



Delay, avoidance and protection in oviposition behaviour in response to fine-scale variation in egg parasitism risk



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Organisms that do not provide parental care are challenged with multiple factors and risks in the selection of an oviposition site. Oviposition site selection greatly affects offspring fitness, but little is known about how females respond to fine-scale variation in environmental cues. The seed beetle, *Mimosstes amicus*, shows remarkable behavioural plasticity in response to variation in egg parasitism cues. When exposed to egg parasitoid adults, females superimpose eggs atop each other to protect bottom eggs from parasitism. Here, we examined egg protection behaviour in response to the microspatial distribution of parasitized eggs. We exposed females to treatments varying in the number and dispersion of parasitized eggs on seed pods. Our results showed that oviposition behaviour was influenced by the evenness of the distribution of parasitized eggs and suggest that *M. amicus* exhibits a conditional strategy on a highly localized 'pod-by-pod' basis. When pods bore no eggs or unparasitized eggs, beetles laid the greatest number of eggs, almost all singly. In contrast, stacking was greatest and oviposition most reduced when parasitized eggs were distributed across all of the five pods provided. Lastly, females avoided ovipositing on seed pods with parasitized eggs when other oviposition sites were available. In general, avoidance behaviour increased, stacking increased and oviposition decreased as the number of pods (one, three or five) with parasitized eggs increased. Our results provide novel evidence of an oviposition strategy that combines both risk avoidance and offspring protection. Avoidance behaviours and reductions in oviposition rate are likely to be obscure among animals and may be more common than has been documented to date.

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In terrestrial, egg-laying organisms that lack parental care, the choice of an oviposition site is a critical one, and mothers may weigh a complex set of factors and risks in deciding where to place their eggs. During oviposition, there are many different cues a female is exposed to; if the maternal environment is predictive of the offspring environment, we would expect that selection would produce mechanisms under maternal control that enhance offspring survival (Mousseau & Dingle 1991; Fox & Mousseau 1998; Mousseau & Fox 1998). These may include alterations to offspring size or development, in which females may impart cytoplasmic factors to eggs, based on the state of the environment and the mother's physiology (Ho & Burggren 2010). Given the importance of offspring survival in achieving reproductive success, the degree to which maternal environment, development and behaviour influence offspring fitness will determine the

likelihood that they will be shaped by natural selection (Mousseau & Fox 1998).

Various biotic and abiotic factors influence oviposition site selection in various taxa. Nest site or oviposition site preferences based on microclimate variables have been documented in birds (Lloyd & Martin 2004), nonavian reptiles (Shine & Harlow 1996; Wilson 1998) and insects (Pincebourde et al. 2007; Potter et al. 2009). Females among various animal taxa exhibit spatially or temporally sensitive egg dispersal mechanisms that guarantee a low probability of egg predation or parasitism, such as mites (Yanagida et al. 2001), mosquitoes (Kiflawi et al. 2003; Blaustein et al. 2004), hydrophilid beetles (Brodin et al. 2006), angelfish (Sakai & Kohda 1995) and treefrogs (Binckley & Resetarits 2002; Rieger et al. 2004). Specifically, herbivorous insects may choose to lay eggs on host plants that are less suitable for offspring development but provide a lower risk of predation or parasitism (e.g. enemy-free space: Jeffries & Lawton 1984; Denno et al. 1990; Berdegue et al. 1996; Mira & Bernays 2002; Heard et al. 2006).

Most studies, however, have not examined the finer spatial scale of variation in biotic risk on oviposition site selection. For example, females may discriminate among host species (Mira & Bernays

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2002) or pools (Blaustein et al. 2004) that harbour natural enemies, but there is also a selective advantage to choosing enemy-free areas within an individual location, such as certain leaves (Lucas & Brodeur 1999) or upper parts of a host plant (Gall et al. 2012), or at deeper depths within a single pool (Hirayama & Kasuya 2009). Furthermore, among herbivores that lay their eggs in fruits and seeds or insect parasitoids that oviposit in insects, opportunities for laying eggs are often constrained by the need to place their offspring in or on a discrete host resource of limited quantity (Diaz-Fleischer & Aluja 2003). The temporal and spatial variability in host resources is predicted to have a major effect on the evolution of egg load (Ellers et al. 2000; Harvey et al. 2001) and ovarian dynamics during a female's life span (Papaj 2000). Lastly, fine-scale oviposition decisions may be difficult to observe when resources of high and low value cannot be easily distinguished, but may bear larger-scale consequences, such as shifts in host population growth and species interactions within the community (Werner & Peacor 2003; Schmitz et al. 2004; Fill et al. 2012).

