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# BENEFITS OF EGG CLUSTERS IN THE EVOLUTION OF LARVAL AGGREGATION IN THE NEOTROPICAL BUTTERFLY *ASCIA MONUSTE ORSEIS*: REDUCTION OF EGG FAILURE AND ENHANCED LARVAL HATCHING

**Additional key words:** decision making, oviposition, hatching, cannibalism

The number of species of phytophagous lepidopterans that aggregate eggs during oviposition indicates that this behavior is rare in nature when compared to those laying single eggs: in North America, only 4.7% of species aggregate eggs (Stamp 1980). Egg clutch size is an important component of these species' life history because it reduces predation risk of newly hatched larvae (Stamp & Bowers 1988, Lawrence 1990) and maintains adequate temperature and relative humidity for proper egg (Clark & Faeth 1998) and larvae development (Stamp 1980, Willmer 1980). In addition, the egg desiccation hypothesis (Stamp 1980, Clark & Faeth 1998) proposes that aggregation of eggs is adaptive per se because it decreases mortality through increasing larvae hatching success.

Cannibalism is described as an important component of behavior in *A. monuste orseis* larval aggregations in the laboratory (Zago-Braga & Zucoloto 2004, Santana et al. 2011) and in the field (Barros-Bellanda & Zucoloto 2005). Despite the associated costs and benefits related to egg aggregation in this species—like biomass gain (Barros-Bellanda & Zucoloto 2001) and removal of potential competitors (Zago-Braga & Zucoloto 2004)—some implications for this behavior have yet to be identified. It has been shown, for example, that egg cannibalism may reduce reproductive potential (Fordyce 2005) for the parental female. Egg clustering can be a risky behavior for the parental female as well as for the deposited eggs. In fact, *A. monuste orseis* females avoid ovipositing on plants with conspecific larvae (Barros-Bellanda & Zucoloto 2005), because older caterpillars can cannibalize eggs (Zago-Braga & Zucoloto 2004, Barros-Bellanda & Zucoloto 2005). On the other hand, egg mortality in nature also happens due to factors like desiccation when abiotic conditions are variable or suboptimal.

Most hypotheses related to the adaptive significance of egg aggregation focus on the consequences observed in larvae, especially in first instars, when they show higher mortality (Stamp 1980, Kagata & Ohgushi 2002, Zalucki et al. 2002). As every larval aggregation is a result of an egg clutch in phytophagous insects (Stamp 1980), we aimed to evaluate whether there is a variation in the vulnerability of egg clutches of different sizes, based on egg cannibalism and egg failure data. It is hypothesized that eggs in larger clutches are more resistant to environmental effects and would show higher hatching rates than eggs in smaller clutches.

*Ascia monuste orseis* eggs were collected from kale (*Brassica oleracea* L. var. *acephala* D.C.) leaves in a pesticide-free garden, situated in the Biology Department of Faculdade de Filosofia, Ciencias e Letras de Ribeirao Preto, USP (FFCLRP / USP) (21°05'S, 47º50'W), Brazil. Leaves with eggs were taken to the laboratory and kept in glass jars (12 cm high × 8 cm diameter) to maintain leaf freshness until larval hatching. Newly hatched caterpillars were placed in plastic boxes (10 × 10 × 4 cm) and were fed with fresh kale taken from the host plant. Leaves were offered ad libitum and replaced daily. Boxes were kept in a climate-controlled chamber ELETROLAB® (93,5 × 50 × 51 cm): temperature: 29±1ºC, humidity: 75% e photoperiod: 10 light: 14 dark (Barros-Bellanda & Zucoloto 2005, Santana & Zucoloto 2011).

Twenty-four hours after hatching, a butterfly pair, raised in controlled abiotic conditions during the larval period, was placed in an aluminum cage, covered with white tulle, for reproduction. The cage was in an external greenhouse under semi-natural conditions, excluding predators and rain. Kale plants, that were approximately 2 months old, measuring 80cm high and grown in the same substrate, were available for ovipositions and a liquid diet of water and sugar (3:1). The plants were replaced every couple of days. Plants were also checked daily for the presence of eggs; once detected, eggs were separated for observation. Plants with eggs were also kept in the greenhouse under the same conditions.

