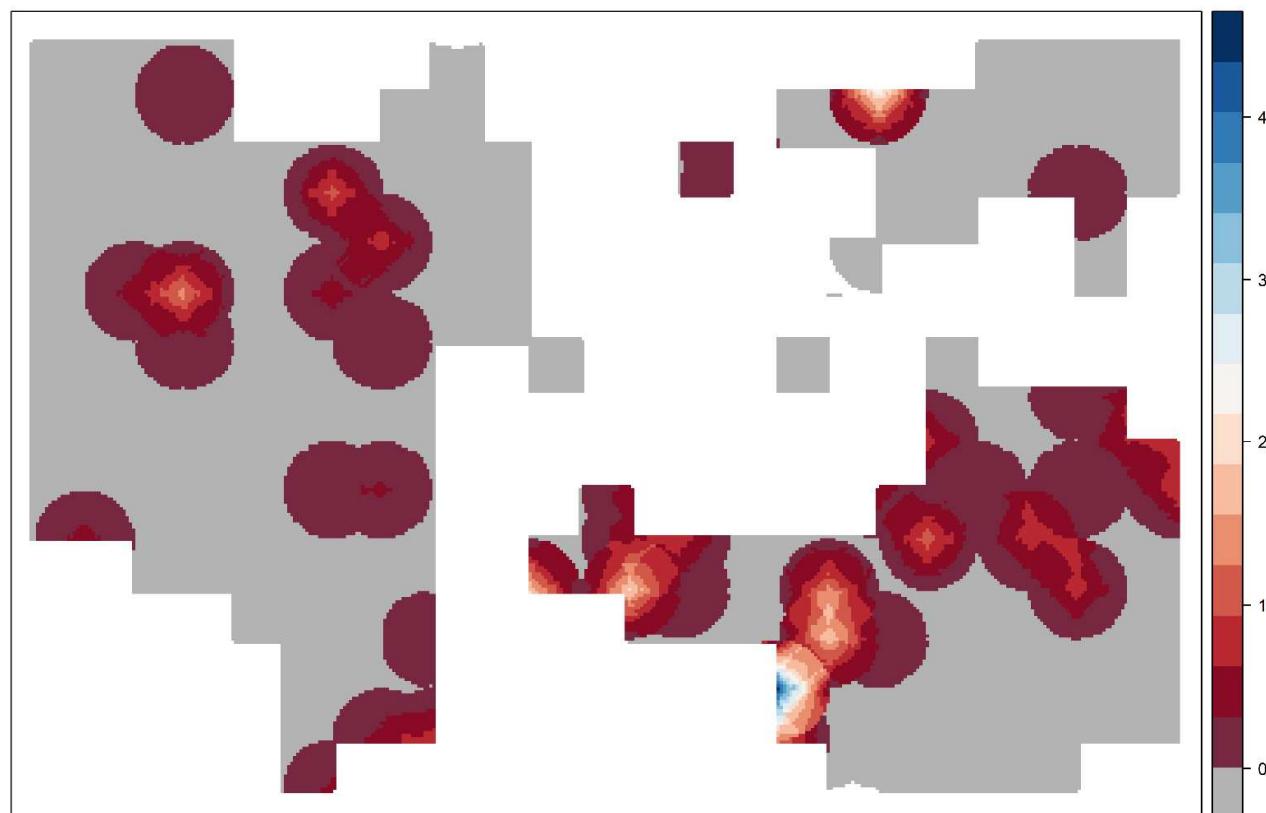


Figure S2: Result of the inverse distance weighted interpolation for abundance of *Myrmica scabrinodis* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

