Egg laying by a butterfly on a fragmented host plant: a multi-level approach

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Egg placement by herbivorous insects is an important step in their interaction with their host plants, and is the result of processes operating at different spatial and temporal scales. Although several studies have examined egg-placement patterns at different scales, this has rarely been achieved simultaneously using a multi-scale hierarchical approach. We studied egg placement in a rare European butterfly, *Iolana iolas*, whose larvae specifically feed on seeds of plants of the genus *Colutea,* using a hierarchical approach and Generalised Linear Mixed Modelling. The study was carried out in 2002 and 2003 in a ca 60 km^2 area in southern Madrid province, Spain, where the host plant, *Colutea hispanica*, has a highly fragmented distribution. We monitored in detail 132 plants in 24 patches and estimated the abundance of butterflies over the whole reproductive period of *C. hispanica*. We measured phenological, morphological and landscape variables potentially affecting egg-placement at three hierarchical levels: fruit, plant and host plant patch. Using egg presence–absence on mature fruits as the response variable, we found that eggs were more likely to be laid on fruits aged 1–2 weeks at the middle of the flowering period (fruit level), on large plants with a small number of shoots at the base (plant level), and in well connected host plant patches (patch level). Our results suggest that egg-placement is a process determined by factors operating at different levels: fruit, plant and host plant patch. Because egg-placement studies are often made with spatially correlated data, neglecting their intrinsic hierarchical nature could lead to equivocal conclusions.

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Egg laying is one of the major processes involved in the ecology and evolution of interactions between insects and plants. In the case of the order Lepidoptera, females may exhibit preference and specificity for plant species, individual plants within populations, and different parts within plants (Thompson and Pellmyr 1991). Because the hatching larvae are often relatively immobile, their growth and survival depend on the choice of food plant by the adult female. Thus, egg laying is considered a major force in the evolution of behaviour in Lepidoptera (Renwick and Chew 1994, but see Rausher 1979).

Within a population of a single host plant species, female Lepidoptera are able to discriminate among plants due to different size (Forsberg 1987, Sparks et al. 1994), physiological condition (Bourn and Thomas 1993), flowering status (Wiklund and Åhrberg 1978, Courtney 1982), spatial position within the patch (Courtney and Courtney 1982, Dennis 1984), microhabitat (Rausher 1979, Thomas 1983, McKay 1991, Grundel et al. 1998, Roy and Thomas 2003), distance to nectar sources (Murphy 1983, Grossmueller and Lederhouse 1987), presence of conspecific eggs (Dempster 1992), and occurrence of mutualists (Jordano et al. 1992, Seufert and Fiedler 1996, Wagner and Kurina 1997, Van Dyck et al. 2000). Likewise, within a single plant, females may exhibit preference for

particular types of plant modules, such as leaves, stems, flowers or fruits (Thompson and Pellmyr 1991), or even for specific modules within a particular type (Williams 1981, Dennis 1984, Rodríguez et al. 1993, Ellis 2003).

However, most studies consider the determinants of egg laying in a single or at best a few sites and do not explicitly assess the effects of the landscape on egg laying. Frequently data from different populations are pooled for analyses or treated separately for each site (Bourn and Thomas 1993, Rodríguez et al. 1993, Sparks et al. 1994, Floater and Zalucki 2000). During the 1990s, it became increasingly clear that the plant-insect inter-actions within a site may partly depend on processes occuring at larger spatial scales (Tscharntke and Brandl 2004). This could be particularly important in those monophagous Lepidoptera species that feed on rare host plants with fragmented distributions consisting of small and isolated plant patches. In these cases, egg laying patterns could be the result of patch size, connectivity and landscape matrix as well as of local characteristics. Two theoretical approaches, metapopulation theory and landscape ecology, have underlain recent research on the occurrence of herbivorous insects in fragmented land-scapes (Tscharntke and Brandl 2004, see also Wiens 1997, Hanski 1999), including butterflies (Thomas and Hanski 1997). However, the consequences of habitat fragmentation on egg laying patterns remain almost unexplored. One exception is the study of Förare and Solbreck (1997), who found that egg density of a monophagous moth (*Abrostola asclepiadis*) decreased with patch size of its host plant, *Vincetoxicum hirundi-naria*. More recently, a study on the butterfly *Cupido minimus* has shown that eggs or a high number of adults were found on all 70 *Anthyllis vulneraria* patches in northern Germany, suggesting that area and isolation are not important on the occurrence of this species (Krauss et al. 2004).

Egg laying involves processes that occur at different spatial scales in a hierarchical fashion (e.g. selection of parts within plant, plants within host plant patches, patches within landscape). Nevertheless, empirical stu-dies have mostly focused on one particular level (plant, modules), or, if simultaneously more than one level was considered, they have rarely considered an explicit multiscale perspective (see above). Standard single-level modelling (e.g. plant parts over several plants, or individual plants over several populations) can pose problems of independence of data points due to spatial autocorrelation and the influence of predictors assigned to different levels (Bukley et al. 2003). To our knowledge, only Kéry et al. (2001), using hierarchical mixed model-ling, evaluated the determinants of presence–absence of *Maculinea rebeli* eggs on populations and genets of its rare host plant, *Gentiana cruciata*, and demonstrated that variables at genet level were more important than those at host plant patch level.

