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ORIGINAL ARTICLE

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Ladybird mothers mitigate offspring starvation risk by laying trophic eggs

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Abstract A large proportion of ladybird beetle (Coleoptera: Coccinellidae) eggs are apparently infertile—they do not develop an embryo and are consumed by larvae hatching within the egg batch. The predicted benefits of egg consumption for larvae are empirically well supported. An important question, however, remains: are these eggs a maternal strategy to feed offspring (i.e., trophic eggs) *or* did egg eating evolve to exploit unavoidably infertile eggs? We investigated the adaptive value of infertile eggs in laboratory experiments with multicoloured Asian ladybirds (*Harmonia axyridis*). Female *H. axyridis* were assigned to low and high resource environments for brief intervals; we predicted that tactics to facilitate egg cannibalism, such as infertile egg production and hatching asynchrony, would be adopted in low food environments in which starvation risk for offspring is greater. We conducted two experiments in this manner that provided females with information about resource levels through prey feeding or scent. We also observed female oviposition patterns and tested for infertile egg distributions that departed from random. Females produced 56% more infertile eggs in the low vs. the high food treatment; however, hatching synchrony did not change. We consider a potential confound between information and nutrition state unlikely because ladybirds are well able to tolerate low food for 24 h, the duration of trials, and because females were in good condition when trials began. Results suggest that ladybirds use information from prey encounter to manipulate the proportion of trophic eggs in a manner consistent with the adaptive hypothesis, the first evidence of trophic egg plasticity in a non-eusocial insect.

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Introduction

Parents in many species face the problem of high starvation risk for their offspring. Furthermore, some parents (e.g., most insects) do not interact with their offspring following oviposition; hence mothers in this situation are limited to starvation-reduction behaviors that are expressed at the egg production or deposition stage. In the extreme case, a mother's best option might be to provide food for offspring in the form of eggs themselves (Alexander 1974; Mock and Forbes 1995). Mothers should sacrifice some offspring to others when they gain more offspring from the increased survival of the cannibals than they lose as victims (Crespi 1992)—thus, when offspring starvation risk is high and eating a sibling provides a large benefit.

One way for mothers to ensure that offspring have a sibling to eat is to produce 'trophic' offspring that serve as a meal (Alexander 1974). This hypothesis provides a foundation for the study of trophic eggs—non-developing, ovariole-produced structures that are formed to feed offspring (Crespi 1992). In studying hypothesized trophic eggs, then, it is necessary to ask the functional question: is there an adaptive maternal strategy to feed offspring? The alternative hypothesis is that some infertile eggs are unavoidably produced through some constraint (e.g., sperm limitation), and that offspring are adapted to consume such eggs. In some taxa, the adaptive nature of trophic eggs seems clear; for example, when they have a unique morphology, clearly different from viable eggs (e.g., West and Alexander 1963; Henry 1972), or when parents actively feed them to offspring (e.g., Nakahira 1994; Heying 2001). In ladybird beetles, offspring consume undifferentiated, apparently infertile eggs that occur within their natal egg batch (e.g., Kawai 1978; Osawa 1992). For ladybirds and similar taxa (e.g., Valerio 1977; Frechette and Coderre 2000), the adaptive nature of trophic eggs must be empirically investigated.

A second way for mothers to sacrifice some offspring to others is to manipulate hatching synchrony (O'Connor 1978; Forbes et al. 2002). If cannibalism is directed towards siblings still in the defenceless egg stage, as in many insects (e.g., Branquart et al. 1997; Via 1999; Sigsgaard et al. 2002), then mothers can decrease hatching synchrony in low-food environments to allow early-hatching offspring to cannibalize (otherwise viable) siblings. For example, mothers might alter hatching synchrony by staggering the rate of egg development or by creating eggs that vary in mass (and, hence, development time). Mothers in some insect [e.g., lacewings (Frechette and Coderre 2000)] and bird [e.g., American kestrels (Wiebe and Bortolotti 1994)] species appear to manipulate hatching synchrony to facilitate sibling cannibalism.

