

Eat or be eaten: prevalence and impact of egg cannibalism on two-spot ladybirds, *Adalia bipunctata*

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Abstract

Ladybirds commonly engage in cannibalistic behaviour. Egg cannibalism by first instars is considered advantageous to the cannibal, because it not only results in direct metabolic gain but also a reduction in potential competitors. In this study, we quantified the effect of cannibalism on the development rate and survival of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) larvae through development to the adult stage. We also assessed the synchrony of egg-hatching in relation to laying order and compared the proportion of eggs cannibalized in egg batches laid as clusters or linearly. Larvae that had consumed a conspecific egg after hatching reached the adult stage 1.65 days earlier than those larvae that had not. Larval and pupal mortality was lower for cannibals compared to non-cannibals; only 46% of non-cannibalistic individuals reached the adult stage whereas 81% of cannibals pupated successfully. Egg cannibalism is undoubtedly advantageous to *A. bipunctata* larvae both in terms of faster development and increased survival. There is a positive correlation between laying and hatching order for eggs laid linearly or in a cluster. There was no significant difference in the proportion of eggs hatching in clusters or in a line (80 and 77%, respectively). The remaining eggs were either cannibalized or did not hatch. The ecological implications of these results are discussed with particular reference to trophic egg plasticity.

Introduction

Cannibalism, the partial or complete ingestion of a conspecific, is taxonomically widespread but for many species is a rare phenomenon (Pfennig et al., 1998). Coccinellids commonly engage in cannibalistic behaviour, consuming both sibling and non-sibling conspecifics (Hodek & Honěk, 1996). Such behaviour is evident at both adult and larval stages but most sibling cannibalism occurs before newly hatched larvae disperse from the egg clutch when the first instars cannibalize unhatched eggs from the same clutch (Majerus, 1994).

Cannibalism accrues important fitness benefits to the individual because cannibals gain a superior balance of nutrients for growth and maintenance compared to individuals that prey on heterospecifics (Crump, 1990; Majerus, 1994). Indeed, it has been reported that coccinellid larvae find conspecific eggs more palatable than those of other

species (Yasuda & Ohnuma, 1999). It is critical that a first instar finds prey rapidly after hatching and the inability to do so accounts for the high mortality observed at this stage (Wratten, 1976). Larvae that consume a sibling egg prior to leaving the egg clutch are more likely to survive the first instar compared to non-cannibalistic siblings (Banks, 1955). A recent study has demonstrated that harlequin ladybirds, *Harmonia axyridis*, increase the proportion of infertile eggs produced in response to low prey availability (Perry & Roitberg, 2005); a concept termed 'trophic egg plasticity'.

Ladybirds have few natural enemies because they contain species-specific toxins that make them unpalatable (Majerus, 1994), therefore, cannibalism plays an important role in structuring coccinellid and other communities (Mills, 1982; Polis & Holt, 1992; Fincke, 1994; Wagner & Wise, 1996; Wissinger et al., 1996). Many studies have noted the widespread occurrence of cannibalism in coccinellids but the effect of cannibalism on larval survival and development beyond the first instar has received little attention (Yasuda & Ohnuma, 1999). In this article, we consider the effect of egg cannibalism on the development

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rate and survival to adult of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) larvae. The high prevalence of egg cannibalism by newly hatched larvae would be a strong selection pressure for early hatching and so we consider whether the order in which eggs hatch is simply related to the order in which they are laid or whether other factors, such as position within the cluster, determine hatch order. Finally, we assess the prevalence of cannibalism.

Materials and methods

Pea aphids [*Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae)] were cultured on dwarf broad bean plants [*Vicia faba* L. cultivar The Sutton (Fabaceae)] at 20 ± 2 °C and an L16:D8 photoperiod. Adult apterous *A. pisum* were used in all the experiments.

