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Mothers modify eggs into shields to protect offspring from parasitism

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Eggs are an immobile, vulnerable stage of development and their success often depends on the oviposition decisions of the mother. Studies show that female animals, and sometimes males, may invest parental resources in order to increase the survival of their offspring. Here, we describe a unique form of parental investment in offspring survival. The seed beetle *Mimosestes amicus* may lay eggs singly, or may cover eggs with additional egg(s). This egg stacking serves to significantly reduce the mortality of the protected egg from parasitism by the parasitic wasp, *Uscana semifumipennis*. The smaller top eggs serve only as protective shields; they are inviable, and wasps that develop in them suffer negative fitness consequences. Further, we found egg stacking to be inducible; *M. amicus* increase the number of stacks they lay when parasitoids are present. However, stacking invokes a cost. When wasps are absent, beetles lay more single eggs, and produce more offspring, highlighting the adaptive value of this extraordinary example of behavioural plasticity in parental investment.

Keywords: egg stacking; offspring quality versus quantity; life-history trade-offs; seed beetles; *Mimosestes amicus*; offspring defence

1. INTRODUCTION

As the sedentary stage of an insect's life cycle, eggs are the most vulnerable of all developmental stages. Because of the variety of biotic and abiotic sources of mortality risk (e.g. desiccation, cannibalism and predation) for eggs in the environment, oviposition behaviour by the mother may be an important determinant of their success. Whether females invest in offspring survival through increasing egg size, or by hiding or protecting them, females may adjust investment in offspring in order to match the severity of environmental conditions [1]. In general, the reproductive value of offspring increases as investment per offspring increases [2]. Typically, high-investment eggs result in increased survival to reproduction through a variety of mechanisms [3] such as an increase in the ability to overcome or detoxify low-quality resources [4] or provide a competitive advantage when resources are scarce or conditions are crowded [5–7]. Low-investment eggs are advantageous typically when environmental conditions are less severe and the demands on offspring quality are reduced.

Generally, these patterns of parental investment correspond to adjustments to egg size or offspring phenotype. Egg placement also confers benefits such as increased offspring survival. In studies on the water strider *Aquarius palidum*, females were shown to lay eggs deeper in the water to keep them out of reach of parasitoids, but at an increasing cost of mortality owing to water pressure [8]. Female golden egg bugs lay eggs on conspecific males or females to protect them from parasitoids, but the bright, yellow eggs make the egg-carriers significantly more conspicuous to predators [9]. In this context, parental investment is costly and occurs at any stage of oviposition (e.g. site selection or placement and adjustments to size or development).

In this study, we describe a behaviour that may be unparalleled in the animal kingdom, in which females deposit some of their eggs as shields for eggs below; these top eggs serve no other purpose than to protect other eggs from being attacked by natural enemies. We tested whether the unique 'egg-stacking' behaviour of the seed beetle *Mimosestes amicus* influenced offspring survival in response to the principle mortality threat, high rates of egg parasitism by *Uscana semifumipennis*. In the field, *M. amicus* lays eggs either singly or in stacks (figure 1a). The observed variation in stacking behaviour, and the apparent use of the same currency (eggs) for defence and reproduction led us to examine this trait in the context of parental investment in offspring survival, and phenotypic plasticity in offspring quality [1,5,10,11]. We asked first about the adaptive value of this behaviour: (i) does egg stacking protect eggs from parasitism and/or desiccation? We then investigated the nature of these top egg 'shields' compared with bottom eggs, and asked: (ii) do top and bottom eggs differ in size or quality? Finally, we were interested in whether female beetles showed plasticity in stacking with respect to the risk of parasitism, and asked: (iii) does the presence of parasitoids induce the stacking behaviour in *M. amicus*? We used field observations and laboratory experiments to answer these questions.

