Population Ecology

Population ecology and egg laying strategy in the ‘cruciata’ ecotype of the endangered butterfly Maculinea alcon (Lepidoptera: Lycaenidae)

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Abstract: Population dynamics studies in insects mostly focus on a specific life stage of a species and seldom consider different stages. In the framework of our research we studied the population demography of a protected Maculinea alcon ‘cruciata’ population and the factors that could influence the distribution of eggs. The results of the mark-recapture survey showed a relatively short flight period between mid-June and mid-July with clearly marked early peak period. Unlike in many other butterflies, proterandry was not strong. The total population of M. alcon ‘cruciata’ was estimated at 699 individuals. The survival rate, and consequently the average life span, was relatively low. Generally, males proved to be more mobile than females. Eggs showed a highly aggregated pattern, and egg numbers was positively related to general shoot size, while the number of flower buds and the features of the surrounding vegetation did not display any effect on egg laying. Based on our findings the studied population appears viable, but specific management techniques could ensure optimal conditions for egg laying in this protected butterfly.

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Abstract

Population dynamics studies in insects mostly focus on a specific life stage of a species and seldom consider different stages. In the framework of our research we studied the population demography of a protected *Maculinea alcon ‘cruciata’* population and the factors that could influence the distribution of eggs. The results of the mark-recapture survey showed a relatively short flight period between mid-June and mid-July with clearly marked early peak period. Unlike in many other butterflies, proterandry was not strong. The total population of *M. alcon ‘cruciata’* was estimated at 699 individuals. The survival rate, and consequently the average life span, was relatively low. Generally, males proved to be more mobile than females. Eggs showed a highly aggregated pattern, and egg numbers was positively related to general shoot size, while the number of flower buds and the features of the surrounding vegetation did not display any effect on egg laying. Based on our findings the studied population appears viable, but specific management techniques could ensure optimal conditions for egg laying in this protected butterfly.

Key-words: host plant; mark-recapture; sex ratio; species conservation; survival; vegetation characteristics
Introduction

Dynamics of insect populations, mostly in the case of pests, and more recently also in protected species, has been the subject of wide range of studies (Hassell et al. 1991, Way and Heong 1994, Hunter 2001, Yamamura et al. 2006, Thomas et al. 2009). Most of these studies though concentrate on a single life stage of an insect (e.g., adults, larvae), while usually neglecting the parallel investigations into other developmental stages, or the connection between them (Jones and Sullivan 1982, Elkinton and Liebhold 1990, Yamamura et al. 2006, Ordano et al. 2015). Admittedly, it is much easier, and therefore much more practical to determine the viability of any population based solely on the abundance of adults, consequently many pest control and species conservation actions primarily rely on such information (Jones and Sullivan 1982, Katsoyannos 1992, Steytler and Samways 1995, Sunderland and Samu 2000, Thomas et al. 2009, Vrezec et al. 2012). However, linking the dynamics of adults with e.g., egg laying patterns can offer a more precise picture of the sustainability of certain populations in a given area, since the viability of a population is primarily determined by the number of offspring produced in the study area, i.e., in the case of insects by the number of eggs and/or larvae (Begon et al. 1996). In winged insects, such as butterflies, which disperse very efficiently, this combined information is vital, since the mere presence of adults might not imply the persistence of a population in that area at all, it could merely be a sign of efficient dispersal of adults.

The population dynamics of adult butterflies is frequently connected to weather conditions and environmental stochasticity (Melbourne and Hastings 2008, Nowicki et al. 2009, Cormont et al. 2013), while adult egg laying decisions, thus the fate of their
offspring primarily relies on the condition of host plants, the strength of intraspecific competition, predatory pressure or other habitat parameters (Stamp 1980, Wiklund 1984, Bergman 2001, Czekes et al. 2014). Ovipositing females have to choose the optimal site for their offspring, as well as the best available host plant within the site. Additionally, the survival of the offspring can also be affected by the host plants’ direct or indirect responses to the presence of eggs and/or larvae as well (see Hilker and Fatouros 2015 for a review). In the present study we investigated the within-season dynamics of adult butterflies in the ‘cruciata’ ecotype of the endangered Maculinea alcon (featured in many former studies as Maculinea rebeli) while linking it to egg laying patterns and preferences.