As females deposited different numbers of eggs, we categorized the clutch sizes as: small (1–9 eggs); intermediate (10–19) and large (more than 20 eggs), according to the frequency of ovipositions distribution (data not shown). Average numbers of hatched larvae from small, intermediate and large clutches were 2.1, 11.5 and 22.6, respectively. Number of deposited eggs, failed eggs and the number of hatched larvae were recorded. Larvae fed from the same plant in which oviposition occurred. There was no manipulation of egg or larvae number to form treatment groups; the natural variation deposited by the female was maintained. The

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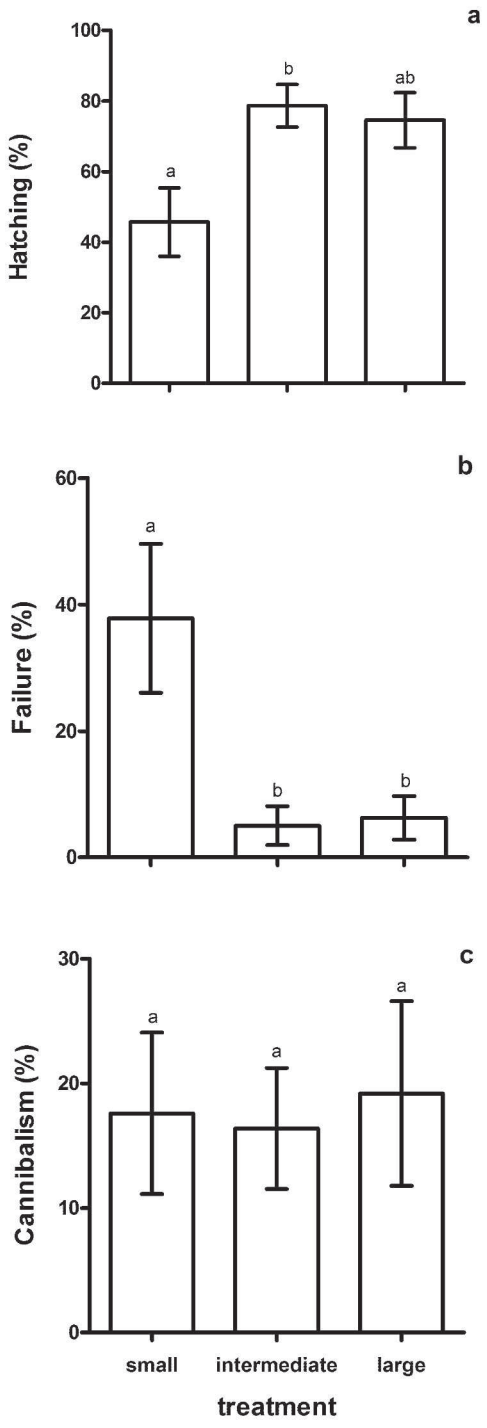



FIG. 1. Larval hatching rate (a), egg failure (b) and eggs/larvae cannibalism (c) (mean ± standard error) of *A. monuste orseis* in different clutch sizes. Small clutch: 1–9 eggs; intermediate: 10–19 eggs and large: more than 20 eggs. N=16 (small), N=14 (intermediate), N=11 (large). In each graph, different letters on the top of bars indicate significance difference among treatments. ANOVA, Tukey Multiple Tests, $P<0,05$.

parameters analyzed were: hatching rate (number of hatching larvae/number of eggs), cannibalism rate ([number of deposited eggs – number of hatching larvae]/number of deposited eggs) and failure rate (number of failed eggs/number of deposited eggs). Larvae mortality by failure and cannibalism were compared among treatments to check the relevance of each in the control of aggregation size.

Hatching, failure and cannibalism rates were compared by Kolmogorov-Smirnov normality tests with consideration given to the homoscedasticity of variances by Bartlett tests. Percentage data were transformed using the arcsin function. After transformation, the data were analyzed using analysis of variance (one factor), followed by Tukey multiple tests, using $\alpha = 0.05$. We used $\chi^2$ tests to analyze failure and cannibalism mortality of eggs and/or larvae for multiple comparison between treatments. To account for the consequences of making multiple comparisons, we used a Bonferroni correction for $\alpha = 0.01$.

