



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

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11 inputs from JE. AV and JE wrote the manuscript. Both authors gave final approval for

12 publication.

13

14

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 (Jaenike 1978, Scheirs and De Bruyn 2002, Gripenberg et al.
43 2010). The suitability of host plants, in turn, might depend on factors such as host plant
44 nutritional quality (Chen et al. 2004), size (Rabasa et al. 2005), microclimatic conditions (Eilers
45 et al. 2013) or enemy-free space (Kaminski et al. 2010). Most consumers depend on multiple
46 resources throughout their life cycle, e.g. different sources of food, shelter and resting sites
47 (Dennis et al. 2003, 2006). The availability of one resource might thus influence the use of other
48 resources. For example, the availability of nectar resources for adults (Öckinger 2008) or
49 secondary hosts for larvae (Wynhoff et al. 2015) might determine host plant choices in
50 butterflies.

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

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

76 Myrmecophilous butterflies of the genus *Phengaris* need two sequential hosts to
77 complete their development: the caterpillars are predispersal seed predators during their first
78 instars and parasitize ant nests (*Myrmica* spp.) during later stages. Butterflies are very specific
79 regarding both host plant and host ant species and the developmental stage of host plants (Elmes

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

92 In this study, we investigated how the quality, in terms of the developmental stage of the
93 reproductive structures, of one resource, the focal host plant *Gentiana pneumonanthe*, the density
94 and quality of neighboring conspecific plants, and the abundance of host ants influence resource
95 use, in terms of egg-laying, by the consumer butterfly *Phengaris alcon*. We used an extensive
96 field data set to test two hypotheses: (1) Effects of plant quality and ant abundance on egg
97 distribution patterns are interdependent: butterflies choose plants for oviposition that
98 simultaneously maximize the availability of both resources, i.e. they have a stronger preference
99 for high-quality early-flowering host plants where host ants are abundant, and (2) The probability
100 of a plant receiving at least one egg, as well as the number of eggs in oviposited plants, decreases
101 with increasing conspecific neighbor density (i. e. a dilution effect), and this decrease is stronger

102 when neighbors are of high quality.

103 MATERIALS AND METHODS

104 Study system

105 The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, occurring in
106 open habitats, such as wet heathlands and grasslands (Simmonds 1946). Plants can have one to
107 many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by
108 bumblebees. The species is self-compatible and flowers in July and August in the study area (SW
109 Sweden). Fruits are capsules containing a high number of minute, wind-dispersed seeds (mean
110 seed number per fruit \pm SD = 490 ± 243 , mean seed weight = 0.041 mg, authors' unpublished
111 data). *Gentiana pneumonanthe* is the host plant of the Alcon Blue butterfly (*Phengaris alcon*), a
112 specialist predispersal seed predator which oviposits on young flower buds in July and August
113 (Appelqvist and Bengtsson 2007). During an oviposition event, adult female butterflies usually
114 lay more than one egg on the same plant and even on the same bud, and there is no evidence of
115 avoiding plants that already carry eggs (Van Dyck and Regniers 2010). The caterpillars feed
116 inside the capsule until they reach the fourth instar, when they drop to the ground to be picked up
117 by *Myrmica* ants. Caterpillars mimic the surface chemistry of the ant brood (Nash et al. 2008)
118 and the acoustic signals of queen ants (Sala et al. 2014), and these features cause ants to carry
119 them to their nest, where they spend the rest of their larval period as brood parasites. Females of
120 *Phengaris* detect host ant presence through plant chemicals produced by ants or by plants in
121 response to root tissue damage caused by ants (van Dyck et al. 2000, Patricelli et al. 2015,
122 Wijnhoff and van Langevelde 2017). Contrary to the majority of *Phengaris* species, which prey
123 on ant brood, *P. alcon* is a "cuckoo" species (Als et al. 2004), and caterpillars feed primarily on

124 regurgitants from ant workers, trophic eggs (i.e. nutritious, infertile eggs which are fed to the
125 queens and ant larvae) and prey items brought to the nest by ants. Only ants belonging to
126 *Myrmica* sp. can act as hosts of *P. alcon*. In Sweden, *M. rubra* is considered to be the most
127 common host ant species (Elmes et al. 1994).

128 **Data collection**

129 The study was carried out in a population of *G. pneumonanthe*, where the butterfly *P.*
130 *alcon* was present, located in Tånga Hed nature reserve in the county of Västra Götaland, SW
131 Sweden (58°01'40.0"N 12°49'47.0"E). Field work was conducted between 9 July and 3
132 September 2016. The plant population occupies a fenced meadow that is grazed by cattle at the
133 end of the summer every year. A study plot measuring 69 m × 45 m was established in the
134 meadow. This plot was further divided into a grid comprising 345 3 m × 3 m subplots with
135 corners permanently marked by wooden poles (figure 1A). In 154 of the subplots where *G.*
136 *pneumonanthe* was present, all reproductive shoots of *G. pneumonanthe* were mapped (figure
137 1B), and their phenology and the number of eggs of *P. alcon* were recorded between 29 July and
138 5 August (18 additional occupied subplots were not possible to fully survey due to time
139 constraints). Shoots were mapped by recording their coordinates within subplots. We mapped
140 shoots rather than plant individuals (that may have one or several shoots) because we judged that
141 individual shoots are the relevant unit for the butterfly to assess the food resource quality and
142 because individual shoots act as the unit of attraction for butterfly females. For each shoot,
143 phenology was recorded as the developmental stage of the most advanced bud, according to six
144 ordinal categories: (1) the sepals covering the bud completely, (2) bud becoming visible, (3) bud
145 growing over the sepals, (4) bud turning blue, (5) flower opening, and (6) flower showing signs

146 of wilting (Valdés and Ehrlén 2017a). Each stage corresponds to a time interval of flower
147 development, with higher values indicating a more advanced floral development at the day of
148 recording, i.e. an earlier flowering phenology. The number of eggs was counted on the whole
149 shoot.

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

157 **Interpolations of ant abundances**

158 We also incorporated the locations of the 254 points where the ant community was sampled into
159 the GIS. We used inverse distance weighted interpolation (Pebesma 2004) based on the values at
160 the sampling points to generate values of the abundance of ant species over the surface of all
161 occupied subplots. A maximum distance of 3 m was used because it represents the ground
162 foraging distance of most investigated *Myrmica* species (Elmes et al. 1998). From these
163 interpolated surfaces (see Appendices S1-5), we extracted values of abundance of the different
164 ant species for each of the mapped shoots.

165 **Calculation of neighborhood measures**

166 Field maps were digitized using a Geographic Information System (GIS) created with
167 ArcGIS 10.3.1, assigning relative coordinates to each recorded reproductive shoot of *G.*

168 *pneumonanthe* (figure 1B). For each shoot, we defined a buffer zone with a 3-m radius, centered
169 at the focal shoot (see example in figure 1B), and calculated the number of reproductive shoots
170 of *G. pneumonanthe* per m² within this buffer zone (hereafter, “neighbor density”) as well as the
171 mean phenology of these shoots (hereafter, “neighbor phenology”), excluding in each case the
172 focal shoot.

173 **Statistical analyses**

174 The effects of shoot phenology, host ant abundance, neighbor density and neighbor phenology on
175 *P. alcon* egg distribution were examined using two Generalized Linear Models (GLMs). Effects
176 on the probability of a shoot having at least one egg were evaluated using a binomial GLM, and
177 effects on the number of eggs in shoots with at least one egg were evaluated using a negative
178 binomial GLM. Both models included also the effects of three interactions. First, we included the
179 interaction between shoot phenology and ant abundance in order to test if the effects of the two
180 resources on egg distribution patterns are interdependent. Second, we included the interaction
181 between neighbor density and neighbor phenology in order to test if the effect of host plant
182 density depends on phenology of neighboring host plants. Third, we included the interaction
183 between shoot phenology and neighbor phenology to account for the fact that the relationship
184 between egg distribution and phenology of the focal plant might depend on the phenology of
185 neighboring host plants. As a measure of host ant abundance, we used the summed abundances
186 of *M. rubra* and *M. schencki* (hereafter, “ant abundance”). *M. rubra* is considered to be the most
187 common host ant species of *P. alcon* in Sweden (Elmes et al. 1994), and *M. schencki* has been
188 cited as host in several places in Europe (Vilbas et al. 2016). Our data agreed with this previous
189 knowledge, as the combined abundance of *M. rubra* and *M. schencki* had the strongest positive

190 effect on the probability of host plants receiving eggs (Appendix S6).

