

Egg and time limitation mediate an egg protection strategy

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Abstract

The number of mature eggs remaining in the ovaries and the time left for oviposition determine the reproductive decisions of the hyperdiverse guild of insects that require discrete and potentially limiting resources for oviposition (such as seeds, fruits or other insects). A female may run out of eggs before all available oviposition sites are used (egg limitation), or die before using all of her eggs (time limitation). Females are predicted to change clutch size depending on whether eggs or time is the limiting resource. We extend this framework and ask whether the same constraints influence a strategy in which females modify eggs into protective shields. In response to egg parasitism cues, female seed beetles (*Mimosestes amicus*) lay eggs in vertical groups of 2–4, modifying the top 1–3 eggs into shields in order to protect the bottom egg from attack by parasitoids. We made contrasting predictions of how egg and time limitation would influence egg size and the incidence and level of egg protection. By varying access to seed pods, we manipulated the number of remaining eggs a female had at the time she received a parasitism cue. Although egg size was not affected, our results confirm that egg-limited females protected fewer eggs and time-limited females protected more eggs. Female body size explained the number of eggs in a stack rather than host deprivation or the timing of parasitoid exposure. Our results clearly show that host availability relative to female age influences the incidence of egg protection in *M. amicus*. Furthermore, our study represents a novel use of life history theory to explain patterns in an unusual but compelling defensive behaviour.

Introduction

Resource allocation theory assumes that animals have a finite amount of resources, and their various activities, including growth, survival and reproduction, make conflicting demands on the allocation of those resources (Pianka, 1981, 1988; Boggs, 2009). This is particularly important for females, because a substantial proportion of a female's resources are allocated to producing successful offspring. In insects, oviposition opportunities are often constrained by the need to place offspring in or on a discrete and limited package of resources on which their offspring develop, such as seeds or insect hosts (Diaz-Fleischer & Aluja, 2003). The temporal and

spatial variability in these oviposition resources is expected to shape the evolution of egg load, or the number of mature eggs a female is carrying (Ellers *et al.*, 2000; Harvey *et al.*, 2001). There is still not often a perfect match between resource quality and egg maturation rate, so females will be in situations where eggs left to lay or time left to lay eggs constrains the maximum number of high-quality offspring that can be produced. Females will approach egg limitation when they deplete their egg supply before all available hosts can be used (Rosenheim, 1996), and approach time limitation when death or loss of ability to reproduce is imminent and they have yet to deposit all of their eggs (Sevenster *et al.*, 1998). Egg limitation may play a larger role at the end of life when host densities are high, or earlier when females have only matured a few eggs (Driessen & Hemerik, 1992).

Sensitivity to host availability, life expectancy and egg load influences the reproductive decisions in insects

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(Fletcher *et al.*, 1994; Babendreier & Hoffmeister, 2002; Diaz-Fleischer & Aluja, 2003; Xu *et al.*, 2012). Time and egg constraints on females have been argued to be major mediators in the reproductive behaviour of insects (Rosenheim, 2011), and host availability may be the most important factor influencing whether a female is egg limited or time limited (Ellers *et al.*, 2000; Diaz-Fleischer & Aluja, 2003; Xu *et al.*, 2012). Egg and time limitation have been shown to mediate oviposition behaviour among species of herbivorous insects and parasitoids that use discrete units of food for oviposition. When assessing host quality or availability, females are generally more selective when egg limited, and less selective when time limited (Iwasa *et al.*, 1984; Parker & Courtney, 1984; Odendaal & Rausher, 1990; Javois & Tamaru, 2004). For example, when tephritid fruit fly species *Anastrepha* and *Bactrocera* are egg limited, they more often reject lower-quality hosts and lay fewer eggs per fruit; but when time limited, they accept lower-quality hosts and lay more eggs per fruit (Diaz-Fleischer & Aluja, 2003; Xu *et al.*, 2012). Egg-limited solitary parasitoids often avoid ovipositing in hosts that have been parasitized, but superparasitize hosts more often when time limited (van Alphen & Visser, 1990). When eggs are not limiting, even marginal oviposition sites are better than none if females can save time or reduce ovipositor wear (Lalonde & Mangel, 1994; Mangel & Heimpel, 1998).