Large Blue butterflies of the genus Maculinea Van Eecke, 1915 (synonymised lately with Phengaris Doherty, 1891) are one of the most intensively studied butterfly groups in Europe, being considered flagship and umbrella species in nature conservation. They are highly sensitive to habitat changes, and the conservation of their habitats is beneficial to many other threatened species (Thomas et al. 1998a, Thomas and Settele 2004, Nowicki et al. 2005a, Settele et al. 2005). In the past decades severe declines were recorded in most of their Western European populations due to habitat fragmentation and intensification of agriculture (Van Swaay and Warren 1999, Van Swaay et al. 2010). They also raise specific scientific interest due to the intriguing obligate myrmecophylic lifestyle of their larvae (see Witek et al. 2010). Most Maculinea populations are small and isolated (Thomas et al. 1998b, Meyer-Hozak 2000), characterized by density dependent regulation due to intra-specific competition between larvae on host plants and/or in host ant colonies (Hochberg et al. 1992, Nowicki et al. 2009). Long-term surveys have already shown the importance of weather
patterns (Roy et al. 2001, Cormont et al. 2013), but Maculinea populations are also affected by general habitat characteristics (Nowicki et al. 2007), and human activities (e.g., changes in agricultural practices) (Schmitt and Rákosy 2007).

Despite a relatively large number of both field and modelling studies into the ecology of *M. alcon ‘cruciata’* (e.g., Hochberg et al. 1992, Meyer-Hozak 2000, Árnyas et al. 2006, Oskinis 2012, Timuș et al. 2013, Czekes et al. 2014), hardly any of them investigated the adult population size and their egg laying behaviour at the same time (Meyer-Hozak 2000, Körösi et al. 2008). Furthermore, none of these studies combined the information from both sources in a joint analysis. Despite the need for complex information on populations of protected butterflies, such studies are generally rare in case of other butterfly species as well (see Bergman 2001). Consequently, the aims of our research were to (a) study the within-season dynamics of a *M. alcon ‘cruciata’* population, while also (b) examining the temporal changes in the deposition of eggs, and (c) identifying the factors influencing the distribution of eggs.

**Materials and methods**

**Study species and site**

Two major ecotypes of the butterfly *M. alcon* are differentiated based on their host plant: the hygrophilous form feeding on *Gentiana pneumonanthe* (previously treated as *M. alcon*), and the more xerophilous form feeding on *G. cruciata* (previously treated as *M. rebeli*, hereafter referred to as *M. alcon ‘cruciata’*). Recent molecular studies showed that the two forms cannot be regarded as different species (Als et al. 2004, Bereczki et al. 2005, Steiner et al. 2006, Pecsenye et al. 2007). Nevertheless, in addition to habitat and host plant segregation, they typically use different host ant
species, and they also have different flight periods (Bereczki et al. 2005, Pech et al. 2004, Sielezniew et al. 2012). *M. alcon* ‘cruciata’ prefers semi-natural calcareous grasslands (Bálint 1994, Pech et al. 2004, Rákosy and Vodă 2008), and it uses quite a wide range of host ant species from the genus *Myrmica* Latreille, 1804, which adopt them due to their efficient chemical and acoustical mimicry (see Fiedler 2006 and Witek et al. 2014 for a review). Their development continues inside the ant nest, where they are fed by the ant workers (Elmes et al. 1991). The flight period of adult butterflies is from mid-June to mid-July (Meyer-Hozak 2000, Körösi et al. 2008, Timuș et al. 2013).

The conservation status of *M. alcon* is Least Concern according to the IUCN Red List in Europe and Near Threatened in the European Union (Van Swaay et al. 2010).

