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Oviposition in Zerynthia cretica (Rebel, 1904) : loading on leaves, shoots and plant patches (Lepidoptera, Papilionidae)

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Summary

Zerynthia cretica females tend not to lay eggs on small plant patches with small leaves, unless there are few or distant neighbouring plants. On plants chosen for oviposition, females lay proportionately more eggs on large plant patches with large leaves, typically at the plant patch margin, and tend to load eggs on fewer than the expected number of shoots and leaves. In *Z. cretica*, egg loading on plants may be facilitated by variable clutch size and the tendency of females to add to egg batches laid by other females on the same leaves and shoots ; direct observations are required on both these aspects of behaviour. Some of the potential consequences of egg loading on hostplant patches for the population dynamics of the insect are discussed, particularly the influences of drought and parasitism.

Résumé

Les femelles de Zerynthia cretica ont tendance à ne pas pondre leurs œufs sur de petites touffes de plantes avec de petites feuilles, à moins que les plantes des alentours soient peu nombreuses ou éloignées. Sur les plantes choisies pour la ponte, les femelles pondent proportionnellement plus d'œufs sur les grosses touffes de plante avec de grandes feuilles, typiquement à la limite de la touffe de plante. Pour la femelle de Z. cretica, la ponte des œufs sur la plante peut être facilitée par la dimension variable des touffes et la penchant des femelles à ajouter leurs œufs aux paquets pondus par d'autres femelles sur les mêmes feuilles et pousses ; il faut arriver à des observations directes sur ces deux aspects du comportement. L'auteur discute des conséquences possibles de la ponte des œufs sur les touffes de la plante nourricière pour la dynamique de population de Z. cretica, en particulier les influences de la sécheresse et du parasitisme.

Zusammenfassung

Zerynthia cretica-Weibchen legen Eier tendenziell nicht auf kleine Gruppen kleinblättriger Pflanzen, außer wenn es insgesamt nur wenige oder nur weit entfernte Nachbarpflanzen gibt. Auf den für die Ablage ausgewählten Pflanzen legen die Weibchen verhältnismäßig mehr Eier auf große Gruppen großblättriger Pflanzen, insbesondere am Rand der Gruppen, und sie neigen dazu die Eier auf weniger Triebe und Blätter zu legen, als zu erwarten wäre. Bei Z. cretica wird eine Anhäufung von Eiern auf einzelnen Pflanzen möglicherweise dadurch gefördert, daß die Gelegegröße variabel ist und daß Weibchen neue Eier zu bereits vorhandenen Gelegen hinzufügen. Direkte Beobachtungen beider Verhaltensaspekte sind wünschenswert. Einige der potentiellen Konsequenzen des Ablageverhaltens auf Pflanzengruppen für die Populationsdynamik des Insekts werden diskutiert, insbesondere der Einfluß von Trockenheit und Parasitismus.

Introduction

In Crete, the endemic butterfly Zerynthia cretica oviposits on the endemic plant Aristolochia cretica (Lam) (Aristolochiaceae). Two other Aristolochia species are present in the island group (Aristolochia sempervirens L. and A. parvifolia Sm.) but the butterfly only occurs near A. cretica (L. Chilton, pers. comm.). A. cretica occurs in two distinct habitats: (i) by shady, cool limestone and dolomitic rocks, typically with a northern aspect or screened by the slopes of cliffs and gorges; (ii) on dry soil under cultivated olives. Z. cretica occurs in both habitats in southern Crete, but in the vicinity of Plakias is more commonly associated with the former habitat (N = 5) than the latter (N = 1). A. cretica is also on the island of Karpathos, but Z. cretica has not been recorded there. Elsewhere, close relatives of Z. cretica use other Aristolochia species. On Rhodes, the supposed sibling species to Z. cretica, Zervnthia cerisy (Godart) must use one or both of Aristolochia guichardii (Davis & Khan) or A. parvifolia (Sm.) (Olivier, 1993). Between April 11-23, 1990, some data were collected on egg distributions of Z. cretica on A. cretica plant patches at Plakias in southern Crete. During that period, few adults were still evident, but the flight season was nearing an end. Thus, the bulk of the eggs had been laid and observations were restricted to determining egg distributions on the hostplant.

