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

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

2   Running head: Resource use in a consumer butterfly

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ABSTRACT

1. The effects of consumers on fitness of resource organisms are a complex function of the spatio-temporal distribution of the resources, consumer functional responses and trait preferences, and availability of other resources.

2. The ubiquitous variation in the intensity of species interactions has important consequences for the ecological and evolutionary dynamics of natural populations. Nevertheless, little is known about the processes causing this variation and their operational scales. Here, we examine how variation in the intensity of a consumer-resource interaction is related to resource timing, resource density and abundance of other resources.

3. Using the butterfly consumer *Phengaris alcon* and its two sequential resources, the host plant *Gentiana pneumonanthe* and the host ants *Myrmica* spp., we investigated how butterfly egg-laying depended on focal host plant phenology, density and phenology of neighboring host plants and host ant abundance.

4. Butterflies preferred plants that simultaneously maximized the availability of both larval resources in time and space, i.e., they chose early-flowering plants that were of higher nutritional quality for larvae where host ants were abundant. Both the probability of oviposition and the number of eggs were lower in plant individuals with a high neighbor density than in more isolated plants, and this dilution effect was stronger when neighbors flowered early.

5. Our results show that plant-herbivore interactions simultaneously depend on the spatio-temporal distribution of a focal resource, and on the small-scale spatial variation in the

37   abundance of other herbivore resources. Given that consumers have negative effects on

38   fitness and prefer certain timing of the resource organisms, this implies that processes

39   acting at the levels of individuals, populations and communities simultaneously

40   contribute to variation in consumer-mediated natural selection.

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42 Keywords: butterflies, flowering phenology, myrmecophily, plant-herbivore interactions,

43 resource use, spatial variation

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45   INTRODUCTION

46   Resource-consumer interactions often have important effects on the performance of both partners

47   and the distribution and abundance of consumers is expected to match the spatio-temporal

48   distribution of their resources. Resource use by insect herbivores is often conditioned by

49   oviposition site selection by females. As larval stages usually show low mobility, insect

50   oviposition preferences are predicted to match the spatial and temporal distribution of host plants

51   suitable for offspring development (Gripenberg, Mayhew, Parnell, & Roslin, 2010; Jaenike,

52   1978; Scheirs & De Bruyn, 2002). The suitability of host plants, in turn, might depend on factors

53   such as host plant nutritional quality (Chen, Lin, Wang, Yeh, & Hwang, 2004), size (Rabasa,

54   Gutiérrez, & Escudero, 2005), microclimatic conditions (Eilers, Pettersson, & Öckinger, 2013)

55   or enemy-free space (Ballabeni, Wlodarczyk, & Rahier, 2001). Most consumers depend on

56   multiple resources throughout their life cycle, e.g. different sources of food, shelter and resting

57   sites (Dennis, Shreeve, & Van Dyck, 2003, 2006). The availability of one resource might thus

58   influence the use of other resources. For example, the availability of nectar resources for adults

59   (Öckinger, 2008) or secondary hosts for larvae (Wynhoff, Bakker, Oteman, Arnaldo, & van

60   Langevelde, 2015) might determine host plant choices in butterflies.

61   The quantitative relationship between the abundance of consumers and the abundance of

62   resource organisms, and thus resource exploitation, will depend both on the birth and death

63   processes and on consumer behavior. Consumer behavior may specifically cause consumer

64   density and resource exploitation to increase (cf. resource concentration hypothesis, e.g.

65   Hambäck & Englund, 2005; Root, 1973; Stephens & Myers, 2012) or decrease (cf. resource

66   dilution, e.g. Otway, Hector, & Lawton, 2005) with increasing patch size and resource

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67   abundance. Observed differences in the relationships between resource exploitation and patch

68   size or patch density among different consumers have been suggested to be the result of

69   differences in how emigration and immigration rates depend on patch size and patch density, and

70   on the scale of consumer selection (Andersson, Löfstedt, & Hambäck, 2013; Hambäck &

71   Englund, 2005). For example, a strong negative relationship between consumer density and

72   patch area observed in visual searchers like butterflies might be the result of that immigration is

73   diameter-dependent while emigration is perimeter-dependent. Not only should the abundance of

74   the resource organism be important for consumer responses, but also its timing and the temporal

75   matching with the activity period of the consumer. For example, in herbivores that depend on a

76   specific developmental stage of their host plant, the density of plants that are in suitable

77   developmental stages at the time when the herbivore is active, rather than the overall density, is

78   expected to influence host use. Although the effects of resource density on plant-insect

79   interactions (Kim & Underwood, 2015; Kula, Castillo, Dudash, & Fenster, 2014; Verschut,

80   Becher, Anderson, & Hambäck, 2016; Yamamura, 2002) are well studied, the role of resource

81   timing and the effects of the availability of other resources have rarely been considered in these

82   studies. Moreover, while the ecological and evolutionary effects of variation in consumer-

83   resource interaction intensities have been documented for many systems (e.g. Chamberlain,

84   Bronstein, & Rudgers, 2014; Kolb, Ehrlén, & Eriksson, 2007; Vanhoenacker, Ågren, & Ehrlén,

85   2013), the simultaneous effects of factors at different levels: individuals (e.g. timing),

86   populations (e.g. resource density), and communities (e.g. availability of other resources) have

87   rarely been explored. This is particularly true for small spatial scales, such as variation in the

88   intensity of herbivory among individuals within plant populations.

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

90   complete their development: the caterpillars are predispersal seed predators during their first

91   instars and parasitize ant nests (*Myrmica* spp.) during later stages. Butterflies are very specific

92   regarding both host plant and host ant species and the developmental stage and phenology of

93   host plants (Elmes et al., 1998; Thomas & Elmes, 2001; Valdés & Ehrlén, 2017). Female

94   butterflies oviposit preferentially on buds in early phenological stages, probably because these

95   constitute higher-quality food for the caterpillars; the developing seeds might become tougher,

96   drier and more difficult to digest as buds develop into flowers and fruits (Arnaldo, Gonzalez,

97   Oliveira, Langevelde, & Wynhoff, 2014). In some species, host plant flowering time determines

98   oviposition and individuals flowering early in the season receive more eggs because the presence

99   of the preferred bud developmental stages overlaps more with the period of butterfly oviposition

100   in early- than in late-flowering plants (Valdés & Ehrlén, 2018). Moreover, the mobility of

101   caterpillars after leaving their host plants is low (Hayes, 2015), and further development is

102   possible only if the foraging range of suitable host ants is sufficiently close to the host plant

103   individual (usually a few meters). Butterflies are therefore expected to oviposit on host plants in

104   suitable development stages only if plants occur at micro-sites where ant hosts are present

105   (Wynhoff, Grutters, & Langevelde, 2008; Wynhoff & van Langevelde, 2017).

106   In this study, we investigated how the phenology, in terms of the developmental stage of

107   the reproductive structures, of one resource, the focal host plant *Gentiana pneumonanthe,* the

108   density and phenology of neighboring conspecific plants, and the abundance of host ants

109   influence resource use, in terms of egg-laying, by the consumer butterfly *Phengaris alcon.* We

110   used an extensive field data set to test two hypotheses: (1) Effects of plant phenology and ant

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111   abundance on egg distribution patterns are interdependent: butterflies choose plants for

112   oviposition that simultaneously maximize the availability of both resources, i.e. they have a

113   stronger preference for early-flowering host plants where host ants are abundant, and (2) The

114   probability of a plant receiving at least one egg, as well as the number of eggs in oviposited

115   plants, decreases with increasing conspecific neighbor density (i. e. a dilution effect), and this

116   decrease is stronger when neighbors show the preferred phenology (i.e. flower early).

117   MATERIALS AND METHODS

118   **Study system**

119   The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb, occurring in

120   open habitats, such as wet heathlands and grasslands (Simmonds, 1946). Plants can have one to

121   many, up to 45 cm high, shoots and produce deep blue flowers that are pollinated by

122   bumblebees. The species is self-compatible and flowers in July and August in the study area (SW

123   Sweden). Fruits are capsules containing a high number of minute, wind-dispersed seeds (mean

124   seed number per fruit $\pm$ SD $= 490 \pm 243$, mean seed weight $= 0.041$ mg, authors' unpublished

125   data). *Gentiana pneumonanthe* is the host plant of the Alcon Blue butterfly (*Phengaris alcon*), a

126   specialist predispersal seed predator which oviposits on young flower buds in July and August

127   (Appelqvist & Bengtsson, 2007). During an oviposition event, adult female butterflies usually

128   lay more than one egg on the same plant and even on the same bud, and there is no evidence of

129   avoiding plants that already carry eggs (Van Dyck & Regniers, 2010). The caterpillars feed

130   inside the capsule until they reach the fourth instar, when they drop to the ground to be picked up

131   by *Myrmica* ants. Caterpillars mimic the surface chemistry of the ant brood (Nash, Als, Maile,

132   Jones, & Boomsma, 2008) and the acoustic signals of queen ants (Sala, Casacci, Balletto,

133   Bonelli, & Barbero, 2014), and these features cause ants to carry them to their nest, where they

134   spend the rest of their larval period as brood parasites. Females of *Phengaris* detect *Myrmica*

135   presence through plant chemicals produced by ants or by plants in response to root tissue damage

136   caused by ants (Patricelli et al., 2015; van Dyck et al., 2000; Wynhoff & van Langevelde, 2017).