In our study, we aimed to generalize beyond the current application of the egg and time limitation framework and explore how a unique but integral protective oviposition behaviour is influenced by costs accrued due to highly variable opportunities to lay eggs. We used the principles of egg and time limitation theory to generate predictions on how host availability ultimately affects the frequency and level of protective oviposition behaviour. Then, we tested the effects of unlimited host access and host deprivation over time on the protective 'egg stacking' behaviour in the seed beetle, *Mimosestes amicus* (Deas & Hunter, 2012). When exposed to egg parasitism cues, females respond by superimposing one to three protective eggs on top of a viable egg, thus reducing or preventing its access to the egg parasitoid, *Uscana semifumipennis*. In contrast to other studies where laying multiple eggs per oviposition is triggered by changes in host availability or quality, egg stacking behaviour is triggered by a parasitism cue whenever it occurs, regardless of previous host experience (Deas & Hunter, 2012). This beetle system thus provides an opportunity for a novel test of general theory in which physiological state can be manipulated independently of the cue that triggers the behaviour. Given the evidence that females make adjustments to clutch size depending on their degree of egg or time limitation, we expected similar patterns to apply to the incidence and level of egg protection. We generated two predictions about how egg limitation and time limitation should mediate the egg stacking response:

- 1 *Allowing females unlimited host access to impose egg limitation:* When female beetles are supplied with unlimited access to oviposition sites (seed pods), those exposed to parasitism cues late in oviposition will invest in less offspring protection by (i) protecting fewer eggs, (ii) using fewer eggs for each multiegg stack and (iii) lay smaller eggs compared to females exposed to parasitism cues early in oviposition.
- 2 *Depriving females of hosts to impose time limitation:* When female beetles are deprived of hosts and then supplied with access to seed pods and a parasitism cue simultaneously, females deprived for longer will invest in more offspring protection by (i) protecting more eggs, (ii) using more eggs for each multiegg stack and (iii) lay larger eggs compared to females deprived for fewer days.

Materials and methods

Study system

Mimosestes amicus is a seed beetle (Chrysomelidae: Bruchinae) distributed from the south-western United States through Mexico and in Costa Rica (Kingsolver & Johnson, 1978). The legumes *Parkinsonia florida* (blue palo verde), *P. microphyllum* (foothill palo verde) and *Prosopis velutina* (velvet mesquite) are the host plants commonly infested by *M. amicus* in the collection area around Tucson, Arizona, but *M. amicus* attacks 22 other legume species as well (Kingsolver & Johnson, 1978). Because *P. microphyllum* seeds confer the highest beetle survivorship, we used these seeds for maintaining laboratory colonies and experiments. *Mimosestes amicus* individuals are synovigenic, maturing eggs throughout their 2-week laboratory lifespan, and lay eggs for an average of 8 days (maximum ~12 days). Upon hatching, larvae burrow through the pod and into one of the interior seeds, where they feed, pupate and from which they emerge as adults. In the field, eggs are often under threat of parasitism by *Uscana semifumipennis* (Trichogrammatidae), a solitary parasitoid that is sympatric with *M. amicus* and specializes on seed beetle eggs (Fursov, 1995).

Mimosestes amicus females may lay single eggs or produce multiegg 'stacks', in which 1–3 eggs are laid, one after another, directly on top of a single egg. Each egg in a stack is laid before a female moves on to lay her next stack or single egg. Upper eggs in a stack protect the bottom egg from parasitism by *U. semifumipennis* (Deas & Hunter, 2012). They do not produce beetle progeny even in the absence of parasitoids, but may support the development of a parasitoid. Female beetles do not generally protect their eggs in the absence of parasitism cues. However, females do protect eggs in the presence of adult parasitoids and parasitized eggs, or parasitized eggs alone (Deas & Hunter, 2013). Although we have not identified the mechanism of the

detection of parasitism cues by female beetles, we think they may be exploiting host discrimination cues left behind by parasitoid females. We have observed female wasps wiping their hind legs against the ventral side of their abdomen after parasitizing an egg (perhaps to discourage other parasitoid females from superparasitism), and female beetles drumming eggs with their maxillary palps while foraging for an oviposition site. Although parasitized eggs alone are a sufficient cue, we used adult parasitoids to induce egg stacking; the wasps start to oviposit in beetle eggs as soon as beetles begin laying them in experimental containers.

Insect rearing

During late June of 2010 and 2012, *P. microphyllum* trees in Tucson, AZ, were inspected for pod infestation by seed beetles. We collected uninfested (= no visible eggs) seed pods from trees and stored them at -20°C to exterminate any undetected, developing seed beetle larvae and parasites of larval/adult seed beetles such as the straw itch mite, *Pyemotes tritici* (Southgate, 1979). All beetles used in experiments were descended from developing beetles collected from foothill palo verde seed pods in central Tucson (in the vicinity of Sentinel Peak) early to mid-August of 2010 and 2011. Seed pods were supplied to beetles in plastic containers without supplementary food or water. All adults of the trichogrammatid egg parasitoid *U. semifumipennis* used in experiments descended from individuals that emerged from *M. amicus* eggs collected in the field in August 2010. They were reared on the eggs of *Callosobruchus maculatus*, another seed beetle species, and these beetles were reared on cowpea (*Vigna unguiculata*) seeds.

