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## EGG DISTRIBUTION OF THE SOUTHERN FESTOON (*ZERYNTHIA POLYXENA*) (LEPIDOPTERA, PAPILIONIDAE)

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We investigated environmental factors grouped along hierarchical spatial scales influencing the egg density of a monophagous papilionid, the southern festoon (*Zerynthia polyxena*) on its food-plant, the birthwort (*Aristolochia clematitis*). Two patch level variables were considered: habitat type (black locust plantation, clearing and hummock) and food-plant patch size. We measured several plant variables at the egg-bearing shoots. We counted the number and measured the mean height of birthworts within the microenvironment of the egg-bearing shoots. We also measured the height and counted the leaves of each egg-bearing shoot itself. Plant apparency was defined as the height difference between egg-bearing shoots and the surrounding ones. Two variables were measured on the egg-bearing shoots (hence at food-plant scale): number of leaves and position of eggs on the leaf-storeys. Habitat type affected the distribution of eggs; black locust plantations and hummocks were preferred against clearings. At a smaller scale, neither shoot density, nor food-plant apparency affected egg distribution. At the plant level, the number of eggs increased with the number of leaves, and the position of eggs also co-varied with egg density, having fewer eggs in clusters positioned higher on shoots. We conclude that spatially correlated data on butterfly egg distribution should be analysed considering the intrinsically hierarchical structure of environmental factors.

Key words: *Zerynthia polyxena*, oviposition, plant–insect interaction, *Aristolochia clematitis*, food-plant, spatial scale

### INTRODUCTION

Egg-laying is a particularly important ecological interaction between phytophagous insects and their food-plants (RABASA *et al.* 2005), therefore, oviposition preference and larval performance are central topics in insect–plant biology (XUE *et al.* 2007). Female imagoes can discriminate among sites based on characteristics like climatic regimes, food quality and potential levels of competition and predation (BERNARDO 1996). Variation in these aspects of offspring environments affects offspring performance as well. In case of butterflies, egg-laying females may exhibit biased preferences toward particular plant species, toward particular plant

individuals and even toward certain parts of the food-plant, which will determine the physical and chemical attributes to which insects respond (THOMPSON & PELLMYR 1991, BERNAYS & CHAPMAN 1994). Since the emerging caterpillars are relatively immobile, the key to their survival and development is the food-plant choice of the female (PORTER 1992).

Beside experimental studies (e.g. SINGER *et al.* 1993), there are two widespread methods to study the egg-laying preferences of female butterflies. One of them is based on tracking the female imagoes and direct observation of oviposition (e.g. GRUNDEL *et al.* 1998, BERGMAN 1999, ZIMMERMANN *et al.* 2005, KÖRÖSI *et al.* 2008). The other method takes the presence-absence, or the distribution of observable eggs on food-plants (e.g. FLOATER & ZALUCKI 2000, ELLIS 2003, RABASA *et al.* 2005). This indirect method is more flexible allowing the researcher to plan the sampling design and to have a much larger sample size (depending on the visibility and identification ability of eggs). However, the evident disadvantage of the latter method compared to the first one is that it takes only the known host-plants into account and leaves out of consideration the acceptable non-host plant species (DE BOER & HANSON 1984).

Several studies have investigated the factors influencing egg distribution at different spatial scales. MCKAY (1991) studied the egg-laying requirements of Brimstone butterfly (*Gonepteryx rhamni*) in wet woodlands considering different food-plant-related spatial, physical and chemical factors, and found that most eggs were laid on juvenile host-plant trees growing in sunny sites. Further, butterflies appeared to prefer host-trees with low concentration of secondary compounds. Similarly, FLOATER and ZALUCKI (2000) explored the host-tree quality and apparency preferences of an Australian moth, *Ochrogaster lunifer*. They found more egg batches on high-quality trees in open homogeneous habitats, while in diverse mixed-species habitats, more egg batches were laid on low-quality highly apparent trees. In the case of Northern Brown Argus butterfly's (*Aricia artaxerxes*) oviposition, ELLIS (2003) described the effects of different food-plant characteristics, microenvironmental factors, such as food-plant versus bare ground cover, and sward height. The eggs of this butterfly were more common on younger and larger food-plant leaves and on unmanaged *vs* managed sites (shorter vegetation), while food-plant density and bare-ground cover did not affect oviposition. Furthermore, FARTMANN (2006) studied the effects of food-plant, microenvironment and microclimate on the egg distribution of the Duke of Burgundy Fritillary (*Hamearis lucina*). He found a lower deposition height of eggs on the host-plant (*Primula veris*) and that majority of egg clutches situated at sites that receive direct insolation between 09:00–17:00, and which have more than 60% herb cover. DENNIS (1996) studied the oviposition in *Zerynthia cretica* in relation to food-plant leaves, shoots

