SHORT COMMUNICATION

Larger clutches of chemically defended butterflies reduce egg mortality: evidence from Battus philenor

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Abstract. 1. Many toxic butterflies lay eggs in clusters and their eggs are aposematically coloured to warn predators. The pipevine swallowtail, Battus philenor (L.) (Papilionidae), is a specialist herbivore on plants in the genus Aristolochia, from which it sequesters toxic alkaloids (aristolochic acids, AAs). Eggs of this group of butterflies are laid singly or in clusters of different sizes, are aposematic and can possess AAs.

2. A field study was conducted during three consecutive summers in Tennessee where we manipulated the exposure of B. philenor eggs of different clutch sizes to predators to assess the defensive role of egg clustering.

3. It was found that larger egg clutches suffer less predation compared with small clutches. No relationship between clutch size and AA content in the eggs was detected. Crawling predators seem to play the most important role in egg mortality.

4. This study suggests that for toxic eggs there is a clear benefit in laying eggs in large clusters in areas where there are high levels of predator threat.

Key words. Aristolochic acid, Battus philenor, clutch size, egg predation.

Introduction

Oviposition strategies of butterflies and other insects vary broadly. Generally, studies examining oviposition behaviour focus on the location where clutches are laid by females and variation in egg clutch sizes (Clark & Faeth, 1998; Desouhant et al., 2000; Faraji et al., 2002). After encountering a host plant, a female must choose when, where, and how many eggs to oviposit. Female butterflies oviposit eggs either singly or in clusters with many eggs, and clutch size can vary within and among species (Stamp, 1980). Variation in clutch size is thought to be an adaptive mechanism to reduce larval mortality, thereby increasing females’ realised fecundity (Courtney, 1984; Fordyce & Nice, 2004). Aggregative feeding of larvae often occurs as a consequence of egg clustering. A number of hypotheses have been proposed to explain the evolution of egg clustering and the adaptive value of aggregative feeding (Stamp, 1980; Fordyce, 2005).

Many species of butterflies that lay eggs in clusters are unpalatable and aposematically coloured (Stamp, 1980; Sillén-Tullberg & Leimar, 1988; Hunter, 1991). It has been proposed that in chemically defended insects, grouping has the potential to increase the individual per capita survivorship of the prey, even though a group might be more apparent to predators (Clark & Faeth, 1998; Hunter, 2000). One explanation of this behaviour might be enhanced aposematism, because a group of prey that are aposematically coloured display a more conspicuous signal to predators compared with a single aposematic prey, thereby enhancing learning in predators (Sillén-Tullberg & Leimar, 1988; Gagliardo & Guilford, 1993).

The present study aims to further our understanding on the potential role clutch size might play for egg survival. By conducting field observations and experiments during three consecutive years we ask: (i) Do large egg clutches reduce the risk of predation in this toxic specialist herbivore? (ii) Broadly, what are the important invertebrate predators (crawling or flying) of B. philenor eggs?

Material and methods

Species description

The pipevine swallowtail, Battus philenor L., is a butterfly in the tribe Troidini (Papilionidae) that specialises on plants in the genus Aristolochia (Aristolochiaceae) (Racheli & Oliverio, 1993). Aristolochia contain toxic alkaloids called aristolochic...
acids (AAs) that serve as defences against most herbivores (Chen & Zhu, 1987). Battus philenor sequester these chemicals as larvae, rendering both larvae and adults chemically defended against many invertebrate and vertebrate predators (Fordyce, 2000; Sime et al., 2000). Eggs of B. philenor are bright orange, possess AAs on their surface, and are often laid in clusters (Sime et al., 2000). Although eggs are chemically defended, the highest mortality occurs during the egg and first instar larval stages, due in part to predation (Tatar, 1991; Fordyce & Nice, 2004). Clutch size can vary within and among populations (Fordyce, 2003; Fordyce & Nice, 2004). The average clutch size of B. philenor in Tennessee is 8.4 eggs per clutch ranging from 1 to 26 eggs per clutch (R. D. Dimarco, pers. obs.), whereas in Texas the average clutch size is 5 eggs/clutch with a range of 1–17 eggs/clutch (Fordyce & Nice, 2004), and in California is 13 eggs/clutch ranging from 1 to 86 eggs/clutch (Fordyce, 2003, 2005). Although B. philenor has been widely studied, there is a paucity of empirical studies on the relationship between clutch size of B. philenor and egg predation.

**Study area**

Norris Dam State Park is a 1634-ha park, located in east Tennessee (36°13′N, 84°5′W). Aristolochia macrophylla is the common host plant of B. philenor in this area. Oviposition by B. philenor in Tennessee begins in May and continues until September.

**Clutch size and egg predation experiment**

To assess if clutch size explains variation in mortality of B. philenor eggs, we conducted a field experiment over three consecutive summers (2009–2011) where clutch size and accessibility to predators was manipulated. Accessibility to predators was manipulated through the application of Tanglefoot pest barrier (Tanglefoot Company, Grand Rapids, Michigan) to the stem below each clutch. The application of Tanglefoot allowed us to elucidate the foraging strategy of important egg predators in this system. Hereafter, we refer to crawling predators as those that are effectively excluded by the application of Tanglefoot, and flying/jumping predators as those that could circumvent the sticky barrier. From extensive observations in the area and in our experiments, we found that velvet mites, Trombidium holosericeum (Trombiidiidae), are the most common generalist crawling predator of B. philenor eggs at our study site.