We focus on the egg laying patterns of the butterfly *Iolana iolas* Ochsenheimer (Lycaenidae) on its host plant in central Spain *Colutea hispanica* (Leguminosae). *Iolana iolas* is an extremely rare lycaenid in Europe that has very specific egg laying requirements: the females lay their eggs nearly exclusively in the calyx of fruits of plants of the genus *Colutea* (Munguira 1989, García-Villanueva et al. 1996). In central Spain, *I. iolas* mostly breeds on *C. hispanica*, which shows a neatly patchy structure in the landscape with small patches of only tens or up to a few hundred individuals. This plant-butterfly system conforms to a clearly hierarchical structure in space with three nested levels: fruits within plants, plants within patches and host plant patches in the landscape. Our main aim is to model the occurrence of *I. iolas* eggs on fruits considering potential explana-tory fruit characteristics within plants (such as flowering moment), plant attributes within host plant patches (size, flower and fruit production, and flowering duration), and patch features within the landscape (size and connectivity).

Methods

Study site

The study was conducted in 2002 and 2003 in a gypsum outcrop area located near Chinchón, ca 45 km southeast of Madrid, central Spain (centred on 40°08′N, 3°28′W; Fig. 1). The 60 km² study area is a northeast-southwest band of gypsum vegetation mostly covered by sparse perennial vegetation dominated by *Stipa tenacissima* tussocks with some remnants of Mediterranean wood-land with *Quercus coccifera* and *Quercus rotundifolia*. This area is sharply bounded to the south by extensive olive groves and to the north by irrigated fields where *C. hispanica* does not occur. The climate is semi-arid mesomediterranean with a mean annual temperature of 13.8°C and average annual rainfall of 428 mm (unpubl. data from Arganda del Rey thermopluviometric station, N = 16 yr). Mean daily maximum and minimum tem-peratures are, respectively, 11.9°C and 0.8°C in January, and 34.0°C and 13.3°C in July. There is a pronounced summer drought from May to September.

Study species

Iolana iolas occurs with a patchy distribution through-out southern Europe and Magreb (Tolman and Lewing-ton 1997). This species is classified as "endangered" in the Iberian Lepidoptera Red Data Book (Viedma and Gómez-Bustillo 1985), and included in the regional list of endangered flora and fauna of the Autonomous Community of Madrid as a "species sensitive to the alteration of its habitat" (Anon. 1992, see also Munguira
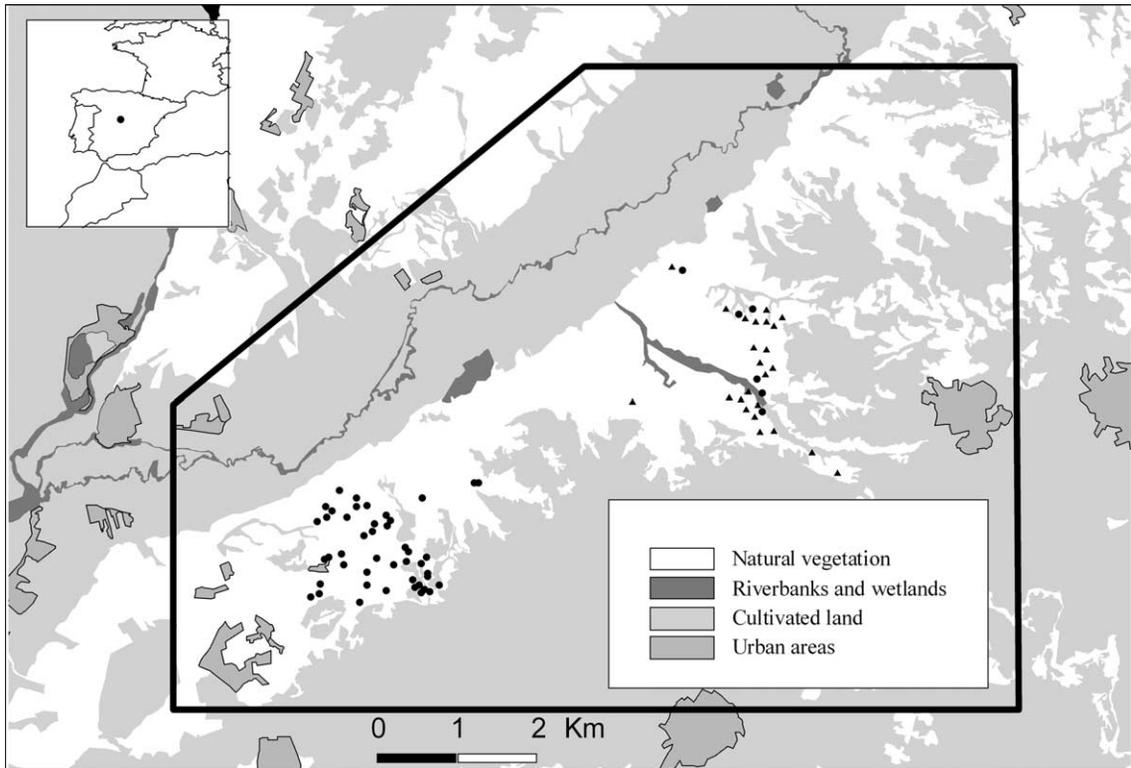
Fig. 1. Map of the study area depicting the different land covers in the study area in southern Madrid (Spain) where the 75 *C. hispanica* study patches were located. Black triangles indicate the 24 patches selected for intensive studies of egg laying and black dots the remaining populations. The bold line indicates the area comprehensively searched for *C. hispanica* patches. The study system was separated from the nearest known *C. hispanica* patch by ca 5 km.