and patches. He found that females laid more eggs on large plant patches with large leaves, typically at the plant patch margin.

We found only two studies that focused on monophagous butterflies and used the presence-absence data of butterfly eggs in relation to factors at different spatial scales in hierarchical nested models. KÉRY *et al.* (2001) studied the presence of *Maculinea rebeli* eggs across *Gentiana cruciata* fruits, genets and populations, and found that the factors measured at the genet level were more important than those measured at population level. RABASA *et al.* (2005) used a similar nested design to investigate the egg presence of *Iolana iolas* on the *Colutea hispanica* shrubs at fruit, plant and patch levels. They showed important factors influencing the egg presence in each level.

In the present study we aimed to investigate hierarchically structured environmental factors influencing the density and distribution of *Z. polyxena* eggs in a black locust – poplar plantation complex, where the food-plant of this butterfly was abundant.

## MATERIALS AND METHODS

### *Study species*

The southern festoon (*Zerynthia polyxena* DENIS et SCHIFFERMÜLLER, 1775) is a papilionid species that reaches its northern range in Central Europe (TOLMAN 1997). The species is protected by law in Hungary. It is a monophagous species in Hungary feeding on a herbaceous plant, the birthwort (*Aristolochia clematitis* LINNEAUS, 1753, Aristolochiaceae). This food-plant is common on disturbed habitats, like flood plains, orchards, roadsides or black locust (*Robinia pseudoacacia* LINNEAUS, 1753, Fabaceae) and hybrid poplar (*Populus × euramericana*, Salicaceae) plantations. The flight period of the butterfly starts from mid/end April and lasts until the mid/end of May. The females oviposit on the abaxial surface of the food-plant leaves, laying either a single egg, or a smaller or larger cluster of eggs. Contrary to *Zerynthia rumina* LINNEAUS, 1758 which has two food-plant species (*Aristolochia baetica* LINNEAUS, 1753, Aristolochiaceae and *Aristolochia longa* LINNEAUS, 1753, Aristolochiaceae), the *Z. polyxena* is monophagous at least in Hungary; both eggs and larvae were observed only on *A. clematitis* (ROTHSCHILD *et al.* 1972, JORDANO & GOMARIZ 1994, ÖRVÖSSY *et al.* unpubl.)

### *Study area and sampling design*

The study area was situated on the Hungarian Great Plain near Csévharaszt (Central Hungary, 47°18'N, 19°26'E), in a landscape comprising of tree plantations, mainly black locust and poplar cultivar plantations interrupted by clearings. The 0.02–0.03 km<sup>2</sup> large plots were separated by hummocks originating from the last harvest of the plantations and consisted of stumps and roots covered by soil, providing an ideal place for the birthwort. A clump of food-plants consisting of at least five shoots per m<sup>2</sup> and separated by at least 10 m from other food-plants was considered as a patch. We chose 4–4 food-plant patches for sampling in the three available habitat types, i.e. in black locust

plantations, in clearings and in hummocks. In our earlier study on the same study area, we found that the imagoes avoided the poplar plantations (ÖRVÖSSY *et al.* 2005). Each of these food-plant patches were covered by several thousands of birthwort shoots. We randomly selected 10 points within each patch, where we checked food-plant shoots for eggs in a 5-meter radius circle. Since relatively few food-plant shoots had leaves loaded by eggs, we stopped further searching after finding the first shoot with eggs. These circles were covered by an average of 775 food-plant shoots. From the total of 120 randomly chosen circles, 98 contained eggs, and circles without eggs were excluded from the analyses. The flight period (26 April to 15 May) ended before the egg searching (17–23 May), thus new ovipositions during the sampling period could not cause a bias. No larvae were found during the egg survey.