Experiment 1: Egg limitation conditions

Emerging adult beetles were sexed, weighed (in milligrams), paired for matings and separated into four treatment groups ($n = 15$ replicates each) before allowing them to lay eggs. Female body weight was measured after adult emergence but before any mating could occur. In the three experimental treatments, the beetles were exposed to five mated, female parasitoids for 48 h on days 2 through 4 (early), 4 through 6 (middle) or 6 through 8 (late) post-emergence of an experimental period that lasted 12 days. Control beetles were not exposed to parasitoids. We chose 48-h exposure intervals to give females time to switch behaviours, and chose time points that did not extend beyond the 8th day because most females lay few eggs beyond 8 days. If beetles laid any eggs at all during the experiment, we assumed they had been mated. All beetle mating pairs were placed in 60-mm Petri dishes and provided with five fresh seed pods daily throughout the experiment. Five seed pods were chosen so that beetles could lay eggs continuously and we would have enough time

points to compare daily changes in reproductive output. After a 2-day parasitoid exposure interval, treatment beetles were placed in a clean dish with fresh pods. For every day, we recorded the (i) incidence of stacking, or the proportion of stacked eggs (number of stacks/total depositions; depositions = sum of singles and individual stacks), (ii) the level of stacking, or the proportion of multi-egg stacks (2+ protective egg stacks/total stacks), and (iii) the mean mass of a single egg. To show the effect of time on the number of depositions made per day and a possible mechanism for changes in stacking behaviour, we tracked depositions made per day across the oviposition period. We only analysed the responses of general stacking, multi-egg stacking and changes in egg mass occurring within each level of timing of parasitoid exposure.

Experiment 2: Time limitation conditions

Emerging virgin beetles were sexed and placed in a 0.25-dram vial sealed with cotton to restrict movement and access to seed pods. Beetles were then isolated for 1, 4 or 8 days before mating pairs were established and beetles were allowed to oviposit. Each of these groups was further divided into groups that were exposed or not exposed to parasitoids. Each treatment combination in the 2×3 factorial design had 35 replicates. The exposed group received 5 mated, female parasitoids in a ventilated 9-ounce cup with enough seed pods to contain 20 seeds. The size of the container and the number of seeds given ensured that beetles had ample hosts for oviposition. The experiment lasted 5 days from the initial exposure to hosts, after which we recorded the same data as in Experiment #1 (proportion of eggs that were protected, proportion of multi-egg stacks and the mean mass of a single egg). To show the effect of host deprivation on egg load and the mechanism for changes in female behaviour, we also calculated the egg load of unmated females that had not been exposed to parasitoids, but were deprived of hosts for up to 8 days. We isolated females and dissected them at the same time that we measured stacking in the time limitation experiment (1, 4 and 8 days) in addition to 0, 2 and 6 days, and counted the total number of mature (freely floating) oocytes stored in the lateral oviducts.

Statistical analyses

Experiment 1

For the egg limitation experiment, separate GLMs (quasibinomial distribution, logit link) were used to analyse the effect of timing of parasitoid exposure (explanatory variable), body size (covariate) and the interaction between timing of parasitoid and body size on the proportion of stacks (number of stacks/total depositions = response variable) and multi-egg stacks (2+ protective egg stacks/total stacks = response vari-

able) laid by females. An F-test was used to test the statistical significance of the terms in the quasibinomial model (Crawley 2007). The full model was run to evaluate the difference among all treatments and control, and the model was also analysed without the control, in order to evaluate the differences in stacking among the experimental treatments. To analyse the effect of timing of parasitoid exposure on the number of egg depositions laid across the oviposition period, we square-root-transformed the number of egg depositions and applied a linear mixed model for repeated measures, using SAS version 9.3 (SAS Institute Inc, Cary, NC, USA 2010). We included timing of parasitoid exposure, day of eggs laid, the interaction between these variables and female body weight as explanatory variables and female ID as a random effect. We fitted a power covariance error structure onto the data across time in order to account for our unequally spaced observations. To analyse the effect of treatment on mean egg weights, we used a GLM (Gaussian error distribution, identity link).

