Phengaris (Maculinea) alcon butterflies deposit their eggs on tall plants with many large buds in the vicinity of *Myrmica* ants

IRMA WYNHOFF,[1] RALDI B. BAKKER,[1,2] BAS OTEMAN,[1,2,3] PAULA SEIXAS ARNALDO[4] and FRANK VAN LANGEVELDE[2]   [1]Dutch Butterfly Conservation, Wageningen, The Netherlands, [2]Resource Ecology Group, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands, [3]Spatial Ecology Department, Royal Netherlands Institute for Sea Research (NIOZ-Yerseke), Yerseke, The Netherlands and [4]CITAB - Center for Research and Technology in Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Abstract. 1. The survival of eggs and larvae is dependent on the oviposition site selection of their mothers. In obligate myrmecophilic butterflies, both host plant phenology and host ant presence are expected to affect the decision where to deposit eggs. The importance of ant nest presence in the oviposition site selection of *Phengaris* butterflies is, however, highly debated.

2. We studied oviposition in the largest *Phengaris (Maculinea) alcon* population in Portugal, exploiting *Gentiana pneumonanthe* as the host plant and *Myrmica aloba* as host ant. We collected phenological plant data and recorded the presence and number of eggs on plants with and without *Myrmica* ants nearby during the flight period of the butterfly.

3. Females oviposited on tall plants with many tall buds, while the presence of host ant nests weakly affected oviposition on plants where the probability of finding ants at close range was high. Moreover, larger plants with many tall buds close to host ant nests received more eggs.

4. A density-dependent shift in oviposition was not found as the proportion of buds not infected with eggs did not differ between plants with or without ants, whereas plant characteristics did have an effect. Tall plants with many large buds were associated with earlier oviposition.

5. Our results suggest that females of *P. alcon* in Portugal choose gentian plants for oviposition mainly based on plant characteristics whereas the vicinity of ants had a weak effect. Moreover, our study shows that testing the ant-mediated oviposition hypothesis requires baiting ants more than once.

Key words. Ant-mediated oviposition, *Gentiana pneumonanthe*, host plant phenology, myrmecophilic butterflies, *Myrmica aloba*, Portugal, random oviposition.

Introduction

In most insect species females produce a high number of offspring by depositing hundreds or even thousands of eggs. However, females are usually very selective when choosing oviposition sites, facilitating the access to food resources for the hatching larvae, ensuring protection of eggs and larval instars or finding microclimatic conditions for optimal development (Obermaier & Zwölfer, 1999; Scheirs & de Bruyn, 2002; Gullan & Cranston, 2010). Optimal oviposition decisions support a high survival probability during larval instars to reach the adult stage and reproduce (Jaenike, 1978; Scheirs & de Bruyn, 2002). Hence oviposition preferences are expected to correlate

with host suitability for offspring development. Butterflies typically lay eggs on or in the vicinity of plants the caterpillars feed on (Fartmann & Hermann, 2006). Some species prefer plants with high nutritional values (Baylis & Pierce, 1991; Chen *et al.*, 2004), a certain host plant size (Wiklund, 1984; Küer & Fartmann, 2005; Nowicki *et al.*, 2005; Rabasa *et al.*, 2005; Reudler Talsma *et al.*, 2008), suitable microclimatic conditions (Shreeve, 1986; Eilers *et al.*, 2013), avoidance of competition (Wiklund & Friberg, 2008; Sielezniew & Stankiewicz-Fiedurek, 2013) or presence of specific mutualists (Pierce & Elgar, 1985).

Over 50% of lycaenid butterfly species have an ant-associated lifestyle known as myrmecophily, where the species have a neutral, mutualistic or parasitic interaction with ants (Fiedler, 1991; Fiedler *et al.*, 1996). The genus *Phengaris* Doherty, 1891 (the senior synonym of *Maculinea* van Eecke, 1915) forms a specific group within this family with an extraordinary life cycle where the caterpillars parasitise on *Myrmica* ants. Many of these butterfly species have experienced severe declines over the last few decades (Van Swaay *et al.*, 2011). As adoption by ants is obligate for caterpillar survival (Elmes *et al.*, 1991; Akino *et al.*, 1999; Als *et al.*, 2001), one might expect that a *Phengaris* female butterfly is capable of including ant presence into her oviposition decisions and adjust the oviposition locations accordingly, which is however highly debated. Some studies found support of this ant-mediated oviposition (Van Dyck *et al.*, 2000; Wynhoff *et al.*, 2008; Van Langevelde & Wynhoff, 2009; Van Dyck & Regniers, 2010; Patricelli *et al.*, 2011), whereas others did not (Thomas & Elmes, 2001; Nowicki *et al.*, 2005; Musche *et al.*, 2006; Fürst & Nash, 2009).

Phengaris butterflies fly for 1–2 months during summer, depositing their eggs on specific host plants. After hatching, the caterpillars bore themselves into the flower buds and feed on the soft internal parts where they quickly develop to the fourth instar and leave the plant (Akino *et al.*, 1999; Als *et al.*, 2001). By mimicking a *Myrmica* ant larva, the caterpillar ensures adoption by foraging *Myrmica* ants. The caterpillar spends around 10–11 or even 22–23 months in the ant nest, being fed by ants or feeding on ant brood (Elmes *et al.*, 1991; Thomas *et al.*, 1998; Schönrogge *et al.*, 2000; Als *et al.*, 2001; Witek *et al.*, 2006). To date, thirteen species of *Myrmica* have been described as hosts for *Phengaris* in Europe (Als *et al.*, 2001; Steiner *et al.*, 2003; Tartally *et al.*, 2008; Arnaldo *et al.*, 2011; Witek *et al.*, 2010). However, depending on location, the survival rate of the caterpillars in the ant nests differs between ant species. Best survival is achieved in primary hosts, but lower survival in secondary host ant nests may be important in years with a high butterfly population density (Thomas *et al.*, 2005, 2013). *Myrmica* ants are ground foragers, usually searching up to 2 m from the nest. The probability of a caterpillar being located by a foraging worker is directly dependent on the density of the workers in the colony, and proximity of the host plant to the ants nest (Elmes *et al.*, 1998).

The decision where to oviposit is crucial for *Phengaris* with their dependence on both specific host plants as food source for the first three instars, and their need for host ants. Usually only a small developmental window in plant phenology is accepted for oviposition (Thomas & Elmes, 2001; however see Van Dyck & Regniers, 2010). Since plants grow during the flight season while ant nests remain at the same location, it is possible that temporal differences where butterflies oviposit can be observed following the best combination of plants as a food source and the presence of *Myrmica* ants (Patricelli *et al.*, 2011). On the one hand, there could be a density-dependent shift in oviposition preference when suitable plants near ants already contain high egg loads (Van Dyck *et al.*, 2000). On the other hand, a female butterfly might be attracted by plant characteristics such as the height of a plant, the number of buds and the size of the buds, rather than the presence of ants close to the plant (Thomas & Elmes, 2001; Fürst & Nash, 2010; Czekes *et al.*, 2013).

To get further insight into the relationship between *Phengaris* and *Myrmica* ants, we studied the largest known population of *Phengaris alcon* (Denis & Schiffermüller, 1775), commonly known as the Alcon Blue, in Portugal (Rodrigues *et al.*, 2010; Arnaldo *et al.*, 2011). The local population of *P. alcon* is dependent on the marsh gentian (*Gentiana pneumonanthe* L.) as its single host plant and *Myrmica aloba* Forel, 1909 as the single host ant (Arnaldo *et al.*, 2011). Our goal was to identify the influence of plant characteristics and the presence of *Myrmica* ant nests on oviposition choices over time. We hypothesised that ant presence influences the choice of oviposition location, in addition, to plant characteristics. We expected to find more eggs on plants with ants in the proximity than on plants outside the ants' foraging range, and that plant characteristics (i.e. plant height, flower bud length and the number of buds) also have a positive influence on the presence and number of eggs (hypothesis 1). We also expected that in the beginning of the season, the eggs will be concentrated on suitable plants with ants in the proximity until all suitable plants with ants are used and then suitable plants without ants will be also selected (hypothesis 2). These hypotheses were tested in a field study, where we selected host plants with and without ants in the proximity.