The field study was performed on a 9252 m$^2$ semi-natural calcareous dry grassland of southeastern exposure in the surroundings of Rimetea village (N46°27’51.45”, E23°33’46.26”, ca. 620 m a.s.l., Romania). The grassland is a plant species-rich meadow dominated by *Brachypodium pinnatum*, *Carex humilis* and *Festuca rupicola* with other characteristic species like *Dorycnium pentaphyllum*, *Cytisus albus*, *Hieracium bauhinii*, *Teucrium montanum* and *Thymus serpyllum*, and it is intensively grazed by goats and sheep. The meadow is partially surrounded by a mixed forest and shrubs of *Crataegus monogyna*, *Prunus spinosa*, *Pyrus pyraster* and *Rosa canina*. The site constitutes a part of the ROSCI0253 ‘Trascău’ Natura 2000 protected site.

**Data collection**

*a) Population dynamics survey*
A mark-recapture study of adult *M. alcon ‘cruciata’* butterflies was conducted between 15 June and 16 July 2012 covering the entire flight period. The survey plan followed the requirements of the Pollock’s Robust Design approach (Pollock 1982; Pollock et al. 1990), i.e. relatively infrequent but highly intensive capture days were established, which constituted primary sampling periods. The sampling took place on every fourth day, with a single exceptional case in which the interval between consecutive capture days was reduced to three days due to the forecast of unfavourable weather conditions on the following days. Butterflies were surveyed between 10 AM and 5 PM during five one-hour capture sessions, regarded as secondary sampling periods, and were separated by 30 min breaks to allow free mixing of butterflies between the secondary sampling periods. Captured individuals were marked on the underside of their hind-wing with unique identity numbers using a fine-tipped waterproof pen (© Schneider GmbH), and then immediately released at the place of capture. For each capture we recorded the date, the exact time and the position of each capture (GPS coordinates), as well as the identity number and the sex of the adult.

*b) Distribution of butterfly eggs*

Prior to the adult butterfly survey we randomly placed out 22 sampling plots within the study site. The plots were circles of 2 m radius, as generally applied in the case of *Maculinea* species based on the average foraging radius of the host ant *Myrmica* (see Elmes 1975, Elmes et al. 1998), with a focal *G. cruciata* plant in the middle. Within the plots we recorded the number of all *G. cruciata* host plants, and the number of their shoots. Shoots were considered to belong to the same plant when they were obviously connected either above the soil surface. In order to minimise disturbance, we
recorded the number of eggs on the focal host plant within each plot (n = 22) only at the end of each mark-recapture sampling day. At the end of the whole study period we counted all eggs found on all host plant shoots within the sampling plots in addition to the characteristics of the host plants and general vegetation features. The following parameters were recorded: (a) the total number of butterfly eggs laid on the host plant shoots, and separately on different verticils, (b) shoot height as the length of the shoot (cm), (c) number of shoot leaves, and (d) number of flowers (only flower buds with coloured sepals were taken into account since small green flower buds are impossible to count sometimes) on separate verticils of shoots, (e) the number of host plants in each plot, (f) the maximum height of the surrounding vegetation (cm), and (g) the proportion of vegetation cover visually estimated to the nearest 5%.

Data analysis

Mark-recapture data was analysed with the use of Mark 7.0 program (White and Burnham 1999) applying the Robust Design (RD) model (Pollock 1982; Kendall et al. 1995). The RD model allows relatively high precision of population estimates, and it has proved its applicability in butterfly population studies (Nowicki et al. 2008). The analyses were conducted separately for males and females, because sex-specific population parameters were of interest for our study. The data from capture sessions (i.e. secondary periods of the RD model) within sampling days were used to estimate daily population sizes for these days \( (N_i) \). In the estimation we accounted for individual heterogeneity in capture probabilities, since its existence was revealed by the tests for violations to equal catchability assumption (Otis et al. 1978; Chao 1988). In turn, the data pooled together within capture days (i.e. primary periods of the RD model) were
used for assessing survival rate between these days ($\phi_i$). The model variant assuming no
time variation in survival rate performed the best as indicated by its lowest value of the
Akaike Information Criterion corrected for small sample ($\text{AIC}_c$; Hurvich and Tsai
1989), which implies that adult survivorship was fairly constant throughout the flight
period. Subsequently, we calculated the average adult lifespan as $e = (1 - \phi_i)^{-1} - 0.5$
(Nowicki et al. 2005b).