Egg clutches where obtained by confining individual, wild-caught B. philenor females in cages where portions of A. macrophylla stems were provided. Clutch sizes range from 1 to 26 eggs, the range observed for naturally laid clutch in the field at this location. Each stem with clutches of a variable size were placed back in the field attached to a branch of an A. macrophylla plant. In total, we had 54 clutches ranging from 2 to 26 eggs, and 20 singleton clutches without Tanglefoot, and 37 clutches and 16 singleton clutches with the addition of Tanglefoot. Clutches were assigned to the Tanglefoot and no Tanglefoot treatment haphazardly. Egg survival was estimated as the proportion of eggs that successfully hatched. The clutches were observed once a day until larvae hatched (on average 7 days after eggs were laid), and predation was recorded by looking at the characteristic predator damage on the eggs (i.e. a small hole in the egg chorion).

**Chemical analysis of egg’s aristolochic acid content**

To determine if AA content of B. philenor eggs varies depending on whether eggs were laid in large or small clutches, we analysed the AA content of individual eggs that were laid in the field in clutches of various sizes (n = 25 clutches). An individual egg from each clutch was dried under reduced pressure and weighted to the nearest 0.1 μg. AAs were extracted twice from each egg in 0.75 ml of 100% ethanol and sonicated once for 20 min at 50°C. The resultant extract from each egg was passed through a 0.45-μm filter and dried under reduced pressure. The residue was resuspended in 0.04 ml of 100% methanol and placed into a total recovery autosampler vials for high-performance liquid chromatography (HPLC) analysis. HPLC analyses were performed according to Dimarco et al. (2012).

**Statistical analysis**

Data on clutch size and egg survival were analysed using logistic regression in R 2.14.2 (R development core team 2012). The model included the presence or absence of the Tanglefoot barrier and log clutch size as factors and egg survival as the dependent variable. We also ran a linear regression to see if there was a relation between clutch size and AA content.

**Results**

**Clutch size and egg predation experiment**

The full model detected an effect of predator exclusion treatment on egg survival (z = −4.607, P < 0.001); however, the interaction between predator exclusion and clutch size was significant (z = 2.473, P = 0.013) and, thus, we ran two separate models to examine how clutch size might affect egg survival. For clutches where crawling predators were excluded, there was no evidence that clutch size explained the probability of egg survival (z = −0.513, P = 0.61). However, when crawling predators had access to clutches, clutch size affected the probability of egg survival (z = 5.792, P < 0.001) with larger clutches having a higher probability of survival (Fig. 1). The percentage of eggs eaten in the treatment where eggs were protected against crawling predators was 4.6% whereas the percentage of eggs eaten in the treatment when crawling predators were not excluded was 55.1%. Based on the parameter estimates of the model, we estimated that egg survival exceeds 50% when a clutch is larger than 6.9 eggs (SE = 0.16).
Chemical analysis of eggs

We failed to detect a relationship between clutch size and total AA content per egg \( F_{1,23} = 1.118, P = 0.301 \). We also failed to detect a relationship between clutch size and AA concentration per egg (µg of AAs/mg of dry weight) \( F_{1,23} = 0.206, P = 0.654 \).

Discussion

We found that, for *B. philenor*, egg predation decreased as a function of clutch size (Fig. 1). Further, we found that crawling predators, those that were successfully excluded by the Tanglefoot barrier, are the important predators in this system. Our results are consistent with the hypothesis that larger clutches frequently observed in toxic butterflies play a defensive role (Stamp, 1980). Although enhanced aposematism is frequently championed as the function of aggregation for chemically defended prey (Stamp, 1980; Sillén-Tullberg & Leimar, 1988; Hunter, 1991); the role vision plays for the important predators that were observed in our study is uncertain. For example, it is unclear what role enhanced aposematism might play against the velvet mite (*T. holosericeum*). One possible explanation for the lower incidence of predation on larger clutches might be satiation of the predator. However, this explanation is perhaps not likely given that our experimental clutches remained in the field for a long enough period of time (7 days on average) so there should have been ample opportunity for multiple predator encounters. Moreover, for many large clutches (with more than 20 eggs), only on average 20% of the eggs suffered predation, leaving most of the siblings untouched. We found no evidence that egg toxicity varies with clutch size. This suggests that larger clutches of toxic eggs might suffer less predation not because of increased toxicity associated with larger clutches, rather because predators reject larger clusters of unpalatable eggs after sampling few members of the clutch (Alatalo & Mappes, 1996; Hunter, 2000). More research is needed to elucidate the importance of these different factors in explaining the behaviour of egg predators.

The existence of variability in egg clutch sizes in different regions and species, and geographical variation in the benefit of being gregarious and toxic at the first instar larval stage among populations of *B. philenor* (Fordyce & Nice, 2004) suggests that the benefit of being in aggregation might vary. These differences in oviposition strategies could be affected by ecological factors (e.g. climate, presence of other species of Troidini), and likely maintained by variation in the types and abundance of local predators.

Our results suggest that predation rate is reduced as a function of increased clutch size, but this fact does not imply that maximising clutch size is the optimal egg-laying strategy. It is possible that egg clutch size might also affect other factors that can have detrimental effects, such as competition among larvae for food resources. However, evidence that larger clutches benefit toxic eggs can also be found in the fact that non-toxic species of Lepidoptera often lay eggs solitarily and rely on crypsis for defence (Hunter, 1991). In species with toxic eggs, it might be expected that under conditions of high levels of predator threat, larger clutches might be adaptive. The variation observed in clutch size within and between Troidini species, might be a response to different levels of predation risk. This study suggests that in toxic organisms, in some circumstances, egg clustering is an effective strategy to reduce predation risk. In areas where egg predation is common, as in our study site, there appears to be a clear defensive benefit in laying eggs in large clusters.

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**References**


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