One way to investigate whether mothers adaptively use these two provisioning strategies—trophic eggs and hatch synchrony adjustment—is to test whether they are adjusted to offspring starvation risk (Frechette and Coderre 2000), which determines the benefit of sibling cannibalism from a mother's perspective. The multicolored Asian lady beetle *Harmonia axyridis* is a good candidate species for examining flexible use of the behaviors for several reasons. First, females produce many apparently infertile eggs (15.2%, Kawai 1978; 24.5%, Osawa 1992) that are consumed by larvae hatching in the same egg batch. Second, first instar larvae face a high starvation risk due to oviposition sites that are some distance from aphid prey (Majerus 1994) and their poor predatory abilities (Hodek and Honek 1996). Furthermore, egg cannibalism dramatically reduces the risk of starvation in *H. axyridis* (up to 226%, Osawa 1992, see also Kawai 1978) and other ladybirds (reviewed by Hodek and Honek 1996). Finally, female ladybirds frequently disperse to new sites between bouts of egg laying (Evans 2003) and are thus likely to deposit offspring at sites that vary in resource availability. Therefore, female ladybirds should be selected to use plastic strategies to mitigate offspring starvation risk.

Two possibilities, then, are that the infertile eggs of *H. axyridis* are trophic offspring adaptively designed to be eaten (Osawa 2003); or that sibling cannibalism behavior takes advantage of an available food source. We also looked for evidence that females use hatching synchrony to alter the opportunity for sibling cannibalism among offspring. We hypothesized that if the behaviors are plastic, female ladybirds would lay clutches with fewer trophic eggs and more synchronous hatching when food resources for offspring were high, and more trophic eggs and less synchronous hatching in low food environments. We conducted two experiments to test the hypotheses that differed in how ladybirds acquired information about food levels, through direct contact with prey or indirect cues such as prey scent. Female ladybirds used in experiments were in similar physiological condition, minimizing a potential confound between information about resource levels and physiological state. Finally, if the infertile eggs are adaptively produced then deposition of such eggs should optimize their contribution to sibling survival. Thus, we examined the spatial distribution and order of oviposition of infertile eggs, suggesting that a uniform distribution indicates adaptive production (e.g., if it maximizes the chance that cannibals attack an infertile egg first rather than a viable offspring).

Methods

Study system and experimental animals

Harmonia axyridis is an aphidophagous ladybird beetle, native to Asia. It was introduced to parts of North America for the biological control of aphid pests, and is now the dominant coccinellid in many areas (Koch 2003). After overwintering, females emerge in the spring and lay clusters of eggs on plants infested with prey (aphids or similar phloem-feeding insects, Koch 2003). Females produce a clutch of 10–50 eggs every 1–2 days for their adult lives of 1–3 months. Larvae progress through four instars in 3–5 weeks. Several generations per summer are usually possible (Koch 2003).

Harmonia axyridis and other ladybirds produce three types of eggs: apparently infertile eggs, viable eggs, and 'inviable' eggs where a larva develops but does not emerge from the egg capsule (Ng 1986). In this study, we investigate the trophic function of infertile eggs, excluding inviable eggs because some embryo development occurs. Larvae are soft-bodied upon emergence. After a period of quiescence (mean: 141 min; 95% CI: 126–155; N=76; unpublished data) following hatching, they are sclerotized enough to consume any unhatched eggs in the natal clutch. Egg-eating larvae apparently do not discriminate between viable and infertile or inviable eggs (Banks 1956; Brown 1972; Osawa 1992). Other unhatched eggs are normally hatched or consumed by the time a cannibal is ready to eat a second egg. Larvae remain at the egg batch for about 24 h, then disperse in search of aphid prey (Osawa 1989).

Newly eclosed adult *H. axyridis* were obtained from Applied Bionomics (Victoria, British Columbia, Canada). Females were maintained individually in petri dishes and supplied daily with an excess of pea aphids (*Acyrthosiphon pisum* reared on broad bean, *Vicia faba*) and a water source. They were mated to males from laboratory colonies every 10 days.

Resource level experiments

The goal of these experiments was to test the hypothesis that ladybirds would increase trophic egg production and/or hatching asynchrony in low resource conditions. Ladybirds might assess resource levels for offspring by (1) the internal cue of their own food intake (because adults and offspring consume the same prey), and/or (2) an external cue, e.g. the scent of aphids or their honeydew excretions (Evans and Dixon 1986). We conducted two experiments that provided ladybirds with information about food abundance through internal and external cues, in a paired design such that females experienced both low and high food treatments in randomized order.