To examine the extent of fine-scale variation in parasitism cues on oviposition behaviour, we chose to study a beetle that deploys modified eggs as protective shields to reduce mortality by egg parasitism. *Mimosestes amicus* lays eggs on the outside of seed pods of legumes, and when exposed to parasitism cues, females superimpose eggs atop one another, shielding bottom eggs in the stack from parasitism by the trichogrammatid wasp, *Uscana semifumipennis* (Deas & Hunter 2012). In previous experiments, we discovered that parasitoid adults trigger the egg-stacking response (Deas & Hunter 2012), but, because these adults started to parasitize beetle eggs as soon as they were introduced, we could not determine whether beetles responded to the parasitoid adults or to the parasitized beetle eggs, or both. We predicted that parasitized eggs would be a reliable cue in nature (and in this experiment), because our casual observations of behaviours of both the parasitoid and the beetle suggested both the deposition of a cue by the parasitoid after parasitizing an egg and the reception of that cue by ovipositing beetles.

We tested whether parasitized eggs triggered the response and then compared oviposition behaviour across laboratory environments that varied in the probability of a female encountering parasitized eggs. We asked two specific questions. (1) Do beetles increase their stacking response (add proportionately more stacks to single eggs and more eggs per stack) when exposed to more parasitized eggs? (2) Do beetles increase their stacking response when parasitized eggs are more dispersed across pods? We predicted that a beetle's stacking response would increase when parasitized eggs were either more numerous or more widely dispersed across pods.

METHODS

Study System

Seed beetles are an ideal system for examining the fine-grained spatial scale of oviposition decisions because they lack parental care (J. B. Deas, personal observation) and thus selection of high-quality oviposition sites can have enormous consequences for offspring fitness (Gall et al. 2012). Additionally, seed beetles require legumes for oviposition that vary temporally and spatially in accessibility (J. B. Deas, personal observation). Finally, and notably, different seed beetle species exhibit ovipositional and egg size plasticity in response to different aspects of habitat quality (Prevedt 1966; Messina & Renwick 1985; Fox et al. 1997; Teixeira et al. 2009; Deas & Hunter 2012).

Mimosestes amicus is a seed beetle (Chrysomelidae: Bruchinae) distributed from the southwestern United States throughout

Mexico and Costa Rica (Kingsolver & Johnson 1978). *Parkinsonia florida* (blue palo verde), *Parkinsonia microphyllum* (foothill palo verde) and *Prosopis velutina* (velvet mesquite) are the host plants most commonly attacked by *M. amicus* populations in central Arizona, but we used *P. microphyllum* pods to maintain laboratory colonies and experiments because these pods confer higher beetle survivorship (J. B. Deas, unpublished data). *Mimosestes amicus* lay eggs and egg stacks directly on seed pods, placing eggs on pods containing seeds. Upon hatching, larvae burrow through the pod and into the seed below, where they develop, pupate and emerge as adults. Eggs may be laid in stacks of two or more eggs. Top eggs protect the bottom egg from parasitism in both laboratory and field settings (Deas & Hunter 2012). Mitchell (1977) observed the stacks and originally speculated that females were responding to risk of parasitism or desiccation of eggs, given his observations of parasitized eggs and unexplained embryonic mortality in top eggs. Our results supported the role of protection against parasitism but not against desiccation; even in the absence of parasitism, all top eggs are smaller and inviable, and larvae die before hatching (Deas & Hunter 2012). Desiccation is not ruled out as a selective pressure involved in the evolution of egg stacking behaviour, but our results suggest that the inviability of these eggs is not due to desiccation. *Uscana semifumipennis* (Trichogrammatidae) is a solitary egg parasitoid that co-occurs with *M. amicus* in southern Arizona and belongs to a genus that parasitizes the eggs of seed beetles (Fursov 1995).

General Methods

During late June of 2010 and 2012, we collected apparently uninfested seed pods from *P. microphyllum* trees in Tucson, Arizona, U.S.A. All pods were used for rearing, but the newest pods (June 2012) were used for experiments. Seed pods were stored at -20°C to exterminate larvae of *M. amicus*, and lethal, bruchid parasites such as the straw itch mite, *Pyemotes tritici* (Southgate 1979). Beetles and wasps used in experiments were descended from individuals collected in early to mid-August of 2010 and 2011 and were reared on stored seed pods. Laboratory populations were reared at 30°C , 50% relative humidity. Emerging female and male beetles were collected from laboratory populations and kept in breeding containers for 1–2 days before being used in experiments. Seed pods of *P. microphyllum* vary between one and three seeds per pod, so except for each of three replicates in which we had to use one two-seed pod and three one-seed pods, we used only one-seed pods in our experiments. The egg parasitoid *U. semifumipennis* used in experiments originated from parasitized eggs of *M. amicus* collected in the field, which were reared in the laboratory on eggs of *Callosobruchus maculatus*, which were, in turn, reared on cowpea seeds, *Vigna unguiculata*. After emergence, wasps were kept in 100 mm test tubes at 12°C and 65% relative humidity with drops of honey until needed for experiments.

Do parasitized eggs alone trigger the stacking response?