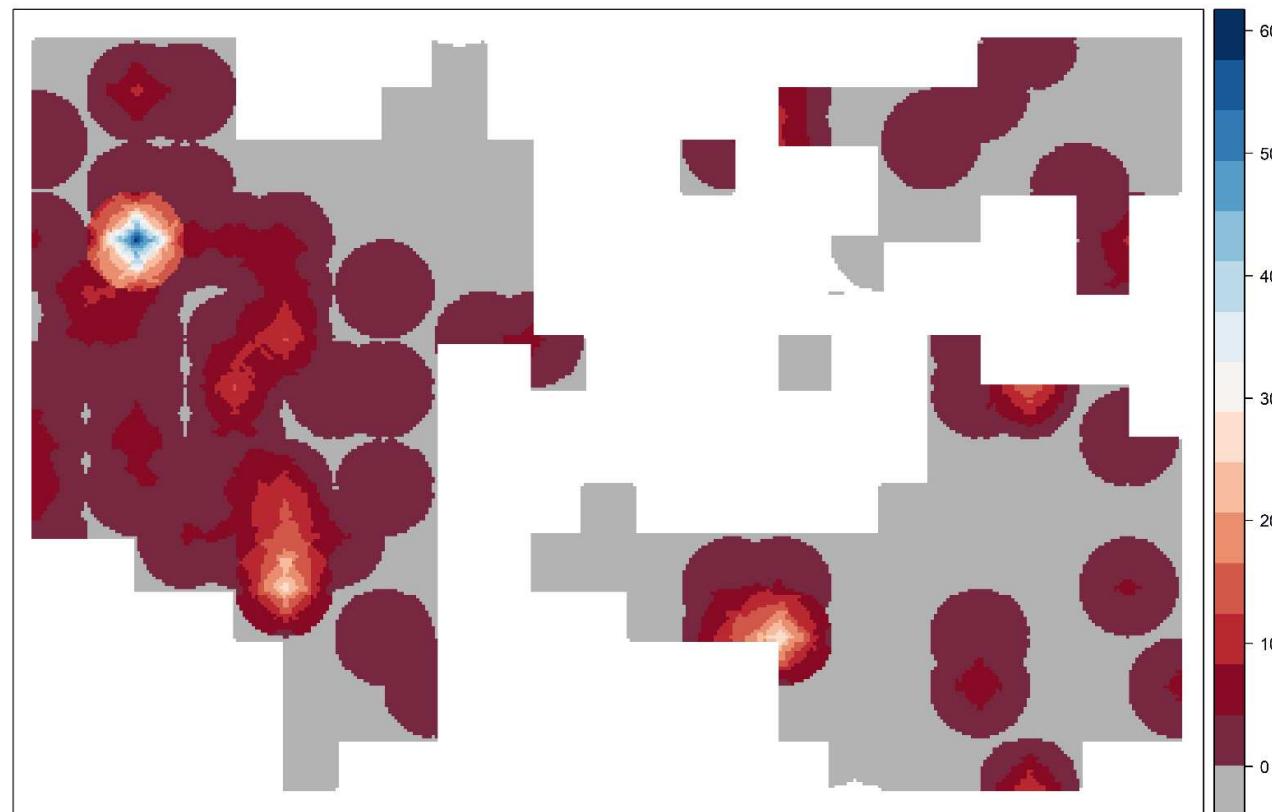


Figure S3: Result of the inverse distance weighted interpolation for abundance of *Myrmica ruginodis* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