and Martín 1993). *Iolana iolas* is a single-brooded species, flying from late April to early July (Munguira 1989). The larvae feed exclusively on developing seeds of *Colutea arborescens* and *C. hispanica* (Munguira and Martín 1993), and possibly *C. brevialata*. In our study area, adults daily move 98 m on average, with a maximum distance of 1792 m (median 29 m; SD = 192 m; n = 717; unpubl. mark-release-recapture data). This suggests that migration between the furthest points of the study area would be rare, and consequently, its size is large enough for metapopulation processes to occur (see Fig. 1). *Colutea hispanica* is a relatively rare shrub that occurs in limestone and gypsum soils (Talavera and Arista 1998), and is the only species of the genus in Madrid. This species is almost a complete summer semideciduous shrub, which reaches 2–3 m in height and flowers between April and June.

Host plant patch characteristics

During January–April 2003, we exhaustively mapped the distribution of *C. hispanica* in the study area using a GPS hand-held receiver and 1:5000 maps, and estimated plant population size by counting directly the total number of shrubs in each patch. Distinct patches were defined if their boundaries were separated by 40 m or more of terrain with no *C. hispanica*, or in a few cases, by a shorter distance if there was a distinct land cover change (e.g. cultivated land). For patches consisting of more than one plant, we entered the geographical position of the outermost plants of the patches in the GPS receiver, and exported their UTM coordinates to a GIS software package (X-Tools Extension in ArcView GIS, ver. 3.1 for Windows) to calculate areas and distances. In the case of patches consisting of only one plant, we calculated patch areas from field measures. Given that plant patches were relatively small compared to the geographical area covered by the study system, we estimated connectivities using distances among patch centroids. Connectivity for patch i (S_i) was defined as (Hanski 1994)

$$S_i = \Sigma p_j \exp(-\alpha d_{ij}) A_j^b, \qquad j \neq i \qquad (1)$$

where p_j equals 1 for occupied and 0 for empty patches, α is a constant setting the distance dependence of migration rate (the slope of the negative exponential dispersal kernel), d_{ij} is the distance between centroids of patches i and j, A_j is the area of each patch, and b scales how the per capita emigration rate declines with

increasing patch area. α is a species-specific parameter describing the dispersal ability of the species. We used a value of 1.5 on the basis of the comparison of *I. iolas* mobility with that of other butterfly species with associated α values (Cabeza 2003, Schneider 2003, James et al. 2003). Nevertheless, to test the robustness of analysis, we also calculated connectivities using values of α of 1 and 2.

Following previous studies, and in the absence of detailed data for *I. iolas*, parameter b was fixed at 0.5 based on the patch perimeter to area scaling (Hanski et al. 1994). To determine the occupancy status of *C. hispanica* patches (p_j) in 2003, we walked standard butterfly transects (Pollard and Yates 1993) in all patches between 3 and 6 June, during the peak of the flight period. In addition, we carried out extensive searches for *I. iolas* eggs in all patches during the same period, and for those patches in which no eggs were found, at the end of the flight period. Patches were judged unoccupied if neither eggs nor adults were detected in any of the surveys; otherwise, they were considered occupied. Connectivity measures were calculated considering all 75 patches found in the study area.

To study the phenology of adult *I. iolas*, we walked at each patch weekly standard butterfly transects of length approximately proportional to patch size. In the case of patches consisting of only one plant, where transects could not be walked, we stood in front of the plant searching for butterflies for one minute.

Plant and fruit characteristics

Out of the total of 75 patches of *C. hispanica* found in the study system, we selected a sample of 24 patches from the eastern half of the system covering a wide range of sizes and connectivities (Fig. 1). We selected 16 small patches in the range from 1 to 5 reproductive plants, 6 medium-sized patches from 5 to 100 plants, and 2 large patches with >100 plants.

In order to estimate the reproductive and phenological plant characteristics which could potentially affect egg occurrence on fruits, we marked all plants in the small patches, 10 plants in the medium patches, and 20 plants in the large patches, totalling 132 marked plants. For each marked plant, we measured its height and maximum crown diameter and counted the number of shoots at the base. Likewise, we counted the number of open flowers at weekly intervals during the entire flowering period and the number of mature fruits at the end of the flowering period. Flowers remain open for ca 1 week.