To produce parasitized eggs for experiments, we collected adult beetles as they emerged, allowed the females to mate and lay eggs for 48 h, and then exposed approximately 75% of each female's eggs to 1–3-day-old *U. semifumipennis*. The remaining 25% of the eggs were untouched and used as a control for the female's response to the presence of conspecific eggs ($N = 47$ sets). Seed pods bearing parasitized eggs were then split into two treatments. Parasitized eggs were either left intact ($N = 23$ sets), or removed to control for the presence of cues left on the seed by the female ($N = 46$ sets). Eggs were removed in this treatment in order to distinguish between the females' responses to parasitism cues associated with the eggs themselves and their possible responses to cues left by the

adult parasitoid or parasitized egg on the seed pod. We used approximately 35 parasitized eggs per set. Experimental beetles were 1 day old. The experiment was run for 10 days. In each replicate of each treatment, five pods were enclosed in a 60×15 mm petri dish with a single mating pair of beetles. Halfway through the experiment, parasitoids were ready to emerge from the parasitized eggs used in the experiment, so we replaced these eggs with pods bearing newly parasitized eggs. Newly laid beetle eggs were left in place and counted at the end of the experiment.

Does number or dispersion of parasitized eggs influence oviposition behaviour?

To produce parasitized eggs for experiments, we repeated our method from the first experiment. We established 20 mating pairs per treatment using 1-day-old beetles. Experimental beetles were inspected for up to 2 days to ensure they were laying only single eggs at the start of the experiment. Females were then assigned to one of six treatments and placed into 60×15 mm petri dishes with five pods, which differed according to the number, distribution and condition of beetle eggs (Fig. 1): (1) no eggs ($N = 16$); (2) 10 parasitized eggs on 20% of pods (i.e. all on one pod; $N = 19$); (3) two parasitized eggs on 20% of pods (i.e. two on one pod; $N = 21$); (4) six parasitized eggs on 60% of pods (i.e. two each on three pods; $N = 12$); (5) 10 parasitized eggs on 100% of pods (i.e. two per pod; $N = 19$); and (6) 10 unparasitized eggs on 100% pods (i.e. two per pod; $N = 19$). To test whether the number of parasitized eggs encountered per pod influences the stacking response of beetles, we held egg dispersion constant (20% of pods) and compared the number of parasitized eggs (10 versus 2 eggs) per pod. To test whether beetles would increase the stacking response when parasitized eggs were more evenly dispersed across pods, we controlled egg number (10 eggs) and compared eggs that were clustered (all on one pod, 20% of pods) versus evenly spread (two per pod, 100% of pods). We also compared the stacking response among treatments that varied in both parasitized egg number and dispersion across pods, while controlling for egg number per pod (two parasitized eggs on 20% pods versus six parasitized eggs on

60% of pods versus 10 parasitized eggs on 100% of pods). Finally, the no-egg and unparasitized egg treatments served as control conditions in which parasitism cues were absent. Beetle cages were checked at 4, 8, 12, 24, 36 and 48 h (0800–0800 hours) after exposure of females to the various treatments. Recorded data included the oviposition rate, stacking rate, numbers of eggs in a stack and the number of newly laid beetle eggs per pod.

Statistical Analyses

Logistic regression was used to analyse the proportion of stacks laid by beetles across parasitism cue treatments (no eggs, parasitized eggs present, parasitized eggs removed), while a univariate ANOVA was used to compare the average number of eggs per stack across treatments. A GLM (generalized linear model) repeated measures analysis was performed to assess changes in stacking rate and oviposition rate over time in the experiment where parasitized egg number and distribution were manipulated. The variances in stacking rate and oviposition rate among time points were not equal (i.e. did not meet the assumption of sphericity and increased type I error rate), so we applied the Greenhouse–Geisser correction to each analysis, which adjusted our degrees of freedom and validated our F ratio for within-subject (time, time \times treatment) effects. We determined statistical significance among the mean oviposition rates of each treatment (eggs/day) using Tukey–Kramer multiple comparisons procedure by ANOVA: no eggs; two parasitized eggs on 20% pods; 10 unparasitized eggs on 100% of pods; 10 parasitized eggs on 20% pods; six parasitized eggs on 60% of pods; and 10 parasitized eggs on 100% of pods. We then compared the number of newly laid beetle eggs per day among the following treatments: (1) 10 versus two parasitized eggs on 20% of pods; (2) 10 parasitized eggs on 100% versus 20% of pods; (3) two parasitized eggs on 20% pods versus six parasitized eggs on 60% of pods versus 10 parasitized eggs on 100% of pods. All of these analyses were performed using IBM SPSS v.21 (IBM Corp., Armonk, NY, U.S.A., 2012).