Study area and methods

Observations were made in a small area $(70 \times 40m;$ Fig. 1) on Kakomouri headland, Plakias. The area was mapped, divided into a



Fig. 1. Study site on Kakomouri headland, Plakias, for Zerynthia cretica. The hostplant, Aristolochia cretica, is also found on the surrounding cliffs.

number of topographically distinct zones and the number of Aristolochia cretica plant patches enumerated. Some 110 plant patches were identified in 11 zones. Of these, 33 patches (30%) marked for measurement, were selected so as to represent an unbiased sample of the plants accessible for survey on this part of the headland. The mean aspect of the plant patches was north-north-west $(339^\circ \pm 29^\circ)$. A number of measurements were made on each plant patch : maximum length and breadth (PATCH SIZE = length × breadth, cm^2); maximum patch height (PLANT HEIGHT, cm); the number of shoots (SHOOT NUMBER); the number of leaves (LEAF NUMBER, those on large plant patches were estimated from a sample of shoots along the axes taken for plant patch length and breadth); the maximum leaf size (LEAF SIZE, $= \pi \frac{1}{2} \frac{l}{2} b \text{ cm}^2$, where l and b are respectively the length and maximum breadth of the largest leaf); the mean distance to the three nearest neighbouring patches (MEAN DISTANCE, cm) and the number of patches within a 5m radius (DENSITY). The degree of exposure to sunlight (EXPOSURE, length of time, h) and the toughness of leaves (LEAF TOUGHNESS) of each plant were determined on a three and four-point ranked scale respectively. Each shoot and leaf was examined for the number of juvenile Z. cretica (eggs, cases, larvae),

providing data on the number of Z. cretica for individual leaves, shoots and plant patches. Also observed were egg colour, the position of eggs on each shoot (leaf number from shoot tip) and the position of eggs on leaves (upper- or undersurface).

The distributions of all variables were tested for normality (Kolmogorov Smirnov test ; Shapiro-Wilks' W test) and transformed as necessary. Some variables could not be normalised (EXPOSURE, LEAF TOUGH-NESS) and thus distribution-free tests were generally used. Correlations are Spearman's r_s . Multiple regression applied stepwise entry criteria controlled by significant F values and limited to four variables in any analysis. The data reduction technique applied principal factors analysis with VARIMAX orthogonal rotation of the factor axes (Nie *et al.*, 1975).

Results

Between April 11-23, all juvenile stages of Z. cretica were found from recently laid eggs up to 4th instar larvae. Adults were sparse. In the previous year, the peak adult flight period occurred in late March (Dennis, pers. obs.). The eggs are large, spherical and prominent on the hostplant (see Hensle, 1993). At first, they are yellow, but become brown. On hatching, the larvae do not consume the egg case, which remains attached to the plant (Fig. 2). Larvae typically eat a hole through the leaf to produce characteristic feeding damage (Fig. 2) to continue feeding on the upper surface. A number of larvae can occur exposed on the hostplant simultaneously and are highly apparent (Fig. 3) though there is no obvious gregarious behaviour as in the case of *Pieris brassicae* L. (Pieridae) or *Aglais urticae* L. (Nymphalidae) (see Dennis, 1992 : 119). Defence mechanisms include rows of spines and an orange forked osmaterium (Fig. 4).

Some 4696 leaves were examined. Of these, 4353 leaves had no eggs and 343 had one egg or more (mean, 0.125; variance 0.154). The distribution of eggs on leaves has been compared with a poisson distribution; this reveals that there is a significant excess of leaves without eggs (observed 4353 vs expected 4142) and with two eggs or more (observed 133 vs expected 37) and a deficit of leaves with just one egg (observed 210 vs expected 517) ($\chi^2_{(2)} = 442.1$, P < 0.001). As there is a possibility that these results may be biased by including data from plant patches which received no eggs at all, the comparison was repeated excluding leaves from eight patches apparently lacking oviposition events. The previous observation was confirmed ($\chi^2_{(2)} = 437.7$, P < 0.001). As many as nine eggs were found on a single



Fig. 2. Five egg cases of Zerynthia cretica and early larval feeding damage on a leaf of Aristolochia cretica.

leaf, but it was evident that when large numbers (N > 3) were found on leaves, they were probably the result of separate oviposition events by the same female on different occasions or by different females : (i) varying egg colour, cases and larvae indicated that they had been laid at different times, days if not weeks apart ; (ii) eggs also occurred, loosely, in separate "batches", occasionally on different leaf surfaces. However, as it is possible that eggs of the same or different colour could have been laid by different females and that eggs laid apart on the same leaf could have been laid by the same female at much the same time, it is not possible to make an accurate assessment of "brood" size, not without following females and directly observing oviposition events.