We considered the following independent variables for modelling: plant size (estimated as plant height multiplied by maximum plant diameter), number of shoots at the base, total fruit production, flowering duration

(estimated as the number of days elapsed between the first and last open flower), and flowering intensity (estimated as the number of flowers at the flowering peak).

We considered the time in the season at which a fruit was formed, the major variable that potentially could affect egg occurrence at fruit level. We structured this variable as the date when the fruit was an open flower (flowering moment henceforth), because it was an easily identifiable and short-time stage. To study flowering moment, we tagged a maximum of 10 flowers per plant at weekly intervals throughout the flowering season (from April to June) using a week-specific tag. This resulted in 10 census intervals and 7803 tagged flowers. For each census date, we examined the tagged flowers and fruits for *I. iolas* eggs, and additionally marked individually those fruits bearing one or more eggs. We estimated flowering moment as the difference in days between the date when the fruit was an open flower and 1 April. This date was a baseline for comparison because no flowers were open at that moment. Because we marked those fruits bearing eggs at each census date, we were able to determine whether a particular fruit received a new egg between two consecutive census dates. This allowed us to examine whether females oviposit on fruits of a specific age at one particular date. Fruit age at census i was the difference in weeks between the date at census i and the date at which the fruit was an open flower.

We collected all marked fruits in late June, immediately before fruit dispersal, giving a sample size per plant ranging from 1 to 40 fruits, depending on total fruit production. The collected fruits were placed separately in paper bags, and taken to the laboratory, where they were checked again for *I. iolas* eggs under a stereomicroscope. This allowed us to count the number of *I. iolas* eggs for each individual fruit.

To assess the inter-year variability in *I. iolas* egg occurrence, in June 2002 we collected a maximum of 50 fruits per plant from 40 plants immediately before fruit dispersal in the two largest patches (>100 plants) of the study system, and estimated the total fruit production for each plant. These 40 plants were the same individuals studied in 2003. Fruits were examined for eggs, in the same way as mentioned above.

Statistical analyses

To test whether *I. iolas* females disproportionately laid eggs on fruits of different ages at particular census dates, we classified fruit ages into three categories: 1 week, 2 weeks and >2 weeks old. We used 2×3 contingency tables in which each cell represents the number of fruits with or without recently laid eggs in a given age category to test whether the proportion of fruits bearing eggs

Table 1. Description of the independent variables used in the GLMM analyses for the probability of *I. iolas* eggs presence on host fruits in 2003.

Level	Variable	Description
Patch	Patch size	Number of plants
	Connectivity	Connectivity index (S_i, eq. 1)
Plant	Plant size	Product of plant height by maximum crown plant diameter (m^2)
	Number of shoots	Number of shoots at the base
	Flowering intensity	Number of flowers at peak of flowering period
	Flowering duration	Time elapsed between the first and last open flower (d)
	Fruit production	Total number of fruits at the end of the season
Fruit	Flowering moment	Difference between the date when the fruit was an open flower and 1 April (d)

changes with fruit age at each census date. Contingency tables were analysed using the G-test.

Our data on the occurrence of eggs on fruits had an unbalanced and hierarchical structure, with fruits nested within plants and plants nested within patches. Hierarchical data structure implies correlation between data points at different scales, inflating the error degrees of freedom and increasing the chance of making a Type I error. In order to overcome these problems, we analysed the data using a multilevel approach considering fruits, plants and patches as random factors and applying mixed modelling (Verbeke and Molenberghs 1997). Because the response variable was the presence–absence of eggs on a fruit, which clearly departs from the standard normality assumption, we used generalized linear mixed models (GLMM; McCullagh and Nelder 1989, Breslow and Clayton 1993). GLMMs for egg occurrence were performed assuming a binomial distribution of errors with logit link and using the restricted maximum-likelihood (REML) method (Patterson and Thompson 1971). Because our data were unbalanced, we used Satterthwaite's method to determine the approximate denominator degrees of freedom for tests (Verbeke and Molenberghs 1997).

In GLMMs, the response variable, which is a (link) function from the original data, is a linear combination of fixed and random effects (Littell et al. 1996). To analyse the effects at different levels on egg occurrence on fruits, we considered fruits within plants, plants within patches and patches as random factors (variance components), and flowering moment, plant size, number of shoots, flowering duration, flowering intensity, fruit production, patch size and connectivity as fixed effects (Table 1). Because we expected a potentially greater egg laying rate around the flight peak of *I. iolas*, we included in the model a quadratic term for flowering moment. Due to the complexity of the model structure and the relatively large number of potential explanatory variables, all possible combinations of interactions and polynomials could not be fitted and the model was kept relatively simple considering main effects only. Effects of random factors were tested using Wald Z-statistic tests, which are appropriate for large samples, and those of fixed factors were tested with F-tests, which

are preferable when the structure of the variance-covariance matrix depends on an unknown scale parameter (Littell et al. 1996). We first fitted an unconditional means model to examine the variation associated with each hierarchical level, fruit plant and patch. This model exclusively made up of random variables provided a baseline against we could compare a more complex model with fixed effects (Singer 1998). The unconditional means model expresses the probabilities of a fruit bearing an egg as the sum of the grand mean and random error terms associated with each level.