Based on the estimates of daily population sizes and survival rates, we also
estimated the recruitment ($B_i$), i.e. the numbers of individuals eclosing from pupae and
entering the adult population during the intervals between consecutive capture days. As
the adult life span was relatively short when compared with the length of these intervals
($d_i$), we used the formula of Nowicki et al. (2005a; see this reference for the rationale),
which accounts for the individuals eclosing and dying within the same intervals: $B_i' = d_i$
$\times (N_{i+1} - N_i \cdot \phi^d) \times (\phi - 1) / (\phi^d - 1)$. The sum of recruitment for the entire flight period
makes up seasonal population size ($N_{\text{total}}$). In a similar way, by summing female
recruitments prior to each capture day, we derived the total numbers of females present
until these days.

To compare the distances covered by female and male butterflies we measured
the distance between the two furthermost points where an individual was captured. We
also measured the area of the polygon marked by the capture points of an individual in
order to compare the area covered by female and male butterflies. The measurements
were based on recorded GPS coordinates and conducted using Garmin Mapsource
software (version 6.16.3, Garmin Ltd. 1999-2010). The flight distances and areas could
be quantified only in the case of individuals which were captured at least twice (for
distance), and three times (for area), therefore, due to the low recapture rate, a relatively
small data set was available for analysis (n = 11 females and 59 males for distance; n = 5 females and 28 males for area). Wilcoxon signed rank test were used for the comparison of flight distances between males and females. Due to the low sample size a similar analysis was not performed for the area covered.

Poulin’s discrepancy index (Rózsa et al. 2000) was used to characterize the distribution of eggs on all host plants within sampling plots recorded at the end of the study period. Biases in the distribution of eggs among different host plant verticils were checked with Generalized Linear Mixed Model approach (GLMM, Poisson error, maximum likelihood approximation; n = 133). The number of eggs laid on different verticils of egg bearing plant shoots was introduced as dependent variable, while the ID of verticil as independent factor. Sampling plot and plant IDs were introduced as nested random factors to handle dependency of data. Only egg data regarding the top four verticils were taken into account since no eggs were recorded on lower verticils.

We tested the relationship between the estimated number of females present before each sampling day, and the total number of eggs laid in the same period (n = 8) in order to reveal whether the number of eggs laid is related to the number of female butterflies. Spearman rank correlation analysis was applied due to the lack of normality of both variables. In addition, the effect of the abundance of eggs already present on oviposition was checked by testing the relationship between the number of eggs present and the number of newly laid eggs in the following period for seven consecutive periods between the eight sampling days. Again, Spearman rank correlation analysis was applied in this case.
The effects of host plant and vegetation characteristics on egg distribution were analyzed with the use of GLMM approach (Poisson error, maximum likelihood approximation; n = 410). Correlation between host plant characteristics were checked using Spearman rank correlation analysis due to non-normality of datasets. A principal component analysis (PCA) was applied to obtained uncorrelated derived variables for plant characteristics, and the principal components were used as independent variables in the GLMM analysis. The number of eggs laid on each focal host plant was introduced as a dependent variable, while independent variables were the host plant morphological characteristics (PC1 [correlated plant height and number of leaves] and PC2 [correlated number of flower buds]), the number of host plants in sample plots, the maximum height of the surrounding vegetation, and vegetation cover. Sampling plot and host plant IDs were introduced as random factors to handle dependencies. Automated model selection procedure was carried out, and the effects of different explanatory variables were averaged across the supported models with delta AIC<sub>c</sub> < 4, i.e. those with the AIC<sub>c</sub> differing by less than 4 from the best model (see Grueber et al., 2011).