Before trials, females were each fed a set mass of pea aphids daily for 5 days (0.0310 g±10% if alone; 0.0445 g±10% if kept with a male for mating), to ensure a similar physiological condition. This amount is approximately as much as an adult female can consume in 24 h (Soares et al. 2001). Trials began on the sixth day after initiation of controlled feeding. Each female was placed in an empty petri dish (90 mm×23 mm) for 1 h to emphasize the transition from maintenance to experimental conditions. Females were then transferred to a new petri dish that contained the randomly assigned treatment (low or high aphid masses), a piece of cotton wick moistened with distilled water, and filter paper as an oviposition surface. During the 24-h trials, egg batches were removed from petri dishes every 3 h and aphid levels were replenished every 6 h (in the internal cue experiment (described below), by adding three second or third instar aphids to the low food treatment and a small mass of aphids to the high food treatment; in the external cue experiment, by adding three second or third instar aphids to both treatments). After first trials, females were fed the aphid masses mentioned above daily for 5 days, before the second trial with the reverse treatment.

During hatching (c. 80 h after each trial), we monitored egg batches every 10 min, removing emerged larvae to prevent egg cannibalism. Larvae were considered emerged when all six legs were free of the egg capsule.

Internal cue experiment

In this experiment, females were permitted to contact and feed upon aphids in both the low and high food treatments. The 'low food' treatment consisted of the smallest mass of aphids necessary to stimulate oviposition (0.0040–0.0070 g in preliminary trials). The 'high food' treatment was over nine times that amount, 0.0516 g±10%. Two replicates were conducted, with 106 and 70 females, respectively.

External cue experiment

In this experiment, ladybirds could smell, but not contact, aphids in high food treatment. The experimental chamber consisted of two petri dish bottoms (90 mm×23 mm) separated by nylon mesh, which was secured to the bottom dish with a rubber band. To minimize absorbed odors, the mesh was washed and rinsed with 70% ethanol followed by distilled water. The upper dish was secured to the lower with cellulose transparent tape. The lower compartment contained a 1-dram vial of distilled water with a bean leaf pair inserted through Parafilm (Pechiney Plastic Packaging, Wisconsin, USA). The leaf pairs were cut with a sharp razor and inserted through the Parafilm under distilled water within 2 h prior to the experiment, to minimize distress odors released by the plant (Petrescu et al. 2001).

For the high food treatment, a large mass of aphids (0.0806 g±10%) was placed in the lower compartment, whereas the low food treatment was no aphids. Females were placed individually in the upper compartment with a small mass of aphids (0.0040–0.0070 g) present in both treatments to stimulate oviposition. We conducted one replicate with 116 females.

Statistical analyses

We excluded egg batches from analysis when: (1) they were laid during the first 6 h of a trial, because ladybirds may have been responding to the food levels of maintenance conditions; (2) there were more than 40% non-developing eggs, because *H. axyridis* females can carry a male-killing bacteria (Majerus et al. 1998) and killed male embryos appear as infertile eggs (taking 40% as a conservative level for exclusion); (3) they had fewer than 5 eggs, because small egg batches are scattered rather than clumped (the typical arrangement); (4) females produced more than one egg batch during a trial (which happened only a few times); then we chose one batch randomly to avoid non-independence. The qualitative pattern of results did not change when data from categories 1–3 were included.

We used analysis of variance (ANOVA) to analyze the effect of food level treatment on several response variables: the number of eggs per batch, the proportion of trophic eggs weighted by clutch size, and three measures of hatching synchrony (described below). Because the experiments had a paired design, we used a cross-over analysis to account for multiple samples within females. This approach has the advantage of being able to handle missing values, which occurred when females oviposited under one food treatment only. The models included the factors treatment (low or high food), order of treatment (low food first or high food first), the treatment by order interaction, and female nested within order as a random factor. Exceptions to this form are noted. The models were built using the restricted maximum likelihood function in the program JMP 5.0 (SAS 2002).