137   Contrary to the majority of *Phengaris* species, which prey on ant brood, *P. alcon* is a "cuckoo"

138   species (Als et al., 2004), and caterpillars feed primarily on regurgitants from ant workers,

139   trophic eggs (i.e. nutritious, infertile eggs which are fed to the queens and ant larvae) and prey

140   items brought to the nest by ants. Only ants belonging to *Myrmica* sp. can act as hosts of *P.*

141   *alcon*. In Sweden, *M. rubra* is considered to be the most common host ant species (Elmes et al.,

142   1994), although *M. ruginodis* has also been reported as a host (Nash & Andersen, 2015).

143   **Data collection**

144   The study was carried out in a population of *G. pneumonanthe*, where the butterfly *P.*

145   *alcon* was present, located in Tånga Hed nature reserve in the county of Västra Götaland, SW

146   Sweden (58°01'40.0"N 12°49'47.0"E). Field work was conducted between 9 July and 3

147   September 2016. The plant population occupies a fenced meadow that is grazed by cattle at the

148   end of the summer every year. A study plot measuring 69 m × 45 m was established in the

149   meadow. This plot was further divided into a grid comprising 345 3 m × 3 m subplots with

150   corners permanently marked by wooden poles (Fig. 1A). *G. pneumonanthe* was present in 172 of

151   these subplots. In 154 of the subplots where *G. pneumonanthe* was present, all reproductive

152   shoots of *G. pneumonanthe* were mapped (Fig. 1B), and their phenology and the number of eggs

153   of *P. alcon* were recorded between 29 July and 5 August (18 additional occupied subplots were

154   not possible to fully survey due to time constraints). Shoots were mapped by recording their

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155   coordinates within subplots. We mapped shoots rather than plant individuals (that may have one

156   or several shoots) because we judged that individual shoots are the relevant unit for the butterfly

157   to assess the timing of the food resource, and because individual shoots act as the unit of

158   attraction for butterfly females. For each shoot, phenology was recorded as the developmental

159   stage of the most advanced bud, according to six ordinal categories: (1) the sepals covering the

160   bud completely, (2) bud becoming visible, (3) bud growing over the sepals, (4) bud turning blue,

161   (5) flower opening, and (6) flower showing signs of wilting (Valdés & Ehrlén, 2017). Each stage

162   corresponds to a time interval of flower development, with higher values indicating a more

163   advanced floral development at the day of recording, i.e. an earlier flowering phenology.

164   According to the observed average duration of the stages, a one-unit increase in this phenology

165   measure roughly corresponds to 1 week earlier development (Valdés & Ehrlén, 2017, 2018).

166   The number of eggs was counted on the whole shoot.

167   The ant community was sampled at each corner of the occupied subplots (a total of 254

168   points) at two occasions, 14 July and 3 September (Fig. 1B). Baits consisted of a 50-ml plastic

169   tube in which we placed a sugar cube. The tubes were left open on the ground in the evening,

170   and collected the next morning. Ants collected were transferred to 70º ethanol and the number of

171   ants of different *Myrmica* species were counted in the lab (using Douwes, Abenius, Cederberg,

172   & Wahlstedt, 2012 for identification). Ants belonging to other genera where not identified to

173   species, but counted as "other ants".

174   **Interpolations of ant abundances**

175   We also incorporated the locations of the 254 points where the ant community was sampled into

176   the GIS. We used inverse distance weighted interpolation (Pebesma, 2004) based on the values at

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177   the sampling points to generate values of the abundance of ant species over the surface of all

178   occupied subplots. The ground foraging distance of most investigated *Myrmica* species is up to 2

179   m, although some species can forage further afield (Elmes et al., 1998). For our interpolations,

180   we therefore used a maximum distance of 3 m because we considered it to include most foraging

181   events. From these interpolated surfaces (see Figs. S1-S5), we extracted values of abundance of

182   the different ant species for each of the mapped shoots.

183   **Calculation of neighborhood measures**

184   Field maps were digitized using a Geographic Information System (GIS) created with

185   ArcGIS 10.3.1, assigning relative coordinates to each recorded reproductive shoot of *G.*

186   *pneumonanthe* (Fig. 1B). For each shoot, we defined a buffer zone with a 3-m radius, centered at

187   the focal shoot (see example in Fig. 1B), and calculated the number of reproductive shoots of *G.*

188   *pneumonanthe* per m^2 within this buffer zone (hereafter, "neighbor density") as well as the mean

189   phenology of these shoots (hereafter, "neighbor phenology"), excluding in each case the focal

190   shoot.

191   **Statistical analyses**

192   The effects of shoot phenology, host ant abundance, neighbor density and neighbor phenology

193   on *P. alcon* egg distribution were examined using two Generalized Linear Models (GLMs).

194   Shoot phenology was treated as a discrete numeric variable taking values of 1, 2, 3, 4, 5 or 6. We

195   chose to treat phenology as a numeric variable rather than an ordinal variable because the

196   relationship between oviposition probability and phenological stage was monotonic and

197   approximately linear (Fig. S6). Neighbor phenology could take any values between 1 and 6 and

198   was therefore treated as a continuous numeric variable. In order to minimize effects of

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199   multicollinearity, we checked for correlations between explanatory variables, which were

200   generally low (Table S1). Effects on the probability of a shoot having at least one egg were

201   evaluated using a binomial GLM, and effects on the number of eggs in shoots with at least one

202   egg were evaluated using a negative binomial GLM. Both models included also the effects of

203   three interactions. First, we included the interaction between shoot phenology and ant abundance

204   in order to test if the effects of the two resources on egg distribution patterns are interdependent.

205   Second, we included the interaction between neighbor density and neighbor phenology in order

206   to test if the effect of host plant density depends on phenology of neighboring host plants. Third,

207   we included the interaction between shoot phenology and neighbor phenology to account for the

208   fact that the relationship between egg distribution and phenology of the focal plant might depend

209   on the phenology of neighboring host plants. As a measure of host ant abundance, we used the

210   abundance of *M. rubra* (hereafter, "ant abundance"). *M. rubra* is considered to be the most

211   common host ant species of *P. alcon* in Sweden (Elmes et al., 1994), although *M. ruginodis* has

212   also been reported as a host in areas near our study site (Nash & Andersen, 2015). As we have no

213   direct information on host ant use from our study site (no nests have been opened), we cannot

214   exclude either of these two species as being used as hosts by *P. alcon*. Our decision to use the

215   abundance of only *M. rubra* in our models was based on that it was the only species that showed

216   positive effects on the probability of host plants receiving eggs and the number of eggs in plants

217   with at least one egg (*M. rugnodis* had in fact a negative effect, Table S2). However, as we have

218   no conclusive evidence that *M. rubra* serves as the only or main host at our site, we also

219   performed alternative analyses using the abundance of *M. ruginodis* and the summed abundances

220   of both *M. rubra* and *M. ruginodis* (Table S3).

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221   All predictors were standardized by subtracting the mean and dividing by the standard

222   deviation before the analyses. We checked for spatial autocorrelation in the residuals of models

223   by plotting spatial correlograms and by calculating global Moran's I with a permutation test

224   (1000 random permutations). Moran's I was calculated based on a connectivity matrix of

225   pairwise Euclidean distances among the shoots up to a distance of 30 m. Residual spatial

226   autocorrelation could lead to biased model estimates and invalid statistical inference (Dormann

227   et al., 2007). A significant spatial autocorrelation was found in the residuals of the binomial

228   GLM, but not for the negative binomial GLM (global Moran's $I = 0.0148$, $p < 0.001$ and global

229   Moran's $I = -0.0013$, $p = 0.479$, respectively, Figs. S7-S8). We therefore applied Moran's

230   eigenvector mapping (MEM), which translates the spatial arrangement of data points into

231   explanatory variables (eigenvectors) that capture spatial effects, to the binomial model

232   examining effects on the probability of a shoot having at least one egg (Dray, Legendre, & Peres-

233   Neto, 2006; Thayn & Simanis, 2013). The model was refitted including two eigenvectors issued

234   from MEM as spatial predictors, and this strongly reduced the residual spatial autocorrelation

235   (global Moran's $I = 0.0004$, $p = 0.004$, Fig. S7). Although residual spatial autocorrelation was

236   still significant, the low value of Moran's I and visual inspection of the spatial correlogram (Fig.

237   S7) indicated that almost all of the residual spatial autocorrelation was removed by the spatial

238   predictors. The results of this model were similar to the results of the binomial GLM not

239   including the spatial predictors (Table S4). Statistical analyses were carried out in R 3.4.0 (R

240   Core Team 2017).

241   RESULTS

242   Eggs of *P. alcon* were found on 731 (8.3%) of 8848 reproductive shoots of *G.*

243 *pneumonanthe* recorded in 154 subplots. On shoots with at least one egg, the mean number of

244 eggs was 5.03 ± 0.20 SE.