2. MATERIAL AND METHODS

(a) *Study system*
All seed beetles are within the subfamily Bruchinae (Coleoptera: Chrysomelidae) and lay eggs on the outside of seed pods or on the seeds themselves. The larvae then hatch and burrow into the centre of the seed to complete development. Generally, after 25–30 days, adults cut holes in the seeds, emerge and mate. *Mimosestes amicus* lay eggs on the outside of the seed pods of one of 14 North and South American species within

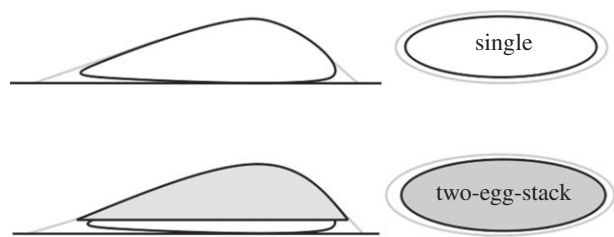* Author for correspondence (jbdeas@email.arizona.edu).

Figure 1. Cartoon side-view and top-view (labelled) of single eggs and a two-egg-stack. Grey lines represent attachment glue.

the legume genera *Acacia* (5), *Parkinsonia* (5) and *Prosopis* (4) [12]. When we sampled *M. amicus* eggs from *Parkinsonia florida* (blue palo verde) and *Parkinsonia microphyllum* (foothill palo verde) trees from three sites in southern Arizona (USA), we observed an average level of 71 per cent parasitism by *U. semifumipennis* (Hymenoptera: Trichogrammatidae), a solitary parasitoid that is a specialist on seed beetle eggs [13]. We also observed egg stacks. These stacks are formed when one egg (less often, two or three) is laid directly on the top of another egg laid by the same female (figure 1*a*). The top egg is flattened such that it completely covers the egg underneath. The bottom egg is hidden so well that a freshly laid egg stack at first appears simply to be a slightly larger egg.

Egg stacking in *M. amicus* was first documented by Kunhikannan [14], and was later confirmed by Swier [15], who observed it in *M. amicus* and other seed beetle species that attacked *Prosopis* spp., and speculated that the behaviour served to protect against parasitism. It was most recently noted by Mitchell [16], who observed the death of top eggs that were unparasitized, and hypothesized that egg stacking served to protect against parasitism and desiccation. He also hypothesized that the behaviour was a bet-hedging strategy in which beetles laid single eggs until parasitism of single eggs reached some threshold, and then produced a mixture of both single eggs and stacks.

(b) *Insect rearing*
Uninfested seedpods for rearing beetles were collected from *P. microphylla* (foothill palo verde) trees in Tucson, AZ, USA and stored at −20°C for at least one week to exterminate other insects. Foothill palo verde is a better host for *M. amicus* than blue palo verde (*P. florida*) but both are commonly used by the beetles in nature [17]. All beetles used in experiments were the offspring from eggs laid in the field by beetles. All parasitoids used in experiments were reared on the eggs of *Callosobruchus maculatus*, another seed beetle species, and these beetles were reared on cowpea seeds (*Vigna unguiculata*).

(c) *Does egg stacking protect eggs from parasitism and/or desiccation?*
(i) *Effect of egg type on rates of parasitism in the field*
One hundred infested blue palo verde seed pods were collected from each of three sites in Tucson (Oro Valley, 32°23′35.76″ N, 110°57′15.72″ W; Sentinel Peak, 32°12′49.08″ N, 110°59′57.75″ W; St Mary's Road, 32°13′40.58″ N, 111°00′15.54″ W). Relatively large (approx. 4 mm) and circular emergence holes in the seed pods, or *M. amicus* eggs on the surface of the seed pods, were used as confirmation of infestation by this species. In the laboratory, eggs were scored for whether or not they were in stacks and for evidence of parasitism. Parasitized eggs were identified by the appearance of the red eyes of a developing wasp pupa, or a dark brown to black coloration of the eggs. Unparasitized

eggs were identified by their cloudy, yellow coloration, the head capsules of beetle larvae in various stages of development, or white frass that had been defecated into the empty eggshell during larval penetration into the seed.