We used three indices of hatching synchrony (Frechette and Coderre 2000): (1) Total hatch time, standardized to mean batch size by the equation: Standardized hatch time= Mean batch size (25.9) × (Total hatch time)/(Number of eggs). (2) The average interval between two sequential hatching larvae. (3) The proportion of eggs per batch, weighted by batch size, that were vulnerable to sibling cannibalism (i.e., because they emerged later than the 141 min quiescent period after the first larva in their egg batch, and thus could have been attacked by that larva; see *Study system and experimental animals*). We refer to this variable as the 'proportion of delayed hatch eggs'.

We report least squares means, which account for variation caused by all other factors in the ANOVA model, ± standard error. As an indication of the magnitude of difference between treatments, we report the estimated difference and the 95% confidence interval of the difference in the format: mean difference (lower estimate, upper estimate).

In the first replicate of the internal cue experiment, females produced similar numbers of egg batches under both food treatments (77 low food, 78 high food). One hundred batches were excluded from analysis for reasons listed above. Of the 55 remaining batches, 27 were produced under low food and 28 under high food. In the second replicate, 50 egg batches were laid (17 low food, 33 high food) and 34 were excluded, leaving 6 low food and 10 high food batches. Some batches had a combination of excluded features. The factor 'Replicate' did not affect any result, so it was dropped from the models. In this experiment, the order of treatment significantly affected synchrony—that is, there was a day effect of duration of hatching. We interpret this to mean that temperature affected hatch synchrony, because temperature was slightly warmer during hatching in the second trial of the first replication (first trial: 24.4°C±0.6, N=116; second trial: 25.8°C±0.6, N=116). For this reason, we analyzed hatch synchrony data for the first replication only; however, analyzing both replicates together did not change the pattern of results. We additionally analyzed hatch synchrony data for day one of replicate one, to entirely remove the day effect; the model for this analysis included the factor 'treatment' only. In some batches, a few larvae hatched much later than the rest; we excluded their hatch times from analysis.

In the external cue experiment, females produced a similar number of egg batches under both treatments (74 low food, 70 high food); after excluding some egg batches, 38 remained for analysis (17 low food, 21 high food). Because we were unable to monitor eggs for the entire hatching period, we obtained hatching synchrony data for only 17 batches (11 low food, 6 high food). As there were too few hatching synchrony data to include all factors in the nested models, the Order × Treatment interaction term was removed from the model.

Oviposition patterns

Spatial patterns

To examine the spatial distribution of infertile eggs, we collected egg batches from 40 females maintained individually in petri dishes and supplied with a water source and an excess of pea aphids daily. Females used in these observations were not used in the above experiments. The egg batches were photographed using a digital microscope (Scalar USB Microscope M2, 50× magnification). Larvae were removed from the egg batch after all viable eggs hatched and egg cannibalism occurred. It is easy to distinguish trophic eggs from embryonated eggs because trophic eggs contain visible yolk, or traces of yolk if they have been eaten. It is unlikely that consumed infertile eggs would have developed an embryo because viable and inviable eggs show visible embryo development by the time emerged larvae are ready to eat an egg.

Following Avilés et al. (1999), we use the program NIH Image 1.62 (available on the Internet at http://rsb.info.nih.gov/nih-image/) to calculate the distance in pixels between each pair of eggs in each batch. We then determined the average distance between all pairs of the N infertile eggs. Using a computer-coded bootstrap procedure, we sampled randomly with replacement from all pairwise distances to generate average distances between N randomly drawn eggs. We repeated the sampling 10,000 times to generate a null distribution and calculated the proportion of randomly generated values that were greater or less than the observed average distance between infertile eggs, following usual randomization test procedure (Edgington 1995). Using α=0.05, proportions <0.025 indicate a clumped distribution and values >0.975 indicate a uniform distribution. We checked for the occurrence of any significant P-values within the list of P-values by the procedure described by Neuhäuser (2004), for both spatial and oviposition patterns.

Oviposition patterns

To examine the dispersion of infertile eggs within the oviposition sequence, we videotaped ovipositing females kept individually in petri dishes. When the eggs hatched, we associated each type of egg with its position in the oviposition sequence. We tested for non-random pattern with a runs test, which uses the null hypothesis of randomness in the order of occurrence of members of two groups (Zar 1999). As above, we checked for the presence of any significant P-values following Neuhäuser (2004).

Results

Resource level experiments

Internal cue experiment

Egg batches were of similar size under both treatments [low food: 25.7±2.3; high food: 26.4±2.1; mean difference: 0.7 eggs (−6.0–7.4)].