During the egg-survey several environmental variables were measured in the close proximity of the egg-bearing shoots. We grouped these variables according to spatial scales. The habitat type of a given food-plant shoot (black locust plantation, clearing or hummock) was interpreted as a patch level variable, and incorporated into the model as a factor. To characterise the habitat types we counted the food-plant shoot density on 20 randomly selected 2×2 m squares in each food-plant patches. We measured the size of patches. To characterise the microenvironment of the selected food-plant shoots, we counted the number of shoots and measured the mean shoot height in a 1×1 m square. We also measured the height and counted the leaves of egg-bearing shoots. We expressed food-plant apparency as the height difference between an egg-bearing shoot and the mean of surrounding shoots in the 1×1 m square. On the egg-bearing shoots two variables were measured; the number of food-plant leaves and the position of the egg or the egg cluster (hence we call the scale of these variables as food-plant level to differentiate from the scale of variables measured in the direct proximity of the egg-bearing shoots, i.e. microenvironment level). The latter one was expressed as the number of leaves below the leaf bearing the egg(s) divided by the total number of leaves.

### *Statistical analysis*

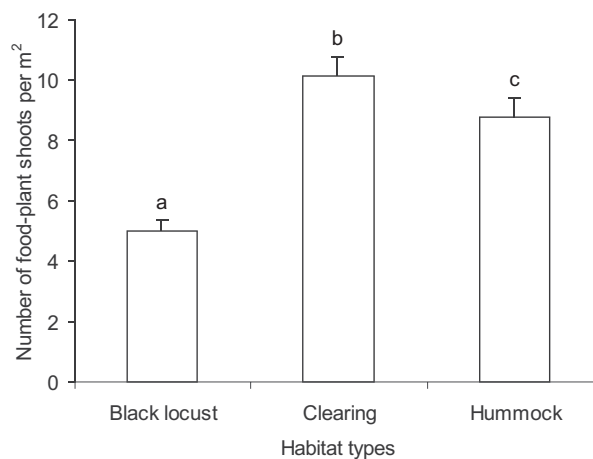
The effects of above mentioned environmental variables on egg distribution were analysed in general linear mixed-effects models with the Restricted Maximum Likelihood method. The normality of the distribution of eggs per leaf was assessed using normal quantile plots. Log-transformation was applied to handle non-normal distribution. The following non-correlated variables were considered in the statistical model: 1) habitat type as a factor and patch size as a co-variable at patch level; 2) at the microenvironment level, the food-plant shoot density around the egg-bearing shoot and apparency; 3) the number of food-plant leaves and the position of eggs at food-plant level. Since food-plants were nested within food-plant patches, the latter was used as a random factor. Although food-plant leaves were also nested within food-plants, we could not include food-plant as a random factor in the model, because it would have had too large effect relative to the residual. Altogether there were only five food-plant shoots with eggs on two leaves and one food-plant shoot with eggs on three leaves. Leaves without eggs (1036) were excluded from the analysis. Furthermore, we compared models with and without the inclusion of food-plant as a random factor. We found that the two models were significantly different (L-ratio = 59.8;  $P < 0.0001$ ), and the model without food-plant had smaller AIC value, indicating that this model was more supported. By right of these, we decided to apply the latter model. The calculations were made using R (version 2.2.1; R DEVELOPMENT CORE TEAM 2006) and the *nlme* package for R (version 3.1, PINHEIRO *et al.* 2007).

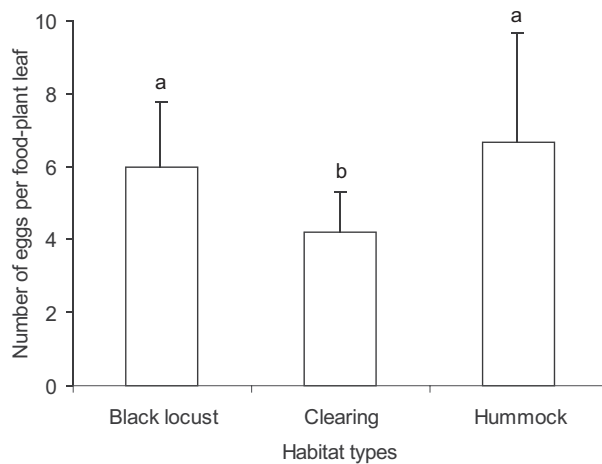
**Table 1.** Linear mixed models for testing the effects determining the egg-laying preference of *Z. polyxena* at different levels. Bold p values indicate significant effects.