All statistical analyses were carried out using the R 3.1.1 Statistical Environment (R Development Core Team 2014) and Quantitative Parasitology 3.0 (Rózsa et al., 2000). Normality of datasets was regularly checked with the Shapiro-Wilk test. Relevel function was used in order to carry out post-hoc sequential comparisons among factor levels when performing GLMM. GLMMs were carried out with the use of glmer function in lme4 package (Bates et al. 2014), and dredge function in MuMIn package (Barton 2015) was applied for automated model selection. Table-wide Bonferroni-Holm correction was applied in the case of sequential comparisons, such as Spearman rank
correlations and comparison of factor levels in the GLMM analysis concerning the location of eggs on different verticils.

Results

Demography of adult butterflies

During the entire study we captured and marked 152 (67.5%) males and 73 (32.5%) females, out of which 85 males and 14 females, respectively, were recaptured at least once. The total adult population was assessed at 699 individuals, with a relatively balanced sex ratio (55% males vs. 45% females) (Table 1). The estimated survival was fairly low, which translates in rather short adult lifespan of ca. 2 days with no major inter-sexual difference (Table 1).

The butterfly had a relatively short flight period between mid-June and mid-July, with a clearly pronounced peak occurrence in the early part of the period (Fig. 1). More than 50% of individuals emerged within the first week, and more than 80% within the first two weeks (Fig. 1). Besides, in comparison to many other species of butterfly, we found rather weak protandry (cf. Pfeifer et al. 2000; Nowicki et al. 2005b): the number of females peaked only three days after the peak of males.

Most of the butterflies clearly preferred the close proximity of shrubs (Fig. 2). The mean distance covered by males was 81.61 m (SD = ±56.99, min = 9, max = 217), and the mean area was 1766.98 m² (SD = ±2260.67, min = 25, max = 8091). Females were less mobile, with a mean flight distance of 53.9 m (SD = ±57.72, min = 14, max = 213), and with a mean area of 193 m² (SD = ±289.19, min = 5, max = 684). Males covered significantly longer distances, than females (Wilcoxon signed rank test W = 454, p < 0.05).
Egg laying dynamics and preferences

At the end of the study altogether 94 eggs were recorded on a total of 410 G. cruciata shoots of 201 plants within the 22 study plots. More than 90% of the shoots lacked eggs, and the maximum number of eggs was 23 on a single shoot. The overall mean egg density was 0.47 eggs/plant, and 0.23 eggs/shoot (4.48 eggs/plant and 4.09 eggs/shoot only for plants with eggs); while the mean host plant density was 0.72 plants/m² (9.05 plants/plot), and 1.48 shoots/m² (18.6 shoots/plot). The distribution of eggs on plants showed a highly aggregated pattern (Fig. 3) as indicated by Poulin’s discrepancy index (D = 0.97). Eggs were laid only on the top four verticils of the plants. Most eggs were laid on the 2nd verticil (33.93% of total), but no significant differences were revealed between the number of eggs on the different verticils (GLMM, z ≤ 1.487, p = NS, n = 133).

Eggs were recorded even during the first part of the study period on the focal host plants of the sampling plots (n = 22), even if less than 10% of the focal plants bore eggs on the 2nd sampling day (22.06). By the 6th sampling day (08.07) 63% of the plants had eggs, after this the percentage of egg bearing plants decreased (Fig 4.). The number of eggs laid before each capture period did not correlate with the number of females recruited in the same period (Spearman r = 0.53, p = 0.13, n = 8). In turn, the number of newly laid eggs correlated negatively with the number of eggs already present on host plants, the negative correlations between the two variables reached statistical significance level during all but two sampling periods (Table 2).