(ii) *Laboratory effects of parasitism and humidity on eggs laid singly versus in stacks*
Female and male beetles emerging from field-collected foothill palo verde seed pods were sorted into groups of 20–30 beetles each in 50 ml Falcon tubes without hosts. Eggs for the experiment were produced by 15 females placed together with 15 males in a container holding 300 uninfested foothill palo verde seed pods. Owing to variation in the timing of beetle emergence, the experiment was run in three blocks. In each block, beetles were allowed to mate and lay eggs for 2 days before seed pods were inspected for eggs. Then seed pods were separated into 9 ounce plastic cups with approximately 30 *M. amicus* eggs per cup and covered with lids with a fine-mesh fabric centre. Pods were assigned to either a high (65%) or a low (35%) relative humidity (RH) treatment and either a 'parasitoids present' or 'parasitoids absent' treatment, for 15 replicates in each treatment combination. 'Parasitoids present' cups had one female and two male *U. semifumipennis* added. After 3 days, at which time eggs became unsuitable for attack, the pods were removed and eggs were scored for presence/absence of parasitism, stack position and the cause of larval beetle mortality.

(d) *Do top and bottom eggs of a stack differ in size or quality?*
(i) *Egg size*
Beetles emerging from field-collected foothill palo verde pods were isolated in one ventilated plastic container, in an environmental chamber set to 27°C at 60 per cent RH with paper towels and water. After 24 h, the paper towels were removed and replaced with 200 uninfested blue palo verde seed pods. Beetles were allowed to mate and lay eggs for 24 h. Afterwards, single, bottom and top eggs were collected from the container. Eggs of each type were weighed in groups; each data point represents the average of five eggs ($n = 6$).

(ii) *Effect of top versus bottom eggs on wasp fitness*
Parasitoid fitness depends on both host egg size and nutritional quality, and thus provides an indirect measure of egg quality. Parasitized eggs from the laboratory experiment described above were removed from the pods and placed in a 0.25 dram vial in an environmental chamber at 27°C and 60 per cent RH until emergence. Development time (in days) was recorded and a sub-sample of emerged wasps was dissected to measure hind tibia length.

(e) *Does the presence of parasitoids induce the stacking behaviour in* Mimosestes amicus*?*
Beetles emerging from field-collected foothill palo verde pods were isolated in one ventilated plastic container with paper towels and water. After 48 h, male and female pairs of beetles were placed in 9 ounce containers with 20 foothill palo verde seed pods, in an environmental chamber set to 27°C and 60 per cent RH. Fifty pairs received 10 parasitoids each, while the other 50 received no parasitoids. Female beetles were allowed to lay eggs for 10 days. For each female, we recorded the total reproductive effort, the proportion of all eggs laid that were in stacks and the number of eggs per stack. Because we had discovered that top eggs were inviable, we considered each egg stack or single egg as one unit of reproductive effort.
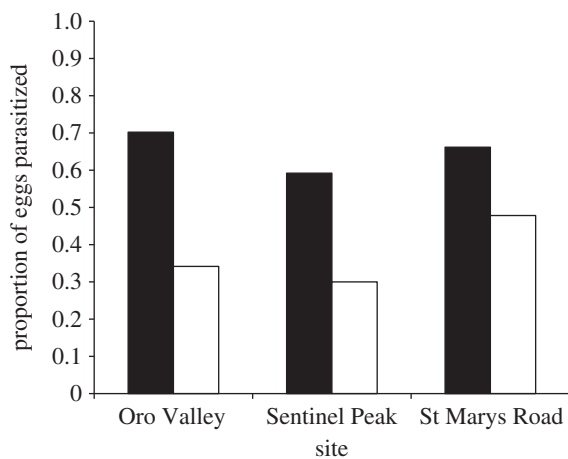
Figure 2. Field evidence of the effectiveness of egg stacking against parasitism. Unprotected eggs (i.e. single eggs) experienced proportionally higher parasitism than protected eggs (i.e. bottom eggs in a stack) at all sites visited. As indicated in the text, sites are variable in both the level of stacking and the level of parasitism. (Oro Valley, $n = 208$; Sentinel Peak, $n = 416$; St Marys, $n = 160$). Filled bars, unprotected; open bars, protected.