Larval hatching rate was directly proportional to the increase of egg clutch size (ANOVA, Tukey Multiple Testes, $P=0.03$) (Fig. 1a), while egg failure decreased with increasing clutch size (ANOVA, Tukey Multiple Tests, $P=0.007$) (Fig. 1b). Although cannibalism rate did not differ among treatments (ANOVA, $P=0.93$) (Fig. 1c), it was a significant cause of mortality (Table 1), especially in intermediate and large clutches. In small clutches, failure was the main mortality cause (Table 1). These results support the idea that the evolution of larval aggregation in *A. monuste orseis* possibly occurred already in the egg phase through decreased egg failure and increased egg hatching in larger clutches.

Egg and newly hatched larvae cannibalism was detected in a similar rate in all clutch sizes tested, supporting the development of this behavior in this species. In previous work from Barros-Bellanda and Zucoloto (2005), the difference between cannibalism rates shown by larvae from different clutch sizes was meaningful only above 60 eggs per clutch. This supports the importance of cannibalism as a strong regulation agent in larger clusters.

Despite small clutches having disadvantages to *A. monuste orseis* survival, due to the higher failure rate in this group, they are found in nature. Female vulnerability to predators during oviposition and conspecific males (in the search for copulation) could explain the presence of smaller clutches. Frequently *A. monuste orseis* males were seen in the field interrupting female oviposition (A.F.K. Santana pers. obs.). These females would not necessarily return to the same place to deposit their remaining eggs, resulting in small clutches.

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TABLE 1. Relative rate of *A. monuste orseis* egg mortality in greenhouse by failure and by cannibalism in different treatments. Small clutch: 1–9 eggs; intermediate: 10–19 eggs and large: more than 20 eggs.

| Clutch size/mortality | N | by failure (%) | by cannibalism (%) |
|---|---|---|---|
| small | 16 | 59,52 [a] | 40,48 [a] |
| intermediate | 14 | 21,15 [b] | 78,85 [b] |
| large | 11 | 25,00 [b] | 75,00 [b] |
| Statistic | | $\chi^2$= 25,35; gl=2; p<0,0001 | $\chi^2$= 13,78; gl=2; p=0,001 |

Different letters in the same columm indicate significant difference between treatments. Mortality by failure: S vs I= 1,85; P<0,0001, S vs L=14,09; *P*=0,0002 e I vs L=0,32; *P*=0,57. Mortality by cannibalism: S vs I= 12,32; *P*=0,0004, S vs L=10,32; *P*=0,001 e I vs L=0,09; *P*=0,75. Binomial tests, $\alpha$ = 0,01.

While in some species [e.g. *Chlosyne lacinia* (Geyer)] relative humidity influences directly larval hatching rate (Clark & Faeth 1998)], in others [e.g. *Grammia geneura* Strecker (Arctiidae); *Manduca sexta* Johannson (Sphingidae)], hatching rates are high even when humidity is near zero (Woods & Singer 2001). If nymphalids are taken into consideration, a higher number of North American species aggregate eggs when compared to Neotropical and subtropical species (Stamp, 1980), suggesting that dry and/or cold weather favored the selection of this behavior. Even some Neotropical species, in which water conservation is not apparently a limiting factor for development, benefits for large clutches were also noticed for *A. monuste orseis*. Our data clearly demonstrated that benefits of larval aggregations already happen during the egg phase through the increase of larval hatching and the decrease of egg failure in larger clutches. In some insect species, oviposition in clutches may be a strategy to save time and energy searching for deposition places (Stamp 1980, Courtney 1984). Predation is considered a strong selective pressure not only for eggs (Dimarco & Fordyce, 2013), but also for females in reproductive period (Burger & Gochfeld 2001). Future studies considering the costs of large clutches (predation rates) are extremely important because they influence the decision making process of the female during oviposition period .

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