Experiment 2

For the time limitation experiment, separate GLMs (quasibinomial distribution, logit link) were used to analyse the effect of host deprivation period on the proportion of stacks and the proportion of multiegg stacks. A GLM (Gaussian distribution, identity link) was used to analyse the effect of host deprivation period and parasitoid presence/absence on mean egg weights. Lastly, we used a GLM (Poisson distribution, log link) to analyse the effect of host deprivation period on egg load. We included female body weight as a covariate and an interaction between host deprivation period and female body weight for this analysis. For our analyses on proportions of stacks, proportions of multiegg stacks and egg load, we determined differences among treatments using Tukey's HSD post hoc tests as a part of the analysis. Letters denoting significant differences among treatments are shown in the figures. Unless otherwise noted, all analyses in Experiments 1 and 2 were performed using R version 3.0.2 (R Development Core Team, 2013, Vienna, Austria).

Results

Experiment 1: Egg limitation conditions

Those females exposed to parasitoids earlier in the experiment laid a higher proportion of stacks (as opposed to singly laid eggs) compared to females exposed to parasitoids later (Fig. 1). Among the experimental treatments only ('no parasitoid' control removed), the timing of exposure to parasitoids (treatment) affected the proportion of egg stacks laid (GLM, quasibinomial distribution, logit link function, $F_{2,31} = 8.04$, $P = 0.0019$), but neither body size nor an interaction between treatment and

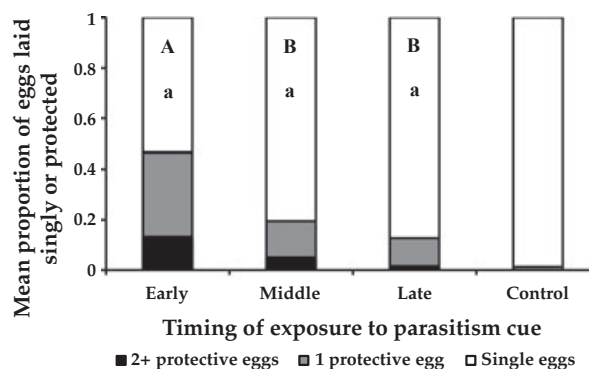


Fig. 1 When egg limitation is imposed, females exposed to parasitoids earlier protected more eggs than females exposed later. Uppercase and lowercase letters indicate pairwise differences among exposure treatments in the mean proportion of stacks laid and mean proportion of multiegg stacks, respectively (acquired through post hoc multiple comparisons of means using Tukey's contrasts). White represents the proportion of single eggs laid, whereas grey and black together equal the proportion of eggs allocated to protection. Grey represents stacks with one protective egg, whereas black represents multiegg stacks.

body size influenced the propensity to stack eggs (body size, $F_{1,31} = 0.06$, $P = 0.8071$; treatment X body size $F_{2,31} = 1.35$, $P = 0.2772$). The effect of treatment is not different when the control is included (exposure time, $F_{1,44} = 10.65$, $P < 0.0022$; body size, $F_{1,44} = 0.05$, $P = 0.8261$; exposure time X body size, $F_{1,44} = 0.27$, $P = 0.6073$). Although there appeared to be a trend for younger beetles to lay a greater proportion of multiegg stacks (2 + protective eggs) than older beetles (see Fig. 1, black bars), this effect of treatment on the proportion of multiegg stacks was not statistically significant (GLM, quasibinomial distribution, logit link function; $F_{2,20} = 0.25$, $P = 0.7815$). However, body size influenced the proportion of multiegg stacks laid ($F_{1,20} = 5.93$, $P = 0.0278$). There was no significant interaction between treatment and body size on the proportion of multiegg stacks that were laid ($F_{2,20} = 0.36$, $P = 0.7061$). These patterns did not change when the control treatment was included (treatment, $F_{3,24} = 0.79$, $P = 0.5148$; body size, $F_{1,24} = 6.72$, $P = 0.019$; treatment X body size, $F_{3,24} = 0.27$, $P = 0.8467$). The weight of eggs did not vary among treatments (Fig. 2, GLM, Gaussian, log link, $\chi^2 = 5.55$, $P = 0.1357$, $n = 55$).

Single egg or stack depositions were recorded daily, but given that we could not record data from beetles during their exposure period to parasitoids, our analysis includes only the days on which we could collect data from every treatment (7 days in total). There was no effect of timing of parasitoid exposure (treatment) on the transformed response of number of egg depositions (linear mixed model, $F_{3,48} = 0.11$, $P = 0.9516$), and no interaction between treatment and time on this response ($F_{18,306} = 0.87$, $P = 0.6120$), which shows that

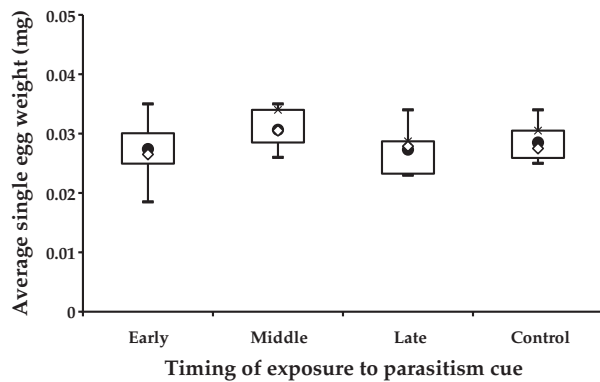


Fig. 2 Among females given access to hosts throughout their lifetime, single egg weight did not vary among treatments. For each symbol, black circles indicate the mean, white diamonds indicate the median, top and bottom borders of the box indicate 25% and 75% quartiles of the mean, and the lines above and below the boxes indicate the minimum and maximum values.