Trophic egg laying. Females produced 56% more trophic eggs in a low compared to a high food environment (Fig. 1a; Table 1). There was little effect of the order in which females experienced treatments (low food first, 18.1%±1.8%; high food, 20.0%±2.3%; $F_{54,\ 57}$=0.38; P=0.54) and the interaction term was not significant ($F_{13,\ 57}$=0.95; P=0.35).

Hatch synchrony. None of the three measures of hatching synchrony was statistically different under low or high food treatments (Table 1); however, hatching was less synchronous in the low food treatment for all measures, as predicted. The wide range of values in the 95% confidence intervals indicates a low ability to detect differences (Table 1). There was an order effect, such that females that experienced 'low food' first produced egg batches that took significantly longer to hatch, though intervals between hatching eggs were longer, this was not statistically significant (Low vs. high food: Total hatch time: 227 min±22

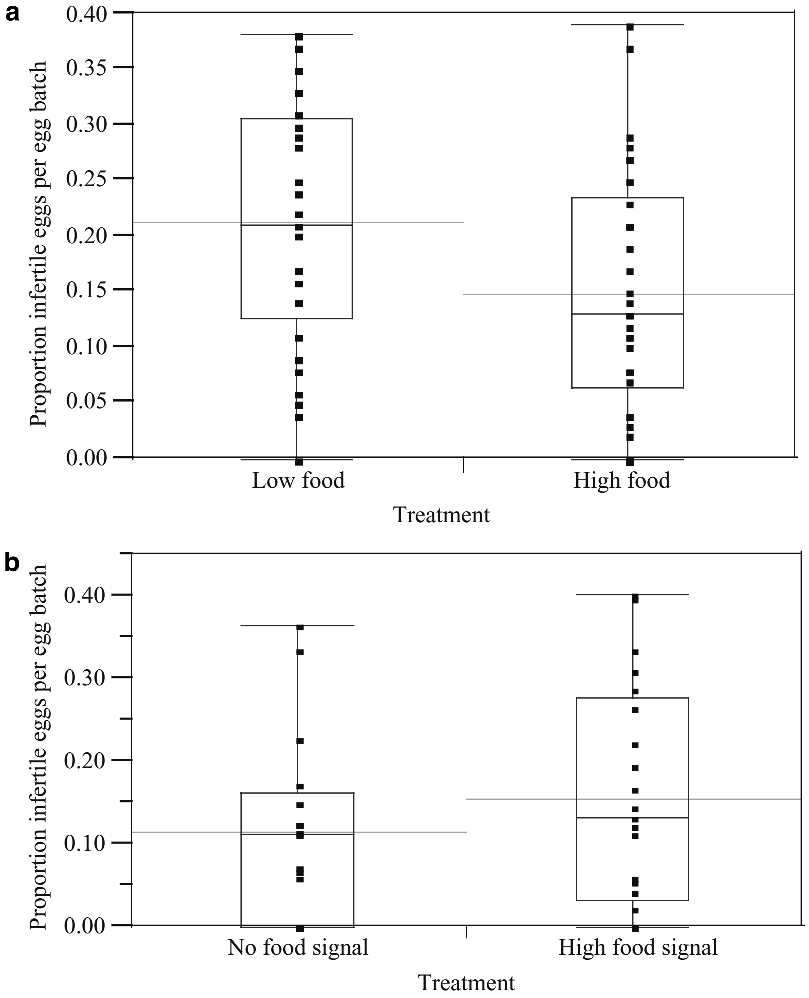

Fig. 1 Proportion of trophic eggs produced when females were given information that there was low or high food availability through (**a**) the internal cue of prey encounter and consumption or (**b**) the external cue of prey scent; see text for details. Least squares means are presented, which account for variation in proportion of trophic eggs caused by order of treatment, female, and the order × treatment interaction. The proportion of trophic eggs was smaller under the low food treatment in the internal cue but not the external cue experiment (see Table 1). Horizontal lines represent means. Whiskers indicate the range of observations

Table 1 Effect of food level (low or high) on the proportion of trophic eggs and hatching synchrony (using three measures of synchrony; see text) when females were given information about food conditions through feeding (internal cue) or other senses (external cue)