(f) Statistical analyses

To determine whether stacking protects against parasitism, the proportion of eggs parasitized was analysed in a logistic regression framework. Similarly, logistic regression was used to analyse the proportion of all reproductive events that consisted of stacks ('proportion of stacks') in different 'parasitoid treatments' (parasitoids present or absent). In logistic regressions, 'beetle mortality' [1/0], 'proportion parasitized' or 'proportion of stacks' were treated as dependent or response variables, and 'humidity' (high/low), 'site' (Colossal Cave/Oro Valley/St Marys), 'egg type' [bottom/top/single], 'protection' [1/0] or 'parasitoid treatment' [1/0] were treated as independent or explanatory variables. Two-tailed t-tests were used to test whether wasp size or development time was significantly different when wasps developed on top eggs as opposed to single eggs, as well as to determine whether the number of eggs in a stack varied with respect to treatment. Contingency table analyses were performed on the level of stacking across field sites, as well as on the survivorship of top versus single eggs in the laboratory. An ANCOVA was performed on the number of reproductive events (stacks and single eggs) in which explanatory variables were both continuous (proportion of stacks) and categorical (parasitoid treatment). All statistical analyses were performed using JMP v. 7.0 (SAS Institute Inc., Cary, NC, USA, 1989–2007).

3. RESULTS

(a) Does egg stacking protect eggs from parasitism andlor desiccation?

(i) *Effect of egg type on parasitism rates in the field*

Overall, across three sites, whether or not an egg was protected (i.e. the bottom in a stack versus an egg laid singly) had a strong effect on whether or not it was parasitized (figure 2; logistic regression, $\chi^2 = 57.6$, $p < 0.0001$, $n = 784$). 'Site' as an independent variable contributed significantly to the fit of the model (likelihood ratio (LR) test, $\chi^2 = 7.05$, $p = 0.03$), suggesting among-site variation in the level of egg parasitism. We found no
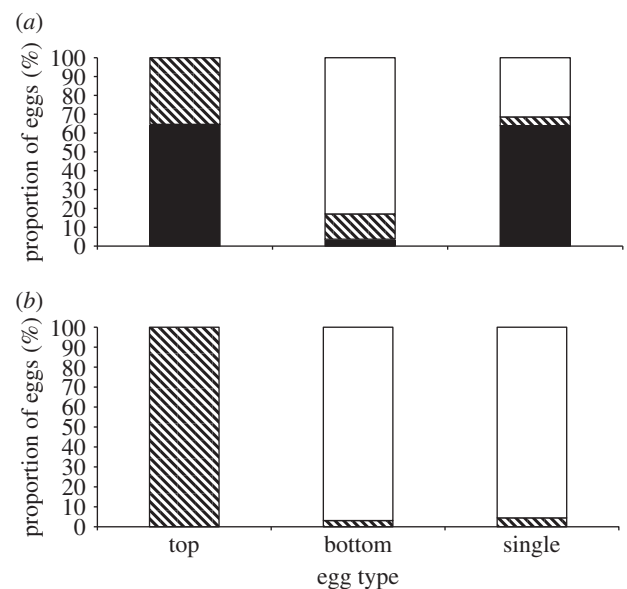
Figure 3. Laboratory evidence of performance of egg types, in particular the complete inviability of top eggs. Humidity data were pooled, as it had no effect on mortality among egg types. Data are presented by parasitoid treatment: (a) presence or (b) absence. All top eggs died, regardless of humidity treatment, while survivorship of bottom or single eggs was similar for both humidity treatments. Similar to our field observations, protected eggs (bottom) suffered proportionately less parasitism than unprotected (single) eggs. (a) Filled bars, parasitized; bars with striped lines, other mortality; open bars, eggs hatched. (b) Bars with striped lines, other mortality; open bars, eggs hatched.

interaction between 'site' and 'protection', indicating little variation in the effectiveness of stacking across sites. Interestingly, sites differed overall in the proportion of protected eggs (contingency table analysis, LR test, $\chi^2 = 203.0$, $p < 0.0001$, $n = 787$) and they parallel the variation in parasitism of single eggs at these sites, with Sentinel Peak having the lowest proportion of protected eggs (0.22) and lowest parasitism rate of single eggs (0.59), followed by St Mary's (0.57 protected eggs and 0.66 single-egg parasitism), and then Oro Valley (0.78 protected eggs and 0.70 single-egg parasitism).