To test the inter-year variation in egg occurrence, we performed a GLMM with data from the two largest patches in 2002 and 2003. We consider fruits nested within plants, plants within patches, and patches as random factors, and year, plant size and number of shoots as fixed effects. All GLMM computations were performed using SAS Macro program GLIMMIX, which iteratively calls SAS Procedure Mixed until convergence (GLIMMIX ver. 8 for SAS/STAT; available on <http://www.sas.com.techsup/download/stat/ >).

Results

The flowering phenology of C. *hispanica* pooled for the 24 patches in 2003, lasted approximately from early April to mid-June, and shows a clear peak in mid-May (Fig. 2). The flight period of *I. iolas* in 2003 lasted barely two months and stretched from late April to mid-June. It showed a clear delay relative to the flowering phenology of C. *hispanica*. Based on butterfly transects and extensive egg searches, we found that 63 (85%) out of 75 C. *hispanica* patches were occupied by *I. iolas* in 2003.

Fruit age selection

Tests for fruit age selection were performed for census 7 (20 May), 8 (27 May), 9 (3 June) and 10 (10 June) because no recently laid eggs were observed on earlier dates. The proportion of fruits with eggs was relatively low for all age classes, ranging from 0 to ca 0.04 (Table 2). Nevertheless, the analyses of contingency
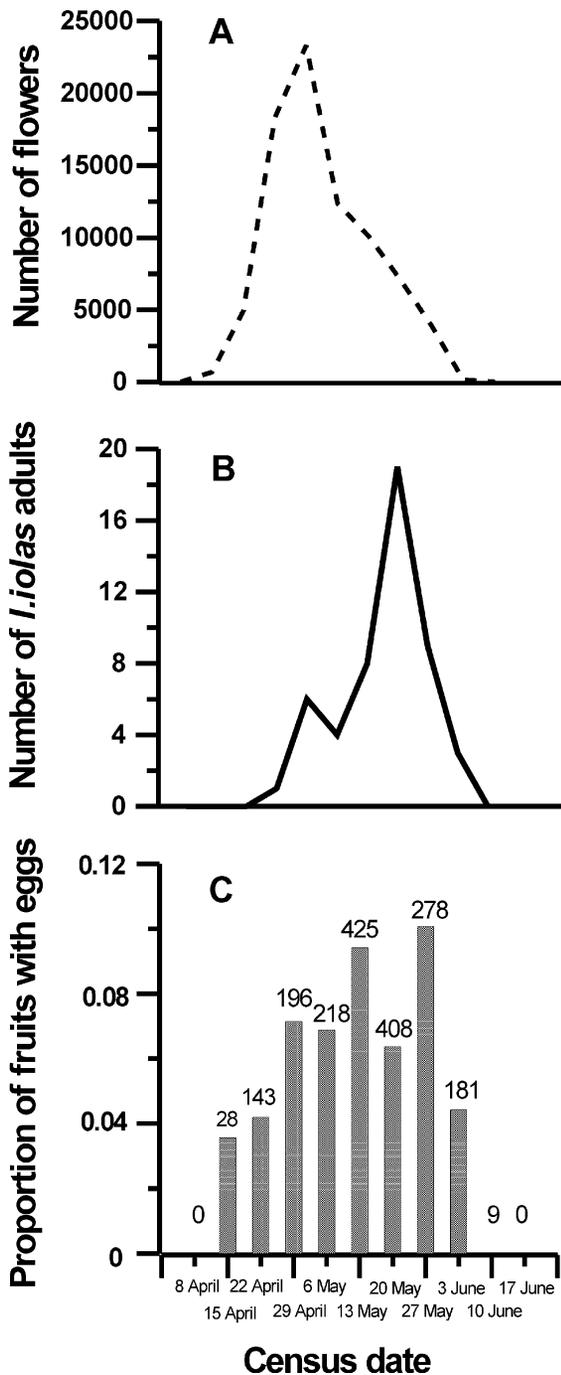
Fig. 2. Temporal variation in: a) the number of open flowers of *C. hispanica* (dashed line); b) the number of adult *I. iolas* (solid line); and c) the proportion of fruits with eggs coming from flowers opened at different dates in the season (figures on bars are the number of fruits at each date). (See also Table 1 and Methods for details.) Note that flowering moment indicates the date when the fruit was an open flower, not the date when eggs were laid. Plant data were from weekly counts for 132 plants, butterfly data from transects walked weekly, and egg incidence data from 1886 marked fruits from 122 plants. All data were collected in the 24 patches represented in Fig. 1 in 2003. Total transect length summed across all patches was 1210 m.

tables showed that there was a significant effect of fruit age on the proportion of fruits with eggs in all census dates; *I. iolas* females tended to lay eggs disproportionately on younger fruits (1–2 weeks) relative to older fruits (>2 weeks) over the four census dates.