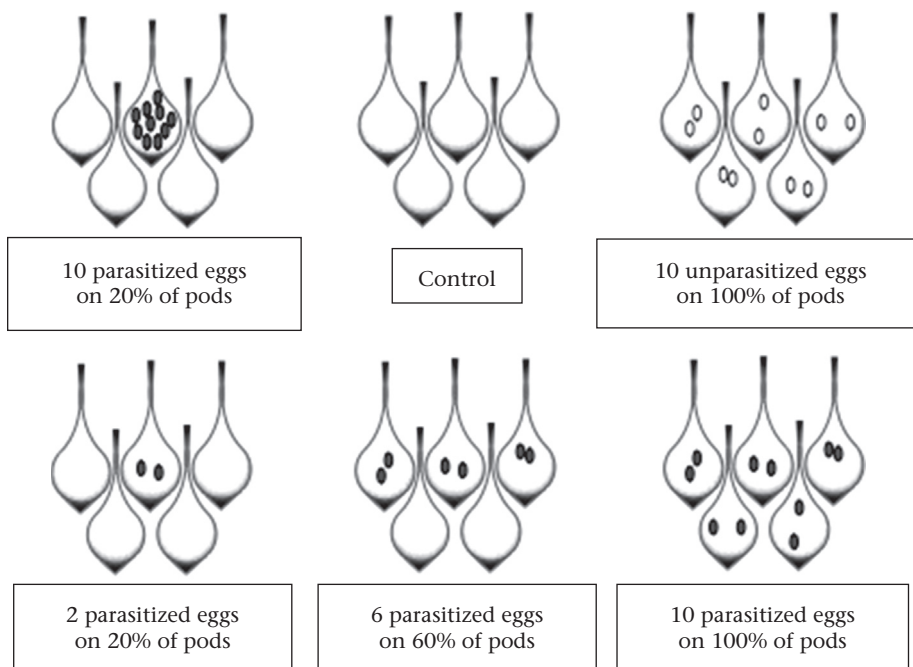


Figure 1. Spatial arrangement of seed beetle eggs on foothill palo verde seed pods in each treatment.

Seed beetles are well known to distribute eggs evenly among seeds, presumably to reduce larval competition per seed. This has been observed for *M. amicus* (J. B. Deas, unpublished data) as well as for other seed beetle species (Avidov et al. 1965; Mitchell 1975; Wright 1983; Messina & Renwick 1985; Messina & Mitchell 1989; Shimada & Ishihara 1990). If females distribute their eggs evenly, then 20% of the eggs should have been laid on each of the five pods. We used the above data to measure how females disperse eggs among seed pods in response to treatments that had mixtures of fresh pods and pods bearing parasitized eggs. We performed another experiment in which we measured females' responses to the same dispersion treatments (10 eggs on 20% of pods, two eggs on 20% of pods and six eggs on 60% of pods) but using unparasitized conspecific eggs. We then performed goodness-of-fit tests to test the null prediction that the observed and expected distributions of eggs were statistically similar. We performed these analyses using IBM SPSS v.21.

RESULTS

Egg Stacking in Response to Parasitized Eggs

Parasitized eggs alone were sufficient to induce the egg-stacking response in *M. amicus*. Beetles stacked more of their eggs in the presence of parasitized eggs (0.62) than in the presence of unparasitized eggs (0.02) (logistic regression: $\chi^2_{150} = 221.03, P < 0.0001$; Fig. 2). Low levels of stacking (0.11) also occurred in the treatment where parasitized eggs were removed (Fig. 2). In addition, although almost all stacks consisted of two eggs across treatments, beetles in the parasitized egg treatment were more likely to lay two or more protective eggs per stack (1 cover egg = 0.905; 2+ cover eggs = 0.095) than when there were no eggs present (1 cover egg = 0.994; 2+ cover eggs = 0.006) or when the parasitized eggs had been removed (1 cover egg = 0.995; 2+ cover eggs = 0.005; ANOVA: $F_{2,80} = 8.68, R^2 = 0.18, P < 0.0001$; Fig. 2).

Oviposition Behaviour in Response to the Number and Distribution of Parasitized Eggs across Pods

All of the female beetles laid single eggs prior to the experiment, but exposure to an environment in which 100% of pods bore parasitized eggs led to a rapid transition to egg stacking (Fig. 3).

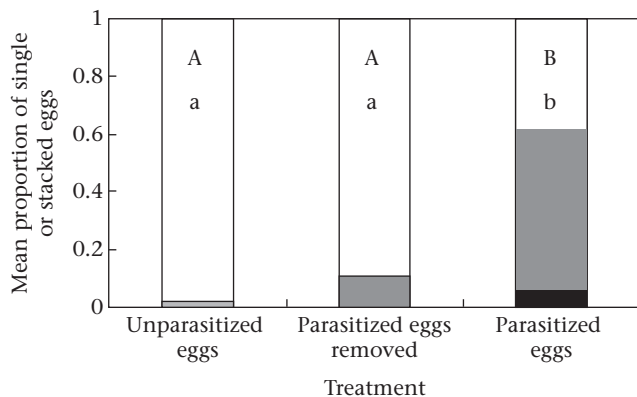


Figure 2. Mean proportion of single eggs or stacked (cover) eggs laid by female seed beetles in response to the presence or absence of parasitized eggs: unparasitized eggs ($N = 47$); parasitized eggs removed ($N = 23$); parasitized eggs ($N = 46$). □: single eggs; ■: 1 egg cover; ■: 2+ egg covers. Letters indicate statistical differences between treatments in the mean proportion of stacks (uppercase) and the number of eggs laid per stack (lowercase) using Tukey–Kramer multiple comparisons procedure based on an ANOVA.