(ii) *Laboratory effects of parasitism and humidity on eggs laid singly versus in stacks*

Beetle egg mortality differed significantly among egg types (logistic regression using 'beetle mortality' as the response variable, and 'humidity level' and 'egg type' as explanatory variables, $\chi^2 = 355.9$, $p < 0.0001$). There was no interaction between 'egg type' and 'humidity level' ($F_2 = 0.04$, $p = 0.98$), suggesting that the relationship between beetle mortality and egg types was not affected by humidity. Therefore, data from low- and high-humidity treatments were pooled for further analyses. Across blocks, there were differences in parasitism among top, bottom and single eggs (stratified by block using Cochran–Mantel–Hanzel test: $n = 918$, d.f. $= 2$, $\chi^2 = 113.1$, $p < 0.0001$). Differences in parasitism between single (unprotected; $n = 754$) eggs and bottom (protected; $n = 82$) eggs in the 'parasitoids present' treatment (figure 3a) are similar to the field results (figure 2). Top eggs in this treatment are either parasitized or

Table 1. A comparison of wet weight, larval (beetle and wasp) survivorship, parasitoid development time and parasitoid body size for the different egg types laid by *M. amicus* females. (Beetle survivorship proportions are from the 'parasitoids-absent' treatment. Different letters following values in a particular row indicates statistically significant differences (Tukey–Kramer multiple comparisons method).)

egg type	single egg	top egg (stack)	bottom egg (stack)
mean egg weight (mg)	0.036 ± 0.002 [a], $n = 6$	0.021 ± 0.002 [b], $n = 6$	0.043 ± 0.003 [a], $n = 6$
beetle survivorship	0.93, $n = 1099$	0.00, $n = 93$	0.93, $n = 143$
parasitoid survivorship	0.93, $n = 483$	0.74, $n = 53$	1.0, $n = 3$
mean parasitoid development time (days)	11.44 ± 0.05 [a], $n = 451$	13.63 ± 0.30 [b], $n = 39$	11.83 ± 0.33 [a], $n = 3$
mean parasitoid body size (micrometre)	24.53 ± 0.18 [a], $n = 51$	22.62 ± 0.24 [b], $n = 39$	24.50 ± 0 [ab], $n = 3$

inviable. Single and top eggs were parasitized at similar rates. All top eggs ($n = 64$) from the 'parasitoids absent' treatment (figure 3b) were also inviable, while bottom ($n = 64$) and single eggs ($n = 827$) experienced high survivorship. In the parasitism treatment, top eggs and single eggs generally both supported the development of parasitoids, (but see §3b(ii), below and table 1). Some unparasitized top eggs showed signs of larval development (i.e. head capsules and a characteristic larval form) before dying, suggesting that at least some top eggs are fertilized, but this was not tested experimentally.

(b) Do top and bottom eggs differ in size or quality?
(i) *Egg size*
Egg weights differed significantly among top eggs of a stack, bottom eggs and single eggs (one-way ANOVA, $F_{2,15} = 31.4$, $p < 0.0001$). The average top egg in a stack was approximately half the weight of single or bottom eggs (table 1). The weights of single and bottom eggs were not significantly different from each other (using Tukey–Kramer test for multiple comparisons).

(ii) *Effect of top versus bottom eggs on wasp fitness*
Survivorship of wasp parasitoids was significantly lower in top eggs than in single eggs (table 1; LR test, $\chi^2 = 17.1$, $p < 0.0001$). Only three out of 82 bottom eggs were parasitized; these eggs all produced parasitoid wasps but were too few to be included in the likelihood ratio test and ANOVA. Wasps that survived in top eggs had significantly longer development time (two-tailed t-test, $t_{39} = 7.05$, $p < 0.0001$) and smaller body size (two-tailed t-test, $t_6 = 73.5$, $p < 0.0001$) than wasps that developed in single eggs (table 1).

(c) Does the presence of parasitoids induce the stacking behaviour in Mimosestes amicus?
Exposure to parasitoids caused an increase in the proportion of egg stacks a beetle laid (figure 4; logistic regression, $\chi^2 = 58.6$, $p < 0.0001$) as well as an increase in the average number of eggs per stack (two-tailed t-test, $t_{37} = 6.04$, $p < 0.0001$). Unexposed beetles laid no more than two eggs per stack (mean of 2 ± 0 s.e. eggs per stack), whereas stacks laid by exposed beetles were often composed of two to three eggs (mean 2.23 ± 0.04 s.e. eggs per stack).