245   The effects of shoot phenology and ant abundance on *P. alcon* oviposition were

246 interdependent. The probability of a shoot of *G. pneumonanthe* having at least one egg increased

247 with an earlier phenology (i.e. with a more advanced floral development at the day of recording,

248 Table 1, Fig. 2A, Fig. S6). This increase was stronger in shoots where *M. rubra* was more

249 abundant (the effect of the interaction shoot phenology × ant abundance being significant in the

250 model without spatial predictors and marginally significant after including the spatial predictors,

251 Table S3, Fig. 2A, Table 1). In analyses based on the abundance of *M. ruginodis* or the summed

252 abundance of *M. rubra* and *M. ruginodis*, the probability of a shoot of *G. pneumonanthe* having

253 at least one egg instead decreased with a higher ant abundance (Table S2). The number of *P.*

254 *alcon* eggs on individuals with at least one egg increased with an earlier shoot phenology and

255 with a higher host ant abundance, and the effect of the interaction between phenology and ant

256 abundance was marginally significant and in the same direction as in the model for the

257 probability of having eggs (Table 1, Fig. 2B and C).

258   High densities of host plant shoots resulted in a dilution effect, and the strength of this

259 effect depended on the quality of neighboring plants. Both the probability of having at least one

260 egg and the number of eggs on shoots with at least one egg decreased with the overall density of

261 neighboring host plant shoots (Table 1, Fig. 3). In both cases, the decrease was stronger when

262 neighboring host plants showed the preferred phenology, i.e. had an earlier phenology

263 (significant effect of the interaction neighbor density × neighbor phenology, Table 1, Fig. 3).

264 DISCUSSION

265   The results of this study show that resource use in the myrmecophilous butterfly *P. alcon*

266   is shaped by both the spatial overlap of the two key larval resources and by a dilution effect.

267   Specifically, we found that butterflies tend to prefer to oviposit on early-flowering plants that

268   have a high abundance of potential host ants, that plants surrounded by many conspecific

269   neighbors receive fewer eggs, and that the dilution effect is stronger when neighbors show the

270   preferred phenology.

271   We found that both the probability of *P. alcon* butterflies laying at least one egg on a host

272   plant as well as the number of eggs increased with an earlier phenology of its host plant *G.*

273   *pneumonanthe*. This agrees with previous studies showing that insects usually prefer plants with

274   a particular phenology (Elzinga et al., 2007), and more specifically, that antagonists

275   preferentially attack early-flowering plants (König, Wiklund, & Ehrlén, 2015; Parachnowitsch &

276   Caruso, 2008; Valdés & Ehrlén, 2017, 2018). The timing of butterfly oviposition relative to plant

277   phenology is likely to vary among years, depending on temporal variation in both plant and

278   butterfly phenology. Higher temperatures associated with climate change are expected to cause

279   significant advances in the phenology of many organisms (Parmesan, 2006), and the synchrony

280   of plant-insect interactions, like the one examined in this study, might be affected if interacting

281   species respond differently to warming. Anyway, our results, together with previous studies

282   (Valdés & Ehrlén, 2017, 2018) provide convincing evidence of the important role of the timing

283   of the plant resource (i.e. host plant phenology) relative to the oviposition period of *P. alcon*.

284   Interestingly, in our study the strength of this preference for an early plant phenology did

285   depend on the abundance of a possible second resource for the antagonist, i.e. the ant species

286   *Myrmica rubra*. The preference of *P. alcon* for early-flowering host plants tended to be stronger

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287   at locations where *M. rubra* was more abundant. For example, the probability of a shoot with the

288   earliest possible phenology receiving at least one egg was 0.07 at sites where *M. rubra* was

289   absent, and 0.62 at sites with the maximum *M. rubra* abundance observed (n = 34 ants). This

290   suggests that the butterflies are able to simultaneously track the distribution of the two resources,

291   and to preferentially oviposit on plants where both the availability of high-quality food for the

292   caterpillars and the probability of adoption by a suitable species of *Myrmica* are higher. When

293   suitable host ants were absent or very scarce, host plant phenology did not seem to affect egg-

294   laying, and the probability that early-flowering plants should receive eggs was very low. Our use

295   of abundance of *M. rubra* as a measure of host ant abundance was not based on actual host

296   identification but on correlations between abundance and occurrence and number of eggs.

297   Although the other *Myrmica* species investigated had a negative effect on egg occurrence (Table

298   S2), we cannot completely rule out the possibility that these species act as hosts in our study site,

299   and that the correlations with *M. rubra* abundance are due to the effect of some unmeasured

300   environmental variable, rather than to butterflies directly tracking the abundance of this ant

301   species. Previous observational studies have suggested that oviposition is host-species dependent

302   (van Dyck et al., 2000). Experimental studies have found *Phengaris* oviposition to be dependent

303   on *Myrmica* ant presence, but found no effect of species identity (Patricelli et al., 2015;;

304   Wynhoff & van Langevelde, 2017). Lastly, there are several studies that have found no evidence

305   of ant-dependent oviposition (Fürst & Nash, 2010; Nowicki, Witek, Skorka, & Woyciechowski,

306   2005; Thomas & Elmes, 2001). Still, in combination with previous evidence of *M. rubra* being

307   the most common host ant species of *P. alcon* in Sweden (Elmes et al., 1994), the results of our

308   analyses suggest that *M. rubra* serves as a host species at our study site.

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309   Spatial overlap between the distribution of host plants and host ant nests at larger scales

310   has previously been shown to increase the density and population persistence of the congeneric

311   species *P. arion* (Casacci et al., 2011; Thomas, Simcox, & Clarke, 2009). Other studies with

312   *Phengaris* butterflies have demonstrated effects of host plant phenology (Thomas & Elmes,

313   2001; Valdés & Ehrlén, 2017, 2018), *Myrmica* presence (van Dyck et al., 2000; Wynhoff et al.,

314   2008; Wynhoff & van Langevelde, 2017) or both of these factors (Casacci et al., 2011) for

315   oviposition site selection. Our study suggests that the spatial overlap between these two

316   resources also at the scale of the host plant individual is associated with butterfly oviposition

317   preference, and that the effects of plant phenology and ant abundance are interactive. An

318   important general implication of these findings is that differences in availability of a resource

319   over very small spatial scales might influence the use of other resource by consumers that

320   depend on multiple resources. The results also suggest that the effects of factors at the levels of

321   the individual (plant phenology) and the community (availability of the ant resource) on small-

322   scale spatial variation in consumer-resource interactions are interactive.

323   Our results show both that there is a dilution effect of local host plant density on butterfly

324   resource use, and that the strength of this effect depends on the phenology of neighboring plants.

325   Higher densities of neighboring host plants were associated with both a decreasing probability of

326   a focal plant receiving at least one egg and a lower number of eggs in plants with eggs, i.e. a

327   lower consumer pressure. The results of previous studies with this species (Van Dyck &

328   Regniers, 2010) suggest that females usually lay more than one egg on the same plant, and that

329   they do not avoid oviposition on plants that already carry eggs. If this is true also in our study,

330   then the eggs observed on an individual plant correspond to the offspring of either a single or

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331   several females (but this is something we cannot determine because oviposition was not directly

332   observed in this study). Because we cannot be sure whether the lower number of eggs was the

333   result of fewer females visiting the plant, or of each visiting female laying fewer eggs, it is more

334   appropriate here to talk about "consumer pressure" than about "consumer abundance". In any

335   case, the observed negative relationship between consumer pressure and overall resource

336   abundance was stronger when the surrounding plants flowered early in the season, i.e. they had

337   buds in early developmental stages during the period of butterfly oviposition. In other words, at a

338   given conspecific density more eggs were found in *G. pneumonanthe* plants surrounded by non-

339   attractive neighbors (i.e. late-flowering plants). In our study, plant phenology thus appears to

340   influence butterfly oviposition not only because the phenology of a focal plant interacts with host

341   ant availability to determine plant suitability for oviposition, but also because the phenology of

342   neighbors modulates the strength of host plant density effects on oviposition. Therefore, not only

343   factors at the levels of individuals and communities, but also at the level of the plant population,

344   drive variation in resource use.

345   Our results suggest that oviposition decisions in myrmecophilous butterflies are taken at

346   two levels. When flying over an area populated with host plants, females might primarily use

347   visual cues (Van Dyck & Regniers, 2010) to evaluate host plant availability (i.e. density) and

348   identify host plants with the preferred phenology (i.e. early-flowering). When butterflies

349   approach host plants, or after alighting on a plant, females may use both visual cues to evaluate

350   plant phenology and chemical cues to detect ant presence (Patricelli et al., 2015; Wynhoff et al.,

351   2015; Wynhoff & van Langevelde, 2017), thereby evaluating the spatial overlap of the two

352   resources needed by their offspring. If females use plant developmental stage as a cue indicating

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353 the availability of high-quality food for the caterpillars, host plants surrounded by late-flowering

354 neighbors would be more attractive to butterflies than host plants surrounded by early-flowering

355 neighbors. Negative density-dependence in antagonists has been documented also in other

356 systems, where egg clumping on plants with few conspecific neighbors was explained by a

357 disproportionate attraction of insect herbivores to more isolated host plants (Hasenbank &

358 Hartley, 2015; Shea, Smyth, Sheppard, Morton, & Chalimbaud, 2000). For herbivores feeding on

359 several plant species, associational effects linked to the surrounding plant community have been

360 documented more frequently (e.g. Bergvall, Rautio, Kesti, Tuomi, & Leimar, 2006; Hjältén,

361 Danell, Lundberg, & Hjalten, 1993). Our study shows that associational effects are present also

362 for a specialized herbivore using a single host species, and that the strength of these density-

363 dependent effects depends on the phenology of conspecific neighboring host plants.