Field-collected *A. bipunctata* adults were placed in a ventilated box (16 cm in diameter, 18 cm in height) and fed an excess of *A. pisum*. The ladybirds were fed daily and egg clusters were collected (laid on rose leaves) and placed in a 9-cm triple-vented Petri dish. Continuous observations were made at the onset of hatching as the eggs turned from yellow to grey. Larvae were left with the egg clutch for 2 h after hatching, and those that consumed a sibling egg were termed ‘cannibals’ and those that did not were termed ‘non-cannibals’. Excess mixed instar pea aphids were provided as an alternative food source for the newly emerged larvae but none were consumed during the 2-h post-hatching period. Each larva was transferred to a 9-cm Petri dish where it was kept individually at 20 ± 2 °C and an L16:D8 photoperiod, and fed an excess of pea aphids. The larvae were maintained under these conditions and observed daily until adult emergence. All mortality was recorded.

Effect of cannibalism on rate of development and survival to adult

In total, 26 cannibals and 26 non-cannibals were monitored for the following parameters: length of time required for completion of each instar measured in days (development time) and percentage cumulative survival (survival). Cannibals were defined as larvae consuming one sibling egg and non-cannibals were those that did not consume a sibling egg.

Assessment of the synchrony between laying and hatching of eggs

Individual laboratory-cultured mated *A. bipunctata* females were placed in 9-cm triple-vented Petri dishes and maintained at 20 ± 2 °C and an L16:D8 photoperiod with an excess of pea aphids. The oviposition behaviour of 17 females was continuously observed to provide the following details: order in which an egg was laid, pattern of oviposition (egg batches were classified as either linear,

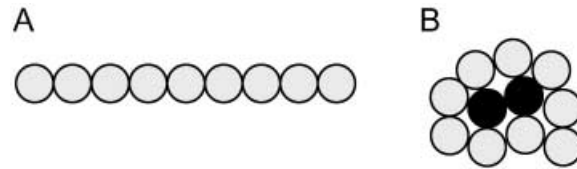


Figure 1 Typical arrangement of *Adalia bipunctata* egg batches laid in (A) a linear pattern, and (B) a cluster. The eggs depicted in black would be defined as ‘inside’ the cluster and those in grey as ‘outside’ the cluster.

when oviposition was in an egg width line, or cluster; Figure 1). In addition, for batches of eggs laid in a cluster the position of an egg was noted, an egg within a cluster was defined as ‘inside’ if there were more than four eggs surrounding it and ‘outside’ if less than four eggs were surrounding it. Egg batches were maintained at 20 ± 2 °C and an L16:D8 photoperiod, and observed at eclosion. Each egg was classified as hatched (neonate larva emerged; eclosion order recorded), cannibalized (consumed by sibling neonate larva), or not hatched (no emergence of neonate larva; egg usually shrivelled over time). In total, 17 egg batches (seven linear; 10 clusters) were monitored constituting 237 eggs (101 linear; 136 clusters). The mean number of eggs was 14.4 and 13.6 per linear or cluster egg batch, respectively. Each egg batch was produced by a different female.

Statistical analyses

Differences in survival and time to complete each developmental stage between cannibals and non-cannibals were tested using t-tests. The relationship between laying and hatch order was tested using linear regression.

The difference in survival of cannibals and non-cannibals was tested using χ^2 .

The relationships between cannibalism and egg batch type (cluster or linear) and position in the egg cluster (inner or outer egg) were tested using binary logistic regression. Minitab, version 13 (Minitab, 2000) was used for all analyses.

Results

Effect of cannibalism on rate of development and survival to adult

Cannibals completed the first, second, third and fourth instars significantly faster than non-cannibals ($t_{31} = 2.887$, $P = 0.007$; $t_{31} = 2.815$, $P = 0.008$; $t_{31} = 3.686$, $P = 0.001$; and $t_{31} = 2.618$, $P = 0.014$, respectively; Figure 2). There was no significant difference in pupation time for cannibals compared to non-cannibals ($t_{31} = 1.369$, $P = 0.181$; Figure 2).