Reproductive tactic	Least squares mean (SE)		Mean difference (95% CI)	*F* (*P*)
	Low food	High food		
Internal cue experiment				
Proportion of trophic eggs	0.232 (0.02)	0.150 (0.02)	0.082 (0.03–0.13)	$F_{13. 57}$=12.3 **(0.004)**
Hatching synchrony				
Total hatch time (min)	203 (26)	167 (26)	36.2 (−43–116)	$F_{1, 42}$=0.98 (0.34)
Interval between hatching eggs (min)	15.3 (2.3)	10.4 (2.4)	4.9 (−1.6–11.4)	$F_{1, 42}$=2.68 (0.13)
Proportion of delayed hatch eggs	0.070 (0.011)	0.045 (0.011)	0.024 (−0.008–0.057)	$F_{1, 42}$=2.80 (0.12)
External cue experiment				
Proportion of trophic eggs	0.124 (0.027)	0.135 (0.026)	0.011 (−0.07–0.09)	$F_{1, 34}$=0.175 (0.70)
Hatching synchrony				
Total hatch time (min)	110 (24)	61 (40)	48.8 (−43–141)	$F_{1, 2}$=1.29 (0.27)
Interval between hatching eggs (min)	6.5 (1.4)	8.6 (2.5)	2.10 (−3.7–7.9)	$F_{1, 2}$=0.60, (0.45)
Proportion of delayed hatch eggs	0.041 (0.016)	−0.030 (0.027)	0.071 (0.01–0.13)	$F_{1, 2}$=6.76 **(0.02)**

The least squares mean value gives the effect of food treatment on the response variable, accounting for variation in the response caused by order of treatment, female, and the order × treatment interaction. Proportions are weighted by number of eggs per batch. Total hatch time is standardized to mean number of eggs per batch vs. 143 min±30, $F_{39,\ 42}=5.10$, $P=0.03$; Hatching interval: 16.4 min±2.1 vs. 9.4 min±2.8, $F_{39,\ 42}=3.87$, $P=0.06$).

In a separate analysis, we considered the effect of treatment on hatching synchrony, using data from day one of the first replicate of this experiment. The results were similar to the above analysis: hatching was less synchronous in the low food treatment for all measures, but not significantly so (Low vs. high food: Total hatch time: 264.4±44.4 vs. 141.0±59.2, $F_{1,23}=2.8$, $P=0.11$; Hatching interval: 20.2±3.9 vs. 8.4±5.2, $F_{1,23}=3.4$, $P=0.08$; Proportion of delayed hatch eggs: 9.1%±1.5 vs. 4.3%±2.1, $F_{1,23}=3.5$, $P=0.07$).

There was thus no effect of food availability on any measure of hatching synchrony, though the trend was in the predicted direction; however, more trophic eggs were produced under the low food treatment.

External cue experiment

Egg batches were similar in size for both low and high food treatments [low food: 24.2±3.3; high food: 31.8±3.3; mean difference: 7.5 (−5.1–20.1)].

Trophic egg laying. When information regarding resource availability was provided through an external cue, trophic egg production was not different between treatments (Fig. 1b; Table 1). There was no effect of order of treatment ($F_{31,\ 34}=0.25$; $P=0.62$).

Hatch synchrony. Food level did not affect total hatch time or average hatching interval (Table 1). There was, however, a significantly higher proportion of delayed hatch larvae in the low food treatment (Table 1); that is, more larvae were vulnerable to sibling cannibalism from delayed hatching. Order did not significantly affect total hatch time ($F_{1,\ 2}=1.28$; $P=0.28$), the interval between hatching eggs ($F_{1,\ 2}=0.64$; $P=0.44$), or the proportion of vulnerable eggs ($F_{1,\ 2}=3.35$; $P=0.09$), but there was limited ability to detect differences between the groups [mean differences: total hatching time, 54.6 (−49–158); interval between hatching eggs, 2.3 (−8.4–3.8); proportion of vulnerable eggs, 0.60 (−0.01–13)].

Thus, there was no effect of food availability on trophic egg production in the external cue experiment. And, while there were more larvae with delayed hatch in the low food treatment, as predicted, the statistical model did not include the interaction or nested terms, limiting the confidence that can be placed in the result.