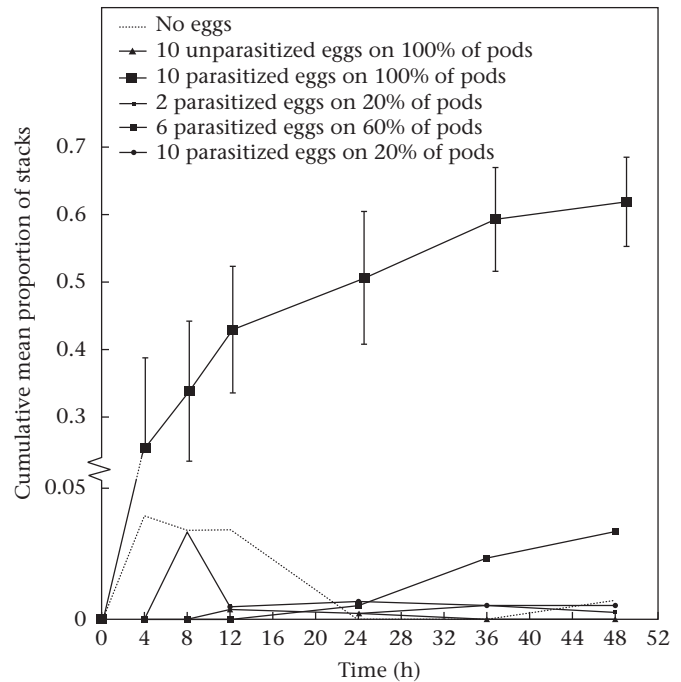


Figure 3. Cumulative mean proportion of egg stacks produced by female seed beetles during the first 48 h of exposure to various treatments. Error bars are shown only for the treatment with 10 parasitized eggs on 100% of pods (top curve: the line break on the Y axis and the associated dotted portion of the curve for this treatment are used to magnify differences between the remaining treatments).

Within 8 h, 10% of oviposition events were stacks, and this rate rose to 34% by 36 h. In contrast, the rate of stacking in the other treatments was near zero throughout the experiment (treatment effect for GLM repeated measures: $F_{5,72} = 15.7, P < 0.0001$; time effect: $F_{2,148} = 11.16, P < 0.0001$; time \times treatment effect: $F_{10,148} = 4.77, P < 0.0001$; Figs 3, 4).

There were strongly significant effects of treatment (GLM repeated measures: $F_{2,101} = 24.96, P < 0.0001$) and time ($F_{2,241} = 394.42, P < 0.0001$; Fig. 5) on mean oviposition rate, and a significant interaction between time and treatment ($F_{12,241} = 9.35, P < 0.0001$; Fig. 5). A comparison of mean daily oviposition rates revealed that beetles laid fewer eggs per day when they encountered more parasitized eggs on the same pod (10 versus two parasitized eggs on 20% of pods), the same number of parasitized eggs dispersed among more pods (10 parasitized eggs on 100% versus 20% of pods) and more pods with parasitized eggs (two parasitized eggs on 20% pods versus six parasitized eggs on 60% of pods versus 10 parasitized eggs on 100% of pods). We determined statistical significance between treatment means using Tukey–Kramer multiple comparisons procedure: no eggs (A), two parasitized eggs on 20% pods (AB), 10 unparasitized eggs on 100% of pods (AB), 10 parasitized eggs on 20% pods (BC), six parasitized eggs on 60% of pods (CD) and 10 parasitized eggs on 100% of pods (D).

Beetles also avoided laying eggs on pods containing parasitized eggs. If newly laid eggs are distributed evenly, then 20% of the eggs should have been laid on each of the five pods. However, for each treatment evaluated, the observed distribution of newly laid eggs differed significantly from the expected uniform distribution (10 parasitized eggs on 20% of pods, 1.98% observed versus 20% expected: $\chi^2_1 = 102.24, P < 0.0001$; two parasitized eggs on 20% of pods, 5.95% observed versus 20% expected: $\chi^2_1 = 101.48, P < 0.0001$; six parasitized eggs on 60% of pods, 15.65% observed versus 60% expected: $\chi^2_1 = 214.73, P < 0.0001$; Fig. 6). This result is