Host plant morphological characteristics were mostly correlated according to the results of the Spearman rank-correlation analysis (n = 410): height vs. number of leaves
$r = 0.5, p < 0.001$; number of leaves vs. number of flower buds $r = 0.25, p < 0.001$; height vs. number of flower buds $r = 0.10, p = 0.050$. The PCA yielded 1st (PC1) and 2nd (PC2) principal components that explained 52% and 31% of the variance, respectively. PC1 represented plant height and number of leaves with loadings of 0.66 and 0.68, respectively, as a measure of general shoot size, while PC2 reflected the number of flower buds with a loading of 0.94. All input variables were retained in the best average GLMM model for egg laying preferences (Table 3), but only the general shoot size (PC1) had a significant positive effect on the number of eggs laid ($z = 4.59, p < 0.001$; Fig. 5), while none of the other variables displayed any significant effects ($z \leq 1.63, p = \text{NS}$)

Discussion

The results of the present study show that the studied butterfly population appears fairly viable based on the comparison with other studies concerning the size of *M. alcon ‘cruciata’* populations (Árnyas et al. 2005, Timuș et al. 2013). During a three year long MRR study Árnyas et al. (2005) found that on a 0.75 ha site the studied *M. alcon ‘cruciata’* population was stable with nearly 1000 individuals, while Timuș et al. (2013) estimated the size of another population in Romania to 1073 individuals for a 40 ha site. In comparison, the size of our studied population (699 individuals on ~1ha) suggests that the population is relatively big. Generally, *Maculinea alcon* populations show very small fluctuations (Hochberg et al. 1994, Elmes et al. 1996), thus there is a considerable chance that our studied population is stable.

Similarly to other studies (Meyer-Hozak 2000, Árnyas et al. 2005), we found that the butterflies fly from mid-June to mid-July. In some cases the flight period takes
less than one month (Timuș et al. 2013), which can reflect differences in habitat or/and meteorological conditions of different populations. Although we found a relatively weak indication of protandry, the peak emergence of males still preceded that of females. This phenomenon is in fact common for all Maculinea species and for butterflies in general. According to Elmes and Thomas (1987) the males pupate a few days before females, and thus during the initial part of the flight period the population is dominated by males. During the entire study we caught roughly twice as many male individuals as female, but the estimated sex ratio was relatively balanced, which corresponds with results of other studies (Árnyas et al. 2005, Timuș et al. 2013). Considerably higher capture and recapture rates of males may be attributed also to the fact that they fly more often and higher searching for the less mobile females. The latter tend to fly lower because they are searching for food plants in the undergrowth (Árnyas et al. 2005).

Earlier mark-recapture and individual tracking studies suggested that Maculinea butterflies are highly sedentary (Hovestadt and Nowicki 2008, Hovestadt el al. 2011, Skórka et al. 2013). Our results concerning adult mobility also support this fact. Keeping close to the place of eclosion can be an adaptation of these butterflies to myrmecophily (Hovestadt and Nowicki 2008). Inter-sexual differences were revealed by our study: males proved to be more mobile than females. On the other hand, similar research on other Maculinea species showed higher mobility in females (Kőrösi et al. 2012, Skórka et al. 2013), which could be attributed to females trying to decrease intra-specific competitive pressure among their offsprings (Nowicki and Vrabec 2011). However, all these studies were carried out on M. teleius and M. nausithous, i.e. species with more restricted habitat requirements (wet meadows) and
higher population densities in comparison to *M. alcon ‘cruciata’*, which prefers semi-dry grasslands and has lower population densities (Meyer-Hozak 2000, Nowicki et al. 2007). In our population the higher mobility of males is likely to stem from the high male/female ratio due to which males may need to cover larger areas in order to find mating partners. Therefore, male mobility may play a crucial role in gene flow in our population (Piaggio et al. 2009, Solmsen et al. 2011).