191 All predictors were standardized by subtracting the mean and dividing by the standard
192 deviation before the analyses. We checked for spatial autocorrelation in the residuals of models
193 by plotting spatial correlograms and by calculating global Moran's I with a permutation test
194 (1000 random permutations). Moran's I was calculated based on a connectivity matrix of
195 pairwise Euclidean distances among the shoots up to a distance of 30 m. Residual spatial
196 autocorrelation could lead to biased model estimates and invalid statistical inference (Dormann
197 et al. 2007). A significant spatial autocorrelation was found in the residuals of the binomial
198 GLM, but not for the negative binomial GLM (global Moran's I = 0.0150, $p < 0.001$ and global
199 Moran's I = 0.0023, $p = 0.613$, respectively, Appendices S7-S8). We therefore applied Moran's
200 eigenvector mapping (MEM), which translates the spatial arrangement of data points into
201 explanatory variables (eigenvectors) that capture spatial effects, to the binomial model
202 examining effects on the probability of a shoot having at least one egg (Dray et al. 2006, Thayn
203 and Simanis 2013). The model was refitted including two eigenvectors issued from MEM as
204 spatial predictors, and this strongly reduced the residual spatial autocorrelation (global Moran's I
205 = 0.0003, $p = 0.03297$, Appendix S7). Although residual spatial autocorrelation was still
206 significant, the low value of Moran's I and visual inspection of the spatial correlogram
207 (Appendix S8) indicated that almost all of the residual spatial autocorrelation was removed by
208 the spatial predictors. The results of this model were similar to the results of the binomial GLM
209 not including the spatial predictors (Appendix S9). Statistical analyses were carried out in R
210 3.4.0 (R Core Team 2017).

211 RESULTS

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

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

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

229 DISCUSSION

230 The results of this study show that resource use in the myrmecophilous butterfly *P. alcon*
231 is shaped by both the spatial overlap of the two key larval resources and by a dilution effect.
232 Specifically, we found that butterflies prefer to oviposit on early-flowering plants that have a
233 high abundance of host ants, that plants surrounded by many conspecific neighbors receive fewer

234 eggs, and that the dilution effect is stronger when neighbors are of a high quality.

235 We found that both the probability of *P. alcon* butterflies laying at least one egg on a host
236 plant as well as the number of eggs increased with an earlier phenology of its host plant *G*
237 *pneumonanthe*. This agrees with previous studies showing that insects usually prefer plants with
238 a particular phenology (Elzinga et al. 2007), and more specifically, that antagonists preferentially
239 attack early-flowering plants (Parachnowitsch and Caruso 2008, König et al. 2015, Valdés and
240 Ehrlén 2017a, 2017b). Interestingly, in our study the strength of this preference for an early plant
241 phenology did depend on the abundance of the antagonist's second resource, i.e. the two host ant
242 species *Myrmica rubra* and *M. schencki*. The preference of *P. alcon* for early-flowering host
243 plants was stronger at locations where the host ants were more abundant. For example, the
244 probability of a shoot with the earliest possible phenology receiving at least one egg was 0.07 at
245 sites where host ants were absent, and 0.40 at sites with the maximum ant abundance observed (n
246 = 34 ants). This indicates that the butterflies are able to simultaneously track the distribution of
247 the two resources, and to preferentially oviposit on plants where both the availability of high-
248 quality food for the caterpillars and the probability of adoption by the right species of *Myrmica*
249 are higher. When suitable host ants were absent or very scarce, host plant phenology did not
250 seem to affect egg-laying, and the probability that early-flowering plants should receive eggs was
251 very low. Spatial overlap between the distribution of host plants and host ant nests at larger
252 scales has previously been shown to increase the density and population persistence of the
253 congeneric species *P. arion* (Thomas et al. 2009, Casacci et al. 2011). Other studies with
254 *Phengaris* butterflies have demonstrated effects of host plant phenology (Thomas and Elmes
255 2001, Valdés and Ehrlén 2017a, 2017b), host ant presence (van Dyck et al. 2000, Wynhoff et al.

256 2008, Wynhoff and van Langevelde 2017) or both of these factors (Casacci et al. 2011) for
257 oviposition site selection. Our study shows that the spatial overlap between these two resources
258 also at the scale of the host plant individual is associated with butterfly oviposition preference,
259 and that the effects of plant phenology and ant abundance are interactive. An important general
260 implication of these findings is that differences in availability of a resource over very small
261 spatial scales can influence the use of other resource by consumers that depend on multiple
262 resources. The results also provide evidence of that the effects of factors at the levels of the
263 individual (plant phenology) and the community (availability of the ant resource) on small-scale
264 spatial variation in consumer-resource interactions are interactive.

265 Our results show both that there is a dilution effect of local host plant density on butterfly
266 resource use, and that the strength of this effect depends on the quality of neighboring plants.
267 Higher densities of neighboring host plants were associated with both a decreasing probability of
268 a focal plant receiving at least one egg and a lower number of eggs in plants with eggs, i.e. a
269 lower consumer pressure. The results of previous studies with this species (Van Dyck and
270 Regniers 2010) suggest that females usually lay more than one egg on the same plant, and that
271 they do not avoid oviposition on plants that already carry eggs. If this is true also in our study,
272 then the eggs observed on an individual plant correspond to the offspring of either a single or
273 several females (but this is something we cannot determine because oviposition was not directly
274 observed in this study). Because we cannot be sure whether the lower number of eggs was the
275 result of fewer females visiting the plant, or of each visiting female laying fewer eggs, it is more
276 appropriate here to talk about “consumer pressure” than about “consumer abundance”. In any
277 case, the observed negative relationship between consumer pressure and overall resource

278 abundance was stronger when the neighbors represented high-quality resources during the period
279 of butterfly flight, i.e. when surrounding plants flowered early in the season. In other words, at a
280 given conspecific density more eggs were found in *G. pneumonanthe* plants surrounded by non-
281 attractive, low-quality neighbors (i.e. late-flowering plants). In our study, plant phenology thus
282 influences butterfly oviposition not only because the phenology of a focal plant interacts with
283 host ant availability to determine plant suitability for oviposition, but also because the phenology
284 of neighbors modulates the strength of host plant density effects on oviposition. Therefore, not
285 only factors at the levels of individuals and communities, but also at the level of the plant
286 population, drive variation in resource use.

287 Our results suggest that oviposition decisions in myrmecophilous butterflies are taken at
288 two levels. When flying over an area populated with host plants, females might primarily use
289 visual cues (Van Dyck and Regniers 2010) to evaluate host plant availability (i.e. density) and
290 identify high-quality (i.e. early-flowering) host plants. When butterflies approach host plants, or
291 after alighting on a plant, females may use both visual cues to evaluate plant quality and
292 chemical cues to detect ant presence (Wynhoff et al. 2015, Patricelli et al. 2015, Wynhoff and
293 van Langevelde 2017), thereby evaluating the spatial overlap of the two resources needed by
294 their offspring. If females use plant developmental stage as a cue indicating plant quality, host
295 plants surrounded by low-quality neighbors would be more attractive to butterflies than host
296 plants surrounded by high-quality neighbors. Negative density-dependence in antagonists has
297 been documented also in other systems, where egg clumping on plants with few conspecific
298 neighbors was explained by a disproportionate attraction of insect herbivores to more isolated
299 host plants (Shea et al. 2000, Hasenbank and Hartley 2015). For herbivores feeding on several

300 plant species, associational effects linked to the surrounding plant community have been
301 documented more frequently (e.g. Hjältén et al. 1993, Alm Bergvall et al. 2006). Our study
302 shows that associational effects are present also for a specialized herbivore using a single host
303 species, and that the strength of these density-dependent effects depends on the quality of
304 conspecific neighboring host plants.