Level	Variable	numerator DF	denomi- nator DF	F	P
Patch	Habitat type	2	8	15.777	<b>0.002</b>
Patch	Food-plant patch size	1	8	0.442	0.525
Microenvironment	Food-plant density	1	89	1.347	0.249
Microenvironment	Food-plant apparency	1	89	1.219	0.273
Food-plant	Number of food-plant leaves/shoot	1	89	5.668	<b>0.019</b>
Food-plant	Egg place	1	89	28.657	<b>&lt; 0.001</b>

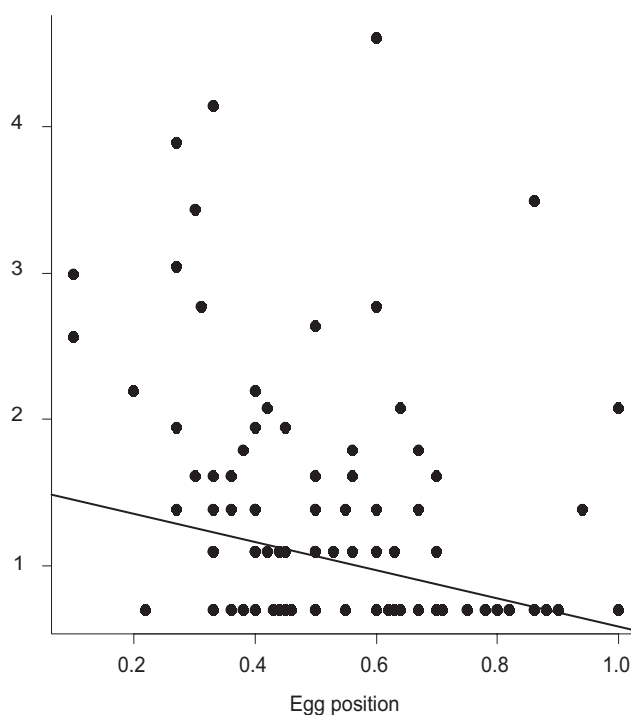
## RESULTS

We registered 597 eggs of *Z. polyxena* laid either singly, or in small (2–8 eggs) or large (10–99 eggs) clusters on the underside of food-plant leaves. During the survey we did not find any larvae nor hatched eggshells. The habitat type significantly affected the distribution of eggs (Table 1). When we compared the food-plant shoot density between habitat types with a one-way ANOVA, we found significant difference between them ( $F = 23.207$ ,  $P < 0.001$ ,  $N = 240$ , Fig. 1, Tukey HSD post-hoc tests showed that all habitat types differed significantly from each other regarding food-plant shoot density). There were significantly more eggs on food-plants in black locust plantations and hummocks than in clearings (Fig. 2). At the microenvironment level, neither food-plant shoot density, nor food-plant appar-

**Fig. 1.** Mean ( $\pm$ SE) number of food-plant shoots per  $m^2$  in the three habitat types. The different letters indicate significant differences at  $P < 0.05$



**Fig. 2.** Mean ( $\pm$ SE) number of eggs of *Z. polyxena* per food-plant leaf in the three habitat types. The different letters indicate significant differences at  $P < 0.05$



**Fig. 3.** Egg position on the plant's leaf-storeys (the number of leaves below the leaf bearing the egg(s) divided by the total number of leaves) is plotted against log number of eggs. Smaller value of egg position indicates that the eggs are on lower leaves. The solid line represents the fix effect of egg position of the fitted model

ency affected the egg distribution (Table 1). At the food-plant level, the number of eggs increased significantly with the number of food-plant leaves/shoot (Table 1). Finally, the position of eggs also affected significantly the number of eggs; there were more eggs on the lower part of the food-plants than upward (Fig. 3).