Phytophagous butterfly species mostly lay their eggs separately one by one or in clusters (Stamp 1980 for a review, Vrinec 1990, Dixon and Guo 1993). Both strategies can influence positively the survival of eggs and larvae. Females can lower the chances of predation and competition for their offspring by depositing their eggs individually. In these cases eggs are usually cryptic (light yellow or green) and are laid on protected parts of the host plants (see Stamp 1980). Laying eggs in clusters can be advantageous when other factors can affect negatively the reproduction, like the patchy distribution of host plants, the scarcity of resources for larvae and adults, low population density or unfavorable weather conditions (Stamp 1980, Karlsson and Johansson 2008, Karlsson et al. 2008). Besides, as clusters of eggs and larvae are more protected from desiccation when clumped together, clusters can ensure higher survivability through lower sensitivity to ambient conditions (Stamp 1980, Clark and Faeth 1998). During our study we found a low mean egg density per host plants (0.47 eggs/plant) compared to that recorded by Czekes et al. (2014) in another population (8.89 eggs/plant). In addition, the distribution of eggs among host plants showed a clearly aggregated pattern, thus most of the eggs were concentrated only on a few host plants. This could suggest the patchy distribution of host ants, however there is no convincing evidence yet that ovipositing

The number of females did not explain the number of new eggs laid, which can also be attributed to the emigration of females. It is possible that some of the females present in the study area laid their eggs in the surrounding land fragments. Another cause of this result could be the perishing of a large amount of eggs during the egg laying season which can be attributed to an increased level of predation (Bergman 2001), but also to meteorological factors and grazing of host plants. There was a negative relationship between the number of eggs already present on the plants and the quantity of newly laid eggs. This result could indicate that females would prefer laying eggs on empty plants or at least with a small amount of eggs present only. However, this evidence is very circumstantial, specifically designed study could only clear the effect of egg abundance on female ovipositing behaviour (see e.g. Kőrösi et al. 2008).

Earlier studies about egg laying preferences showed that the most important factors influencing oviposition are the morphological characteristics of host plants, such as the height of the plant, the number, the size and the phenology of buds, and the number of leaves (Dolek et al. 1998, Nowicki et al. 2005a, Árnyas et al. 2006, 2009, Czekes et al. 2014, Wynhoff et al. 2015). In concordance with the aforementioned studies, our research suggests that females preferred the taller shoots with many leaves for oviposition. A visually conspicuous host plant (i.e. tall ones with many leaves) may be more attractive or more perceptible for females than smaller ones (Nowicki et al. 2005a, Van Dyck and Regniers 2010, Czekes et al. 2014, Arnaldo et al. 2014, Wynhoff et al. 2015). The large number of eggs on tall plants with a high number of leaves can be beneficial for the butterflies due to an increased egg laying surface, decreased larval
competition, or even better climatic conditions. Wynhoff et al. (2015) suggested that larger host plants might provide high quantities of food for the caterpillars because of bearing later many fully developed flower buds. *Maculinea alcon ‘cruciata’* females laid their eggs exclusively on the four top verticils of their host plants, which could be attractive sites for oviposition presumably also because of the lower predation risk for adult females (Van Dyck and Regniers 2010) and the better microclimate for larval development (Alonso 2003). In addition, ovipositing mostly on the 2\textsuperscript{nd} verticil from the top, as suggested by our results, could ensure better climatic conditions to eggs through reduced exposure to sun and wind.

Linking population demography data to oviposition preferences could help the protection of the focal butterfly species not only by offering data to nature conservationists, but also by revealing that specific management techniques could ensure better conditions for egg laying. Specifically, sustaining a low grazing pressure could have a positive effect on the butterfly population (WallisDeVries and Raemakers 2001), and it would also keep shrubs from invading the grassland.