Egg occurrence in relation to fruit, plant and patch characteristics

Because some data were incomplete for 10 plants, they were excluded from analyses, leaving 122 plants and 1886 mature fruits for performing GLMMs. Based on the butterfly transects and the extensive egg searches, we found that 22 (91.7%) out of 24 patches with marked plants were occupied in 2003. However, based on the detailed sampling of marked fruits, we found that marked plants contained eggs in only 20 of the 24 patches. The mean proportion of fruits per plant (±SE) that received *I. iolas* eggs was 0.07 ± 0.01, ranging from 0 to 0.4. Of the 138 fruits where eggs were laid, 124 bore only one egg, 13 two eggs, 1 three eggs, and no fruit contained more than three eggs. Nevertheless, the distribution of the number of *I. iolas* eggs per fruit did not differ from a Poisson distribution (Kolmogorov-Smirnov Test, $z = 0.206$, $p = 1.00$; $N = 1886$ fruits).

Table 3 presents the Spearman rank correlation coefficients for the variables considered as fixed factors at the plant level. All significant correlations were positive, with correlation coefficients ranging from 0.314 to 0.744. Only the number of shoots and flowering duration did not exhibit a significant correlation. At the patch level, the negative correlation between patch size and connectivity was only marginally significant ($r_s = -0.398$, $N = 24$, $p = 0.054$).

In the unconditional means model, hypothesis testing of variance estimates revealed that the three values significantly differed from zero (Table 4). The estimates suggest that there is a disproportionately higher variation among fruits within plants than among higher levels. The results from the GLMM showed that egg occurrence was related to fruit, plant and patch characteristics (Table 5). In the model including fixed variables, Wald-tests for residual variance components resulted in a significant effect among fruits within plants, among plants within patches, but not among patches. At fruit level, the probability of presence of *I. iolas* eggs showed a quadratic relationship with flowering moment (Table 5): fruits from flowers that were open around the middle of the flowering season (late May) were more likely to receive eggs than fruits from flowers than were open either early or late in the season (Fig. 2). At plant level, egg presence was positively related to plant size and negatively to the number of shoots. At patch level, the probability of egg presence increased with increasing connectivity. No significant effects were found for the

Table 2. Proportions of fruits with recently laid eggs in each age category, with sample size in brackets. Likelihood ratio is the value of the G-statistic for testing that fruits with eggs are distributed randomly with respect to fruit age.

Census (date)	Age category			DF	Likelihood ratio (G)	p
	1 week	2 weeks	>2 weeks			
7 (20 May)	0.031 (484)	0.039 (258)	0.024 (426)	2	10 171	0.006
8 (27 May)	0.013 (464)	0.019 (478)	0.003 (672)	2	7937	0.019
9 (3 June)	0.035 (310)	0.070 (460)	0.000 (927)	2	30 984	<0.001
10 (10 June)	0.021 (194)	0.039 (311)	0.012 (1606)	2	18 180	<0.001

remaining fixed variables. Using connectivity estimates based on a value of $\alpha = 1$, we found that this variable was a significant predictor of egg occurrence (p = 0.033). Using connectivity estimates based on a value of $\alpha = 2$, the effect on egg occurrence showed the same trend, but did not attain formal significance (p = 0.055). The effects of the other variables on egg occurrence did not change relative to those using $\alpha = 1.5$ in the connectivity estimates.

Inter-year variation in egg occurrence

We examined for *I. iolas* eggs a total of 2234 fruits from the 40 plants in two patches in 2002 and 2003. We found a significant effect of year on the probability of egg presence (Table 6). Eggs were more likely to be present on fruits collected in 2002 than in 2003 (proportion±SE of fruits with eggs, 0.15±0.01 in 2002 and 0.08±0.01 in 2003). No other variables contributed significantly to the model. We found that fruit production per plant was closely correlated between years 2002 and 2003 ($r_s = 0.732$, N = 122, p <0.001). Likewise, there was year-to-year variation in fruit production per plant. Plants in 2002 produced significantly less fruits than in 2003 (Wilcoxon Signed Ranks Test, Z = −0.543, p <0.001; mean±SE: 65.68±10.05 in 2002, and 208.15±23.54 in 2003).

Discussion

Incidence of *I. iolas* eggs on *C. hispanica* fruits was relatively low, which suggests that its impact on plant reproduction will probably be limited. Nevertheless, there was substantial variation between both study years, and we cannot rule out the possibility that, in particularly favourable years, egg incidence could be much greater. Longer-term studies have shown that fruit-feeding insects in particular undergo substantial variation from year to year due to strong resource fluctuations and weather changes (Solbreck and Sillén-Tullberg 1986, Solbreck 1995). In fact, there was a three-fold variation in fruit production of *C. hispanica* in the two largest patches between consecutive years, 2002 and 2003. The low fruit production in 2002 could be partly responsible for the small number of adults in 2003 and hence the low egg incidence in the same year. On the other hand, if there is no variation in butterfly population among these two years and the egg load is fixed, we would expect a lower incidence of fruits with eggs in the year with a higher production of fruits (2003). A paired-sample test showed that there was no significant difference in the total number of eggs per plant between 2002 and 2003 (mean number of eggs per plant 12.4 and 16.5 in 2002 and 2003, respectively, t = −1.37, p =0.179, N =40 plants), supporting the last hypothesis.