The vast majority of eggs (N = 586) were laid on the underside of leaves (90%); a small fraction were laid on the upper surface of leaves



Fig. 3. Apparency of *Zerynthia cretica* larvae on the hostplant : loading of individuals on a hostplant patch, exposed feeding and thermoregulation on the leaf upper surfaces, obvious feeding damage of leaves and frass accumulation.

(9%), and occasionally eggs were found on stems and on adjacent plants (1%). Even the test difference between leaf surfaces for leaves with eggs, rather than for number of eggs, indicates that the leaf undersurface is highly favoured compared to the leaf uppersurface for oviposition ($\chi^2_{(1)} = 207.8$, P < 0.0001).

Oviposition on shoots reveals much the same bias as for individual leaves. Some 655 shoots were examined. Eggs were found on 217 shoots (mean 0.533; variance 0.856). Compared to a poisson distribution, significantly more shoots than expected had two leaves or more with eggs (observed 87 vs expected 65), and fewer shoots than expected had a single leaf with eggs (observed 130 vs expected 205) ($\chi^2_{(2)} = 56.0$, P < 0.001). This difference remained when the eight plant patches without oviposition records were excluded ($\chi^2_{(1)}$ 19.2, P < 0.001). Such biased loading of eggs on a smaller than expected number of shoots and leaves could be the result of female preference for any differences in host quality, but it may also be influenced by differences among shoots and leaves in accessibility to females. Moreover, any difference would be compounded by 'clutch' laying. Thus, it is notable, that on one circular-shaped hostplant patch, although central plants were not entirely avoided, the eggs were nevertheless loaded around the perimeter



Fig. 4. Mature larva of Zerynthia cretica, illustrating spinal defences and osmaterium.

of the patch. A comparison based on the pooled area of plants within three inner annuli to that in the outer annulus produced $\chi^2_{(1)} = 6.72$, P < 0.001). The distribution corresponds with a characteristic hostplant edge-effect (see Porter, 1992 : 69). There was no bias for aspect in egg distribution around this patch ($\chi^2_{(3)} = 2.41$, P > 0.3).

Hostplant patches surveyed varied in area from 25 cm² to 2400 cm², and comprise from one to 85 shoots. The nine variables measuring hostplant characteristics boil down into three factors using VARIMAX factor analysis, accounting jointly for some 81% of the total variance. The first factor, in particular, is dominated by the close relationship between the three patch size variables (39%), whereas the second and third factors focus on PLANT HEIGHT and LEAF SIZE, and LEAF TOUGHNESS respectively. PATCH SIZE, SHOOT NUMBER and LEAF NUMBER are very highly correlated (r_s minimum, 0.88, P < 0.001). These three patch size variables are not significantly

correlated with plant height (r, maximum, 0.33, P = 0.06), leaf size (r_s maximum, 0.32, P = 0.077) and leaf toughness (r_s maximum, 0.14, P = 0.42) but covary significantly with exposure to sunlight (r_s, 0.44) to 0.63, P = 0.011 to 0.001). Patches with taller plants tend to have larger leaves (r_s , 0.55, P < 0.001) and somewhat softer leaves (LEAF TOUGHNESS r_s , -0.31, P = 0.08) but are not greatly affected by degree of EXPOSURE to sunlight as measured in the study area (r,, -0.09, P = 0.63). Correlations between LEAF SIZE and LEAF TOUGHNESS (r_s , -0.17, P = 0.39), LEAF SIZE and EXPOSURE $(r_s, -0.26, P = 0.15)$ and between LEAF TOUGHNESS and EXPO-SURE (r_s , 0.45, P = 0.009) are as expected in sign, but only the latter relationship is significant. Altogether, hostplant patches that are less shaded from direct sunlight are larger and have more shoots and leaves, but tend to be slightly shorter and have smaller and tougher leaves. In this study area, there is a weak (though not significant) tendency for larger plant patches to have more distant neighbours (r_s, 0.26, P = 0.15), but all hostplant patches surveyed have at least one neighbour each within 5m radius, and the correlation with DENSITY is weaker still (r_s , -0.12, P = 0.49). There is also a tendency for hostplant patches with taller shoots (though not significantly with larger leaves) to be less isolated (PLANT HEIGHT with MEAN DISTANCE r_s, -0.38, P = 0.03; with DENSITY r_s 0.49, P = 0.003; LEAF SIZE with MEAN DISTANCE r_s , 0.16, P =0.38; with DENSITY r_s , -0.18, P = 0.32).