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Table 1. Basic parameters of the investigated *Maculinea alcon ‘cruciata’* population as revealed by the MRR study (95% confidence intervals in brackets).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Captured individuals</th>
<th>Seasonal population [day$^{-1}$]</th>
<th>Survival rate [day$^{-1}$]</th>
<th>Adult lifespan [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>152</td>
<td>382 (305–496)</td>
<td>0.63 (0.57–0.69)</td>
<td>2.2 (1.8–2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>Females</td>
<td>73</td>
<td>317 (219–480)</td>
<td>0.57 (0.34–0.77)</td>
<td>1.8 (1.0–3.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>All</td>
<td>225</td>
<td>699 (565–884)</td>
<td>0.62 (0.56–0.68)</td>
<td>2.1 (1.8–2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
</tbody>
</table>
Table 2. Spearman rank correlations (n = 22 in all cases) between the number of eggs present and the number of newly laid eggs on host plants on different capture dates. Statistically significant values are bolded.

<table>
<thead>
<tr>
<th>capture date</th>
<th>Spearman r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.06</td>
<td>-0.73</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>22.06</td>
<td>-0.21</td>
<td>0.731</td>
</tr>
<tr>
<td>26.06</td>
<td>-0.51</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>30.06</td>
<td>-0.89</td>
<td>&lt; <strong>0.001</strong></td>
</tr>
<tr>
<td>04.07</td>
<td>0.04</td>
<td>0.861</td>
</tr>
<tr>
<td>08.07</td>
<td>-0.74</td>
<td>&lt; <strong>0.001</strong></td>
</tr>
<tr>
<td>12.07</td>
<td>-0.91</td>
<td>&lt; <strong>0.001</strong></td>
</tr>
</tbody>
</table>
Table 3. The supported models according to the results of the automated model selection procedure. See the text for the explanations of the model variables.

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>logLik</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1+PC2</td>
<td>5</td>
<td>-119.61</td>
<td>249.37</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>PC1+PC2+Height</td>
<td>6</td>
<td>-119.30</td>
<td>250.80</td>
<td>1.43</td>
<td>0.12</td>
</tr>
<tr>
<td>PC1</td>
<td>4</td>
<td>-121.47</td>
<td>251.03</td>
<td>1.66</td>
<td>0.11</td>
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<tr>
<td>Cover+PC1+PC2</td>
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<td>-119.51</td>
<td>251.23</td>
<td>1.87</td>
<td>0.10</td>
</tr>
<tr>
<td>Density+PC1+PC2</td>
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<td>-119.56</td>
<td>251.33</td>
<td>1.96</td>
<td>0.10</td>
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<tr>
<td>Density+PC1</td>
<td>5</td>
<td>-121.14</td>
<td>252.43</td>
<td>3.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Cover+PC1+PC2+Height</td>
<td>7</td>
<td>-119.16</td>
<td>252.61</td>
<td>3.27</td>
<td>0.05</td>
</tr>
<tr>
<td>PC1+Height</td>
<td>5</td>
<td>-121.28</td>
<td>252.72</td>
<td>3.35</td>
<td>0.05</td>
</tr>
<tr>
<td>Density+PC1+PC2+Height</td>
<td>7</td>
<td>-119.26</td>
<td>252.79</td>
<td>3.43</td>
<td>0.05</td>
</tr>
<tr>
<td>Cover+PC1</td>
<td>5</td>
<td>-121.37</td>
<td>252.89</td>
<td>3.52</td>
<td>0.05</td>
</tr>
<tr>
<td>Cover+Density+PC1+PC2</td>
<td>7</td>
<td>-119.46</td>
<td>253.20</td>
<td>3.84</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Fig. 1. Dynamics of male and female adult butterflies throughout the study period based on mark-recapture estimates. Error bars represent 95% confidence intervals.

Fig. 2. The (a) outline of the study site and the (b) distribution of male (black) and female (white) butterfly captures (recaptures included).

Fig. 3. The frequency distribution of eggs on host plant shoots.

Fig. 4. Temporal dynamics of eggs laid on the host plants during the flight period. Error bars represent Standard Deviations.

Fig. 5. The number of butterfly eggs laid on host plants in relation to the host plant height and number of leaves (the circle area is proportional to the number of eggs laid).
Figure 1
Figure 3: Proportion of plants with eggs and total number of eggs over sampling days.
Figure 4