The results of this experiment also suggested that egg stacks were costly. Overall, beetles that laid more stacks had lower lifetime reproductive effort (figure 5; ANCOVA, $F_{3,77} = 38.4$, $p < 0.0001$, $r^2 = 0.60$). In our analysis, we found a significant interaction between the proportion
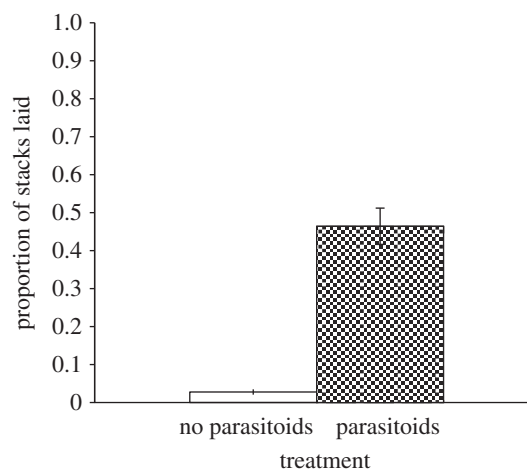

Figure 4. Parasitoid presence induces egg stacking response in beetles. Beetles exposed to parasitoids ($n = 41$) produced a significantly higher proportion of egg stacks during their lifetime than beetles not exposed to parasitoids ($n = 40$).

of stacks and parasitoid treatment (absence/presence; $F_1 = 8.78$, $p = 0.004$), with stacking leading to a steeper decline in reproductive effort in the 'parasitoids absent' treatment than in the 'parasitoids present' treatment.

4. DISCUSSION
Here, we provide evidence for the hypothesis that egg stacking in the seed beetle *M. amicus* is a protective behaviour that significantly reduces egg mortality caused by the egg parasitoid, *U. semifumipennis*. Field and laboratory data show that protected eggs suffer significantly less egg parasitism than unprotected, singly-laid eggs, while humidity differences did not have an effect on egg mortality, regardless of type. To our knowledge, this is the first described example of an egg that has no function other than protecting viable eggs from natural enemies. Top eggs were approximately half the weight of bottom eggs in the same stack or of singly laid eggs, suggesting large differences in egg quality. This is corroborated by the relatively poor performance (measured as survivorship, body size and development time) of parasitoid offspring that developed in these eggs compared with those developing in single eggs. We also found that egg stacking is inducible; beetles respond to the presence of parasitoids by greatly increasing the number of stacks. That stacks are costly is suggested by the relationship between the proportion of stacks a beetle laid and its total lifetime reproductive effort
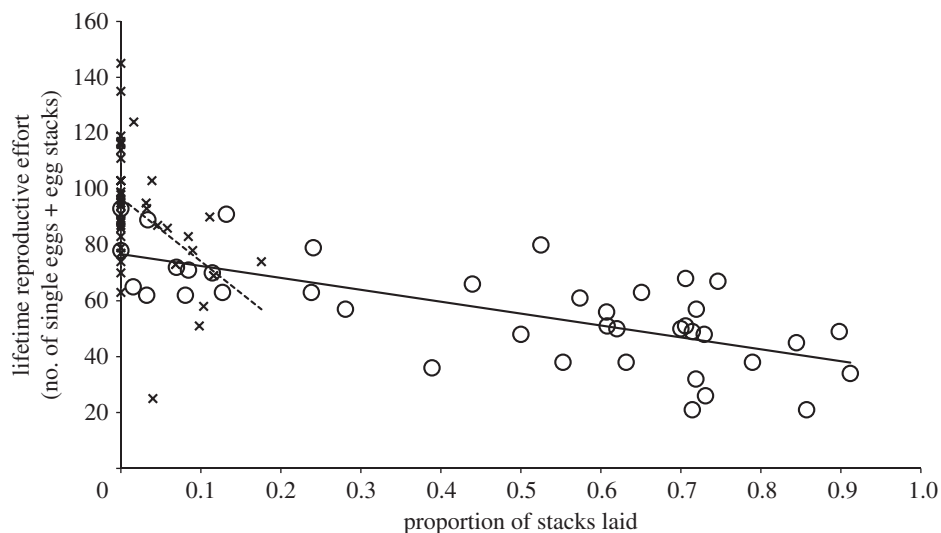
Figure 5. The cost of parental investment in egg protection. Overall, beetles that laid more stacks had lower lifetime reproductive effort. Data points with a cross symbol (×) were in the 'parasitoids absent' treatment while data points with an open circle symbol (○) were in the 'parasitoids present' treatment. The dashed regression line shows the relationship for unexposed beetles and the solid regression line shows the relationship for exposed beetles.