The data collected indicate that Z. cretica lays more eggs on larger plant patches (PATCH SIZE r_s , 0.69, P < 0.001) with more shoots (SHOOT NUMBER r_s , 0.63, P < 0.001) and leaves (LEAF NUMBER r_s , 0.65, P < 0.001). Plant patches with larger leaves also tend to receive more eggs (LEAF SIZE r_s , 0.37, P = 0.033), but hostplant shoot height (PLANT HEIGHT r_s , 0.24, p = 0.19), hostplant exposure to sunlight (EXPOSURE r_s -0.19, P = 0.29) and hostplant leaf condition (LEAF TOUGHNESS r_s , 0.06, P = 0.76) seem to have little influence on the number of eggs laid on hostplant patches. In part, this could be owing to the dominating influences of hostplant patch size. Egg numbers on hostplant patches are not greatly influenced by the number and distance of nearest neighbours (DENSITY r_s , -0.21, P = 0.23; MEAN DIS-TANCE r_s , 0.19, P = 0.29). The inference from the correlations is that more isolated patches receive more eggs, but this could be an artefact of larger patches tending to be more isolated in the survey area.

Applying multiple regression to the set of transformed (normalised) variables, some 43% of the variation in egg load can be explained

by the two variables, SHOOT NUMBER (one of three highly correlated patch size measures) and LEAF SIZE, that correlate significantly with numbers of eggs on plant patches (Table 1). These two variables are effectively the key variables (with highest loadings) on the first two factors of a VARIMAX factor analysis of the nine measurements on hostplant condition (see earlier). It is possible that some of the residual variation in egg numbers on hostplant patches is accounted for by the length of time that hostplant patches have been accessible to Z. cretica females, particularly as the butterfly had been on the wing for at least a month prior to the survey. Those hostplant patches available or accessible for oviposition for longer periods are more likely to have more larvae. It was found that in a simple linear regression of numbers of eggs on hostplant patches against hostplant patch size (SHOOT NUMBER or PATCH SIZE), hostplant patches with an excess of eggs tended to have larvae compared to hostplant patches with a deficit of eggs (Fisher exact test, P = 0.022). The total number of eggs on hostplant patches also correlates significantly with the percent of larvae on patches (r_s , 0.595, P = 0.001) as does the number of unhatched eggs with the number of larvae on hostplant patches with oviposition records (r_s , 0.635, P = 0.0007). In multivariate mode, the PERCENT OF LARVAE account for some 11% of the residual variation from the regression of numbers of eggs on hostplant patches against patch size (SHOOT NUMBER) and LEAF SIZE. When all three independent variables are entered simultaneously, then some 49% of the variation in the numbers of eggs on hostplant patches can minimally be "explained" (Table 1).

Table 1

Multiple regression parameters for the distribution of Zerynthia cretica on hostplant patches

Dependent variable : Number of Z. cretica
(i) Independent variables : NUMBER OF SHOOTS ; LEAF SIZE
$R = 0.68$; $R^2 = 0.46$; Adj $R^2 = 0.43$ F = 12.96; df 2,30; $P = 0.00009$; SE estimate = 0.50 Number of shoots, beta = 0.56, $P = 0.0003$ Leaf size, beta = 0.31, $P = 0.027$
(ii) Independent variables : NUMBER OF SHOOTS ; LEAF SIZE ; PERCENT LARVAE
$R = 0.73$; $R^2 = 0.54$; Adj $R^2 = 0.49$ F = 11.17; df 3,29; $P = 0.00005$; SE estimate = 0.47 Number of shoots, beta = 0.49, $P = 0.0008$ Leaf size, beta = 0.27, $P = 0.046$ Percent larvae, beta = 0.28, $P = 0.041$

Eight of the plant patches received no eggs at all. When coded as present or absent on hostplant patches, Z. cretica correlates significantly with the same set of variables as does the number of Z. cretica eggs (i.e., SHOOT NUMBER, Gamma 0.51; PATCH SIZE, Gamma 0.54; LEAF NUMBER, Gamma 0.50; z = 2.49 to 2.52, P < 0.01; LEAF SIZE, Gamma 0.52, z = 2.58, P = 0.009), but also correlates significantly with hostplant patch isolation measures (DENSITY, Gamma -0.48; MEAN DISTANCE, Gamma, 0.42, $z > \pm 2.09$, P < 0.04). The more apparent are hostplant patches and the fewer are neighbouring patches, the more likely it is that Z. cretica will lay eggs on those plant patches. Application of logistic regression (LOGIT model) indicates that some 29% of the variance in presence or absence of Z. cretica on hostplant patches can be accounted for by the size of hostplant patches (i.e., SHOOT NUMBER, LEAF SIZE) and isolation (i.e., MEAN DISTANCE) from neighbours. Whether plants are used as a resource or not may also depend on how long they have been available or accessible, but the percent of larvae on hostplant patches, which may indicate the length of time they have been available to females, accounts for little of the residual variance (r, 0.243, P = 0.17) and has little explanatory power (c. 6%).