Material and methods

Set-up of field study

The study site was located near Lamas de Olo (41°22′N, 7°48′W; 974 m AMSL) inside Alvão National Park, Portugal. The field work was conducted between 3 July and 10 October of 2012, covering the entire flight period of *P. alcon* which lasted 6 weeks from mid-July till late August. A plot inside a meadow was fenced in to exclude large grazing animals. The plot was split into seven rows, each of one metre wide and 12.5 m in length,

separated by 30 cm of path (we did not find an effect of row in our analysis). All marsh gentians found inside this plot were marked with a number on a plastic label placed next to the plant. The density of marsh gentians was 7.2 plants per m². Only the apical bud of the plant was monitored, because this is the most likely one to receive eggs (Fürst & Nash, 2010; Van Dyck & Regniers, 2010; Arnaldo *et al.*, in press). As one plant can have more than one shoot, a string was tied around the apical bud. This also prevented mistakes while measuring plants. To avoid selecting shoots from the same plant, every new shoot had to be at least 10 cm away from the already selected plants.

Data collection

Every day during the flight period, the apical bud of each plant was checked for new eggs, which gave us the date that the first egg was laid on the apical bud. The field study was designed to study caterpillar competition in the flower buds as well (B. Oteman, R.B. Bakker, P.S. Arnaldo, I. Wynhoff and F. van Langevelde, unpubl. data). As it takes approximately 7 days for a *P. alcon* egg to hatch (Bos *et al.*, 2006), a hood made of fine meshed gauze (approximately 5 by 10 cm in size) was placed over the apical bud 1–6 days after a plant received its first apical egg. The gaze was closed with a noose made of plasticised wire to ensure caterpillars could not leave their hostplant. More ovipositions after day 6 were therefore impossible. For this study, we analysed only the number of eggs on those buds which were encapsuled after exactly 6 days ($n = 147$, this represented 52% of the encapsuled buds). Plant characteristics (i.e. plant height in cm, number of buds and length of the apical bud in cm) were recorded weekly. The means of plant height, length of the apical bud and the number of buds were calculated by interpolation using the data collected closest to the day the very first egg was laid and the data closest to the day the very last egg was laid, thus including the entire oviposition period of *P. alcon*.

At the base of each selected plant, ants were baited three times during the field period, in the middle of July, the beginning of August and the beginning of September. Ant baiting took place in the morning around 10:00 am and evening around 18.00 pm when ants are most active (Elmes *et al.*, 1991; Fürst & Nash, 2010). A small paper cup with a sugar cube inside was placed within five centimetres of the stem of each plant in the early morning. The sugar baits were checked every 2 hours starting at 10:00 am. We kept the attracted ants in 70% ethanol for later identification in the laboratory. Baits without ants were left in the field and were checked regularly every 2 hours until the end of the day. Ants were later identified using a binocular microscope and literature (Radchenko & Elmes, 2010). Plants were considered to grow inside the foraging range of a *Myrmica* ant nest when ants were found in one or more of the ant capturing events. In this study, the only *Myrmica* species we found was *Myrmica aloba*. Furthermore, the spatial distribution of ant presence was tested by calculating the distance from each plant to the closest plant with ants and the closest plant without ants.

Statistical analyses

We tested the effects of the presence of *Myrmica*, the plant characteristics and their interaction due to the presence and absence of eggs as a binary response variable, with a Generalised Linear Model (GZLM) with a logit link function based on a binomial distribution (hypothesis 1). This analysis was followed by a GZLM where we only included plants where at least twice ants were found and plants where we did not find ants. With this analysis we could evaluate oviposition on plants for which we were more sure that they are located nearby ant nests. Then we replaced in each model the presence of *Myrmica* by the distance to the closest plant with ants to test the effect of the spatial distribution of ant presence. We removed the non-significant interaction effects to reduce the number of parameters in the final models. As we had many independent variables, we applied the False Discovery Rate approach (Benjamini & Hochberg, 1995) by calculating the expected proportion of rejected null hypotheses that are falsely rejected. This False Discovery Rate approach can assign a lower *P*-value threshold α than the often used α = 0.05, based on the number of independent variables and their *P*-value. Secondly, we tested the effects of the presence of *Myrmica*, the plant characteristics and their interaction on the number of eggs, using a GZLM with a log-linear link function based on a Poisson distribution. Again, we replaced the presence of *Myrmica* by the distance to the closest plant with ants.

Based on the results of the GZLMs, a regression-based path analysis was conducted to examine the direct and indirect effects of the independent variables on both the presence and the number of eggs (Jaccard & Wan, 1996). A recursive conceptual path model was constructed (Fig. 1a). The final parsimonious path models were constructed based on the W-statistic and χ^2 tests (Epstein *et al.*, 1994). Path coefficients were calculated using a fully standardised (logistic) regression coefficient to compare the strength of the effects on continuous and binary endogenous variables (Menard, 2004). Finally, we tested the spatial autocorrelation of the residuals of the GZLMs (De Knegt *et al.*, 2011) using SAM: Spatial Analysis in Macroecology (Rangel *et al.*, 2010).

An independent samples *t*-test was used to test the difference between the date of first oviposition on plants with and without the presence of *Myrmica*. To show the time it took plants to receive eggs depending on whether they were growing inside or outside the range of ants (hypothesis 2), we used Cox regression tests, where we also included plant characteristics as independent variables. A Cox regression model produces a function that
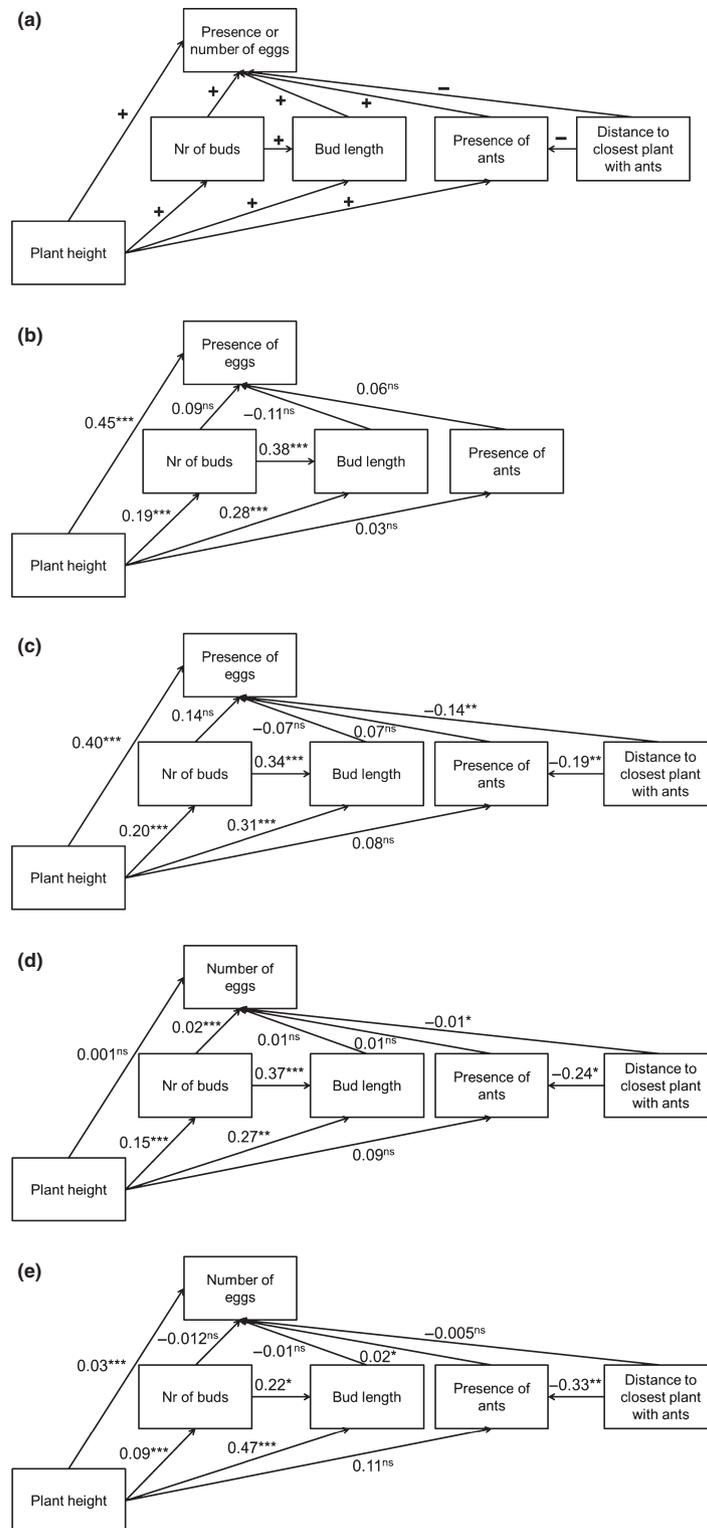
Fig. 1. The recursive conceptual path model for the effects of the plant and ant characteristics on the presence or number of eggs (a), the results of the analysis explaining the presence of eggs for all data (b), the presence of eggs for the plants without ants and the plants for which at least two times ants were found (c) and the number of eggs per bud for all data (d), and the number of eggs for the plants without ants and the plants for which at least two times ants were found (e). The values in (b–e) represent fully standardised regression coefficients. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns, non-significant.