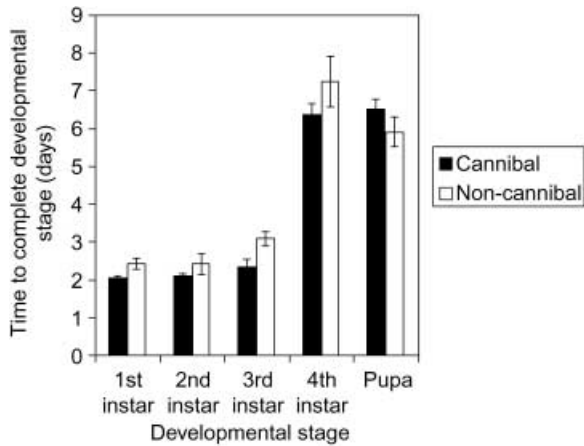


Figure 2 Mean time (days) taken for cannibalistic and non-cannibalistic *Adalia bipunctata* to complete the immature developmental stages (first, second, third, fourth instar, and pupa). Error bar = SE.

There was a significant difference in the proportion of cannibals compared to non-cannibals surviving to adult ($\chi^2 = 6.72$, d.f. = 1, $P < 0.01$; Figure 3). A greater percentage of cannibals (0.81) survived to adult compared to non-cannibals (0.46). This difference in survival was mainly attributed to the higher mortality of non-cannibalistic neonate (first-instar) larvae compared to cannibalistic neonate larvae (42 and 8%, respectively). Mortality at other stages was similar for both cannibalistic and non-cannibalistic neonate larvae.

Assessment of the synchrony between laying and hatching of eggs

Mean number of eggs (\pm SE) for clusters was 13.6 ± 0.7 and for linear batches it was 14.4 ± 1.1 . There was a significant positive correlation between the order in which eggs were laid and the subsequent hatching order ($R^2 = 0.68$,

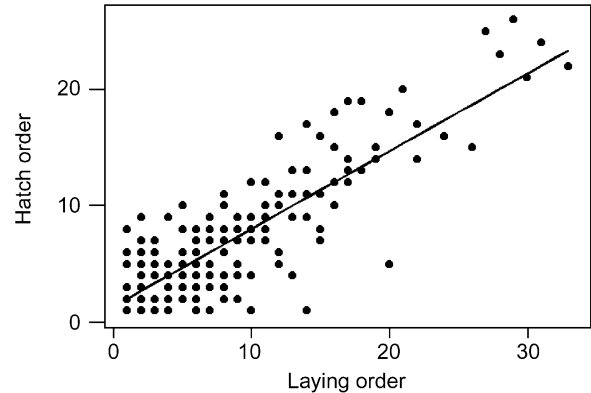
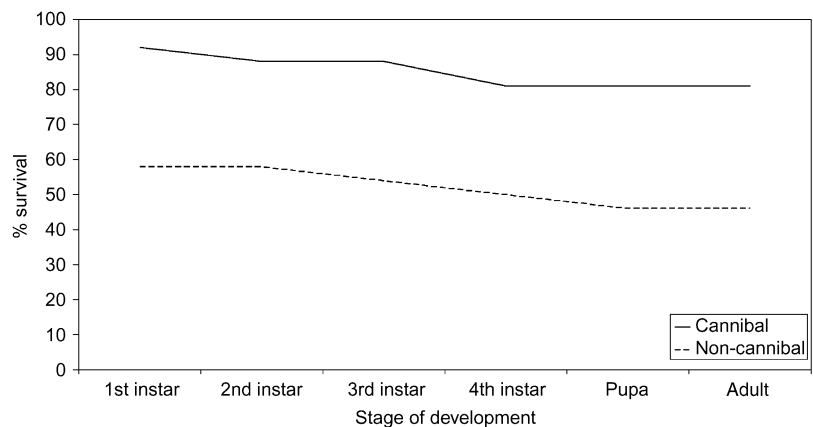


Figure 4 Relationship between laying and hatch order of *Adalia bipunctata* eggs; $R^2 = 0.68$, $P < 0.001$.