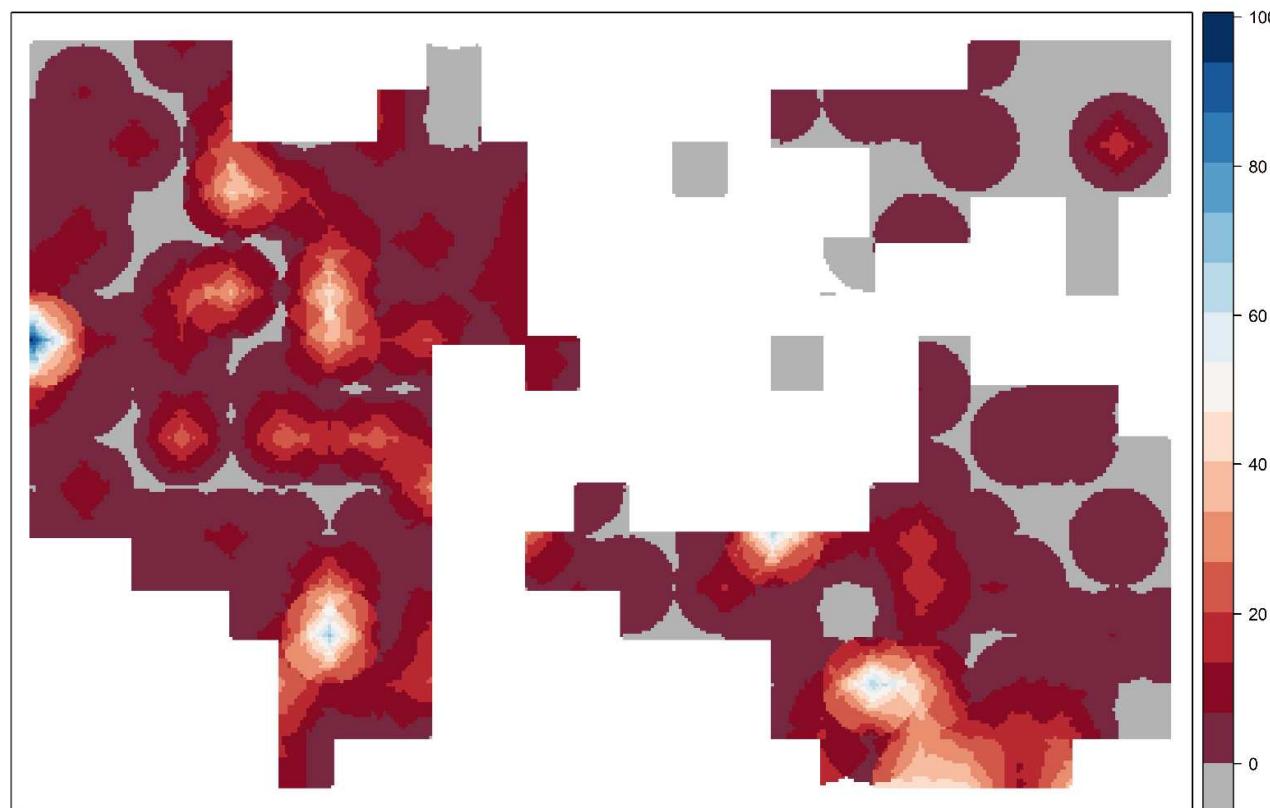


Figure S4: Result of the inverse distance weighted interpolation for abundance of *Myrmica schencki* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

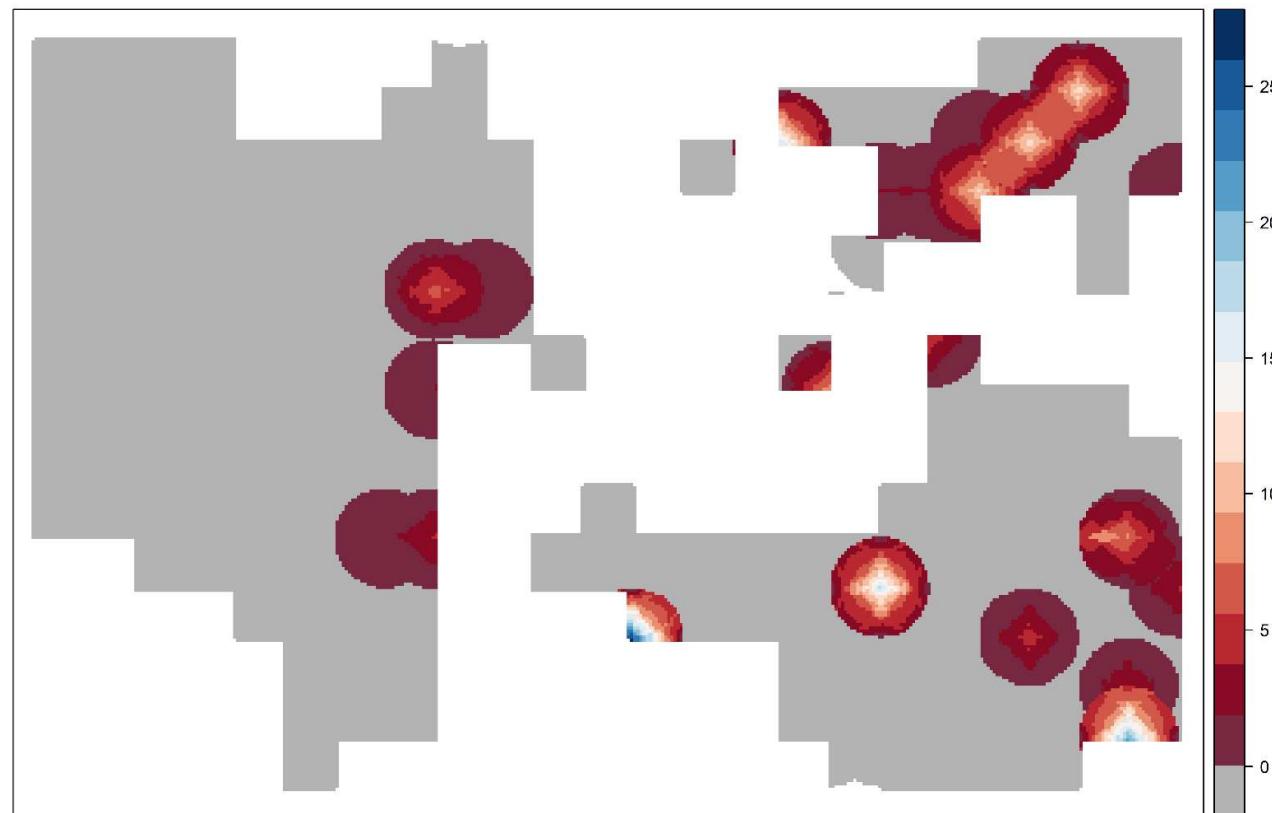


Figure S5: Result of the inverse distance weighted interpolation for abundance of non-*Myrmica* ants based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