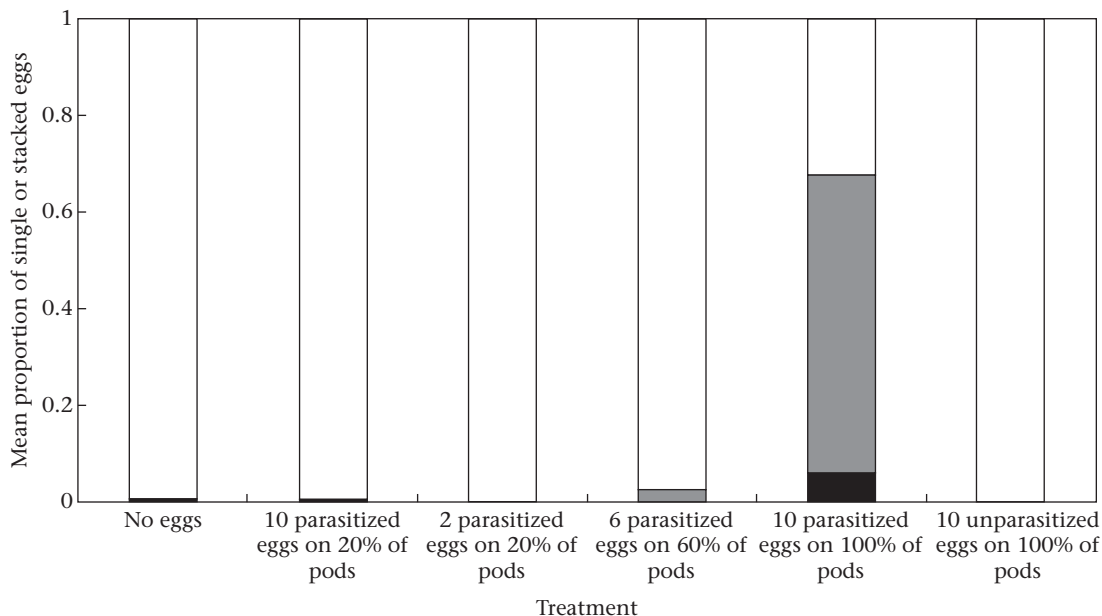


Figure 4. Mean proportion of eggs laid by female seed beetles within 48 h as protection (1 or 2+ egg covers) against parasitoid wasps in each treatment. □: single eggs; ■: 1 egg cover; ▒: 2+ egg covers.

in contrast to our experiment with unparasitized conspecific eggs, where the observed distribution of eggs did not differ from the expected uniform distribution for two parasitized eggs on 20% of pods (14.47% observed versus 20% expected: $\chi^2_1 = 1.451$, $P = 0.288$) and six parasitized eggs on 60% of pods (48.48% observed versus 60% expected: $\chi^2_1 = 1.82$, $P = 0.177$). Beetles laid fewer eggs than expected when they were exposed to a high density of eggs on one seed pod (10 parasitized eggs on 20% of pods, 5.13% observed versus 20% expected: $\chi^2_1 = 16.17$, $P < 0.0001$).

DISCUSSION

The temporal and spatial variability in oviposition resources has a major effect on oviposition behaviour and ovarian development during a female’s life span (Papaj 2000). Likewise, abiotic and biotic factors that increase offspring mortality have been a source of selection for females to evolve mechanisms to increase offspring survival. Here, we studied how fine-scale variation in parasitism

cues influences offspring protection by female seed beetles. We found, unexpectedly, that female *M. amicus* use a flexible, tripartite oviposition strategy for reducing parasitism risk to their offspring. After encountering parasitized conspecific eggs, females began egg stacking, but primarily when parasitized eggs were encountered on every pod in their environment. Otherwise, females appeared to delay oviposition and/or simply avoided ovipositing on hosts with parasitized eggs. Eggs are potentially a very costly resource to use for purposes other than creating viable offspring (Perry & Roitberg 2006), so we might expect beetles to have evolved sensitive behavioural mechanisms to minimize the energetic costs of defensive egg laying.

Parasitized Eggs Trigger Egg Stacking

We found that seed pods with parasitized eggs were sufficient to elicit egg stacking by female beetles, even in the absence of parasitoid individuals searching to oviposit (Fig. 2). The nature of cues

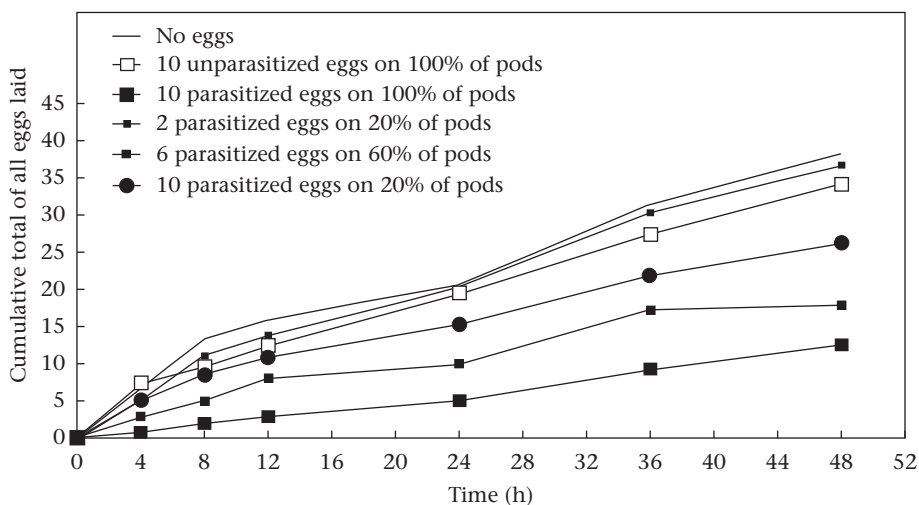


Figure 5. Mean oviposition rates of seed beetles across treatments.