$P < 0.001$; Figure 4). The order of eggs being laid had no effect on the likelihood of being cannibalized ($G = 0.143$, d.f. = 1, $P = 0.706$). There was no significant difference in the proportion of eggs hatched, cannibalized, or unhatched in the linear batches compared to the clusters of eggs ($G = 0.184$, d.f. = 1, $P = 0.668$; Figure 5). The proportion of eggs hatching in the linear batches was 0.77 and in the clusters 0.80. There was also no difference in the proportion of eggs cannibalized in egg batches laid in a cluster (0.17) compared to those laid in a linear pattern (0.12), or in the proportion of eggs that did not hatch in the linear (0.10) and the cluster (0.03) batches.

There was no significant difference in the proportion of eggs cannibalized in the inside of a cluster compared to those on the edge of the cluster ($G = 0.985$, d.f. = 1, $P = 0.321$). However, from observations on the position of eggs within clusters, it was evident that only 23.1% of the cannibalized eggs were classified as being on the inside of the cluster and the remaining 76.9% were on the edge of the cluster.

Figure 3 Cumulative percentage survival of cannibalistic and non-cannibalistic *Adalia bipunctata* reaching each stage of development (first, second, third, fourth instar, pupa, and adult).



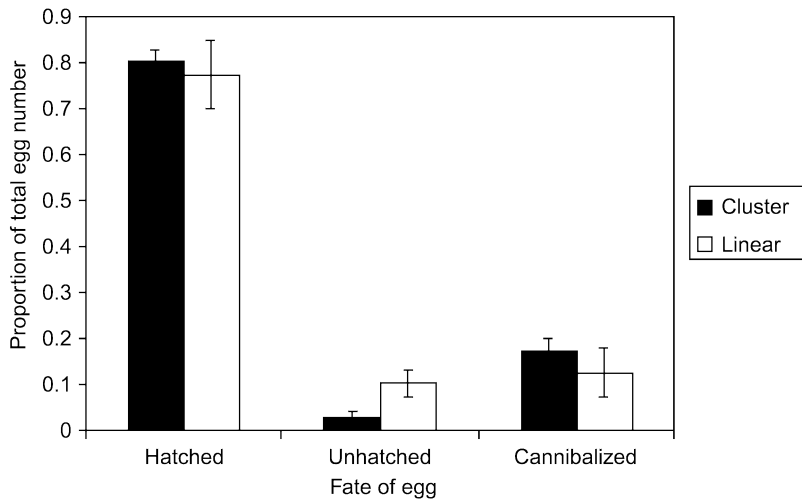


Figure 5 Mean proportion of eggs that either eclosed (hatched), failed to eclose (unhatched), or were cannibalized in *Adalia bipunctata* egg batches laid as either clusters or in a linear pattern. Error bar = SE.

Discussion

Coccinellid eggs are protected from predation by defensive alkaloids, pyrazines, and quinolones synthesized de novo (Cottrell, 2005). However, coccinellids, particularly neonate first instars, commonly engage in egg cannibalism (Yasuda & Ohnuma, 1999), and the consumption of a sibling egg is known to be beneficial when aphid density is low (Agarwala, 1991; Perry & Roitberg, 2005). Previous studies have demonstrated that the consumption of sibling eggs is advantageous to larval development and survival (Kawai, 1978; Agarwala & Dixon, 1992). Our studies support this and demonstrate that egg cannibalism dramatically reduces the mortality of first instars even when aphids are in abundant supply. We therefore agree that sibling eggs are superior prey in terms of survival. This could be attributed to the nutritional value of sibling eggs compared with aphids; Kawai (1978) reports that eggs are better food than aphids for young larvae. However, the energy expended in consuming a sibling egg is less than that expended in the capture and consumption of an aphid (Majerus, 1994), and this will undoubtedly impact on larval survival. It is interesting to note that a proportion of larvae (non-cannibal treatment) did not consume a sibling egg within the 2-h post-hatching period. This could suggest that larvae have a genetic predisposition to cannibalize. Indeed, in this study, our treatment groups were based on the innate tendency of some larvae to consume a sibling egg. It would be interesting to repeat this study by randomly selecting the larvae for the treatment groups (cannibal or non-cannibal) thereby ensuring that observed trends were not the consequence of confounding variables such as nutritional status of emerging larvae. However, for the purpose of this study, we were interested in examining

the differences between innately cannibalistic larvae compared with innately non-cannibalistic larvae.