predicts the time to oviposition of the first egg as a function of the independent variables. We only included plants that received eggs and related the timing of this event as the dependent variable to the presence of *Myrmica* ants and the plant characteristics measured at the moment of oviposition as independent variables. Apart from the spatial autocorrelation, all statistical analyses were performed using IBM SPSS statistics version 20 (IBM Corp., 2011).

Results

In our field study, we monitored 447 plants that had at least one bud. From the selected plants, 165 (36.9%) were found to grow within the foraging range of *Myrmica aloba* while 282 (63.1%) plants grew outside the foraging range. These two groups did not differ in the mean plant height (with *Myrmica* 21.7 cm $\pm$ SE 0.5, n_1 = 282, without *Myrmica* 21.3 cm $\pm$ SE 0.4, n_2 = 165; *t*-test, $t = -0.592$, d.f. = 445, $P = 0.554$), mean bud length (with *Myrmica* 1.65 cm $\pm$ SE 0.07, n_1 = 282, without *Myrmica* 1.54 cm $\pm$ SE 0.05, n_2 = 165; *t*-test, $t = -1.239$, d.f. = 445, $P = 0.216$) or mean number of buds (with *Myrmica* 2.9 $\pm$ SE 0.2, n_1 = 282, without *Myrmica* 2.7 $\pm$ SE 0.1, n_2 = 165; *t*-test, $t = -1.162$, d.f. = 445, $P = 0.246$). In three baiting sessions, we collected ants one time underneath 125 plants (76%), two times underneath 35 plants (21%) and three times underneath 5 plants (3%).

We found that the mean distance from plants where ants were captured to other plants with ants was shorter than the mean distance to plants without ants (distance to plants with ants 29.7 cm $\pm$ SE 1.3, n_1 = 222, distance to plants without ants 35.0 cm $\pm$ SE 1.5 n_2 = 222; *t*-test, $t = 2.65$, d.f. = 434, $P = 0.008$). The mean distance from plants without ants to plants where ants were captured was longer than the mean distance to plants without ants (distance to plants with ants 41.3 cm $\pm$ SE 1.2, distance to plants without ants 20.6 cm $\pm$ SE 0.8: *t*-test, $t = -14.49$, d.f. = 700.1, $P < 0.001$). The frequency distributions show that, in general, distances are short (Fig. 2). Moreover, this analysis reveals that plants with ants occur clustered.

In total, we found that 1735 eggs have been deposited on 282 plants from a total of 447 plants, which were monitored during our study. For these 282 plants, 170 (60.3%) plants had no *Myrmica* ants in the proximity and 112 (39.7%) plants did. We found 948 eggs (54.6%) on plants without ants, whereas 787 eggs (45.4%) were found on plants with ants. Again there were no differences in the mean plant height (with *Myrmica* 23.5 cm $\pm$ SE 0.5, n_1 = 170, without *Myrmica* 23.3 cm $\pm$ SE 0.4, n_2 = 112; *t*-test, $t = -0.297$, d.f. = 280, $P = 0.767$), bud length (with *Myrmica* 1.70 cm $\pm$ SE 0.08, n_1 = 170, without *Myrmica* 1.69 cm $\pm$ SE 0.06, n_2 = 112; *t*-test, $t = -0.104$, d.f. = 280, $P = 0.917$) or number of buds (with *Myrmica* 3.4 $\pm$ SE 0.2, n_1 = 170, without *Myrmica* 3.3 $\pm$ SE 0.2, n_2 = 112; *t*-test, $t = -0.470$, d.f. = 280, $P = 0.638$) between these two groups. Using a GZLM with a logit link function, we found that plant height, the number of buds and bud length had a positive effect on the probability that eggs were oviposited on the host plant (Table 1a). The presence of *Myrmica* did not have an effect on whether a plant would receive an egg or not. The same was found for the effect of the distance to the closest
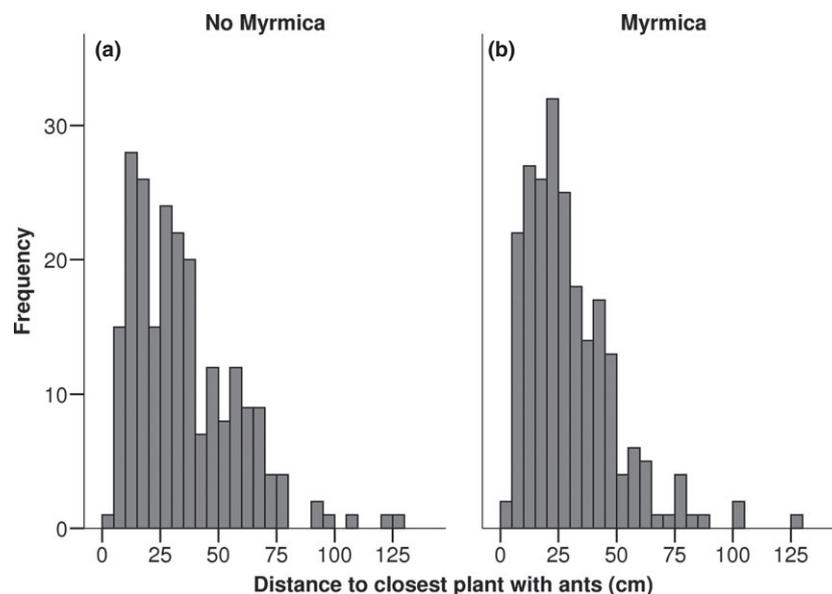

Fig. 2. Frequency distribution of the distance to the closest *Gentiana pneumonanthe* plant with *Myrmica aloba* ants to gentian plants without (a) or with (b) *Myrmica* ants in their proximity.

Table 1. Results of the effect of *Myrmica* presence, the distance to the closest plant with ants, the plant characteristics and their interaction on the presence or absence of eggs using a Generalised Linear Model with a logit link function based on a binomial distribution. The coefficient, its standard error, the Wald statistic and associated *P*-value are given. We removed the non-significant interaction effects to reduce the number of parameters in the models. The first analysis was done using all data (a: *n* = 447), whereas the second analysis was done using the plants without ants and the plants for which at least two times ants were found (b: *n* = 322 from which ants were found at least twice underneath 40 plants). The False Discovery Rate approach assigned the *P*-value threshold α = 0.039.