364 Our results are important to understand the factors causing variation in the intensity of

365 interactions between consumers and their resources. Given that consumers often have important

366 effects on fitness in the consumed organisms and that they show preferences for timing of the

367 resource organisms, identifying the factors that influence the intensity of interactions is also

368 important to understand the mechanisms underlying variation in natural selection. In our study

369 system, the butterfly seed predator has strong negative effects on plant fitness and shows a

370 preference for plants with an early flowering phenology, leading to butterfly-mediated selection

371 for later flowering (Valdés & Ehrlén, 2017). The results of the current study strongly suggest that

372 the strength of butterfly preferences for early-flowering plants is influenced by both population

373 context, in terms of population density and the trait distribution with the population, and by

374 community context in terms of the abundance of the ant species that function as the second host

18

375   of butterfly caterpillars. Taken together, these findings illustrate that observed spatial variation in

376   intensities of interactions and consumer-mediated selection on traits of the resource organisms

377   can be the result of processes acting at the levels of individuals, populations and communities.

378   ACKNOWLEDGEMENTS

384   REFERENCES

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

385 *pneumonanthe* receiving at least one egg from the butterfly *Phengaris alcon* (binomial GLM)

386 and the number of eggs in shoots with at least one egg (negative binomial GLM), of shoot

387 phenology, ant abundance (measured as abundance of *Myrmica rubra*), neighbor density and

388 neighbor phenology, as well as three two-way interactions of these predictors. In the binomial

389 GLM, two eigenvectors issued from Moran's eigenvector mapping (MEM) are also included as

390 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 = 0.23)		
	Estimate	SE	P	Estimate	SE	P
Shoot phenology	1.476	0.079	<0.001	0.251	0.036	<0.001
Ant abundance	-0.028	0.051	0.588	0.117	0.067	<0.001
Neighbor density	-1.673	0.101	<0.001	-0.351	0.067	<0.001
Neighbor phenology	-1.154	0.123	<0.001	-0.105	0.040	0.009
Shoot phenology × Ant abundance	0.116	0.064	0.071	0.057	0.031	0.062
Shoot phenology × Neighbor phenology	0.100	0.072	0.163	0.002	0.034	0.943
Neighbor density × Neighbor phenology	-0.480	0.087	<0.001	-0.089	0.042	0.034
MEM Vector 1	0.281	0.030	<0.001			
MEM Vector 2	0.356	0.050	<0.001			

FIGURE LEGENDS

Figure 1: Sampling design. A) Study plot (69 × 45 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.
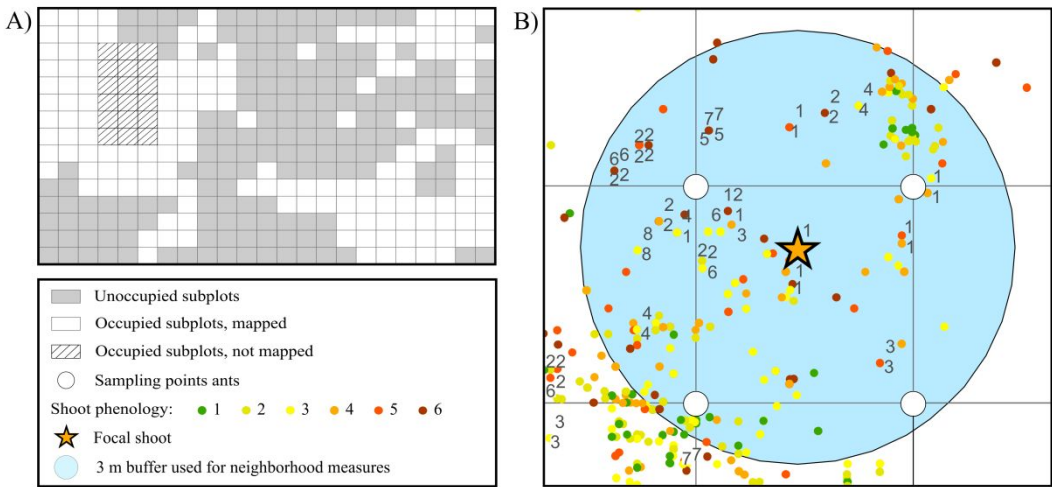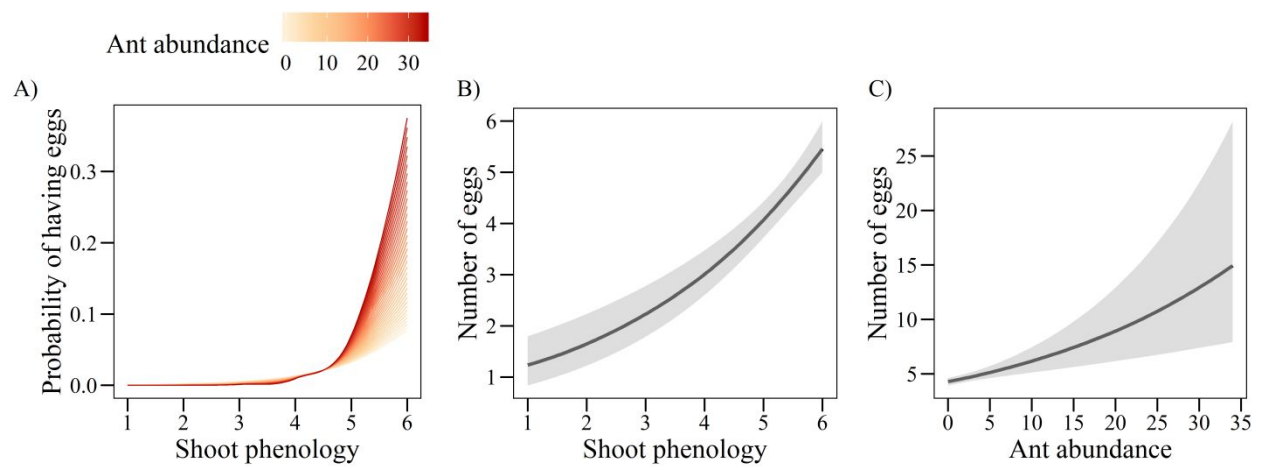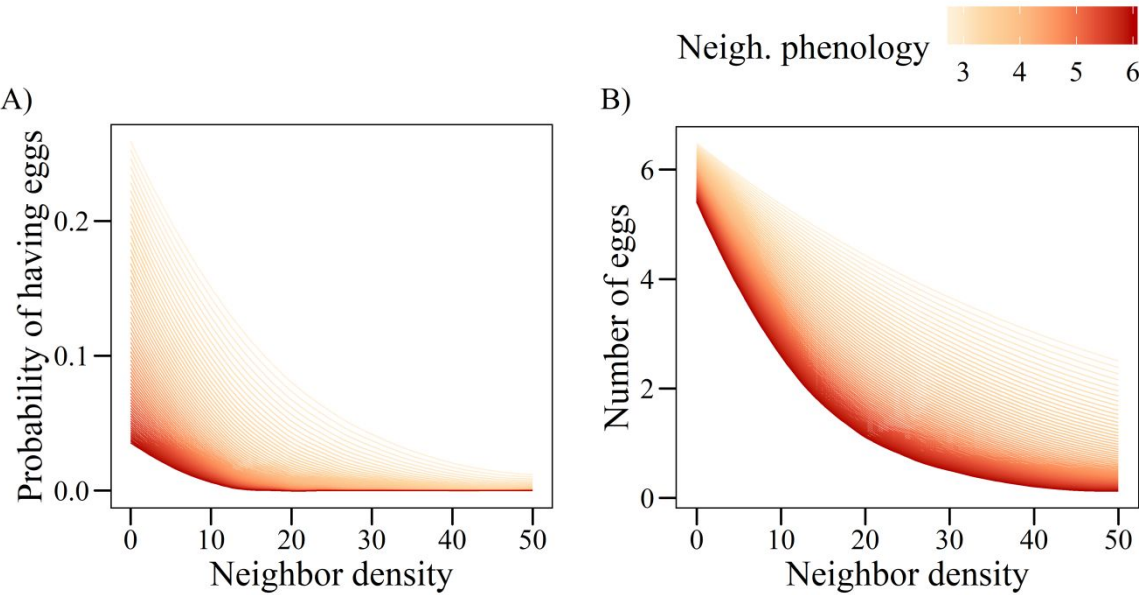
Figure 1

Figure 2

Figure 3

Supporting Information

Table S1: Correlations among the explanatory variables included in the GLMs. Values shown are Pearson correlation coefficients.

	Shoot phenology	Ant abundance (*M. rubra*)	Ant abundance (*M. ruginodis*)	Ant abundance (*M. rubra + ruginodis*)	Neighbor density	Neighbor phenology
Shoot phenology	1.000					
Ant abundance (*M. rubra*)	0.007	1.000				
Ant abundance (*M. ruginodis*)	0.074	0.078	1.000			
Ant abundance (*M. rubra + ruginodis*)	0.071	0.377	0.953	1.000		
Neighbor density	-0.242	-0.020	-0.010	-0.015	1.000	
Neighbor phenology	0.400	0.038	0.185	0.183	-0.584	1.000

1

Table S2: 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 that might act as hosts of *P. alcon* in the study site.