305 Our results are important to understand the factors causing variation in the intensity of
306 interactions between consumers and their resources. Given that consumers often have important
307 effects on fitness in the consumed organisms and that they show preferences for traits of the
308 resource organisms, identifying the factors that influence the intensity of interactions is also
309 important to understand the mechanisms underlying variation in natural selection. In our study
310 system, the butterfly seed predator has strong negative effects on plant fitness and shows a
311 preference for plants with an early flowering phenology, leading to butterfly-mediated selection
312 for later flowering (Valdés and Ehrlén 2017a). The results of the current study show that the
313 strength of butterfly preferences for early-flowering plants is influenced by both population
314 context, in terms of population density and the trait distribution within the population, and by
315 community context in terms of the abundance of the ant species that function as the second host
316 of butterfly caterpillars. Taken together, these findings illustrate that observed spatial variation in
317 intensities of interactions and consumer-mediated selection on traits of the resource organisms
318 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* receiving at least one egg from the butterfly *Phengaris alcon* (binomial GLM) and the number of eggs in shoots with at least one egg (negative binomial GLM), of shoot phenology, ant abundance, neighbor density and neighbor phenology, as well as three two-way interactions of these predictors. In the binomial GLM, two eigenvectors issued from Moran's eigenvector mapping (MEM) are also included as predictors, in order to reduce residual spatial 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

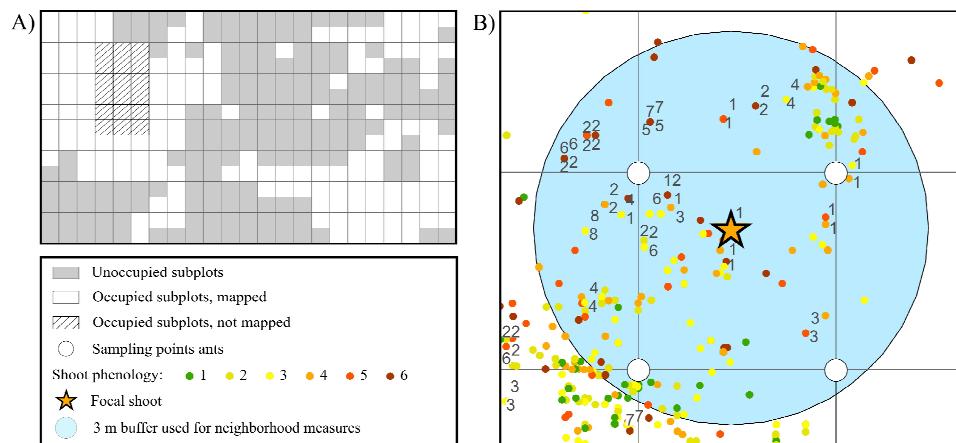


Figure 2

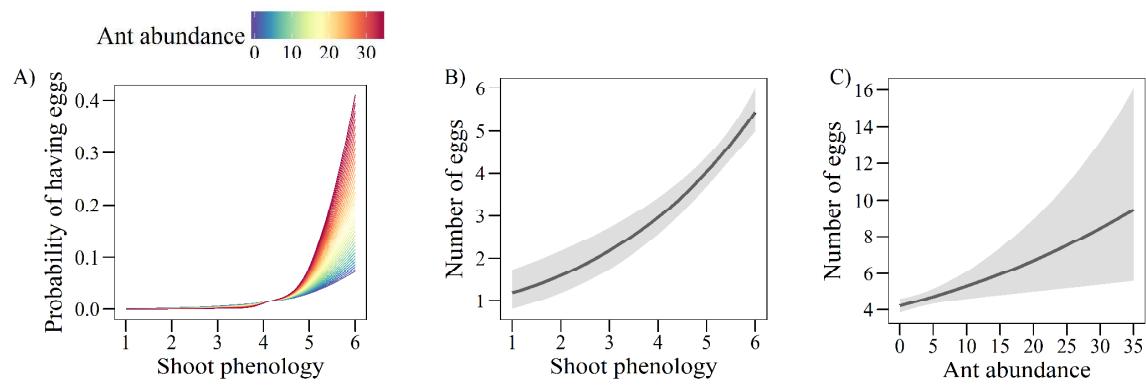
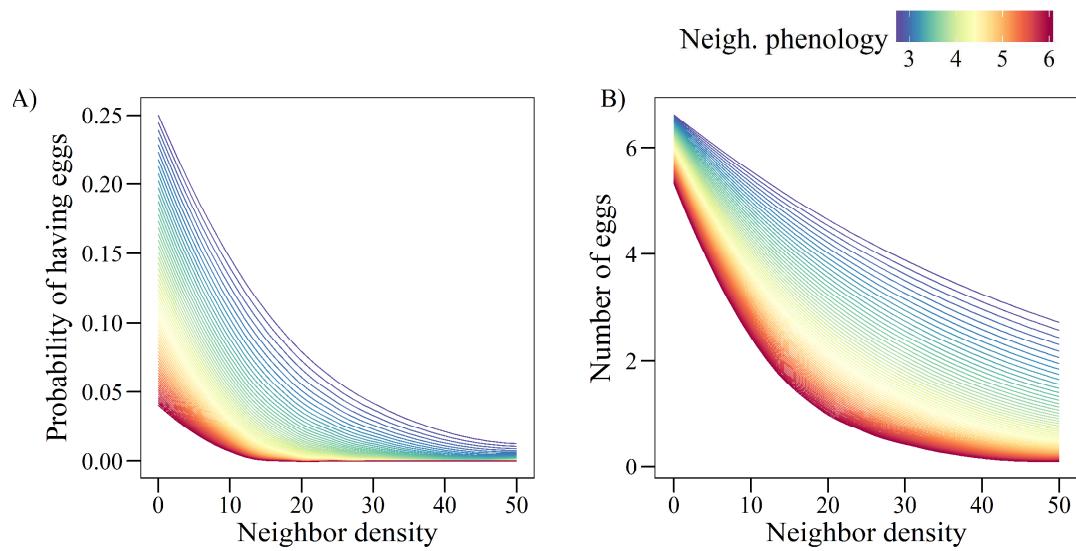