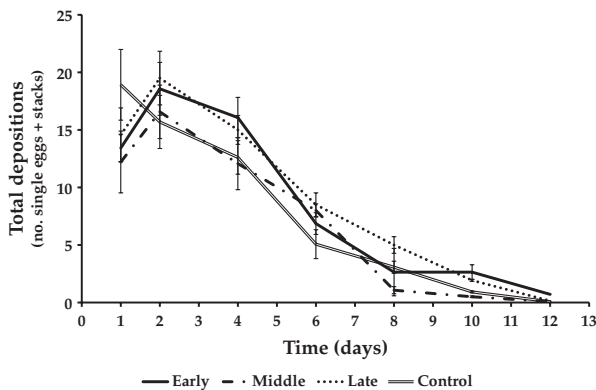


Fig. 3 Among females given access to hosts throughout their lifetime, the total number of depositions made per day decreased significantly over the lifetime of a female. Standard error bars largely overlap until the 6th time point, day 10.

the response did not differ among treatments and the pattern of response across time did not differ among treatments. Time did have an effect ($F_{6,305} = 56.68$, $P < 0.0001$), and a visual pattern of egg laying clearly shows a gradual decrease in depositions made per day (Fig 3). Body weight also influenced the number of depositions a female made ($F_{1,305} = 4.33$, $P = 0.0384$).

Experiment 2: Time limitation conditions

When exposed to parasitoids, females deprived from hosts for 4 or 8 days laid a higher proportion of stacks than females deprived of hosts for 1 day (Fig. 4a,b), and there was no differences among host deprivation periods (treatment) when females were exposed to parasitoids (Fig. 5a,b). Among females exposed to parasi-

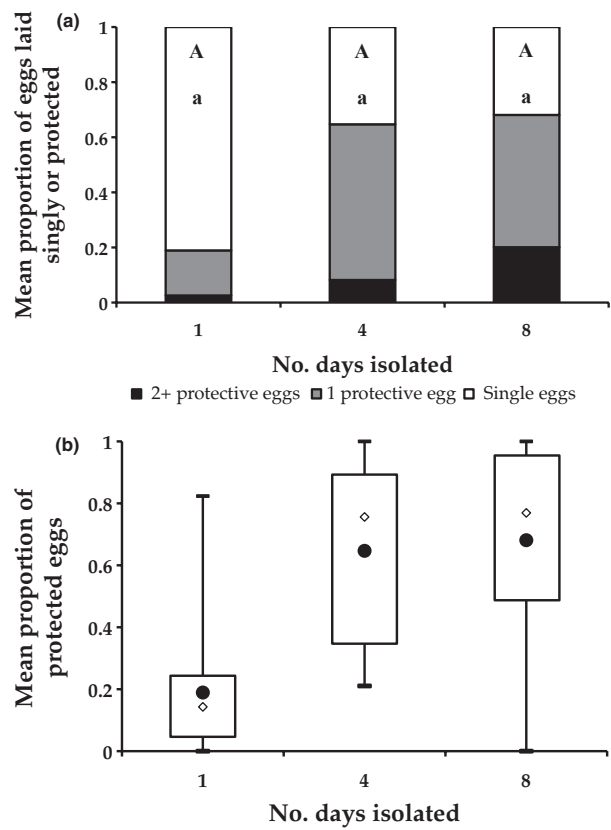


Fig. 4 When exposed to parasitoids, females that were deprived of hosts for longer protected significantly more eggs than females deprived for fewer days. (a) Shows the proportion of stacks and viable eggs. (b) Shows the variation in stacking response. In (a), uppercase and lowercase letters indicate pairwise differences among deprivation treatments in the mean proportion of stacks laid and mean proportion of multiegg stacks, respectively (acquired through post hoc multiple comparisons of means using Tukey’s contrasts). In (b), for each symbol, black circles indicate the mean, white diamonds indicate the median, top and bottom borders of the box indicate 25% and 75% quartiles of the mean, and the lines above and below the boxes indicate the minimum and maximum values.

toids, treatment affected the proportion of stacks laid (GLM, quasibinomial, log link, $F_{1,93} = 50.81$, $P < 0.0001$), but not the proportion of multiegg stacks ($F_{1,88} = 1.23$, $P = 0.2699$). Among females not exposed to parasitoids, treatment did not change the proportion of stacks (GLM, quasibinomial, log link, $F_{1,97} = 1.49$, $P = 0.2252$) or the proportion of multiegg stacks laid ($F_{1,43} = 2.08$, $P = 0.1566$).