Independent variables	Coefficient (SE)	Wald	*P*-value
(a) Models based on all data			
Myrmica (=0)	−0.272 (0.216)	1.595	0.207
No. of buds	0.372 (0.070)	28.429	<0.001
Distance	−0.008 (0.004)	3.686	0.055
Nr of buds	0.379 (0.070)	29.609	<0.001
Myrmica (=0)	−0.296 (0.210)	1.998	0.157
Bud length	0.042 (0.012)	12.177	<0.001
Distance	−0.007 (0.004)	3.095	0.079
Bud length	0.043 (0.012)	12.882	<0.001
Myrmica (=0)	−0.329 (0.230)	2.060	0.151
Plant height	0.019 (0.002)	68.671	<0.001
Distance	−0.012 (0.005)	6.369	0.012
Plant height	0.020 (0.002)	71.240	<0.001
(b) Models based on data for the plants without ants and the plants for which at least two times ants were found. Plants where ants have been found only once are excluded			
Myrmica (=0)	2.193 (1.418)	2.390	0.122
Nr of buds	2.280 (0.912)	8.595	0.003
Myrmica (=0) × Nr of buds	−1.873 (0.917)	4.177	0.041*
Distance	0.006 (0.008)	0.675	0.411
Nr of buds	1.078 (0.213)	25.485	<0.001
Distance × Nr of buds	−0.010 (0.003)	13.617	<0.001
Myrmica (=0)	−0.965 (0.421)	5.259	0.022
Bud length	0.052 (0.015)	12.443	<0.001
Distance	−0.013 (0.005)	6.890	0.009
Bud length	0.056 (0.015)	13.870	<0.001
Myrmica (=0)	−0.808 (0.442)	3.338	0.068
Plant height	0.019 (0.003)	47.326	<0.001
Distance	−0.018 (0.006)	9.968	0.002
Plant height	0.020 (0.003)	52.031	<0.001

*Not significant given the threshold α = 0.039 based on the False Discovery Rate approach.

plant with ants, except for the model with plant height: here a negative effect of distance to the closest plant with ants was found, suggesting that the probability to find eggs increases when ants are near.

The path analysis confirmed the GZLM results for explaining egg presence (Fig. 1b): plant height appeared to be the most important independent variable that has a direct, positive effect on egg presence (total effect is 0.45). Ant presence had no significant effect on egg presence. The non-significant effect of the number of buds and bud length is due to the high correlation between the plant

characteristics. Adding the variable distance to the closest plant with ants to the model would cause the variable bud length to change sign and was therefore omitted. For these models, the spatial autocorrelation was found to be low (−0.019 < Moran's I < 0.015 for the first 10 lags).

When selecting the plants without ants and the plants for which at least on two occasions ants were found (*n* = 322 from which ants were found underneath 40 plants), we found a similar effect of the plant characteristics (Table 1b). However, in most of the cases, the presence of ants or a short distance to the closest plant with ants increased the probability that eggs are deposited. The interaction between the number of buds and the distance to the closest plant with ants implies that when the plant has a high number of buds, the probability of finding eggs increases when it is also growing close to plants with ants (Fig. 3). The path analysis showed that indeed distance to the closest plant with ants had a negative direct effect on the presence of eggs, but the effect was not very strong, given the relatively low standardised coefficient, whereas the positive direct effect of plant height was larger (Fig. 1c). We again found a very low spatial autocorrelation for these models (−0.02 < Moran's I < 0.026 for the first 10 lags).

In total, 890 eggs were distributed over 131 plants (Fig. 4), with on average 6.8 eggs on the apical bud (median 5.0, SE 0.54). Again there were no differences in the mean plant height (with *Myrmica* 24.6 cm ± SE 0.8, n_1 = 81, without *Myrmica* 24.0 cm ± SE 0.6, n_2 = 50; *t*-test, *t* = −0.658, d.f. = 129, *P* = 0.512), bud length (with *Myrmica* 1.84 cm ± SE 0.09, n_1 = 81, without *Myrmica* 1.78 cm ± SE 0.09, n_2 = 50; *t*-test, *t* = −0.395, d.f. = 129, *P* = 0.693) or number of buds (with *Myrmica* 3.9 ± SE 0.4, n_1 = 81, without *Myrmica* 3.5 ± SE 0.3, n_2 = 50;
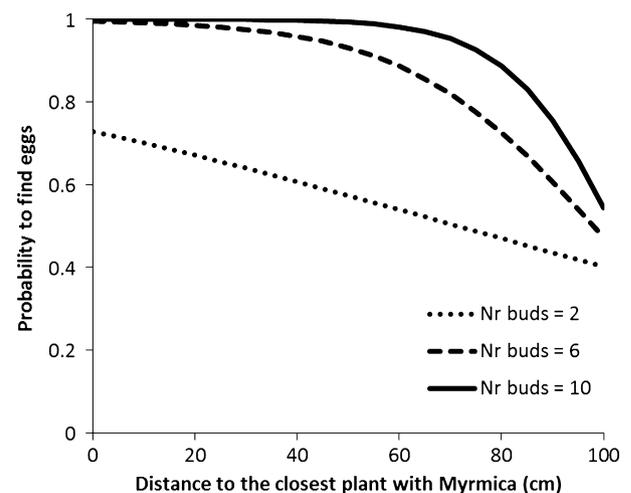
Fig. 3. Predicted relationships between bud length and the probability that eggs were oviposited in relation to the distance to the closest *Gentiana pneumonanthe* plant with *Myrmica aloba* ants in the proximity, for different number of buds per plant (see text for statistics).
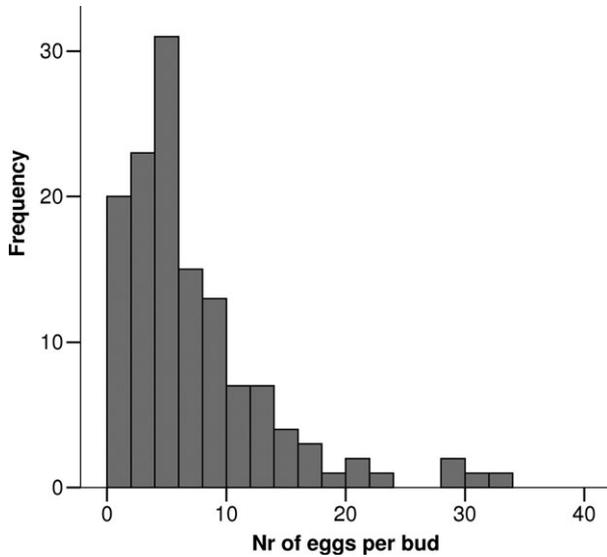
Fig. 4. Frequency distribution of the number of eggs laid by *Phengaris alcon* females per apical gentian flower bud within 6 days after the first egg was laid.

t-test, $t = -0.747$, d.f. = 129, $P = 0.457$) between plants with and without *Myrmica* ants. Using GZLM with a log-linear link function, both plant characteristics and the presence of *Myrmica* ants as well as the distance to the closest plant with ants significantly affected the number of eggs (Table 2a). The interactions between the plant characteristics and the distance to the closest plant with ants imply that when the value of a plant characteristic is high, the predicted number of eggs increases when it is also growing close to plants with ants. The path analysis showed that the number of buds had the largest effect (direct effect size of 0.02), meaning that plants with more buds would have more eggs (Fig. 1d). Besides, the distance to the closest plant with ants had a negative effect (a small direct effect size of -0.01), and plant height only indirectly positively affected the number of eggs (through the number of buds: a small, indirect effect size of 0.004). Again, the non-significant effects of plant height and bud length were due to the high correlation between the plant characteristics. The spatial autocorrelation of the models was low ($-0.062 <$ Moran's I < 0.039 for the first 10 lags).