	A) Probability of having eggs (n = 8848)			B) Number of eggs (n = 731)		
	Estimate	SE	P	Estimate	SE	P
M. rubra	0.174	0.029	<0.001	0.137	0.031	<0.001
M. ruginodis	-0.215	0.046	<0.001	-0.016	0.033	0.620
M. rubra + M. ruginodis	-0.101	0.042	0.015	0.043	0.032	0.180

2

Table S3: 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. Ant abundance was measured as abundance of *Myrmica ruginodis* in A) and as the summed abundance of *M. rubra* and *M. ruginodis* in B). In the binomial GLMs, 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 = 0.20)		
	Estimate	SE	P	Estimate	SE	P
A) Shoot phenology	1.486	0.081	<0.001	0.250	0.036	<0.001
Ant abundance	-0.211	0.086	0.014	-0.030	0.032	0.349
Neighbor density	-1.698	0.104	<0.001	-0.394	0.068	<0.001
Neighbor phenology	-1.173	0.127	<0.001	-0.110	0.041	0.007
Shoot phenology × Ant abundance	0.044	0.085	0.603	0.013	0.039	0.744
Shoot phenology × Neighbor phenology	0.109	0.071	0.124	0.004	0.034	0.906
Neighbor density × Neighbor phenology	-0.484	0.090	<0.001	-0.111	0.043	0.010
MEM Vector 1	0.328	0.032	<0.001			
MEM Vector 2	0.337	0.050	<0.001			
B) Shoot phenology	1.489	0.081	<0.001	0.251	0.036	<0.001
Ant abundance	-0.219	0.085	0.010	0.019	0.031	0.554
Neighbor density	-1.704	0.104	<0.001	-0.381	0.068	<0.001
Neighbor phenology	-1.187	0.127	<0.001	-0.105	0.041	0.010
Shoot phenology × Ant abundance	0.088	0.085	0.302	0.027	0.037	0.459
Shoot phenology × Neighbor phenology	0.105	0.071	0.137	0.005	0.034	0.885
Neighbor density × Neighbor phenology	-0.498	0.090	<0.001	-0.105	0.043	0.014
MEM Vector 1	0.326	0.032	<0.001			

3

MEM Vector 2	0.348	0.050	<0.001

Table S4: 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	P
Shoot phenology	1.401	0.076	<0.001
Ant abundance	0.044	0.050	0.383
Neighbor density	-1.954	0.091	<0.001
Neighbor phenology	-0.982	0.118	<0.001
Shoot phenology x Ant abundance	0.123	0.061	0.044
Shoot phenology x Neighbor phenology	-0.002	0.068	0.971
Neighbor density x Neighbor phenology	-626	0.083	<0.001