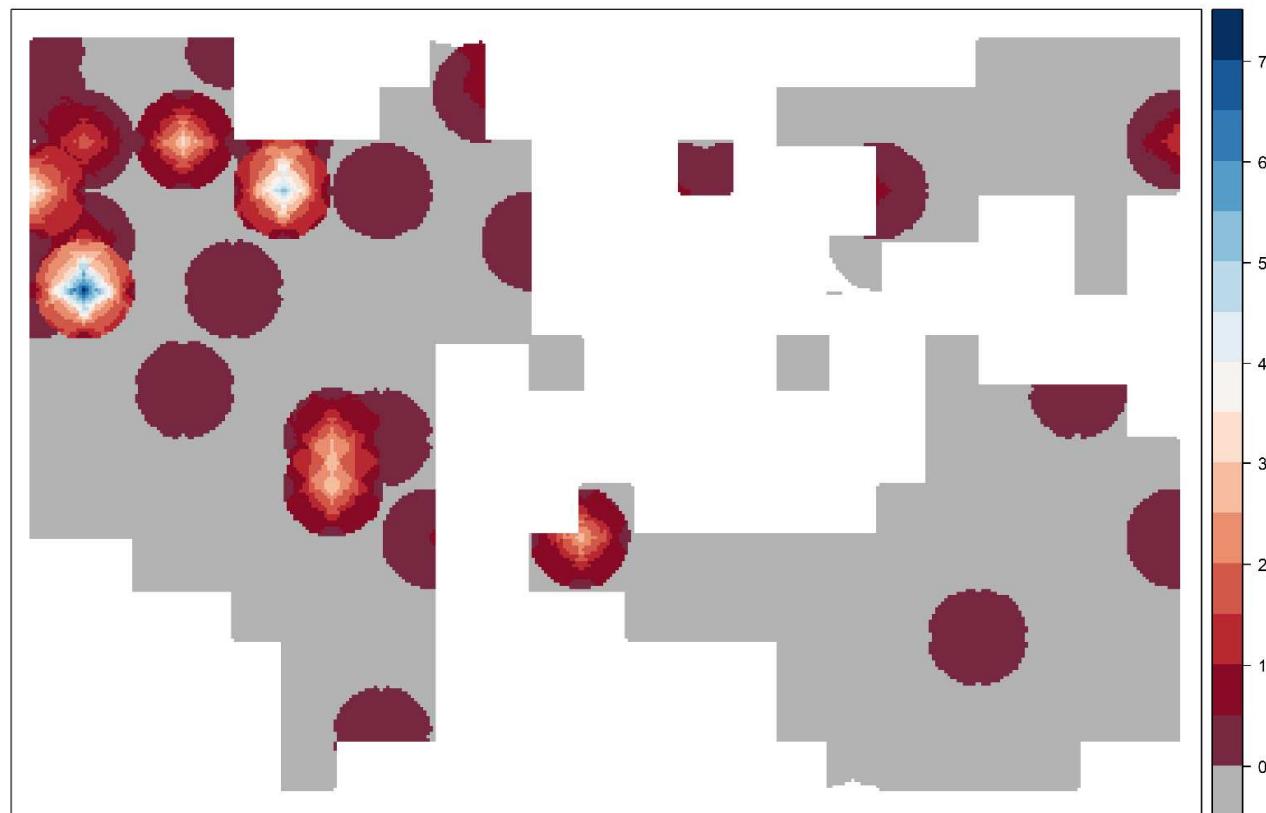


Figure S6: Spatial correlogram of the residuals of the binomial GLMs for the probability of a shoot having eggs before (red) and after (blue) including the two eigenvectors issued from MEM.

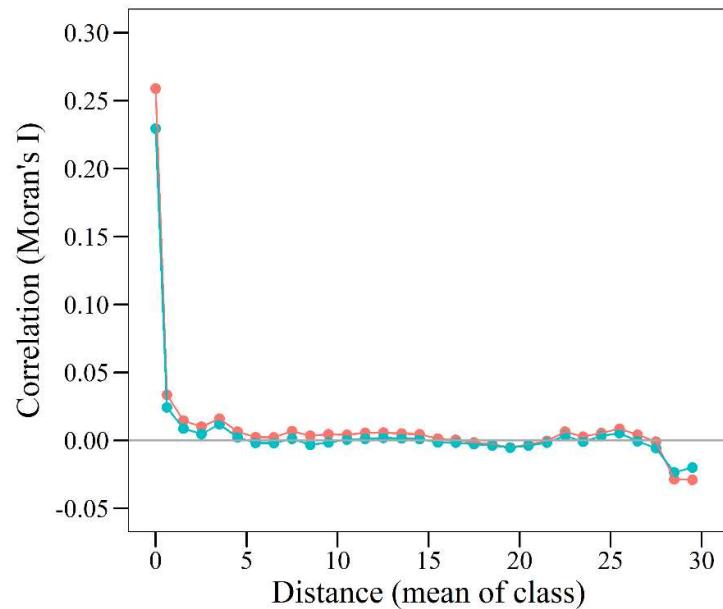


Figure S7: Spatial correlogram of the residuals of the negative binomial GLM for the number of eggs in shoots with at least one egg.