When only selecting the plants without ants and the plants for which at least two times ants were found ($n = 94$ from which ants were found underneath 13 plants), we found similar effects of the plant characteristics and ant presence as well as the distance to the closest plant with ants (Table 2b). The interaction effects should be interpreted as for Table 2a. The path analysis showed that again the positive direct effect of plant height was the most important for explaining the number of eggs, whereas the presence of ants had a small, direct positive effect and the distance to the closest plant with ants had a

Table 2. Results of the effect of *Myrmica* presence, the distance to the closest plant with ants, the plant characteristics and their interaction on the number of eggs deposited until day 6 after the first egg was found, using a Generalised Linear Model with a log link function based on a Poisson distribution. Buds encapsuled between day 1 and 5 after the first egg was removed from the analysis. The coefficient, its standard error, the Wald statistic and associated *P*-value are given. We removed the non-significant interaction effects to reduce the number of parameters in the models. The first analysis was done using all data (a: $n = 131$), whereas the second analysis was done using the plants without ants and the plants for which at least two times ants were found (b: $n = 94$ from which ants were found at least twice underneath 13 plants). The False Discovery Rate approach assigned the *P*-value threshold $\alpha = 0.039$.

Independent variables	Coefficient (SE)	Wald	*P*-value
(a) Models based on all data			
Myrmica (=0)	0.127 (0.120)	1.125	0.289
Nr of buds	0.097 (0.017)	30.803	<0.001
Myrmica (=0) × Nr of buds	−0.067 (0.023)	8.622	0.003
Distance	0.001 (0.003)	0.271	0.603
Nr of buds	0.103 (0.029)	27.779	<0.001
Distance × Nr of buds	−0001 (0.0005)	6.695	0.010
Myrmica (=0)	0.517 (0.170)	9.288	0.002
Bud length	0.032 (0.006)	11.449	0.001
Myrmica (=0) × Bud length	−0.036 (0.008)	19.925	<0.001
Distance	0.012 (0.004)	11.478	0.001
Bud length	0.047 (0.008)	35.795	<0.001
Distance × Bud length	−0.001 (0.0002)	23.454	<0.001
Myrmica (=0)	−0.169 (0.068)	6.197	0.013
Plant height	0.002 (0.0006)	7.102	0.008
Distance	0.023 (0.006)	12.640	<.001
Plant height	0.006 (0.001)	27.838	<0.001
Distance x Plant height	−0.0001 (2.4×10^{-5})	18.300	<0.001
(b) Models based on data for the plants without ants and the plants for which at least two times ants were found. Plants where ants have been found only once are excluded			
Myrmica (=0)	0.015 (0.212)	0.514	0.473
Nr of buds	0.113 (0.034)	14.981	<0.001
Myrmica (=0) × Nr of buds	−0.083 (0.037)	5.047	0.025
Distance	0.004 (0.003)	1.544	0.214
Nr of buds	0.093 (0.024)	15.243	<0.001
Distance × Nr of buds	−0.001 (0.0006)	5.053	0.025
Myrmica (=0)	0.687 (0.358)	3.675	0.055
Bud length	0.037 (0.013)	5.241	0.022
Myrmica (=0) × Bud length	−0.042 (0.014)	8.359	0.004
Distance	0.012 (0.005)	6.648	0.010
Bud length	0.033 (0.010)	10.353	0.001
Distance × Bud length	−0.001 (0.0002)	9.581	0.002
Myrmica (=0)	1.544 (0.543)	8.072	0.004
Plant height	0.008 (0.002)	22.684	<0.001
Myrmica (=0) × Plant height	−0.007 (0.002)	11.864	0.001
Distance	0.032 (0.008)	17.360	<0.001
Plant height	0.008 (0.002)	33.334	<0.001
Distance × plant height	−0.0001 (3.0×10^{-5})	20.675	<0.001

negative indirect effect (through the ant presence; Fig. 1e). Again, the level of spatial autocorrelation of the models was low (-0.081 < Moran's I < 0.074 for the first 10 lags).

The 282 plants received the first eggs between the 22nd of July and the 27th of August (Fig. 5). No significant difference between the date of first oviposition for plants with (33.1 days ± SE 0.63, n_1 = 170) and without the presence of *Myrmica* ants (34.4 days ± SE 0.49, n_2 = 112) was detected (*t*-test, t = 1.643, d.f. = 280, P = 0.110). Using the Cox regression test, we did not find a difference in time of first oviposition between plants with or without ants in the proximity, whereas the plant characteristics did have an effect (Table 3; Fig. 5). Tall plants with many
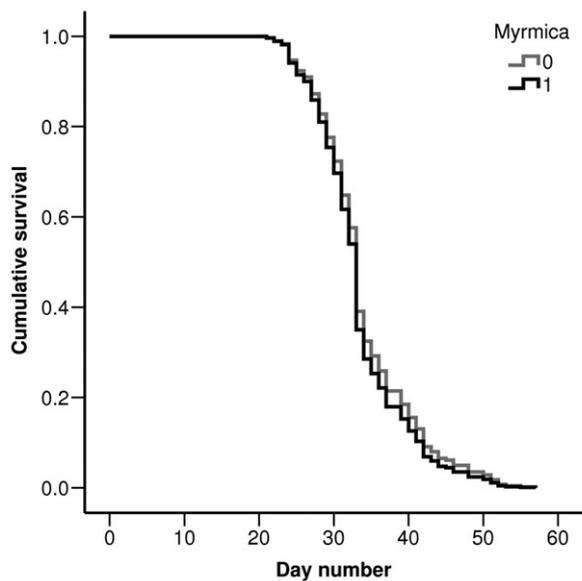

Fig. 5. Proportion of buds not infected with eggs over time for *Gentiana pneumonanthe* plants inside (grey line) and outside (black line) the home range of *Myrmica aloba* ants (Day 1 = 1 July), plotted for the mean plant height (21.45 cm). The plot is based on the Cox regression with presence/absence of *Myrmica aloba* ants and plant height as independent variables (Table 3).

Table 3. Results of the effect of *Myrmica* presence and the plant characteristics on the timing of depositing the first egg (n = 282) using a Cox regression model. The coefficient, its standard error, the Wald statistic and associated *P*-value are given. A positive coefficient means that higher values of independent variables is associated with later oviposition.

Independent variables	Coefficient (SE)	Wald	*P*-value
Myrmica (=0)	−0.069 (0.124)	0.311	0.577
Nr of buds	0.112 (0.022)	25.529	<0.001
Myrmica (=0)	−0.119 (0.123)	0.933	0.334
Bud length	0.031 (0.008)	16.048	<0.001
Myrmica (=0)	−0.109 (0.123)	0.788	0.375
Plant height	0.005 (0.001)	23.859	<0.001

large buds were associated with shorter oviposition time (see coefficients of Table 3: a high value of a positive coefficient implies that the drop in the proportion of not-infected buds is fast). Restricting the data to the plants without ants and the plants where at least two times the ants were found, did not change the results of the Cox regression (data not shown).