Figure 3



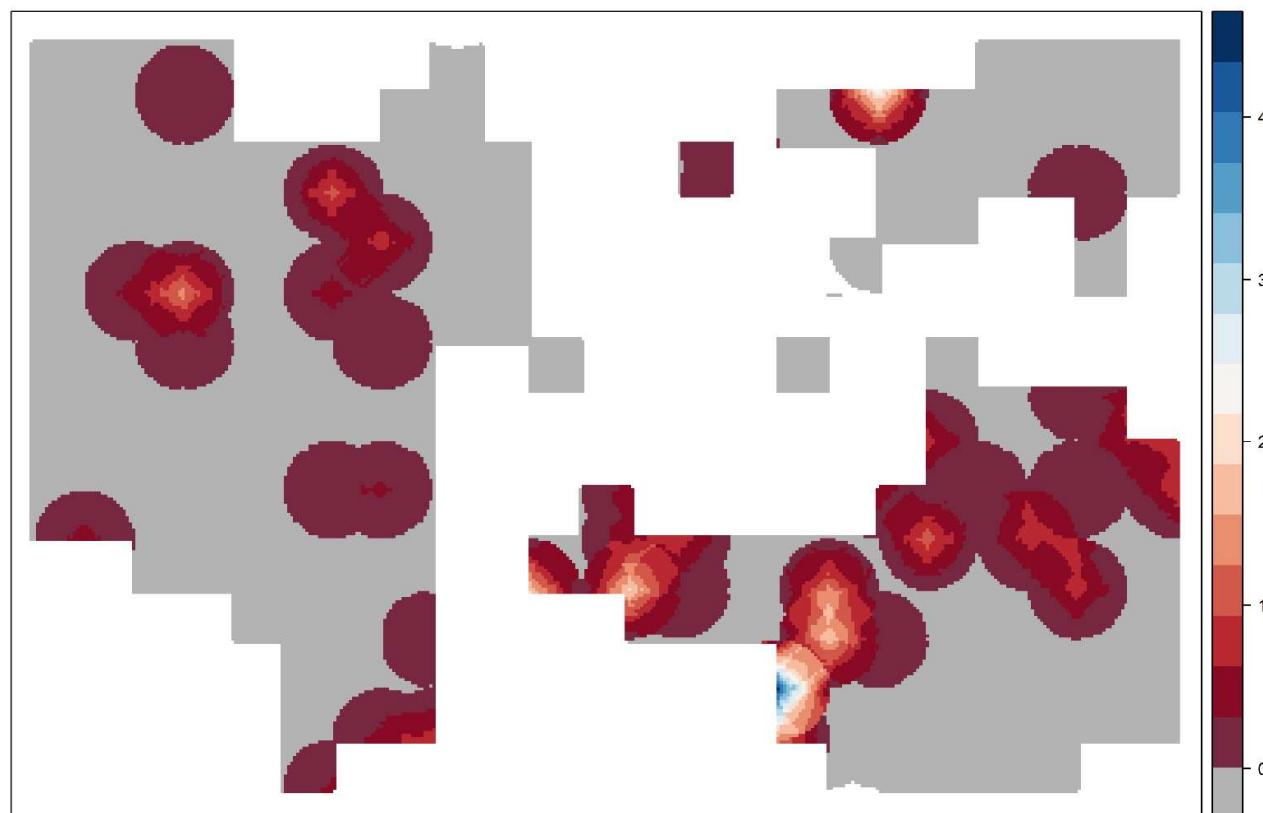
326

Appendices

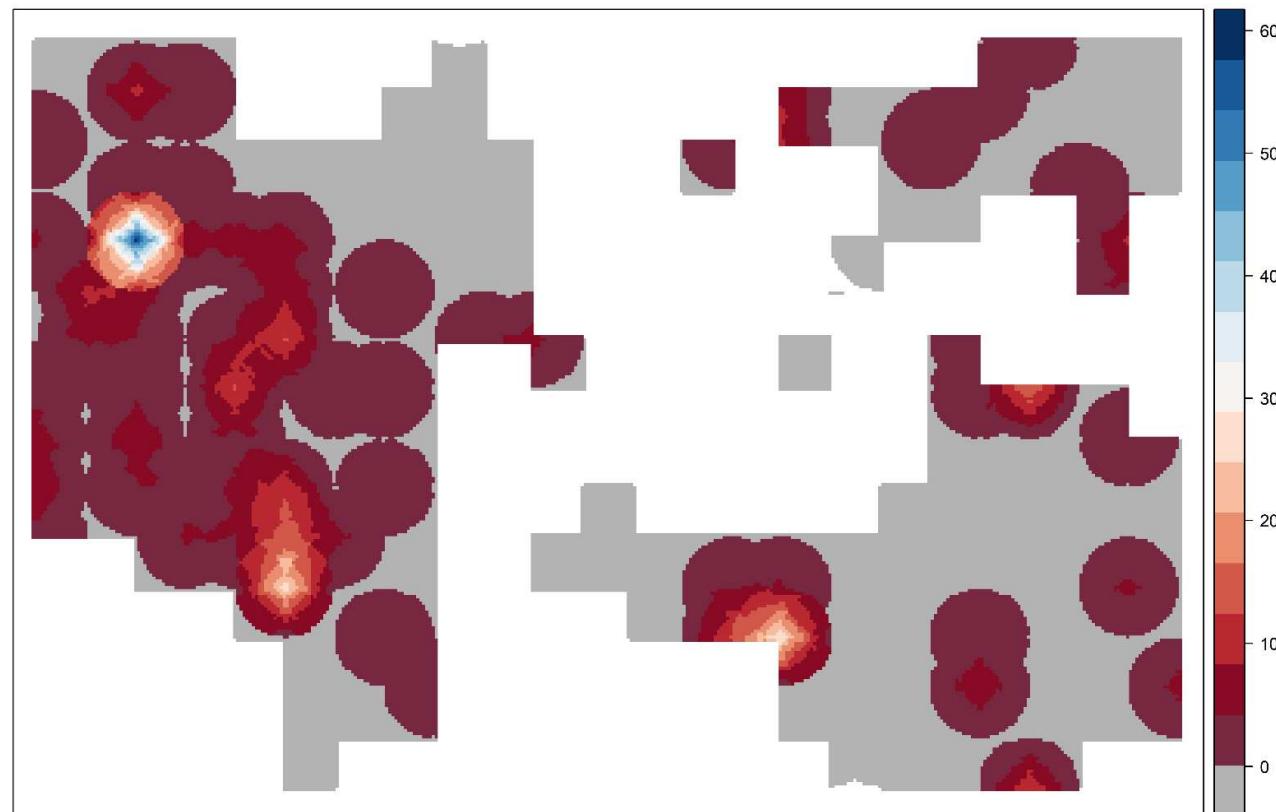
327

For Review Only

Appendix 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.