Analysis of egg distribution patterns revealed that most eggs were found singly on fruits, and the number of eggs did not exceed three per fruit. However, this pattern did not differ from a Poisson distribution. Thus, there appears to be no avoidance of conspecific eggs, and the pattern probably simply reflects the low egg incidence in 2003.

Fruit age selection

The *I. iolas* butterfly has an obvious preference for fruits aged 1–2 weeks. This pattern is in line with occasional oviposition sightings seen by us in the field. Although we do not have relevant data to support a specific hypothesis, this behaviour probably has an important adaptive significance. By selecting young fruits, females might avoid fruit and seeds that are too hard to be penetrated by the tiny young larvae. Likewise, this behaviour might

Table 3. Pairwise Spearman rank correlation coefficients between the independent variables at plant level shown in Table 1. **: p < 0.001. ns: non-significant. N =122 plants for all correlations.

	Number of shoots	Fruit production	Flowering intensity	Flowering duration
Plant size	0.548**	0.585**	0.652**	0.314**
Number of shoots	–	0.470**	0.416**	0.171 ns
Fruit production		–	0.744**	0.337**
Flowering intensity			–	0.458**

Table 4. Unconditional means model for the probability of presence of *I. iolas* eggs (1886 fruits from 122 plants in 24 patches). Analysis based on a GLMM with binomial errors and logit link using REML estimation. Random effects were tested using Wald tests.

Variable	Estimate ($\pm$SE)	Z-value	p
Patch	0.531 ± 0.292	1.82	0.034
Plant (patch)	0.319 ± 0.142	2.25	0.012
Fruit (plant)	0.774 ± 0.026	30.00	<0.001

avoid seeds that mature before the larva completes its development. In the last case, selecting young fruits might not be so important because last instar larvae can move among fruits within the same plant. Nevertheless, moving to other fruits carries with increased risk of predation, parasitism or even starvation if no other suitable fruit is located (Courtney 1982). On the other hand, selecting young flowers or buds for oviposition could carry an increased risk that flower does not set fruit, which invariably would drive offspring to fail. In *C. hispanica*, fruit set per plant is 0.28 ± 0.01 on average (mean $\pm$ SE, unpubl. data), which means that two thirds of flowers usually fail to become fruits. Thus, there are several important reasons for selection to restrict ovipositional choice to young fruits. This sort of time constraint also operates on other butterflies that feed on short-lived resources, for instance *Cyaniris semiargus* in Doñana in Spain. In this case, prediapause larvae must attain a minimum viable size to enter diapause before the dry season, and this is only succesfully achieved by feeding on late flowering heads in hygrophytic host plant patches (Rodríguez et al. 1993, 1994).

The preference of ovipositing females for fruits of 1–2 weeks in age could help to explain the phenological patterns shown in Fig. 2. Apparently, there is a time laying delay of the *I. iolas* flight period in relation to *C. hispanica* flowering period. However, this delay is approximately two to three weeks long, which is precisely the age of fruits selected by females for oviposition. Thus, this suggests that there actually was a tight synchronisation between the flight period of *I. iolas* and the phenology of fruits suitable for oviposition, at least in 2003.

Egg occurrence in relation to fruit, plant and patch characteristics

Our study involves a complex model that explicitly tests the spatial context of data at different nested levels (from smallest to largest scale): fruit, plant and patch. In terms of Kotliar and Wiens (1990), the hierarchy in the system is based on qualitative properties of resources because the contrast (i.e. the degree of difference between resource portions) is relatively high. In our system, the grain (i.e. the smallest scale at which the organism responds to resource structure, Kotliar and Wiens 1990) is organism-defined and is the fruit. In the unconditional means model, we found that the three variance components were significantly different from zero. These estimates suggest that patches, plants within patches and fruits within plants differed in *I. iolas* egg incidence. Fruit-to-fruit variation within plants was identified as the most important source of spatial variation in the probability of a fruit bearing eggs, whereas variation among plants within patches was relatively small, but significant (Table 4). These results support the use of mixed modelling for analysing egg incidence.

The inclusion of fixed potential predictors in the model diminished the variance components representing variation among patches and plants within patches, whereas the component for fruits remained virtually unchanged. In the case of patches, the residual variance component was not significant after including fixed variables. Nevertheless, we found significant predictors of egg occurrence at all three levels, supporting the importance of using a multilevel approach to appropriately analyse hierarchical data. Those predictors explained different fractions of the total variation within each level.

Table 5. Generalized linear mixed model for the probability of presence of *I. iolas* eggs (1886 fruits from 122 plants in 24 patches). Analyses were based on a GLMM with binomial errors and logit link using REML estimation. Random effects were tested with Wald tests and fixed effects with Type III F-tests. Variable descriptions are shown in Table 1.