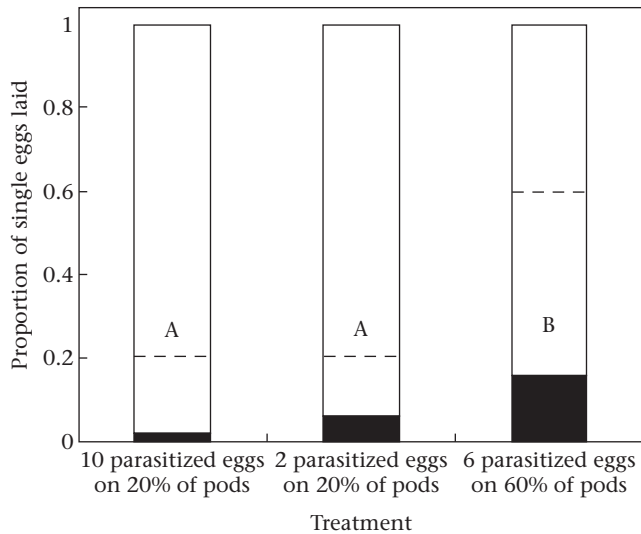


Figure 6. Proportion of single beetle eggs laid on pods with (solid bar) and without (open bar) parasitized eggs when both the number and distribution of eggs across pods were varied. The dotted line in each bar indicates the null expectation of the proportion of eggs laid on pods bearing parasitized eggs if beetles laid eggs uniformly. Letters indicate statistical differences in mean proportions across treatments (using Tukey–Kramer multiple comparisons procedure).

left by parasitoid adult wasps, or produced by their developing offspring is unknown, but casual observations of wasp oviposition behaviour suggest that adult females may mark eggs they have parasitized. After parasitizing an egg, *U. semifumipennis* females rub their hind tibia against their abdomen as they walk over the egg. Parasitoid females often discriminate between parasitized and unparasitized hosts through the detection of marking pheromones deliberately left by an ovipositing female, which serves to reduce superparasitism (Vinson 1976; Bakker et al. 1985). We have also observed that female beetles in turn thoroughly walk over pods before ovipositing. When they encounter another beetle egg, females briefly drum it with their maxillary palps before continuing to walk over the pod. It has been shown previously that in *Callosobruchus* seed beetles, females primarily use their maxillary palps to discriminate between seeds with and without eggs (Messina et al. 1987b) as well as between different host legumes (Messina et al. 1987a). Given our observations and work on related species, it seems likely that beetles may also use their maxillary palps to detect compounds left by parasitoid wasps on previously laid beetle eggs.

Cues from eggs are often a source of important information across taxa. They may permit recognition of eggs sired by male sticklebacks that might otherwise cannibalize conspecifics (Frommen et al. 2007), trigger competitive behaviour in male squid by indicating female receptivity (Buresch et al. 2003; King et al. 2003), orient egg predator sculpins towards their prey (Dittman et al. 1998; Mirza & Chivers 2002), or induce defensive pathways against herbivores in pine trees (Hilker et al. 2002). Brood parasitism may be detected in host birds, in which females may use contrasting areas of the egg (Polacikova et al. 2007) or differences in UV colour reflectance (Avilés et al. 2004) to discriminate cuckoo eggs from their own. We are, however, unaware of another example of a conditional strategy in which mothers detect conspecific egg parasitism and use that information to delay oviposition, to avoid ovipositing near parasitism cues, or to defend the eggs they lay. Lastly, we note that we did not test whether the presence of adult wasps in addition to parasitized eggs would have enhanced the stacking response. Recent results indicating that *Drosophila* may avert risk associated with visual detection of parasitoid wasps

(Kacsoh et al. 2013) suggest this would be an interesting possibility to test in the *M. amicus*–*U. semifumipennis* system.

Ovipositional Response to Seed Pod Variation in Parasitism Cues

When exposed to host pods that each bore parasitized eggs, female beetles began stacking their eggs within 4 h, and continued to increase stacking until 36 h, after which the rate stabilized at about 60% (Fig. 3). Although egg stacking started rapidly, and could be easily induced by the presence of parasitized eggs, it appeared to be a last resort for beetles that had no alternative oviposition site. Beetles stacked little or not at all unless every seed pod bore parasitized eggs. In treatments where clean pods were present, the very few eggs produced (three across all replicates) were laid only on seed pods with parasitized eggs (J. B. Deas, unpublished data).