## DISCUSSION

The behaviour leading to oviposition, i.e. selection of oviposition site, is a complex process, because successful oviposition greatly contributes to individual fitness (SCHOWALTER 2006). However, females do not always select the most appropriate host and newly hatched larvae may reject the plant on which they hatch (BERNAYS & CHAPMAN 1994). So egg-laying females can make errors and larvae can correct it to some degree, but if they are to survive, i.e. to maximise their fitness, the females' preference and the larvae's performance should overlap as much as possible. In the current study, the egg distribution of *Z. polyxena* was affected on two levels, basically by habitat type and also by characteristics at food-plant level, but not by factors at the microenvironment level.

The habitat differences found in the present study, i.e. the black locust plantation and hummocks were more preferred than clearings, may well be interpreted as the butterflies' preference against areas that were more exposed to direct sunshine or cold. FARTMANN (2006) reported that *H. lucina* preferred those sites for oviposition in calcareous grasslands, where the food-plant (*Primula veris*) potentially received direct insolation between 09:00 and 17:00. MCKAY (1991) found that most eggs of *G. rhamni* were laid on isolated juvenile trees (*Frangula alnus*) growing at sunny sites. These studies show that oviposition site preferences may ensure an optimal microclimate for the development of the larvae.

In our case, patch size did not affect the number of eggs laid, while in the case of *Z. cretica* DENNIS (1996) showed that larger food-plant patches had more eggs. However, we have to call the attention to the fact that in the latter study the patches were mapped on a much finer scale (food-plant patch size range: 25 to 2400 cm<sup>2</sup>) than in our case (food-plant patch size range: 661 to 11954 m<sup>2</sup>, mean: 3384 m<sup>2</sup>).

At the microenvironment level, neither food-plant shoot density, nor food-plant apparency seemed to act as a limiting factor on egg density. However, we have to mention that food-plant shoots occurred in a very large number in each habitat. Moreover, food-plant shoot density was highest in the clearings, generally two times higher than in the black locust plantation and about 15% higher than in the hummocks (Fig. 1). This also suggests that the habitat type effect is not connected with the food-plant availability. In contrast to our results, DENNIS (1996)

found in his fine scale study that food-plant shoot number was one of the most important factors affecting the egg density of *Z. cretica*. Regarding food-plant apparency, the height of egg-bearing shoots were significantly higher than those in the microenvironment (paired t-test,  $t = 8.956$ ,  $P < 0.001$ ), however, it probably influences only the food-plant selection of butterflies, but not the egg load.

The number of food-plant shoot leaves showed a positive effect on egg numbers and more eggs were found on the lower leaves of food-plant shoots. In our earlier study, we observed that at the beginning of flight period the food-plants were quite scarce and just started to develop, therefore, the first females had limited choices (ÖRVÖSSY *et al.* 2005). This probably means that early sprouting food-plants have some advantage compared to the late sprouting ones, therefore, they could be more apparent. Since food-plant shoots receive eggs at a younger stage, the eggs are situated on lower leaves. Moreover, at this period the females have large egg load, which could also cause that they lay eggs in clusters rather than single eggs. This result suggests that temporal aspects are also important in oviposition. DENNIS (1996) also emphasized the importance of the duration of food-plant patches; he suspected that food-plant patches available for longer periods are more likely to have more larvae. Investigating a congeneric species, *Z. rumina*, JORDANO & GOMARIZ (1994) found that the freshly hatched larvae consumed the younger and softer leaves of the food-plants. This could stand behind that the observed *Z. polyxena* females also laid the eggs on young leaves, however, with egg searching it was not possible to investigate the temporal effects. Another potential explanation for the young leaf selection could be that females select these because of lower concentrations of some defence chemicals. However, ROTHSCHILD *et al.* (1972) described that *Z. polyxena* contained and stored efficiently two aristolochic acids which were presumably present in its food-plant, in *A. clematidis*.

In this short study we showed that the egg distribution of *Z. polyxena* was affected by several components acting at different spatial levels. We have to emphasise that such a complex process, like oviposition, should also be investigated by the direct tracking of females, which could illuminate further aspects of egg distribution. As a conclusion, we also underline that spatially correlated data in egg distribution studies need to be analysed considering an intrinsically hierarchical structure of environmental factors (RABASA *et al.* 2005).

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