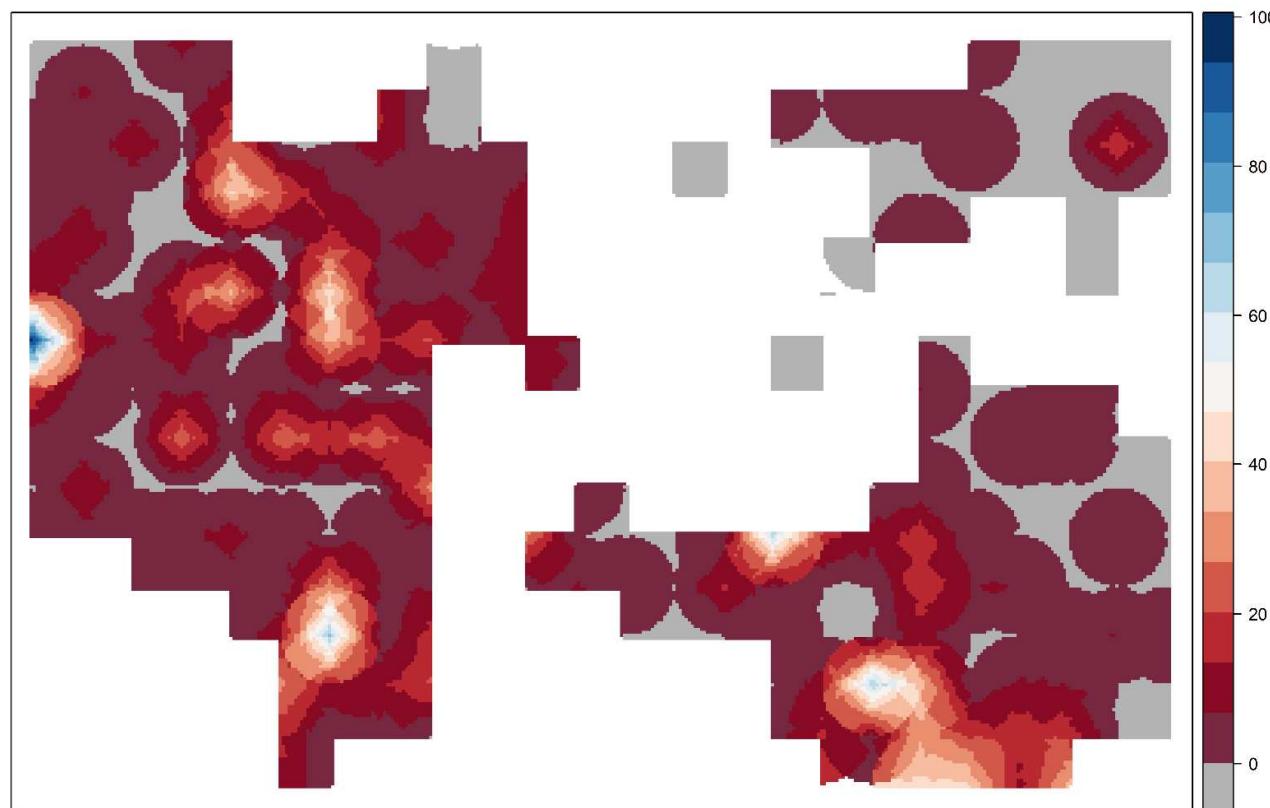


329 Appendix S2: Result of the inverse distance weighted interpolation for abundance of *Myrmica scabrinodis* based on abundance values (sum of
330 individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.



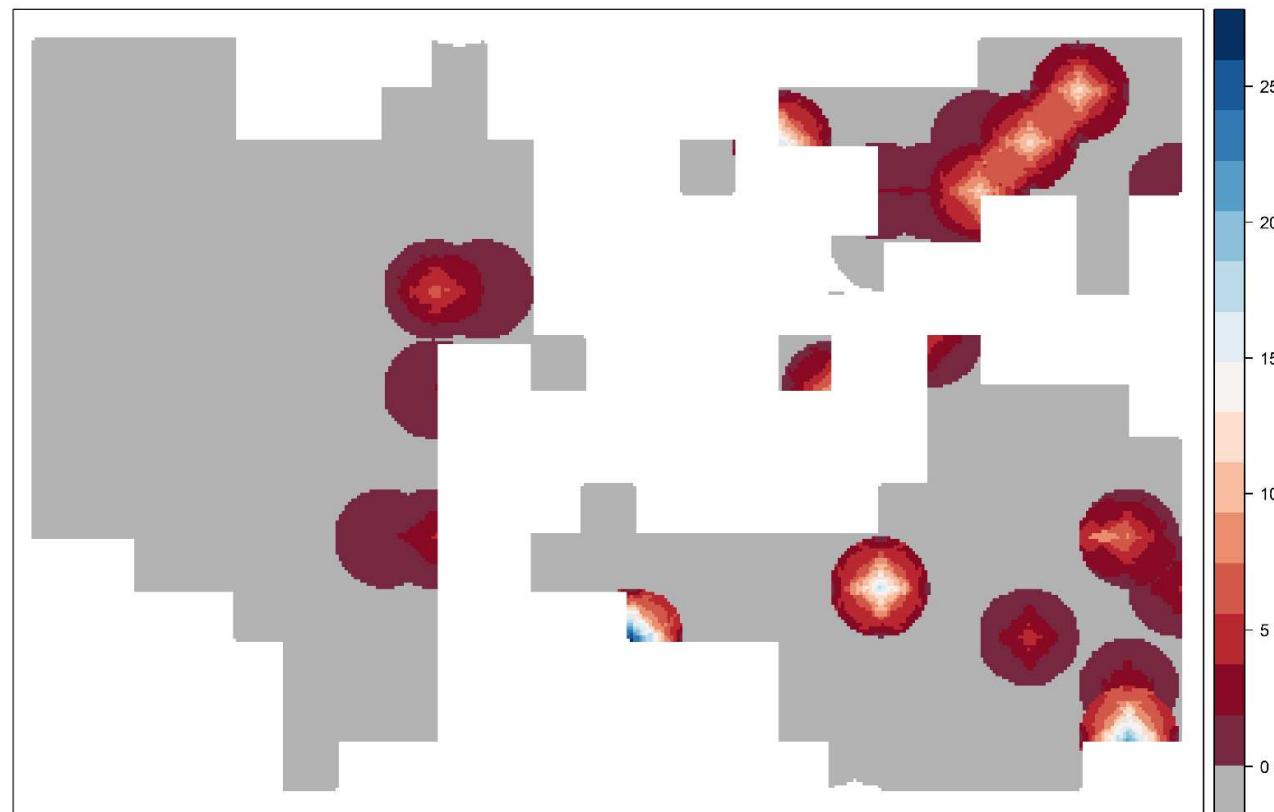
331

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



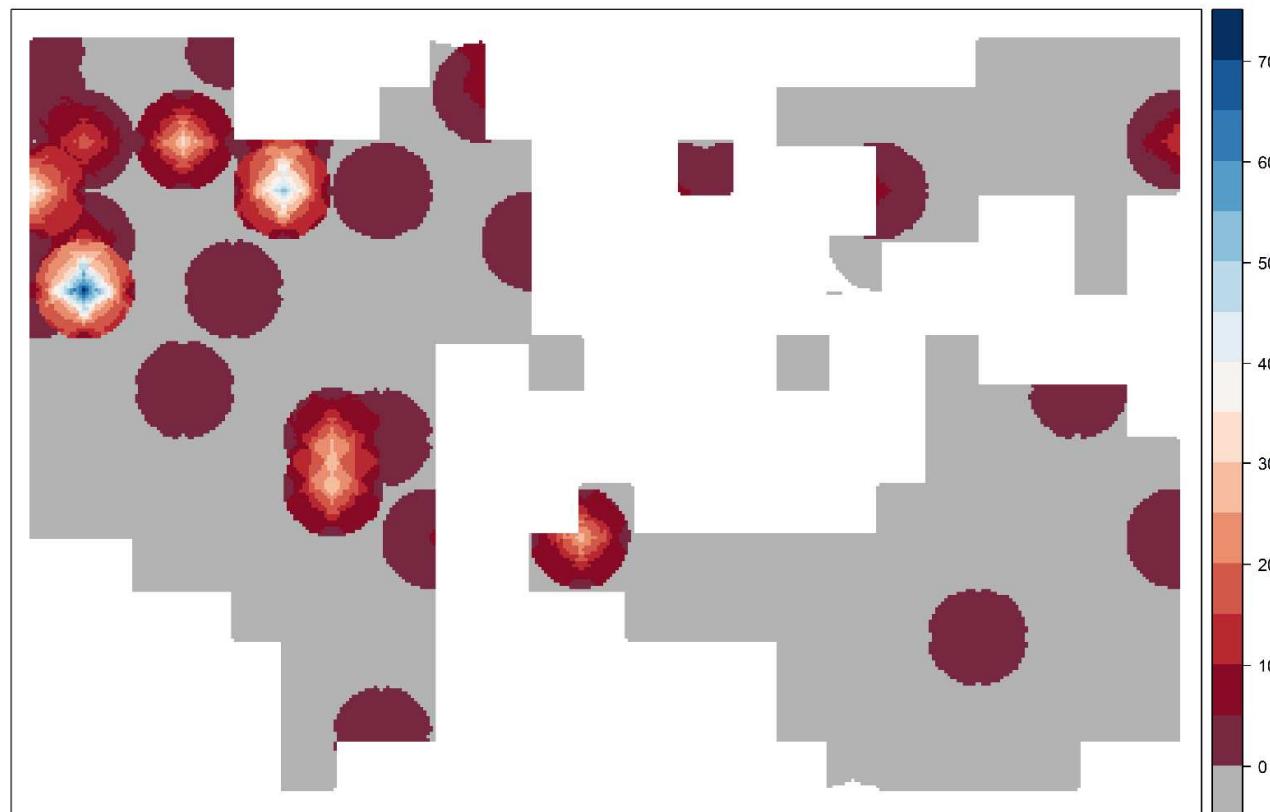
334

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



337

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



340

341 Appendix S6: Coefficients from univariate GLMs relating the probability of having
342 eggs (A, binomial model) and the number of eggs in plants with at least one egg (B,
343 negative binomial model) to the abundance of different ant species.