(number of individual stacks + individual eggs). The higher the proportion of stacks a beetle laid, the lower the number of viable eggs that were produced. In this analysis, we also found an interaction between the proportion of stacks a beetle laid and whether or not they were exposed to parasitoids (figure 5; beetles unexposed to parasitoids had a steeper slope). The shallower slope in beetles exposed to parasitoids represents a lower number of viable eggs produced per stack, which suggests that there may be some extra cost incurred during egg stacking (e.g. stress or time taken protecting eggs). However, this pattern has not been repeated in recent experiments. The apparent cost of stacking underscores the adaptive value of the plastic response to the presence of parasitoids; beetles match offspring quality (a single egg versus a protected egg) to the risk of parasitism.

We found that *M. amicus* can lay distinctly different eggs contemporaneously, some of which serve an exclusively defensive function. Among animals, the best-described eggs with a non-reproductive function are called trophic eggs, and they have been noted in mostly descriptive studies on the reproductive ecology of sharks, amphibians, insects, spiders and snails (reviewed in Perry & Roitberg [18]). Their adaptive value seems apparent in systems in which, compared with viable eggs, they have a different morphology or colour [19–21], are underdeveloped [22] or are actively fed to offspring by the parents [23]. The two main hypotheses regarding the functions of trophic eggs include offspring feeding when starvation risk is high or resources are scarce and/or reducing sibling cannibalism [24,25]. The fact that at least some top eggs laid by *M. amicus* have been fertilized brings up an interesting question about the classification of eggs that contain DNA, but function solely to protect other eggs or satiate other offspring. In eusocial insects, trophic eggs may be considered another 'sterile caste', in that they have a specific function that increases some aspect of colony survivorship (e.g. workers that provide brood care and soldier morphs that defend habitats and individuals from predation) [26]. However, in solitary insects, the production of non-reproductive eggs would seem to fit better under the broader category of polyphenism, and a classification system for these eggs would benefit from knowing the mechanisms that control their production in different systems.

Research on parental care in subsocial heteropterans provides an interesting perspective on the use of eggs for a defensive function. Various species lay clutches of eggs, and then shield them with their bodies. In *Elasmucha ferrugata* (Heteroptera: Acanthosomatidae), centrally located eggs are larger than eggs laid at the periphery of the clutch, have higher survivorship just owing to their size, and also receive greater protection from predation [27]. In *Adomerus triguttulus* (Heteroptera: Cydnidae), fertile egg clutches are covered with inviable eggs that serve to provision offspring and may secondly protect them from natural enemies, but only the parental guard behaviour has been specifically tested as being an effective deterrent against predation [28]. In these systems, predation risk to eggs does not depend on size, but on their position [29]. Females may have been selected to invest less in peripheral eggs because it is less costly. It has been suggested that egg size variation may be owing to physiological constraints during oogenesis [30], and if this is the case, trophic eggs may be the exaptation of oocytes that are too immature to be fertilized. Females may then bias the placement of eggs that differ in resource quality (or some other abiotic or biotic factor) using simple behavioural rules [31].

That there are few records of animals producing eggs solely for defence is perhaps not surprising when one considers the nutrients required to produce these reproductive units. Why does *M. amicus* sacrifice costly eggs for improving individual offspring quality instead of using other materials? Faecal matter is used as a protective covering in many insect taxa at all life stages, and in nearly 20 per cent of species in the family Chrysomelidae, this trait is well-developed [32,33]. In terms of allocating resources to reproduction, producing faeces seems less costly to offspring and maternal fitness to produce.