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

5

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.
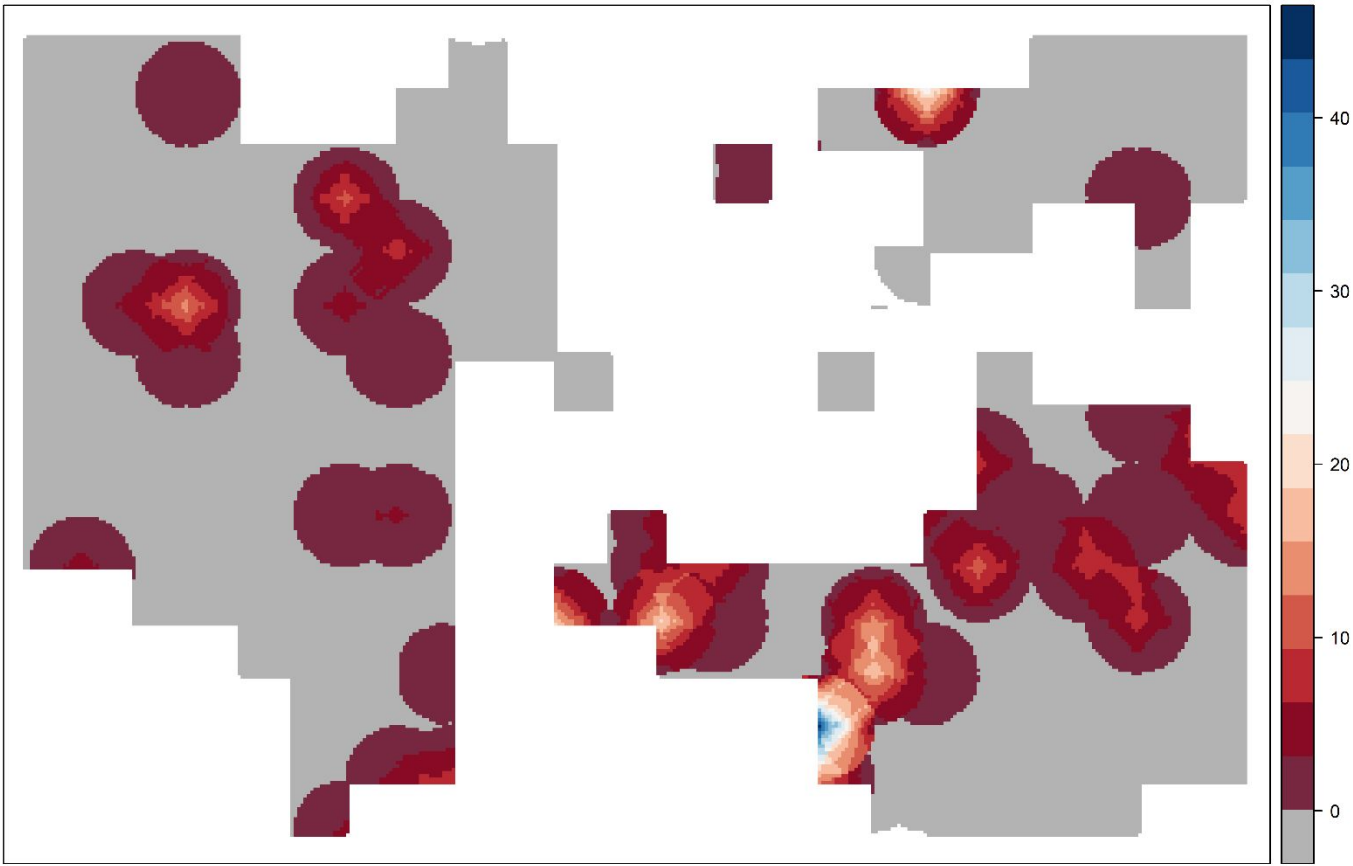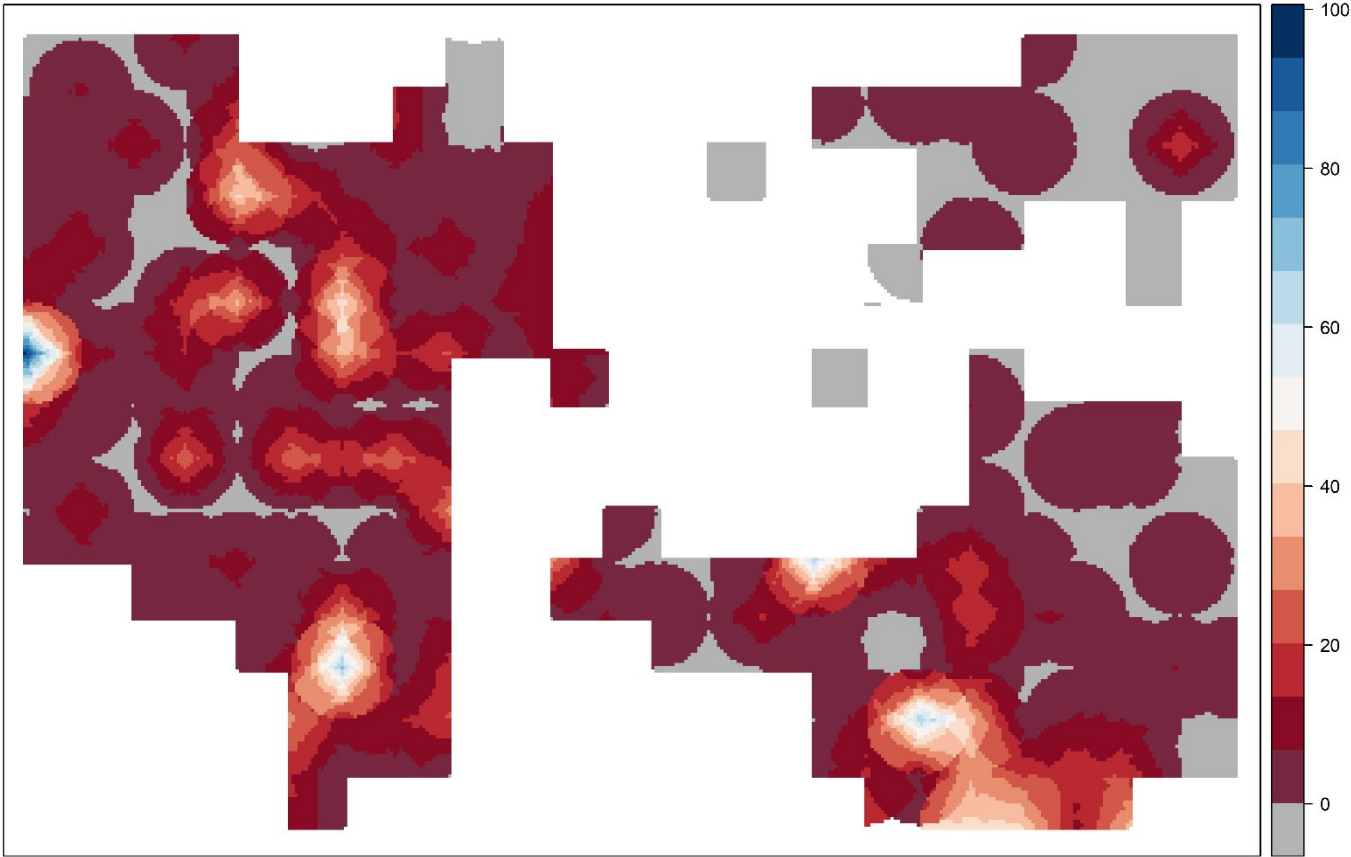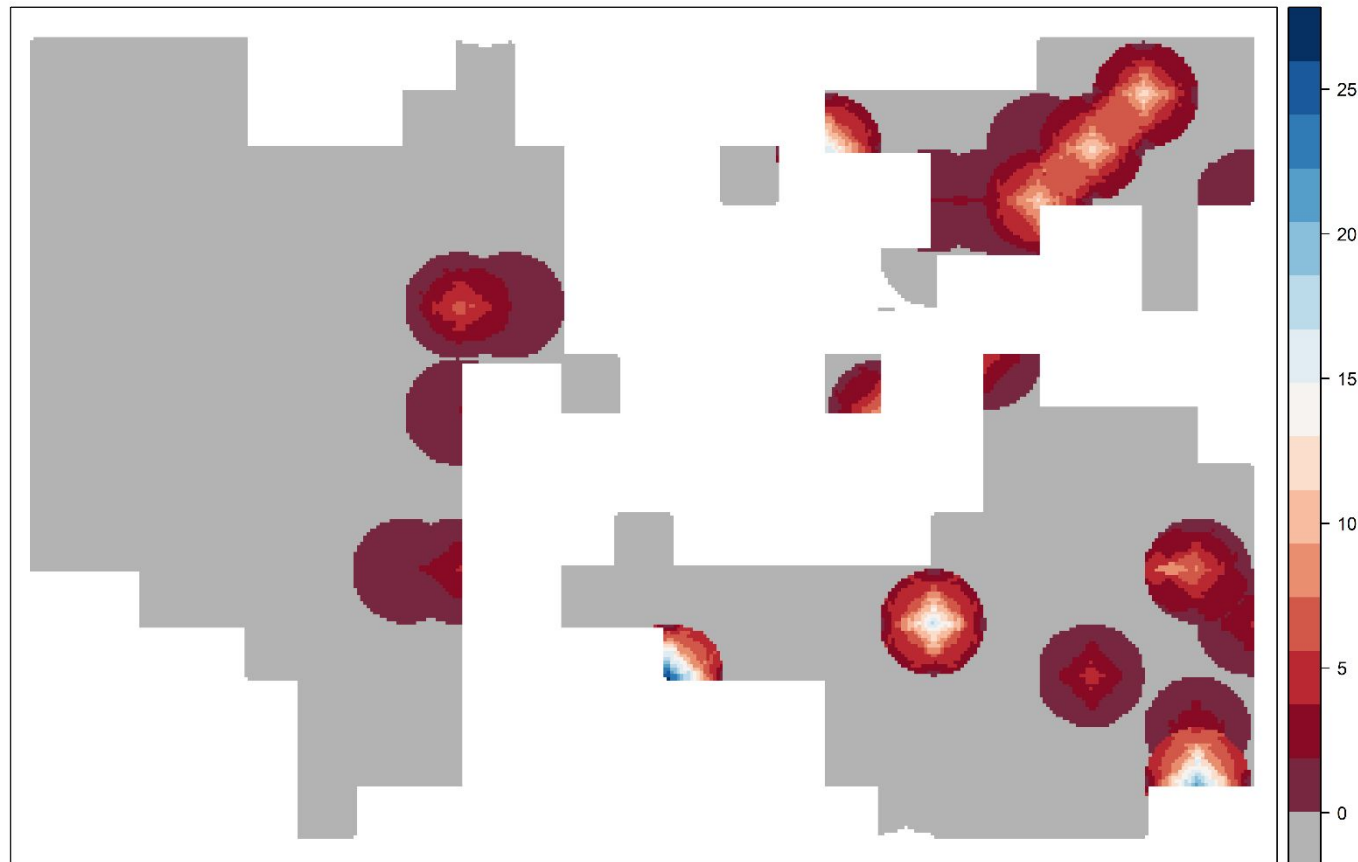
Figure S2: Result of the inverse distance weighted interpolation for abundance of *Myrmica scabrinodis* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.
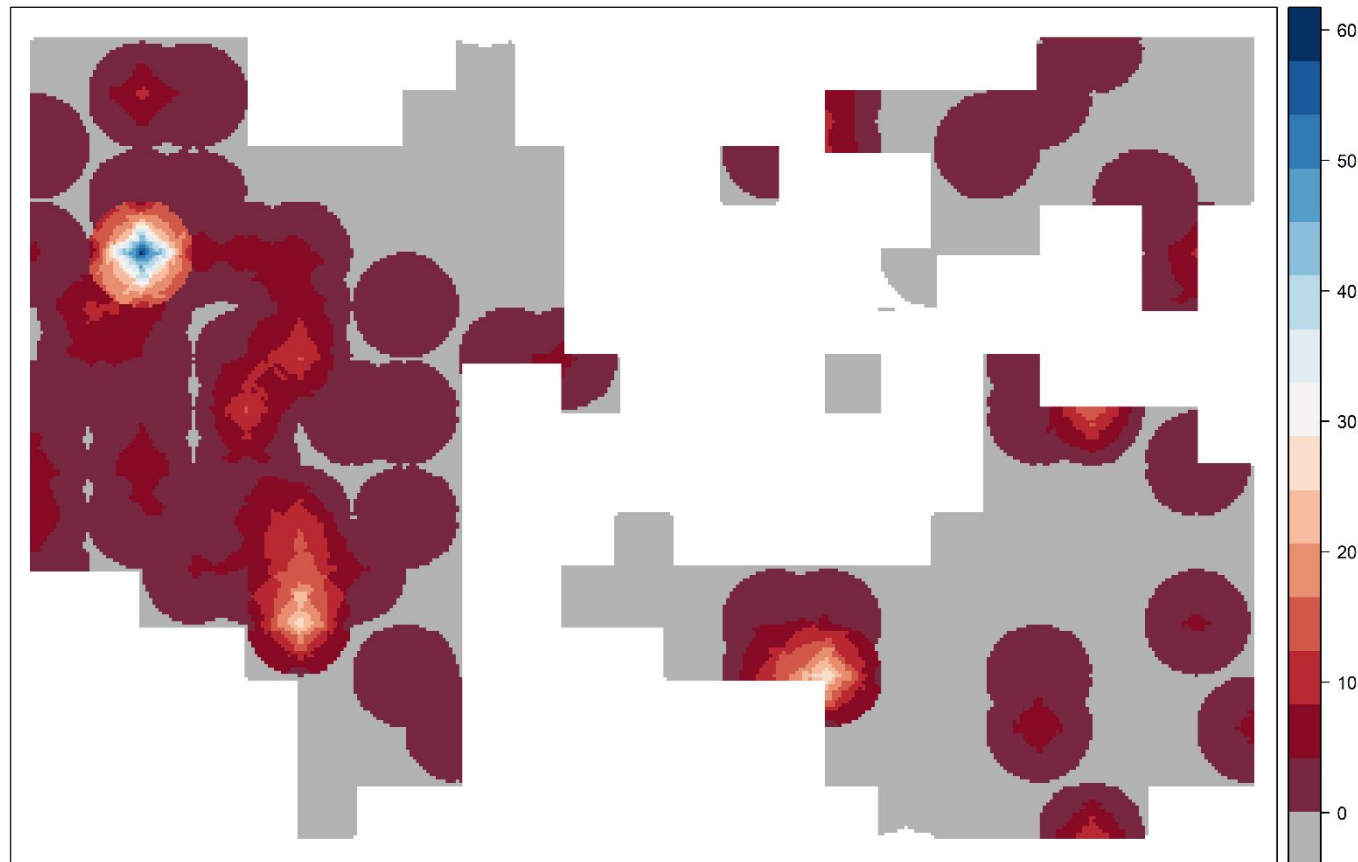
Figure S3: Result of the inverse distance weighted interpolation for abundance of *Myrmica ruginodis* based on abundance values (sum of individuals collected in 2 sampling sessions) at 254 sampling points. The color scale indicates the interpolated abundance values.