Level	Variable	Estimate ($\pm$SE)	DF	Test-value	p
Patch	Patch size	-0.001 ± 0.002	1, 8.52	0.52	0.491
	Connectivity	0.882 ± 0.404	1, 17.6	4.77	0.043
	Residual patch variance component	0.356 ± 0.250		1.43	0.076
Plant	Plant size	0.312 ± 0.104	1, 118	9.01	0.003
	Number of shoots	-0.038 ± 0.015	1, 114	6.82	0.010
	Flowering intensity	-0.0001 ± 0.001	1, 95.6	0.01	0.925
	Flowering duration	0.020 ± 0.015	1, 103	1.83	0.179
	Fruit production	-0.001 ± 0.001	1, 92.4	1.50	0.224
	Residual plant (patch) variance component	0.304 ± 0.148		2.06	0.020
Fruit	Flowering moment	0.125 ± 0.048	1, 1872	6.78	0.009
	(Flowering moment)^2	-0.002 ± 0.001	1, 1871	7.53	0.006
	Residual fruit (plant) variance component	0.777 ± 0.026		30.00	<0.001

Table 6. Generalised linear mixed model for the temporal variation of probability of presence of *I. iolas* eggs (2234 fruits from 40 plants in two patches in 2002 and 2003). Analyses were based on a GLMM with binomial errors and logit link using REML estimation. Only fixed effects are shown and they were tested using Type III F-tests.

Variable	Estimate ($\pm$SE)	DF	F-value	p
Year (2002)	0.741 ± 0.158	1, 2207	21.91	<0.0001
Plant size	0.018 ± 0.067	1, 38.3	0.07	0.7923
Number of shoots	-0.017 ± 0.015	1, 45.7	1.28	0.2643

The results of the GLMM analyses showed that there was a unimodal relationship between egg presence and flowering moment (Fig. 2). Hence, fruits arising from flowers open in mid-late May 2003 were more likely to receive eggs than fruits coming from earlier or later dates. This pattern is likely the result of the higher density of adult *I. iolas* during that period, as well as of its short flight period, which restricts the duration of oviposition activity. Accordingly, we should expect a smaller incidence of seed predation by *I. iolas* at the beginning and the end of the flowering period.

The absence of conspecific egg avoidance behaviour, suggested that larger plants will receive larger numbers of eggs (Wiklund 1977, Mckay 1991, Porter 1992, Webb and Pullin 2000) because they are visually apparent. Our results showed a positive relationship between plant size and egg occurrence. However, this finding does not necessarily imply that females are choosing larger plants to oviposit. Ovipositing females could actually discriminate between hosts of different size, or simply oviposit on larger plants more often because they occupy a larger fraction of space. Larger plants have smaller perimeter-to-area ratios and this could result in a smaller probability of a female leaving a plant. In addition, although some potential cues for oviposition such as flowering intensity and fruit production were related to plant size, they were not significantly related to egg incidence. This suggests that visual attractiveness does not seem to be the reason to oviposit more frequently on larger *C. hispanica* plants. Another possibility is that larger plants are more likely at any time to contain fruits of an appropriate age for oviposition. In fact, we found that the coefficient of variation of flowering moment was significantly positive related to plant size, but not to fruit production (p = 0.006, multiple stepwise regression).

It has frequently been suggested that patch size is an important determinant of butterfly presence in highly fragmented landscapes since small patches are expected to have a higher risk of extinction (Hanski 1994, 1999, Thomas and Hanski 1997, James et al. 2003). Nevertheless, we did not find a significantly higher probability of egg presence on larger patches. Predictions have also been made for isolation among patches. According to metapopulation theory, isolation, the opposite of connectivity, limits the likelihood that an individual moves between patches and colonises empty patches (Hanski 1994, 1999, Thomas and Hanski 1997), and consequently, isolated patches have a lower probability of occupancy. This relationship depends on the dispersal capacity of the species relative to distance among patches. We found that the probability of egg occurrence on fruits increased with increasing connectivity. This could be the result of smaller probability of occupancy in less connected patches as well as of reinforced population size in better connected patches via rescue effect (Brown and Kodric-Brown 1977, see Hanski et al. 1994 for an empirical case). The longest distance from one empty patch to the nearest occupied patch was 1200 m, whereas the maximum observed distance for *I. iolas* was 1792 m (unpubl. data). This is in line with a system in which metapopulation processes are taking place. Thus, it is expected that some empty habitat will occur within the dispersal range of the species (Hanski 1999). Finally, is worth mentioning here that our results concerning connectivity effects were partly dependent on the α value used. However, the trend was the same in the three cases (α = 1, 1.5 and 2) and the slight changes in significance was probably the result of a smaller range in connectivity values when using higher values of α.

Our study illustrates that a multi-scale approach is needed for understanding the egg laying patterns of a rare butterfly. This overcomes the difficulties arising from traditional approaches and leads to a complete assessment of factors operating at different hierarchical levels. Consequently, the incidence of a herbivore in its host plant should not be viewed in isolation for each level, but using a hierarchical framework incorporating potential predictors and accounting for covariance within each level. Such an approach will facilitate the understanding of heterogeneity in the interaction between a herbivore and its host plant.

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