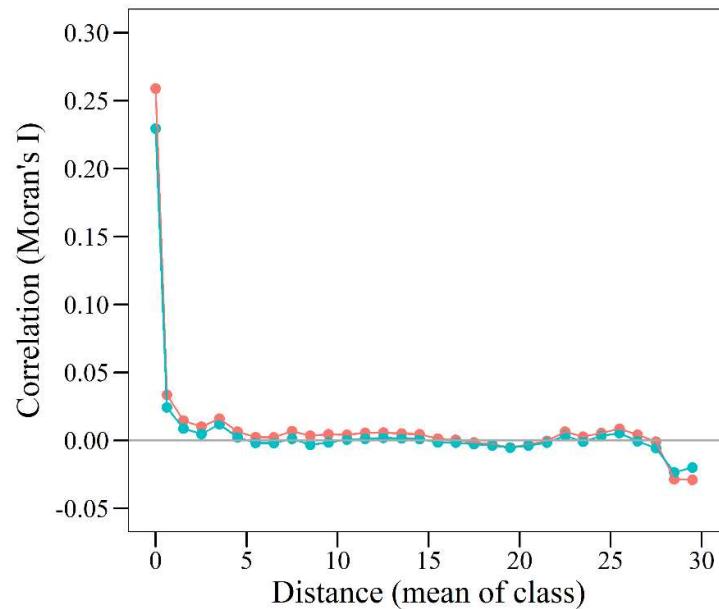
	A) Probability of having eggs (n = 8848)	B) Number of eggs (n = 731)
All <i>Myrmica</i>	-0.522 ***	-0.006
<i>M. rubra</i>	0.174 ***	0.137 ***
<i>M. scabrinodis</i>	-1.364 ***	-0.120 ***
<i>M. ruginodis</i>	-0.215 ***	-0.016
<i>M. schencki</i>	0.358 ***	0.006
Other ants	-0.403 ***	-0.061
<i>M. rubra +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

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

344

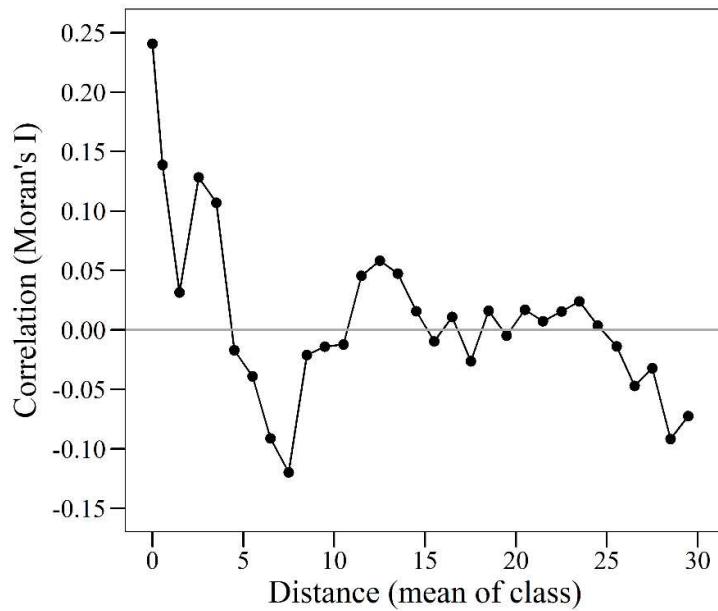
345

346 Appendix S7: Spatial correlogram of the residuals of the binomial GLMs for the
347 probability of a shoot having eggs before (red) and after (blue) including the two
348 eigenvectors issued from MEM.



349

350 Appendix S8: Spatial correlogram of the residuals of the negative binomial GLM for the
351 number of eggs in shoots with at least one egg.



352 Appendix S9: Results of the binomial GLM ($n = 8848$, $R^2 = 0.42$) relating the
353 probability of a shoot of *G. pneumonanthe* having eggs of *P. alcon* to shoot phenology,
354 ant abundance, neighbor density and neighbor phenology, and to three different two-
355 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

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

```
#Load packages
library(MASS)
library(fmsb)
library(spdep)
library(rasterVis)
library(ncf)
library(maptools)
library(gstat)
library(ggplot2)
library(ggthemes)
library(effects)
library(cowplot)

#Read data ants
data_ants_paper<-read.table("C:/Users/User/Dropbox/SU/Projects/neighbourhood_effects/data/clean/data_ants_paper.txt",header=T,sep="\t",dec=".")
head(data_ants_paper)
str(data_ants_paper)

#Defining coordinates and coordinate system
coordinates(data_ants_paper) <- c("x", "y")
project1<-"+proj=utm +zone=33 +ellps=GRS80 +towgs84=0,0,0,0,0,0,0 +units=m
+no_defs" #RT90 0 gon (spatialreference.org)
proj4string(data_ants_paper) = CRS(project1) #assign CRS with projected
coordinates

#Preparing a prediction grid
plot(data_ants_paper)
locator(4)

min_x =1499923 #minimum x coordinate
min_y =-51.043612 #minimum y coordinate
x_length = 149995 - min_x #easting amplitude
y_length = -2.330070 - min_y #northing amplitude
cellsize = 0.18 #pixel size
ncol = round(x_length/cellsize,0) #number of columns in grid
nrow = round(y_length/cellsize,0) #number of rows in grid

grd1<-GridTopology(cellcentre.offset=c(min_x,min_y),cellsize=c(cellsize,cellsize),cells.dim=c(ncol,nrow))

#Convert GridTopology object to SpatialPixelsDataFrame object.
grd1<-SpatialPixelsDataFrame(grd1,
                               data=data.frame(id=1:prod(ncol,nrow)),
                               proj4string=CRS(project1))
plot(grd1)

#Cut grid with shapefile "mask" (20 cm out of the borders of the occupied
plots)
mask<-readShapePoly("C:/Users/User/Dropbox/SU/projects/neighbourhood_effects/
gis/shapefiles/mask_20cm.shp", IDvar=NULL,proj4string=CRS(project1))
plot(mask)
mask<-mask@polygons
mask<-SpatialPolygons(mask, proj4string=CRS(project1))
plot(mask)
grd1<-grd1[!is.na(over(grd1, mask)),-]
plot(grd1)

#Inverse distance weighting (IDW) interpolation based on the values at the
```

```

sampling points to generate values of the abundance of ant species
#over the surface of all occupied subplots.
#A maximum distance of 3 m was used because it represents the ground foraging
distance of most investigated Myrmica species (Elmes et al. 1998).
#From these interpolated surfaces (see electronic supplementary material
figures S1-5), we extracted values of abundance of the different ant
#species for each of the mapped shoots.

idw1_Mrub_sum<-idw(Mrub_sum ~ 1, data_ants_paper, grdl, idp = 1, maxdist=3)
idw1_Msca_sum<-idw(Msca_sum ~ 1, data_ants_paper, grdl, idp = 1, maxdist=3)
idw1_Mrug_sum<-idw(Mrug_sum ~ 1, data_ants_paper, grdl, idp = 1, maxdist=3)
idw1_Msch_sum<-idw(Msch_sum ~ 1, data_ants_paper, grdl, idp = 1, maxdist=3)
idw1_oth_sum<-idw(oth_sum ~ 1, data_ants_paper, grdl, idp = 1, maxdist=3)

#Figures inverse distance weighted interpolation for ant abundances (Figures
S1-5)
myTheme=rasterTheme(region=c("#B3B3B3",brewer.pal('RdBu', n=11)))
levelplot(raster(idw1_Mrub_sum), par.settings=myTheme,
margin=F,scales=list(draw=FALSE))
levelplot(raster(idw1_Msca_sum), par.settings=myTheme,
margin=F,scales=list(draw=FALSE))
levelplot(raster(idw1_Mrug_sum), par.settings=myTheme,
margin=F,scales=list(draw=FALSE))
levelplot(raster(idw1_Msch_sum), par.settings=myTheme,
margin=F,scales=list(draw=FALSE))
levelplot(raster(idw1_oth_sum), par.settings=myTheme,
margin=F,scales=list(draw=FALSE))

#Read data shoot phenology, neighbor density and phenology
data_plants_paper<-read.table("C:/Users/User/Dropbox/SU/Projects/neighbourhood_effects/data/clean/data_plants_paper.txt",header=T,sep="\t",dec=".")
head(data_plants_paper)
str(data_plants_paper)

#Defining coordinates and coordinate system
coordinates(data_plants_paper) <- c("x", "y")
proj4string(data_plants_paper) = CRS(project1) #assign CRS with projected
coordinates
plot(data_plants_paper)

head(data_plants_paper)
tail(data_plants_paper)
str(data_plants_paper)

#Extrapolate ant data to every plant
data_plants_paper$Mrub_sum<-over(data_plants_paper,idw1_Mrub_sum)$var1.pred
data_plants_paper$Msca_sum<-over(data_plants_paper,idw1_Msca_sum)$var1.pred
data_plants_paper$Mrug_sum<-over(data_plants_paper,idw1_Mrug_sum)$var1.pred
data_plants_paper$Msch_sum<-over(data_plants_paper,idw1_Msch_sum)$var1.pred
data_plants_paper$oth_sum<-over(data_plants_paper,idw1_oth_sum)$var1.pred

head(data_plants_paper)
str(data_plants_paper)

#Summed abundance of all Myrmica species
data_plants_paper$allM_sum<-data_plants_paper$Mrub_sum+data_plants_paper$Msca_
_sum+data_plants_paper$Mrug_sum+data_plants_paper$Msch_sum

#Summed abundance of M. rubra and M. schencki
data_plants_paper$Mrub_sch_s<-data_plants_paper$Mrub_sum+data_plants_paper$Ms