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.
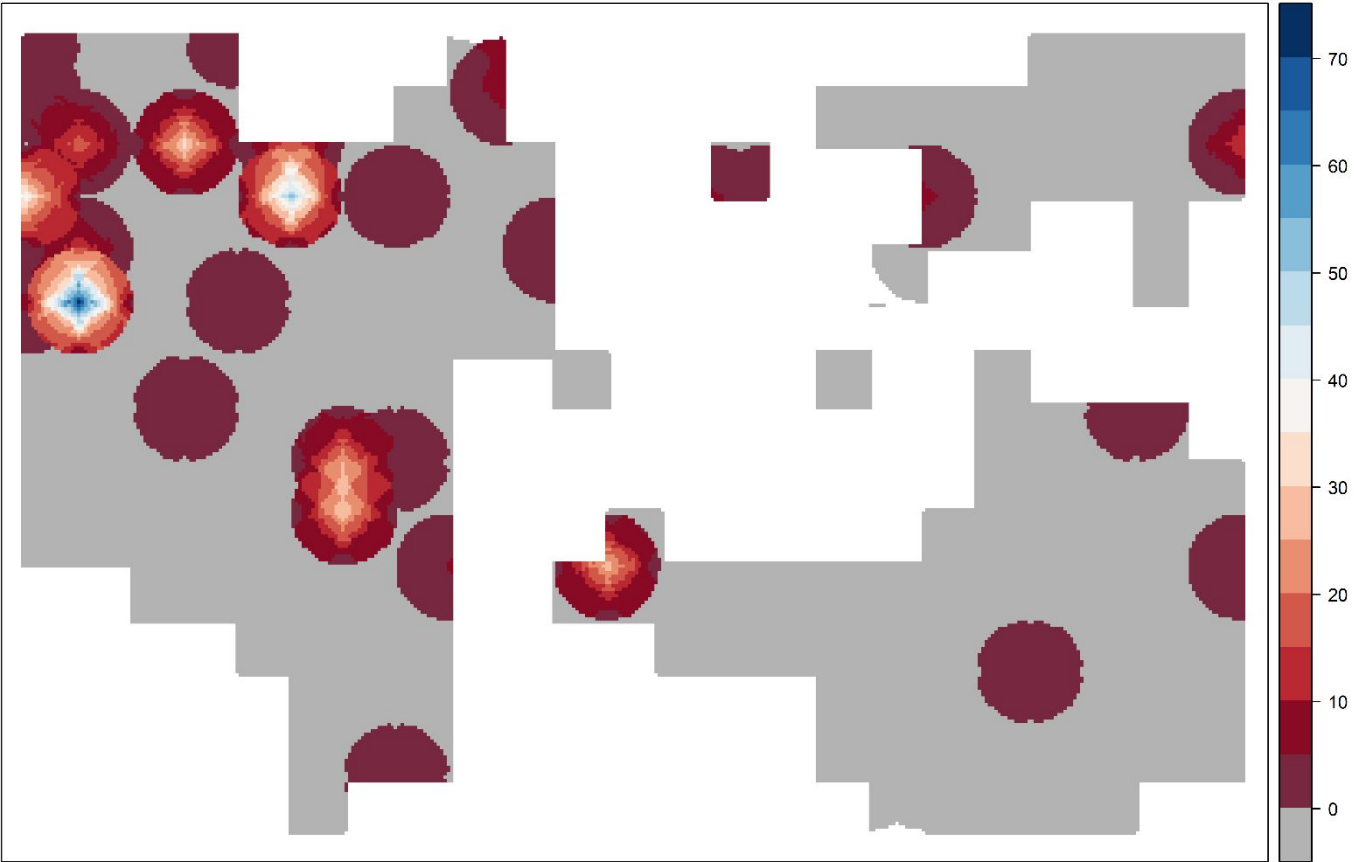
Figure S6: The probability of a shoot having at least one egg plotted against phenology values representing each phenological stage. As there is a monotonic increase in oviposition probability with phenological stage, we assumed the relationship between egg distribution and shoot phenology to be linear.
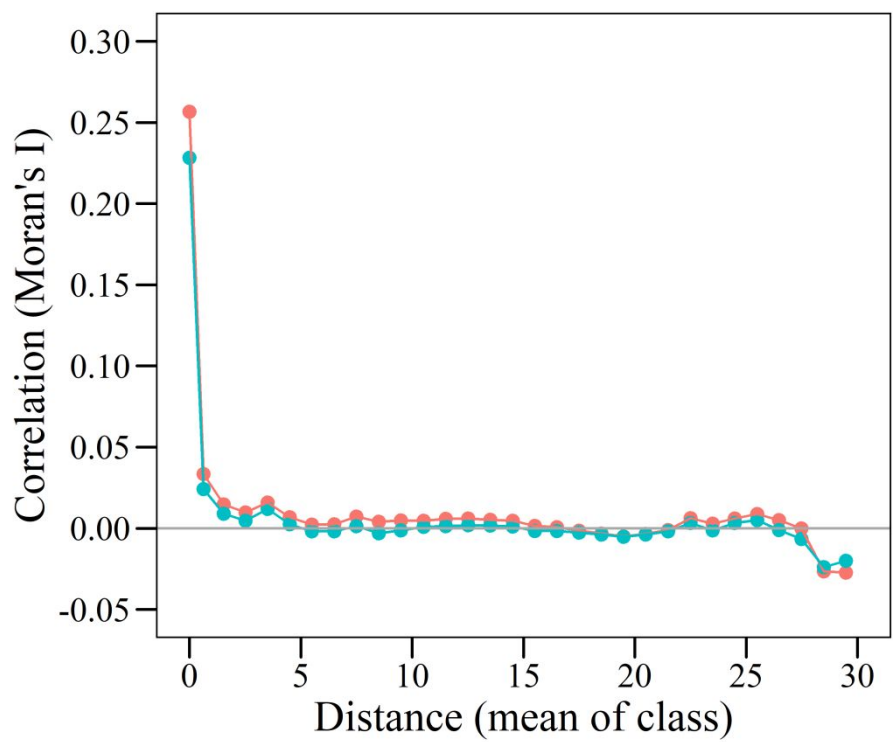