When we analysed single egg weights by host deprivation period and parasitoid treatments, only parasitoid exposure contributed significantly to the model (GLM, Gaussian, identity link, $F_{1,166} = 5.65$, $P = 0.0186$), with beetle eggs laid while parasitoids were present being larger on average (absent = 0.028 ± 0.0006 mg, pres-

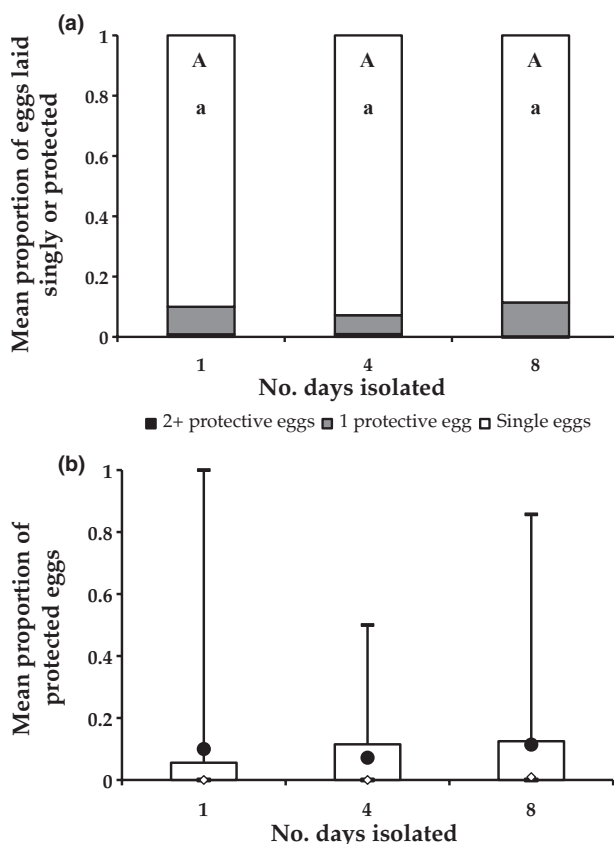


Fig. 5 Host-deprived females not exposed to parasitoids did not vary in their stacking response among treatments. (a) Shows the proportion of stacks and single eggs. (b) Shows the variation in stacking response. In (a), uppercase and lowercase letters indicate pairwise differences among deprivation treatments in the mean proportion of stacks laid and mean proportion of multi-egg stacks, respectively (acquired through post hoc multiple comparisons of means using Tukey's contrasts). In (b), for each symbol, black circles indicate the mean, white diamonds indicate the median, top and bottom borders of the box indicate 25% and 75% quartiles of the mean, and the lines above and below the boxes indicate the minimum and maximum values. While the median number of egg stacks in this treatment was zero, stacking was variable and a very few females stacked most or all of their eggs.

ent = 0.030 ± 0.0004 mg). Host deprivation period did not have a significant effect ($F_{1,166} = 0.55$, $P = 0.4579$), but there was a slightly significant interaction between parasitoid treatment and host deprivation treatment ($F_{1,166} = 3.91$, $P = 0.0497$). Lastly, the number of days a beetle was deprived of hosts also had a significant effect on its egg load (Fig. 6, GLM, Poisson, log link, $\chi^2 = 15.49$, $P < 0.0001$, $n = 227$). Female body weight had a significant effect on egg load ($\chi^2 = 9.60$, $P = 0.0019$). There was also a significant interaction between deprivation time and female body weight ($\chi^2 = 4.35$, $P = 0.037$). Regardless of the number of days isolated, most beetles carried no mature eggs