```

```

ch_sum

#Calculation of the probability of a shoot having at least one egg (0/1)
data_plants_paper$attack<-ifelse(data_plants_paper$n_eggs_max>0,1,0)

#Univariate GLMs relating the probability of having eggs to the abundance of
different ant species (Table S1A)
summary(glm(attack ~scale(allM_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #-0.52232
summary(glm(attack ~scale(Mrub_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #0.17423
summary(glm(attack ~scale(Msca_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #-1.36392
summary(glm(attack ~scale(Mrug_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #-0.21499
summary(glm(attack ~scale(Msch_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #0.35843
summary(glm(attack ~scale(oth_sum),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #-0.40260
summary(glm(attack ~scale(Mrub_sch_s),
subset(data_plants_paper,!is.na(phen)),family="binomial")) #0.36009

#Univariate GLMs relating the number of eggs in plants with at least one egg
to the abundance of different ant species (Table S1B)
summary(glm.nb(n_eggs_max~scale(allM_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #NS
summary(glm.nb(n_eggs_max~scale(Mrub_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #0.13664
summary(glm.nb(n_eggs_max~scale(Msca_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #-0.11955
summary(glm.nb(n_eggs_max~scale(Mrug_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #NS
summary(glm.nb(n_eggs_max~scale(Msch_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #NS
summary(glm.nb(n_eggs_max~scale(oth_sum),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #NS
summary(glm.nb(n_eggs_max~scale(Mrub_sch_s),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))) #0.11490

#Binomial GLM 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 (Table S2)
modell1<-glm(attack ~ scale(phen) + scale(Mrub_sch_s) + scale(pldens_3) +
    scale(phen_n3) + scale(phen):scale(Mrub_sch_s)+
    scale(phen):scale(phen_n3) + scale(pldens_3):scale(phen_n3),
    subset(data_plants_paper,!is.na(phen)),family="binomial")
summary(modell1)
NagelkerkeR2(modell1)

#Checking for spatial autocorrelation in the residuals of modell1
res_modell1<-residuals(modell1) #Get residuals of model

#Spatial correlogram
correlog_modell1_1 <- correlog(subset(data_plants_paper,!is.na(phen))$x,
                                subset(data_plants_paper,!is.na(phen))$y,
                                res_modell1,increment=1, resamp=100)

df5<-data.frame(cbind(distance=as.vector(correlog_modell1_1$mean.of.class[1:31
]),,
```

```

correlation=as.vector(correlog_modell_1$correlation[1:31]),
  p=as.vector(correlog_modell_1$p[1:31]))
ggplot(data=df5,aes(x=distance, y=correlation)) +
  geom_point(colour = "black", size = 2) +
  geom_line(colour = "black") +
  scale_x_continuous("Distance (mean of
class)",limits=c(0,30),breaks=c(0,5,10,15,20,25,30)) +
  scale_y_continuous("Correlation (Moran's I)",limits=c(-0.05,0.3),breaks =
c(-0.05,0,0.05,0.1,0.15,0.20,0.25,0.30))+ 
  geom_hline(aes(yintercept=0), colour="darkgrey")+
  theme_base() + theme(plot.background=element_rect(fill="white", colour=NA))

#Calculation of 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.
data_plants_paper.nb1 <- dnearneigh(subset(data_plants_paper,!is.na(phen)), 0,
30) #Create neighbours matrix (30 m)
data_plants_paper.listw1 <- nb2listw(data_plants_paper.nb1)

moran_modell1<- moran.test(res_modell1, listw=data_plants_paper.listw1)
moran_modell1 #Significant autocorrelation in the residuals of modell1

#Moran's eigenvector mapping of modell1
ME.modell1 <-ME(attack ~ scale(phen) + scale(Mrub_sch_s) +
scale(pldens_3)+scale(phen_n3) + scale(phen):scale(Mrub_sch_s)+
scale(phen):scale(phen_n3) +
scale(pldens_3):scale(phen_n3),listw=data_plants_paper.listw1,
data=subset(data_plants_paper,!is.na(phen)),family=binomial,alpha=0.05,verbos
e=T)

#Repeat modell1 with Moran's eigenvectors as predictors (Table 1A)
vector1<-ME.modell1$vectors[,1]
vector2<-ME.modell1$vectors[,2]
modell_ME<-glm(attack ~ scale(phen) + scale(Mrub_sch_s) + scale(pldens_3) +
scale(phen_n3) + scale(phen):scale(Mrub_sch_s)+
scale(phen):scale(phen_n3) + scale(pldens_3):scale(phen_n3) +
scale(vector1)+scale(vector2),
subset(data_plants_paper,!is.na(phen)),family="binomial")
summary(modell_ME)
NagelkerkeR2(modell_ME)

#Checking for spatial autocorrelation in the residuals of modell_ME
res_modell_ME<-residuals(modell_ME) #Get residuals of model

#Spatial correlogram
correlog_modell_ME <- correlog(subset(data_plants_paper,!is.na(phen))$x,
subset(data_plants_paper,!is.na(phen))$y,
res_modell_ME,increment=1, resamp=100)

df6<-data.frame(cbind(distance=as.vector(correlog_modell_ME$mean.of.class[1:3
1]),

correlation=as.vector(correlog_modell_ME$correlation[1:31]),
  p=as.vector(correlog_modell_ME$p[1:31])))

df5$type<-"modell1_1"
df6$type<-"modell1_ME_1"