Relative to other experiments conducted in our laboratory with different individuals, and in contrast to our observations in the field (where the proportion of stacking may reach 71%; Deas & Hunter 2012), the average rate of stacking across the entire experiment was modest (11.3%). It is likely that beetles in our previous experiments encountered proportionately more pods that bore parasitized eggs, due to the consistent laying of fresh eggs and the presence of wasps to parasitize them. Furthermore, in another study, we found that beetles stacked more as they aged when limited host access increased their egg load (J. B. Deas & M. S. Hunter, unpublished data). In other systems, females with low host access still have eggs by the time of death (i.e. time limitation) and switch to laying larger clutches per host (Iwasa et al. 1984; Parker & Courtney 1984; van Alphen & Visser 1990; Ellers et al. 2000; Xu et al. 2012). In contrast, in the present study, we used newly mated beetles with unlimited access to hosts; thus, we expected the beetles to be more egg limited than time limited and to stack relatively fewer eggs (J. B. Deas & M. S. Hunter, unpublished data). Lastly, the reduction in oviposition rate that we observed might be age dependent. A delay in oviposition is also likely to increase egg load, inflicting a reproductive cost to beetles by imposing a time limitation, but this cost may be offset by the benefit of the delay.

Exposure to parasitized eggs slowed the rate of beetle oviposition, with the greatest reduction evident when every seed bore parasitized eggs, and the least reduction seen in treatments with no eggs, unparasitized eggs or only two parasitized eggs present on a single pod (Fig. 5). The treatment in which oviposition rate was most depressed, when all pods bore parasitized eggs, was also the treatment that showed the highest rate of egg stacking. Thus, one might question whether the reduction in oviposition rate is due to the time and resource costs associated with stacking eggs, or a second strategy adopted by beetles when the probability of egg parasitism is high. That there was a reduction in oviposition rate even in the treatment with virtually no stacking (10 parasitized eggs on 20% of pods; Figs 3, 4) supports the latter explanation. In general, we might predict reductions in activity in response to predator or parasite risk among victims, as this would conserve energy and lessen the risk of being detected by exploiters. However, plasticity in oviposition rate in response to natural threats has only recently been documented in *Neoseiulus*, a phytoseiid mite (Montserrat et al. 2007), and *Drosophila* (Lefèvre et al. 2012). This dampening of oviposition rate is likely to be cryptic to an observer and may in fact be much more common than has been documented to date.

In addition to delaying oviposition, *M. amicus* beetles also actively avoided pods with parasitized eggs (Fig. 6). In general, seed beetles lay eggs uniformly among hosts (Avidov et al. 1965; Mitchell 1975; Wright 1983; Messina & Renwick 1985; Messina & Mitchell 1989; Shimada & Ishihara 1990), but in our study, after 48 h, beetles laid significantly fewer eggs than expected on pods with

parasitized eggs and more eggs than expected on pods with no eggs (Fig. 6). This result is in contrast to our experiment with unparasitized conspecific eggs, where the observed distribution of eggs did not differ from the expected uniform distribution for our treatments (two parasitized eggs on 20% of pods versus six parasitized eggs on 60% of pods). However, female beetles laid fewer eggs than expected in the treatment with 10 parasitized eggs on 20% of pods, suggesting that they also avoid seed pods with unparasitized eggs, but to a lesser degree. Females in our study allocated eggs to fresh pods in close proximity to pods with parasitized eggs in small arenas, suggesting a fine-grained spatial scale of oviposition decisions by this species.

Our results show that females assess the probability of encountering a parasitized egg on a per-pod basis. If parasitism cues were present on one pod, females attempted to lay on a 'clean' pod nearby; if there were few or none of these pods, then they started to stack eggs. We did not control the distances among pods within the small containers provided in this study, nor did we organize the pods in three dimensions. In nature, we do not know whether beetles would ordinarily move farther from the parasitism cue before ovipositing than they did in the laboratory. Depending on the collection site (and even the tree), foothill palo verde seed pods may be organized in tight clumps of pods with one to six seeds per pod, and they may be closer together or farther apart depending on how many pods have shed their seeds or fallen to the ground. Given the variety of legume hosts that *M. amicus* uses, it is likely that the spatial complexity of the oviposition response we observed may be nested within more complexity at larger spatial scales. *Mimosstes amicus* uses 22 species in five to six legume genera (Kingsolver & Johnson 1978), over which there is substantial variation in the number of seeds per pod and the number of pods that group together on a branch. When seeds are nested within large pods, do beetles extrapolate from the presence of parasitized eggs on one seed to make decisions about the rest of the seeds in the pod? Furthermore, does seasonal variation in host diversity and variation in egg parasitism patterns among tree species, populations, or even trees cause beetles to seek enemy-free space on other host plants (Heard et al. 2006)?

In conclusion, these results, along with other recent studies, support the emerging understanding that solitary insect hosts that do not exhibit parental care may use a sophisticated means of assessing risk and protecting offspring. On a larger scale, oviposition avoidance behaviours may represent nonconsumptive effects imposed by natural enemies, in which natural enemy presence causes a costly change in host (or prey) behaviour, physiology or life history, and influences the growth of the host population, which affects the larger community (Werner & Peacor 2003; Schmitz et al. 2004; Fill et al. 2012). Investigating the stacking strategy at larger spatial scales among seed beetles using the same hosts (e.g. *Stator limbatus*: Johnson & Kingsolver 1976; *Mimosstes ulkei*: Kingsolver & Johnson 1978) would begin to address some of these issues.

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