Spatial patterns

Of the 40 egg batches, 21 had at least two infertile eggs, a condition necessary to examine their distribution. Infertile eggs were significantly clumped in one of the 21 batches (Table 2); however, the combined *P*-value for all batches (Neuhäuser 2004) was 0.22, indicating that the significant value was expected by chance alone.

Table 2 Results of randomization tests for deviations from a random spatial distribution of infertile eggs within egg batches produced by different female ladybirds

Number of eggs	Number of infertile eggs	*P*
18	1	**0.01**
43	15	0.05
11	2	0.11
29	9	0.14
40	2	0.15
34	6	0.20
30	2	0.24
37	6	0.37
37	2	0.40
45	5	0.42
35	13	0.44
24	2	0.50
20	2	0.53
36	4	0.56
24	3	0.61
19	4	0.67
27	4	0.78
30	7	0.79
16	3	0.81
34	7	0.84
35	2	0.96

P values were generated from simulations of possible distributions, given the spatial arrangement of each batch. A clumped distribution is indicated by $P<0.025$; $P>0.975$ indicates a uniform distribution

Table 3 Runs tests (Zar 1999) for non-random distributions of infertile eggs within oviposition sequences of different females

Number of eggs	Number of infertile eggs	Test statistic	*P*
9	3	−1.63	0.05
58	6	−1.29	0.10
21	8	1.13	0.13
39	7	0.56	0.29
27	2	−0.33	0.37
32	8	−0.29	0.39

Oviposition patterns

Oviposition sequences from four females were recorded. The distribution of infertile eggs was not different from random in any sequence (Table 3); the combined *P*-value across all tests was 0.26.

Discussion

This study is the first to test the hypothesis that the infertile eggs of ladybirds are an evolved maternal strategy (Osawa 2003; Perry 2004). We found evidence that trophic egg production is a plastic maternal behavior, used adaptively depending on resource availability: in low food conditions, mothers produced 56% more trophic eggs than in high food conditions (Fig. 1; Table 2). Our results are consistent with models of siblicide (O'Connor 1978; Mock and Parker 1997) that predict that when offspring starvation risk is great enough, and the relative benefit of eating a sibling exceeds 100%, mothers should facilitate cannibalism among their offspring. One way to ensure cannibalism is to lay a trophic egg (i.e., to neglect to fertilize an offspring). Our results are also consistent with the empirical work of Osawa (1992), which used survival data from a field experiment with *H. axyridis* to show that sibling cannibalism often supported the inclusive fitness interests of mothers. Our contribution is showing maternal control of a behavior that appeared adaptive. Taken together, these studies suggest some amount of infertility is an adaptation in this species—a maternally controlled sacrifice of some offspring to improve the survival of others. This suggestion does not claim that all ladybird infertility is adaptive for mothers, for some level of infertility may be unavoidable.

The potential for confound between a female's nutritional and information states merits consideration. A female might lay more infertile (trophic) eggs in a low food environment because she is in poor condition, not due to an offspring provisioning strategy. This 'condition hypothesis' suggests that the short period in the low food treatment in this study made females so physiologically weak that fertility plummeted. We consider this explanation unlikely for ladybirds, which feed on an ephemeral prey and should be well adapted to surviving a day with less food. For example, female *Coccinella septempunctata*, another aphidophagous ladybird, survived 91.4±27.2 days without food (Tanaka and Ito 1982). Furthermore, to ensure good condition, we fed all females an equal mass of aphids for 5 days prior to each trial.

We tested another hypothesis about the level of control of trophic egg production, predicting that trophic eggs would be uniformly distributed to maximize the probability that a cannibal attacks a trophic rather than a viable egg. The lack of evidence for any non-random pattern may indicate that, while females manipulate the proportion of trophic eggs in an egg batch, they do not control fertility on an egg-by-egg basis. It is possible that the distribution of trophic eggs is not important for provisioning offspring or preventing the cannibalism of viable eggs.

A second way mothers may facilitate egg consumption is to decrease hatch synchrony so that emerged offspring can consume siblings with delayed hatching. We did not find a statistical difference for most measures of hatching synchrony between low and high food conditions; there was, however, a significantly higher proportion of eggs with delayed hatch under the low food treatment in the external cue experiment (Table 1). We take this result with caution, since no other measure of hatching synchrony yielded a significant difference. However, for most measures of hatching synchrony, the difference between low and high food groups was in the predicted direction. More work is needed to make a conclusion about the plastic use of this tactic in ladybirds.