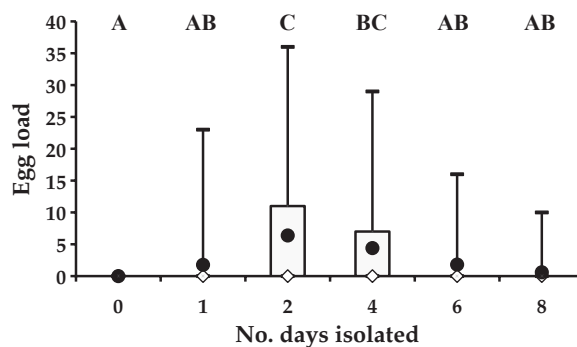


Fig. 6 Among females isolated from hosts throughout their lifetimes, egg load rose, then fell as host deprivation time increased. Most isolated beetles had no eggs (median egg load for all treatments = 0). For each symbol, black circles indicate the mean, white diamonds indicate the median, top and bottom borders of the box indicate 25% and 75% quartiles of the mean, and the lines above and below the boxes indicate the minimum and maximum values. Uppercase letters indicate pairwise differences among deprivation treatments in the mean egg load (acquired through post hoc multiple comparisons of means using Tukey's contrasts).

before encountering hosts (median egg load for all treatments = 0). After two days of isolation, the mean number of mature eggs drops, suggesting that beetles may be resorbing eggs in the interval between two and four days of host deprivation (Fig. 6).

Discussion

Our results provide evidence that the constraints of egg and time limitation in *M. amicus* mediate the egg stacking response to parasitism risk. We show contrasting patterns of stacking behaviour with beetle age, depending on limitations imposed by variation in oviposition opportunities, as predicted by an extension of theory on clutch size decisions in insects. In the egg limitation experiment, females exposed to a parasitism cue late during the oviposition period protected fewer eggs than younger females. Conversely, in the time limitation experiment, females kept without hosts until late in life protected more eggs. We did not find that our other measure of egg protection, the level of stacking, was not variable among treatments in either experiment. When parasitism was absent, the incidence of egg protection was minimal and not variable among host deprivation treatments. Interestingly, we did not find that the timing of exposure to parasitoids (egg limitation experiment) or the duration of host deprivation (time limitation) had an effect on egg size. To our knowledge, this is the first study showing that egg and time limitation mediate an offspring protection behaviour among insects that use discrete resources for oviposition.

Egg size

We predicted that more time-limited females would produce larger eggs, whereas more egg-limited females would produce smaller eggs. Instead, egg size did not vary with treatment (egg limitation experiment, Fig. 2). In general, changes in egg size may require time or may be subject to physiological constraints. In *Stator limbatus*, another seed beetle species that co-occurs with *M. amicus* in *P. florida* and *P. microphyllum*, females respond to host plant quality (represented by seed suitability for offspring development) by modifying egg size, but it takes 72 h before the change in size is complete (Savalli & Fox, 2002). Beetles changing egg size in response to one host imposes a costly time delay (C.W. Fox, personal communication), and this may be due to changes in egg composition these beetles might be making (Fox, 2006). In *M. amicus*, a mechanism for modifying egg size could interfere with the mechanism by which protective eggs are produced, potentially causing a mismatch of investment in top vs. bottom eggs: top eggs might be produced at too small a size to effectively protect the bottom egg, or too large a size and reduce fecundity. Alternatively, our results may simply indicate a nonadaptive physiological constraint. In other studies, authors have more commonly observed a gradual decrease in egg size, number and absolute provisioning over time in insects (Wiklund & Karlsson, 1984; Begon & Parker, 1986; Fox & Czesak, 2000; Geister *et al.*, 2008; Pöykkö & Mänttari, 2012). Some have explained this trend as an unavoidable constraint of dwindling reproductive resources (Wiklund & Karlsson, 1984). It would be difficult to separate these nonadaptive and adaptive explanations for egg provisioning without performing a thorough physiological analysis of absolute (i.e. amount of nutrients) vs. relative (i.e. composition of nutrients) provisioning of nutrients to eggs across time, and tracking changes in offspring development, as has been done in some studies (Giron & Casas, 2003; Karl *et al.*, 2007; Sloggett & Lorenz, 2008). We did find that eggs were slightly heavier in the parasitoid treatments of our host deprivation experiment, but we suspect this may be a simple consequence of changes to the metabolic physiology of eggs due to the presence of parasitoid offspring, as has been found in other systems (Potter & Woods, 2012).

Variation in egg protection

Interestingly, variation in the level of protection per stack was not explained by timing of parasitoid exposure in the egg limitation experiment. Rather, it was the heaviest females (carrying the most eggs) that laid more eggs per stack. This fecundity–body size relationship has been well established for many insect taxa (Honěk, 1993). Further, the incidence of stacking was

not explained by female body size, but by the timing of parasitoid exposure relative to female age. These results suggest that the incidence and level of stacking have not been shaped by the same evolutionary dynamics. Although we did not vary parasitism risk continuously in this experiment, we have found in our previous study that beetles lay larger stacks as parasitism risk increases, and larger stacks confer better protection in environments where wasps are able to attack bottom eggs (Deas & Hunter, 2012). In environments of high parasitism risk, there is an advantage to having higher potential fecundity, and we should see selection for larger female body size. While the expression of both egg protection traits are initially dependent on parasitism cues, body size mediates the level of protection an egg receives, and the number of eggs a mother actually protects is dynamic (female age) and context dependent (host availability).