Discussion

The female *Phengaris alcon* butterflies are expected to make optimal decisions when depositing eggs to support high survival of their offspring. We found that tall plants with many tall buds are most likely to be chosen for oviposition (hypothesis 1). This is consistent with earlier studies (Dolek *et al.*, 1998; Küer & Fartmann, 2005; Nowicki *et al.*, 2005; Árnyas *et al.*, 2006, 2009; Van Dyck & Regniers, 2010). Árnyas *et al.* (2009) also showed that females avoid plants infested with aphids while they do not mind rust. When the caterpillars hatch and bore themselves into the flower buds, they find a high quantity of food as resource during their first three larval instars. Sheltered and hidden from enemies inside the flower bud, the only competition by conspecifics or other organisms using the same host plant, such as the micromoth *Nemophora violellus*, could reduce the probability of reaching the final larval instar, and subsequently leave the host plant and spend the winter in a *Myrmica* nest (Bräu *et al.*, 2006; Arnaldo *et al.*, in press). Females are known to occasionally deposit many eggs on one single host plant, with a high number of eggs per bud resulting in competition which negatively affects the condition of the caterpillars (Bräu *et al.*, 2006). The chance of being successfully adopted by worker ants and surviving until leaving the ants' nest as an adult is dependent on the caterpillar's body condition (Nash *et al.*, 2011).

An effect of the presence of *Myrmica* ants close to plants on the probability of finding eggs of *P. alcon* was found only for the plants for which we were sure that ants are in the proximity, although the effect was weak (hypothesis 1). Our results show that females in search of an oviposition site were mainly attracted by the size of the plants (plant height is the most explaining variable in the path analyses). Indeed, Van Dyck and Regniers (2010) suggested that tall plants represent a visually attractive target to the female. Moreover, tall host plants might provide high quantities of food for the caterpillars as they have many large buds. Even though the amount of food consumed in the period of time needed to reach the L4 instar is undoubtedly small, effects of competition are well known. The survival rate of the caterpillars is mostly between 40% and 60%, depending on the length of the bud and the phenological stage (Bräu *et al.*, 2006; Arnaldo *et al.*, in press). The question remains how it is possible that female butterflies do not respond to ant presence or absence at a certain host plant individual, but then have a higher likelihood to lay eggs on plants if ants

are nearby on neighbouring plants although the effect size is small given the relatively low standardised coefficient.

Our finding that more eggs are found on tall plants with many large buds is consistent with several other studies that showed that *P. alcon* would only select for the developmental stage of the flower (bud) for oviposition (Küer & Fartmann, 2005; Nowicki *et al.*, 2005; Fürst & Nash, 2010). In contrast, we found that more eggs were laid on plants that had *Myrmica* ants present or were growing close to plants with *Myrmica* than on plants where the ants were absent, which indicates that the presence of ants influences the egg load of *P. alcon*. This is consistent with previous research of Patricelli *et al.* (2011) and Wynhoff *et al.* (2008), who also found more eggs being deposited by females of *P. arion, P. teleius* and *P. nausithous* where ants were present. We found an effect of *Myrmica* ants on egg load, even though egg load was limited to only 6 days due to our experimental design. While females of *P. arion* spread their eggs over plants in the home ranges of all present *Myrmica* species, *P. teleius* and *P. nausithous* preferred host plants within the home ranges of their specific host ants, *M. scabrinodis* or *M. rubra* respectively. It is often assumed that an effect of ant presence on oviposition can be expected when the host plant is situated in the home range of an ants' nest. In the case of *Myrmica* ants, workers move up to a distance of about 2 m from the nest site to collect food. Given the host plant density and the spatial distribution of host plants with ants on the baits, all host plants are expected to grow within the home ranges of the host ants' nests at the study site. However, only for 165 out of 447 plants ants were found and, moreover, only for 40 out of 165 plants ants were captured more than once. If females would be able to detect ants based on the assumption that ant cues are present in the whole home range of their nest, no effect of ants would be expected. However, we found the effects of ant presence on the presence and amount of eggs the females deposit per bud. Apparently, when selecting a bud for oviposition, visual cues (likely plant height) are most important and decisive, as was observed by Van Dyck and Regniers (2010). Only after the plants with low probability of ants' nests nearby were removed from the analysis, effects were found. Later, probably after alighting or only at a very close distance to host plants, chemical cues maybe involved as well, resulting in longer oviposition bouts under favourable conditions. The distances involved are much shorter than the home range size of the *Myrmica* ants. While an effect of ants on egg load could be shown, the nature of this effect is unknown. A direct effect is possible; in this case, it is expected to be caused by the ant colony itself rather than markings of the worker ants in the home ranges, because distance to the nearest neighbours with ants are rather short. It is also possible that a plant-derived cue affects the behaviour of the females. Gentian plants growing close to *Myrmica* ant nests could react to this presence by emitting certain herbivore-induced plant volatiles (Dicke *et al.*, 2009) which are detectable by the females. Root lice which are often visited and tended by *Myrmica* ants might induce such a chemical defense reaction.

In our study, no clear indication was found that oviposition on plants without ant nests changed over time (hypothesis 2). Van Dyck *et al.* (2000) did find that the egg load for gentians without the ants present increased over time. We expected a similar change in oviposition, because at the beginning of the flight period of *P. alcon,* only green buds were available and butterflies would have no choice but to oviposit on them, leaving only the presence or absence of ants as a valuable decision for the females to make. Later in the season, all possible phenological stages of buds are present, giving females more choice to ensure the brood has enough food to develop into the fourth instar (Nowicki *et al.*, 2005). When *P. alcon* would select plants where ants are present early in the season, egg density build-up could be a reason that butterflies would later choose plants further away from ants to avoid competition inside the flower buds. However, no temporal change in oviposition preference was found. It could mean that in our case there was no density-dependent shift and butterflies did not need to oviposit more on plants where ants were absent, which is consistent with the study of Wynhoff *et al.* (2008) where a density-dependent shift only occurred in years with high butterfly densities. In our study year, the butterfly density seemed to be much lower than in the years before.

Van Dyck *et al.* (2000) found a density-dependent shift, but they considered a gentian in the home range of the ant nests when a nest was found within a 3 m radius around each gentian. We captured ants using sugar cubes as bait, and used the capturing of ants next to the plant as an indication of ant presence or the distance to the closest plant with ants was used. These differences in interpreting ant presence could account for differences in results since an assumed radius is not necessarily the ants' actual range or covers the spatial dimension in which cues are expected to occur. Moreover, an effect of ants was found to be stronger when the plants where ants have been found only once were removed from the analysis, showing that repeatedly capturing ants is of importance too. Our study shows that testing the ant-mediated oviposition hypothesis requires baiting ants more than once. When reducing the spatial scale by taking the distance to the closest plant with ants into account, effects were even stronger and more consistent. In addition, at our study site, the density of gentians of 7.2 plants per m^2 is much higher than reported from the other sites in Europe, where it is usually below 0.5 plants per m^2 (Maes *et al.*, 2004; WallisdeVries, 2004; Küer & Fartmann, 2005; Nowicki *et al.*, 2005; Bräu *et al.*, 2006; Van Dyck & Regniers, 2010; Czekes *et al.*, 2013). Fewer suitable plants in the area for ovipositing females might have caused the density-dependent shift in oviposition which was not seen in our study site.

When caterpillars are dependent on being found by worker ants which are active only within a limited distance from their colony, their adoption chance is related

to the distance between the host plant and the closest ant nest. Given that *P. alcon* in the majority of its geographical range encounters low gentian density, selection for ant-mediated oviposition is less likely to occur in areas with relatively low plant density than in areas with high plant density where many more plants might be sinks for the caterpillars, assuming that ant nest density is similar in these areas (which is often not measured). In populations with high host plant densities, the probability of a caterpillar to survive until the L4 larval instar might be high. However, later many caterpillars will be taken into a limited number of ant nests. Once adopted and taken into the nest, caterpillars experience contest competition with high mortality rates in the first weeks in the nest (Thomas *et al.*, 1993).

If host plant density is low and host ant nest densities high, selecting oviposition sites only in response to plant characteristics without considering the ants still yields a high probability that caterpillars will be found by worker ants. If so, then this could explain the variation in ant-mediated oviposition in *P. alcon* from undetectable to clearly present, whereas it supports ant-mediated oviposition in *P. teleius* and *P. nausithous* (Wynhoff *et al.*, 2008) and in closed populations of *M. arion* (Patricelli *et al.*, 2011) that encounter high host plant densities. Given the very high density of gentians with only half of them found by *Myrmica aloba* ants, ant-mediated oviposition was found in the investigated population, especially for plants where ants were frequently found. With these findings, this study contributes to the discussion about ant-mediated oviposition versus random oviposition because we found that oviposition choice of *P. alcon* is determined by both plant characteristics and ants but in different ways.