Discussion

Observations from this brief survey indicate a significant loading on *Aristolochia cretica* hostplants by *Z. cretica* females, (i) on leaves; (ii) on shoots; and (iii) on the edges of patches. Egg loading on hostplant patches has several components in *Z. cretica*: (i) plant patch characteristics of size, quality and spacing that influence selectivity; (ii) indications of potential for variable clutch egg-laying by females that affords flexibility in their response to plant characteristics; and (iii) the possibility of the deposition of multiple batches by different females on the same hostplant patch.

Although there may be benefits to group living (e.g., aposematism; Sillén-Tullberg & Leimar, 1988; Guilford, 1988) of the kind noted in Z. cretica (see Fig. 3), egg loading has at least two potential consequences for the population dynamics of a butterfly such as Z. cretica, both tending towards density-dependent 'regulation' of the upper bound to population size. First, excessive egg loading on hostplant patches could result in defoliation of the host and force mature larvae to search for new hosts to complete development. Such searching behaviour could lead to increased mortality among Z. cretica larvae. This has been observed to occur in Moroccan Zerynthia rumina (L.) (S. P. Courtney,

pers. comm.). Secondly, concentrations of eggs and larvae may enhance host-location by parasitoids (Godfray, 1994).

Although large hostplant patches acquire significantly more eggs than small patches, this in itself does not lead to excessive loading of eggs on large patches compared to small patches. In fact, the correlation of hostplant patch egg :shoot ratio (Z. cretica eggs/shoot number) with patch size variables is low and negative (r, max, -0.148, P = 0.48) and, if anything, points to relatively more eggs being loaded on small hostplant patches. Some hostplant patches seem to have much higher egg :shoot ratios than others (range : 0.06 - 5.8), and larvae developing on these patches may be forced to search for complementary resources. The deterioration of hostplant quality (toughening of leaves) in the arid climate of southern Crete, as summer progresses, may also act as a further stimulus for larvae to seek out new resources. It is noteworthy, then, that Z. cretica females tend to avoid laying eggs on the smallest patches, but may be induced to do so when neighbouring plants are few or distant. The second consequence of egg loading on hostplant patches may well have more impact on Z. cretica located on large plant patches. This will depend on how parasitoids are cued into their Z. cretica hosts. The larger the hostplant patch, the more apparent will be both hostplant cues and host cues (direct and indirect) to parasitoids, especially as the numbers of Z. cretica increase much in proportion to patch size. There is the possibility that parasitism will be greater on large plant patches, since larger plants may be more likely (though not invariably) to have larvae at any time (r, min, 0.42, P = 0.014), and the presence of larvae could substantially enhance cues for parasitoids seeking early stadia (Fig. 3).

These potential outcomes of female behaviour are worth more detailed study, especially as they invoke questions about negative feedbacks associated with population size and density. Additional survey work is needed over a wider area to test the limited observations made here. The present survey was carried out, of necessity, in a small relatively accessible area. Thus, the effects of plant patch isolation have not been fully accounted ; for instance, more isolated plants may be more heavily burdened with egg loads regardless of patch size.

Although a substantial amount of the variance in the distribution of *Z. cretica* has been determined (49%), still much remains unaccounted. It is feasible that hostplant chemistry plays an important part in host choice; much as *Zerynthia polyxena* (D & S), slow flying *Zerynthia cretica* is probably a chemically defended aposematic butterfly (Brower, 1984). Other parameters (e.g. predation) may also be at work; eggs

and larvae may already have been lost before or during the survey dates and such mortality may be uneven over plant patches. Some eggs may have been lost from rainwash; a torrential storm occurred overnight midway through the survey. Also, without information on adult numbers, their distribution and movement, it is not possible to determine how egg distribution relates to population parameters or chance influences. Nevertheless, the present brief survey indicates that there is much to be gained from a more detailed examination of Z. *cretica* ecology and of the comparative biology of different Zerynthia species and populations which use a variety of hostplants in different terrains (De Freina, 1979; Hensle, 1993; Jordano & Gomariz, 1994). However, this work will need to incorporate data from directly tracing female behaviour.

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