Although our data do not seek to link individual variation in egg maturation rate and egg protection behaviour, the maximum rate of egg maturation has been argued to be the fundamental constraint on reproductive success in synovigenic species (Rosenheim, 1996; Casas *et al.*, 2000; Rosenheim *et al.*, 2000) and may be a substantial source of variation in the incidence of egg protection we observe in *M. amicus*. We found wide variation in the mean proportion of eggs a particular beetle protects regardless of the presence or absence of parasitism risk. Even when parasitism risk was absent, we observed some individuals protecting most of their eggs (see maximum proportion of stacks, Fig. 5b). Conversely, when parasitism risk is present, some individuals never protect eggs (Fig. 4b). We found highly variable egg loads as well (Fig. 6): while the median egg load of females isolated without hosts was consistently zero, some females had as many as 35 eggs in their ovaries. In stacking females, higher egg maturation rates when parasitism risk is lower may still trigger high egg stacking frequency, and low egg maturation rates when parasitism risk is higher may dampen stacking rate.

Reproductive costs associated with synovigeny

In this seed beetle system, the use of eggs for protection may be especially costly. None of the newly emerged *M. amicus* females carried any eggs, indicating that this beetle has an ovigeny index of zero (Fig. 6). The ovigeny index, calculated as the number of mature eggs at emergence divided by the total eggs produced, was devised to portray the range of egg maturation schedules between strict synovigeny (0) and strict pro-ovigeny (1) (Jervis *et al.* 2001), and by any scale, *M. amicus* is strictly synovigenic. Synovigeny has been shown to confer reproductive plasticity to females, allowing them ample time to modify reproductive effort in response to variation in resource availability (Ellers & Jervis, 2003;

Jervis & Ferns, 2004). However, the flexibility in resource allocation that is representative of synovigeny may expose females to other types of costly egg limitation (Rosenheim *et al.*, 2000). Among females given access to hosts, oviposition rate decreased with time (Fig. 3), suggesting that females may experience transient egg limitation later in life, where the rate of oocyte maturation is so low that they must periodically wait for more eggs to mature before they can lay them (Tatar, 1991; Casas *et al.*, 2000; Rosenheim *et al.*, 2000). This waiting phenomenon is found in strongly synovigenic (and reproductively plastic) species and may impose additional reproductive costs, namely increased risk of mortality, the progression of senescence and lost oviposition opportunities for hosts available for a short period (Rosenheim *et al.*, 2000).

We also found that beetles resorbed eggs in response to low host availability, which is also commonly found in synovigenic parasitoids when host access is restricted (Flanders, 1942; Droste & Carte, 1992; Rosenheim *et al.*, 2000; Asplen & Byrne, 2006). Although egg load seemed to increase initially under host deprivation (Fig. 6), it declined after two days and the mean number of eggs in these host-deprived beetles was many fewer than the mean number of eggs laid by beetles with hosts (Fig. 3). Subsequent experiments using host deprivation have revealed substantial losses of eggs near 8 days of deprivation (unpublished data, JBD). As oviposition opportunities decrease over time, eggs may be consistently resorbed to maintain metabolism, which allows extra search time for more or better quality hosts, but depletes eggs that can be laid when oviposition opportunities become available.

In conclusion, we determined that when exposed to parasitism risk, female senescence and host availability appear to influence when and how much a female will invest in offspring protection. The results of this study suggest that egg and time limitation mediate the egg stacking strategy in *M. amicus* in contrasting ways, similar to the effects of these constraints on clutch size decisions and the inclination to superparasitize in other systems. When more egg limited, younger female beetles showed a stronger stacking response than when exposed to the same level of parasitism risk at an older age. When more time limited, the stacking response is stronger in older females than in younger females. Additionally, we have evidence that prior to stacking eggs, females may decrease oviposition rate and avoid laying on seed pods with parasitized eggs (Deas & Hunter, 2013). The results of that study also suggested that *M. amicus* is very sensitive to cues indicating parasitism risk; stacks accounted for approximately 25% of ovipositions by 4 h and 60% of ovipositions by 48 h, in response to the presence of parasitized eggs (Deas & Hunter, 2013). Taken together, our results suggest that beetles' perceptions of host abundance, quality and nat-

ural enemy cues all interact to produce complex patterns of oviposition behaviour. That these patterns can be largely predicted by extending the theory of time and egg limitation shows the durability of this framework and the remarkable reproductive plasticity of this species.

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