Acknowledgements

References

Akino, T., Knapp, J.J., Thomas, J.A. & Elmes, G.W. (1999) Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 1419–1426.

Als, T.D., Nash, D.R. & Boomsma, J.J. (2001) Adoption of parasitic *Maculinea alcon* caterpillars (Lepidoptera: Lycaenidae) by three *Myrmica* ant species. *Animal Behaviour*, **62**, 99–106.

Arnaldo, P., da Conceição Rodrigues, M., Gonzalez, D., Oliveira, I., Van Langevelde, F. & Wynhoff, I. (in press) Influence of host plant phenology and oviposition date on the oviposition pattern and offspring performance of *Phengaris (Maculinea) alcon*. *Journal of Insect Conservation*.

Arnaldo, P., Wynhoff, I., Soares, P., da Conceição Rodrigues, M., Aranha, J., Csősz, S., Maravalhas, E. & Tartally, A. (2011) *Maculinea alcon* exploits *Myrmica aloba* in Portugal: unusual host ant species of a myrmecophilous butterfly in a peripheral region. *Journal of Insect Conservation*, **15**, 465–467.

Árnyas, E., Bereczki, J., Tóth, A., Pecsenye, K. & Varga, Z. (2006) Egg-laying preferences of the xerophilous ecotype of *Maculinea alcon* (Lepidoptera: Lycaenidae) in the Aggtelek National Park. *European Journal of Entomology*, **103**, 587–595.

Árnyas, E., Bereczki, J., Tóth, A., Varga, K., Pecsenye, K., Tartally, A., Kövics, G., Karsa, D. & Varga, Z. (2009) Oviposition preferences of *Maculinea alcon* as influenced by aphid (*Aphis gentianae*) and fungal (*Puccinia gentianae*) infestation of larval host plants. *Ecological Entomology*, **34**, 90–97.

Baylis, M. & Pierce, N.E. (1991) The effect of host plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Ecological Entomology*, **16**, 1–9.

Benjamini, Y. & Hochberg, Y. (1995) Controlling the False Discovery Rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)*, **57**, 289–300.

Bos, F., Bosveld, M., Groenendijk, D., van Swaay, C., Wynhoff, I. & De Vlinderstichting (2006) *De dagvlinders van Nederland, verspreiding en bescherming (Lepidoptera: Hesperioidea, Papilionoidea)*. Nederlandse fauna 7, Nationaal Natuurhistorisch Museum Naturalis, KNNV Uitgeverij & European Invertebrate Survey – Nederland, Leiden.

Bräu, M., Gros, P., Nunner, A., Stettmer, C. & Settele, J. (2006) Der verlustreiche Weg in die Sicherheit eines Wirtsameisen-Nestes - neue Daten zur Entwicklungsbiologie und zur Mortalität der Präimaginalstadien von Maculinea alcon sowie zum Einfluss der Mahd = New data on the evolutionary biology and mortality of the immature stages of Maculinea alcon and the impact of mowing. *Larvalökologie von Tagfaltern und Widderchen in Mitteleuropa. (Abhandlungen aus dem Westfälischen Museum für Naturkunde 68. Jahrgang Heft 3/4) Landschaftsverband Westfalen-Lippe* (ed. by T. Fartmann and G. Hermann), pp. 197–219. Westfälisches Museum für Naturkunde, Münster, Germany.

Chen, Y.-Z., Lin, L., Wang, C.-W., Yeh, C.-C. & Hwang, S.-Y. (2004) Response of two *Pieris* (Lepidoptera: Pieridae) species to fertilization of a host plant. *Zoological Studies*, **43**, 778–786.

Czekes, Z., Markó, B., Nash, D.R., Ferencz, M., Lázár, B. & Rákosy, L. (2013) Differences in oviposition strategies between two ecotypes of the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae) under unique syntopic conditions. *Insect Conservation and Diversity*, **7**, 122–131.

De Knegt, H.J., Van Langevelde, F., Skidmore, A.K., Delsink, A., Slotow, R., Henley, S., Bucini, G., De Boer, W.F., Coughenour, M., Grant, C.C., Heitkönig, I.M.A., Henley, M., Knox, N.M., Kohi, E.M., Mwakiwa, E., Page, B.R., Peel, M., Pretorius, Y., Van Wieren, S.E. & Prins, H.H.T. (2011) The spatial scaling of habitat selection by African elephants. *Journal of Animal Ecology*, **80**, 270–281.

Dicke, M., Van Loon, J.J.A. & Soler, R. (2009) Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology*, **5**, 317–324.

Dolek, M., Geier, A. & Bolz, R. (1998) Distribution of *Maculinea rebeli* and host plant use on sites along the river Danube. *Journal of Insect Conservation*, **2**, 85–89.

Eilers, S., Pettersson, L.B. & Öckinger, E. (2013) Micro-climate determines oviposition site selection and abundance in the butterfly *Pyrgus armoricanus* at its northern range margin. *Ecological Entomology*, **38**, 183–192.

Elmes, G.W., Thomas, J.A. & Wardlaw, J.C. (1991) Larvae of *Maculinea rebeli*, a large-blue butterfly, and their *Myrmica* host ants: wild adoption and behaviour in ant-nests. *Journal of Zoology*, **223**, 447–460.

Elmes, G.W., Thomas, J.A., Wardlaw, J.C., Hochberg, M.E., Clarke, R.T. & Simcox, D.J. (1998) The ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. *Journal of Insect Conservation*, **2**, 67–78.

Epstein, L.H., Wisniewski, L. & Weng, R. (1994) Child and parent psychological problems influence child weight control. *Obesity Research*, **2**, 509–515.

Fartmann, T. & Hermann, G. (ed.) (2006) *Larvalökologie von Tagfaltern und Widderchen in Mitteleuropa*. Landschaftsverband Westfalen-Lippe, Westfälisches Museum für Naturkunde, Münster, Germany.

Fiedler, K. (1991) Systematic, Evolutionary, and Ecological Implications of Myrmecophily Within the Lycaenidae (Insecta: Lepidoptera:Papilionoidea). *Bonner Zoologische Monographien*, **31**, 1–210.

Fiedler, K., Hölldobler, B. & Seufert, P. (1996) Butterflies and ants: the communicative domain. *Experientia*, **52**, 14–24.

Fürst, M.A. & Nash, D.R. (2010) Host ant independent oviposition in the parasitic butterfly *Maculinea alcon*. *Biology Letters*, **6**, 174–176.

Gullan, P.J. & Cranston, P.S. (2010) *The Insects: An Outline of Entomology*, 4th Edition. Wiley-Blackwell, Oxford.

IBM Corp. (2011) *IBM SPSS Statistics for Windows*, Version 20.0, Armonk, NY.

Jaccard, J. & Wan, C.K. (1996) *LISREL Approaches to Interaction Effects in Multiple Regression*. Sage Publications, Thousand Oaks, California.

Jaenike, J. (1978) On optimal oviposition behaviour in phytophagous insects. *Theoretical Population Biology*, **14**, 350–356.

Küer, A. & Fartmann, T. (2005) Prominent shoots are preferred: microhabitat preferences of *Maculinea alcon* [(Denis & Schiffermüller), 1775] in Northern Germany (Lycaenidae). *Nota Lepidopterologica*, **27**, 309–319.

Maes, D., Vanreusel, W., Talloen, W. & van Dyck, H. (2004) Functional conservation units for the endangered Alcon Blue butterfly *Maculinea alcon* in Belgium (Lepidoptera: Lycaenidae). *Biological Conservation*, **120**, 229–241.