11
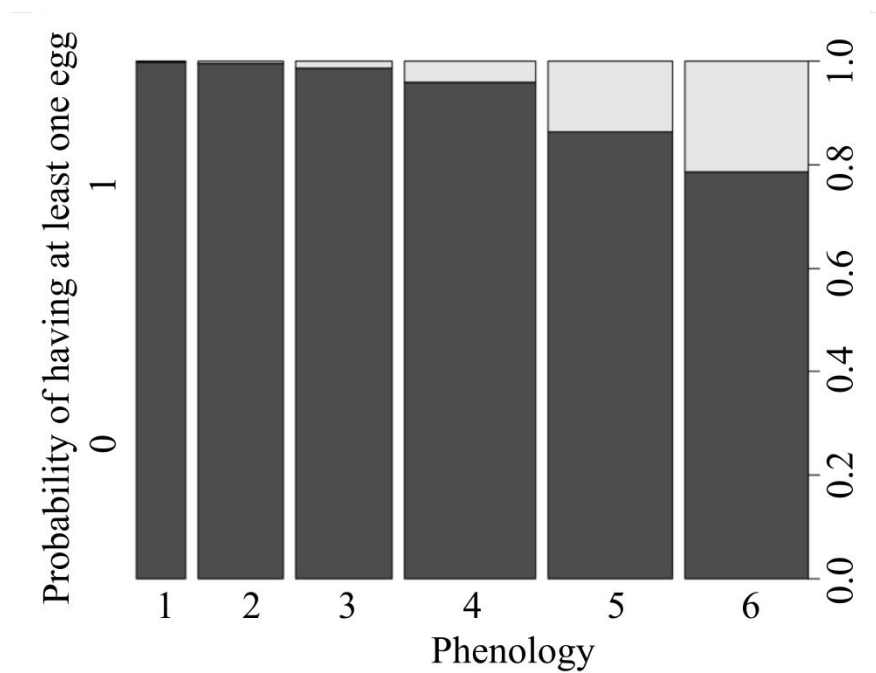
Figure S7: Spatial correlogram of the residuals of the binomial GLMs for the probability of a shoot having eggs before (red) and after (blue) including the two eigenvectors issued from MEM.
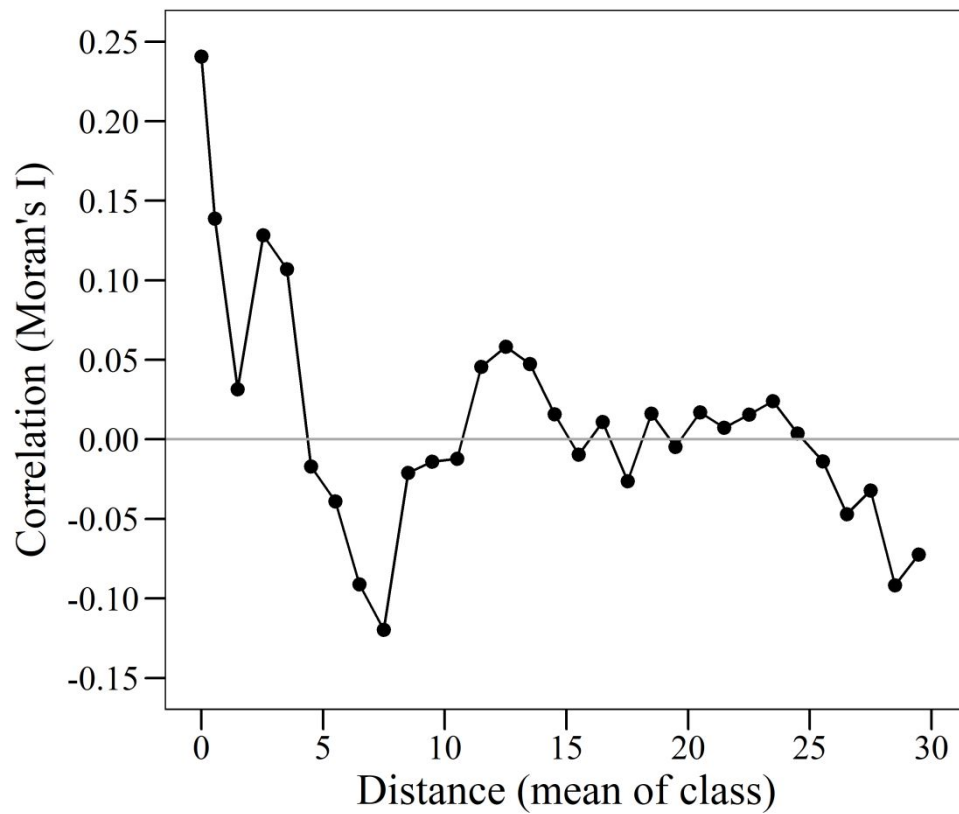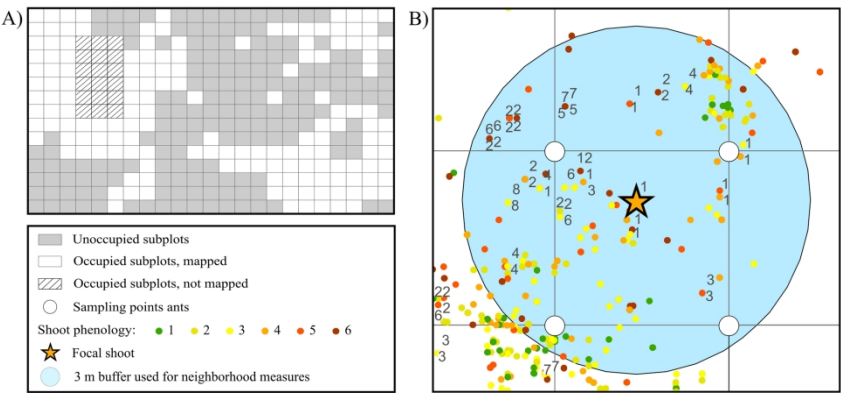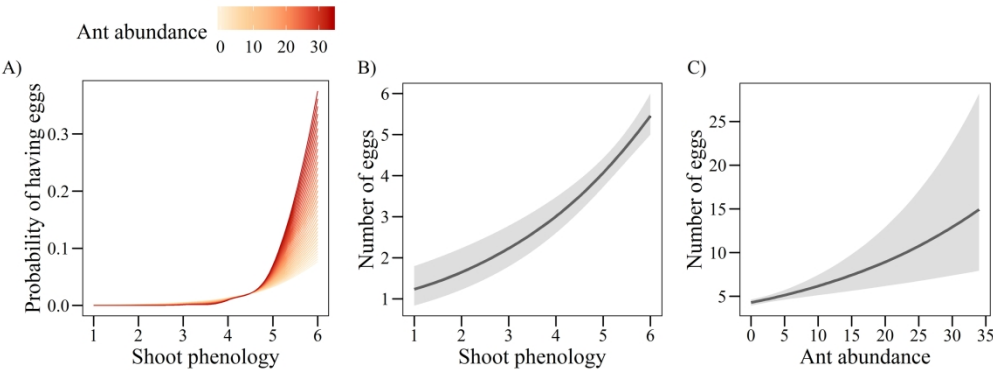
Figure S8: Spatial correlogram of the residuals of the negative binomial GLM for the number of eggs in shoots with at least one egg.

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A)

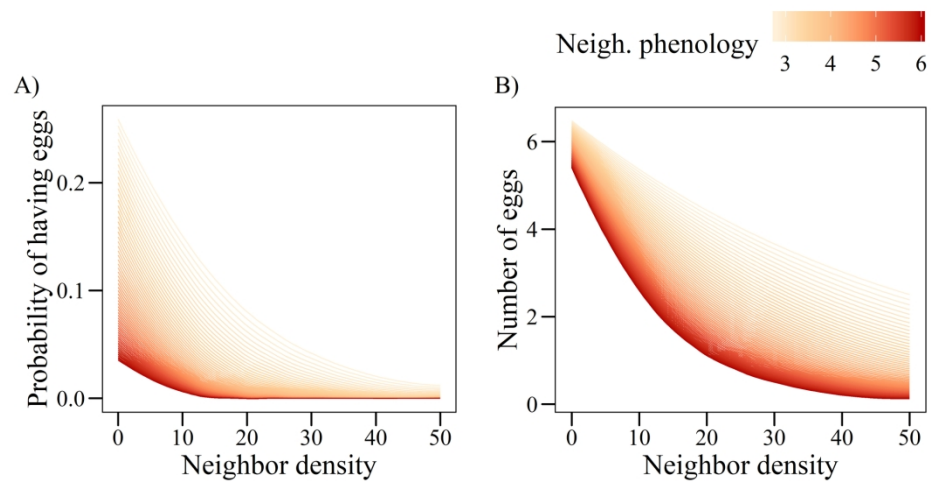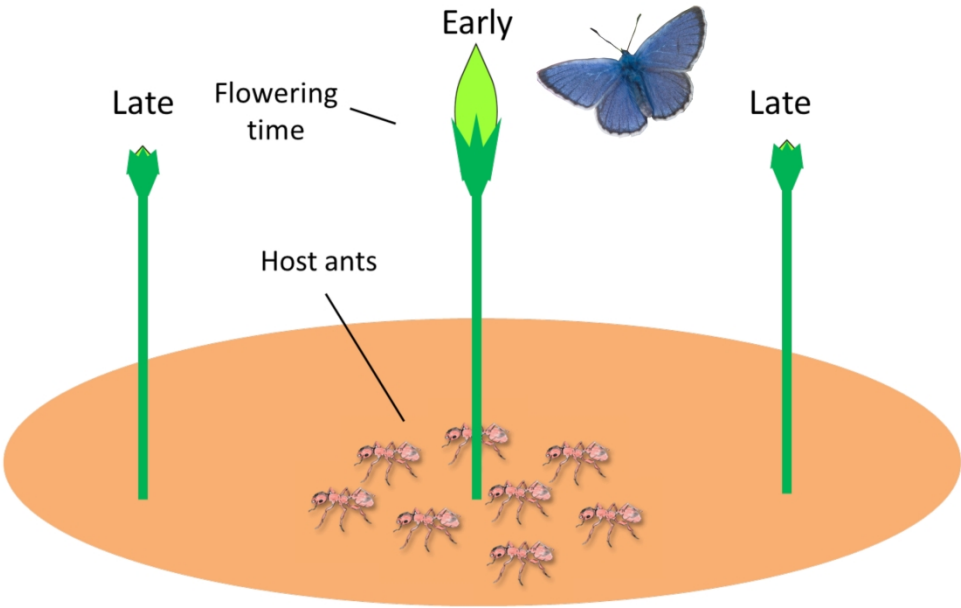| | Unoccupied subplots |
	Occupied subplots, mapped
	Occupied subplots, not mapped
○	Sampling points ants
Shoot phenology:	● 1 ● 2 ● 3 ● 4 ● 5 ● 6
★	Focal shoot
	3 m buffer used for neighborhood measures

B)

209x85mm (300 x 300 DPI)

259x99mm (300 x 300 DPI)

199x99mm (300 x 300 DPI)

Phengaris alcon butterflies preferred to oviposit on plants that simultaneously maximized the availability of both larval resources in time and space, i.e., they chose early-flowering plants that were of higher nutritional quality for larvae where host ants were abundant. Both the probability of oviposition and the number of eggs were lower in plant individuals with a high neighbor density than in more isolated plants, and this dilution effect was stronger when neighbors flowered early. This shows that plant-herbivore interactions simultaneously depend on the spatio-temporal distribution of a focal resource, and on the small-scale spatial variation in the abundance of other herbivore resources.

179x118mm (300 x 300 DPI)

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247x193mm (300 x 300 DPI)

Phengaris alcon butterflies preferred to oviposit on plants that simultaneously maximized the availability of both larval resources in time and space, i.e., they chose early-flowering plants that were of higher nutritional quality for larvae where host ants were abundant. Both the probability of oviposition and the number of eggs were lower in plant individuals with a high neighbor density than in more isolated plants, and this dilution effect was stronger when neighbors flowered early. This shows that plant-herbivore interactions simultaneously depend on the spatio-temporal distribution of a focal resource, and on the small-scale spatial variation in the abundance of other herbivore resources.

194x128mm (300 x 300 DPI)