```

```

df7<-rbind(df5,df6)

#Figure S8
ggplot(df7,aes(x=distance, y=correlation)) +
  geom_point(aes(colour=type),size=2) +
  geom_line(aes(colour=type)) +
  scale_x_continuous("Distance (mean of
class)",limits=c(0,30),breaks=c(0,5,10,15,20,25,30)) +
  scale_y_continuous("Correlation (Moran's I)",limits=c(-0.05,0.3),breaks =
c(-0.05,0,0.05,0.1,0.15,0.20,0.25,0.30))+ 
  geom_hline(aes(yintercept=0), colour="darkgrey")+
  theme_base() + theme(plot.background=element_rect(fill="white", colour=NA)) +
  theme(legend.position="none") + theme(text=element_text(family="serif"))

#Calculation of 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.
moran_modell_ME<- moran.test(res_modell_ME, listw=data_plants_paper.listw1)
moran_modell_ME

#Negative binomial GLM relating the number of eggs in shoots with at least one
egg to shoot phenology, ant abundance,
#neighbor density and neighbor phenology, and to three different two-way
interactions of these predictors (Table 1B)
model2<-glm.nb(n_eggs_max ~ scale(phen) + scale(Mrub_sch_s) + scale(pldens_3)
+
  scale(phen_n3) + scale(phen):scale(Mrub_sch_s) +
  scale(phen):scale(phen_n3) + scale(pldens_3):scale(phen_n3),
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0))
summary(model2)
NagelkerkeR2(model2)

#Checking for spatial autocorrelation in the residuals of model1_ME
res_model2<-residuals(model2) #Get residuals of model

#Spatial correlogram
correlog_model2 <-
correlog(subset(data_plants_paper,!is.na(phen)&n_eggs_max>0)$x,
subset(data_plants_paper,!is.na(phen)&n_eggs_max>0)$y,
res_model2,increment=1, resamp=100)

df8<-data.frame(cbind(distance=as.vector(correlog_model2$mean.of.class[1:31]),
correlation=as.vector(correlog_model2$correlation[1:31]),
p=as.vector(correlog_model2$p[1:31])))

#Figure S7
ggplot(df8,aes(x=distance, y=correlation)) +
  geom_point(size=2) +
  geom_line() +
  scale_x_continuous("Distance (mean of
class)",limits=c(0,30),breaks=c(0,5,10,15,20,25,30)) +
  scale_y_continuous("Correlation (Moran's I)",limits=c(-0.15,0.25),breaks =
c(-0.15,-0.1,-0.05,0,0.05,0.1,0.15,0.20,0.25))+ 
  geom_hline(aes(yintercept=0), colour="darkgrey")+
  theme_base() + theme(plot.background=element_rect(fill="white", colour=NA)) +
  theme(legend.position="none") + theme(text=element_text(family="serif"))

```

```

#Calculation of 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.
data_plants_paper.nb2 <-
dnearest(subset(data_plants_paper,!is.na(phen)&n_eggs_max>0), 0, 30) #Create
neighbours matrix (30 m)
data_plants_paper.listw2 <- nb2listw(data_plants_paper.nb2)

moran_model2<- moran.test(res_model2, listw=data_plants_paper.listw2)
moran_model2 #NO Significant autocorrelation!

#Figures paper
interaction1<-data.frame(effect(term="scale(phen):scale(Mrub_sch_s)",
mod=model1_ME,
xlevels=list(Mrub_sch_s=seq(0,35,1),
phen=1:6)))
interaction2<-data.frame(effect(term="scale(pldens_3):scale(phen_n3)",
mod=model1_ME,
xlevels=list(phen_n3=seq(2.8,6,0.05),pldens_3=0:50)))
effect1<-data.frame(effect(term="scale(phen)",
mod=model2,xlevels=list(phen=seq(1,6,0.01))))
effect2<-data.frame(effect(term="scale(Mrub_sch_s)",
mod=model2,xlevels=list(Mrub_sch_s=seq(0,35,1))))
interaction3<-data.frame(effect(term="scale(pldens_3):scale(phen_n3)",
mod=model2,
xlevels=list(phen_n3=seq(2.8,6,0.05),pldens_3=0:50)))

myPalette <- colorRampPalette(rev(brewer.pal(11, "Spectral")))

p1<-ggplot(interaction1, aes(phen,fit, group = as.factor(Mrub_sch_s))+
  geom_smooth(method=loess,se=F,size=0.3,aes(phen,fit,color=Mrub_sch_s))+ 
  xlab("Shoot phenology")+ylab("Probability of having
eggs") +theme_base() +scale_colour_gradientn(colours = myPalette(100)) +
  theme(legend.position="top") +labs(colour="Ant
abundance") +scale_x_continuous(breaks=c(1,2,3,4,5,6)) + 

  theme(text=element_text(family="serif")) +theme(plot.background=element_rect(f
ill="white", colour=NA))

p2<-ggplot(interaction2, aes(pldens_3,fit, group = as.factor(phen_n3)))+
  geom_smooth(method=loess,se=F,size=0.3,aes(pldens_3,fit,color=phen_n3)) +
  xlab("Neighbor density") +ylab("Probability of having eggs") +
  theme_base() + scale_colour_gradientn(colours = myPalette(100)) +
  theme(legend.position="none") +labs(colour="Neighbor phenology") + 

  theme(text=element_text(family="serif")) +theme(plot.background=element_rect(f
ill="white", colour=NA))

p3<-ggplot(effect1, aes(phen,fit))+
  geom_smooth(method=loess,se=T,size=1,color="black",aes(phen,fit)) +
  xlab("Shoot phenology") +ylab("Number of eggs") +theme_base() + 

  scale_x_continuous(breaks=c(1,2,3,4,5,6)) +scale_y_continuous(breaks=c(1,2,3,4
,5,6)) + 

  theme(text=element_text(family="serif")) +theme(plot.background=element_rect(f
ill="white", colour=NA)) +
  geom_ribbon(aes(ymin = lower, ymax = upper), alpha = 0.5,fill="grey")

p4<-ggplot(effect2, aes(Mrub_sch_s,fit))+

```

```
geom_smooth(method=loess, se=T, size=1, color="black", aes(Mrub_sch_s, fit)) +
  xlab("Ant abundance") + ylab("Number of eggs") + theme_base() +
  scale_x_continuous(breaks=c(0, 5, 10, 15, 20, 25, 30, 35)) +
  scale_y_continuous(breaks=c(0, 2, 4, 6, 8, 10, 12, 14, 16)) +
  theme(text=element_text(family="serif")) + theme(plot.background=element_rect(fill="white", colour=NA)) +
  geom_ribbon(aes(ymin = lower, ymax = upper), alpha = 0.5, fill="grey")
p5<-ggplot(interaction3, aes(pldens_3, fit, group = as.factor(phen_n3))) +
  geom_smooth(method=loess, se=F, size=0.3, aes(pldens_3, fit, color=phen_n3)) +
  xlab("Neighbor density") + ylab("Number of eggs") +
  theme_base() + scale_colour_gradientn(colours = myPalette(100)) +
  theme(legend.position="top") + labs(colour="Neigh. phenology") +
  theme(text=element_text(family="serif")) + theme(plot.background=element_rect(fill="white", colour=NA))

#Figure 2
ggdraw() +
  draw_plot(p1, 0.02, 0.01, 0.3, 1) +
  draw_plot(p3, 1/3+0.04, 0.01, 0.3, 0.81) +
  draw_plot(p4, 2/3+0.04, 0.01, 0.3, 0.81) +
  draw_label(label="A", x=0.01, y=0.8, fontfamily = "serif", fontface = 1) +
  draw_label(label="B", x=0.39, y=0.8, fontfamily = "serif", fontface = 1) +
  draw_label(label="C", x=0.73, y=0.8, fontfamily = "serif", fontface = 1)

#Figure 3
ggdraw() +
  draw_plot(p2, 0.02, 0, 0.45, 0.83) +
  draw_plot(p5, 1/2, 0, 0.45, 1) +
  draw_label(label="A", x=0.01, y=0.81, fontfamily = "serif", fontface = 1) +
  draw_label(label="B", x=0.53, y=0.81, fontfamily = "serif", fontface = 1)
```