Menard, S. (2004) Six approaches to calculating standardized logistic regression coefficients. *The American Statistician*, **58**, 218–223.

Musche, M., Anton, C., Worgan, A. & Settele, J. (2006) No experimental evidence for host ant related oviposition in a parasitic butterfly. *Journal of Insect Behavior*, **19**, 631–643.

Nash, D.R., Als, T.D. & Boomsma, J.J. (2011) Survival and growth of parasitic *Maculinea alcon* caterpillars (Lepidoptera, Lycaenidae) in laboratory nests of three *Myrmica* ant species. *Insectes Sociaux*, **58**, 391–401.

Nowicki, P., Witek, M., Skorka, P. & Woyciechowski, M. (2005) Oviposition patterns in the myrmecophilous butterfly *Maculinea alcon* Denis & Schiffermuller (Lepidoptera: Lycaenidae) in relation to characteristics of foodplants and presence of ant hosts. *Polish Journal of Ecology*, **53**, 409–417.

Obermaier, E. & Zwölfer, H. (1999) Plant quality or quantity? Host exploitation strategies in three Chrysomelidae species associated with Asteraceae host plants. *Entomologia Experimentalis et Applicata*, **92**, 165–177.

Patricelli, D., Barbero, F., La Morgia, V., Casacci, L.P., Witek, M., Balletto, E. & Bonelli, S. (2011) To lay or not to lay: oviposition of *Maculinea arion* in relation to *Myrmica* ant presence and host plant phenology. *Animal Behaviour*, **82**, 791–799.

Pierce, N.E. & Elgar, M.A. (1985) The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilus Lycaenid butterfly. *Behavioral Ecology and Sociobiology*, **16**, 209–222.

Rabasa, S.G., Gutiérrez, D. & Escudero, A. (2005) Egg laying by a butterfly on a fragmented host plant: a multi-level approach. *Ecography*, **28**, 629–639.

Radchenko, A.G. & Elmes, G.W. (2010) *Myrmica Ants (Hymenoptera: Formicidae) of the Old World*. Natura Optima Dux Foundation, Warsaw, Poland.

Rangel, T.F.L.V.B., Diniz-Filho, J.A.F. & Bini, L.M. (2010) SAM: a comprehensive application for spatial analysis in macroecology. *Ecography*, **33**, 46–50.

Reudler Talsma, J.H., Biere, A., Harvey, J.A. & van Nouhuys, S. (2008) Oviposition cues for a specialist butterfly-plant chemistry and size. *Journal of Chemical Ecology*, **34**, 1202–1212.

Rodrigues, M.C., P. Soares, A. J. & Arnaldo, P.S. (2010) Characterization of a *Maculinea alcon* population in the Alvão Natural Park (Portugal) by a mark-recapture method. pp. 404–408. In Proceedings of the IUFRO Landscape Ecology Working Group International Conference, Bragança, Portugal.

Scheirs, J. & de Bruyn, L. (2002) Integrating optimal foraging and optimal oviposition theory in plant-insect research. *Oikos*, **96**, 187–191.

Schönrogge, K., Wardlaw, J.C., Thomas, J.A. & Elmes, G.W. (2000) Polymorphic growth rates in myrmecophilous insects. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 771–777.

Shreeve, T.G. (1986) Egg-laying by the speckled wood butterfly (*Pararge aegeria*): the role of female behaviour, host plant abundance and temperature. *Ecological Entomology*, **11**, 229–236.

Sielezniew, M. & Stankiewicz-Fiedurek, A.A. (2013) Behavioural evidence for a putative oviposition-deterring pheromone in the butterfly, *Phengaris (Maculinea) teleius* (Lepidoptera: Lycaenidae). *European Journal of Entomology*, **110**, 71–80.

Steiner, F., Sielezniew, M., Schlick-Steiner, B., Höttinger, H., Stankiewicz, A. & Górnicki, A. (2003) Host specificity revisited: new data on *Myrmica* host ants of the lycaenid butterfly *Maculinea rebeli*. *Journal of Insect Conservation*, **7**, 1–6.

Tartally, A., Nash, D.R., Lengyel, S. & Varga, Z. (2008) Patterns of host ant use by sympatric populations of *Maculinea alcon* and *M. 'rebeli'* in the Carpathian Basin. *Insectes Sociaux*, **55**, 370–381.

Thomas, J.A. & Elmes, G.W. (2001) Food–plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **268**, 471–477.

Thomas, J.A., Elmes, G.W., Sielezniew, M., Stankiewicz-Fiedurek, A., Simcox, D.J, Settele, J. & Schönrogge, K. (2013) Mimetic host shifts in an endangered social parasite of ants. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **280**, 20122336. doi: 10.1098/rspb.2012.2336.

Thomas, J.A., Elmes, G.W. & Wardlaw, J.C. (1993) Contest competition among *Maculinea rebeli* butterfly larvae in ant nests. *Ecological Entomology*, **18**, 73–76.

Thomas, J.A., Schönrogge, K. & Elmes, G.W. (2005) Specializations and host associations of social parasites of ants. *Insect Evolutionary Ecology* (ed. by M.D.E. Fellowes, G.J. Holloway and J. Rolff), pp. 479–518, Proceedings of the Royal Entomological Society's 22nd Symposium. CABI Publishing, Wallingford, UK.

Thomas, J.A., Simcox, D.J., Wardlaw, J.C., Elmes, G.W., Hochberg, M.E. & Clarke, R.T. (1998) Effects of latitude, altitude and climate on the habitat and conservation of the endangered butterfly *Maculinea arion* and its *Myrmica* ant hosts. *Journal of Insect Conservation*, **2**, 39–46.

Van Dyck, H., Oostermeijer, J.G.B., Talloen, W., Feenstra, V., Van Der Hidde, A. & Wynhoff, I. (2000) Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 861–866.

Van Dyck, H. & Regniers, S. (2010) Egg spreading in the ant-parasitic butterfly, Maculinea alcon: from individual behaviour to egg distribution pattern. *Animal Behaviour*, **80**, 621–627.

Van Langevelde, F. & Wynhoff, I. (2009) What limits the spread of two congeneric butterfly species after their reintroduction: quality or spatial arrangement of habitat? *Animal Conservation*, **12**, 540–548.

Van Swaay, C., Maes, D., Collins, S., Munguira, M.L., Šašić, M., Settele, J., Verovnik, R., Warren, M., Wiemers, M., Wynhoff, I. & Cuttelod, A. (2011) Applying IUCN criteria to invertebrates: how red is the Red List of European butterflies? *Biological Conservation*, **144**, 470–478.

WallisdeVries, M.F. (2004) A quantitative conservation approach for the endangered butterfly *Maculinea alcon*. *Conservation Biology*, **18**, 489–499.

Wiklund, C. (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia*, **63**, 23–29.

Wiklund, C. & Friberg, M. (2008) Enemy-free space and habitat-specific host specialization in a butterfly. *Oecologia*, **157**, 287–294.

Witek, M., Sliwinska, E.B., Skórka, P., Nowicki, P., Settele, J. & Woyciechowski, M. (2006) Polymorphic growth in larvae of *Maculinea* butterflies, as an example of biennialism in myrmecophilous insects. *Oecologia*, **148**, 729–733.

Witek, M., Nowicki, P., Sliwinska, E.B., Skórka, P., Settele, J., Schönrogge, K. & Woyciechowski, M. (2010) Local host ant specificity of *Phengaris* (*Maculinea*) *teleius* butterfly, an obligatory social parasite of *Myrmica* ants. *Ecological Entomology*, **35**, 557–564.

Wynhoff, I., Grutters, M. & van Langevelde, F. (2008) Looking for the ants: selection of oviposition sites by two myrmecophilous butterfly species. *Animal Biology*, **58**, 371–388.