Mothers who lay eggs and leave their offspring are limited in avenues for parental care. Still, in addition to facilitating sibling cannibalism, mothers might reduce offspring starvation risk by adjusting egg size to put more yolk resources into eggs in high starvation risk environments. However, there is no evidence of egg size plasticity in the ladybird *Coccinella septempunctata* (Dixon and Guo 1993), and other studies in ladybirds suggest that egg size is an inflexible species trait (Stewart et al. 1991a, b; Soares et al. 2001), perhaps inflexibly tied to ovariole size. If this is correct, then egg size should increase with increasing starvation risk for offspring across species or populations. As an example, female goby fish lay larger eggs in a population with high offspring starvation risk, compared to a population with low risk (Maruyama et al. 2003). Furthermore, if egg size is highly constrained, mothers have little ability for adaptive adjustments between environments; in comparison, the strategy of manipulating the opportunity for egg cannibalism offers the advantage of plasticity between environments of differing starvation risk.

We, therefore, expect the use of the trophic egg tactic in taxa for which the use of other potential tactics is constrained. For example, ladybird mothers may have limited control over hatching synchrony if hatching is temperature dependent, as it appeared to be in our study. With imperfect control, hatching may be too synchronous, preventing cannibalism when it works for a mother's interests. Then the solution is to ensure a meal for offspring by laying trophic eggs.

Our study should be compared with the work of Frechette and Coderre (2000), who found that female lacewings increased the hatching synchrony of egg batches in the presence of aphid prey, but did not change the proportion of infertile eggs. Infertile egg production was low in both the presence (3%) and absence (4%) of aphid prey; thus one possibility is that these animals produced too few infertile eggs to allow detection of a difference. Lacewings and ladybirds have similar life histories—both are batch-laying aphid predators showing sibling cannibalism behavior—and both seem to have evolved maternal strategies to promote sibling cannibalism among offspring. Taken together, their study and ours suggest that manipulating hatching synchrony and producing trophic eggs are alternate solutions to the problem of reducing offspring starvation risk when there is no or little parental care. Moreover, because hatching asynchrony and trophic egg production achieve the same end of facilitating egg eating, the tactics might be used interchangeably or simultaneously within a given system.

We have focused on a scenario of parent–offspring agreement over sibling cannibalism, proposing that mothers help cannibal offspring by neglecting to fertilize some eggs (Mock and Forbes's (1995) trophic hypothesis; Crespi 1992). An alternative explanation for trophic egg evolution is the conflict reduction hypothesis (Crespi 1992), which proposes that trophic eggs may have evolved to reduce parent–offspring conflict over cannibalism of viable eggs, in systems where trophic eggs are less costly to produce than viable offspring. However, parent–offspring agreement is a more likely explanation for the trophic eggs of ladybirds for two reasons. First, the survival benefit to cannibals of consuming eggs is substantial (reviewed by Hodek and Honek 1996), and in most studies exceed 100%, the threshold for mothers to benefit from sibling cannibalism (O'Connor 1978). Second, there is no evidence that ladybird trophic eggs are energetically cheaper to produce than viable eggs. Ladybird trophic eggs and viable eggs appear identical to the eye (until the last few hours before hatching when the developing embryo becomes visible in viable eggs) and there is no difference in their mass (unpublished data). It thus seems reasonable to assume that they require similar energy input from mothers, though this assumption requires biochemical analysis to be tested. If trophic and viable eggs truly have equal cost, then mothers should produce trophic eggs only if sibling cannibalism is also in their interests, because producing a trophic egg has the same effect as a mother killing a potentially viable offspring. Thus, there should be no conflict over the cannibalism of viable offspring—mothers and cannibals agree that it should proceed.

Our study is the first to test the assumption that trophic eggs are an adaptive maternal strategy in a non-social insect. Another approach to demonstrating the adaptive nature of trophic eggs would be a comparative analysis of infertile egg production in species with relevant characteristics, such as those that do or do not lay eggs in batches and where larvae do or do not cannibalize siblings.

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