The larvae in our experiments had consumed only one sibling egg but even this low level of cannibalism resulted in an advantage in terms of increased development rate in comparison to non-cannibalistic larvae. Indeed, the four larval stages were faster for cannibals compared to non-cannibals. Overall, cannibals reached the adult stage 1.65 days ahead of the non-cannibals. Insects are generally considered to be more vulnerable during immature stages compared to adult stages for a number of reasons: reduced mobility, increased predation and cannibalism (Dixon, 2000), and increased vulnerability to abiotic factors (Costa & Pierce, 1997). Therefore, a reduction in the time spent as a larva or pupa is likely to benefit fitness. Osawa (2002) demonstrated that egg cannibalism by first-instar *H. axyridis* not only reduced development time but also resulted in heavier adults. Increased weight could be advantageous to fecundity and mating success.

It is widely accepted that it is selectively advantageous for an individual aphidophagous coccinellid larva to hatch early because of the prevalence of sibling egg cannibalism (Dixon, 2000). Our study provides some insights into this prediction. First, it was evident that the order in which an egg was laid corresponded to the subsequent order in which it hatched. This suggests that there was limited variability in the rate of embryonic development. From our data, it was apparent that the overall proportion of eggs hatching was similar in both the linear egg batches and the clusters. Marginally higher levels of cannibalism were observed in the clusters compared to the linear egg batches but correspondingly fewer eggs failed to eclose in the clusters compared to the linear egg batches. Assuming that all egg batches have a similar proportion of infertile

eggs, we could extrapolate that the eggs cannibalized in the clusters were infertile and would not have contributed directly to the maternal fitness. Indirectly, the infertile egg contributes to inclusive fitness by increasing the survival chances of its cannibalistic sibling.

The concept of 'trophic eggs' has been proposed as a maternal strategy for feeding offspring (Perry & Roitberg, 2005). In a recent study, Perry & Roitberg (2005) observed that female coccinellids (*H. axyridis*) can increase the proportion of infertile eggs within a cluster in response to reduced prey availability. The adult ladybirds used in our research had been fed to excess. It would be interesting to examine whether trophic egg plasticity is a widespread phenomenon in coccinellids. *Harmonia axyridis* is considered to be more voracious and cannibalistic than other coccinellids (van Lenteren et al., 2003; Ware et al., 2006), such as *A. bipunctata*, and so the selection pressure to manipulate trophic egg production could be greater for *H. axyridis* than for less voracious coccinellid species. This aspect requires further work.

It is advantageous for a female ladybird to produce a proportion of infertile eggs within a cluster, assuming infertile eggs are less costly to produce than fertile eggs and that cannibalism is widespread. Endosymbiotic male-killing bacteria manipulate this scenario elegantly (Majerus & Hurst, 1997); the male-killing bacterium depends on the female carrier surviving and the probability of survival is increased in the presence of unviable male embryos that can be cannibalized (Majerus & Hurst, 1997). Ladybirds appear to have the sensory ability to assess the benefits and costs associated with varying oviposition behaviour (Perry & Roitberg, 2005). A female ladybird can not only vary the proportion of infertile eggs within a batch but it can also manipulate the position of eggs and the final batch formation (linear or cluster). A number of simple factors could determine whether an individual oviposits in a cluster or linearly: prey availability, age, time since last mating, frequency of mating, egg load, parasite burden, etc. Further detailed research into these could provide intriguing insights.

In conclusion, our study highlights the enormous benefits of egg cannibalism to neonate larvae. The ecological and evolutionary implications of this seemingly simple act are far reaching. Aposematic coccinellids are reported as having few natural enemies and so cannibalism is likely to be a major determinant in regulating coccinellid populations. We predict that the high prevalence of cannibalism by *A. bipunctata* will act as a major selection pressure giving rise to traits that ensure an individual is a cannibal and not cannibalized. We support the theory that a female ladybird can theoretically reduce the impact of cannibalism by interspersing infertile trophic eggs among its viable offspring.

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