However, faeces may be highly effective at concealing eggs from one natural enemy, and a highly effective attractant for other natural enemies that can circumvent the defence [34]. Additionally, in species where the adults are non-feeders and may use most food consumed as larvae for growth and reproduction (as in *M. amicus*), or the faecal material is not substantial or malleable, faeces may not be the best material for egg concealment. Other secretions could also potentially provide protection. In seed beetles, eggs are completely covered by, and cemented to a seed or seed pod by a secretion originating from the follicle cells of each egg or female accessory glands [35]. It is unclear, however, how costly this secretion is, whether any ingredients are limiting for the beetles, or how much females would need to produce to secure attachment or deter parasitism of an egg. Concealing the eggs rather than protecting them in place would seem like another alternative. Hiding eggs in cracks and small holes has been observed in the Bruchinae [35]. Alternatively, some bruchines lay eggs directly on the seeds, concealed within the closed pods, using exit holes of other bruchines for ingress [36]. However, hiding eggs outside the pods is only possible if the oviposition substrate has cracks in which to conceal eggs, and natural enemies cannot find or reach them. Further, laying eggs within the pods excludes beetle access to a potentially large resource of undamaged pods and seeds. Finally, it is possible that the use of an egg for defence is advantageous because it exploits the oviposition behaviour of the attacker. If wasps anticipate a single opportunity for oviposition when encountering an egg, they may leave after ovipositing in the top egg, while coatings or protective secretions might simply increase wasp tenure time and persistence. Alternatively, if the wasp–beetle interaction is highly localized, the 'waste' of a wasp egg in a low-quality beetle egg may pay fitness benefits to the beetle in terms of reduced threat to other offspring.

In general, seed beetles (Chrysomelidae: Bruchinae) show remarkable reproductive plasticity in a number of traits. Female seed beetles have been shown to make adjustments to egg quality in response to variation in host plant quality [4,37,38], resource availability or population size [7]. The reason for the extraordinary plasticity in this group is not known, but one might speculate that some aspect of allocation of resources to eggs is under less stringent control in seed beetles than in other systems. For whatever reason, this group of organisms appear to be excellent models for the study of plasticity of parental investment in offspring survival.

For *M. amicus* in particular, the classic Smith & Fretwell [2] trade-off between offspring quality and quantity is embedded in a novel ecological context; where quality is improved not by a continuous size adjustment, but rather by discrete egg increments that contribute to a higher probability of surviving parasitism. In *M. amicus*, top eggs contribute to the fitness of bottom eggs but never become offspring, thus a stack equals an egg with higher parental investment. Further, field data suggest that each supernumerary protective egg reduces the probability of parasitism. While the laboratory experiment showed almost perfect protection from parasitism with a two-egg stack, recent field data averaged over two collection times showed reductions in parasitism of bottom eggs with each additional egg in the stack (proportions of bottom eggs parasitized for Colossal Cave and sample sizes: single (0.47, $n = 124$), two-egg stack (0.35, $n = 88$), three-egg stack (0.1, $n = 51$) and four-egg stack (0, $n = 3$); proportions for Sentinel Peak: single (0.45, $n = 383$), two-egg stack (0.41, $n = 280$), three-egg stack (0.08, $n = 291$) and four-egg stack (0.05, $n = 21$); contingency table analyses using LR tests—Colossal Cave, LR $\chi^2 = 27.24$, $p < 0.0001$; Sentinel Peak, LR $\chi^2 = 148.96$, $p < 0.0001$). Stacks with four eggs only appeared in the second collection, late in the season when rates of parasitism of bottom eggs were higher than at the first collection (J. B. Deas 2010, unpublished data). By increasing both the stacking rate and the number of eggs in a stack, beetles responded to wasp presence in a manner likely to protect the most offspring from parasitic wasp attack. These observations are suggestive of coevolutionary dynamics of beetle egg stacking defence and wasp countermeasures to find and parasitize protected eggs. Future work will address how beetles assess parasitism risk, as well as how the level of risk is matched with an appropriate level of offspring defence.

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